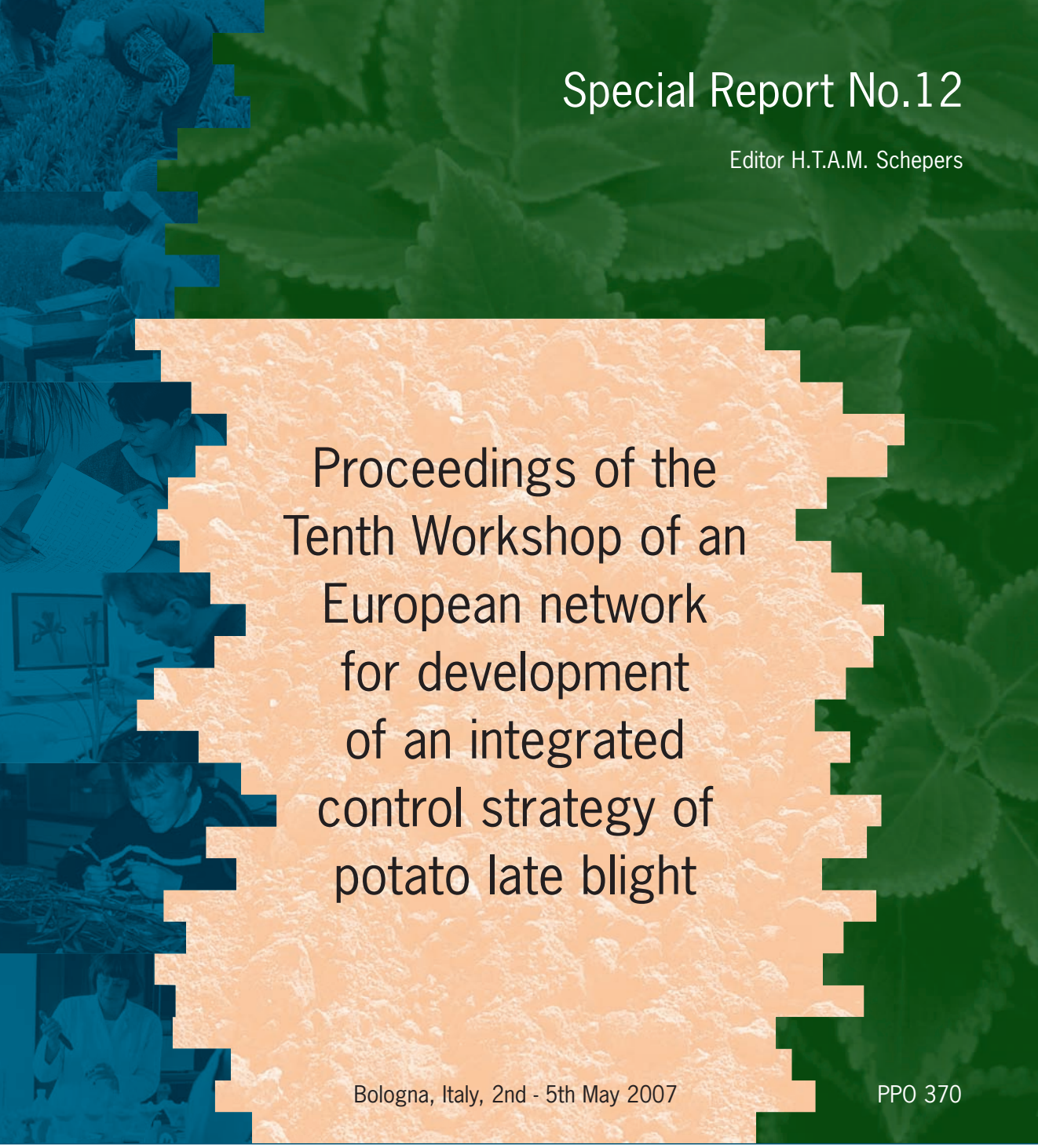

Special Report No.12

Editor H.T.A.M. Schepers

Proceedings of the Tenth Workshop of an European network for development of an integrated control strategy of potato late blight

Bologna, Italy, 2nd - 5th May 2007

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Preface

Integrated control of potato late and early blight

A Concerted Action entitled "European network for development of an integrated control strategy of potato late blight (EU.NET.ICP)" encouraged participants to a yearly Workshop. After four years and four Workshops (Proceedings comprised in four PAV-Special Reports: 1, 3, 5 and 6) the Concerted Action came to an end, but through enthusiastic participants and sponsoring by companies active in late blight control the series of Workshops continued. In 2000, 2001, 2002, 2004 and 2005 the fifth, sixth, seventh, eighth and ninth Workshop were organised in Munich (Germany), Edinburgh (Scotland), Poznan (Poland), Jersey (Channel Islands) and Tallinn (Estonia). The Proceedings of these Workshops are published in PPO-Special Reports (7, 8, 9, 10 and 11). The Proceedings are now also available on the internet www.EuroBlight.net.

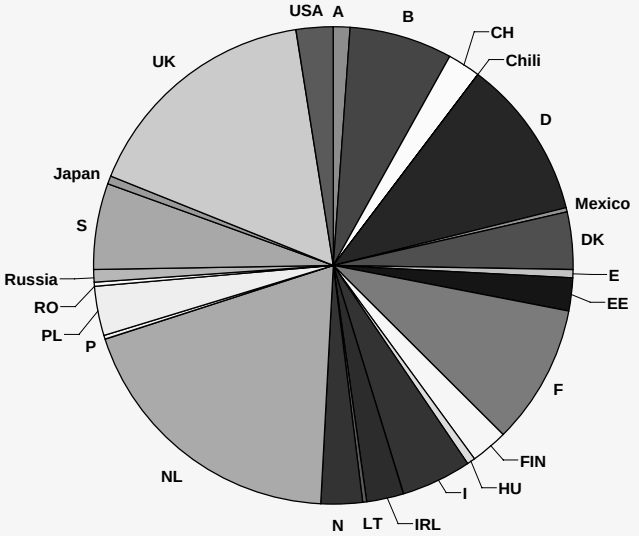
The Plant Protection Service of Regione Emilia-Romagna and the Department of Agri-food Protection and Improvement (Diproval) of the Bologna University organised the tenth Workshop in Bologna, Italy from 2-5 May 2007.

BASF, Belchim, Bayer, Certis, Dacom, DuPont, Germicopa, Nissan and Syngenta sponsored the Workshop.

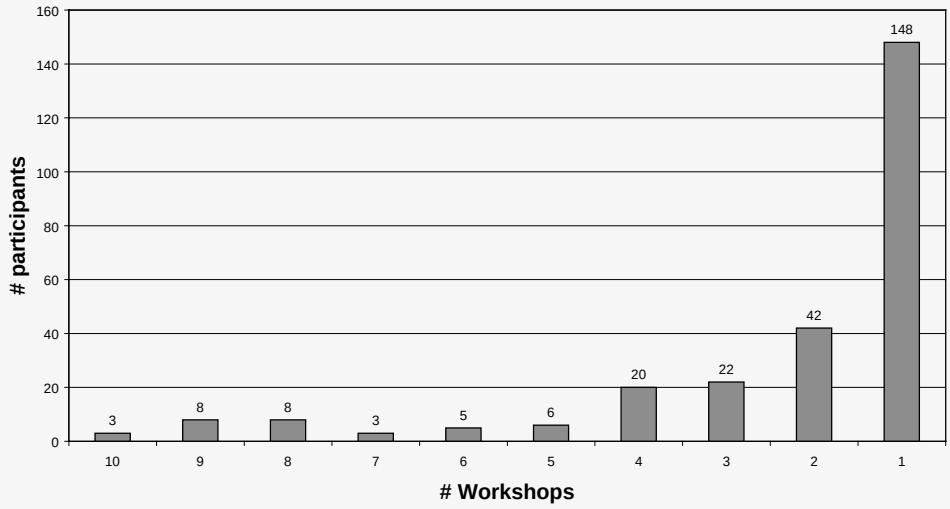
The Workshop was attended by 94 persons from 16 European countries, the United States of America, Chile, Russia and Japan. Representatives from all countries presented the late blight epidemic in 2006 and recent research results regarding integrated control, decision support systems, resistance of varieties and population biology of the late blight pathogen in potatoes. Since early blight seems to be an increasing problem in Europe, also reports on this disease are included.

In Figure 1 and 2 the participation in the 10 Workshops is visualised.

Number of participants per country in 10 Workshops



Frequency of Workshop participation



The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, PPO-Special Report no. 12.
For further information please contact the network secretariat where also additional copies of this Proceedings can be ordered.

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Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

The development and control of *Phytophthora infestans* in Europe in 2006

JENS GRØNBECH HANSEN (DENMARK)[1], BJÖRN ANDERSSON (SWEDEN), RUAIRIDH BAIN (SCOTLAND), GOTTFRIED BESENHOFER (AUSTRIA), NICK BRADSHAW (ENGLAND & WALES), LINDA BUCENA (LATVIA), RICCARDO BUGIANI (ITALY), EMEL ÇAKIR (TURKEY), LOUISE COOKE (NORTHERN IRELAND), LUDOVIC DUBOIS (FRANCE), ALEXEY FILIPPOV (RUSSIA), ASKO HANNUKKALA (FINLAND), HANS HAUSLADEN (GERMANY), ERVIN HAUSVATER (CZECH REPUBLIC), JAN HELDAK (SLOVAKIA), ARNE HERMANSEN (NORWAY), JOZEFA KAPSA (POLAND), MATI KOPPEL (ESTONIA), RENATA LEBECKA (POLAND), ALISON LEES (UK), TOMKE MUSA (SWITZERLAND), WIM NUGTEREN (THE NETHERLANDS), ANTANAS RONIS (LITHUANIA), HUUB SCHEPERS (THE NETHERLANDS), HARRO SPITS (THE NETHERLANDS), PIETER VANHAVERBEKE (BELGIUM)

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Introduction

During the previous EU.NET.ICP workshops, each country briefly presented an overview on late blight disease development, fungicide use and control. Before the Bologna workshop it was decided to survey the late blight situation in Europe before the workshops and then do one review presentation. Recently it was also proposed that the European late blight surveys and the GILB country profiles should be integrated. The web application for generating the Euroblight late blight country profiles was therefore launched in 2007 to keep track of the development of late blight and its control in Europe in individual countries and over years. All of the information collected was analysed in the context of Euroblight and was presented and discussed at this Euroblight workshop. Euroblight is available at www.euroblight.net.

Methods

One or more editors are appointed for each country. They upload information to the Euroblight database via a dedicated web-page on the restricted side of the Euroblight website. The country profiles have the following structure and content:

PPO-Special Report no. 12 (2007), 13 - 25

Summary

- Write a short summary (max 200 words) about late blight development, fungicide use and control of late blight in the country and year selected. This section will be used to generate a summary report covering all countries. Additionally, this will be the starting point for the summary report about late blight, fungicide use and effectiveness of control measures, published after each Euroblight workshop.

Early outbreaks of potato late blight

- Select the date of first observation of late blight in covered or very early planted potatoes
- Disease source for these attacks (options: Seed, Cull pile, Volunteer plants, Covered crop, Waste pile, Oospores, Indications of Oospores, Other, Not known)
- Select the date when first infections were reported in more than 5 conventional, normally planted potato fields. This is the date when late blight is recorded in more than a few fields for the first time. After this event – and if the weather is continuously blight favorable - there will be a risk of epidemic developments in non-treated (and especially in susceptible) cultivars.
- Disease source for these attacks (options: Seed, Cull pile, Volunteer plants, Covered crop, Waste pile, Oospores, Indications of Oospores, Other, Not known)
- Write a short text (max 100 words) about early attacks. The report generator will include dates and disease sources in texts. Enter additional information in the text window (press Save button and select 'Show country report' to see how Euroblight has generated text sections)

Weather conditions and late blight development

- Weather based risk of late blight. Select whether the weather-based risk for late blight development was low, medium or high for the months May to September. Or, select 'Not known'.
- Write a short text (max 100 words) about the weather conditions related to late blight development. Mention if the information about weather conditions is general for the country, related to a specific region and if the risk is qualitative or based on calculations with a model or a DSS.

Use of fungicides and control strategies

- Enter the number of fungicide applications used in ware potatoes. What do the majority of conventional farmers do to control late blight in ware potatoes?
- Enter the number of fungicide applications used in all potatoes. Sometimes quantitative information is available as a mean of all types of potatoes e.g. in DK as calculated Treatment Frequency Index based on amounts of fungicide sold (normal dosage) and related to the total area of conventional grown potatoes
- Write a short text (max 100 words) about fungicide use and control of late blight.

Organic potatoes

- Select when outbreaks were recorded in fields with organic potatoes (Options: early, medium, late or not known compared to normal)
- Select the level of attack (Options: low, medium, high or not known compared to normal).
- Select the mean yield level in organic potato fields (Options: <20 t/ha, 20-30 t/ha, 30-40 t/ha, >40 t/ha or not known)
- Write a short text (max 100 words) about the situation in organic potatoes.

Tuber blight
- Select the level of tuber blight attacks (Options: low, medium, high or not known compared to normal).
- Write a short text (max 100 words) about tuber blight.

Alternaria spp
- Select when outbreaks were recorded (Options: early, medium, late or not known compared to normal).
- Select the level of attack (Options: low, medium, high or not known compared to normal).
- Write a short text (max 100 words) about Alternaria.

Characteristics of *Phytophthora infestans*
- Write a short text (max 100 words) about pathogen characteristics.

Use of cultivars
- Write a short text (max 100 words) about use of cultivars.

Several questions are associated with predefined options for answers. This is helpful in generating harmonized and comparable information. Storing each section separately in a database makes it possible to generate reports by country but also by subject. Options in the first instance will be country reports with all information from one country in one report, a summary report with all summary sections from all countries in one report and a weather report including all country sections about weather conditions and late blight development, and a graph of the seasonal risk of late blight for all countries. These features are only possible when the database approach is used. At present most information will be subjective and qualitative. Ways to generate, organize and integrate more objective and quantitative information will be discussed at a later stage with GILB. One important motivation for partners to upload data into the Euroblight database will be that the results are analysed and presented "on a real time basis" in a pan-European context, and the reports can be edited dynamically by a group of editors. When data are available over more years, it will be possible to analyse the data across years and between countries.

Country reports for the 2006 growing season

The reports published below are taken directly from the database with only slight editing. The information and descriptions are the same as can be found on the Euroblight web site under the menu item 'Summary report'. More detailed information in national reports can be found under the menu item 'Country reports'.

Turkey
The disease level was low in 2006 as compared with 2005. Ullrich Schrödter and Winstel models were tested in this region in 2005 and again in 2006. The plant emergence was completed on 15 May in 2006. After first warning of model Winstel on June 7, the spores of disease were determined on suspicious plants collected from some neighbouring fields of our research area in the laboratory analyses while the disease could not be determined during the field observations after first warnings in the previous years. The reason of seeing the infections in second warnings, most probably, might be the result of low spore densities in the selected areas for research. The spores spreading very large area

infected the potato research areas from neighbouring fields after reaching a certain density during the second warning. The Winstel model gave four warnings in 2006 and the temperature and humidity conditions were favourable for the disease during this period. Therefore, farmers were warned for fungicide applications. The studies were conducted in the areas of ware potato, and the fungicide applications were made by using Trooper 72 WP, Dithane and Ridomil MZ. The season concerning harvest being three weeks after application began on July 24, 2006 and completed on August 31, 2006. The potato cultivars Marfona, Agria and Satina were used in this study. According to our knowledge the early potato cultivars have been more sensitive than the other in our locality. In the laboratory studies conducting to determine the mating types, both mating types A1 and A2 were found in our region.

Italy
Potato emergence in Northern Italy was earlier than previous year, from 20/4 to 5-7/5 for early and late varieties respectively. First outbreaks were recorded on unsprayed plots on 20/5 while on commercial plots on 24-25/5. Disease pressure was low in the North, but higher in the south and Islands. Control of the disease was easy to achieve in the north while problems occurred in the south with yield losses ranging from 10 to 20 %.

Switzerland
In 2006, with the exception of the southern part, springtime was cold and wet in Switzerland. Therefore many potatoes were planted relatively late in the beginning of May. Eighteenth of May, the first LB-outbreak was recorded in the western part of Switzerland in an early planted potato field. Due to rather conducive weather conditions the LB-epidemic spread over the main potato growing regions until beginning of June. During June and July weather was very dry and LB-pressure was reduced. The August was very wet and LB-pressure increased again. Often LB attacks occurred late in the fields and destroyed the haulm very fast. Due to rainy weather, harvest conditions were unfavourable and several cases with heavy tuber blight attacks were observed. However, compared to previous years, LB-pressure was rather low in 2006.

Austria
In Austria the weather conditions 2006 were very variable. Days with intensive rains at the end of May, end of June und beginning of August were followed by sunny and rather dry weeks. Due to the fact that the heavy precipitation was followed by dry weather the first symptoms of late blight occurred only on a few sites between beginning of June and beginning of July only on a low level. The weather based risk increased then from beginning to end of August to a high level - infections of late blight in some middle to late cultivars occurred. In Austria on average 7 fungicides applications were used (range 4 - 10) to control late blight.

Germany
The second half of May was very cold and wet. The first outbreak of late blight was observed beginning of May (early planted and covered potatoes). June and July was very dry and hot. There was no further development of late blight during this period. The weather changed at the end of July. The August was very wet. The disease pressure was very high in August. The weather changed again and the September was dry. The harvest conditions were good. The high disease pressure in August in combination with the occurrence of *Phytophthora infestans* resulted in tuber blight in many cultivars.

France
The country 2006 is marked by a rather strong epidemic at the level of the mildew in the zones of

productions of the big North, with sometimes difficulties of control of the disease. The location of treatments, the choice of the product as well as the conditions of realization of these influenced the control of the disease a lot. The evolution of the origins of late blight also confirms this.

Belgium

Unusual cold temperatures in March and the beginning of April led to delayed planting of early crops; most of the fields were planted during the second half of April. Between April 27 and May 10 a lot of diseased plants were found on dump piles. Combined with the unstable weather conditions in May, the (very) wet soil and sometimes strong winds, the disease risk for emerging crops was very high. By June 5 late blight attacks were reported both in early crops and ware potatoes. The rest of this month, however, weather conditions were mostly sunny and dry, leading to a welcome break in the disease pressure. The tropical summer in July brought the disease to a standstill. The increasing soil water deficit caused a lot of fields to mature early. The summer got a ducking in August, with showers and high rainfall and - consequently - very high disease pressure. In the second half of this month a lot of disease outbreaks were reported and a permanent high protection level was necessary, but not always possible. The problem of infected tubers, however, was overshadowed by the poor quality due to second growth, and the difficult storage of the crop.

Netherlands

The time of planting was rather average in 2006. At the end of April all potatoes had been planted. Due to the cold spring and a dry and sunny period the first half of May the disease pressure was low in the period of emergence of ware potatoes. The first late blight was reported on May 10th on a dump. Disease did not spread out very much during May and June due to low disease pressure. In August the disease pressure was extremely high. This month had a very high precipitation rate. In some regions the precipitation exceeded the 350 mm in this month. After this rainy month September and October turned out to be dry and resulted in good harvest conditions. In general the yield was moderate with major quality problems as a result of second tuber formation after the hot period in July. In a few areas there were problems with tuber blight (Flevoland and south-eastern part of the country).

England & Wales

Most of the crops in England & Wales had been planted by the end of May having recovered from earlier delays caused by heavy rain in several areas. First early plantings were 70-90% ground cover in unfleeced crops and 100% ground cover in fleeced crops. Growth and development of second early and maincrops varied considerably as would be expected at that stage of the season. The spring of 2006 was generally considered to be late and crop development was 7-10 days behind 'normal'. Conditions during early May were not considered conducive for blight activity due to low night (and sometimes day) temperatures. Nevertheless, there were four confirmed outbreaks on dumps in Kent (2) on 10/11 May, in Suffolk on 20 May and in Lincolnshire on 24 May. Apart from the southwest and the south coast of England, conditions were not considered favourable for blight at the start of the season. Very hot dry weather in June and the first half of July was a feature of the 2006 season with temperatures over 30°C at times clearly halting the development of foliar blight. Another important feature of 2006 also resulting from the dry weather was the predominance of stem lesions. This gave the disease the potential for survival during the unfavourable conditions. By mid August the weather had returned to 'normal' and in the unsettled conditions blight activity revived. Over the period 17-20 August, BlightWatch reported Smith Criteria being met in up to 396 of the 652 postcode areas for England, Scotland & Wales. The earlier dry conditions also caused ridges to develop large cracks and as the end of the season approached, tubers were exposed in some crops and there was concern that this may lead to significant tuber infection should the epidemic continue to develop and conditions remained

wet. (British Potato Council funded fungicide trials have shown that even small amounts of foliar blight can result in high levels of tuber infection). During 2006, there were a total of 142 outbreaks confirmed in the BPC's Fight against Blight Campaign, which was on a par with the previous highest total recorded in 2004.

Scotland

In 2006 in Scotland blight outbreaks and development of the disease were generally limited. The number of confirmed outbreaks on the BPC blight map was very low: five outbreaks in July, four in August and one in September. All of these reported outbreaks were in crops. The first outbreak was confirmed on the 13th of July. Unusually the early outbreaks were concentrated in the far northeast of the country. The only confirmed outbreaks not in crops were observed in the middle of October on volunteers at three locations in Tayside. There were two unusual aspects to the outbreaks in 2006. Firstly that such a high proportion of the Scottish total was in Aberdeenshire, which is in the generally cooler northeast of the country, and secondly the very low number of outbreaks in Scotland compared with southern England. Perhaps the distribution of outbreaks partly reflected the key weather event of 2006, i.e. the very high temperatures in most parts of GB at times in July. It is most likely that the hot conditions in July in Scotland helped prevent outbreaks just at the time many would be expected to occur. In contrast, in England there were many outbreaks prior to the very hot weather. The low number of new confirmed outbreaks suggests that crops were well protected when challenged during periods of high-risk.

Northern Ireland

Planting was delayed by a cold, very wet March, and high rainfall continued into April and May. This was followed by unusually dry weather in June and most of July. As a result, crops were slow to establish and by the time that they were growing well, the dry conditions did not favour late blight spread and development. Apart from one very early report of blight in a covered crop, the first outbreaks were not observed until mid-July, when reports were received from most potato-growing areas. Warm, dry weather during much of July and August dried up any blight lesions in crops and there were many fewer outbreaks than in most years (20 reported by mid-September, less than 50% of the usual number). A very wet autumn gave difficult harvest conditions leading to a few cases of tuber blight. Investigation showed that stretched application intervals, product choice or poor desiccation regimes were the likely causes.

Poland

Based on weather conditions in 2006 - risk of late blight development was medium in May. The next period did not induce the disease development as the end of June and entire July were characterized by lack of rainfalls. Nevertheless, the first late blight spots were observed in the fields around Poland at the end of June. Then development of the disease stopped because of summer drought. In addition, the low precipitation in the beginning of August was not only unfavourable for late blight development but also caused earlier senescence in plants. Finally late blight started its development again upon rainfalls at the end of August and beginning of September. The rates of late blight development and infection pressure in that time were very high. During 10-12 days plant destruction was complete. The level of tuber blight in conventional potatoes was high as compared to normal. The number of fungicide applications in ware potatoes ranged from 1 to 8, mostly 1-2 applications. The most popular was fungicide using: metalaxyl M+mzb, chlorothalonil, fenamidone+mzb, iprovalicarb+mzb, propamocarb+chlorothalonil, propineb, cymoxanil+mzb.

Czech Republic

In 2006 potato late blight onset of epidemics was very late in the Czech Republic, after that the disease spreading was intensive; however, weather conditions were not favourable to tuber infection in the end of vegetation. The decisive factors that affected occurrence and disease progress were a lack of infection sources in seed potatoes from the year before, delayed planting and mainly unfavourable weather conditions for late blight in June and July. Sum of rainfalls in June exceeded the normal; however, their distribution did not allow long-lasting crop wetness that is a condition of formation of infection foci and epidemic disease spreading. Late blight occurrence was only rare at this time and when the disease was spread, it was only limited in the surrounding of infection sources. A substantial rainfall deficit in July and high temperatures hindered blight spreading also in risky localities and in susceptible varieties. Intensive rainfalls in the first August decade initiated pathogen spreading and in the end of the second August decade the first occurrences of secondary infections were found in most crops. Further disease development was very fast and foliage was totally killed in the untreated or not well-treated crops in the first days of September. Although relatively high occurrence of the disease in foliage of many crops was found, a lack of intensive rainfalls in the second half of August and September did not support tuber infection. In general, late blight control was well managed in agricultural practice and no important yield losses were recorded. Tuber infection was also rare, exceptionally problems rose in the varieties susceptible to tuber blight infection. Vegetation of propagation crops was mostly ended before blight infection. Differences in the efficacy of fungicides were smaller than in other years in relation to shorter period of intensive disease distribution. A relatively short season and low number of pathogen generations were also a cause of absence of metalaxyl-resistant strains. The highest efficacy in 2006 was found in fungicides Sereno (fenamidone + mancozeb), Infinito (fluopicolide + propamocarb), Ridomil Gold MZ 68 WP (metalaxyl M + mancozeb), Altima 500 SC (fluazinam) and Casoar (chlorothalonil + propamocarb). In the pathogen populations distribution of mating types A2 was found in 11 % (33 % in 2005, 44 % in 2004).

Slovakia

Very early potatoes were planted on the end of March and beginning of April, seed potatoes and maincrop on the end of April and first decade of May. Seed potatoes and maincrop emerged by the beginning of June. First outbreaks in very early potatoes were recorded by the mid of June, in seed potatoes and maincrop by the beginning of July. Average of fungicide spraying: 5 per vegetation. Fungicide used: Tattoo, Casoar, Acrobat, Dithane, Altima, Curzate, Ridomil and Tanos

Latvia

The spring of 2006 was dry with low temperatures. Potato emergence was delayed. Crop emergence was only completed by the 5th of June. The first warning of the development of *P. infestans* was received on the 23rd of June when the temperature and humidity conditions were favourable for the development of the disease. The first warning of the development of *Alternaria solani* was also received at the same time. Due to late emergence in some fields, crops were not closing in rows. The first application was recommended and made with the (systemic + contact) fungicide. Farmers did not wait until the first visual symptoms appeared and usually sprayed protectively. Due to a warm July, more *Alternaria* type leaf lesions were present in potato plants. Fungicide treatments (translaminar or contact) were continued after the start of the rainy period at the end of July. The late blight development and infection pressure in August was very high. The first symptoms of late blight were observed at the beginning of August. At that period 2-3 fungicide applications were made. Mancozeb and fluazinam were the most frequently used fungicides in the second half of the season. Unprotected crops and those that were the most susceptible were totally killed by mid-August. Fungicide applications made

per season: seed 2-4, ware: 2-5, starch: 1-3. The use of fungicides resulted in good to excellent control in all farms when the first application was done at the end of June/ beginning of July.

Estonia

Cool weather in May delayed planting of potatoes until the second half of May. The spring and beginning of summer were rather cold, delaying the emergence of plants. Potatoes emerged only one month after the planting in mid June. First late blight outbreaks were observed in covered early potatoes around June 25. These infections were stopped in a dry July, which was extremely unfavorable for infection and spread of potato late blight. The rainy season starting in first decade of August created favorable conditions for development of late blight for the rest of growing period. Numerous disease outbreaks were observed in mid of August in Southern Estonia. The infection spread to the Northern Estonia for the end of August. Essential part of early and maincrop potatoes were harvested before the establishment of late blight. The first half of September with rainy and warm weather favored fast development of foliage blight in late potato varieties. Some farmers started with fungicide treatments in potatoes of 20 cm height in the end of June, but stopped the fungicide applications at the extreme drought in the beginning of July. Fungicide treatments were continued again after the start of rainy period in beginning of August. Majority of farmers waited until the first infection and made 1-2 treatments thereafter. The use of fungicides resulted in good control. A large part of the farmers harvested potatoes before the late blight infection and did not use any fungicide treatments. There were only minor infections of tuber blight.

Lithuania

Most of the potato crops in Lithuania had been planted by the middle of May. The weather conditions at the beginning of the growing season were not favourable for crop emergence and establishment. Due to the relatively low temperature, crop development was behind normal for about one week. According to the information provided by State Plant Protection Service, the first blight onset was recorded on 22 July in eastern part of Lithuania. In most regions of the country blight attack was recorded about a month later then heavy rain started. Due to the dry weather, growers started to spray crop later and depending on the cultivar 1 - 4 applications were performed, whereas in irrigated plots 4 - 10 applications.

Russian Federation

A severe development of late blight in the European part of Russia was registered in several central and eastern regions (Ryazan, Kursk, Lipetsk, Bashkortostan); the first symptoms of the disease appeared when rows were closed. In the north-west regions late blight occurred shortly before the harvesting. A very high amount of rains before and during the harvesting in these regions caused serious problems with tuber blight (up to 12% of infected tubers). In other regions the long period of a hot and dry weather caused very unfavourable conditions for *P. infestans.* The disease was detected on a small scale in some allotment gardens, but its symptoms on the farmer potato fields were very scarce. A severe or moderate early blight attack was registered on some potato cultivars in central and eastern parts of European Russia. In most cases early blight symptoms were caused by *Alternaria alternaria*. The most commonly used fungicides were: Mancozeb, Chlorothalonil, Copper, Fluazinam, Cymoxanil, Dimethomorph, and Phenylamide. The average number of fungicide applications was 2 - 4. The number of agricultural enterprises, that produced potato, basing on the crop protection technologies, accepted in Western Europe, and using the greater number of fungicidal treatments, was less than 10%. The owners of allotment gardens did not use any fungicides.

Denmark
Crop emergence was normal, May 20-30. During this period weather was wet and cold. First outbreaks were recorded late June (few) or early July (several) - all BBCH > 30. In last week of July, the weather turned into very blight favourable conditions and very severe epidemics started, including in more resistant starch cultivars. Due to heavy rain in August and September, tuber blight was a big problem. Fungicide applications: seed 4-5, ware: 6-7, starch: 8-12 (Mean all=7,4). Most popular fungicide was Dithane NT. Other fungicides used were Shirlan, Tattoo, Acrobat, Ranman and Ridomil. In organic potatoes attacks started in first week of July. The yield was medium as compared to normal.

Norway
There were few late blight favourable days both in June and July, but from medium August the risk of late blight was very high. Use of fungicides was a bit lower than normal and Shirlan was the most commonly used fungicide. A lot of fields were attacked during the last part of the growing season and more tuber blight was found than normal. However there was no significant yield reduction in treated fields.

Sweden
The planting of the potato was late in 2006 due to the cold spring. This delayed the emergence with about two weeks compared to normal. First attacks of late blight were reported 7 June in early potato grown under fleece (about two weeks later than in last years). In the following weeks there were a few additional reports of infected fields, both in early potato and in main crops. June and July was warm and dry and this stopped any further significant disease development. However, heavy rain in August and September resulted in very blight favourable conditions. The high amounts of rain made it difficult to spray in some areas due to inaccessibility of the fields. Late in the season late blight could be found in many fields, especially in the south. There were also severe tuber attacks in this area. Fungicides used were mainly Shirlan, but Ranman, Tanos and Electis were used. Epok is mainly used in the early season, but there was some curative use. Mean number of sprays in ware potato varies significantly from south to north in Sweden. A total average number of sprays could be estimated to 7 with a range between 5 and 18 times. In starch potato the number of sprays is lower (mean 6, range 5 – 10).

Finland
Summer 2006 was exceptionally dry and warm and in general there was very little leaf blight. The first blight attack was reported 3rd of July at a covered crop. Thereafter there were no blight outbreaks until the second half of August, which is approximately one month later than normally during the 2000s. The last week of August was rainy and some blight was present in many crops before harvest. Though there was very little leaf blight, tuber blight was more common than usual. Growers started to spray at the normal time at the beginning of July. Due to dry weather usually only 1-3 applications were done. At the end of season many fields were unprotected, which resulted in more tuber blight than normally.

Comments
Blight was observed in early planted potatoes in France 26 April, in Switzerland and Italy 18-20 May, in North-West Europe around 1 June and in July to August in the Baltic countries and Finland (Figure 1). Widespread late blight in conventional fields was reported to have developed very late in the season in most countries (Figure 2), probably due to the very dry and hot weather in July (Figure 4). The interval between the attacks in early potatoes and the attacks in conventional fields was 1½-2 month in several countries e.g. The Netherlands, Finland, Northern Ireland and Estonia. In other

countries this interval was less than a week e.g. England, Belgium and Italy. When the weather became more favourable for blight development in August, epidemics were extremely severe, and control was difficult due to heavy rain. In ware potatoes the highest number of fungicide applications was estimated for Belgium (14), France (13) and the Netherlands (15). The lowest number of fungicide applications was estimated for Estonia (2), Finland (3) and Poland (2).

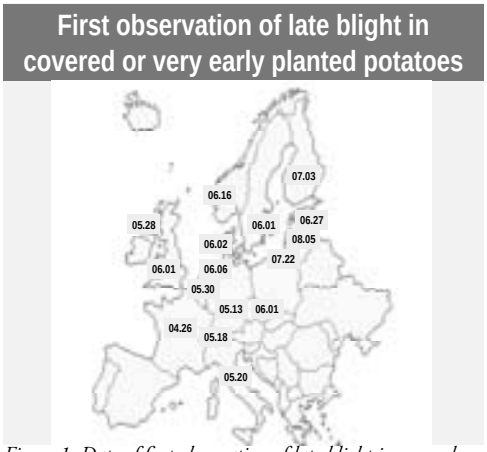

Figure 1. Date of first observation of late blight in covered or very early planted potatoes.

Figure 2. Date when first infections were reported in more than five conventional, normally planted potato fields.

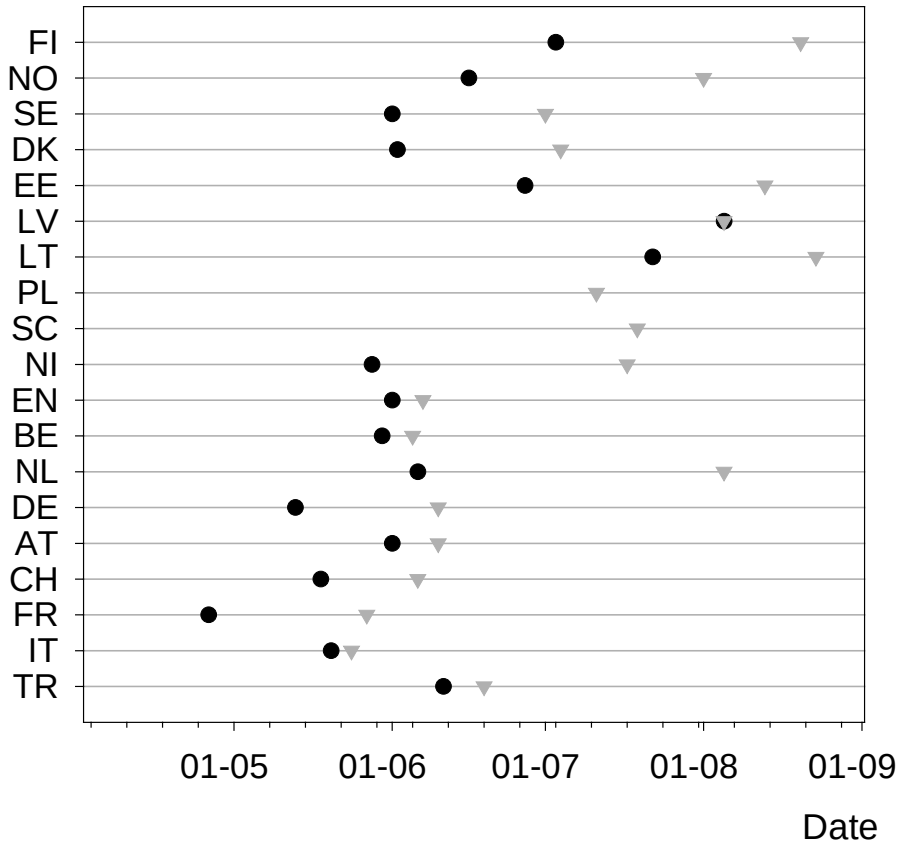

Figure 3. Date of first observation of late blight in covered or very early planted potatoes (dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (triangles).

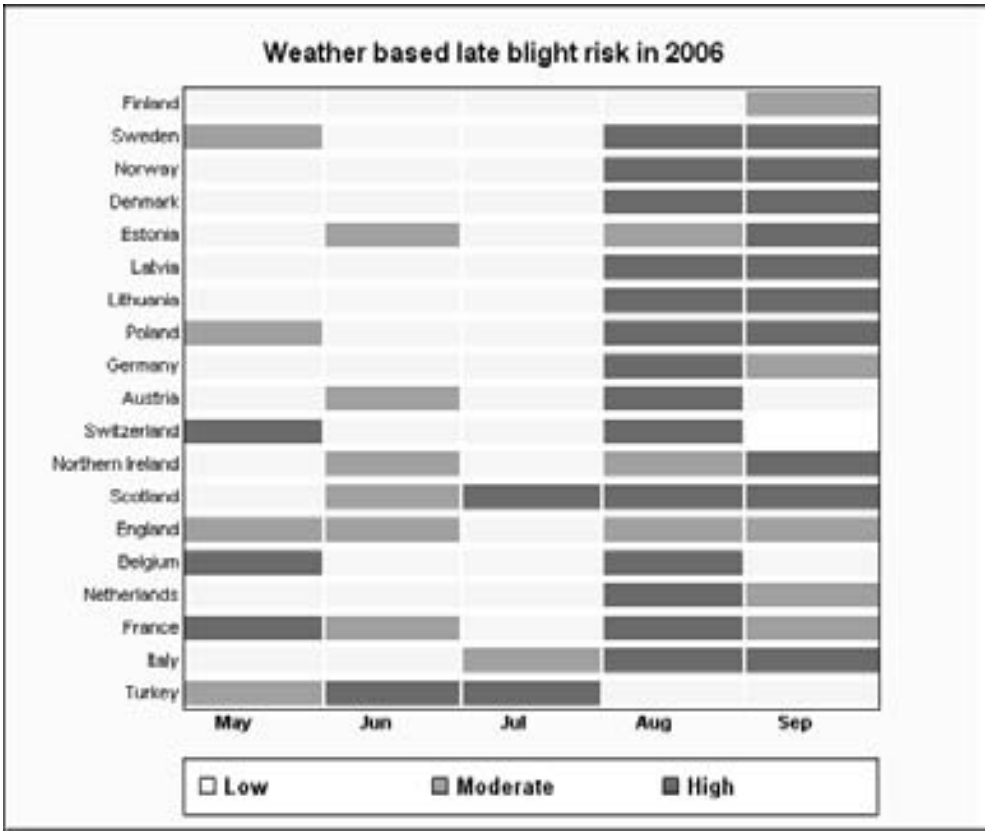

Figure 4. The weather-based risk of late blight development in Europe in 2006.

Table 1. Number of fungicide applications used in 2006 in ware potatoes and all types of potatoes respectively.

	Number of fungicide applications used in ware potatoes			Number of fungicide applications used in all types of potatoes		
Country	min	max	mean	min	max	mean
Austria	4	10	7	4	10	7
Belgium	12	16	14			
Czech Republic						
Denmark	4	7	5	5	16	7.5
England & Wales	8	12	10	8	12	10
Estonia		6	2		6	2
Finland	1	4	3		5	3
France	8	16	13			
Germany	4	12	7			
Italy	5	9	7	5	11	8
Latvia	2	5	3	2	5	3
Lithuania				1	4	2.5
Netherlands	7	20	15	6	20	13
Northern Ireland	5	14	9	5	14	9
Norway	2	8	5	2	8	5
Poland	1	8	2			
Russian Federation						
Scotland						
Slovakia						
Sweden	5	18	8	5	18	8
Switzerland			7			
Turkey	2	6	4	2	6	4

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Occurrence of late blight in the USA

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Late blight caused by the oomycete pathogen, *Phytophthora infestans* (Mont.) de Bary is one of the most important potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) diseases worldwide due to rapid asexual reproduction of the pathogen under conducive weather conditions. In the past 25 years, dramatic changes in populations of this pathogen have been associated with increased problems in controlling late blight on both crops. In the United States, the potential impact of late blight on commercial potato production is reflected in the fact that most crops receive several applications of foliar fungicides sprays to protect them from foliar and tuber infection by *P. infestans.* In the eastern and mid-western states, the chief genotype remains the US-8. However, the US-11 genotype was the main pathogen isolated from disease samples from Washington, Oregon, and Alaska. The US-14 and variants of that genotype were frequently isolated from blighted potato and tomatoes from Florida.

Late blight has suddenly reemerged as a major concern in most tomato producing areas of the United States. Epidemics occurred in 2004 when over 50% of the commercial crop was destroyed in eastern states from New York to Florida; 80 to 90% of early seedbeds in Florida were a complete loss from November 2004 to April 2005. Most growers reported that no available fungicides would control the rapid spread of the epidemic. Heavy losses occurred in transit; symptoms occurred on infected but symptomless fruit within 5 days of harvest. Tomato late blight has become an increasingly important problem to agriculture in the United States in the past decade as more aggressive, fungicide-resistant, and tomato-specialized isolates have appeared. Identification of several of the genotypes has been quite challenging.
For instance, *P. infestans* isolates obtained from late blighted tomatoes fields in New Jersey in 2003 were all A2 mating type, metalaxyl-resistant and mtDNA haplotype Ia. These isolates were homozygous at the loci coding for both glucose-6-phosphate isomerase and peptidase, having *Gpi 122/122, Pep 100/100*. However, RG57 analysis showed that all the tomato isolates from New Jersey had a unique and previously unreported fingerprint. Although severe late blight symptoms were present in nearby potato crops, the isolates were typical of the US-8 genotype.

Similarly, single-lesion isolates from late blighted tomatoes fields in Pennsylvania were also all A2 mating type, metalaxyl-sensitive, mtDNA haplotype Ia and were *Gpi 100/122, Pep 100/100*, characteristics atypical of isolates of *P. infestans* from potato in the same area, which were all of the US-8 genotype.

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The tomato isolates had the allozyme banding pattern and mating type associated with a variant of the US-14 genotype. As with the late blighted tomatoes in New Jersey, RG57 analysis showed that the tomato isolates had a unique fingerprint (different from that found in the New Jersey isolates and not that of US-14) which does not appear to have been reported previously.

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End of season management of tuber blight

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Summary
The effect of different end of season treatments of the haulm on tuber blight has been investigated in a five year project. Haulm killing reduced the amount of inoculum in the haulm and soil and the amount of tuber blight compared to harvest on green haulm treated with fungicide. The infectivity of the soil was reduced to about the half in three weeks and the infectivity persisted longer in dry soil than in wet soil. Most of the inoculum washed down from the haulm remained in the top soil, at 15 mm precipitation in pot experiments.

Keywords
Phytophthora infestans, tuber blight, haulm killing, soil infectivity.

Introduction
Tuber blight, caused by *Phytophthora infestans,* is an important problem in the potato production in Norway. The general recommendation to reduce the problem with tuber infection at harvest is to kill the haulm 2 – 3 weeks prior to harvest. The growing season is relatively short in most potato production areas in Norway. Thus it is important to keep the haulm green as long as possible to obtain high yield. Different aspects of end of season management related to tuber blight have been investigated in a five year project, 2003-2007.

Materials and Methods

End of season management of tuber blight
Artificially inoculated field trials with the cultivar Folva were conducted at three locations for four years. The trials had three replications in blocks and a combination of four end of season management strategies and two maturity classes, which were obtained by planting pre-sprouted (225 day degrees) or cold stored (4 °C) seed potatoes. Until 6 weeks before harvest the haulm was protected with late blight fungicides. Inoculation was done 4 weeks before harvest with a spore suspension of *P. infestans*. To encourage blight development the field was sprinkled with water 12 times per day, a total of 5 mm per day, on days with less than 10 mm of rain from 16 days to 2 days before harvest. The four end of season management treatments were: 1) harvest on untreated green haulm, 2) harvested on

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green haulm treated with fungicide (Shirlan 300 ml/ha, fluazinam 150 g.a.i./ ha) 14 and 7 days before harvest, 3) chemically desiccated (Reglone 3 l/ha, diquat 600 g.a.i./ha) 14 days before harvest, 4) half cut (the top was chopped with a tractor mounted rotary chopper leaving ca 25 cm of the haulm) and chemically desiccated (Reglone 1,5l/ha, diquat 300 g.a.i./ha) 14 days before harvest.

Samples to assess the amount of sporangia in the haulm and infectivity of the soil were taken 14 days before harvest and at harvest. Plants, 5 stems per plot, were shaken in a plastic bag containing 500 ml water for half a minute. The number of spores was counted in a haemocytometer. The infectivity of the soil was assessed using the tuber slice method described by Lacey (1965). Tuber blight was recorded in two samples from each plot. One sample of 5 kg potatoes was harvested into plastic bags and analysed after 3-4 weeks at 15°C and another sample of 5 kg was harvested into paper bags and analysed after 7 days at 10-15°C.

End of season management in different cultivars
Field trials with five cultivars in combination with two end of season strategies were conducted at three locations for four years. They were arranged as complete randomised blocks with three replications. The cultivars were Folva (3, 5), Saturna (5, 6), Asterix (3, 7), Beate (6, 7) and Peik (7, 7) (the numbers in the brackets are the cultivars foliar resistance and tuber resistance score to late blight on the scale from 1 to 9, where 9 is the most resistant). The two different end of season strategies were: 1) harvest on green haulm treated with fungicide (Shirlan 300 ml/ha, fluazinam 150 g.a.i./ ha) 14 and 7 days before harvest, 2) chemically desiccated (Reglone 3 l/ha, diquat 600 g.a.i./ha) 14 days before harvest.

Tuber blight was recorded in two samples from each plot. One sample of 5 kg potatoes was harvested into plastic bags and analysed after 3-4 weeks at 15°C and another sample of 5 kg was harvested into paper bags and analysed after 7 days at 10-15°C.

Large plot field trials comparing end of season strategies
Field trials with three different end of season strategies and three replications were conducted at 20 locations for four years. 1) Harvest on green haulm treated with fungicide (Shirlan 300 ml/ha, fluazinam 150 g.a.i./ ha) 14 and 7 days before harvest. 2) Treated with fungicide (Shirlan 300 ml/ha, fluazinam 150 g.a.i./ ha) 14 days before harvest and chemically desiccated (Reglone 3 l/ha, diquat 600 g.a.i./ha) 7 days before harvest. 3) Chemically desiccated (Reglone 3 l/ha, diquat 600 g.a.i./ha) 14 days before harvest.

Tuber blight was recorded in two samples from each plot. One sample of 5 kg potatoes was harvested into plastic bags and analysed after 3-4 weeks at 15°C and another sample of 5 kg was harvested into paper bags and analysed after 7 days at 10-15°C.

Persistence of infectivity of P. infestans in different soil types and at different humidity
Lab experiments with three soil types in combination with three humidity levels, Pf 4 (dry), Pf 3, Pf 2 (wet), were conducted for two years. The soil types were silt, sand and light clay. Air dried soil was adjusted to the right humidity by adding water, and then kept in ventilated plastic bags. The calculation of the amount of water to add to each sample was based on soil organic content and particle size distribution (Riley, 1996). The soils humidity was monitored with a tetra probe. Three sets of ventilated bags with soil (inoculated and control) were prepared with three replicates of each set. Two of the sets were inoculated with late blight sporangia (1000 sporangia/g soil) and one set was uninoculated control. The soil was sprayed with a spore suspension (10 ml of 10^5, sporangia/ml per kg soil) or water in the control, and mixed by tumbling the bags in a potato washing machine (without

water in the machine). The ventilated bags with soil (inoculated and control) were incubated at 12°C in the dark. Samples were taken 1, 7, 14, 21 and 28 days after inoculation from one of the inoculated sets and the soil infectivity were tested with the tuber slice method (Lacey, 1965).

The two other sets were used to a test that simulates infection of tubers at harvest. To each bag 50 tubers were added 14 days after inoculation. 50% of the tubers were superficially wounded by rolling the tubers over a group of 10 nail points, 1 mm high, 1 cm apart, pointing upwards from a wooden base. The bags with soil and tubers were tumbled in the potato washing machine (without water in the machine) for 3 minutes, to simulate the process of harvest. The bags were incubated at 12°C in the dark for 24 hours, and cut open to let the soil dry and then further incubated at 12°C in the dark. After approximately 3 weeks the tubers were assessed for blight development.

Infectivity of soil after different end of season treatments and rain
Pot trials with four end of season treatments in combination with two simulated rain strategies were conducted with four replications for two years. Potato plants grown in sandy soil in 1,5 litre pots in the glasshouse for 4-5 weeks were inoculated with *P. infestans* in the stems and leaves. The plants were covered with plastic to promote infection. One week after the inoculation, when the lesions had started to sporulate, the plants were given four different end of season treatments.1) Untreated control, 2) Mechanical haulm killing (the haulms were cut into 5 cm long pieces and 25% of the haulms were put on the top of the pot), 3) Chemical desiccation (Reglone 3 l/ha, diquat 600 g.a.i./ha) and 4) Treated with fungicide (Shirlan 300 ml/ha, fluazinam 150 g.a.i./ ha). The plants were kept in a growth room with 15 °C and 16 hours light / 8 hours dark. The night before the rain simulation the plants were sprayed with water and covered with plastic bags to promote sporulation. The pots were subjected to 15 mm simulated rain either 1 or 5 days after the end of season treatments. Soil samples were taken at 0, 3 and 6 cm depths 7 and 14 days after the end of season treatments. The soil infectivity was tested with the tuber slice method (Lacey, 1965).

Results and Discussion

End of season management of tuber blight
Fungicide treatment of the haulm reduced the amount of sporangia in the haulm and the infectivity of the soil, compared to the untreated control. Haulm killing, either by chemical desiccation or by a combination of mechanical haulm killing and chemical desiccation, reduced the amount of sporangia in the haulm and the infectivity of the soil more than treating the haulm with fungicide. However, the end of season treatments did not significantly reduce the amount of tuber blight compared to control. There was less tuber blight in the samples harvested into paper bags than in samples harvested into plastic bags. This could both be an effect of dryer conditions in the infection period, lower temperatures and a shorter incubation period in the paper bags. Maturity class did not have any effect on the different blight parameters.

End of season management in different cultivars
Blight developed in five of the twelve field trials. In the five field trials with blight there were no difference in tuber blight between the end of season strategies, but there was a tendency to more tuber blight in the cultivar with low blight resistance, for the samples stored in paper bags. The amount of soft rot was correlated to the amount of foliage blight at the end of season treatment (r=0,663 p=0,000) so most of the soft rot was probably a secondary rot in late blight infected tubers. In the samples harvested into plastic bags there were no significant differences between the end of season strategies or cultivars in the amount of tuber blight or soft rot.

Large plot field trials comparing end of season strategies
Tuber blight developed in twenty two of the eighty field trials. The amount of soft rot was correlated to the amount of foliage blight at the end of season treatment (r=0,283 P=0,000) so much of the soft rot was probably a secondary rot in late blight infected tubers. There was significant less soft rot in the treatments that were chemically desiccated 14 or 7 days before harvest than in the treatment harvested on green haulm, and it was the same tendency for tuber blight. There was nearly no infection in the samples harvested into paper bags.

Persistence of infectivity of P. infestans in different soil types and at different humidity
The results from the tuber slice test (Lacey 1965) and the harvest simulation were similar. The late blight infectivity persisted longer in dry soil. There were small significant differences between the soil types. In soil with low moisture holding capacity, sandy soil, the infectivity persisted longer. In average of all soil types and humidity levels the infectivity of the soil was roughly reduced to about 90% in one week, 75 % in two weeks and 50% in three weeks, which is similar to approximately a 40% reduction in the effective spore number in 1 week, 75 % reduction in 2 weeks and 95% reduction in 3 weeks.

Infectivity of soil after different end of season treatments and rain
Most of the inoculum washed down from the haulm remained in the top soil (0-3 cm depth). In the pots with mechanical destruction of stems and leaves, 25% of the haulm pieces were put on the top of the pots, this gave a high level of infectivity of the surface soil. In the pot with chemically desiccated haulm, the leaves were hanging shivered down from the stems, and in these pots more inoculum tended to be washed deeper into the soil. None of the end of season treatments reduced the infectivity of the soil significantly compared to the untreated control. Hence, in this experiment it looks like the infectivity of the soil was more affected by the water flow path than by any direct effect of the end of season treatment on the viability of the inoculum.

Conclusions
Haulm killing reduced the amount of inoculum in the haulm and soil, and hence the potential for tuber infection compared to harvest on green haulm treated with fungicide. There were generally low levels of tuber blight in the different experiments and moderate differences between the different end of season treatments. However development of tuber blight is strongly effected by infection conditions before, during and after harvest. These relationships will be studied more in detail, and are still in progress. Lab experiments showed that that the infectivity of the soil persisted for several weeks and longer in dry soil than in wet soil.

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Riley H., 1996. Estimation of physical properties of cultivated soils in southeast Norway from readily available soil information. Norwegian Journal of Agricultural Sciences. Supplement No. 25, 1996. Ås Science Park Ltd., Norway. ISSN 0802-1600

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Modelling Tuber blight with Plant-Plus

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Summary
Phytophthora infestans is a threat to potato growing around the world. During the growing season, the most visible signs appear in the foliage. Infections cause a loss of photosynthesis resulting in a yield loss. However, under specific conditions spores generated by the infections in the foliage will infect tubers. This results in a direct loss of quality and yield in the field or later on in storage. Sometimes these losses go up to 100%.
Dacom Plant Service (Netherlands) has develop a prediction model for the possible infection of potato tubers with *P. infestans*. The development of the model was possible with the data that has been gathered by the Wageningen institute Plant Research International.
The model is not commercially available but mainly used by consultants to analyze the growing season of potato fields that have shown signs of tuber infection. Based on this experience, it can be concluded that it is possible to identify the moments where the protection strategy has failed and the tubers got infected. However, precise quantification of the amount of tuber infection that will show up in storage proved to be difficult. This could be due to unknown differences in storage conditions.
The model has been proven to be a good tool towards a better understanding of the right strategy for protecting a potato crop against *P. infestans* infections.

Keywords
Potato, *Phytophthora infestans*, tuber infection, modelling, Plant-Plus

Introduction
The model was developed by Dacom Plant Service in the Netherlands. Dacom is the leading provider in Decision Support Systems for crop management with the Plant-Plus system. A good relation with the research community in Wageningen combined with her own knowledge of the disease and a pragmatic approach results in new and useful models.
The model is build into the Plant-Plus system of Dacom. Plant-Plus is an integrated system that combines all kinds of input into operational advice. The input consists of local weather data as measured by automatic weather stations and a site specific 10 day hourly weather forecast. Also the crop conditions are recorded in Plant-Plus. Scientific knowledge about the physiology of plant development and the diseases is also added to the information (see Figure 1). All this information is validated and stored in a central databank at Dacom. The main output modules using the information are a set of disease control models, models for insect control, an irrigation management module and a fertilizing system.

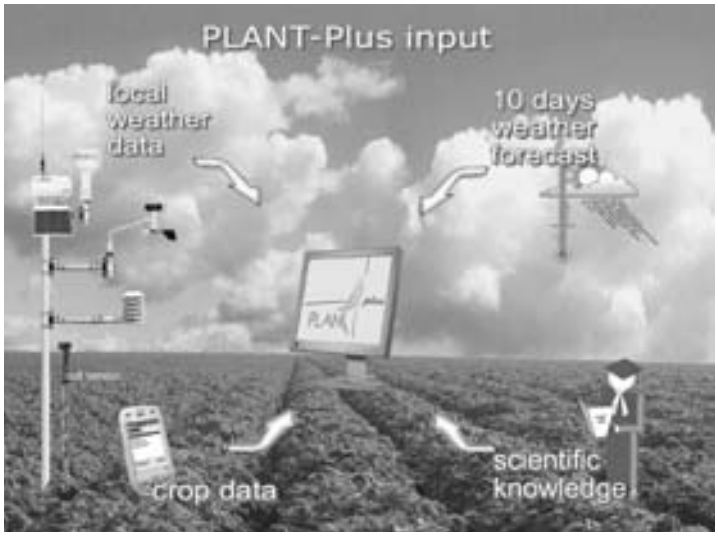

Figure 1.

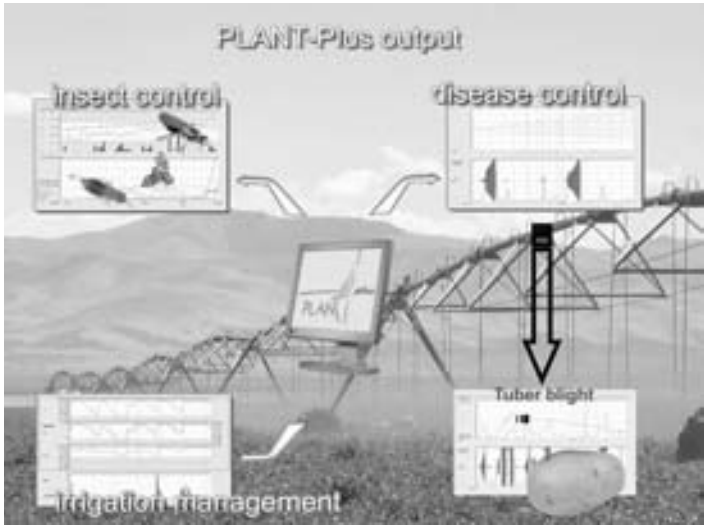

Figure 2.

A sub model of the disease module is the tuber blight model (see Figure 2).

Irrigation

The infection of potato tubers with *P. infestans* is strongly influenced by the amount and the moment of rain and irrigation and the soil moisture situation. Over the last years, Dacom has developed an extensive irrigation management module. The information and output generated by the module can be used as one of the parameters for the tuber blight model. One option is to use the ET0 module to calculate the soil moisture content. The other option is the moisture reading devices for a very precise determination of the water content in the soil. These measurements can be done at different depths including the tuber growing zone. The readings are continuous, meaning at least every hour. This information is ideal for the determination of the status of the soil and the duration of a 'tuber wetness' situation.

The model

The tuber blight infection model uses the same infection events as calculated in the Dacom Plant-Plus foliage infection model. All calculations are done on an hourly base. Starting point for the tuber blight model is the possible spore production on an active lesion that can lead to an infection event in the foliage ("A" in Figure 3). Based on variety characteristics, temperature and RH the number of viable spores is calculated. The grower has to record whether he has determined an infection in the foliage. Basically the model assumes that if no P. infestans is found, no tuber infection will take place. The next action of the model is to determine a rainfall event or an irrigation ("B" in Figure 3). The calculated viable spores will start washing off from the lesion at a precipitation intensity of more than one mm

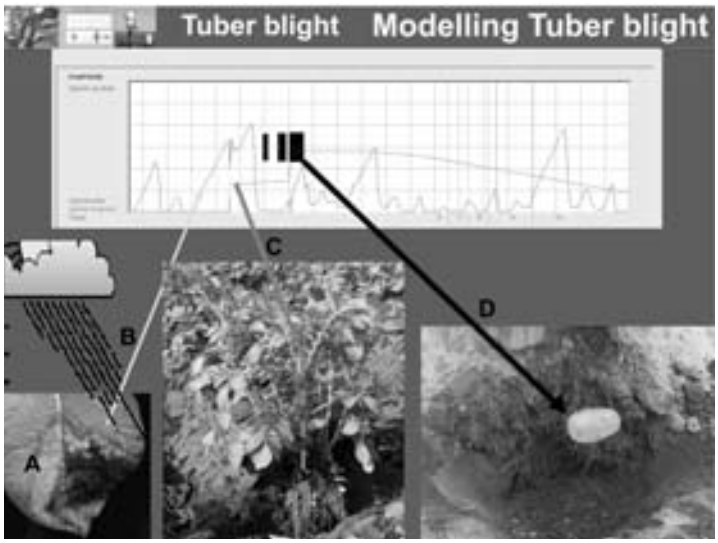

Figure 3.

per hour. The spores wash down along the stem into the soil near the tubers. The calculated number of spores in combination with the precipitation intensity and duration of the precipitation determine the number of spores that wash down into the soil ("C" in Figure 3). Based on time elapse and the soil moisture condition, the model will calculate the dying of these spores. The next phase is for the spores to actually infect the tuber. For this infection the parameters of the tuber resistance, the duration that the tuber skin is calculated to be wet and the temperature are being used. If these combined factors are above a certain threshold, the infection of the tuber has started (see "D" in Figure 3). In the graph this period is indicated by the black block(s).

It should be noted, that between the moment the spores enter into the soil and the actual moment of tuber infection, there could be a shorter or longer period of time. If the tubers are harvested in this period under normal dry conditions, no tuber infection will take place. If infected tubers will show signs of infection later on in storage depend also on the variety and the storage conditions.

Using the model

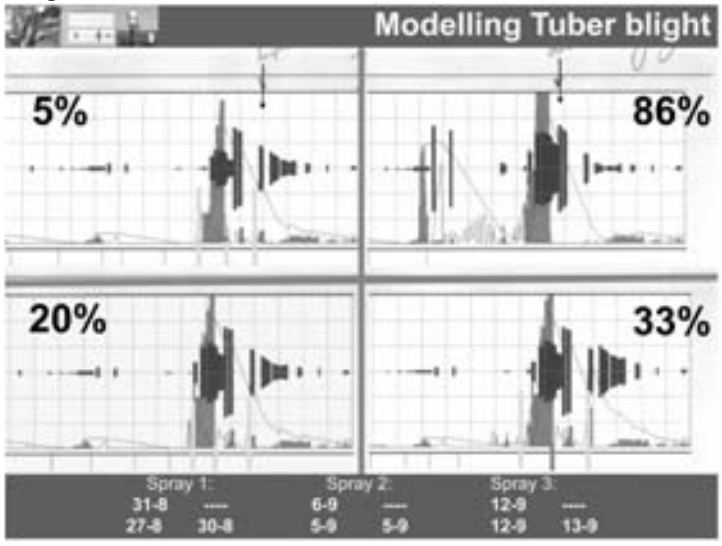

Figure 4.

The model was used in identifying the season of 2001 when stored potatoes had many problems. Dacom was supplied with the basic information as needed for the calculation: spraying date, product and amount, weather conditions, variety and amount of infection in storage. In Figure 4 an overview of four fields is presented. The long vertical bars indicate the spraying moments that matter to the control of tuber blight. The field at

the top left shows 5% tuber rot. The fungicide applications were, according to the model, carried out as optimal as possible. Both were done right before the rain events and subsequent tuber infection periods, therefore killing the spores before they came into the soil. The first application to field at the lower left with 20% tuber rot was carried out just after the first rain event. Therefore some spores be washed down. In the field in the lower right with 33 % tuber rot, it was the 2nd spray that was done after a rain event to cause the problems. In the field at the top right, with 86 % tuber rot, no sprays were done at all during the critical period.

Summary on tuber infections:

- Lesions have to be present in the foliage of the field
- Viable spores have to be present
- Sufficient rain is needed to get spores dissolved
- Sufficient rain is needed to wash spores down the stem into the soil near the tuber.

In the soil:

- Viable spores have to be present near the tuber
- A sufficient period of tuber wetness is needed in relation to temperature and variety

Conclusions

Parameters NOT to get tuber blight infection:

- No infection (= no lesions) present in foliage of field
- All viable spores killed on lesions
- All viable spores killed on the way down the stem

Harvest

- Harvest tubers before a tuber wetness period under normal to dry conditions

If one of these four actions can be carried out, no tuber blight will occur.

Conclusion on Modelling

- Occurrence can be modelled accurate
- Quantity of occurrence difficult to predict
- Model proved to be a good research and analyzing tool
- Effect fungicide treatment to be modelled

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Tuber blight control: effect of foliar applied fungicides such as Infinito on viability of sporangia of *Phytophthora infestans*

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Summary
The results presented in this paper illustrate significant differences between the fungicides tested to reduce the formation of sporangia through translaminar activity. Contact products such as mancozeb and fluazinam without translaminar activity demonstrated not to be able to reduce sporangia formation. Infinito showed to have strong translaminar effect. In addition there are marked differences between the fungicides tested to reduce spore viability and consequently together with antisporulant effect, infectivity. Two fluorochrome stains, fluoresceine diacetate (FDA) and propidine iodine (PI) were selected as rapid indicator of *Phytophthora infestans* sporangia viability using fluorescence microscopy techniques.
This multiple action combined with the sporicide effect of Infinito explains the outstanding activity the product confirmed under field conditions on tuber blight control.

Introduction
Tuber infection from late blight, *Phytophthora infestans* is a threat to all potato crops affecting not only marketable yield but also their storage potential. Tuber infection in crops occurs under conditions of favourable soil moisture and temperature when *P. infestans* spores produced on the foliage, have been washed down into the soils by rain or irrigation (Sato, 1979). It has been observed that sporangia in soil remain viable for a long period of time (15 – 77 days) and they can infect potato tubers from as early at the first onset of tuber formation (Andrivon, 1995; Dubey and Stevenson, 1996; Evenhuis, 2005).
Tuber blight is basically managed by foliar application of fungicides. The foliar applications affect tuber infection in soil through interaction of different factors (Dubey and Stevenson, 1998)

- the ability of a fungicide to limit sporulation of *P. infestans* in plant tissue
- the potential fungicidal effect of a compound on spores (sporicide effect); where the sporangia comes into contact with the fungicide and sporangia or zoospores are killed before they can infect tubers. Rainfall and irrigation has an important role in the redistribution or dilution of fungicide and the transport of sporangia into the soil.
- the ability of fungicides to affect viability and infectivity of sporangia during their formation, leading to reduced infectious inoculum

Bayer CropScience conducted research to study the effect of late blight fungicides on the viability

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of newly formed sporangia in relation to translaminar activity and assessment based on fluorescent staining methods of sporangia.

Materials and methods

Fungicides test
To evaluate the translaminar effect of fungicides, products were applied by spray machine only to the upper surface of potato leaflets. The petioles were protected at the application to prevent fungicide penetration through the cut surfaces. To avoid any direct contact between fungicidal residues and sporangia *P.infestans* was inoculated on the lower surface. Inoculations were made with sporangia suspensions adjusted at 40000 sporangia/ml either 3h, 24h and 48 hours after the treatment or 48 hours before treatment. Two droplets of 10µl were deposited on the lower surface of each leaflet. Assessments were carried out 5 to 8 days after inoculation. The percentage of infected and sporulated surface was recorded to establish an index of infection relative to the untreated control (Townsend Heuberger formula).
Newly formed sporangia were collected on the lower surface of the leaflets and their viability was assessed.

Products

Table 1: products and tested dose rates

Active Ingredient (brandname)	Formulation	Dose product tested /ha	Corresponding dose active g a.i. /ha
fluopicolide + propamocarb (Infinito)	62.5 + 625 g/l SC	1.2 L	75 +750
		1.4 L	87.5 +875
		1.6 L	100+1000
mandipropamide (Revus)	250 g/L SC	0.6 L	150
propamocarb + chlorothalonil (TatooC)	375 + 375 g/L SC	2 L	750+750
dimethomorph + mancozeb (Acrobat M)	90 + 600 g/kg WP	2 kg	180 + 1200
cymoxanil + mancozeb	40 + 400 g/kg WG	2 kg	80 +800
fenamidone	500 g/l SC	0.3 L	150
fluazinam (Shirlan)	500 g/l SC	0.4 L	200
Mancozeb (Dithane Neotech)	750 g/kg WG	2.14 kg	1600

Viability:
Spore viability (eg sporangia or oospores) can be evaluated by using different techniques such as:
- vital stains which differentiate between live, dormant or dead spores
- germination tests in aqueous media
- infectivity tests on foliar discs or tubers slices.

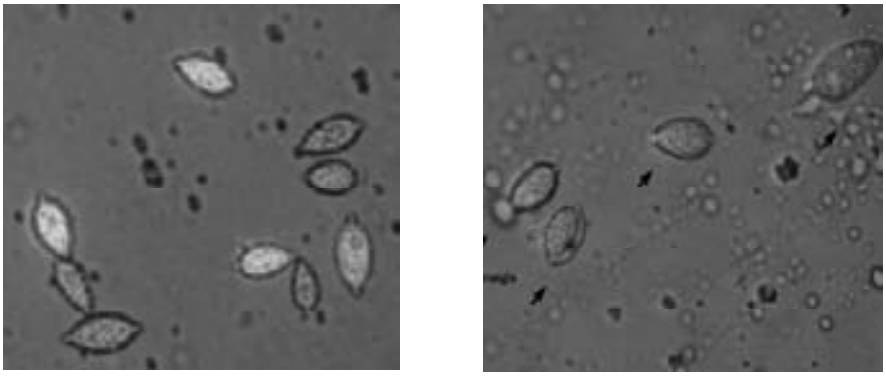
Research groups working on vital stains under optimised conditions observed a positive correlation between the number of vital spores and the germination rate in vitro (El –Hamalawi, 1986). However the obtained results were influenced by type, age and cell-wall of the spore, the permeability of the cell-wall and the vital stain used.

Of the vital dyes available, two fluorochrome stains, fluoresceine diacetate (FDA) and propidine iodine (PI) were selected as the most appropriate and rapid indicator of fungal spore viability using

fluorescence microscopy techniques (Schading et al., 1995; Stewart & Deacon, 1995). FDA fluoresces bright green in viable spores and PI fluoresces red - orange in non-viable spores when viewed under blue light using specific fluorescence microscope.
The LIVE/ DEAD BacLight Bacterial Viability Kit manufactured by Molecular Probes, Inc which was successfully used for determining the viability of the hyaline spores of *Colletotrichum gloeosporioides, Leptosphaeria maculans* and *Sclerotinia sclerotiorum* and the thick-walled spores of *Alternaria brassicae* (Chen and Seguin-Swartz, 2002) was used to determine the viability of *Phytophthora infestans* sporangia.
The stains were used either in combination (Kit L7007) or alone (FDA). The results from vital staining were validated with standard sporangial germination tests in vitro.

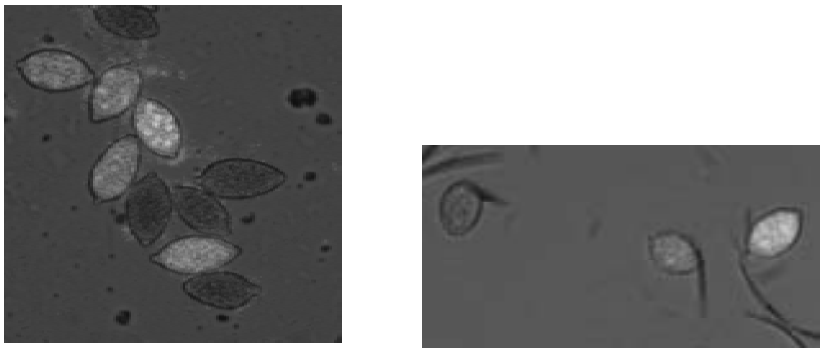
For each fungicide studied, sporangia were harvested at 5, 6, 7 or 8 days after inoculation and suspended in demineralized water and mixed volume to volume with the dyes (according to the experimental protocols for the Viability Kit, Molecular Probes, and with Fluorescein diacetate alone adjusted at 0.1 mg/ml final concentration).
This mixed suspension was incubated in darkness for 5 minutes before assessment.

In each test, half of the sporangia collected on the untreated leaflets were treated with ethanol (95%) to kill them and to represent the negative control (100% dead sporangia) compared to the other half with around 100% live fluoresced green sporangia.



A. B.

Figure1. LIVE/ DEAD Viability Kit (L7007) A: alive fluoresced green (clear) sporangia, B dead orange (dark) sporangia after treatment with ethanol (95%)



A. B.

Figure 2: Comparaison between the A. LIVE/ DEAD Viability Kit Molecular Probes (L7007) and B. fluorescein diacetate alone (FDA).

The advantages / disadvantages to recommend either the use of FDA alone or in mixture with PI (propidium iodide) Viability Kit Molecular Probes (L7007) depend on two criteria:

- LIVE/ DEAD Viability Kit formulation is stable for up to a year when protected from light and for observation all day long, even when FDA has to be resuspended regularly between two observations.
- However PI is a potential mutagen and needs to be handled with appropriate caution whereas the FDA suspension could be used routinely more easily.

No significant difference was observed in sporangia viability using the FDA or the Kit L7007 but in both cases, intermediate green fluoresced sporangia may be scored as alive or as dead depending on the time of sporangia harvest in relation to the beginning of the sporulation. This question requires further investigation.

In vitro germination test

The sporangial suspension was distributed in 96-well microplates with 50 µl of suspension per well and 3 wells per factor.

Assessments were carried out by counting the sporangia that released zoospores or directly germinated compared to the number of sporangia that stayed intact. The % of empty sporangia or germinated sporangia indicates the viability of the zoosporangia in the suspension.

A. B.

Figure 3: In vitro germination of sporangia A: intact sporangia are apparently dead or dormant. B: Empty sporangia or directly germinated means sporangia are alive

Infectivity test
A foliar disc test was preferred to a tuber slices test to evaluate infectivity of sporangia to avoid confusing bacterial symptoms on potato tubers, and because a foliar test is quicker and easier to implement.

In addition to the *in vitro* germination test, the sporangial suspensions were used to inoculate untreated leaf discs to evaluate their infectivity. Assessments were carried out by evaluating the % sporulated area of the leaf discs or by counting the number of sporangia produced on the leaf disks. Sporulating discs were placed in a bottle with 2.5 ml of distilled water and energetically shaken before counting. Five counts were made per new sporangial suspension corresponding to one Petri dish with 10 discs.

Results
The viability of the new formed sporangia at the lower face of the potato leaves which escaped the influence of the fungicide treatment sprayed at the upper face, were measured by fluorescein diacetate (FDA) alone or in mixture with propidium iodide (Viability Kit L7007).
Figures 4, 5 and 6 show the level of disease obtained (% infection /untreated control) related to the different time intervals between treatment and inoculation from 3 hours to 48 hours, which demonstrates protectant activity against late blight following translaminar movement across the potato leaves.
The number of sporangia subsequently produced depends not only on the ability of the compounds applied to the upper surface to inhibit mycelial growth and development of sporangia, but also their penetration kinetics.

Infinito (SC) provided significant translaminar activity (Figure 4). Mandipropamid does not have strong translaminar properties compared to Infinito.
The translaminar activity of dimethomorph increased when the time between treatment and inoculation increased, reflecting the penetration of this active into the tissues to stop the fungal invasion.
Cymoxanil penetration and metabolism was also well illustrated. A rapid penetration into the tissues was observed when intervals between preventive treatment and inoculation were short, but when the interval was extended to 24 hours the level of protection was significantly reduced due to the rapid metabolism of cymoxanil in the plant tissue (Klopping and Delp, 1980; Forbes *et al.,* 1996).
Fluazinam as a contact product showed no translaminar control.

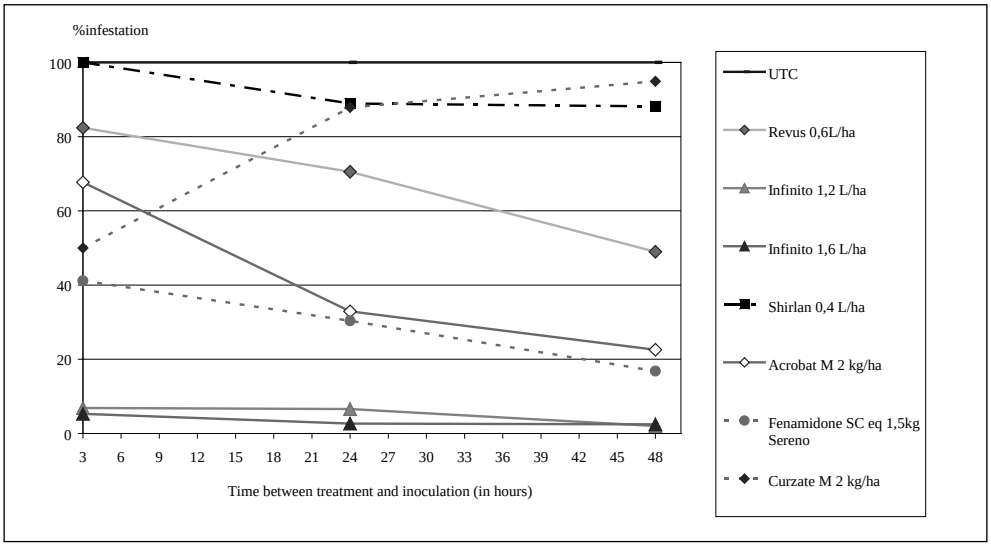


Figure 4: Translaminar effect of fungicides with different intervals between preventive treatment and inoculation (assessment 6 days after inoculation)

The results as presented in Figure 4 were reproduced in a test where fungicides were applied preventative 24 hours before inoculation (See Figure 5).
The results confirm the activity and penetration kinetics of the fungicides as presented in the first experiments in Figure 4. Infinito confirmed its strong translaminar profile and outperformed all the other tested fungicides. Fenamidone (SC) showed a relevant translaminar effect as also illustrated in Figure 4.

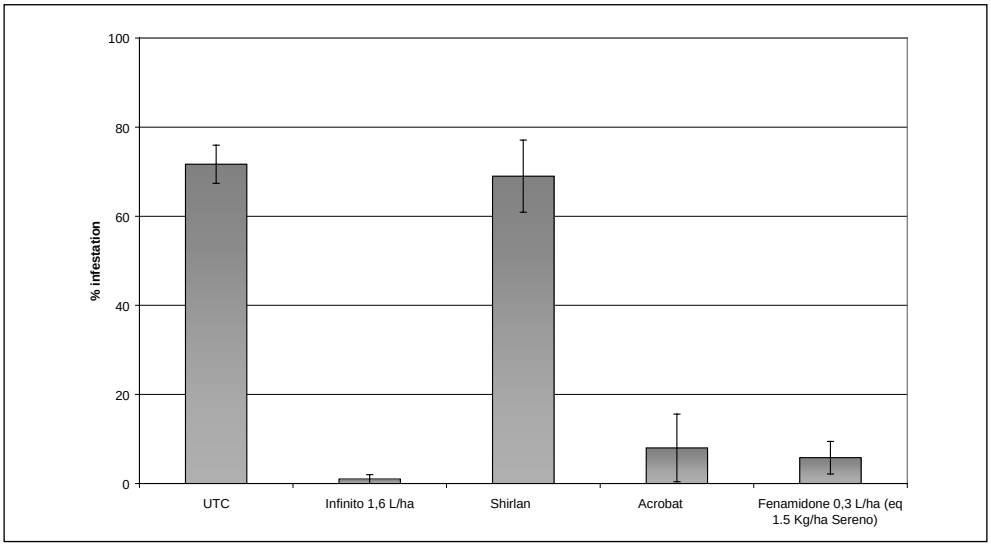


Figure 5: Translaminar effect of fungicides with 24 hours intervals between preventive treatment and inoculation (assessment 6 days after inoculation)

Viability of sporangia

The viability of sporangia collected was very variable from one trial to another and was linked to the ability of products to reduce their production. When no sporangia were produced, it was not possible to study the impact of the fungicide treatment on the viability of new formed sporangia. This was the case for Infinito (Figure6) that showed to have strong antisporulant properties (Latorse M.P.and all, 2006). However it was possible to collect enough even few sporangia for staining by applying fungicides either preventive or curative (2 days before or after inoculation Figure7 & 8).The spore viability followed the similar trends when applied preventative or curative as shown in Figures 7 and 8.

Figure 6: Translaminair effect of Infinito 1.2L/ha and 1.6L/ha at application 24 or 48 hours before inoculation

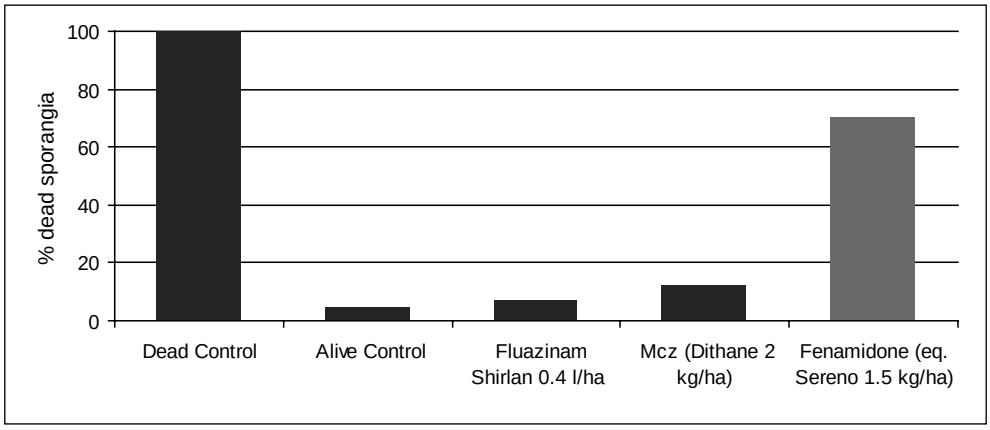


Figure 7: Viability of sporangia (assessment: FDA+PI staining method 7 days after inoculation fungicides applied 48 hours before inoculation).

Tests with newly formed sporangia showed that the majority of the sporangia collected on fluazinam or mancozeb treated leaves as well as untreated control leaves were fluoresced green (live) compared to the ethanol treated one which fluoresced red-orange (dead) . On leaflets treated with a translaminar product such as fenamidone only a limited amount of sporangia could be harvested. The majority of these sporangia fluoresced red orange indicating they were no longer viable (see Figure 7.). Due to the strong translaminar properties of Infinito when applied preventative, no sporangia were formed on the leaflets. As a result the viability of sporangia could not be assessed in these tests as presented in Figure 7.

The viability of sporangia after treatment with Infinito could be evaluated when the product was applied curative 48 hours after inoculation (see Figure 8). The percentage of dead sporangia when assessed under curative conditions was lower compared to preventative treatment (see fenamidone Figure 7 and 8). Even though the percentage of dead sporangia was lower compared to the preventative application, the translaminar products such as Infinito showed a significant effect on the viability of newly formed sporangia. A significant fraction of the sporangia up to ca. 40% fluoresced red-orange (dead). The contact products fluazinam and mancozeb had no effect on spore viability.

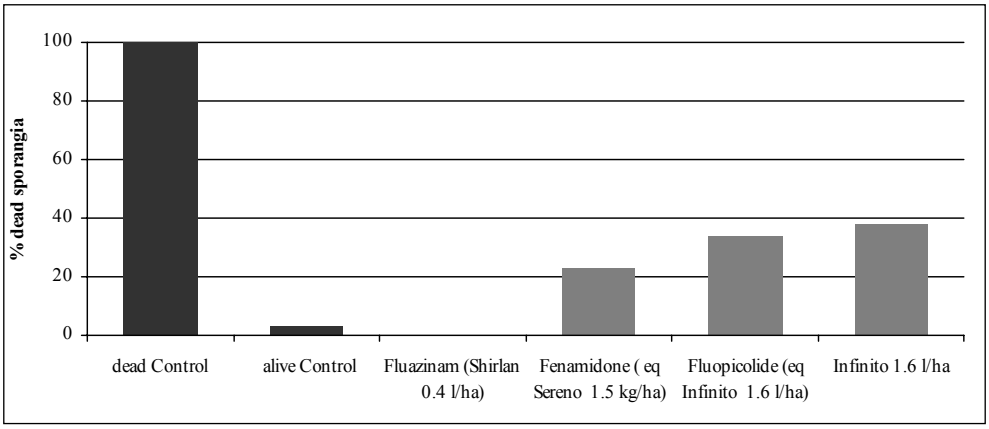


Figure 8: Translaminar effect of fungicides applied curatively 48 hours before inoculation on viability of sporangia. (FDA+PI staining method, assessment 7 days after inoculation).

Infectivity test

In addition to the tests on sporangia viability, new sporangia collected from the leaf underside (Figure 9) were used to inoculate untreated leaf disks to evaluate their infection success. Assessments were carried out by evaluating the percentage sporulated area of the leaf disks or by counting the number of sporangia produced on the leaf disks.

To be sure to collect sporangia for infectivity test, Infinito was applied curatively; it significantly reduced the quantity of sporangia produced due to the combined fungicidal activity of both fluopicolide and propamocarb in comparison to the reference product Tattoo C (propamocarb + chlorothalonil) Figure 9.

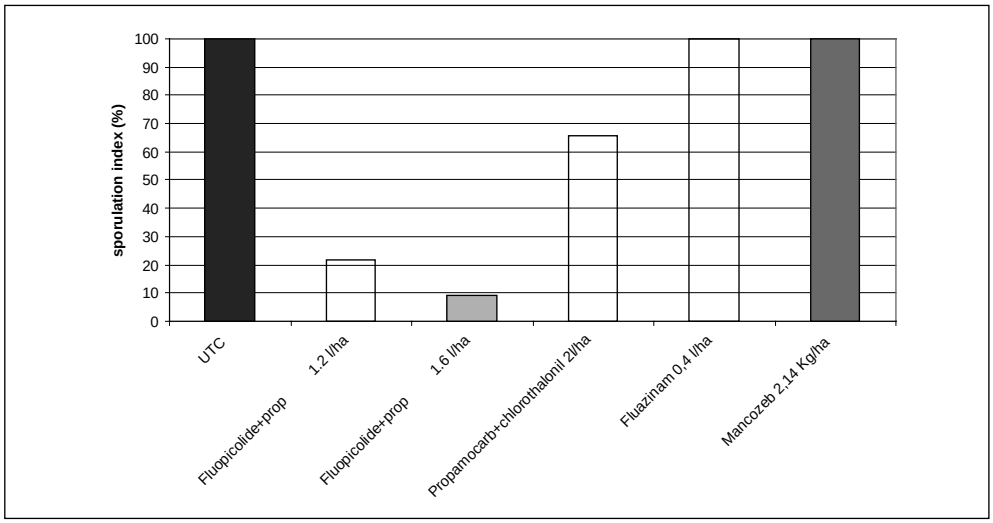


Figure 9. Antisporulant effect of fluopicolide based product Infinito when applied curatively on young lesions

In Figure 10, it was observed that the infection success of the inoculum of new sporangia collected from Infinito treated leaves was affected, even more a significant reduction of infection success was achieved at a dose rate of 1.6 liter per ha of Infinito.

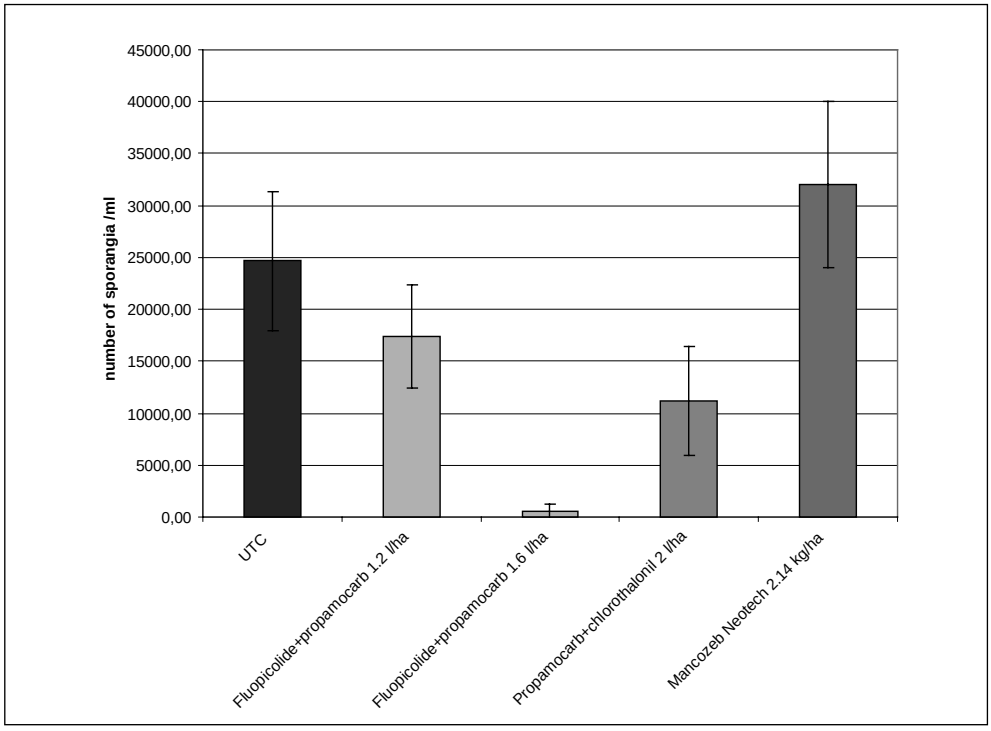


Figure 10. Infinito influence on infection success of secondary sporulation of P. infestans

The results of the tests on infection success indicates the loss of infectivity of the secondary sporulation of leaves treated with Infinito compared to standard fungicides such as Tattoo C and Dithane. The combination of translaminar mobility and antisporulant properties provide contribute to the outstanding activity of Infinito to control late blight.

Discusssion- conclusions

The results presented in this paper illustrate significant differences between the fungicides tested to reduce the formation of sporangia through translaminar activity. Contact products such as mancozeb and fluazinam without translaminar activity demonstrated not to be able to reduce sporangia formation. Infinito showed to have strong translaminar effect. In addition there are marked differences between the fungicides tested to reduce spore viability and consequently together with antisporulant effect, infectivity. Infinito acts on the different steps in the formation of sporangia: reduction of sporangia formation, viability and so infectivity. This multiple action combined with the sporicide effect of Infinito explains the outstanding activity of the product confirmed under field conditions. Infinito has a strong impact in reducing foliar blight (Tafforeau, 2005) and tuber blight (Cooke, 2007). The reduction of the inoculum potential (lower number of sporangia in combination with reduced viability and infectivity) has a significant effect on the apparent infection rate of *P. infestans* as described by Skelsey et al. (2005) and Segarra (2001). It is suggested to consider the properties of fungicides on the apparent infection rates for incorporation into Decision Support Systems for late blight control.

Infinito is a key to farmers to achieve both optimal yield and tuber quality is successful protection of leaves and stems and therefore prevention from infectious sporangia dissemination into the crop.

The results described above are in line with results achieved during intensive field testing of Infinito in 2003-2006. Best results on tuber blight were achieved when Infinito was applied mid season. Infinito is able to strongly reduce the blight on foliage and stems and in addition the viability of spores. As a result the potential of tuber infection is dramatically reduced during the most critical time of the season. These field data underline that modern strategies start at mid season to control tuber blight.

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Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

Epidemic fitness of *Phytophthora infestans* in foliage and tubers during growing season and harvest

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Summary
This papers describes laboratory and field experiments studying competition between *P. infestans* isolates A, B and C during an epidemic in three potato cultivars. The aim was to determine whether a trade off exists between aggressiveness towards foliage and towards tubers. Three *P. infestans* genotypes were inoculated (solo and as a mixture) on three potato cultivars during a replicated field experiment. Each cultivar supported the *P. infestans* isolates that were best adapted to the specific cultivar. The *P. infestans* isolates differed in their capability to establish following inoculation, in their competitive ability during the foliar epidemic and in the efficiency of transition to the tuber. In general, the B isolate was best in establishment and the A isolate was best during the foliar epidemic and the transition to tubers. None of the isolates was best at all three capabilities indicating 'room for improvement' and a future direction for adaptation within *P. infestans* populations.
A trade off between aggressiveness towards foliage and tubers was not found.

Keywords
Potato late blight, potato, aggressiveness, fitness.

Introduction
Following the re-introduction of *Phytophthora infestans* in the Netherlands around 1976, genetic variability increased and the sexual cycle was shown to be functional. An increase of aggressiveness and highly complex virulence spectra (Flier *et al.*, 2003; Flier & Turkensteen, 1999) were two of the consequences that caused drastic changes to the daily practice of potato late blight control.
In a genetically heterogeneous population, the various *P. infestans* genotypes are engaged in a struggle for survival through competition for a 'limited' amount of substrate (potato foliage and tubers). Successful genotypes will dominate both, the foliar and tuber population and survive the winter to be an important part of next years (early) population.
Dominance of the foliar population can be achieved through a superior level of fitness as compared to competing genotypes. Dominance in the tuber population can be achieved through an efficient transition to the tuber (production of high numbers of sporangia, washing down of sporangia by rain, survival in the soil and efficient infection of tubers). In both cases high levels of foliar aggressiveness (high infection efficiency (IE), lesion growth rate (LGR), sporulation intensity (SI) and a short latent

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period (LP)) are beneficial for a specific genotype.
Once in the tuber however high levels of aggressiveness may not be beneficial for survival to the next season. Highly aggressive genotypes will quickly destroy the tuber and with it their survival capsule to the next growing season. There may thus be a trade off between foliar aggressiveness and aggressiveness in the tubers. In support of this theory, bio-assays aimed at quantifying *P. infestans* aggressiveness towards foliage and tubers may display differences in the level of aggressiveness between both tissue types. In support of the alternative hypothesis (the aforementioned trade off does not exist) are the modern, low temperature, storage regimes and the idea that heavily infected tubers during and shortly after harvest may contaminate much more healthy tubers on their way to the storage facilities leading to high levels of latently infected tubers.
Competition between *P. infestans* genotypes was investigated earlier in conjunction with fungicide resistance (Cohen & Samoucha, 1990; Kadish & Cohen, 1988) and host specificity (Lebreton & Andrivon, 1999). This papers describes laboratory and field experiments aimed at testing the hypothesis that a trade off exists between aggressiveness towards foliage and tubers.

Materials & Methods

Potato cultivars and P. infestans isolates
During the first stage of this project, isolate – cultivar combinations were selected displaying differential compatibility. An overview of the cultivars and isolates selected and their characteristics is given in Table 1 and Table 2. Isolates NL01900, NL01096 and NL00228 will be further referred to as isolates A, B and C respectively.

Table 1. Potato cultivars and their characteristics concerning potato late blight resistance as used in the laboratory and field experiments.

Cultivar	Earliness	Foliar resistance	Tuber resistance	Purpose
Karakter	4	6	5	Starch
Mondial	4.5	4.5	5.5	Ware
Remarka	5	6.5	9	Ware

Table 2. *P. infestans* isolates and their characteristics as used in the laboratory and field experiments.

Isolate	Location of origin	Host of origin	Year of origin	Haplo type[1]	Mating type
NL01900 (A)	Wageningen (NL)	*S. sisymbriifolium*	2001	Ia	A1
NL01096 (B)	Katshaar (NL)	*S. tuberosum*	2001	IIa	A2
NL00228 (C)	Dinteloord (NL)	*S. tuberosum*	2000	Ib	A2

Aggressiveness to foliage and tubers
Aggressiveness of the selected isolates towards the foliage of the selected cultivars was determined in laboratory bio-assays by quantifying compatibility parameters 'infection efficiency' (IE), 'latent period'(LP), 'radial lesion growth rate' (LGR) and 'sporulation intensity' (SI).
Aggressiveness towards tubers was determined in bio-assays by quantifying the infection efficiency on tuber eyes.

Field experiments
A field experiment was carried out in 2005 and 2006. Each field experiment contained 36 potato plots measuring 10x10.5m organized in four 'isolate blocks'. Each isolate block contained all three cultivars in three replicates. Isolate blocks were separated by bare soil and maize. Each isolate block was inoculated with one of the *P. infestans* isolates or a mixture of all three isolates.
P. infestans was inoculated by spraying nine single plants per plot with the appropriate sporangial

suspension. Potato late blight severity was assessed twice a week in the following weeks.
The composition of the *P. infestans* population in each plot was determined at three points in time during the epidemic: in the foliage at the start of the epidemic following inoculation, in the foliage just before desiccation and in the tubers following harvest. At each of these sampling times, leaf or tuber samples were taken, *P. infestans* was re-isolated and the haplo type was determined to be able to identify the *P. infestans* isolate. All three isolates used in the experiments could be distinguished based on the mitochondrial haplo type (Griffith & Shaw, 1998, Table 2).

Results

Aggressiveness to foliage and tubers in bio-assays
Based on the results of bio-assays quantifying IE, LP, LGR and SI, two composite parameters were calculated as estimates for R_0, the foliar net life time reproduction, and r, the foliar apparent infection rate according to Skelsey *et al.* (2005). Results are given in Table 3. The higher R_0 and r, the more aggressive, or fit, the isolate on this cultivar.

Table 3. Calculated estimates for foliar aggressiveness; the net life time reproduction (number of daughter lesions per mother lesion) "R_0" and the apparent infection rate "r" for a total of nine cultivar – isolate combinations.

Parameter / Isolate	Karakter	Remarka	Mondial
R_0 [-]			
Isolate A	1158	133	1543
Isolate B	231	167	920
Isolate C	63	0	635
r [day^{-1}]			
Isolate A	0.75	0.39	0.78
Isolate B	0.55	0.45	0.68
Isolate C	0.27	0	0.71

Results on infection efficiency on tuber eyes are given in Table 4. In this experiment, a cultivar effect was the only statistically significant factor. Mondial clearly is the most susceptible cultivar after inoculation of tuber eyes. Remarka and Karakter are equally resistant to infection by the isolates included in this study.

Table 4. Cultivar effect on tuber infection after inoculation of tuber eyes with a sporangial suspension. Means followed by the same letter are not statistically different according to an LSD test at P = 0.05.

	Karakter (5)	Mondial (5.5)	Remarka (9)
Tuber infection (%)	2.5 a	26.5 b	2.2 a

Field experiments
In accordance with the laboratory results, analysis of the levels of tuber infection (incidence) in the field experiments resulted in a significant cultivar effect. The average level of tuber infection was found to be 23.8% for Karakter, 14.1% for Mondial and 4.9% for Remarka ($LSD_{0.05}$ = 9.2%). In contrast to the laboratory results however, Karakter tubers are the most susceptible in the field followed by Mondial and Remarka. This discrepancy possibly indicates additional infection sites, such as lenti cells, apart from the tuber eyes which were specifically targeted in the bio-assay.

P. infestans population dynamics
Analysis of *P. infestans* population dynamics focuses on the cultivar Mondial. The time course of the

epidemics on Mondial in both years and for all inocula is given in Figure 1. Differences between the inocula exist within each year but are not consequent between years. Overall, the course of the epidemic is more or less the same for all inocula. In 2006 however the epidemic is delayed by approximately 1 – 2 weeks as compared to 2005. This is due to a warm and dry period in July 2006 which stopped epidemic development for 1 – 2 weeks but did not succeed in killing *P. infestans*.

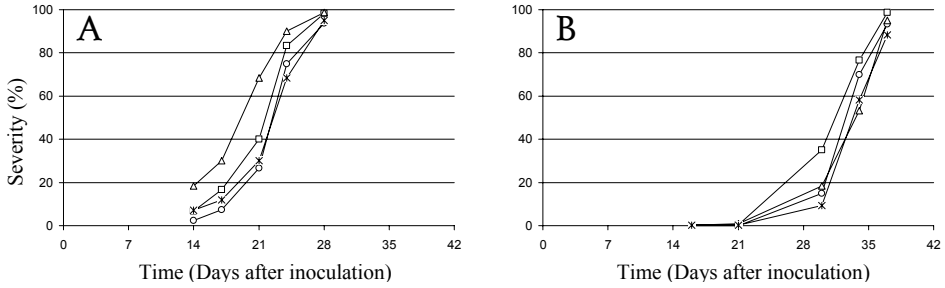

Figure 1. Time course of foliar epidemics in 2005 (A) and 2006 (B) on Mondial for each of the four inocula: A isolate (○), B isolate (∗), C isolate (Δ) en Mixed inoculum (□). The first and second leaf sampling took place on day 7 and day 24 in 2005 en on day 7 en day 34 in 2006.

The result of the competition between *P. infestans* isolates on potato cultivar Mondial are given in Figure 2. The top three rows in this Figure represent the results after inoculation with one of three *P. infestans* genotypes. The fourth and last row of two graphs represents the results after inoculation with a mixture of all three *P. infestans* genotypes. The population composition is given for each of the three sampling times. In general, the genotype used for inoculation dominates the population after inoculation with 1 genotype. Due to incoming inoculum from other isolate blocks, the A isolate is however always able to establish itself and become a significant part of the population during the foliar epidemic, also in plots where it was not inoculated. Although the reverse is true for the B and C isolate, their contribution to the population remains marginal when not inoculated.

When the plots are inoculated with a mixture of all three genotypes, all three establish and contribute significantly to the total population. During the course of the foliar epidemic however, the A isolate becomes more dominant at the expense of mostly the C isolate.

During the transition from foliage to tuber, the A isolate gains an even more dominant position in the tuber population than was to be expected based on its contribution to the foliar population.

On both other cultivars, Karakter and Remarka, the C isolate does not establish and can be considered non-compatible. The A isolate behaves in a similar way as described above for Mondial. On Remarka and Karakter however, the B isolate is better in establishment following inoculation than the A isolate.

Overall we can thus say that potato cultivars support those *P. infestans* genotypes that are best adapted to a cultivar. Furthermore, the B isolate seems to be better in establishment than the A and C isolate, at least on Karakter and Remarka. The A isolate is a better competitor during the subsequent foliar epidemic and is more efficient than the other two *P. infestans* genotypes during the transition to tubers.

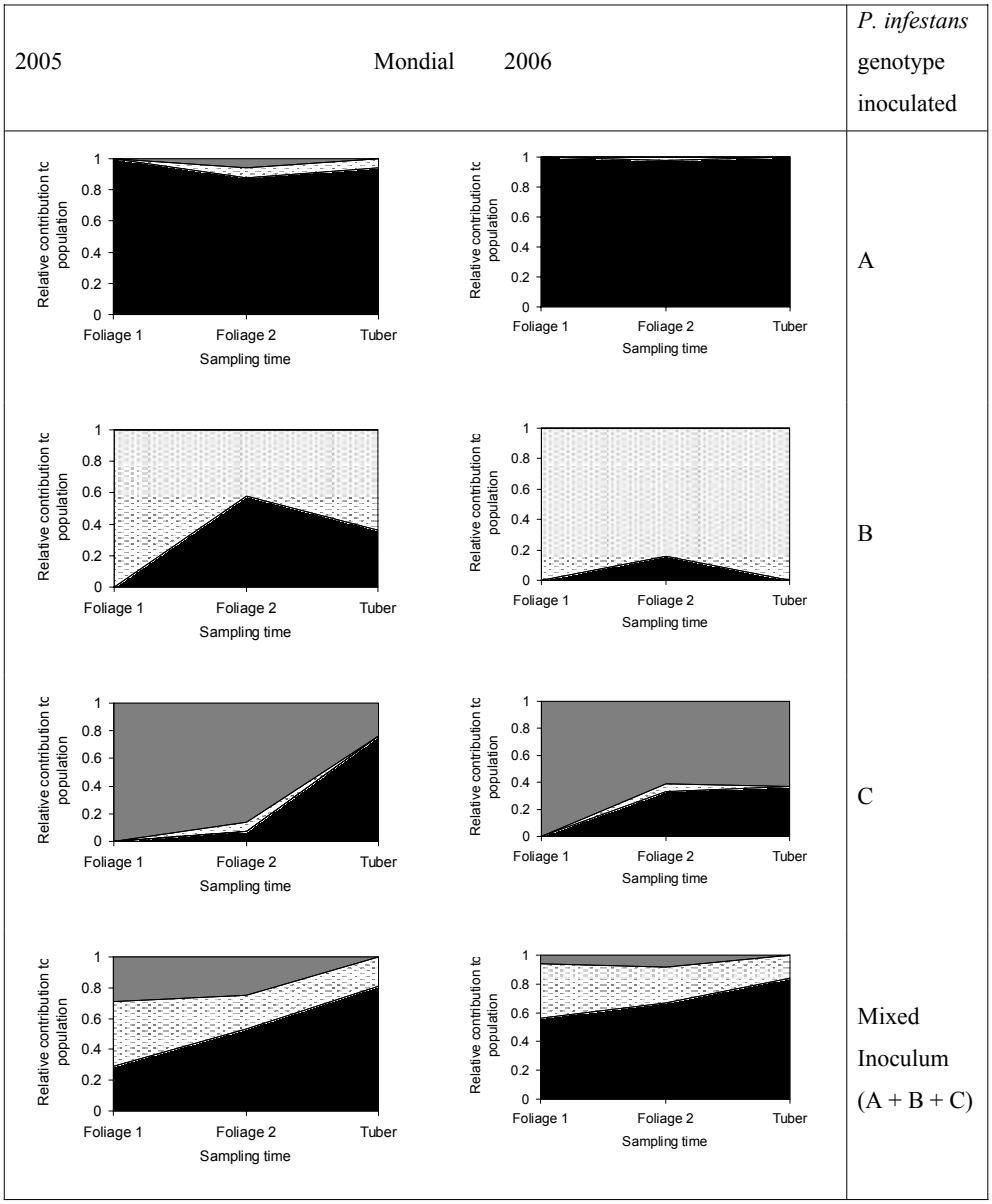

Figure 2. Relative contribution of P. infestans genotypes following solo inoculation and following inoculation with a mixture of the three genotypes. ■ : P. infestans A genotype; ▒ : P. infestans C genotype; ▨ : P. infestans B genotype.

Discussion and Conclusions

This papers describes laboratory and field experiments studying competition between *P. infestans* genotypes (isolates) during an epidemic in three potato cultivars. The aim was to determine whether a trade off exists between aggressiveness towards foliage and towards tubers. Three *P. infestans* genotypes were inoculated (solo and as a mixture) on three potato cultivars. Each cultivar supported the *P. infestans* isolates that were best adapted to the specific cultivar. The *P. infestans* isolates differed in their capability to establish following inoculation, in their competitive ability during the foliar epidemic and in the efficiency of transition to the tuber. In general, the B isolate was best in establishment and the A isolate was best during the foliar epidemic and the transition to tubers. None of the isolates was best at all three capabilities indicating 'room for improvement' and future direction for developments within *P. infestans* populations.

A trade off between aggressiveness towards foliage and tubers was not found. The most aggressive isolate in foliage was also the most prominent isolate in the tuber population. Thus, this isolate is in the best position to survive both the summer and winter (storage) period. Although the effect of the storage period on the composition of the *P. infestans* population could not be investigated, it is expected that the environmental conditions of a modern storage regime will neutralize the situation as it was found two weeks after harvest and thus preserve the population composition for the new growing season.

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Estimated yield losses caused by potato late blight in Finnish fungicide trials in 1992-2006

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Summary
Potato late blight epidemics in Finland have shown to start 3-4 weeks earlier in 2000s in comparison to 1980s. The impact of earlier epidemics on yield losses, tuber blight and performance of fungicide programs was examined from standard fungicide efficacy trials carried out in 1992-2006. The efficacy of fungicide programs against leaf blight was constantly reasonable. There was no increase in tuber blight incidence during the period. More marketable yield was obtained by all fungicide programs in 2000s than in 1990s in comparison to untreated control. There was no constant difference between fungicide programs in leaf or tuber blight control or yield increase.

Keywords
Phytophthora infestans, tuber blight, chemical control

Introduction
During the 1980s a new population of *Phytophthora infestans* containing both mating types A1 and A2 thus capable in sexual reproduction migrated from South America in Europe and replaced the old clonal lineage (Fry *et al.*, 1993). Circumstantial evidence for oospores as a primary source of inoculum has been published (Drenth *et al.*, 1995, Andersson *et al.*, 1998, Hannukkala & Lehtinen, 2004).

In Finland it has been shown that late blight epidemics in 2000s have started 3-4 weeks earlier than in 1980s and early 1990s probably due to oospores and short crop rotations. Simultaneously the use of fungicides against late blight has increased considerably (Hannukkala *et al.*, 2007).

The aim of this study was to examine if the yield losses due to earlier epidemics have increased, is there an increase in tuber blight and how does mancozeb products perform in comparison to systemic and translaminar compounds in fungicide programs.

Material and Methods
The data was collected from standard fungicide efficacy trials carried out in 1992-2006 at two experimental sites, Jokioinen and Lammi, in Southern Finland. There were 44 individual experiments included in the dataset. All trials were randomized complete block design with four replicates. In

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Jokioinen the trials were carried out according to GEP (Good Experimental Practise) standards since 1999. The fungicides were applied according to standard procedures at 7-10 day intervals. Only treatments with on label full dose were included in the comparison. The number of applications varied between four and eight per season depending on the blight risk and the type of fungicides in program. In this data the average number of applications per season was not increased from 1990s to 2000s as has happened in commercial potato production.

Fungicides in trials varied from year to year. Only mancozeb and untreated control were included in all trials in each year. Therefore the treatments were classified as follows:

1. Untreated control
2. Mancozeb program
3. New contact fungicides (fluazinam, zoxamide, cyazofamid)
4. 2 x translaminar (propamocarb, dimethomorph, fenamidone) + 3-4 x contact (mancozeb or fluazinam)
5. 1-2 x metalaxyl + 3-5 x contact (mancozeb or fluazinam)

Results and Discussion

The percentage of infected leaf area at the end of season in untreated plots was normally close to 100 %. In average fungicide programs provided reasonable control against leaf blight. Performance of mancozeb programs was equal to any other programs. However in recent years new contact fungicides were more effective against leaf blight than mancozeb. In early 1990s, when metalaxyl resistant isolates were widely present in the population (Lehtinen *et al.,* 2007) metalaxyl programs sometimes totally failed in leaf blight control.

Tuber blight was commonly present in the experiments in most years. There was no trend towards increase of tuber blight from 1990s to 2000s. There was huge variation between years and fungicide programs in the incidence of tuber blight. New contact fungicides were clearly more effective against tuber blight than mancozeb products.

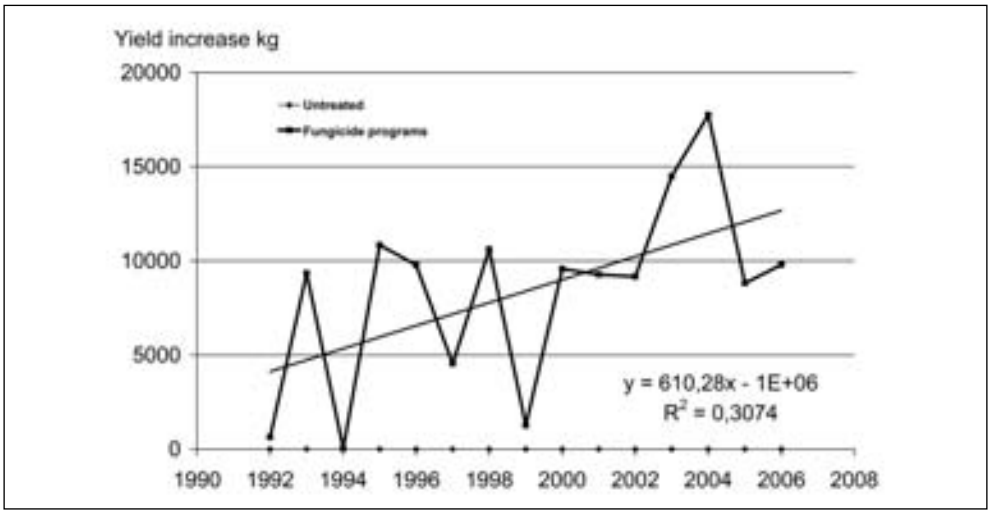

Figure 1. The average increases in healthy marketable yield obtained by different fungicide programs in comparison to untreated control in 1992-2006.

There was a clear trend to obtain more marketable healthy yield by all fungicide programs towards the end of the period 1992-2006 (Figure 1). The increase in yield compensates the cost of increased fungicide use due to earlier epidemics. None of the fungicide programs gave constantly higher yield increases than any other.

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Tenth Workshop of an European Network for development of an
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Amisulbrom (NC-224): Performance of new fungicide for potato late blight control

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Summary
NC-224 (amisulbrom; ISO proposed) is a new fungicide for control of Oomycete diseases. In the field trials carried out in 6 different European countries from 2002 through 2006, NC-224 provided excellent control against late blight on leaves and stems of potatoes. In addition, NC-224 was shown to be effective against tuber blight. Greenhouse tests revealed that NC-224 had strong anti-sporulation activity and inhibited secondary infestation, and exhibited good rain stability. NC-224 has good toxicological, ecotoxicological and environmental profiles. NC-224 is now in progress of commercial development under the representative trade name Reimay® in Europe and regulatory approval is scheduled in 2007 and after in the world.

Keywords
NC-224, Amisulbrom, *Phytophthora infestans*, late blight, stem blight, tuber blight

Introduction
NC-224 (Amisulbrom; ISO proposed) is a Nissan Chemical's proprietary compound belonging to sulfonamide derivatives, which was discovered in 1999 and has been developed since 2002 as an Oomycete specific fungicide. Mode of action is inhibition of respiration by binding Qi center of complex III on mitochondrial electron transport system. Regulatory approval is scheduled in 2007 and after in the world. This paper describes fungicidal characteristics of NC-224 and summary of field trials conducted in 6 different European countries from 2002 through 2006.

Materials and Methods
NC-224 20SC was formulated as a solo product of 200 g/l suspension concentrate (SC) with adjuvant incorporated, and used for greenhouse tests and field trials. Registration of the formulation has been intended under the representative trade name Reimay® in Europe.

Plants and fungal strains
Potato plants (cv. Danshaku) were grown in a greenhouse in 10×10×13 cm pots for 21 days. *Phytophthora infestans* (strain TK-301) was grown on rye A agar plates at 20 °C for 10 days. Zoosporangia suspension was prepared by pouring distilled water over the fungal colonies and gently rubbing the surface with a brush. The suspension was filtered through two layers of gauze and zoosporangia concentration was adjusted to 10^5 sporangia/ml with a haemocytometer. This suspension was maintained at 15 °C for 1 hour to release zoospores from the sporangia and used as inoculum for greenhouse tests.

Anti-sporulation activity
Potato plants were sprayed NC-224 20SC at 100 ppm after inoculation of *P. infestans* zoospores, incubation in a dew chamber (20 °C, humidity 100%) for 1 day and kept in a greenhouse for 4 days. Anti-sporulation activity was evaluated 24 hr after application on the basis of the following rates; 100: no sporulation, 99: little sporulation, 50: sporulation on only lower surface, 0: sporulation on both surfaces.

Inhibition of secondary infestation
Six pots of potato plants were inoculated with *P. infestans* and maintained in a dew chamber. Two days after inoculation, these pots were sprayed with NC-224 20SC at 100 ppm and placed forming a circle. A healthy untreated potato plant pot was placed in the middle of the circle 1 day after application. The plants were kept in the dew chamber again for 6 days and the plant placed in the middle was evaluated for efficacy against late blight.

Rainfastness
Potato plants were sprayed with NC-224 20SC at 100 ppm and reference products at practical doses for the following rainfastness experiments.

Rainfall strength experiment
Two hours after fungicide application, the pots were subjected to 40 mm/hr of artificial rain for 1 or 3 hr (total rainfall: 40 mm or 120 mm respectively). One day after the rainfall, the plants were inoculated and kept in a dew chamber for 1 day and then placed in a greenhouse for 5 days. Efficacy was evaluated on the basis of disease severity.

Post-spray timing of rain experiment
The fungicide-applied pots were subjected to 10 mm of artificial rain for 2 hr from at 1, 2, 3 or 6 hr after application, respectively. Inoculation and evaluation were conducted by the same procedure described above.

Field trials
Field trials were carried out from 2002 through 2006 in total 64 trial sites in UK, Netherlands, Germany, Finland, Austria and France. All the trials (except a specific trial described below) were conducted in line with EPPO guidelines for registration, focusing on protection against foliar, stem and tuber blight.

Specific stem blight field trial
NC-224 20SC and reference products were applied at July 6, 13 and 20 in 2006 followed by Shirlan SC. Inoculation was carried out by injecting 20-50 zoospores superficially in the leaf axil of potato

plants. Efficacy was evaluated on the basis of disease severity at 7 day intervals from August 11 until October 5 and AUDPC (Area Under Disease Progress Curve) value was calculated.

Results and Discussion

Safety and environmental profile

NC-224 20SC has very good safety and environmental characteristics. It was demonstrated that NC-224 20SC had low toxicity to non-target organisms, which were carps, daphnia, algae, bees, earthworms and natural enemies (Table 1 and Table 2). Persistency of NC-224 in soil was confirmed relatively short, showing DT50 3-13 and DT90 9-42 in European field studies. Level of NC-224 accumulation in groundwater was less than 0.001 µg/L in all the scenarios.

Table 1. Toxicity data

Oral rat	LD50 >5000mg/kg
Dermal rat	LD50 >5000mg/kg
Inhalation rat	LC50 >6.4mg/L
Skin irritation rabbit	Not irritating
Eye irritation rabbit	Moderately irritating
Sensitization	Not sensitizing

Table 2. Ecotoxicity data

Acute - Carp	LC50=12 mg/L
Acute - Daphnia	EC50=0.31 mg/L
Growth inhibition - Alga	EbC50=0.37 mg/L
Bees (oral, contact)	LD50 (48hrs) >0.1mg as/bee
Acute NTA - *T. pyri*	LR50 (7d) >1000g as/ha
Acute NTA - *A. rhopalosiphi*	LR50 (48hrs) >1000g as/ha
Acute - Earthworm	LC50 >1000ppm

Greenhouse test

Anti-sporulation activity

Effect of NC-224 20SC against zoosporangia sporulation on pre-inoculated potato leaves was evaluated. High level of inhibition against sporulation on the leaves was observed at 100 ppm (Table 3). The result suggested that translaminar activity of the compound also contributed to a high level of inhibition of sporulation on leaves.

Table 3. Anti-sporulation activity

Product	Dose (ppm)	Efficacy
NC-224 20SC	100	75

Inhibition of secondary infestation

Leaf area infested in the plant surrounded by the NC-224 20SC-treated plants was 12%, whereas control was 80% (Table 4). It was suggested that zoospore viability was significantly affected by the contact with NC-224 on leaf surface.

Table 4. Inhibition of Secondary infestation with physical contact of zoospores

Product	Dose (ppm)	Disease severity of the placed plant
NC-224 20SC	100	12
Control	-	80

Rainfastness

Efficacy of NC-224 20SC against late blight was not affected by rainfall at 40 mm/hr for 3 hr as well as cyazofamid TP, whereas disease control by mancozeb reduced from 89.2% to 75.5% at the same condition (Figure 1). When subjected to rainfall at 2 or 3 hr after application, NC-224 20SC provided 58 and 86% control respectively, exceeding the level of cyazofamid TP (Figure 2). At the time of 6 hr after application, efficacy of NC-224 20SC was confirmed no longer affected by the rainfall. This performance was supposed due to preferable formulation besides chemical and physical properties of the compound.

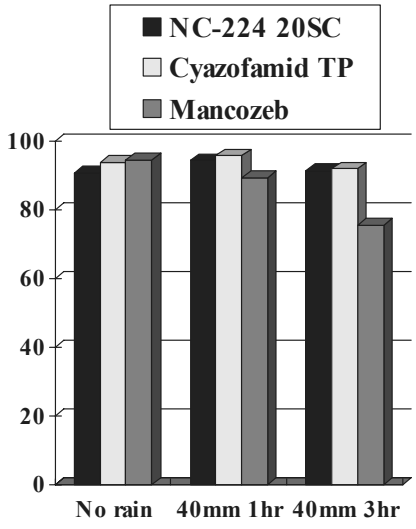


Figure 1. Rainfastness-Greenhouse test in Japan, 2005.

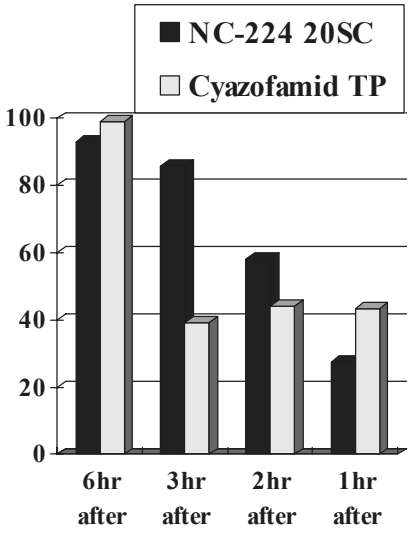


Figure 2. Rainfastness-Greenhouse test in UK, 2005.

Field trials

Efficacy on foliar blight

NC-224 20SC was highly effective against foliar blight at 0.5 l/ha or 100 g a.i./ha (Figure 3). In 48 trials, 90% of data of efficacy values was ranged between 70 and 100 (average 87.4), which is comparable to cyazofamid TP and better than fluazinam SC and cymoxanil + mancozeb.

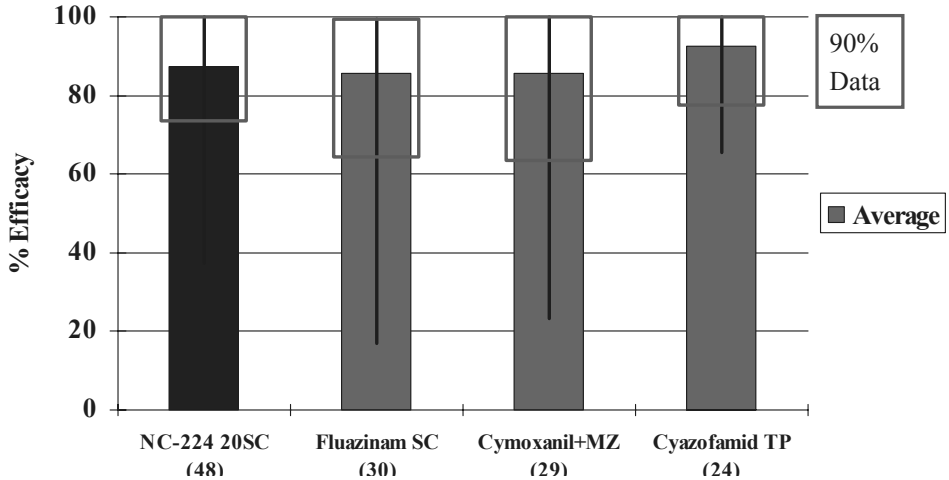


Figure 3. Efficacy on foliar blight.

Efficacy on stem blight

NC-224 20SC demonstrated good protection against stem blight at 0.5 l/ha or 100 g a.i./ha (Figure 4). In addition, specific field trial with artificial inoculation revealed that NC-224 20SC provided excellent stem protection equivalent to mefenoxam + mancozeb and superior to fluazinam and cymoxanil + mancozeb (Figure 5). AUDPC value of NC-224-treated plot was 426 under condition that of untreated control 2,570.

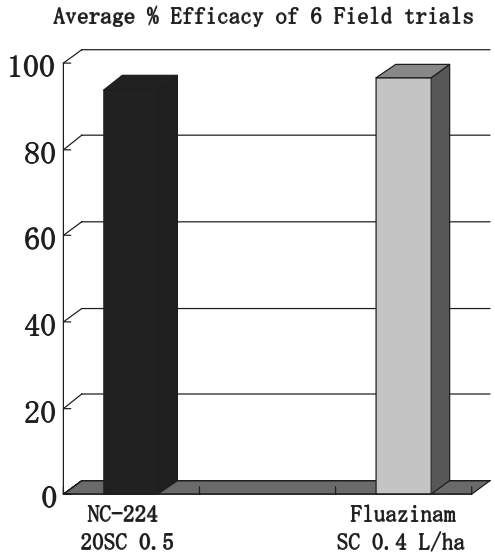
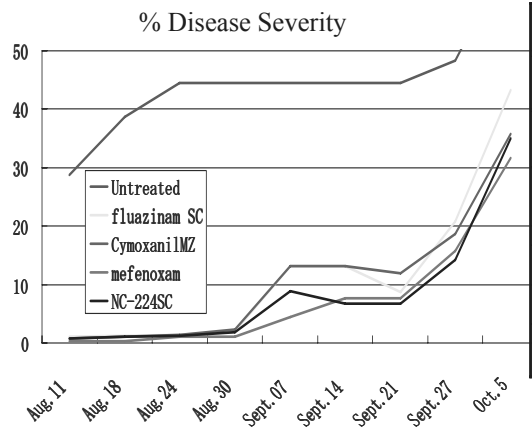


Figure 4. Efficacy on stem blight.

Efficacy on tuber blight

NC-224 20SC significantly reduced tuber blight when applied at 0.5 l/ha or 100 g a.i./ha. An average yield rate of NC-224 20SC-applied plots in 45 trials was 191% compared to untreated control (100%), which was equivalent to fluazinam SC (Figure 6). An average rate of infested tubers in weight of NC-224 20SC-applied plots in 12 trials was 3.9% under condition untreated control was 19.5%, which level was comparable to cyazofamid TP (Figure 7)



Fungicide	AUDPC
NC-224 20SC 0.5L/ha	426
FluazinamSC 0.4L/ha	603
Cymoxanil +MZ WG 2.5kg/ha	578
Mefenoxam +MZ WG 2.5kg/ha	392
Untreated	2,570

Figure 5. Specific stem blight field trial.

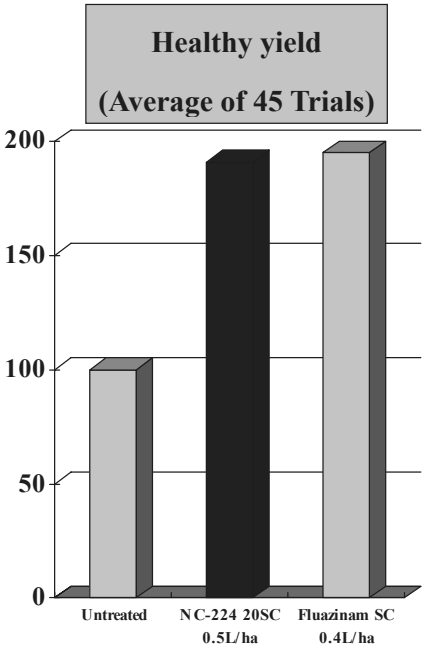


Figure 6. Efficacy on tuber blight

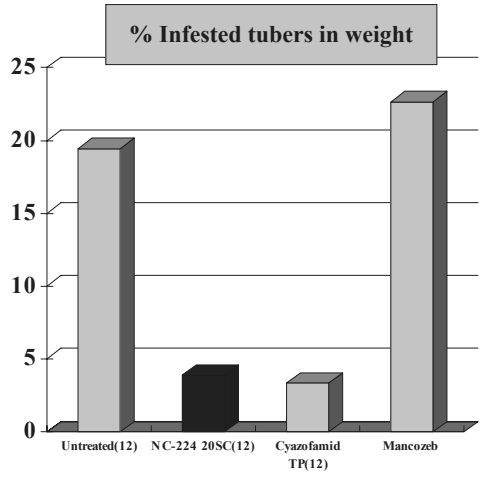


Figure 7. Efficacy on tuber blight

Conclusion

NC-224 20SC is a highly active, protective Oomycete fungicide with good biological properties, strong anti-sporulation activity and good rainfastness. It exhibits a high level of control of foliar, stem, and tuber blight in the field trials. It also has low toxicity to mammals and non-target organisms and is very safe to the environment. These characteristics make NC-224 20SC a good candidate for late blight control.

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Mandipropamid - a new fungicide for the control of late blight in potatoes

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Abstract
Mandipropamid formulated as SC250 and recommended at 150 g a.i./ha is a highly effective new product for the control of late blight in potatoes. It is recommended as preventive treatment. Mandipropamid prevents the germination of spores of *Phytophthora infestans* at very low concentrations. Its affinity to the waxy layer of plant surfaces assures long lasting efficacy and excellent rainfastness. Gradual uptake of small amounts of the active ingredient into the plant tissue provides good translaminar and limited curative and antisporulant activity. These properties of mandipropamid are further documented in this paper. They can explain the consistently excellent disease control achieved with this new product in field trials and large scale farm applications also during periods of high disease pressure and difficult weather conditions with frequent rain. For the potato grower demanding a high standard of control of late blight, mandipropamid is a new tool providing more reliable protection from disease infection independent of weather conditions.

Keywords
Mandipropamid, *Phytophthora infestans*, late blight, rainfastness, field efficacy

Introduction
Mandipropamid is a new fungicide against foliar Oomycete pathogens developed by Syngenta. It is the first derivative of the chemical class of the mandelamide fungicides available commercially. Since its discovery in 1999, it has undergone extensive international evaluation in laboratory tests and in extensive global field testing programs. The chemical and physical properties of mandipropamid, its biological activity and its safety profile were first described in 2005 (Huggenberger *et al.*, 2005; Hermann *et al.*, 2005; Knauf-Beiter and Hermann, 2005).

This paper summarizes results from additional laboratory, greenhouse and field tests to further evaluate the efficacy of mandipropamid against late blight *(Phytophthora infestans)* in potatoes.

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Mode of action and resistance risk
Based on cross-resistance studies with grape downy mildew *(Plasmopara viticola)* mandipropamid, dimethomorph, flumorph, iprovalicarb and benthiavalicarb are grouped in the same mode of action group of the carboxylic acid amide (CAA) fungicides. The CAA-FRAC working group has been established in December 2005 and the CAA-fungicides have been grouped in the mode of action group 40 (CAA FRAC www.FRAC.info).

The mode of action of the CAA-fungicides is not known. Morphological studies indicate that CAA-fungicides inhibit cell wall biosynthesis/assembly (Mehl and Buchenauer, 2002). Biochemical studies suggest alterations in the phospholipid biosynthesis with an inhibition of phosphatidylcholine (Griffith *et al.*, 2003).

The resistance risk to CAA-fungicides has been evaluated in extensive tests in grape downy mildew (*Plasmopara viticola*) and late blight in potatoes (*P. infestans*).

Resistant field isolates against the CAA-fungicides can be found in *P. viticola*. In crossing experiments with resistant and sensitive isolates of *P. viticola* to CAA-fungicides the segregation patterns suggest that resistance to CAA-fungicides is controlled by two recessive nuclear genes. No cross-resistance was found to other mode of action groups (Gisi *et al.,* 2007). After removal of selection pressure resistant isolates in a population of *P. viticola* decline to a non detectable level within one or two seasons. In *P. viticola* the resistance risk to CAA-fungicides is judged as moderate (Gisi *et al.*, 2007). For resistance management a maximum of 4 sprays of CAA-fungicides are recommended against grape downy mildew. Against this pathogen CAA-fungicides should always be used in combination with an effective partner such as multisite protectants or other non cross-resistant fungicides (CAA FRAC www.FRAC.info).

Despite extensive use of CAA-fungicides for many years, no resistant field isolates of *P. infestans* to CAA-fungicides were found to date. Extensive monitoring studies were carried out in different European countries and in Israel since 2001 (Cohen *et al.*, 2007). Resistance in artificial laboratory mutants is not stable and it was not possible to select resistant strains in a series of forced selection experiments with mixed populations of a wide range of isolates. CAA-fungicides are similarly effective against A1 and A2 strains of *P.infestans* as well as against metalaxyl-sensitive and resistant isolates. Based on these extensive tests the resistance risk in *P. infestans* to CAA-fungicides is judged as low (Rubin *et al.*, 2007; Cohen and Gisi, 2007; Cohen *et al.*, 2007). As a resistance management precaution, products containing a CAA-fungicide should not represent more than 50% of the sprays in a spray program against late blight in potatoes. Alternation with fungicides from different mode of action groups should be considered (CAA FRAC www.FRAC.info). Because of its excellent field efficacy mandipropamid is recommended as stand alone product for the control of late blight in potatoes.

Activity of mandipropamid in the life cycle of *P. infestans*
To complement results of studies reported earlier (Knauf-Beiter and Hermann, 2005), Thompson and Cooke (2007) investigated the effect of mandipropamid on the germination of encysted zoospores and the direct germination of sporangia by scanning electron microscope. Greenhouse grown potato plants were treated with spray solutions containing 100 ppm mandipropamid. Treated and untreated leaflets were detached and inoculated with 20 µl droplets of spore suspensions on the adaxial or abaxial leaf surface. Spores were examined under the microscope 24 h (zoospores) or 48 h (sporangia) after inoculation. The zoospores appeared to fail to develop walls during encystment. They failed to produce a germ tube and subsequently disintegrated. Sporangia simply failed to germinate.

Cohen and Gisi (2007) compared the activity of the CAA-fungicides mandipropamid, dimethomorph and iprovalicarb against the various stages in the asexual life cycle of *P. infestans*. Also in these studies the CAA-fungicides had a strong effect on the germination of encysted zoospores and on the direct germination of sporangia. In these tests mandipropamid was clearly the most active molecule and was inhibitory at nM concentrations (EC50 < 0.0005 µg/ml). Dimethomorph and iprovalicarb were about 10-100 times less active. Mandipropamid and dimethomorph induced major ultrastructural changes in encysted zoospores during germination. When applied after germination the CAA-fungicides caused malformation of the germ tubes, inhibited mycelial growth and reduced sporulation. The efficacy of preventive spray applications to potato or tomato plants was fungicide- and dose dependent reflecting the relative efficacy of the different CAA-fungicides against spore germination. The CAA-fungicides also showed limited curative and antisporulant effects.

Uptake and translocation

Mandipropamid has a high affinity to wax layers of plant surfaces. After the spray liquid reaches plant surfaces, a major proportion is adsorbed rapidly into the wax layer and is fully resistant to wash-off by rain as soon as the spray deposit has dried. Gradually a proportion of the active ingredient moves into the plant tissue by translaminar movement. The results of a study by Hermann *et al.* (2005) using C^{14} radiolabelled material showed that the proportion adsorbed into the wax layer was higher for mandipropamid than for dimethomorph. The proportions taken up into the leaf tissue were similar for both molecules (Figure 1).

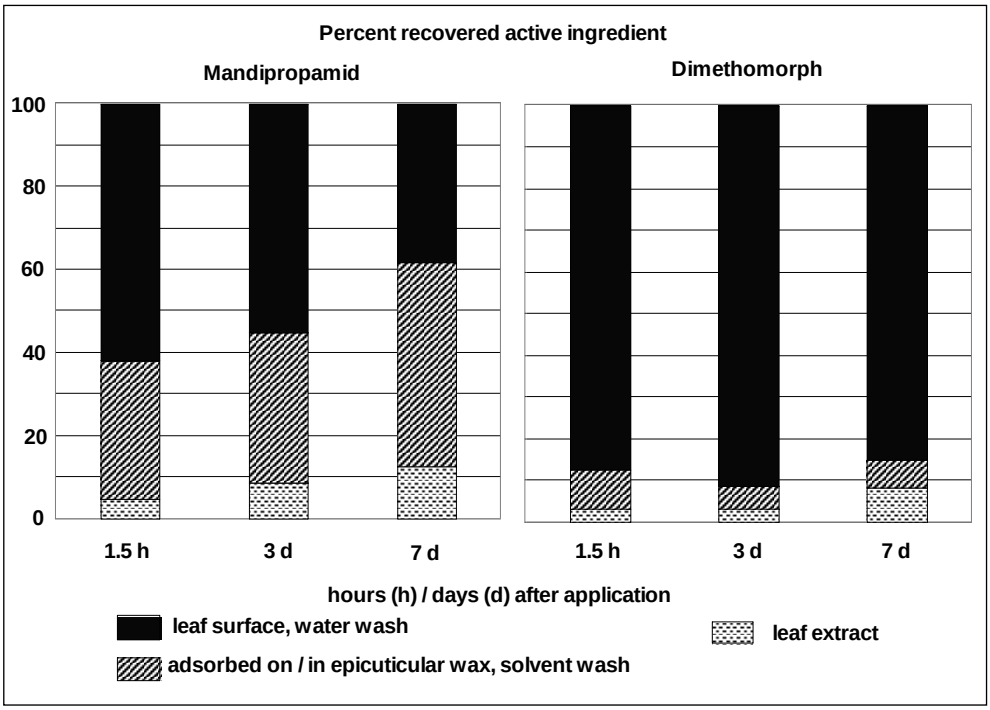


Figure 1. Proportions of mandipropamid and dimethomorph remaining on the leaf surface, adsorbed into the wax layer and taken up into the leaf tissue following foliar application of the products to potato leaves; study with C14 radiolabelled material.

Translaminar activity in potato plants

The results of a test evaluating the translaminar activity of mandipropamid and dimethomorph are shown in Figure 2 (Cohen and Gisi, 2007). The products were applied to the upper leaf surface of potato plants in the greenhouse. Inoculation was carried out on the lower leaf surface of detached leaves. The percent leaf area infected was evaluated 6 days after inoculation. The results of this test show a clearly better translaminar efficacy of mandipropamid, when compared to dimethomorph. Even though only a small amount of mandipropamid is taken up into the plant tissue, the excellent translaminar efficacy can be explained by its high intrinsic activity.

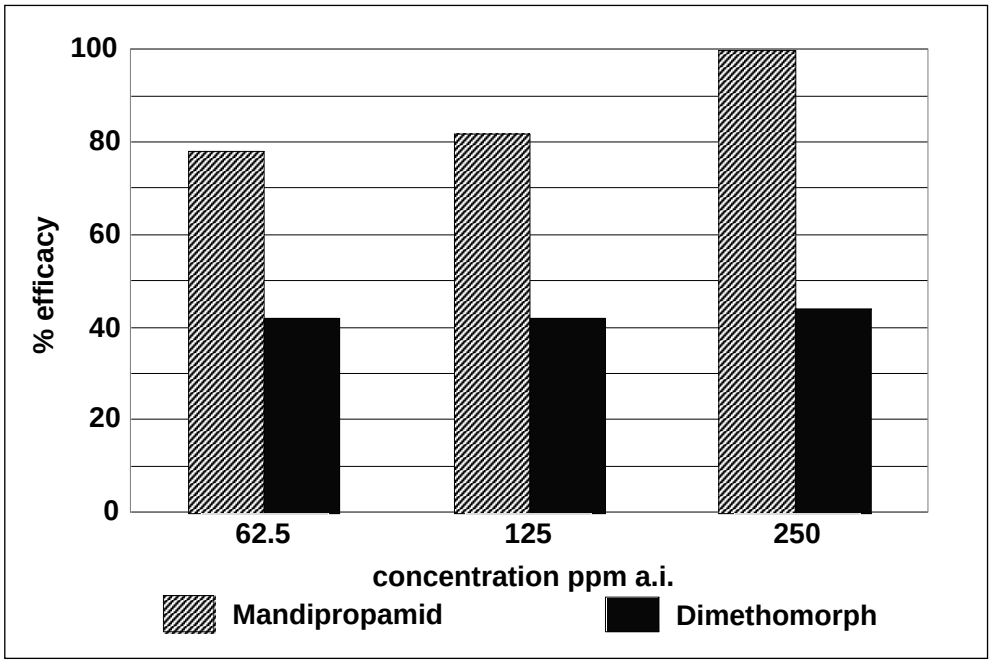


Figure 2. Translaminar activity of mandipropamid and dimethomorph in potato leaves.

Preventive, curative and antisporulant activity in greenhouse tests

Consistent with results of tests carried out to study the activity of mandipropamid in the asexual life cycle of *P. infestans*, the efficacy of mandipropamid was consistently excellent in further greenhouse or field studies, when the product was applied preventively. Mandipropamid also provides excellent persistence of disease control (Figures 5 and 6).

The curative and antisporulant activity of mandipropamid is more variable. The curative and antisporulant activity of mandipropamid is dependent on the speed and the intensity of the epidemic. In greenhouse tests with small plants and optimal conditions for disease development leaves are often completely destroyed by the disease 4-5 days after inoculation. Under these conditions the curative and antisporulant activity of mandipropamid is weak. This is also true for dimethomorph and other translaminar compounds with relatively low water solubility. The activity of mandipropamid can be significantly improved by tankmixing a suitable adjuvant to increase its leaf uptake. However, the consistently high level of curative activity achieved with cymoxanil cannot be reached in standard greenhouse tests with small plants.

In an attempt to more closely simulate field conditions in a controlled greenhouse environment, studies with curative applications of mandipropamid were carried out using larger plants. In these tests the disease progress was clearly slower (Figure 3). The evaluation of the leaf area infected was made 11 days after inoculation. In these tests the curative activity of mandipropamid alone applied one day after inoculation was clearly better than in tests with small plants. Also in these tests the tankmix addition of a suitable adjuvant significantly improved the curative efficacy of mandipropamid. In the test reported in Figure 3 the curative activity of mandipropamid tankmixed with an adjuvant was similar to the best reference standard cymoxanil+mancozeb.

For commercial use mandipropamid based products are recommended as preventive treatments. However, especially under heavy disease pressure, the limited curative and antisporulant activity of mandipropamid may complement its excellent preventive activity and contribute to its overall highly reliable field efficacy.

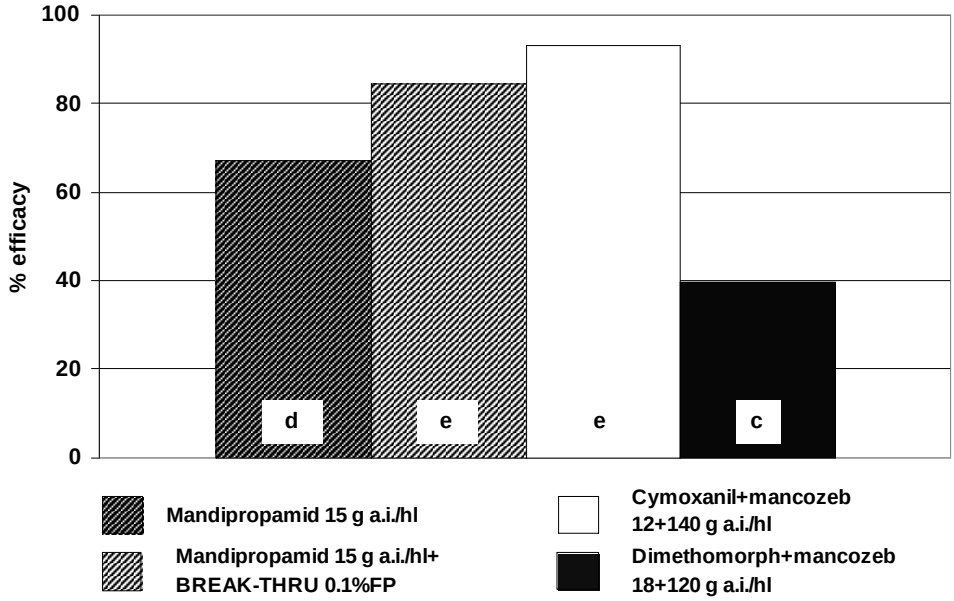


Figure 3. Curative activity of mandipropamid and reference standards, when applied to large potato plants under greenhouse conditions. Inoculation with 10.000 sporangia per ml. Curative treatment one day after inoculation, evaluation 11 days after inoculation (percent leaf area infected). Results with the same letter are not statistically different.

Rainfastness and persistence of activity

Consistent with results from the studies on uptake and translocation (Hermann *et al.,* 2005), the results in Figure 4 confirm, that mandipropamid is rainfast within 1 hour after application or as soon as the spray deposit has dried. The efficacy of mandipropamid was reduced only when the artificial irrigation was applied immediately after the treatment.

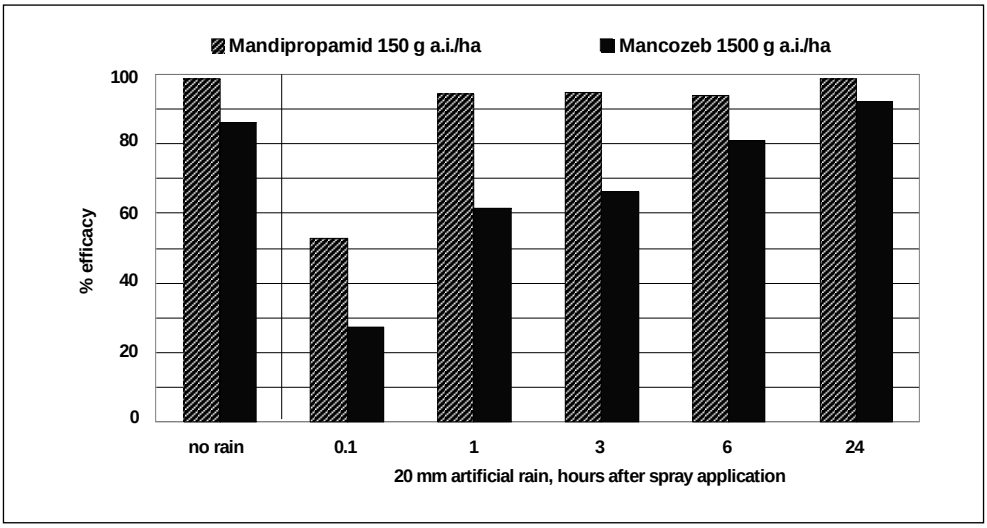
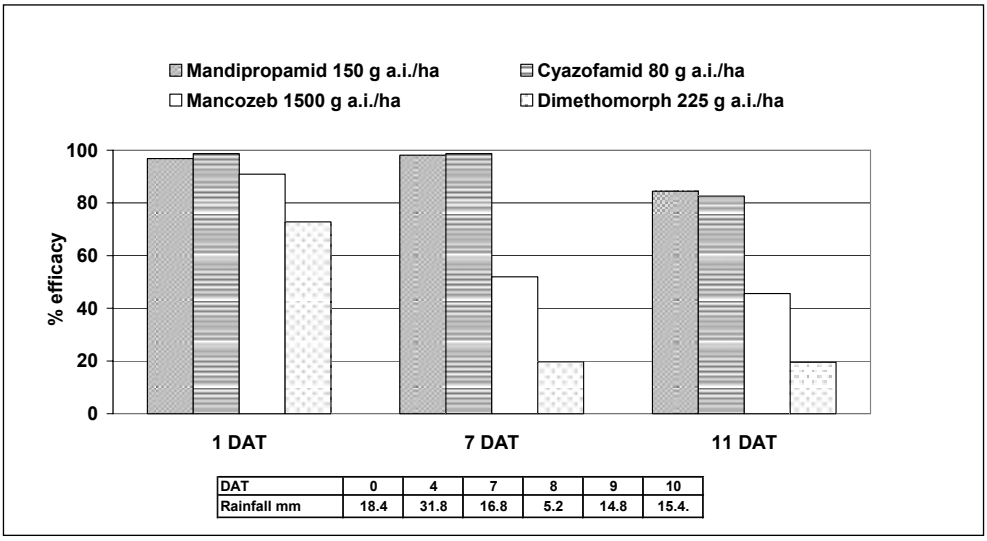
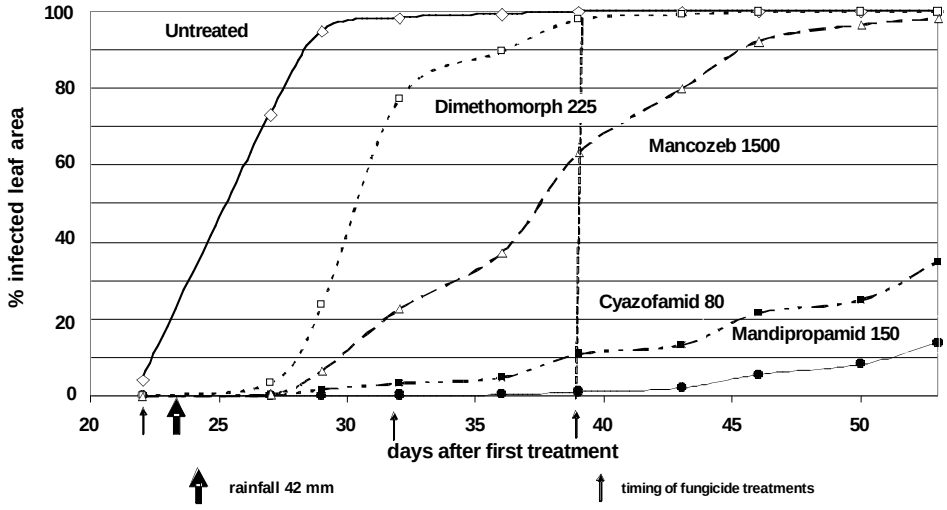


Figure 4. Rainfastness of mandipropamid with overhead irrigation at different times after the treatment in a greenhouse test with large plants, inoculation with 20'000 sporangia/ml after the last rain event, evaluation percent leaf area infected 9 days after inoculation.



DAT	0	4	7	8	9	10
Rainfall mm	18.4	31.8	16.8	5.2	14.8	15.4.

Figure 5. Duration of efficacy and rainfastness of mandipropamid and reference standards. One fungicide spray was applied to field plots. Leaf samples were taken at different times after the treatment. Leaflets were inoculated (5000 sporangia/ml) and evaluated in the laboratory (percent leaf area infected).



DAT1	11	16	19	23	24	28	34	36	42	46	47	49	51
Rainfall mm	10	9.4	16	41.7	12.4	10.5	24.4	23.4	10.2	8	13.3	5.8	9.1

Figure 6. Duration of efficacy and rainfastness of mandipropamid and reference standards.
Field trial with 6 fungicide sprays applied at spray intervals of 6-10 days, natural infection, whole plot rating of percent leaf area infected twice per week. Strong disease epidemic started approximately 3 weeks after the first fungicide treatment. Application rates as g a.i./ha.

The results of the trials summarized in Figures 5 and 6 demonstrate the excellent rainfastness and long lasting activity of mandipropamid under field conditions. In both trials the duration of activity of mandipropamid was clearly better than dimethomorph or mancozeb and slightly better than the best reference standard cyazofamid. In both trials strong thunderstorms occurred between treatments and probably washed off mancozeb and dimethomorph. Wash-off by rain can partly explain the lower activity of the weaker treatments.

In addition to the results reported in Figures 4-6 additional results concerning the rainfastness and duration of activity of mandipropamid in comparison to different reference standards were obtained by independent research institutes during the 2006 season. The methodologies used in these tests are summarized in Table 1 and the results obtained in Table 2 (Spits and Schepers, 2007; Hausladen, 2007; Culiez *et al.*, 2007).

The results of these tests further document the consistently excellent rainfastness and good duration of activity of mandipropamid. In the trial carried out by the Service de la Protection des Végétaux (SPV) in the North of France only mandipropamid provided consistently good disease control, when the lower leaf surface was inoculated. These results confirm the excellent translaminar activity of mandipropamid also under field conditions.

Table 1. Methodologies used in rainfastness tests with mandipropamid and reference standards carried out by independent institutes during the 2006 season.

	PPO Holland	TU Munich	SPV France
Treatment of plants	in greenhouse	in greenhouse	in field
Amount of rain (mm)	0, 20, 40, 80	>80	0, 27, 85 [(1)]
Rain after treatment (hours)	1	3-6	24-48
Sampling of leaves after treatment (days)	3, 7	1	3, 5, 7
Inoculation with droplets of spore concentrations/ml [(3)]	1x104	2x105	1x104-1x105 [(2)]
Results	rainfastness persistence	rainfastness	rainfastness persistence (translaminarity)

[(1)] additional rainstorm of 48 mm occurred five days after the treatment
[(2)] separate inoculation and evaluation on upper and lower leaf surfaces.
[(3)] bioassay in laboratory with detached leaves in all tests

Table 2. Ranking of results obtained in rainfastness tests with mandipropamid and reference standards carried out by independent institutes during the 2006 season.

	PPO Holland	TU Munich	SPV France
Mandipropamid	+++	+++	+++ *
Cyazofamid+adjuvant	+++	+++	+++
Zoxamid+mancozeb			++(+)
Dimethomorph+mancozeb		++(+)	+(+)
Cymoxanil+mancozeb	+	++	
Fluopicolide+propamocarb	++		
Fluazinam	+++		+
Mancozeb		+(+)	+

Mandipropamid used at 150 g a.i./ha, reference standards applied at recommended rates
** only mandipropamid provided consistently good protection of the lower leaf surface by translaminar movement throughout this test*

Conclusions

Mandipropamid formulated as SC250 and recommended at 150 g a.i./ha is a highly effective new product for the control of late blight in potatoes. It is recommended as preventive treatment.
Mandipropamid prevents the germination of spores at very low concentrations. Its affinity to the waxy layer of plant surfaces assures long lasting efficacy and excellent rainfastness. Gradual uptake of small amounts of the active ingredient into the plant tissue provides good translaminar and limited curative and antisporulant activity. Especially under heavy disease pressure, the limited curative and antisporulant activity of mandipropamid may complement its excellent preventive activity and contribute to its overall highly reliable field efficacy.

The properties of mandipropamid documented in this paper explain the consistently excellent disease control observed in the field also under conditions of high disease pressure and frequent rain.
For the potato grower demanding a high standard of control of late blight, mandipropamid is a new tool providing more reliable protection from disease infection independent of weather conditions.

Acknowledgements
The authors would like to thank all colleagues and scientists from independent institutes, who have contributed to the evaluation and successful development of mandipropamid for the control of late blight in potatoes.

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Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

Evaluation of fluopicolide-containing formulations for the control of potato late blight in Northern Ireland, 2003-2006

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Summary
Fluopicolide is a new Oomycete-specific, translaminar fungicide with a novel mode of action, developed by Bayer. Field trials in Northern Ireland over the four years 2003-2006 compared potato late blight control by a standard fungicide programme (two applications of metalaxyl-M + mancozeb followed by eight applications of fluazinam) with programmes which were identical except that fluopicolide + propamocarb hydrochloride was substituted for three of the fluazinam applications. Treatments were applied to the susceptible maincrop cultivar Up-to-Date at 7-day intervals. A severe, uniform infection pressure was provided by inoculating unsprayed spreader rows with recent Northern Ireland isolates of *Phytophthora infestans* (50/50 phenylamide-resistant/-sensitive) and plots were misted at dawn and dusk to encourage infection. A programme in which fluopicolide + propamocarb hydrochloride (87.5 + 875 g a.i./ha) was applied as the final three sprays was evaluated each year. This consistently out-performed the standard programme, significantly reducing foliage and tuber blight and increasing marketable yield. In 2005 and 2006, fluopicolide + propamocarb hydrochloride was also evaluated at a higher rate (100 g + 1000 g a.i./ha) as three mid-season treatments. Mid-season use proved more effective in terms of both foliage and tuber blight control in 2005, but end-of-season application was more effective in 2006. The positioning of the fluopicolide + propamocarb hydrochloride applications within the spray programme needs to take account of the seasonal timing of infection pressure.

Keywords
Phytophthora infestans, potato late blight, fluopicolide, propamocarb.

Introduction
In most years in Northern Ireland, the mild, wet climate encourages the rapid spread of foliage blight between mid-June and September and the high rainfall also often favours tuber infection. As elsewhere in Europe, the vast majority of potato cultivars planted in Northern Ireland have little or no blight resistance and therefore growers rely on fungicides to combat blight, those with translaminar and/or systemic activity being particularly useful. Phenylamide-resistant strains of *P. infestans*, first identified

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in Northern Ireland in 1981, remain present in the population, but in recent years have been found in fewer than 50% of isolates tested, partly due to effective anti-resistance strategies (Cooke and Little, 2006).

The new Bayer fungicide, fluopicolide, is based on novel chemistry and is believed to interfere with the functioning of spectrin, which is involved in stabilising the cytoskeleton (Toquin *et al.*, 2006). It has been placed by FRAC (the Fungicide Resistance Action Group) in a new mode of action group. Fluopicolide is Oomycete-specific and has translaminar activity (Latorse *et al.*, 2006). It was evaluated in a co-formulation with propamocarb hydrochloride (approved for late blight control in the UK in 2006 as 'Infinito') in trials at Newforge Lane, Belfast under conditions of extreme blight pressure over the four years 2003-2006. The trials were designed to evaluate its effectiveness against foliage blight, but particularly against tuber blight. The comparison programme used was metalaxyl-M + mancozeb followed by fluazinam, a programme which has proved very effective in reducing tuber infection (Cooke *et al.*, 1999). The fluopicolide + propamocarb hydrochloride formulation was applied in each year as the final three applications of the spray programme; in 2005 and 2006 a mid-season application was also evaluated.

Table 1. Field trials for the control of potato blight, 2003-2006: dates of field operations

Year	Planting date	Fungicide application dates		Inoculation date	Desiccation	Harvest
		First	Last			
2003	9 May	25 June	27 August	9 July	3 September	24 September
2004	11 May	22 June	25 August	7 July	2 September	28 September
2005	13 May	23 June	26 August	7 July	1 September	27 September
2006	25 May	28 June	30 August	10 July	5 September	9 October

Materials and Methods

Tubers of the blight-susceptible maincrop potato cv. Up-to-Date were planted in May of each year (Table 1) at the Agriculture & Food Science Centre, Newforge, Belfast, Northern Ireland (a location remote from commercial crops) in fully randomised blocks with five replicate plots per treatment. Each plot (2.8 x 3.0 m^2) contained four rows of ten tubers. Pairs of rows of unsprayed plants adjacent to each treated plot served as an infection source and were inoculated in July of each year (Table 1) with recent Northern Ireland isolates. In these rows, two leaves on every fourth plant were inoculated alternately with phenylamide-resistant and phenylamide-sensitive isolates of *P. infestans*, 50% of leaves being inoculated with a mixture of three or more phenylamide-resistant isolates and 50% with a mixture of three or more phenylamide-sensitive isolates. All isolates originated from Northern Ireland potato crops and were obtained within the last three years. The plots were misted daily after inoculation for 2-3 h at dawn and dusk when required to encourage spread of blight.

Table 2. Fungicide programmes evaluated for the control of potato blight, 2003-2006

Number of applications of active ingredients (g a.i./ha)[a]			Abbreviation	Year			
Early season	Mid-season	Late season		2003	2004	2005	2006
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x fluazinam (150)	3 x fluazinam (150)	met+man/fluaz	√	√	√	√
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x fluazinam (150)	3 x fluopicolide + propamocarb HCl (87.5 + 875)	met+man/ fluaz/fluo+prop 1.4	√	√	√	√
2 x metalaxyl-M + mancozeb (76 + 1216)	2 x fluazinam (150); 3 x fluopicolide + propamocarb HCl (100 + 1000)	3 x fluazinam (150)	met+man/ fluaz/fluo+prop 1.6/fluaz	-	-	√	√

a all applications were at 7-d intervals

b fluopicolide+prop 1.4 and fluopicolide+prop 1.6 refer to the 1.4 and 1.6 l/ha application rates of the formulated product ('Infinito')

Fungicide formulations were applied at manufacturers' recommended rates in *c*. 300 litres/ha using a Cooper Pegler CP15 knapsack sprayer. The first applications were made (before inoculation) in the last week of June of each year (Table 1) and ten treatments were applied at 7-day intervals (as far as possible) until late August. In each year, the standard programme comprised two sprays of metalaxyl-M + mancozeb ('Fubol Gold WG', Syngenta) followed by eight sprays of fluazinam ('Shirlan', Syngenta), while the comparison programmes included fluopicolide + propamocarb hydrochloride (subsequently registered as 'Infinito', Bayer) replacing three fluazinam treatments either mid- or late season. Details of programmes are shown in Table 2.

Foliage blight was assessed on each drill of each sprayed plot twice weekly from the time that blight was first seen in them until haulm destruction, using the ADAS key (Anon., 1976) with added 0.01% and 10% categories. Plots were desiccated with diquat dibromide ('Reglone', Syngenta) in early September within 7 days of the final fungicide application and tubers harvested in late September or early October, at least three weeks after desiccation (Table 1). The yield from each plot was graded and recorded; the number and weight of blighted, soft-rotted tubers was recorded and they were then discarded. The number and weight of firm blighted tubers >35 mm was assessed (and diseased tubers discarded) in November-December in each year. The remaining healthy tubers were stored and re-assessed for tuber blight the following January-February.

All data were subjected to analyses of variance with angular transformations of means used for percentage data. Two programmes were used in the trials in each of the four years, viz. the standard metalaxyl-M + mancozeb/fluazinam programme and the metalaxyl-M + mancozeb/fluazinam/ fluopicolide + propamocarb hydrochloride 1.4 l/ha programme. Analyses of variance were carried out to compare the performance of these two treatments over years, using trials/years as blocks.

Table 3. Field trials evaluated for the control of potato blight, 2003-2006: area under the foliar disease progress curve

Treatment	AUDPC[a]			
	2003	2004	2005	2006
met+man/fluaz	696	352	254	236
met+man/fluaz/fluo+prop 1.4	503	175	191	156
met+man/fluaz/fluo+prop 1.6/fluaz	n/a	n/a	96	171
L.S.D. (P<0.05)	153.7	114.2	135.7	95.5

a area under the disease progress curve for foliage blight development
n/a = not applicable; programme not included

Table 4. Comparison of standard and programme ending with fluopicolide + propamocarb hydrochloride over 2003-2006

Treatment	AUDPC[a]	Blight (% ang trans)			Yield (kg/plot)[b]
		Final foliage blight[c]	Tuber blight by number	Tuber blight by weight	
met+man/fluaz	384	35.4	14.8	15.5	48.7
met+man/fluaz/fluo+prop 1.4	256	25.5	12.9	13.5	50.3
L.S.D. (P<0.05)	105.4	3.98	1.80	1.56	1.09

a area under the disease progress curve for foliage blight development
b marketable yield of healthy tubers >35 mm
c in 2003 the penultimate foliage blight assessment was used as by the final assessment there was no significant effect of treatment
n/a = not applicable; no significant effect of treatment (P>0.05)

Results

Foliage blight control

In each of the four seasons, the programmes which concluded with three applications of fluopicolide + propamocarb hydrochloride 1.4 l/ha resulted in less foliage blight than the standard and this was significant (P<0.05) at the end of the season in every year except 2005 (Figure 1). This was reflected in lower areas under the disease progress curve (AUDPC): these were significantly lower than the standard in 2003 and 2004 (Table 3). Analysis of the percentage final foliage blight over the four years (Table 4) showed that there was consistently less foliage blight at the end of the season in plots treated with the fluopicolide + propamocarb hydrochloride programme and that AUDPC value was significantly lower (P<0.05).

In 2005, plots receiving the mid-season fluopicolide + propamocarb hydrochloride had less foliage blight than those receiving it at the end of the season, but the situation was reversed in 2006, with the programme including the end-of-season fluopicolide + propamocarb hydrochloride having less foliage blight than the mid-season one (Figure 1). In both 2005 and 2006, mid-season fluopicolide + propamocarb hydrochloride reduced foliage blight significantly at the end of the season compared with the standard programme, but there was no significant difference between the two fluopicolide + propamocarb hydrochloride programmes.

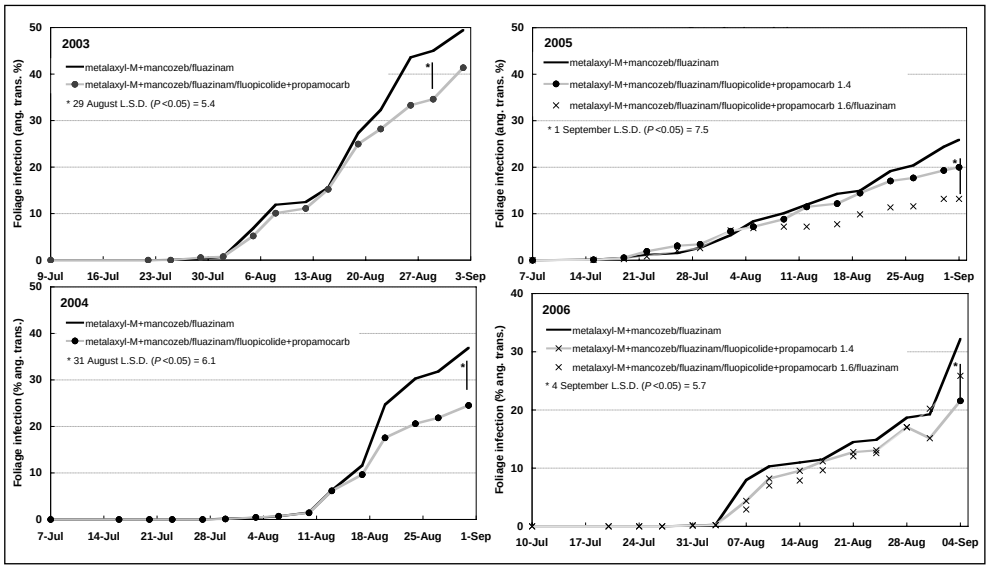

Figure 1. Field trials for the control of potato late blight, 2003-2006: foliage blight assessments (angular transformed percentage)

Table 5. Field trials evaluated for the control of potato blight, 2003-2006: marketable and blighted yield assessments

Treatment	Weight (kg) per plot of tubers >35 mm				
	total[a]	marketable[b]	soft blight[b]	firm blight[b]	total blight[b]
2003					
met+man/fluaz	55.3	47.8	2.0	2.5	4.6
met+man/fluaz/fluo+prop 1.4	56.6	49.8	1.9	1.2	3.1
L.S.D. (P<0.05)	n/a	n/a	n/a	1.27	2.12
2004					
met+man/fluaz	63.7	57.7	0.8	2.3	3.1
met+man/fluaz/fluo+prop 1.4	64.8	59.6	0.5	1.6	2.1
L.S.D. (P<0.05)	n/a	n/a	0.58	n/a	1.76
2005					
met+man/fluaz	59.4	53.8	0.6	0.7	1.3
met+man/fluaz/fluo+prop 1.4	61.3	55.7	0.8	0.1	0.9
met+man/fluaz/fluo+prop 1.6/fluaz	64.9	59.2	0.5	0.2	0.7
L.S.D. (P<0.05)	n/a	n/a	n/a	n/a	n/a
2006					
met+man/fluaz	44.6	35.7	3.4	3.9	7.3
met+man/fluaz/fluo+prop 1.4	46.7	37.7	2.9	4.3	7.2
met+man/fluaz/fluo+prop 1.6/fluaz	46.8	36.3	3.5	5.3	8.8
L.S.D. (P<0.05)	3.16	6.41	n/a	n/a	n/a

n/a = not applicable; no significant effect of treatment (P>0.05)
a at grading
b after final blight assessment

Table 6. Field trials evaluated for the control of potato blight, 2003-2006: tuber blight assessments by weight

Treatment	Blighted tubers (% ang. trans. by weight)[a]		
	total	soft only	firm only
2003			
met+man/fluaz	16.5	10.6	11.8
met+man/fluaz/fluo+prop 1.4	13.4	10.5	8.2
L.S.D. (P<0.05)	3.47	n/a	2.96
2004			
met+man/fluaz	12.1	6.3	10.2
met+man/fluaz/fluo+prop 1.6	9.9	4.8	7.9
L.S.D. (P<0.05)	4.05	2.78	n/a
2005			
met+man/fluaz	6.8	5.7	5.2
met+man/fluaz/fluo+prop 1.4	5.7	6.1	1.8
met+man/fluaz/fluo+prop 1.6/fluaz	4.6	4.6	2.5
L.S.D. (P<0.05)	n/a	n/a	n/a
2006			
met+man/fluaz	23.3	15.2	16.8
met+man/fluaz/fluo+prop 1.4	22.9	14.4	17.2
met+man/fluaz/fluo+prop 1.6/fluaz	25.1	14.9	19.2
L.S.D. (P<0.05)	n/a	n/a	n/a

a based on tubers >35 mm
n/a = not applicable; no significant effect of treatment (P>0.05)

Marketable yield
In each of the four years, plots receiving the programme which concluded with fluopicolide + propamocarb hydrochloride 1.4 l/ha had a greater marketable yield of tubers >35 mm after removal of all blighted tubers (Table 5). This effect was not significant in any one year, but was significant when the data were analysed over the four years (Table 4). The weight of blighted tubers was always less in plots which had received the fluopicolide + propamocarb hydrochloride treatments, except with the mid-season fluopicolide + propamocarb hydrochloride in 2006, but there was no significant difference compared with the standard programme in any individual year (Table 5) or when analysed over years (data not presented).

Tuber blight
The percentage by weight and by number of blighted tubers from plots receiving the programme which concluded with fluopicolide + propamocarb hydrochloride 1.4 l/ha was lower than that from those which received the standard programme in each year (Table 6, Figure 2, respectively). Analysis over years (Table 4), showed a significant reduction in percentage tuber blight by number and by weight by this programme. In 2005, plots receiving the mid-season fluopicolide + propamocarb hydrochloride at 1.6 l/ha had the fewest blighted tubers, but the situation was reversed in 2006 when the mid-season programme had more blighted tubers than the other programmes. However, differences between treatments were were not significant (P>0.05) in 2005 or 2006.

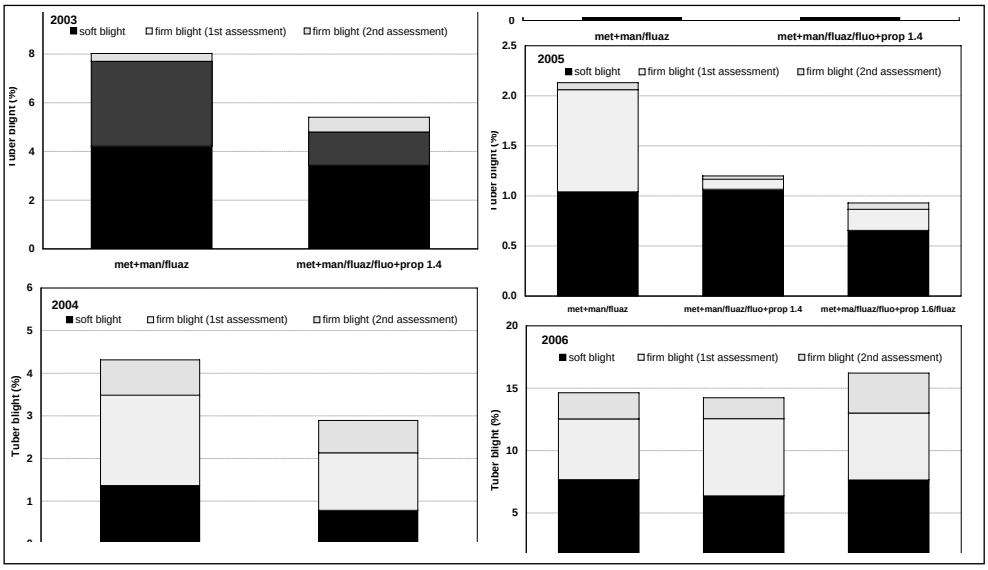

Figure 2. Field trials for the control of potato late blight, 2003-2005: tuber blight assessments (percentage by number)

Discussion

Control of potato late blight is a major problem for growers in regions with high rainfall. The conditions which encourage infection and spread are also those which make it difficult to maintain fungicide spray programmes. Current *P. infestans* genotypes can sporulate within 3 days of infection

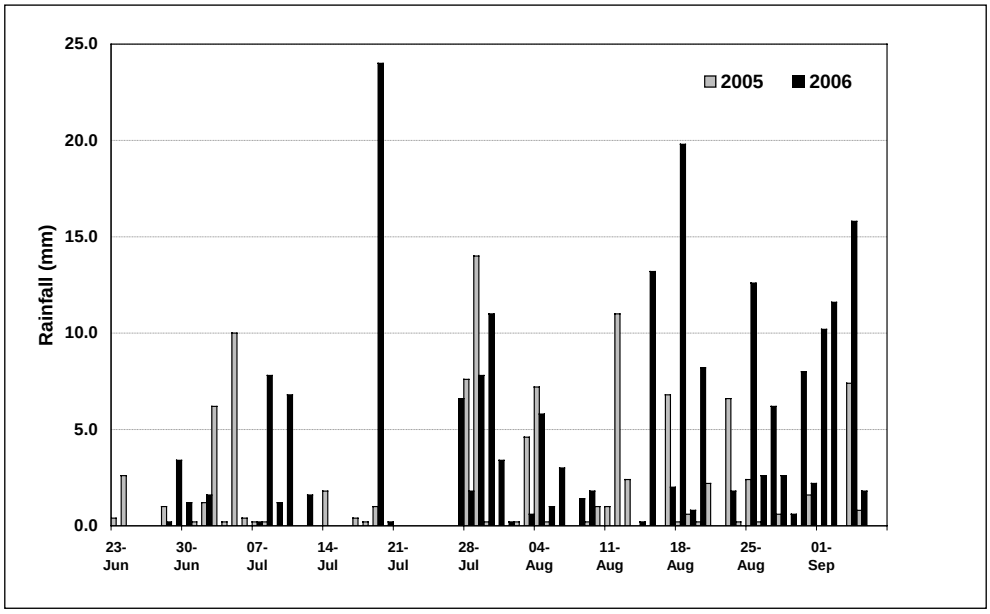

Figure 3. Rainfall in Northern Ireland, 21 June – 7 September 2005 and 2006

under optimum conditions (Carlisle *et al.*, 2002), so that the epidemic can build-up very rapidly with resulting reduction in the photosynthetic capacity of the crop and hence of yield. Even more crucially, high rainfall washes sporangia into the soil and provides favourable conditions for tuber infection. While foliage infection alone reduces yield, infection of tubers has a direct impact on quality and may make the crop completely unsaleable (e.g. if it is intended for seed or for high quality pre-packed ware) or result in very greatly increased costs to the producer in removing blighted tubers. Tuber blight also predisposes tubers to secondary bacterial soft rotting (Sicilia *et al.*, 2002) and this can spread in store leading to total crop loss.

The availability of fungicides with systemic and/or translaminar activity is of particular value in high rainfall areas, as they provide protection within the foliage and also affect the pathogen post-infection in the early stages of its development within the leaf. Since the revocation of the approval for fentin-based fungicides at the end of 2003, there has also been concern in the UK about the availability of fungicides which give effective control of tuber infection. Levels of tuber blight are often not related to the severity of foliage infection. Rapid haulm destruction by *P. infestans* in the absence of adequate foliar protection may result in relatively few tubers being infected, while, conversely, low level foliar infection which remains active in the crop for a prolonged period may be associated with very severe tuber blight.

Fluopicolide has both systemic and translaminar activity (Tafforeau *et al.*, 2005b; Latorse *et al.*, 2006) and in combination with propamocarb hydrochloride can give good control of foliage infection and reduce tuber blight more than the current standard even under very testing conditions, as indicated by the results of these trials. Over the last four years, spray programmes comprising metalaxyl-M + mancozeb followed by fluazinam and concluding with three applications of fluopicolide + propamocarb hydrochloride at 1.4 l/ha consistently out-performed the standard programme (metalaxyl-M + mancozeb followed by fluazinam), significantly reducing foliage and tuber blight and increasing marketable yield. This performance is noteworthy since programmes starting with metalaxyl + mancozeb followed by fluazinam for the remainder of the season have proved very effective in Northern Ireland trials (Cooke *et al.*, 1999). Tafforeau *et al.* (2005a) also reported excellent protection of tubers from infection by *P. infestans* in four trials in the Netherlands, but in this case fluopicolide + propamocarb hydrochloride was applied for the entire programme, whereas in the present study only three applications were used.

The results from the comparison of fluopicolide + propamocarb hydrochloride used mid-season and end-of-season in 2005 and 2006 showed that it performed better mid-season in 2005, but better end-of-season in 2006. In 2006, high rainfall favouring tuber infection occurred during August (Figure 3), whereas rainfall in the 2005 season was lower. It is suggested that the positioning of fluopicolide + propamocarb hydrochloride applications within the spray programme needs to take account of the seasonal timing of infection pressure in order to exploit the properties of the active ingredients most effectively.

In terms of resistance management, fluopicolide adds to the expanding range of fungicides with different modes of action, providing more options for growers and helping to reduce the selection pressure on any group. Most current formulations containing more than one active ingredient combine a systemic and a non-systemic fungicide and, once infection has occurred and the pathogen is within the plant, it is exposed only to the systemic component. The combination of fluopicolide

with propamocarb hydrochloride provides two compounds which have different and unique modes of action and are both able to move into the potato plant: this should provide an effective anti-resistance strategy.

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Sensitivity to fungicides and mating types of *Phytophthora infestans* populations collected in North-Eastern and Central Italy

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Summary
Seventy four *Phytophthora infestans* populations from tomato and potato crops were analyzed *in vitro* for their sensitivity to fungicides and mating type; populations were collected from 2002 to 2006 in the Emilia Romagna, Lombardy, Marche and Abruzzo regions. 56 % of populations showed A1 mating type, 42% were A2 while 2% were found to be self fertile; no strains showed resistance to metalaxyl, cymoxanil, dimethomorph, azoxystrobin, zoxamide and iprovalicarb.

Keywords
Phytophthora infestans, mating type, sensitivity to fungicides, *in vitro* assays.

Introduction
Phytophthora infestans (Mont.) de Bary is the most devastating disease of tomato and potato crops in Italy. Before 1995, very few studies were carried out in Italy on the characterization of *P. infestans* populations by identification of mating types and sensitivity to fungicides. Cristinzio and Testa (1997) reported the first data on A2 mating type occurrence in Southern Italy. In a study on sensitivity of 149 *P. infestans* populations collected in several regions in Central and Southern Italy, they also reported that 82% were Intermediate Resistant to metalaxyl while 5% were classified as Resistant (Cristinzio and Testa, 1998).
In Emilia Romagna (Northern Italy) the disease pressure unexpectedly increased in the late 90's (Bugiani *et al.*, 2001) when climatic conditions extremely favourable to late blight caused early and heavy damage on potato and tomato crops. This study has been carried out since 2002 in order to evaluate the sensitivity to fungicides and the distribution of A1 and A2 mating types in the *P. infestans* populations collected in North Eastern and Central Italy.

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Table 1. Origin of *Phytophthora infestans* populations used in the study

P.infestans population	Italian Region - Province	Host	*P.infestans* population	Italian Region - Province	Host
BO 11	ER - Bologna	potato	AN 9	M – Ancona	tomato
BO 12	ER – Bologna	potato	AN 10	M – Ancona	potato
BO 14	ER – Bologna	potato	AP 6*	M – Ascoli Piceno	tomato
BO 16	ER – Bologna	potato	AP 9*	M – Ascoli Piceno	tomato
BO 17	ER – Bologna	potato	AP 11*	M – Ascoli Piceno	tomato
BO 18	ER – Bologna	potato	AP 12*	M – Ascoli Piceno	tomato
BO 27	ER – Bologna	tomato	AP 2a*	M – Ascoli Piceno	tomato
BO 28	ER – Bologna	tomato	AQ 1*	A – L'Aquila	potato
BO 31	ER – Bologna	tomato	AQ 2*	A – L'Aquila	potato
BO 32	ER – Bologna	tomato	AQ 3*	A – L'Aquila	potato
BO 40	ER – Bologna	potato	AQ 4*	A – L'Aquila	potato
BO 43	ER – Bologna	potato	CH 1*	A - Chieti	tomato
BO 44	ER – Bologna	potato	CH 2*	A - Chieti	tomato
BO 54	ER – Bologna	potato	CH 3*	A - Chieti	tomato
BO 59	ER – Bologna	tomato	CH 4*	A - Chieti	tomato
FE 22	ER - Ferrara	tomato	CH 5*	A - Chieti	tomato
FE 25	ER - Ferrara	tomato	CH 6*	A - Chieti	tomato
FE 26	ER - Ferrara	tomato	CH 7*	A - Chieti	tomato
FE 29	ER - Ferrara	tomato	CH 8*	A - Chieti	tomato
FE 36	ER - Ferrara	potato	CH 9*	A - Chieti	tomato
FE 51	ER - Ferrara	tomato	PE 1*	A - Pescara	tomato
FE 52	ER - Ferrara	tomato	PE 2*	A – Pescara	tomato
FE 53	ER - Ferrara	tomato	PE 3*	A – Pescara	tomato
FE 62	ER – Ferrara	tomato	PE 4*	A – Pescara	tomato
FC 33	ER – Forlì, Cesena	tomato	PE 5*	A - Pescara	tomato
PR 60	ER – Parma	tomato	PE 6*	A – Pescara	tomato
RA 64	ER - Ravenna	tomato	PE 7*	A – Pescara	tomato
RA 57	ER – Ravenna	tomato	PE 8*	A – Pescara	tomato
MN 20	L - Mantova	tomato	PE 9*	A – Pescara	tomato
AP 1	M – Ascoli Piceno	tomato	PE 10*	A – Pescara	tomato
AP 2	M – Ascoli Piceno	tomato	PE 11*	A – Pescara	tomato
AN 3	M – Ancona	tomato	PE 12*	A - Pescara	tomato
AN 4	M – Ancona	tomato	TE 1*	A – Teramo	tomato
AN 5	M – Ancona	tomato	TE 2*	A – Teramo	tomato
AN 6	M – Ancona	tomato	TE 3*	A – Teramo	tomato
AN 7	M – Ancona	tomato	TE 4*	A – Teramo	tomato
AN 8	M – Ancona	tomato	TE 5*	A – Teramo	tomato

ER = Emilia Romagna; L = Lombardia; M = Marche; A = Abruzzo

** = P. infestans populations analysed only for mating type and sensitivity to metalaxyl with Shattock's formula*

Materials & Methods

Seventy four *P. infestans* populations were collected and isolated from several tomato and potato crops in the Lombardy, Emilia Romagna, Marche and Abruzzo regions between 2002 and 2006 (table 1). Populations were purified and cultured in V8 medium (250 mL V8 Campbell Grocery; 2.7 g $CaCO_3$ Fluka; 15 g Agar Grade BBL, distilled water up to 1.0 Litre) containing 100 mgL^{-1} vancomycin, 10 mgL^{-1} neomycin, 10 mgL^{-1} rifampicin, 250 mgL^{-1} ampicillin, 10 mgL^{-1} benomyl and 100 mgL^{-1} PCNB.

Populations were analyzed *in vitro* for mating types assessment and sensitivity to fungicides.

In the mating type test, each unknown population was paired with two isolates of known A1 and A2 mating types in Petri dishes containing V8 medium. Plates were placed at 20°C and 12 hours of photoperiod; after 10 days colonies were observed microscopically to check the presence of oospores in the area where the mycelia crossed. When the unknown isolate paired with A1 mating type produced oospores, it was classified as A2 mating type; the result was confirmed by observation of only sporangia in the other plate.

Sensitivity to fungicides was carried out on V8 medium amended with several fungicides at different concentrations; the amended medium was poured into 9 cm Petri dishes which were inoculated with an inverted mycelium plug (9 mm diameter) taken from the edge of a seven day old colony of *P. infestans* populations. Technical grade of common fungicides applied in late blight control were tested, except for zoxamide used as formulate; metalaxyl sensitivity was assessed at 0/0.001/0.005/0.01/0.05 /0.1/1/5/10 mgL^{-1}, cymoxanil at 0/0.5/0.75/1/1.5/2 mgL^{-1}, dimethomorph at 0/0.1/0.15/0.2/0.25/ 0.5/1 mgL^{-1}, azoxystrobin at 0/0.05/0.75/0.1/0.5/1 mgL^{-1}, zoxamide at 0/0.025/0.05/0.1/0.25/0.5/1 mgL^{-1} and iprovalicarb at 0/0.1/0.15/0.2/0.3/0.4/0.5/1 mgL^{-1}. Stock solutions were prepared for each active ingredient in acetone or water (for zoxamide) (the solvent concentration never exceeded 1% (v/v)).

The mycelial growth was evaluated after 7 days of incubation at 20°C and 12 hours of photoperiod, measuring and averaging the diameters of colonies. EC_{50} values were determined by probits analysis for each compound. All *P. infestans* populations taken from the Abruzzo region and five from the Marche region were only analyzed for sensitivity to metalaxyl, comparing radial mycelial growth between colonies amended with 0.1/1.5/10 mgL^{-1} of active ingredient and the control colonies at, according to the Shattock' s formula (Shattock, 1988) (table 1). The populations in this latest case were classified as Sensitive (% growth <10%), Intermediate (% growth 10-60%) or Resistant (% growth >60%).

Results and Discussion

Among tested *P. infestans* populations, 56% were A1 and 42% were classified as A2 mating types (table 2 and 3). Since 2002, A2 m.t. has been found in Northern and Central Italy, confirming the presence of A2 that was observed for the first time in 1996 (Cristinzio and Testa, 1997). No differences in frequency were found between A2 m.t. isolated from potato and tomato fields. Two populations from the Emilia Romagna region were classified as self fertile: in fact they produced oospores both when paired with A1 and A2 mating types (Tantius *et al.*, 1986). Self fertility *in vitro* is not clear, because it seems to be affected by genetics and cultural factors such as medium compositions, pH, etc. (Smart *et al.*, 2000).

EC_{50} values determined in sensitivity studies to fungicides are shown in table 2. None of the populations tested demonstrated resistance to the fungicides used. For metalaxyl EC_{50} values ranged from <000.1 to 0.3 mgL^{-1} according to data reported by Deahl (1993) for sensitive isolates; for cymoxanil from 0.03 to 2.48 mgL^{-1} (Ronald and Power, 1998); for dimethomorph values ranged from 0.008 to 0.80 mgL^{-1} (Stein and Kirk, 2003); for azoxystrobin from 0.004 to 0.18 mgL^{-1} (Godwin *et al.*, 1992); for

zoxamide from 0.025 to 0.09 mgL^{-1} (Cooke *et al.*, 2002) and for iprovalicarb from 0.07 to 0.28 mgL^{-1} (Stenzel *et al.*, 1998). Strains of *P. infestans* analyzed only for response to metalaxyl using the formula suggested by Shattock were all classified as MS (Metalaxyl Sensitive) (table 3).

Table 2. Mating type and sensitivity to fungicides of *P. infestans* populations of Emilia Romagna, Lombardia and Marche regions

***P.infestans* population**	**M.T.**	**Sensitivity to fungicides (EC_{50}, mgL^{-1})**					
		M	**C**	**D**	**A**	**Z**	**I**
BO 11	A1	< 0.001	1.33	0.01	0.06	0.03	0.25
BO 12	A1	0.3	0.57	0.5	0.08	0.05	0.17
BO 14	A2	< 0.001	0.68	0.17	0.09	0.06	0.28
BO 16	A2	< 0.001	1.37	0.13	0.08	0.03	0.23
BO 17	A2	< 0.001	0.74	0.11	0.08	0.04	0.25
BO 18	A2	< 0.001	0.71	0.15	0.05	0.02	0.22
BO 27	A1	< 0.001	1.89	0.02	0.04	0.02	0.20
BO 28	A2	< 0.001	0.56	0.1	0.1	0.02	0.07
BO 31	A2	0.002	1.71	0.05	0.006	nt	nt
BO 32	A1	0.08	1.19	0.19	0.04	0.04	0.16
BO 40	A1	0.004	1.36	0.21	0.04	0.01	0.23
BO 43	A1A2	< 0.001	1.01	0.11	0.02	0.03	0.12
BO 44	A1	0.02	0.83	0.13	0.10	0.01	0.15
BO 54	A1	nt	nt	nt	nt	nt	nt
BO 59	A1	nt	nt	nt	nt	nt	nt
FC 33	A1	nt	nt	nt	nt	nt	nt
FE 22	A2	< 0.001	1.36	0.13	0.07	0.03	0.18
FE 25	A2	< 0.001	0.94	0.008	0.06	0.02	0.12
FE 26	A1	< 0.001	1.45	nt	0.04	nt	nt
FE 29	A2	< 0.001	1.37	0.47	0.17	0.03	0.16
FE 36	A2	0.03	1.46	0.17	0.05	0.02	0.17
FE 51	A1	0.08	2.48	0.18	0.01	0.01	0.14
FE 52	A1A2	< 0.001	0.42	0.08	0.01	0.02	0.13
FE 53	A1	nt	nt	nt	nt	nt	nt
FE 62	A1	0.002	0.53	0.08	0.13	0.06	0.27
MN 20	A2	< 0.001	1.39	0.01	0.06	0.03	0.26
PR 60	A1	0.004	0.74	0.09	0.14	0.08	0.22
RA 57	A1	0.02	0.98	0.17	0.03	0.04	0.11
RA 64	A1	0.002	0.99	0.18	0.11	0.07	0.17
AP 1	A1	0.03	0.66	0,06	0.004	nt	nt
AP 2	A1	0.02	0.95	0.06	0.008	0.01	0.13
AN 3	A1	nt	nt	nt	nt	nt	nt
AN 4	A1	< 0.001	0.89	0.17	0.04	nt	nt
AN 5	A1	0.11	0.03	0.02	0.02	nt	nt
AN 6	A1	0.02	0.26	0.04	0.009	nt	nt
AN 7	A1	nt	nt	nt	nt	nt	nt
AN 8	A1	0.04	0.39	0.22	0.009	0.03	0.11
AN 9	A1	0.09	0.54	0.14	0.02	0.02	0.16
AN 10	A1	0.04	0.39	0.2	0.18	0.03	0.14

M = metalaxyl; C = cymoxanil; D = dimethomorph; A = azoxystrobin; Z = zoxamide; I = iprovalicarb; nt = not tested because of colony death before sensitivity could be tested

Table 3. Mating type and sensitivity to metalaxyl of *P. infestans* populations tested by Shattock's formula

***P. infestans* population**	**Mating type**	**Concentrations of metalaxyl (mgL^{-1})**		
		0.1	**1.5**	**10**
AP 6	A2	MS	MS	MS
AP 9	A2	MS	MS	MS
AP 11	A2	MS	MS	MS
AP 12	A2	MS	MS	MS
AP 2a	A2	MS	MS	MS
AQ 1	A1	MS	MS	MS
AQ 2	A1	MS	MS	MS
AQ 3	A2	MS	MS	MS
AQ 4	A1	MS	MS	MS
CH 1	A2	MS	MS	MS
CH 2	A2	MS	MS	MS
CH 3	A1	MS	MS	MS
CH 4	A2	MS	MS	MS
CH 5	A1	MS	MS	MS
CH 6	A2	MS	MS	MS
CH 7	A2	MS	MS	MS
CH 8	A1	MS	MS	MS
CH 9	A1	MS	MS	MS
PE 1	A1	MS	MS	MS
PE 2	A1	MS	MS	MS
PE 3	A1	MS	MS	MS
PE 4	A2	MS	MS	MS
PE 5	A2	MS	MS	MS
PE 6	A2	MS	MS	MS
PE 7	A1	MS	MS	MS
PE 8	A2	MS	MS	MS
PE 9	A2	MS	MS	MS
PE 10	A1	MS	MS	MS
PE 11	A2	MS	MS	MS
PE 12	A2	MS	MS	MS
TE 1	A2	MS	MS	MS
TE 2	A2	MS	MS	MS
TE 3	A1	MS	MS	MS
TE 4	A2	MS	MS	MS
TE 5	A1	MS	MS	MS

MS = Metalaxyl Sensitive (mycelial growth < 10% compared to the control)

Conclusions

In *P. infestans* populations collected and analyzed in Northern and Central Italy from 2002-2006, A1 and A2 mating types were present almost at the same ratio. The presence of A2 mating type, as well as self fertile strains, did not influence the sensitivity to the fungicides tested; indeed all populations showed EC_{50} values similar to those of sensitive populations reported in the references. Even though our results showed no resistance to fungicides, it is important to continue the monitoring of *P. infestans* populations to study their sensitivity evolution also because isolates with low sensitivity to metalaxyl were found in Southern Italy in 1995 (Cristinzio and Testa, 1998).

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Tenth Workshop of an European Network for development of an
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The development and control of the late and early blight of potato in the European part of Russia

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Summary
The damage caused by the late and early blights of potato in European Russia has been studied. Within the period of 1994-2004 the most severe late blight was registered for the western and north-western regions; the moderate epidemic was observed in the central, northern, and some southern regions; and the weakest disease development was registered in south-eastern regions. In 2006 the epidemic situation was registered in several central and eastern regions. In recent years the early blight became to be an increasing problem in consequence of the lower fertilization with nitrogen-containing preparations and the poor quality of the seed material.
The yield losses due to the late blight development at favorable weather conditions reach 28% for potato farms and 65 % for allotment gardens. Only small part of farmers (<10%) were able to produce potato, basing on the Western Europe standards and input levels, and now the use of fungicides is low.

Keywords
Potato late blight, early blight, mapping of the late blight damage, potato yield losses

Introduction
Due to its virulence and ability to spread rapidly, oomycete *Phytophthora infestans* is one of the most destructive plant pathogens. Two types of its population have been registered in Russia: (1) Siberia and Far East population, characterized by a very low genotypic variability, and (2) greatly varying European population. In Asiatic Russia, i.e. on the huge territory between Ekaterinburg and Sakhalin island, the predominating clonal lineage is SIB-1, that can be characterized by A1 mating type and IIa mtDNA haplotype, excepting the Khabarovsk territory and the Jewish Autonomous Region, where the SIB-2 genotype predominates. SIB-2 is characterized by A2 mating type and IIa mtDNA haplotype. In contrast to Siberia, populations of many European regions are represented by many clonal lineages, and almost every isolate has its unique multipoint genotype. It is possible that some of such strains are hybrids. For example, MO-4, MO-8, and MO-11 strains (Moscow region), heterozygous in the PEP locus, can be hybrids between the MO-8 strain, which is homozygous in the one allele of PEP locus and has A1 mating type, and MO-12, MO-21, and MO-22 strains, which have A2 mating type and are homozygous in another allele of the same locus, respectively (Elansky *et al.*, 2001; Elansky *et*

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al., 2002). Now the complex races of *P. infestans* prevail in all Russian regions. The average number of specific virulences in isolates from different locations varies from 5.5 to 10.
It has been demonstrated that populations of the pathogen, developing in different geographic regions, essentially differ in their aggressiveness to the same potato cultivars. For example, the level of cultivar resistance to the Tula and Leningrad populations is lower than that of the Moscow and Stavropol ones (Filippov *et al.*, 2004).
Potato is a strategic product for Russia. The food security of millions of rural families strongly depends on this crop. According to the data of the Ministry of Agriculture of Russian Federation, the total area of potato fields is about 3000000 ha, including 170000 ha treated by agricultural organizations (large enterprises), 55000 ha of potato farms, and about 2900000 ha of allotment hardens.
P. infestans continues to be an important pathogen, developing on this culture. In total, the annual yield loss due to late blight is about 4000000 tons (Filippov, 2005). That is why the control measures against late blight are of great importance for the food safety of Russia.
Early blight caused by *Alternaria spp*. became an increasing problem in recent years. The lower nitrogen fertilization and the use of a potato seed material, infected by viruses, strongly influence on the disease progress.
The following investigations were carried out in order to examine the actual damage, caused by potato blights, and to get information about the efficiency of accepted control measures.

Materials and methods
Based on the disease dynamics during a vegetation season, we developed a method for the calculation of yield losses, caused by late blight (Gurevich, Filippov, Tverskoy, 1977). This method is based on the known hypothesis of Van der Plank assuming a direct ratio between the area under the curve, describing the seasonal disease dynamics, and the yield loss (Plank, 1963). It can be expressed as follows: where w is a yield loss (%), caused by the premature death of the potato foliage; S is the area under the curve, describing a season late blight dynamic; q is a number of days between the flower-bud formation phase and the destruction of uninfected potato foliage. The average q value for early, intermediate and mid-late potato cultivars is 46, 52 and 84 days, respectively. When the foliage is killed by frost or the harvesting is carried out earlier than it naturally dies, q is considered as a number of days between the flower-bud formation phase and the day of the actual foliage destruction. It was found that the standard deviation of a calculated yield loss from the actual loss was 9,8% for the independent set (219 disease dynamics curves).
Within the frame of the present investigation, this method has been used to solve the following two problems:
– estimation of the frequency of the late blight epidemics in different parts of European Russia;
– obtaining of information, characterizing an actual damage, caused by the late blight, and the efficiency of control measures on private potato farms and allotment gardens in two districts of Moscow region.
In order to perform a mapping of epidemic frequencies, we used results of the disease monitoring, annually carried out by the regional divisions of the Plant Protection Service on several potato fields in each region (measurements were performed several times per vegetation season). The late blight dynamics was estimated according to the scale of the British Mycological Society (Anonymous, 1947; James, 1971). Dynamics of the disease was converted into the yield losses (%). The map (Figure 1) demonstrates a frequency of years with yield losses, exceeding 20%, in different regions of European Russia. It was considered that the epidemic level or severe late blight development takes place, when the calculated yield loss, caused by the premature death of potato foliage, exceeds 20%.
In 2004, according to the second task, we kept under observation 13 potato farms and 8 allotment gardens plots in two districts of the Moscow region. The size of potato fields was 1.5-2 ha (7 farms)

and 30-63 ha (6 farms). The fields were occupied only with potato cultivars susceptible to late blight: Ukama, Rosamunda, Romano, Sante, Feloks, and Impala. The analysis of the seed material for infection was carried out in all observed fields before the planting. During the vegetation season the fields were sprayed with fungicides (3-4 times), including Ridomil Golden MZ, Section Fenomen, Mancozeb, and polycarbacin.
The size of the observed allotment gardens was 0.01-0.03 ha. All of them were occupied by the local unregistered or unknown potato cultivars.
The weather conditions in that year were favorable for the late blight development. We carried out the estimation of the late blight development, and it allowed us to calculate the yield loss and, as a result, to know the actual efficiency of undertaken control measures.
The situation with the blight development in 2006 was evaluated using our data and the data, presented by the Federal Plant Protection Service.

Results and Discussion
Figure 1 shows that there are three zones in European Russia with different late blight epidemic frequency. The first, second, and third zones include regions, where the epidemic frequency exceeds 75%, 75-50%, and less 50%, respectively. The most severe disease was registered in the western and north-western regions, the less severe epidemic was observed in the central, northern and some southern regions, and the weakest disease development was registered in the south-eastern regions of Russia.
In 2006 the epidemic situation was nontypical (Figure 2). The severe late blight development was registered in several central and eastern regions (Ryazan, Kursk, Lipetsk, Bashkortostan); the first symptoms of the disease appeared when rows were closed. In north-western regions the late blight occurred shortly before the harvesting. A large amount of rains before and during the harvesting in these regions caused serious problems with the tuber blight (up to 12% of infected tubers). In other regions a long period of hot and dry weather caused very unfavorable conditions for *Phytophthora infestans*. The disease was detected on a small scale in some allotment gardens, but its symptoms in the farmer potato fields were very scarce.

Figure 1. The epidemic frequency of Late Blight in European Russia (Spiglazova S., Filippov A., 1994-2003).

Figure 2. The development of Late Blight in European Russia in 2006.

A severe or moderate early blight attack was registered on some potato cultivars in central and eastern parts of European Russia. In most cases the early blight symptoms were caused by *Alternaria alternata*. A low level of nitrogen application and a high level of the viral infection of potato seed material intensified the damage, caused by this disease.
Table 1 shows the situation with the late blight control typical for the private farms and allotment gardens.

Table 1. Potato yield losses, caused by the late blight in observed farms of the Moscow region in 2004 (Filippov *et al.*, 2005)

Type of fields	Number	Size of potato field, ha	Number of fungicide applications	Yield losses, %
Farms	13	12-63	3-4	8.8-28.0 (av. 18.6)
Allotment gardens	8	0.01-0.03	0	25.6-65.0 (av. 46.0)

In spite of the use of fungicides, many Russian farmers lost a considerable part of their yield due to the late blight. There are several reasons for it. The pre-planting analysis of the potato seed material showed that about 3-9% of the seed tubers were infected with *P. infestans*, whereas, according to the Russian standards, the lowest category of the seed material should not contain more than 2% of infected tubers. The high infection level promoted very early disease development on the fields. Moreover, the most observed farmers, due to different reasons, made some mistakes during the choice of the terms and number of fungicide applications and also in the order of the fungicide rotation. The first treatment usually was carried out too late; the intervals between the treatments were too large. As a result, calculated yield losses came up to 28% for the farmer's fields (18.6% on average).
The yield losses in allotment gardens were even higher (up to 65%, 46% on average). That was caused by the absence of any fungicide applications.
In 2006 the situation with the late blight control did not change. The number of agricultural enterprises, that produced potato on the basis of crop protection technologies, accepted in Western Europe and using the greater amount of fungicides, was less than 10%. The owners of allotment gardens did not use any fungicides.
At present there are no rural development agencies or any other organizations, which serve as the agricultural extension agents and provide a natural bridge for the introduction of the knowledge and plant protection technologies.
The training of Russian potato growers is of primary importance to demonstrate how to protect potato using the correct use of fungicides and the cultivation of the late blight resistant cultivars.

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Presentations

Tenth Workshop of an European Network for development of an
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Efficacy of Zoxamide and Mancozeb (Electis) against Late Blight and other diseases of tomato.

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Summary
Electis is a ready to use combination of zoxamide + mancozeb officially launched in Italy in 2005 for the control of downy mildew of grapevine and a range of diseases of tomato and potato. Electis is now an established product in tomato spray programs in Italy where its efficacy against the key fungal pathogens afflicting tomato production is excellent. Extensive testing has shown that field performance of Electis is superior or equivalent to the leading fungicides currently approved for use in tomato. Zoxamide binds strongly to cuticular waxes and is highly resistant to wash-off. This characteristic enhances ability to control disease on the fruit improving finish and consequently the quality of processed products. The Mancozeb component of Electis provides tried and tested broad spectrum protection and is an excellent resistance management tool. Electis has a favorable eco-toxicological profile and is accepted by food chain, processors and integrated pest management organizations.

Keywords
Tomato, zoxamide, mancozeb, control of diseases, early blight, late blight, fungicide efficacy, *Phytophthora infestans*, *Alternaria solani, Septoria lycopersici and Cladosporium fulvum.*

Introduction
Tomatoes grown for the processing industry in Italy are an important crop with around 90,000 ha produced principally in the North (Emilia Romagna, Lombardia) and in the South (Puglia, Campania) (Dongiovanni *et al.,* 2006). Tomato is cultivated in open fields and under covered conditions and is often heavily irrigated (in-furrow, sprinkler, micro-irrigation) which can raise moisture levels in the crop and create a micro climate conducive to disease development. A range of diseases affect the aerial portions (leaf and fruits) of tomato grown in Southern Europe in open field and under cover i.e. *Phytophthora infestans* (Late blight), *Alternaria solani* (Early blight), *Septoria lycopersici* (Septoria Leaf spot), *Cladosporium fulvum* (Leaf mold), *Colletotrichum sp.* (Antrachnose), (Jones *et al.,* 1991).

Chemical management of tomato pathogens utilises several different classes of fungicides including multi-site protectants such as dithiocarbamates (as in mancozeb), chlorothalonil, copper; penetrants such as dimethomorph, iprovalicarb, cymoxanil; systemics as in phosetyl-al, mefenoxam, benalaxyl, QoIs such as azoxystrobin, pyraclostrobin; QiI as in cyazofamid and a new mode of action preventative molecule such as zoxamide.

Where tomato is grown in Northern Italy under favourable conditions for late and early blight, typically 5-6 sprays are required per season. Spray programs are based around the Fungicide Resistance Action Committee (FRAC) recommendations involving mixtures with multi-sites and rotation with different modes of action to prevent resistance issues. Copper and mancozeb are normally sprayed early in the season to control primary infections generated by over-wintering inocculum and also provide additional preventative activity against bacterial diseases. The spray program usually continues with penetrants or systemics in combination with mancozeb or copper applied 2-3 times during canopy formation and across flowering. After fruit set fungicides with a high affinity for cuticular waxes and different mode of action such as zoxamide + mancozeb are recommended twice to improve fruit finish (high quality of raw materials for processed fractions are important).

In Southern Italy fungicide programs on industrial tomatoes are simplified to around 3 sprays with typical multi-site protectants such as mancozeb. Under covered conditions broad-spectrum fungicides such as QoIs are frequently alternated with penetrants and systemics in combinations with copper, chorothalonil, tolyfluanide and zoxamide+mancozeb to improve efficacy against secondary diseases which often appear in combination with early and late blight.

This paper reports results from field trials carried out in Southern Europe over several seasons investigating the efficacy of Electis (83g zoxamide + 667g mancozeb/kg) for control of key tomato pathogens. Zoxamide is highly active against a wide range of oomycete diseases (Olson *et al.,* 2002; Smith *et al.,* 2002; Kemmitt and Green, 2002); and its mode of action (inhibition of β tubulins in mitosis) is unique amongst compounds currently registered to control this important group of plant pathogens (Young and Slawecki, 2001; Egan *et al.,* 1998). Mancozeb is a long established and highly effective broad spectrum protectant fungicide. It has multi site activity and has been successfully used for the past 40 years by tomato growers around the world to control fungal disease in tomatoes with no reported issues of resistance.

Materials and Methods

A water dispersible granule (WG) formulation containing 83 g/kg of zoxamide and 667 g/kg of mancozeb (trade name Electis) was tested in a series of trials against key diseases of tomato. Diseases investigated were *Phythophthora infestans*, *Alternaria solani, Septoria lycopersici and Cladosporium fulvum* on industrial open field tomatoes used for processing and on fresh tomatoes from open field and protected conditions (greenhouse). Trials were carried out across Southern Europe according to the relevant EPPO (EPPO, 1997) and AFPP-CEB guidelines.

The use rate of Electis was between 1.5 and 2.0 kg/ha and water volumes were between 400 -1000 l/ha depending on the crop growth stages. Standard small plot experimental spraying equipment was used to make applications. Products based on cymoxanil, metalaxyl, iprovalicarb, fenamidone, dimethomorph, pyraclostrobin and azoxystrobin and on protectant fungicides such as mancozeb, metiram and copper were also included as references in various trials at local label rates which varied amongst countries. All products were applied season long on a 8-12 day schedule. Total number of applications varied from 4 to 9 depending on trials site and rate of epidemic development.

Visual assessments of percentage infected leaf surface and incidence of infected fruits were made periodically. The results presented in this paper are those obtained at the final observations which were typically conducted at the end of the season (before harvesting). An analysis of variance (ANOVA, p = 0.05) was carried out followed by a Student Newman Keuls (SNK) test to separate the means.

Results and Discussion

Table 1. Efficacy of Electis applied at intervals of 8-12 days against Late blight (*Phytophthora infestans*) of Industrial Tomato at Malborghetto di Boara (Emilia-Romagna - Italy). Italy 2002.

Treatments	Application Code †	Formulation Conc. (g /L or g/Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 10 DAAF *	% of Fruits Infected 10 DAAF
1. Electis	A-G	750 WG	1.8	22.5 f	15.3 f
2. Electis	A-G	750 WG	2.0	18.8 f	10.8 f
3. Dimethomorph+ mancozeb	A-F	690 WG	2.5	26.3 f	25.0 e
4.. Azoxystrobin	A-F	250 SC	1.0	37.5 e	49.5 c
5. Untreated	-	-	-	100 a	100 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 7 or 6 Sequential applications are designated by the letters A through F or G
** DAAF = days after application F*

Table 2. Efficacy of Electis applied at intervals of 8-12 days against *Phytophthora infestans* on Industrial Tomato at Colorno (Emilia-Romagna – Italy). 2002.

Treatments	Application Code†	Formulation Conc. (g /L or g/Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 14 DAAF*	% of Fruits Infected 14 DAAF
1. Electis	A-F	750 WG	1.8	16.9 c	11.5 b
2. Azoxystrobin	A-F	250 SC	1.0	28.9 b	16.7 b
3. Untreated	-	-	-	82.9 a	44.8 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 6 Sequential applications designated by the letters A through F
** DAAF = (days after application F)*

Trials reported in Tables 1 and 2 were designed to investigate control of *P. infestans* using Electis season long. This is not how any fungicide would typically be used in a grower program where different products are alternated, but it indicates the intrinsic efficacy of a product. The data clearly show that Electis applied season long at 1.8-2.0 kg/ha delivers excellent efficacy against *P. infestans* on both leaves and fruits. Performance on fruits was superior to azoxystrobin and dimethomorph + mancozeb whilst the foliar assessments indicated superior performance to azoxystrobin and equivalence with dimethomorph + mancozeb. The excellent level of fruit protection is a result of Electis being highly resistance to wash off due to its affinity for the cuticular waxes. This is particularly well demonstrated by these two irrigated trials.

Table.3. Efficacy of Electis applied at intervals of 8-10 days against *Phytophthora infestans* on Industrial Tomato at Bosco Mesola (Emilia-Romagna - Italy). 2005.

Treatments	Application Code†	Formulation Conc.(g /Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 7 DAAF*	% of Fruits Infected 12 DAAF
1. Electis	A-F	750 WG	2.0	13.7 d	4.3 b
2.Dimethomorph+copper-oxychloride	A-F	460 WP	3.5	26.7 c	4.6 b
3.Metalaxyl-M+copper-oxychloride	A-F	402.4 WP	3.0	11.2 d	1.8 b
4.Iprovalicarb+copper-oxychloride	A-F	398 WP	3.5	27.5 c	5.5 b
5.Fenamidone+copper-oxychloride	A-F	460 WP	3.0	18.0 cd	3.4 b
6. Pyraclostrobin+metiram	A-F	600 WG	2.0	13.7 d	4.8 b
7. Mancozeb	A-F	750 WG	2.0	58.7 b	6.0 b
8. Untreated	-	-	-	100 a	37.9 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 6 Sequential applications designated by the letters A through F
** DAAF = (days after application F)*

Table.4. Efficacy of Electis applied at intervals of 8-10 days against *Phytophthora infestans* on Industrial Tomato at Altedo (Emilia-Romagna - Italy). 2004.

Treatments	Application Code†	Formulation Conc.(g /L or g/Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 20 DAAG*	% of Fruits Infected 20 DAAG
1. Electis	A-G	750 WG	2.0	11.8 a	14.5 b
2.Metalaxyl-M+copper-oxychloride	A-G	402.4 WP	4.0	13.7 a	12.8 b
3.Dimethomorph+copper-oxychloride	A-G	460 WP	3.5	20.6 a	15.6 b
4. Azoxystrobin	A-G	250 SC	1.0	20.0 a	4.3 a
5. Mancozeb	A-G	750 WG	2.0	65.0 b	34.3 c
6. Untreated	-	-	-	99.2 c	54.2 d

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 7 Sequential applications designated by the letters A through G
** DAAG = (days after application G)*

Tables 3 and 4 report data from two trials on *P. infestans* designed to compare efficacy of Electis with other commonly used penetrant or systemic + multi-site combination products. Season long programs with each product where used to measure comparative performance. The data clearly illustrate the efficacy of Electis against infestation of foliage and fruits was always statistically comparable and in some cases superior to the other reference products. Electis at 2kg/ha was superior to 2kg/ha of mancozeb in both trials illustrating the benefits of enhanced efficacy when zoxamide is formulated with mancozeb.

Table 5 Efficacy of Electis applied in Spray Programs against *Phytophthora infestans* on Industrial Tomato at Borgo Montello (Lazio - Italy). 2004.

Treatments	Application Code†	Formulation Conc.(g /L or g/ Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 7 DAAE*	% Leaf Surface Infected 7 DAAG
1a. Copper-oxychloride	A-B	500 WP	3.5	32.5 b	50.0 b
1b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
1c.Dimethomorph +copper-oxychloride	E-F	460 WP	3.5		
1d. Azoxystrobin	G	250 SC	1.0		
2a. Copper-oxychloride	A-B	500 WP	3.5	28.7 b	36.3 cd
2b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
2c. Electis	E-G	750 WG	2.0		
3a. Copper-oxychloride	A-B	500 WP	3.5	22.0 b	41.3 bc
3b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
3c. Dimethomorph+ mancozeb	E-G	690 WG	2.2		
4. Dimethomorph+ mancozeb	A-G	690 WG	2.2	16.2 b	26.3 d
5. Electis	A-G	750 WG	2.0	17.0 b	26.3 d
6.. Tolylfluanide	A-G	500 WP	1.5	71.2 a	92.3 a
7. Untreated	-	-	-	78.4 a	88.8 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 7 Sequential applications designated by the letters A through G
** DAAE = (days after application E)*

Table 5 reports data from a trial on *P. infestans* looking at efficacy of Electis when used as part of a typical grower spray program. In program 2, Electis was used as a spray to protect fruit at the end of the season following applications of Copper oxychloride and metalaxyl M + mancozeb. This programme delivered equivalent performance on leaves and superior performance on fruits over programs 1 and 3 where azoxystrobin and dimethomorph + mancozeb where used at the end of the spray programme.

Table 6. Efficacy of Electis applied at intervals of 8-10 days against Early blight (*Alternaria solani*) of Fresh Tomato at Sueca (Valencia – Spain). 2000.

Treatments	Application Code†	Formulation Conc.(g /L or g/Kg)	Rate Unit(L or Kg PR/ ha)	% of Leaves Infected 7 DAAI*	% Leaf Surface Infected 7 DAAI
1. Electis	A-I	750 WG	1.5	76.0 abc	2.4 b
2. Electis	A-I	750 WG	1.8	74.0 abc	1.95 b
3. Electis	A-I	750 WG	2.0	58.7 cd	1.95 b
4. Mancozeb	A-I	750 WG	2.0	54.7 cd	1.89 b
5. Cymoxanil + mancozeb	A-I	440 WP	3.0	69.3 bcd	1.81 b
6. Chlorothalonil	A-I	750 WG	2.0	48.0 d	0.55 b
7. Azoxystrobin	A-I	250 SC	1.0	47.3 d	0.54 b
8. Untreated	-	-	-	96.7 a	46.25 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 9 Sequential applications designated by the letters A through I.
** DAAI = (days after application I)*

Table 7. Efficacy of Electis applied at intervals of 8-10 days against mixed infection of *Septoria lycopersici* and *P. infestans* on Industrial Tomato at Boara (Emilia-Romagna – Italy). 2000.

Treatments	Application Code†	Formulation Conc. (g/KG)	Rate Unit (L or Kg PR/ha)	PHYTIN % Leaf Surface Infected 7 DAAD*	SEPTLY % Leaf Surface Infected 7 DAAC
1. Electis	A-D	750 WG	1.5	29.4 c	1.1 b
2. Electis	A-D	750 WG	1.8	4.3 d	0.6 b
3.Cymoxanil+copper-oxychloride	A-D	440 WP	3.0	23.41 c	0.8 b
4. Famoxadone+ cymoxanil	A-D	525 WG	0.4	39.1 b	0.7 b
5.Dimethomorph+copper-oxychloride	A-D	460 WP	3.5	4.5 d	0.1 c
6. Untreated	-	-	-	98.2 a	11. 4 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 4 Sequential applications designated by the letters A through D.
** DAAD = (days after application D)*

Table 8. Efficacy of Electis applied in Spray Programs against *Cladosporium fulvum* on Fresh Covered Tomato at Marina di Acate (Sicily). 2004.

Treatments	Application Code†	Formulation Conc.(g /L or g/Kg)	Rate Unit (L or Kg PR/ha)	% Leaf Surface Infected 10 DAAG*	% Leaf Surface Infected 10 DAAH
1a. Copper-oxychloride	A-B	500 WP	3.5	6.8 b	12.7 b
1b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
1c.Dimethomorph + copperoxychloride	E-G	460 WP	3.5		
1d. Azoxystrobin	H	250 SC	1.0		
2a. Copper-oxychloride	A-B	500 WP	3.5	5.6 b	8.4 c
2b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
2c. Electis	E-H	750 WG	2.0		
3a. Copper-oxychloride	A-B	500 WP	3.5	5.7 b	8.8 c
3b. Metalaxyl-M + mancozeb	C-D	680 WP	2.5		
3c. Dimethomorph + mancozeb	E-H	690 WG	2.2		
4. Dimethomorph + mancozeb	A-H	690 WG	2.2	0.8 c	1.9 d
5. Electis	A-H	750 WG	2.0	0..5 c	1.0 d
6. Untreated	-	-	-	16.6 a	28.2 a

Means followed by the same letter do not significantly differ (P=0.05, SNK test)
† 8 Sequential applications designated by the letters A through H.
** DAAG = (days after application G)*

Table 6 shows efficacy data from an early blight (*A. solani)* trial located in Spain which illustrates 2kg/ha of Electis was required to give control equivalent to the commercial references chlorothalonil and azoxystrobin. Performance of Electis was equivalent to straight mancozeb in this trial. Efficacy of Electis was also excellent against *S. lycopersici* when applied at 1.5 -1.8 kg/ha (Table 7). A heavy infection of late blight was observed in the same trial. This situation can occur where the crop is attacked and weakened early in the season by a secondary disease such as *S. lycopersici,* predisposing it to infection by late blight. In this instance Electis treatments provided excellent control of late blight as well as *S. lycopersici*. Table 8 shows efficacy data from a trial against *Cladosporium fulvum* located in Sicily. Electis used season long at 2kg/ha was numerically the most efficacious treatment and provided excellent control. When Electis was used as the last two sprays in a program (Treatment 2) control was significantly reduced illustrating the importance of adequate control of this pathogen early in the season with effective fungicides.

Conclusions

Electis is a new Oomycete fungicide approved in Italy for use in grapevine, tomato and potato. Electis contains the dithiocarbamate mancozeb and zoxamide, a novel benzamide which is highly effective against Oomycete fungi. The combination of zoxamide's high intrinsic efficacy on Oomycetes coupled with the broad spectrum multi site activity of mancozeb delivers excellent protection against late blight, early blight and other key diseases of tomato. Extensive testing on tomato in Italy and other Southern European countries have shown that Electis is an important tool when used as part of a modern disease control program designed to deliver high grade fruit finish. Electis is a useful and flexible addition to fungicide programmes and the novel mode of action of zoxamide coupled with the multi site activity of mancozeb make it a vital component of any effective resistance management

strategy. Electis will provide the grower with broad spectrum activity which is vital when competitive fungicide solutions are evaluated in term of cost vs. benefit. Electis is highly resistant to wash-off and has a high affinity for cuticular waxes which makes it a strong product for preserving fruit finish, a key factor influencing the quality of processed fractions produced from the fruit. The efficacy of Electis against key diseases coupled with its favourable food chain and eco-toxicological profile makes it a vital tool for professional growers of fresh and processed tomato.

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Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
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Report of the fungicide sub-group: Discussion of potato early and late blight fungicides, their properties & characteristics and harmonised protocols for evaluating these

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Objectives

The objectives of the sub-group meeting were:-

- to review and update the ratings given in 2005 for the various properties and characteristics of early and late blight fungicides at the Tallinn workshop in October 2005 (PPO-Special Report No 11 (2005), 95-100).
- to discuss the 2006 field trials using the harmonised protocol for leaf blight control
- to discuss harmonised protocols for the evaluation of tuber blight control, curative activity, rainfastness and *Alternaria* control.

PPO-Special Report no. 12 (2007), 107 - 111

General comments about the ratings tables for late blight fungicides (Tables 1 and 2)

- The ratings given in Table 1 are for blight fungicides currently registered in several EU countries and are based on the label recommendations for commercially available products containing one or two active ingredients as a co-formulated mixture. The ratings are NOT for the active ingredients themselves. Whilst in previous proceeding, the ratings were for all products containing a specific active ingredient, this point may have been misunderstood. As a result, Table 1 has been amended and lists the commercially available mixtures of active substances. The ratings given are for the highest dose rate registered for the control of *P. infestans* in Europe. Different dose rates may be approved in different countries.

- The ratings are intended as a guide only and will be amended in future if new information becomes available. Table 1 will be available for public viewing on the EUROBLIGHT website using the link http://www.euroblight.net/Fungicide/FungicideEffect.asp

- Table 2 gives provisional ratings for 'recently introduced' products and new fungicide formulations. The inclusion of a product in this table is NOT indicative of its registration status either in the EU or elsewhere in Europe. These ratings are based on information from field experiments or minimal practical experience of a product and will be amended at future workshops, as new information becomes available and the body of experience in commercial use increases.

- Before the fungicide ratings were discussed, the group heard presentations on the activity of two new fungicide formulations (mandipropamid and propamocarb+cymoxanil) for the control of late blight.

- Following one season's experience of commercial use in the UK, propamocarb-HCl+fluopicolide was placed in Table 1 and some of the ratings were amended. Before the workshop, Syngenta Crop Protection made available data on the performance of mandipropamid for independent members of the sub-group to assess and give provisional ratings. These appear in Table 2. Belchim Crop Protection presented results from a series of experiments concerning protection of new growth with cyazofamid. On basis of these results the rating for this characteristic was updated in Table 1.

Harmonised protocols

- Three trials following the harmonised protocol for leaf and stem blight control were carried out in the Netherlands, Denmark and the UK in 2006 (see *Testing fungicides for effectiveness against leaf blight using harmonised protocol in 2006, Schepers, Bain and Nielsen, on EUROBLIGHT website*). The proposed methodology for transformation of these results (using the relative Area Under the Disease Progress Curves) into fungicide ratings must have the agreement and 'buy-in' of all stakeholders, in particular the fungicide manufacturers. Because the number of trials generating data using the harmonised protocol (six sites over two years) is small, the possibility of using data supplied by manufacturers' that conforms to the harmonised protocol will be considered and where appropriate will be used to derive the appropriate rating.
- Harmonised protocols for the evaluation of tuber blight control, curative activity, rainfastness and *Alternaria* control were also considered. Comments from the sub-group will be used to amend these protocols which will be available on the EUROBLIGHT website

Definitions and disclaimer are reproduced below from the Tallinn 2005 proceedings for ease of reference.

Phenylamide resistance - The ratings assume a phenylamide-sensitive population. Strains of *P. infestans* resistant to phenylamide fungicides occur widely within Europe. Phenylamide fungicides are available only in co-formulation with protectant fungicides and the contribution which the phenylamide component makes to overall blight control depends on the proportion of resistant strains within the population. Where resistant strains are present in high frequencies within populations the scores for the various attributes will be reduced.

New growth - The ratings for the protection of the new growing point (new growth) indicate the protection of new foliage due to the systemic or translaminar movement or the redistribution of a contact fungicide. New growth consists of growth and development of leaves present at the time of the last fungicide application and/or newly formed leaflets and leaves that were not present.

Protectant activity - Spores killed before or upon germination/penetration. The fungicide has to be present on/in the leaf/stem surface before spore germination/penetration occurs.

Curative activity - the fungicide is active against *P. infestans* during the immediate post infection period but before symptoms become visible, i.e. during the latent period.

Antisporulant activity - P. infestans lesions are affected by the fungicide by decreasing sporangiophore formation and/or decreasing the viability of the sporangia formed.

Stem blight control - effective for the control of stem infection either by direct contact or via systemic activity.

Tuber blight control - activity against tuber infection as a result of fungicide application after infection of the haulm, during mid- to late-season i.e. where there is a direct effect on the tuber infection process. The effect of phenylamide fungicides on tuber blight control was therefore not considered relevant in the context of the table as these materials should not be applied to potato crops if there is blight on the haulm, according to FRAC guidelines. Only the direct (biological) effect of a particular fungicide on the tuber infection process was considered relevant and NOT the indirect effect as a result of manipulation or delay in the development of the foliar epidemic.

Whilst every effort has been made to ensure that the information is accurate, no liability can be accepted for any error or omission in the content of the tables or for any loss, damage or other accident arising from the use of the fungicides listed herein. Omission of a fungicide does not necessarily mean that it is not approved for use within one or more EU countries.

The ratings are based on the label recommendation for a particular product. Where the disease pressure is low, intervals between spray applications may be extended and, in some countries, fungicide applications are made in response to nationally issued spray warnings and/or Decision Support Systems. It is essential therefore to follow the instructions given on the approved label of

a particular blight fungicide appropriate to the country of use before handling, storing or using any blight fungicide or other crop protection product.

Table 1. The effectiveness of fungicide products/co-formulations for the control of *P. infestans* based on the highest rate registered in Europe. These ratings are the opinion of the Fungicides Sub-Group (independent scientists and representatives from the crop protection industry) at the Bologna late blight workshop, 2007 and are based on field experiments and experience of the products performance when used in commercial conditions.

	Effectiveness				Mode of Action			Rain fastness	Mobility in the plant
Product [1]	Leaf blight	New growth	Stem blight	Tuber blight	Protectant	Curative	Anti-sporulant		
benthiavalicarb+ mancozeb	+++	?	+(+)[4]	+(+)	+++	+(+)	+	++(+)	translaminar +contact
chlorothalonil	++	?	(+)	0	++	0	0	++(+)	contact
copper	+	?	+	+	+(+)	0	0	+	contact
cyazofamid	+++	++	+	+++	+++	0	0	+++	contact
dithiocarbamates [2]	++	?	+	0	++	0	0	+(+)	contact
famoxadone +cymoxanil	++	?	+(+)	N/A	++	++	+	++(+)	contact +translaminar
fluazinam	+++	?	+	++(+)	+++	0	0	++(+)	contact
zoxamide+mancozeb	+++	?	+[4]	++	+++	0	0	++(+)	contact +contact
cymoxanil+ mancozeb, metiram or copper	++(+)	?	+(+)	0	++	++	+	++	translaminar +contact
dimethomorph+ mancozeb	++(+)	?	+(+)	++	++(+)	+	++	++(+)	translaminar +contact
fenamidone +mancozeb	++(+)	?	+(+)[4]	++	++(+)	0	+(+)[4]	++	translaminar +contact
benalaxyl+mancozeb [3]	++	++	++	N/A	++(+)	++(+)	++(+)	+++	systemic +contact
metalaxyl-M + mancozeb or fluazinam [3]	+++	++	++	N/A	++(+)	++(+)	++(+)	+++	systemic +contact
propamocarb-HCl+fluopicolide	+++	++	++	+++	+++	++	++(+)	++(+)	systemic + translaminar
propamocarb-HCl+ fenamidone, or +mancozeb or +chlorothalonil	++(+)	+(+)	++	++	++(+)	++	++	+++	systemic +translaminar or+contact

[1] The scores of individual products are based on the label recommendation and are NOT additive for mixtures of active ingredients. Inclusion of a product in the list is NOT indicative of its registration status either in the EU or elsewhere in Europe.:

[2] Includes maneb, mancozeb, propineb and metiram..

[3] See text for comments on phenylamide resistance.

[4] Based on limited data.

Key to ratings : *0 = no effect ; + = reasonable effect ; ++ = good effect ; +++ = very good effect ; N/A = not recommended for control of tuber blight; ? = no experience in trials and/or field conditions.*

Table 2. Provisional ratings for the effectiveness of new fungicide products /co-formulations for the control of *P. infestans* in Europe. These ratings are the opinion of the Fungicides Sub-Group at the Bologna late blight workshop, 2007 and are based on field experiments and not experience
under commercial conditions.

	Effectiveness				Mode of Action			Rainfastness	Mobility in the plant
Product [1]	Leaf blight	New growing point	Stem Blight	Tuber blight	Protectant	Curative	Anti Sporulant		
mandipropamid	+++	?	+(+)	++ 2	+++	+ 3	+(+)	+++	translaminar & contact

[1] *The scores of are based on the label recommendation and are NOT additive for mixtures of active ingredients. Inclusion of a product is NOT indicative of its registration status either in the EU or elsewhere in Europe.*
[2] *Based on limited data in which the direct effect was assessed.*
[3] *In some trials there were indications that the rating was +(+)*
Key to ratings : *0 = no effect ; + = reasonable effect ; ++ = good effect ; +++ = very good effect*

Early blight – *Alternaria solani & Alternaria alternata*

Problems have been experienced in some countries with the early blight disease complex caused by *Alternaria spp (A. solani and A. alternata).* Under field conditions, it is not possible to distinguish symptoms caused by the different species although differences in fungicide effects have been recorded when tested in laboratory conditions. There may be differences in fungicide performance against the two species under field conditions, but currently there are insufficient data to give separate ratings. The ratings are based on fungicides used according to the principles of Good Agricultural Practice (GAP) i.e. at the rates and water volumes recommended on the label and the application timings for control of late and/or early blight.

The information that is available on the efficacy of (late blight) fungicides against this disease complex is presented Table 3. The rating in Table 4 is for products that do not have a registration for control of Alternaria and is based on field experiments and very limited experience.

Table 3. Efficacy of fungicides for the control of early blight caused by *Alternaria solani* and *Alternaria alternata*.

Product	Efficacy [1]
azoxystrobin	+++
fluazinam	(+)
metiram/mancozeb2	++
propineb	++
chlorothalonil	+(+)
famoxadone+cymoxanil	++
fenamidone+mancozeb	++
or propamocarb3	
zoxamide+mancozeb	++(+)

Table 4. Efficacy of new fungicides for the control of early blight caused by *Alternaria solani* and *Alternaria alternata*. The product(s) listed do not yet have a registration for this use

Product	Efficacy [1]
pyraclostrobin+boscalid	+++

[1] ***Key to ratings :*** *0 = no effect ; + = some effect; ++ =reasonable effect ; +++ = good effect ; ++++ very good effect*
[2] *This rating applies to mancozeb containing products when used at the highest dose rates (>1500g/ha). Where less than this rate of mancozeb is used, this rating may not be appropriate particularly where the second active substance is not effective against Alternaria.*
[3] *In some trials there were indications that the rating was ++(+)*

Tenth Workshop of an European Network for development of an
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Protection of new growth with cyazofamid

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Summary
The aim of the project described in this paper was to demonstrate the efficacy of cyazofamid in the protection of the new growth of the potato plant against potato late blight or *Phytophthora infestans.* The efficacy of several fungicides was tested in field trials carried out in the different potato areas of 7 countries in North-Western Europe from 2001 until 2006. The trials showed that cyazofamid (Ranman Twin Pack) has a very good efficacy for potato late blight control in the period of fast vegetative growth despite the fact that it is a contact fungicide.

Keywords
Phytophthora infestans, potato late blight, new growth, cyazofamid

Introduction

General

The protection of the potato plant against an attack by *Phytophthora infestans* during the period of fast vegetative growth is one of the most important challenges for the potato growers. In this period, farmers spray with systemic fungicides or non-systemic in a short interval, following the recommendation of Decision Support Systems.

Definition "New Growth"

A presentation by Evenhuis, Spits & Schepers in Jersey 2004 suggested changes to the definition of 'new growth (new growing point)'. (PPO-Special Report No 10 (Jersey, 2004), 157-160). Further work in the Netherlands since the Jersey workshop has confirmed that contact and translaminar fungicides can give protection of new growth against late blight. As a result, the subgroup agreed that there should be a new definition for the protection of new growth.
"New Growth" consists of growth and development of leaves present at the time of the last fungicide application and/or newly formed leaflets and leaves that were not present. (PPO Special Report No 11 (Tallinn, 2005), 95-100).

Cyazofamid

ISK Biosciences has registered and manufactures an end-use product containing cyazofamid. The end-use product, Ranman 400SC is a suspension concentrate with 400g/l active ingredient (a.i.) used for control of diseases caused by oomycete fungi. Cyazofamid has limited curative activity so it is used as a

protectant fungicide spray. The biochemical mode of action of cyazofamid is inhibition of all stages of fungal development. The product is registered in most of the European countries from 2000 onwards. It is commercialized in a twin-pack containing Ranman 400SC + adjuvant.

Figure 1. Research 'protection new growth' with Ranman

Table 1. Fungicides that were tested in the different trial

Commercial name	**Active ingredient**	**Dose rate** (l or kg/ ha)
Ranman Twin Pack	Cyazofamid + Adjuvant	0,2 + 0,15
Consento	Fenamidone + Propamocarb	2
Shirlan	Fluazinam	1,4 (UK 0,3)
Ridomil Gold	Mefenoxam + Mancozeb	2,5
Curzate	Cymoxanil + Mancozeb	2,5
Tattoo C	Propamocarb + Chlorothalonil	2-2,5
Dithane DG	Mancozeb	2
Sereno	Fenamidone + Mancozeb	1,5
Acrobat (Invader)	Dimethomorph + Mancozeb	2

Materials and methods

Introduction

The contact fungicide Ranman Twin Pack (cyazofamid + adjuvant) was tested in a large number of field trials all over N-W Europe (Figure 1) to prove the efficacy of the product during this fast

growing period and in particular, on the "New Growth". The efficacy of cyazofamid was compared with 8 other preventive fungicides (table 1).

In the period 2001-2006, 13 trials were conducted by several independent institutes in Scotland (1 trial), United Kingdom (1 trial), Belgium (2 trials), France (1 trial), Denmark (1 trial), the Netherlands (3 trials) and Germany (4 trials). The trial method as described in the next chapter is a general one, in the countries there might be some variation in the used methodology. In 2005, the independent members of the fungicide subgroup agreed to prepare a protocol for testing fungicide effectiveness in protecting new growth based on the Dutch methodology.

Trial method

Potatoes were treated 2 times with mancozeb starting at row closing followed by a single preventive application of the test products (table 1) at a normal interval. Treatment included the buds and very small leaves that are going to become the "new growth". In most of the countries, leaflets were tagged or coloured at the application of the test products to identify "new growth".
Next, plots were sampled 7 days after application and subsequently artificially infected with *Phytophthora infestans* (Leaflets inoculated with *Phytophthora infestans* at a sporangia concentration of 50,000/ml). Inoculation and incubation happened in lab conditions. After at least 5 days after incubation, leaflets were assessed for presence/absence of sporulation, and for the percentage leaf area infected.
In the Netherlands in 2004, the inoculation was done in the field to test the relevance of the lab tests. It was concluded that the conditions are similar.

Results and discussion

Because of the diverse trial methods and the different products used in the various trials, we only used the results of the samples that were inoculated 7 days after application to demonstrate the efficacy of Ranman, compared to the other products. We choose the 7 DAA samples because it is assumed that the growing point and small leaves have grown sufficiently to demonstrate our purpose. Another reason was to be able to compare the different trials, as in some countries trials, the only inoculation timing was 7 days after application. This is also the most commonly recommended interval in spraying advices. Systemic, as well as translaminar and contact products were compared.
In Figure 2, the percentage of the infected leaf surface is illustrated. Figures show the average percentage over the different trials in all countries and during the 6 years period. Figures of Shirlan and Dithane were available from all 13 trials, Acrobat from 12, Curzate and Tattoo C from 10, Ridomil from 6, Consento from 3 and finally Sereno from 2 trials.

Conclusion

It is proven in laboratory and field trials that Ranman 400SC + adjuvant, although classified as a contact fungicide Ranman 400SC + adjuvant is a valuable tool to protect a developing growing point. The behaviour of the product sprayed under those growth conditions is still under investigation but for the moment being the hypothesis is that this is due to excellent re-distribution of the product in the wax layer.

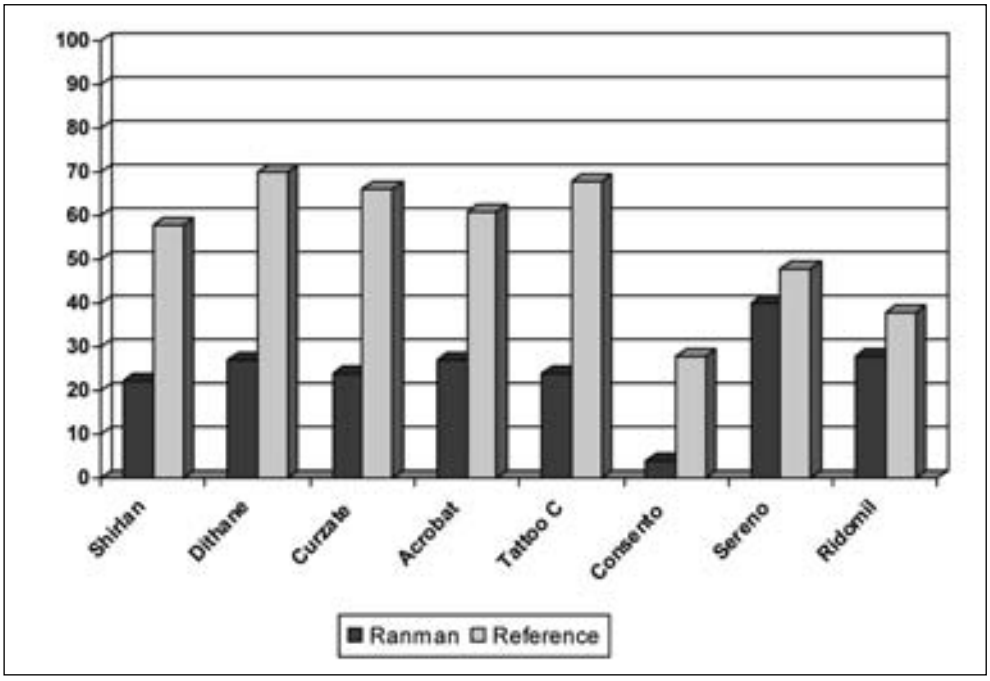

Figure 2. Percentage of the leaf surface attacked by Phytophthora infestans.

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PSA trial, Oldenburg Germany 2005, Dr. J. Kakau
SAC trial, Scotland 2006, R.A. Bain
DLR trial, Bad Kreuznach Germany 2006, Dr. Albert

Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
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Proxanil®: Profile of a new mixture propamocarb + cymoxanil

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Summary
Proxanil® is a new fungicide (combination) developed by Agriphar (a Belgian company) for the control of late potato blight. It combines two well known molecules: Cymoxanil and Propamocarb –HCl.

In field trials conducted throughout Europe since 2004, Proxanil has shown very strong control of late blight in comparison to reference products. Proxanil is a strong mixture combining the different modes of action of two fungicides which is under development in most potato growing countries and will be registered this year in Belgium, with UK registration expected by end 2007, early 2008.

Keywords
Propamocarb-HCl; Cymoxanil, late blight.

Introduction
Proxanil® is a mixture of propamocarb-HCl 400 g /l + cymoxanil 50 g/l SC.
This liquid formulation is very easy to handle and measure out with a rate of 2 L/ha - giving 7 days of protection. Proxanil® has been developed to combine the retroactivity of cymoxanil with the systemicity of propamocarb-HCl for preventive application although Proxanil® can also work under severe disease conditions. The table shows the results of two years field trials - completed for registration purpose. Those trials demonstrate the excellent efficacy of Proxanil® on leaf and stem blight with a certain control of tuber blight.
Proxanil® works at least as well as all the reference products.

What are the features of Proxanil® :

- Easy product to handle - 2L/ha for 7 days protection
- Favorable toxicological, environmental and residue profile
- Anti-sporulant and translaminar properties
- Combination of two different mode of fungicide action
- Protection of new growth
- Rain-fastness
- Preventive and curative activity
- Anti-resistance management

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Materials and methods

Field performance

Fields experiments were conducted in 2004-2005 for the registration of Proxanil® in the United Kingdom, Germany, Netherlands and Belgium in small plot trials in order to compare different commercial fungicides applied at uniform dose rates (Table 1). Foliar applications of the products were made in a 7 day intervals throughout the season on potatoes.

Table 1. Dose rate of Proxanil® and commercial fungicides applied in field trials in U.K. and Germany

Treatments	Dose rates	
	L or kg of product / Ha	g a.i. / Ha
Proxanil® (propamocarb-HCl + cymoxanil)	1.5	600 + 75
Proxanil® (propamocarb-HCl + cymoxanil)	2	800 + 100
Propamocarb-HCl + mancozeb	4	831 + 1206
Famoxadone + cymoxanil	0.7	175 + 175

Results

High level of leaf blight control

Figure 1 shows that Proxanil® gives excellent protection against leaf blight compared to other commercial fungicides. It also shows that Proxanil® at 1.5 litre performs very well. No phytotoxicity symptoms were observed during the trials period.

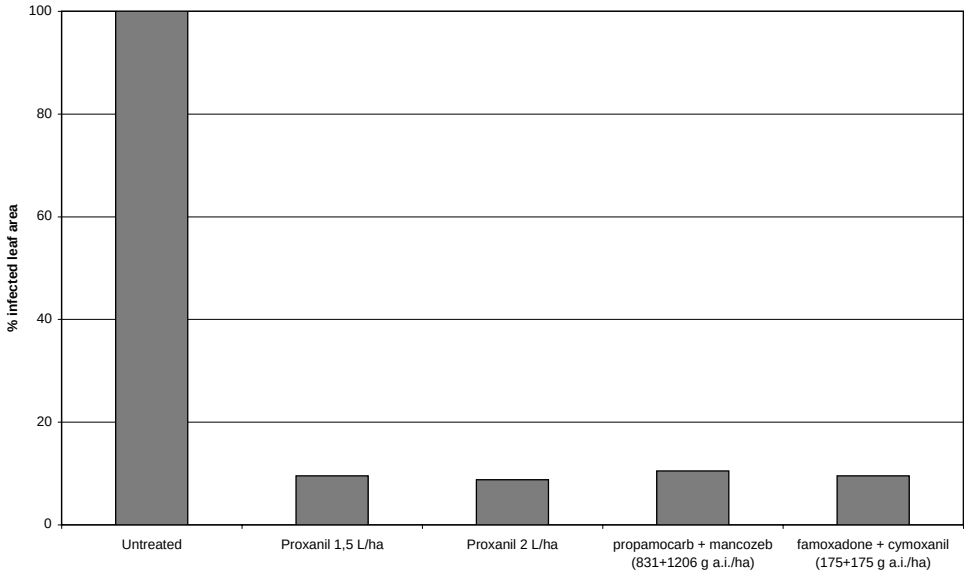

Figure 1. Efficacy of Proxanil® for control of leaf blight

Control of stem blight
Proxanil® performed very well against stem blight.

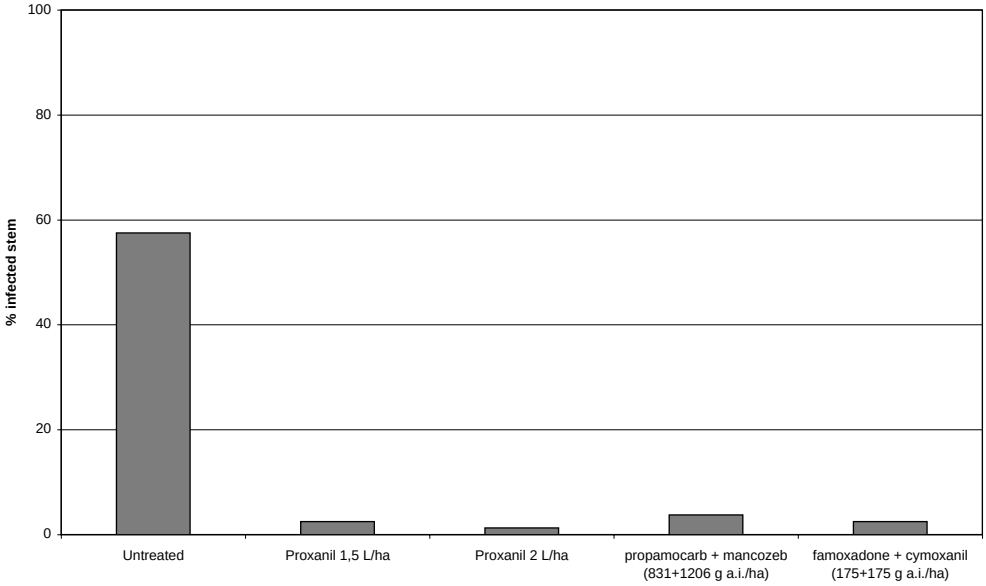

Figure 2. Efficay of Proxanil® against Phytophthora infestans on stems

Protection against tuber blight
Figure 3 shows that Proxanil® gives good results against tuber blight in comparison with the two references.

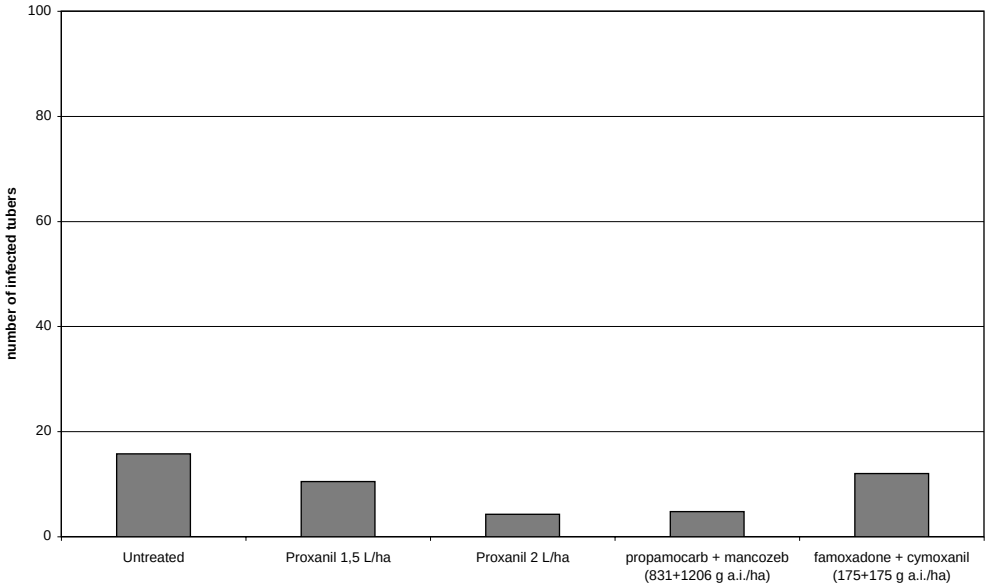

Figure 3. Efficacy of Proxanil® against tuber blight

Activity rating of Proxanil®:
Proxanil® is now being tested by some research institutes in the Netherlands, France, U.K and Belgium in order to get additional information regarding its future rating. Due to the rate of application of each compound per ha, we can already be sure that Proxanil® will get the minimum rating for each of them. This rating will be discussed with the experts by end of 2007.

Conclusion
Proxanil® combines two well known potato blight fungicides at their best respective rates of application (800g/l propamocarb-HCl and 100g/ha Cymoxanil) and thus provides outstanding control of late blight on potato, particularly on leaf and stem blight.
Proxanil® will be a good partner for the anti-resistance management.

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REVUS® – Field performance and product recommendations against late blight in potatoes in Europe

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Summary
REVUS is a potato late blight fungicide containing the new active ingredient mandipropamid. In field trials REVUS provided excellent protection against foliar and stem blight. REVUS showed long lasting activity and rainfastness, equal or superior to the best standards. This resulted in high yields and a good prevention of tuber blight. REVUS is already registered in Austria and the UK. It will be available in Germany and The Netherlands mid 2007 and in other countries from 2008 onwards.

Keywords
Mandipropamid, fungicide, late blight, tuber blight, potato, *Phytophthora infestans*

Introduction
REVUS is formulated as a Suspension Concentrate (SC) containing 250 g/l mandipropamid. The low use rate of 0.6 l/ha and the liquid formulation make it easy to use.

Potato late blight is a highly destructive disease. In order to prevent yield losses and diseased tubers a high level of late blight control is required. Rain events lead to washing off of many fungicides and make it necessary to respray to keep the crop protected. High disease pressure makes it necessary to shorten spray intervals of many fungicides from 7 to 5 days. In this paper we present and discuss field trial results, documenting the following properties of REVUS:

- reliable high level of foliar disease control
- excellent rainfastness
- long lasting activity
- good control of stem blight
- prevention of tuber blight
- high yield enhancement

Results

Efficacy against foliar blight
In the years 2004-2006 a large number of field trials were conducted to compare the efficacy of REVUS to that of commercial standards (Figures 1-4). In each trial, a single representative late season

assessment was selected, when the untreated control was close to complete destruction. The efficacy of REVUS is indicated by the line and the efficacy of the reference product is indicated by a bar. Each bar represents one trial. REVUS provided consistently higher efficacy against foliar blight. The efficacy of REVUS was by far superior to that of mancozeb alone (Figure1) and the combination of cymoxanil and mancozeb (Figure 2). It was clearly superior and more consistent than the efficacy of the mixture of dimethomorph and mancozeb (Figure 3). Cyazofamid (+ adjuvant) delivered the highest level of

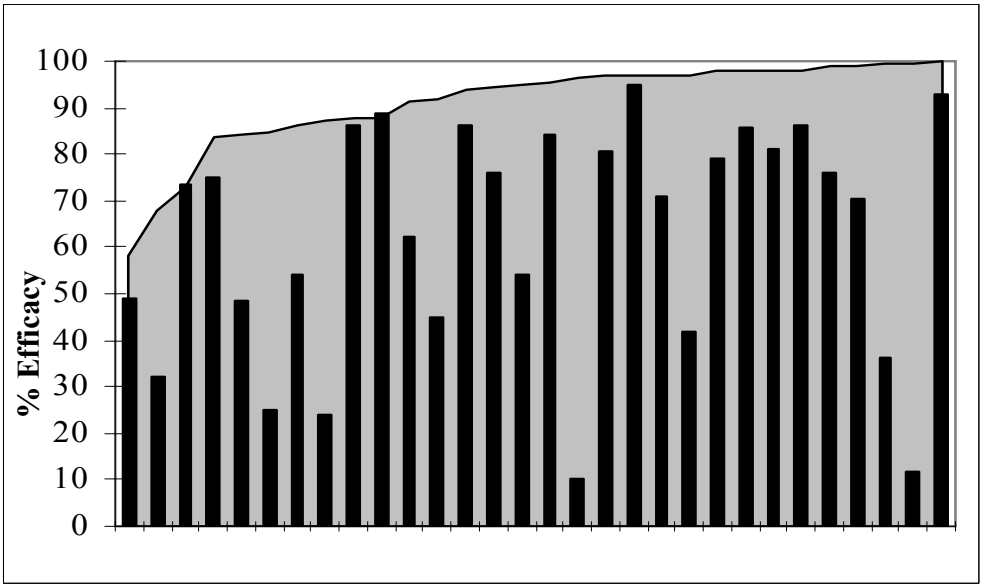

Figure 1. Efficacy of REVUS - 0.6 l/ha (line) compared to Mancozeb - 1600 g/ha (bars). Each bar represents one trial, assessed late season.

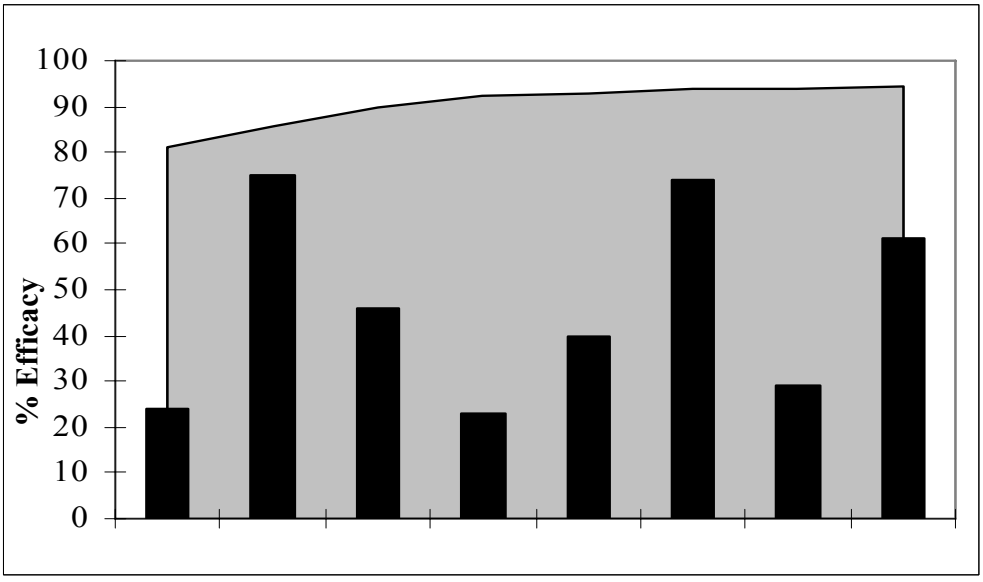

Figure 2. Efficacy of REVUS - 0.6 l/ha (line) compared to Cymoxanil + Mancozeb - 90 + 1300 g/ha (bars). Each bar represents one trial, assessed late season.

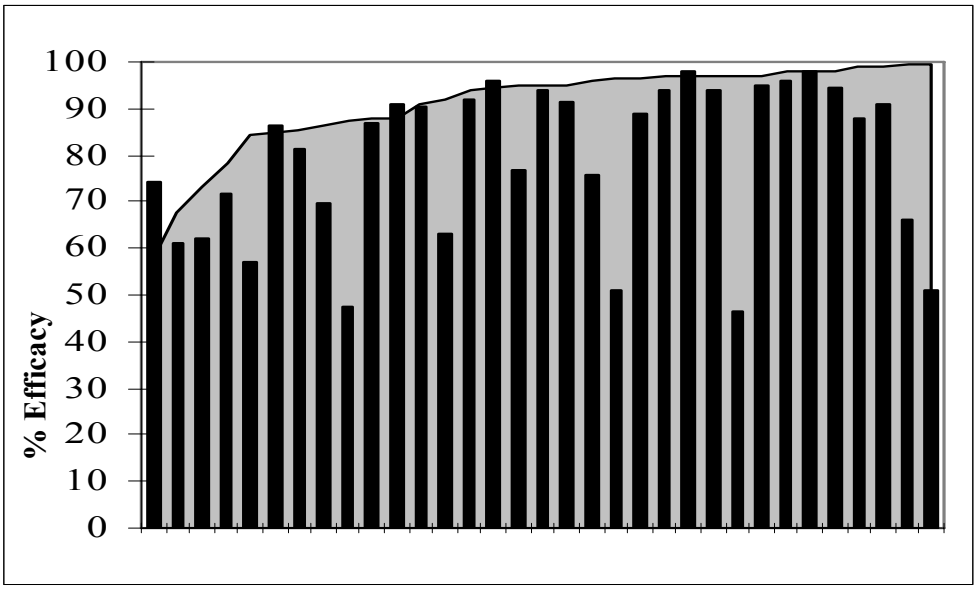

Figure 3. Efficacy of REVUS - 0.6 l/ha (line) compared to Dimethomorph + Mancozeb 180 + 1200 g/ha (bars). Each bar represents one trial, assessed late season.

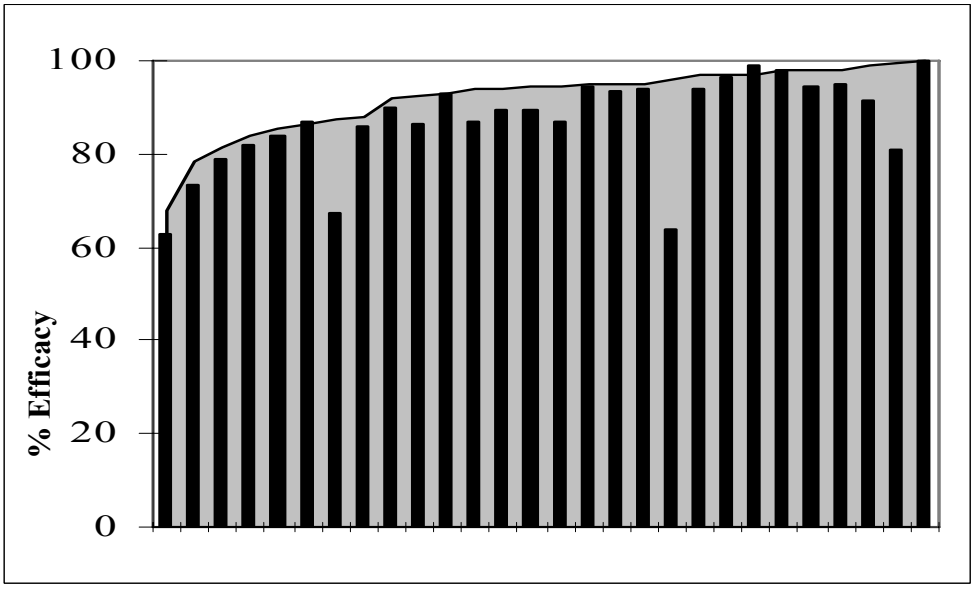

Figure 4. Efficacy of REVUS - 0.6 l/ha (line) compared to Cyazofamid 80 g/ha + Adj. (bars). Each bar represents one trial, assessed late season.

activity of all reference products, but even here the performance of REVUS was superior and more consistent (Figure4).

A representative single trial from Switzerland in 2006 gave similar results (Figure 5). Here the time course of foliar disease progression is shown in curves. Mancozeb alone and cymoxanil plus mancozeb

showed the earliest and most rapid disease progression. They were followed by the combinations of benthiavalicarb or zoxamid with mancozeb. Fluopicolid plus propamocarb, dimethomorph plus mancozeb and cyazofamid plus adjuvant gave a good level of efficacy. REVUS gave the best efficacy and kept the plots healthy for the longest time.

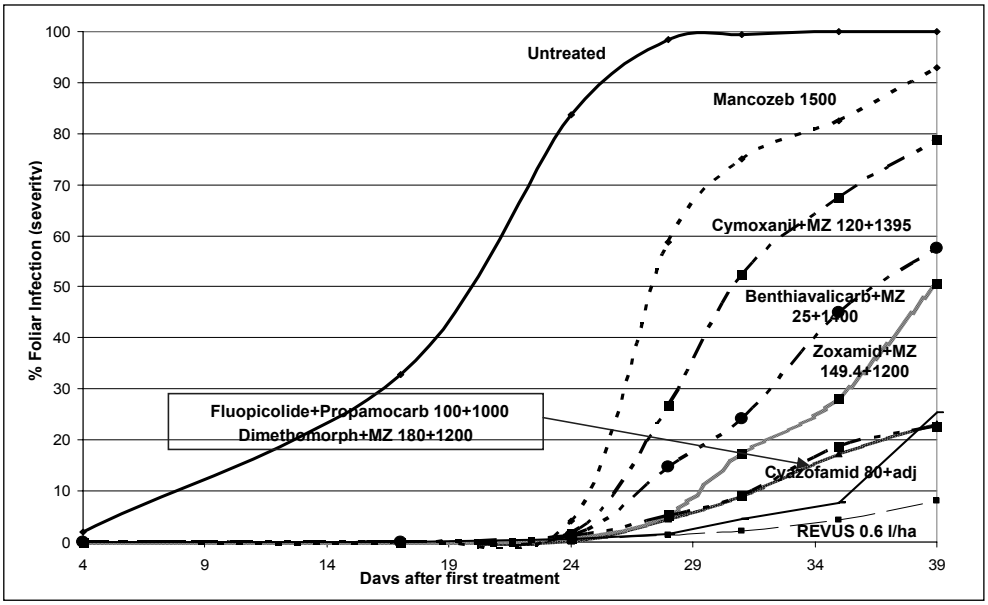

Figure 5. Diesease development in a development trial (Switzerland 2006), comparing REVUS - 0.6 l/ha to commercial reference products.

Rainfastness

Mandipropamid is rapidly absorbed into the foliar waxes, where it is protected from washoff by rain (Huggenberger and Knauf-Beiter, 2007).

A rainfastness trial was conducted in France by SRPV Nord Pas-de-Calais (Culiez and Detourne, 2006) comparing 0 mm, 25 mm and 85 mm of irrigation. 25 mm were applied the night after application, 85 mm were applied split in two doses (45 and 35 mm) one and two nights after application respectively. Leaf discs were sampled 3, 5 and 7 days after application and inoculated in the laboratory. Between the first and second sampling 48 mm of natural rainfall were observed. Inoculation was made either on the upper or lower leaf surface. Results of the 0 mm and 80 mm rain regimes are shown below for the inoculation of the upper surface in Figure 6 and for inoculation of the lower surface in Figure 7.

The performance of products depending on mancozeb for their protectant activity (mancozeb alone and dimethomorph plus mancozeb) suffered severely from rainwashing. This can be explained by the loss of the protectant ingredient. The mixture of zoxamid and mancozeb performed well, when the upper leaf surface was inoculated, but performed less well, when the lower surface was inoculated. This can be explained by the fact that zoxamid is a rainfast protectant but mancozeb is washed off. The activity zoxamid alone is sufficient on the upper surface, it is not sufficient on the lower leaf surface, which is normally not covered as well by the spray treatment as the upper leaf surface. Overall cyazofamid (plus adjuvant) and REVUS were the best treatments in this trial. Mandipropamid also

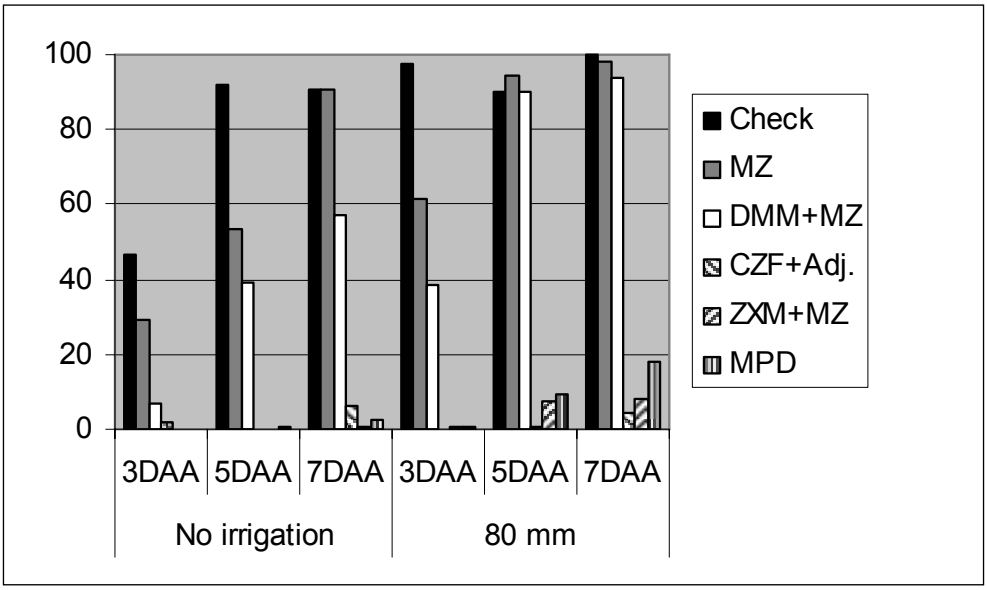

Figure 6. Effect of rainwashing on the protection against foliar late blight. Potatoes were treated in the field. Rainfall was simulated by artificial irrigation. Leaf discs were taken at 3, 5 and 7 days after treatment and inoculated on the upper surface in the laboratory. Between the first and the second sampling 48 mm of natural rainfall were observed.

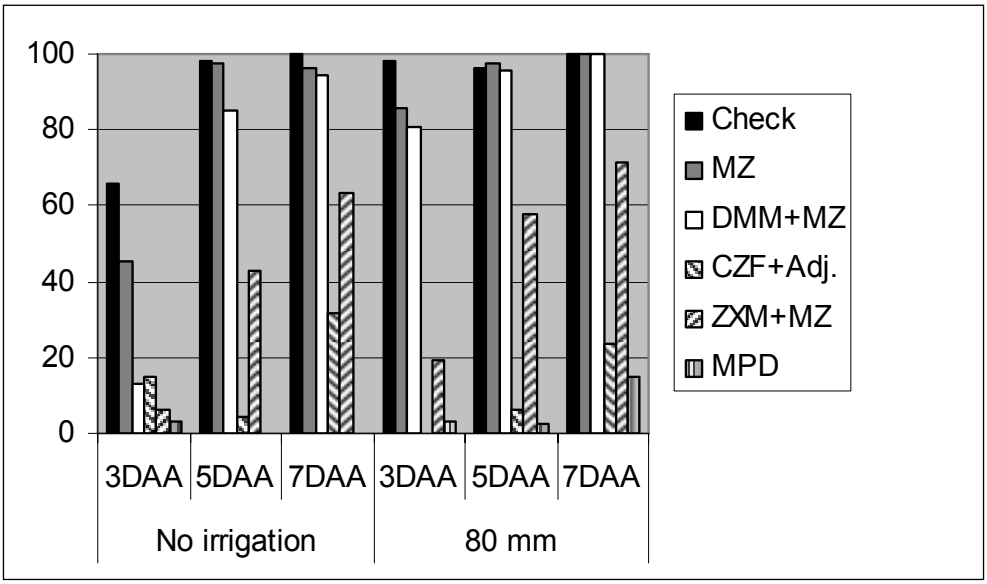

Figure 7. Effect of rainwashing on the protection against foliar late blight. Potatoes were treated in the field. Rainfall was simulated by artificial irrigation. Leaf discs were taken at 3, 5 and 7 days after treatment and inoculated on the lower surface in the laboratory. Between the first and the second sampling 48 mm of natural rainfall were observed.

provided consistently good disease control, when the lower leaf surface was inoculated. These results confirm the excellent translaminar activity of mandipropamid under field conditions.
In an efficacy trial against late blight in potatoes and tomatoes in Indonesia (Figure 8), 300-350 mm

of natural rainfall occurred during 5 weeks. All products depending on mancozeb as a protectant had low levels of efficacy. REVUS and cyazofamid (plus adjuvant) gave excellent disease control. REVUS is highly rainfast as soon as the spray has dried and does not require respraying. There is also no need for shortening the spray intervals under rainy conditions.

Efficacy against stem blight

In development trials in Germany in the years 2000-2006 stem blight was assessed (Figure 9). REVUS gave control of stem blight equal or better than the reference product, a combination of dimethomorph and mancozeb.

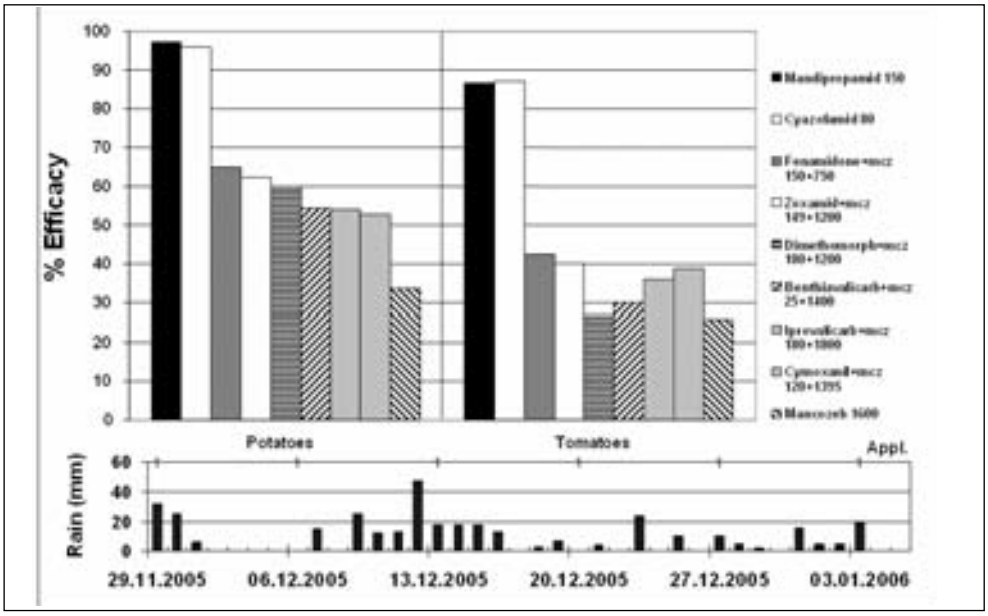

Figure 8. Efficacy trial against late blight in potatoes and tomatoes in Indonesia. In the course of 5 weeks 300-350 mm of natural rainfall occurred.

Yield response and control of tuber blight

In 2003-2006, replicated large plot trials (60 m^2) were conducted for the assessment of yield and tuber blight. In these trials (Figure 10) REVUS provided the best control of foliar late blight, followed by cyazofamid plus adjuvant. Mancozeb gave a much lower level disease control. Yield per ha was closely linked to foliar late blight control. As a result of severe foliar blight, a high percentage of tubers from the mancozeb treated plots were infested with tuber blight. Treatments with REVUS and cyazofamid plus adjuvant gave similar levels of tuber blight protection.

REVUS product recommendations

- Product rate: 0.6 l/ha
- Number of sprays: max 6 sprays per season (CAA products are recommended at max. 50% of total sprays)
- Spray interval: 7 days
- Preharvest interval: 3 days.

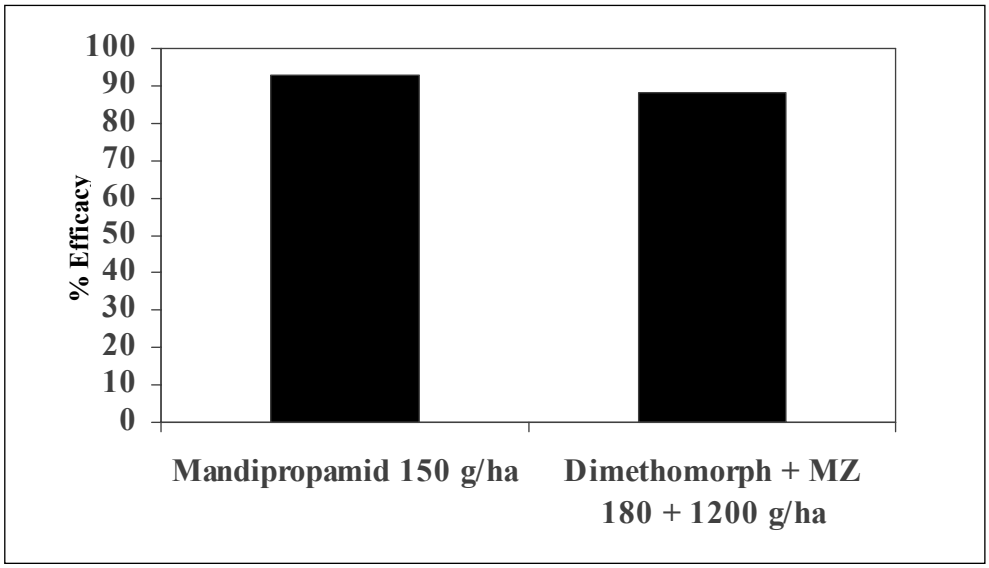

Figure 9. Efficacy of REVUS - 0.6 l/ha compared to Dimethomorph + Mancozeb against stem blight - Germany 2002-2004 (n=6)

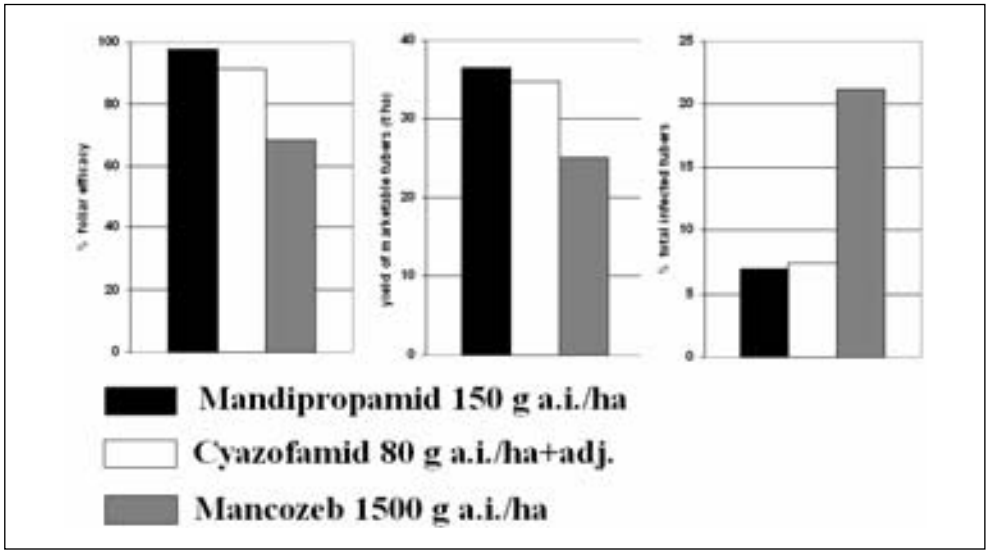

Figure 10. Foliar late blight control, marketable yield and tuber infection in replicated large plot trials. Switzerland 2003-2006, n=5, 60m2 plots

A typical position for REVUS in spray programmes would be at canopy complete, after a systemic product like RIDOMIL GOLD MZ for the protection of new growth and followed by a specialist tuber blight product such as SHIRLAN.

REVUS can be applied in mixture with the commonly used early blight fungicides, insecticides, adjuvants and mineral oils.

Conclusions

REVUS is a new, highly active and reliable potato late blight fungicide, providing excellent protection against foliar and stem blight. The excellent foliar blight protection is a solid base for a high yield of healthy tubers. The robust performance of REVUS can be attributed to the high intrinsic activity and long lasting efficacy of mandipropamid, independent of disease pressure and weather conditions. The rainfastness of REVUS is equal or superior to that of the best commercial standards. Mandipropamid is rapidly absorbed into the foliar waxes, where it is protected from rain. REVUS does not rely on easily washed off mancozeb for preventive activity.

REVUS is currently registered in Austria and UK. It will be available in Germany and The Netherlands late 2007 and in other countries from 2008 onwards.

Acknowledgements

The authors would like to thank the scientists from SRPV Nord Pas-de Calais and all the Syngenta colleagues who contributed the field trials data.

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Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

EUCABLIGHT one year on: an update on the European blight population database

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Summary
This paper builds on a series of updates on the database of *Phytophthora infestans* population data assembled under the EU-funded Concerted Action project 'EUCABLIGHT'. For the background and details we refer readers to the project web site (www.eucablight.org) and publications in previous EU.ICP.NET reports. In this paper the main changes to the database, the database entry tool, some preliminary findings and future plans are presented. The database has continued to expand with an additional 3168 isolates and data from two additional European countries added since the EU.ICP.NET meeting in Tallin bringing the totals to 16806 isolates from 20 countries. A detailed view of the population is emerging and the updates are starting to capture some of the significant changes in the mating type ratios occurring in Western European potato crops. Significant improvements to the "Phytophthora.exe" PC-based data entry tool have been made. For example, to streamline the data entry process, data is now transmitted in blocks defined by year and country to limit the size, and thus time taken, to upload and download data. In line with increasing research interest on in the functional and evolutionary analyses of specific pathogen genes and *P. infestans* phylogeography, the database has been expanded to allow sequence data from multiple loci to be uploaded for any isolate. Lastly, discussion with research teams beyond Europe has resulted in the development of a new version of the data entry tool that will allow data entry from isolates from South and Central America.

Introduction
The EUCABLIGHT project (EUCABLIGHT: A potato late blight network for Europe (2003-2006)) was very successful in combining the expertise of many research teams to harmonise methods and collate information on blight resistance and *P. infestans* populations on a European scale. Comprehensive databases on both aspects were assembled and although the project formally finished in January 2006 the databases were carefully designed to allow updates so that the project did not become 'frozen in time' but continually updated with data from European research labs. The integration of the EU.ICP.NET and EUCABLIGHT projects into a single entity 'EUROBLIGHT' was a logical means of

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combining the activities of both groups with the schedule of meetings acting as a catalyst for updating and reviewing the progress in both pathogen and host EUCABLIGHT databases.

The background information and goals of the pathogen database have been covered elsewhere (Cooke *et al.*, 2006). In brief, the success of management options is influenced by the nature of the pathogen population and the aim of the EUCABLIGHT database is to compile data on the populations on a European scale. For example, if both the A1 and A2 mating types are present in a region there is a significant risk of oospores acting as a source of primary inoculum as well as an increase in the rate at which *P. infestans* adapts, or evolves, to overcome other management strategies. Similarly the resistance to fungicides and the ability of the pathogen to overcome host resistance is important. Such *P. infestans* monitoring thus allows the potato industry to be proactive in adjusting its approaches to late blight management according to the data on contemporary pathogen populations.

All of the above factors are important on a local to regional scale but populations at national and international scales and over longer time periods also need to be considered. A database of international *P. infestans* isolates with RG57 and isozyme data sufficient to define the clones of the pathogen found in several countries was previously developed for this purpose (Forbes *et al.*, 1998). This was, however, relatively limited in scope and it was clear that the more detailed data and the interfaces developed within the EUCABLIGHT project are appropriate for an updated version of this international database. Such global tracking of major lineages of *P. infestans* will enable the early identification of major changes in population structure suggestive of newly introduced exotic strains, breakdowns of significant sources of resistance or the widespread failure of a key chemical active ingredient. There are also aspects of quarantine and international trade to consider.

Materials, Methods and Results

The database

The EUCABLIGHT pathogen database has two main interfaces. Firstly, the data entry tool "Phytophthora.exe"; a program that runs on the users PC for a rapid and accurate means of entering data that can be processed locally or used to update the central database in Denmark. Secondly the results are presented in some detail via a series of web interfaces that allow key parameters of the European population to be examined on a range of spatial and temporal scales. Only secondary or derived data are presented; raw data can only be accessed by the original data submitter.

As "Phytophthora.exe" is used to enter individual *P. infestans* isolate data into the database a key comprising country, year of collection, 'regionID' and 'isolateID' is created. This key data is linked to any further data from approximately 50 database fields for phenotypic, genotypic and cropping data. The database was constructed by DIAS (now part of the University of Arhus) Denmark (Lassen & Hansen 2005; Hansen *et al.*, 2006)) and was carefully designed to remain functional and expandable with minimum maintenance beyond the official end of the project in January 2006. Having said that an upgrade of the "Phytophthora.exe" software to Version 2.0 is underway and the detail of the improvements will be listed on the project website with the new release in autumn 2007. Several changes relate to the geographical expansion to other continents where additional factors need to be considered. Version 2.0 will accommodate, for example, the altitude of the collection point, whether the isolate is from natural vegetation or a crop and any previously defined genotype name (e.g. US-8 according to criteria defined in Forbes *et al.*, 1998). Sequence data may now also be uploaded and linked to each isolate. This software can be downloaded from the project web-site.

The database primarily contains data from the 1990s onwards with older data being important to set the context for studies on contemporary populations. If DNA or isolates of 'old' isolate collections are available their entries may be updated with, for example, SSR or other genotypic data. At SCRI we have been using Phytophthora.exe to manage our local data, recording each isolate as it processed throughout the 2006 and 2007 seasons.

Data submitted to the database server as detailed above is presented via a series of web-based display tools. Firstly an overview of the number of database entries (isolates) is presented as a table arranged by year and country. Selection of tick boxes relating to key traits indicates the extent of the data for a single trait or a combination of traits (Figure 1). Clicking on the Graphic analysis box brings up a powerful and flexible tool that displays a similar table but in this case a single factor or combinations of up to three factors can be presented as graphs. The user may select an overview of the total dataset or select particular combinations of countries and years. The charts are generated directly from the database entries and thus immediately account for any new data entered. Further detail on the genetic make-up of the population is viewed with the Genotype analysis tool in which up to three traits of specific *P. infestans* populations may be displayed. An example is shown in Figure 2 in which the frequency of occurrence of different combinations of mating type and the alleles at the D13 SSR locus are plotted for 677 isolates collected in England in 2006. This indicates that a specific A2 type with a heterozygous 136/154 combination was the most prevalent type comprising over 40% of the population.

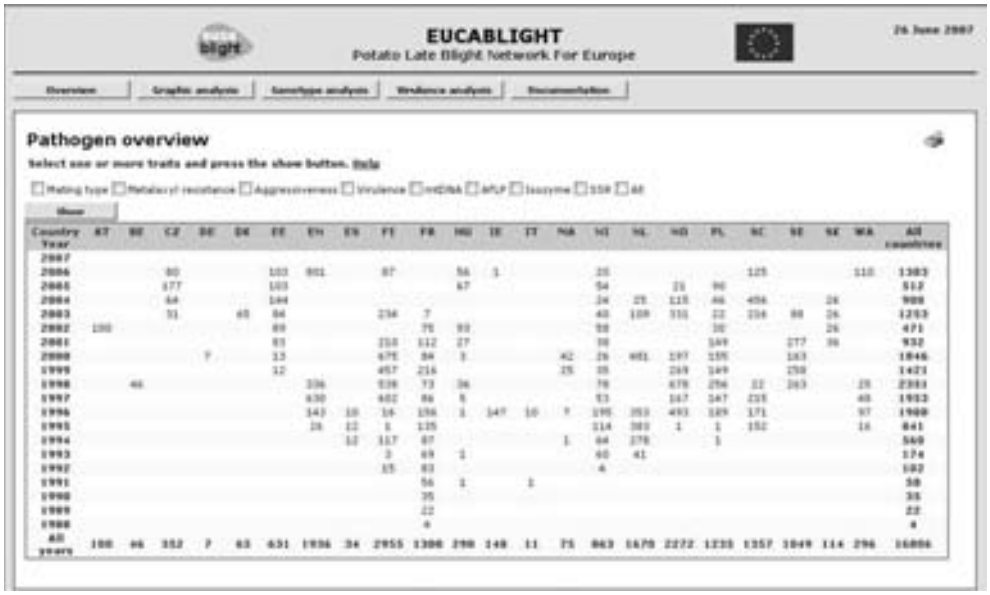

Figure 1. Example of the overview table showing the current status of the database. The numbers in the table indicate the number of isolates per year and country and, in bold, the totals by country and year.

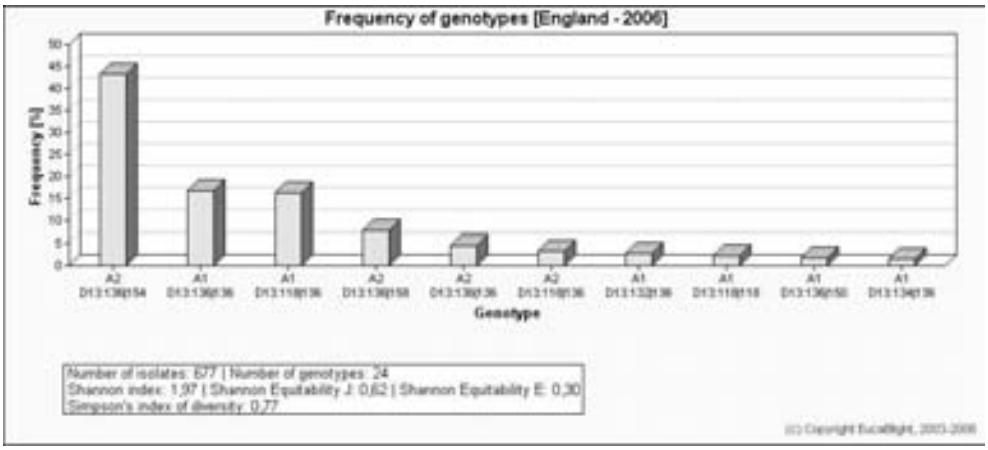

Figure 2 An example of the output from the Eucablight Genotype Analysis tool showing the relationship between mating type and D13 allele combinations for 677 isolates from England in 2006.

On the members side of the web site (available after log-in) additional data on the allele frequencies at each individual SSR loci are plotted by country as a series of pie-charts. This allows a rapid overview of the broad genetic structure of populations from different countries. The example shown (Figure 3) indicates the allele frequencies at the D13 locus for nine countries. The 154 allele which coincides with the A2 genotype shown in Figure 2 is highlighted.

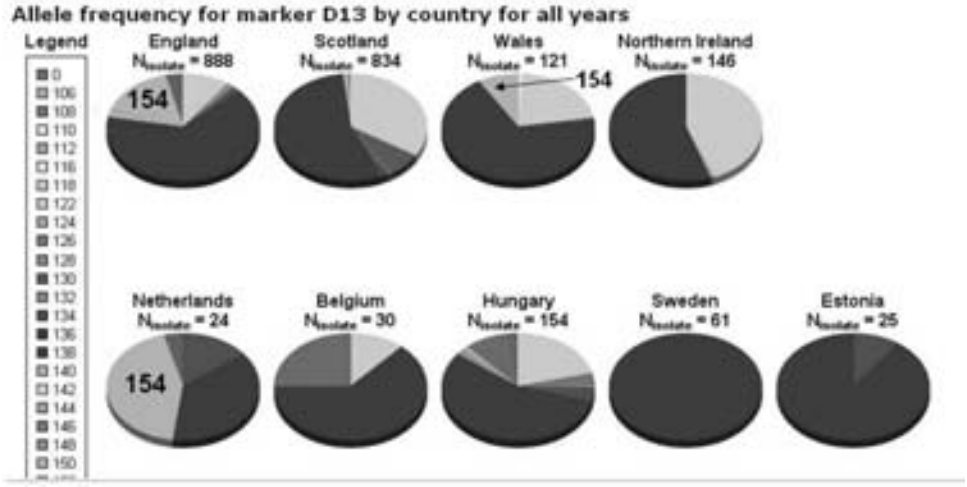

Figure 3 An example of the output from the Eucablight SSR Analysis Tool showing the frequencies of the alleles of SSR marker D13 in a series of 9 countries. Allele 136 is dominant in most states but 154 (labeled) is frequent and increasing (see text).

Discussion

The Eucablight project assembled many experienced research teams across Europe in a co-ordinated project that standardised and collated a wealth of *P. infestans* population data available in state research projects into a single comprehensive database. Data is continually being entered and updated and a total of 20,000 isolates is a realistic target for 2007 as several partners are known to have data awaiting

entry. Clearly there are opportunities to expand the geographical area covered by the database and a great number of ways of exploring and presenting a wealth of results from this comprehensive dataset. The collation and publication of a joint paper by the data contributors is now a key objective.

Within Europe, past population shifts have been widely documented with the arrival of a new population comprising the A1 and A2 mating types reported from the early 1980s onwards that displaced the original A1 population. In Western regions throughout the 90's the frequency of the A2 mating type has, in general, remained low with reports of < 1% in Great Britain (GB), 20% or lower in Scotland and very rare occurrences in Northern Ireland or France (e.g. Day *et al.*, 2004, Cooke *et al.*, 2003; Cooke *et al.*, 2006; Lebreton *et al.*, 1998). In other regions over the same period the A2 has been more prevalent. In the Netherlands, Hungary, Norway and Finland for example, rates of up to 60% were reported in some regions (Zwankhuizen *et al.*, 2000; Bakonyi *et al.*, 2002; Hermansen *et al.*, 2000). Higher levels have been observed in the North and Eastern regions of Europe with Western regions continuing to report low levels (see EUCABLIGHT web page). Over the past 2-3 years, however, a change has been noted. Recent reports from the Netherlands, France and GB document significant increases in the frequency of the A2 mating type up to almost 100% of the population in some regions (van Raaij *et al.*, 2007; Detourne *et al.*, 2007; Shaw *et al* ., 2007; Cooke *et al.*, 2007). In GB, detailed fingerprinting using the SSR markers (Lees *et al.*, 2005; Knapova *et al.*, 2002) has shown that a marked increase in single lineage of the A2 mating type explains much of that change (Cooke *et al.*, 2007). Using the EUCABLIGHT database an allele at the D13 locus (154bp) that is characteristic of this lineage can be seen at a high frequency in England and Wales but also in the Netherlands (Figure 3). The 24 isolates from the Netherlands are all from a single region in 2004. Their genotype is identical to the dominant GB A2 isolates and the fact that this clone was not found in GB until 2005 suggests that the arrival of this lineage into GB from mainland Europe (D. Cooke unpublished data). More analysis of populations from the Netherlands, France and other regions is however required to substantiate this. Wider questions raised by this population shift are: What is the origin of this A2 strain? What are the factors that influence its spread and increasing dominance? Is it more aggressive and if so how will this affect blight management? What will be its impact on oospore formation? Is it recombining with A1 strains to form oospores or is the risk of oospores reducing as the relative proportion of A1 strains decreases in some regions?

A particular advantage of the Eucablight project has been to facilitate discussions on pathogen population differences and the drivers behind such changes. It is beyond the scope of this paper to discuss the main findings of the project but it is clear that having a large collection of reliable and comparable data on *P. infestans* populations will help answer some of the questions posed above. The database will thus be an important instrument for potato breeders, scientists, advisors and policy makers to follow the co-evolution of host and pathogen in Europe and inform the use of appropriate resistance genes and control measures.

Positive feedback from other parts of the world has influenced the database design and an additional field labeled 'ContinentID' added. Regions have now been defined for all countries in Central and South America and a version of the software for this region will shortly be available on the EUCABLIGHT website.

Data submission is open to any group who wish to contribute with data generated according to the standard protocols or their equivalents. The advantages to the submitter are that their data will add resolution to any analysis of the European population structure and they will be part of the project for the planned publications. Furthermore they will have access to the suite of analysis tools that allow

comparison between their submitted data and that of other EU states. Submitters can be assured that no raw data will be passed to other users without their permission. Please contact David Cooke at the address shown above for further details.

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Bologna (Italy), 2007

The 2005 population of *Phytophthora infestans* in Great Britain: the frequency of A2 mating type has increased and new molecular genotypes have been detected.

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Summary
The population of *P. infestans* in Great Britain was sampled in 2005. Mating type, sensitivity to metalaxyl and molecular genotypes were compared to those found in similar samplings in 1995-98. A2 was widely distributed in 2005 and had increased in frequency from 5% to 38% of sites sampled in the last three years. Few of the clonal lineages found in 1995-98 were found in 2005; A1 clonal lineage, RF006, which was common in the 1990s was still present in 2005 and had become the predominant A1 genotype. The most common clonal lineage from 1995-98 (RF039) was not detected in 2005. Most A2 isolates from the 1995-98 collections were of the same clonal lineage, RF040; none of these A2 isolates were fully resistant to metalaxyl. However in 2005, RF040 was not detected and A2 isolates belonged to one of several new clonal lineages. Lineages referred to as blue and green were particularly common; genotype blue (genotype 13 as defined by SSR markers, Cooke *et al.*, this volume) was consistently fully resistant to metalaxyl and was distributed mainly in the east of England whereas isolates of genotype green were resistant, intermediate or sensitive to metalaxyl and had a western distribution. Other, rarer A2 genotypes, were sensitive to metalaxyl.

Keywords
Mating type; clonal lineage; RG57; mtDNA haplotype.

Introduction
Although migration of the A2 mating type of *P. infestans* to Great Britain occurred as early as 1981 its frequency in small samples in the 1980s remained low, not more than a few percent of isolates collected (Tantius *et al.,* 1986). When more intense sampling was conducted in 1995-98, A2 was detected at 9.6% of 354 sites (Day *et al.,* 2004) and up to 20% in Scotland (Cooke *et al.,* 2003). As part of the British Potato Council's Fight Against Blight initiative starting in 2003, Blight Scout volunteers sent in single lesion samples. Of 114 samples received in 2003, 6 were A2 (5.2%) and of 120 samples in 2004, 12 (10%) were A2 (unpublished).

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Isolates collected in 1995-98 from Scotland, England and Wales were characterised for RG57 fingerprint and mitochondrial (mt) DNA haplotype. There was evidence that several clonal lineages predominated, three of A1 mating type and only one of A2 mating type. None of the A2 mating type isolates were fully resistant to metalaxyl (Day *et al.,* 2004).

The aim of the present work was to determine if the modest increase in A2 mating type recorded in 2004 had increased again in 2005 and if the molecular genotypes within the population had changed.

Materials & Methods

Samples of blighted foliage were sent to Henfaes Research Centre via Central Science Laboratory, York by volunteer Blight Scouts recruited by the British Potato Council. *P. infestans* was isolated from one single lesion from each sample on Rye A agar amended with antibiotics (Day and Shattock, 1997). In addition, single or multiple isolates were recovered from samples collected by the authors and by readers of the vegetable growers' magazine, Kitchen Garden; these are referred to as miscellaneous samples/isolates. Mating types of all isolates were determined by pairing each with known A1 and known A2 isolates on Rye A agar.

Sensitivity of the isolates to metalaxyl was determined *in vitro.* Three replicate inocula, each of 4 mm diameter, were placed towards the edge of plates of Pea-water agar containing 0 or 50 µg /ml metalaxyl-m for each isolate. The hyphal extension over 7 days' incubation at 20°C was measured and the mean growth on 50 µg/ml was expressed as a percentage of that made on 0 µg/ml metalaxyl. Isolates showing growth relative to the control of 0 - 20% were classed as sensitive, S, of 20 - 40% were classed as intermediate, I, and of 40 - >100% as resistant, R.

For molecular characterisation, cultures were grown in plates of Pea-water broth to generate approximately 150 mg of dried mycelium which was used for DNA extraction. Mitochondrial haplotypes were determined by the PCR method (Griffith and Shaw, 1998) but using an annealing temperature of 62°C. The determination of RG57 genotype was carried out using the protocol of Pipe and Shaw (1997) as modified by Nagy *et al.,* (2006).

Results

Mating type

Many of the 70 samples provided by Blight Scouts came from commercial fields from the main potato-growing areas of West Midlands and East Anglia. A1 was detected at 47 sites (67.2%) and A2 at 23 sites (32.8%).

More than one (average 6.5, minimum 1 and maximum 16) isolate was made from most of the 29 miscellaneous sites. These were predominantly from the west of England and Wales (Figure 1A). A1 only was detected at 14 sites (48.2%), A2 only at 9 (31.0%) and both mating types were detected at 6 sites (20.6%). In total, of the 99 sites sampled, A2 was detected at 38 (38.3%).

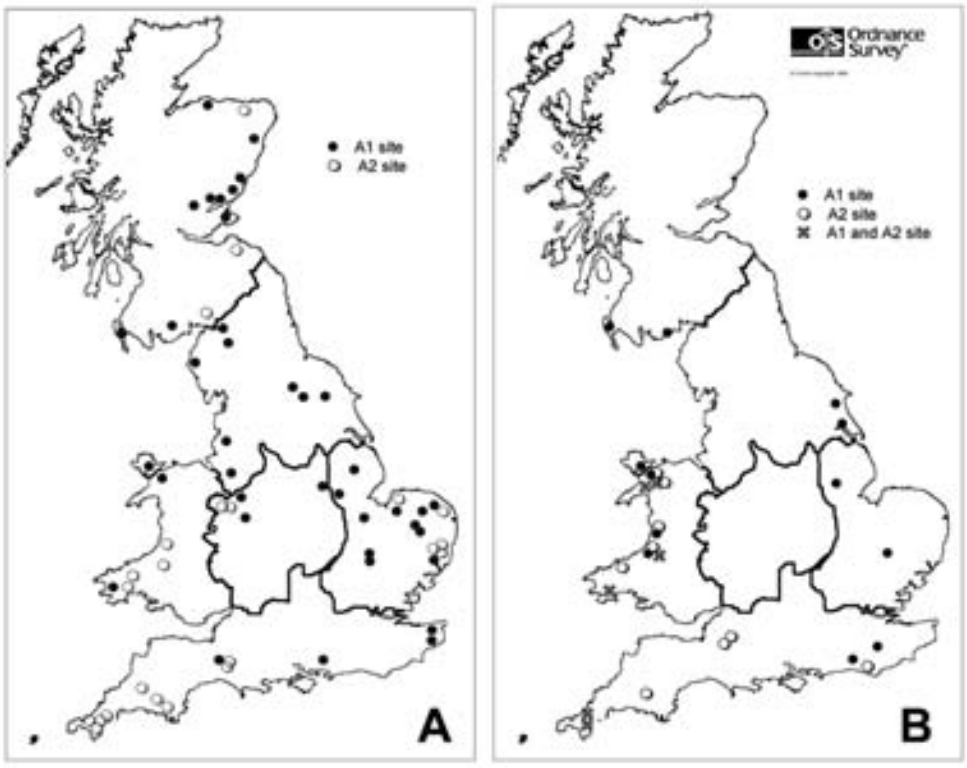

Figure 1. A: sites of isolates collected by Blight Scouts and subsequently fingerprinted. B: sites of miscellaneous isolates.

Characterisation of isolates

Most of the isolates collected by Blight Scouts were characterised for RG57 fingerprint, mitochondrial DNA haplotype and metalaxyl sensitivity (Figure 1A). From the miscellaneous sites, one isolate was characterised when a single mating type was detected and one of each mating type was chosen when both mating types were detected at one site. Results are shown in Table 1.

From a total of 88 isolates tested for RG57, 40 genotypes were detected. The 50 isolates of A1 mating type had 25 different fingerprints and the 38 isolates of A2 mating type had 17 different fingerprints. A large proportion of the A1 isolates (19 of 50) had the same fingerprint, referred to as RF006 in Table 1. Other A1 fingerprints were much less frequent (one new fingerprint was represented by five isolates, RF042 by three isolates and RF008 by two isolates) and most fingerprints were represented by a single isolate. More of the A2 isolates shared a fingerprint: seven fingerprints were each represented by more than one isolate. The eight isolates of the fingerprint labelled green were distributed in the west of Great Britain whereas the seven isolates of the blue genotype clustered in the east. One fingerprint was shared by three A2 isolates and one A1 isolate and another by one A1 and one A2 isolate. Nine A2 fingerprints were represented by a single isolate.

Fifty-four isolates were of mtDNA haplotype Ia and 34 isolates were of haplotype IIa. More of the A1 fingerprints were Ia haplotype (20 of 25) than the A2 fingerprints (10 of 17). A large proportion (19 of 24) of the A1 isolates of haplotype IIa had the common fingerprint RF006.

The ratio of isolates resistant:intermediate:sensitive to metalaxyl was 44:22:21. A1 isolates had a ratio of 20:17:12 and A2 isolates 24:5:9. Twenty-five of the 40 fingerprints had one or more isolates

resistant to metalaxyl. The 19 common RF006 IIa isolates had a ratio of 3:11:5. All seven isolates of the A2 blue genotype were resistant to the fungicide. Resistant isolates were found in 19 of the 28 Ia haplotype genotypes but in only five of the 12 IIa haplotype genotypes.

Table 1. Origins and characteristics of isolates of *P. infestans* collected in Great Britain in 2005.

Type[1]	Fingerprint[2]	mtDNA haplotype	Metalaxyl sensitivity[3]	No. of isolates	Type of site[4]	Region[5]
Isolates of identical fingerprint and mating type A1						
new	1,2,3,5,6,7,10,13,14,16,20,21,22,24,25	Ia	R	1	C	N
RF097	1,2,3,5,6,7,8,10,13,14,16,20,21,24,25	Ia	I	1	C	E
RF042	1,2,3,5,7,10,13,14,16,20,21,22,24,25	Ia	I,R,S	3	O	W
new	1,2,3,5,7,10,13,14,16,20,22,24,25	Ia	I	1	U	N
new	1,2,5,10,13,14,16,17,20,22,24,25	Ia	R	1	O	S
new	1,2,5,10,13,14,16,20,22,24,25	Ia	I	1	O	S
new	1,2,5,13,14,17,20,22,24,25	Ia	S	1	O	S
new	1,2,5,8,10,13,14,20,22,24,25	Ia	R	1	C	Sc
new	1,2,5,9,10,13,14,16,20,21,22,24,25	IIa	R	1	C	N
new	1,2,8,10,13,14,16,17,20,21,22,23,24,25	Ia	R	1	C	E
new	1,3,5,6,7,8,10,13,14,16,20,21,24,25	Ia	R	1	C	M
new	1,3,5,6,7,8,9,10,13,14,16,17,20,21,22,24,25	Ia	R	1	C	M
new	1,3,5,6,7,8,9,10,13,14,16,20,21,24,25	Ia	R (4), S	5	C (4), V	S (2), E (2), M
new	1,3,5,6,7,8,9,10,13,14,17,20,21,24,25	Ia	S	1	C	E
new	1,3,5,6,7,8,9,10,13,14,20,21,24,25	Ia	R	1	C	Sc
new	1,3,5,6,7,9,10,13,14,16,17,20,21,25	Ia	R	1	U	S
RF018	1,3,5,6,7,9,10,13,14,16,20,21,24,25	Ia	R	1	C	S
new	1,3,5,7,9,10,13,14,16,19,20,21,24,25	Ia	R	1	O	W
RF008	1,5,10,13,14,16,20,21,24,25	IIa	S, I	2	C	Sc
new	1,5,13,14,17,20,21,24,25	IIa	S	1	O	E
new	1,5,6,9,10,13,14,17,20,21,24,25	Ia	not tested	1	O	W
RF006	1,5,9,10,13,14,16,20,21,24,25	IIa	I (11), S (5), R (3)	19	C (10), U (5), O (4)	E (7), N (3), Sc (4), W (3), S (2)
new	1,5,9,10,13,17,20,21,24,25	IIa	S	1	C	Sc
Isolates of identical fingerprint and mating type A2						
red	1,2,3,5,7,10,13,14,17,19,20,21,22,24,25	IIa	S	1	O	M
yellow	1,2,3,5,7,9,10,13,14,17,19,20,21,22,24,25	IIa	S	3	O, C	M, W, S
new	1,2,3,5,7,9,10,13,14,17,20,21,22,24,25	IIa	R	1	C	W
new	1,2,3,5,7,9,10,13,14,17,20,21,24,25	IIa	I, R	2	O, U	W, S
new	1,2,5,10,13,14,16,17,20,21,24,25	Ia	I	1	O	S
new	1,2,8,10,13,14,16,17,20,21,22,24,25	Ia	R	1	O	W
blue	1,2,8,10,13,14,17,19,20,21,22,24,25	Ia	R	7	C	E (4), S (2), Sc
new	1,3,5,7,10,13,14,16,17,20,21,24,25	Ia	S, R	2	O, U	W, S
green	1,3,5,7,10,13,14,17,19,20,21,22,24,25	Ia	R (5), I (2), S	8	C (6), O, U	S (6), W (2)
new	1,3,5,7,10,13,14,17,20,21,24,25	Ia	R	3	O	W

new	1,3,5,7,10,13,14,19,20,21,22,24,25	IIa	S	1	C	E
new	1,3,5,7,10,13,14,20,21,22,24,25	Ia	R	1	O	W
new	1,5,10,13,14,17,19,20,21,22,24,25	IIa	S	1	C	N
new	1,5,10,13,14,17,20,21,24	Ia	S	1	C	W
new	1,5,9,10,13,14,16,20,21,22,24,25	IIa	R	1	C	N
Isolates with identical fingerprint but different mating type						
RF064	1,2,3,5,7,10,13,14,16,20,21,24,25[β]	Ia	I	1	U	W
RF064	1,2,3,5,7,10,13,14,16,20,21,24,25[β]	Ia	R	1	U	N
new	1,3,5,7,10,13,14,17,19,20,21,24,25[β]	Ia	R	1	O	W
new	1,3,5,7,10,13,14,17,19,20,21,24,25[β]	Ia	R (2), I	3	O (2), U	W (2), S

[1]Fingerprints designated with Restriction Fragment (RF) number were detected in GB in 1990s and are labelled as in Day et al. (2004).

[2]Band 4 was excluded from the analysis.

[3]R: resistant, I: intermediate, S: sensitive. Numbers in brackets are numbers of isolates.

[4]C: conventional, O: organic, U: unknown. Conventional crop was protected with various fungicides. Organic crop here means any crop where late blight was unprotected or protected with copper fungicide. This category also includes isolates from volunteers or dumps.

[5]E: Eastern England, N: Northern England, S: Southern England, Sc: Scotland, W: Wales. Borders of regions are highlighted on Figure 1. Numbers in brackets are numbers of isolates.

[α]A1 mating type.

[β]A2 mating type.

Discussion

In Great Britain, 2005 was moderately favourable for the development of blight in potato growing areas and as usual more favourable in the wetter west of the country. A2 was detected at just over 30% of the single-isolate sites and at just over half of the miscellaneous sites represented by a variable number of isolates. A higher proportion of A2 in the sites more intensively sampled is to be expected; undoubtedly more A2 isolates would have been detected if more isolates had been taken from the single-isolate sites. Both mating types were detected at one quarter of the 24 sites represented by two or more isolates, all in Wales or S.W. England. These results indicate a substantial increase in A2 mating type over that detected in previous years. It is possible that both mating types were present in most, if not all, crops. If oospores are formed in foliage and have the ability to remain viable in the soil, there would be an increased risk of survival of *P. infestans* over winter.

Characterisation of these isolates indicated a substantial change in variation in the population since it was last studied with the same markers in the 1990s. At that time, A2 mating type was not frequent in the pathogen population and clonal lineages dominated the population with three genotypes of A1 being common. Fingerprint RF039, mt haplotype Ia and variable sensitivity to metalaxyl was found at more than half of all sites, RF 002, Ia, metalaxyl resistant at more than one third of sites and RF006, IIa, mainly metalaxyl sensitive at one in seven sites. A2 isolates were predominantly monomorphic, being RF040, Ia and never fully resistant to metalaxyl (Day *et al.*, 2004).

Within the 88 isolates characterised, the commonest variant, occurring at 19 sites was an A1 genotype with fingerprint identical to RF006 which was relatively common in the 1990s. Four other variants (RF008, RF018, RF042 and RF097) were identical to old variants and could have been asexual descendants of clonal lineages of the 1990s.

The most surprising finding was that A2 isolates had become much more polymorphic being represented by 17 (including the two which had both A1 and A2 isolates) fingerprints. The predominant A2 clonal lineage RF040, Ia, from the 1990s was not detected but one isolate, red fingerprint in Table 1,

differed from RF040 in having band 17 instead of 16 and another three isolates, yellow in Table 1, was identical to the red fingerprint except that it possessed band 9. Both isolates were mtDNA haplotype IIa and not Ia as in RF040. This suggests that genotypes red and yellow might be related and belong to the same or related clonal lineages, derived from the old A2 lineage. Supporting this hypothesis is the fact that the yellow genotype was recovered from the same postcode area as the red genotype. The other A2 isolates belonged to one of 17 genotypes only one of which had been detected before (RF064).

One of the new genotypes (green in Table 1) was present at eight sites. This could be a new clonal lineage or could be related to RF040 to which it is identical but for the absence of band 2. All the isolates of this genotype had the mtDNA haplotype (Ia) also found in the old RF040 clonal lineage. Isolates of this green genotype appear to be distributed to the west of GB (six isolates in Cornwall/ Devon and two isolates in SW Wales).

A clonal lineage with a quite distinct and new fingerprint (blue in Table 1) occurred at seven sites with a cluster in East Anglia, the main potato-growing area in Great Britain. All isolates with this fingerprint were mt haplotype Ia and were metalaxyl resistant. It remains to be seen if this clone is invariably resistant to metalaxyl. This is in contrast to A2 isolates from the 1990s which were never fully resistant to metalaxyl. The blue clone was detected only late in the season (late July and August).

Two fingerprints were represented by both A1 and A2 isolates. This phenomenon was recorded for certain genotypes in the 1990s (Day *et al.,* 2004) and may indicate recombination.

Nineteen A1 and nine A2 genotypes were detected at one site only. These could be rare clonal lineages whose frequency is decreasing in the population or could be emerging lineages whose frequency is increasing. They may have arisen from sexual recombination. Alternatively isolates with these genotypes could be migrants carried from continental Europe in seed tubers.

The increase in the frequency of the A2 mating type detected here is consistent with the increase that has taken place in some other European countries. The increase in The Netherlands and in the Nordic countries took place in the 1990s (e.g. Drenth *et al.,* 1993; Hermansen *et al.,* 2000). In contrast, the increase in N. France was, as in GB, detected in 2005 (Détourné *et al.,* 2006). In N. France, the A2 mating type was detected in 6% of fields in 2003, 20% in 2004 and 37% in 2005. This increase was accompanied by an increase in resistance to metalaxyl and resistance was detected even where little or no application of metalaxyl had been made. Outbreaks were earlier and more severe in 2005 than in the last 20 years and were more difficult to control. The most frequent phenotype characterised was A2 and metalaxyl resistant. It is not known if isolates of this phenotype belonged to one clonal lineage or consisted of a range of different genotypes. Recent surveys in Hungary (Nagy *et al.,* 2006) have detected roughly equal frequencies of each mating type and a high level of variation such that almost all molecular genotypes detected were unique to a single site.

Cooke *et al.* (this volume) have charted the continued rapid evolution of *P. infestans* in Great Britain in 2006 and have provided confirmation of the lineages found here using SSR markers. It remains to be seen if the newly selected genotypes are more damaging of the potato crop or are able to cause disease in formerly resistant cultivars.

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Survey of GB Blight Populations

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Summary
Analysis of samples of late blight collected via the British Potato Council Fight Against Blight (BPC FAB) campaign suggested that the GB population of *Phytophthora infestans* was changing. The presence of the A2 mating type in up to 38% of outbreaks in 2005 raised a concern about oospore production and the BPC thus funded a project to conduct more detailed pathogen monitoring. In this survey the scouts collected up to eight samples per reported blight outbreak and these were subject to genetic analysis using Simple Sequence Repeat (SSR) markers to build a picture of the population structure and assist in determining the source of the primary inoculum. In 2006, 165 outbreaks across GB were sampled and the A2 mating type was present in 65% of these. Targeting of specific outbreaks with clearly defined primary foci by so-called 'superscouts' provided detailed data and up to 32 lesions per outbreak. In combination with the DNA fingerprint data of isolates an assessment of the source of infection was made with a particular emphasis on any role of oospores. However, none of the eight 'superscout' outbreaks had a signature typical of oospore infection. Detailed analysis of the 899 isolates genotyped from all 165 outbreaks also suggested an absence of oospore derived outbreaks as almost 90% of population was represented by only 8 genotypes of *P. infestans*. The widespread involvement of oospores would have generated a considerably more diverse pathogen population. Genotypic analysis also indicated that the marked rise in the A2 type frequency was largely down to an increase in genotype 13_A2 to 41.3% of the population. Analysis of *P. infestans* isolates from the previous three years suggested this genotype arrived late in 2005 crops but rapidly established itself during the 2006 season. To study the threats posed by oospores, work is underway in trial plots of potatoes in polythene tunnels that are being cropped twice a year after generating blight epidemics with combinations of A1 and A2 genotypes commonly found in GB.

Introduction
Primary inoculum carried over from one season to the next is the source of all late blight outbreaks and the avoidance of such sources of *Phytophthora infestans* by using healthy seed and eliminating infected outgrade piles and groundkeepers forms an important part of any management strategy. The

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oospore or sexual stage of *P. infestans* however, represents a potentially more serious and generally less familiar threat to the grower. The implications of oospore production are two-fold. Firstly, soil-borne oospores are an additional and potentially more damaging reservoir of long-lived primary inoculum, increasing the likelihood of crop infection. Oospore-borne infections have been reported to occur earlier in the season (Andersson *et al.*, 1998, Hannukkala *et al.*, 2002) and result in infections in the lower part of the canopy that are more difficult to identify early and treat with fungicides. Secondly, oospores are the result of sexual recombination which may accelerate the rate of pathogen evolution.

Up until the 1980s, the A2 mating type was not found in Europe and despite its presence in Great Britain (England, Wales and Scotland) for around 20 years it has remained at very low levels (e.g. Cooke *et al.*, 2003; Day *et al.*, 2004). BPC-funded reporting of blight outbreaks however, revealed an increase in the proportion of A2 isolates with its presence in up to 38% of sampled outbreaks in 2005 (Shaw *et al.*, 2007). Further examination of these A2 mating type isolates using restriction fragment length polymorphism (RFLP) analysis with the RG57 probe indicated the presence of several RG57-types not recorded in the 1995-1998 GB survey (Day *et al.*, 2004; Shaw *et al.*, 2007). The three most common A2 genotypes characterised in 2005 were labelled blue (7 isolates), green (8 isolates) and yellow (3 isolates) (Shaw *et al.*, 2007).

Genetic analysis tools that allow a low-cost but high-throughput and objective view of the genetic structure of the *P. infestans* populations are now available (Cooke & Lees, 2004; Lees *et al.*, 2006). Such markers, in parallel with more detailed sampling were used to objectively examine the changing population and the threat of oospores to the GB industry in 2006. Comparisons were made with populations sampled from other European countries to determine the population structure on a European scale (Cooke *et al.*, 2007).

Materials and Methods

Outbreaks of blight in GB were sampled, primarily by the BPC's network of volunteer blight scouts. In most cases, up to 8 lesions per outbreak were sent to CSL for confirmation and creation of a blight incident report via the BPC FAB service (see www.potato.org.uk/blight). After placing each sample in a small potato tuber the samples were sent to SCRI for isolation onto agar and further analysis. After mating type testing isolates were grown on pea broth and a small 2mm^3 fragment of freeze-dried mycelium was subject to DNA extraction using a 'Quick and Easy' protocol (submitted to the Eucablight web site (www.eucablight.org) modified from Wang and Cutler (1993). The DNA (1 µl) was subsequently subject to SSR analysis with the previously published primers (Lees *et al.*, 2006) using methods optimised for the ABI3730 48 capillary system. The 11 SSR markers were carefully designed with different fluorescent labels and a minimum overlap in size range across the three panels of multiplexed markers.

An in-depth study of outbreaks in a very early stage of disease development was conducted by 'superscouts' who provided detailed observations of disease in the canopy and up to 32 lesions per outbreak. These samples were sent directly to SCRI and processed as described above.

Additional studies at SRT are aimed at examining the production and survival of oospores. Crops of Maris Piper and Bintje were established in polythene tunnels and infected with A1 and A2 isolates selected from the 2006 *P. infestans* population. Oospore production was monitored in leaves throughout the epidemic, soil samples have been collected and subsequent crops will be inspected for any oospore-borne infection. Isolates will be collected and genotyped at SCRI to determine whether

they represent asexual (parental) or sexual (progeny) strains. The second crop will be planted in autumn 2007 once the risk of blight from surrounding crops has diminished.

Results

Over the course of the 2006 season, 161 FAB blight outbreaks were processed by CSL with 142 of these proving positive for blight. An additional 29 outbreak samples were sent to SCRI and included 8 'superscout' samples, Scottish Agricultural Science Agency (SASA) seed inspector samples and miscellaneous samples from wild solanaceous weeds or garden samples of potato or tomato blight. A total of 1290 isolations were attempted at SCRI yielding 1099 isolates from 165 blight outbreaks from across GB.

The mating type data may either be presented as the percentage of the total number of isolates or broken down and examined per disease outbreak. Since a major objective of this project was to assess the risks of oospore formation that would occur if both mating types are present in the crop, this report focuses on the data per outbreak. The raw mating type data and breakdown according to outbreak class is shown in Tables 1 and 2. In total, 54.2% of the isolates were of mating type A2 with the A2 mating type found in 108 of the 165 outbreaks (65.5%).

Table 1. The numbers and percentages *P. infestans* isolates of each mating type collected during the 2006 season in GB (n= 1014).

Mating type of isolates	Total
A1	464
A2	550
Total	1014
%A1	45.8
%A2	54.2

Table 2. The number and percentage of GB blight outbreaks sampled in 2006 categorised according to the *P. infestans* mating types present.

Outbreak type	Total
A2 only	72
A1 only	57
Mixed	36
Total outbreaks	165
Total with A2 present	108
% outbreaks with A2	65.5

The 'superscouts' sampled eight outbreaks from early June to mid July. Seven of these yielded *P. infestans* isolates of only the A1 (5) or A2 (2) mating type and in each case only a single genotype of each (Table 3). A detailed breakdown of isolates from the four small disease foci in one outbreak showed three were A1-only and the other A2-only. Examination of the data provided by the scouts on, for example the extent of the outbreak, position of lesions in the canopy and evidence of seed tuber infection was combined with evidence from the SSR genotyping and it was concluded that oospores were not implicated in any of the eight outbreaks. The signature of an oospore-derived outbreak would be severe infection in the lower part of the canopy consistent with a below-ground source combined with a mixture of A1 and A2 mating types and unique SSR allele combinations. The right-hand columns of Table 3 indicate that, in each case, the isolates recovered were of clonal

genotypes of *P. infestans* representing between 1 and 41% of the GB population in 2006. For example in outbreak SS2 all 28 isolates were of an A1 genotype, termed 7_A1 that made up 6% of the GB population. The commonly recovered A2 genotype 13 was found in three of the eight outbreaks.

Table 3. Details of the eight GB outbreaks sampled by the superscouts in 2006 showing the mating type and SSR genotype information for the *P. infestans* isolates recovered.

SCRI code	BPC no.	Postcode	Type	Variety	Created	No. samples	No. isolates	A1	A2	Geno types	% of 2006 Pop.
06_SS1	3900	PE34	Single Plant	Maris Piper	05 Jun 2006	6	5	5	0	6_A1	7
06_SS2	3896	IP12	Several Patches	Maris Peer	05 Jun 2006	32	28	28	0	7_A1	6
06_SS3	3988	CT7	Several Patches	Desiree	08 Jun 2006	18	15	11	4	A1/13	10/41
06_SS4	3956	TF6	Patch (1m2)	Unknown	07 Jun 2006	24	23	23	0	8_A1	11
06_SS5	4160	NR16	Patch (1m2)	Unknown	27 Jun 2006	14	9	0	8	13_A2	41
06_SS6	4212	NR17	Patch (1m2)	Maris Piper	29 Jun 2006	20	17	0	17	13_A2	41
06_SS7	4336	AB53	Patch (1m2)	Marfona	18 Jul 2006	12	8	8	0	2_A1	6
06_SS8	4332	AB51	Patch (1m2)	Marfona	18 Jul 2006	18	13	13	0	18_A1	1

For each of 1055 isolates from the 2006 season, 11 SSR loci were PCR amplified and the allele(s) present were scored. The same procedure was completed for a total of 300 isolates from the 2003, 2004 and 2005 seasons. The combination of alleles at the 11 loci observed in a single isolate are used to define its genotype. The 2005 genotypes previously defined by RG57 and colour coded, blue yellow and green (Shaw *et al.*, 2007) were also clearly discriminated by the SSR markers (See Table 4).

High quality genotype data was generated for 1184 isolates and these were sorted in an Microsoft XL spreadsheet revealing 19 genotype categories named arbitrarily using a number and mating type (e.g. 2_A1). An additional category of genotype termed 'miscellaneous' was defined for combinations of alleles that were found at a very low frequency and commonly in only a single blight outbreak. The overall numbers and frequencies of all the different genotypes in each of the four seasons are shown

In 2006, the 899 isolates examined indicated that 96.2% of the isolates belonged to *P. infestans* lineages (genotypes) found in multiple outbreaks and, for the most part, also found in previous years. A single A2 genotype accounted for the marked increase in the A2 mating type. This genotype, termed 13_A2, comprised the greatest single component of the 2006 population and at 41.3% was four times more frequent than the second commonest type (8_A1 RF06). Unlike many of the other genotypes, the 13_A2 genotype was not sampled in GB outbreaks prior to 2005. In 2005 it comprised 12.3% of the population (n=73) but was found relatively late in the season from mid July to late August. The scale of the increase in genotype 13_A2 is not matched by any other genotype over the four seasons and, in general, it appears that the increase in 13_A2 has been at the expense of several A1 lineages (e.g. 12_A1, 5_A1, 7_A1 & 2_A1). The miscellaneous category has not shown any consistent increase over the course of the four seasons.

Discussion

Considerable progress has been made on our understanding of the changing GB *P. infestans* populations. The upward trend in the frequency of the A2 mating type has continued; it was found in over 65% of GB outbreaks in 2006. As discussed by Cooke & Lees (2004) the use of SSR genetic fingerprinting in Table 4.

Table 4. Results of SSR genotyping *P. infestans* isolates recovered from GB outbreaks over each of four years. Isolate numbers and the percentage of each years sample falling into each genotype class are presented with the A2 and miscellaneous categories shown in the lower half of the table. Where possible, reference is made to genotypes defined by other means in other studies (1= Day et al., (2004) 2= Shaw et al (2006)).

SSR genotype	Other genotype name	2003		2004		2005		2006	
		No.	%	No.	%	No.	%	No.	%
21_A1		0	0.0	3	2.2	0	0.0	0	0.0
4_A1		2	2.7	10	7.2	0	0.0	2	0.2
12_A1		3	4.1	6	4.3	5	6.8	6	0.7
20_A1		0	0.0	0	0.0	0	0.0	6	0.7
19_A1		0	0.0	0	0.0	0	0.0	6	0.7
5_A1	RF2[1]	9	12.2	5	3.6	4	5.5	8	0.9
18_A1		0	0.0	0	0.0	0	0.0	13	1.4
7_A1		5	6.8	7	5.1	10	13.7	56	6.2
2_A1	RF39[1]	22	29.7	54	39.1	13	17.8	58	6.5
6_A1		0	0.0	5	3.6	2	2.7	62	6.9
1_A1		2	2.7	10	7.2	0	0.0	89	9.9
8_A1	RF6[1]	23	31.1	19	13.8	10	13.7	103	11.5
17_A2		0	0.0	0	0.0	0	0.0	8	0.9
16_A2		0	0.0	0	0.0	0	0.0	13	1.4
13_A2	Blue[2]	0	0.0	0	0.0	9	12.3	371	41.3
10_A2	Yellow[2]	0	0.0	4	2.9	3	4.1	31	3.4
15_A2		0	0.0	2	1.4	0	0.0	0	0.0
22_A2	RF40[1]	1	1.4	1	0.7	0	0.0	0	0.0
3_A2	Green[2]	4	5.4	7	5.1	13	17.8	33	3.7
Misc.		3	4.1	5	3.6	4	5.5	34	3.8
Total		74	100	138	100	73	100	899	100

has proved particularly valuable in understanding the nature of this changing population and in investigating the role of oospores as primary inoculum. A single A2 genotype has rapidly increased in frequency over the 2006 season but, as yet, oospores do not appear to be making a significant contribution to GB blight epidemics. Studies are underway to examine the generation and impact of oospores in more detail. Further work has also been commissioned to extend our knowledge of the aggressiveness of GB *P. infestans* genotypes.

The increase in the frequency of the 13_A2 genotype from 12 to 41% of the GB population in a single year has been dramatic. Fingerprinting of isolates from the FAB campaign from 2003, 2004 and 2005 showed that other A2 genotypes that have been present in GB crops for 3-4 years decreased in frequency over the same period. This obviously raises many questions in terms of the origins of the new genotype, whether it is harder to control and whether it is combining with A1 isolates to generate sexual oospores.

Although the A2 type has been present in GB since the 1980s, surveys in 1995-1998 indicated a frequency of 3% across GB (Day *et al.*, 2004) and 19% in Scotland (Cooke *et al.*, 2003). In Scotland, subsequent monitoring in 2003-4 has shown a drop in A2 to less than 1% (D. Cooke unpublished

data). In other studies at SCRI a bridge between contemporary and past survey data is being made by examining isolates from previous surveys with the new SSR markers (Lees *et al.*, 2006) used in this study. It was thus shown that the A2 genotype most commonly found in 1995-1998, named RF040 by Day *et al.*, (2004) and known in this study as genotype 22_A2, was found only rarely amongst FAB isolates in 2003 and 2004 and never in 2005 and 2006. This A2 lineage thus no longer plays a significant role in GB blight epidemics. Similarly, an A1 genotype (RF039 equivalent to 2_A1 in this study) that comprised 46% of the 1995-8 GB population (Day *et al.*, 2004) has decreased to only 6.5% of the 2006 population.

Little is known about the origins of the 13_A2 genotype. The only other isolates known to match it in genotype were found in the starch growing region of the Netherlands in 2004 (D. Cooke, unpublished data). Other publications from the Netherlands and France have reported significant increases in the frequency of the A2 mating type (van Raaij *et al.*, 2007; Detourne *et al.*, 2007) but in neither of these cases is genotype data available to confirm whether the 13_A2 genotype is responsible. In GB it was first recorded relatively late in the 2005 season in several counties (Norfolk, Suffolk, Somerset and the Scottish Borders). This widespread distribution suggests it was present earlier in the season but was not detected by the BPC FAB sampling regime. Imported seed or windborne sporangia are the most likely origins of this strain.

The question of whether oospores are contributing to GB blight outbreaks as a source of primary inoculum was also addressed. Indirect evidence was gathered by detailed scouting and genetic analysis of the *P. infestans* isolates. Despite the threats that oospores pose, few conclusive examples of their role in initiating blight have been published. Studies of 20 blight foci in Finnish crops planted in land with blight infected crops in at least one of the previous four years provided strong circumstantial evidence of oospore inoculum. All primary infections involved lower leaves in contact with the soil, in every case A1 and A2 isolates were isolated from such primary infections and soil bioassays confirmed the presence of overwintering soil-borne inoculum (Lehtinen & Hannukkala, 2004). Six discrete foci in a blight outbreak in a Swedish field were examined and 68 isolates of *P. infestans* recovered. Analysis of the mating type, mitochondrial DNA and SSR fingerprinting indicated a diverse mix of genotypes of both mating types and strongly suggested that oospores were the source of the infection (Widmark *et al.*, 2007). In this GB survey data from the eight outbreaks sampled by experienced 'superscouts' led us to conclude that oospores did not cause any of the sampled outbreaks. Although less rigorous, an analysis of the description and eight lesions provided by the standard scouts was also valuable. In no outbreak was a mix of both A1 and A2 isolates and genotypes such as those presented by Widmark *et al.,* (2007) demonstrated. If oospores are present in GB soils then it is either at a very low frequency and not picked up by the intensity of sampling in this project or it could be that any contaminated land has yet to come back into potato production. In a typical GB rotation ware potatoes are planted every four to 5 years. Since the marked increase in A2 was noted over the 2005-6 seasons (Table 4) monitoring over the 2009-2010 seasons may be the most appropriate time to monitor the impact of any soil-borne oospores on the timing and severity of epidemics.

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Genetic variability of *Phytophthora infestans* in Nordic countries

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Abstract
Phytophthora infestans was isolated from potato leaves collected from 200 fields located in different parts of Finland, Denmark, Sweden and Norway in 2003. Sampling was carried out relatively early in the epidemic. The SSR analysis was carried out in Norway. Nine SSR markers were tested; Pi4B, Pi4G, PiG11, Pi02, Pi04, Pi16, Pi26, Pi33 and D13. Based on only 7 SSR markers 190 genotypes were found. Six genotypes occurred twice and one genotype occurred six times. The high genetic variation indicates sexual reproduction in the Nordic late blight population.

Keywords
Phytophthora infestans, SSR analysis, genetic variation

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Phytophthora infestans population in Poland

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Summary
The isolates of *Phytophthora infestans* collected in Poland were characterised by mating type, virulence, resistance to the fungicide metalaxyl, mitochondrial DNA haplotype and Simple Sequence Repeats markers. In a group of 125 *P. infestans* isolates, collected in 2005 and 2006, 83 were of A2 mating type and 42 of A1. The average number of virulence factors per isolate was 8.0 in a group of 97 *P. infestans* isolates from 2006 and 7.4 in a group of 72 isolates from 2005. Virulence for genes *R*1, *R*3, *R*4, *R*7, *R*10 and *R*11 was observed with high frequency (above 88%), the least frequent virulence was observed for genes *R*8 and *R*9. Simple races and complex ones possessing two or three virulence factors were not found. Majority of isolates, 73.3 %, collected both from protected and unprotected potato fields in 2005 and 2006, were sensitive to metalaxyl. Two mitochondrial DNA haplotypes were detected, Ia and IIa, with predominance of Ia isolates in a group of 74 isolates from 2005 (eight isolates were collected in 1997-2004). Considerable genotypic diversity was observed. All 10 applied SSR markers were polymorphic in a group of 88 *P. infestans* isolates tested. From two to 12 alleles for individual markers were determined, which in combinations allowed to discriminate 55 genotypes among 90 tested ones. Five *P. infestans* isolates differing in mating type, metalaxyl resistance, mitochondrial haplotype and genotype based on SSR analysis were assessed for their aggressiveness. There were small differences among isolates for disease parameters evaluated during the assessment of aggressiveness. Mean rAUDPC on tested leaflets under lab condition was 0.303, ranged from 0.242 to 0.338. Mean infection frequency was 99.6%, average for mean latent period was 3.9 days, sporulation intensity ranged from 7700 to 34400 sporangia /cm^2 of infected area.

Keywords
Mating type, metalaxyl resistance, virulence, SSR markers, mitochondrial DNA haplotype, aggressiveness

Introduction
Phytophthora infestans is most destructive pathogen of potato and tomato crop worldwide. European climate favours the disease development in most years and then chemical control is necessary for the protection of the crop. For the last thirty years great changes in *P. infestans* population have been observed. New isolates belonging to both mating types were introduced to Europe and replaced so

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called "old population" representing by A1 mating type lineages (Ristaino *et al.*, 2001; Fry *et al.*, 1992). Control strategies rely both on agronomic practices and chemical control. Information about the pathogen, for example if it is resistant to certain fungicides, if both mating types are present, which virulence factors are common, helps to find the most effective strategy to control the disease.

Materials & Methods

Isolation of *P. infestans* pure cultures
The pathogen was isolated from single lesions on potato leaflets using the procedure described by Śliwka *et al.* (2006).

Mating type determination
The mating type was determined by crossing the assessed isolates with A1 (MP 503) and A2 (US-8 isolate kindly supplied by W. Fry) isolates on rye A agar medium with addition of 40 mg/l of β-sitosterol (Spielman *et al.,* 1990) and observing the oospore formation.

Virulence evaluation
To define the virulence of isolates we used set of 11 Black's differentials, each possessing a single *R*-gene (*R1-R11*) from *Solanum demissum* in detached leaflet assay (Zarzycka, 2001b).This set was offered by SASA, Edinburgh.

M**etalaxyl resistance**
Metalaxyl resistance was tested by measuring diameters of *P. infestans* cultures growing on rye A medium (control) or rye A media amended with metalaxyl (Metalaxyl-M, Syngenta Crop Protection) at final concentrations of 5 or 100 mg/l. Isolates were classified as sensitive, when diameters of cultures on media with both 5 and 100 mg/l of metalaxyl were smaller than 40% of the control. Cultures of intermediate isolates achieved more that 40% of the control size on 5 mg/l medium and less than 40% of the control size, when growing on the medium containing 100 mg/l of metalaxyl. Resistant isolates extended 40% of the control culture diameter on both media with metalaxyl (Bakonyi *et al.,* 2002; Pérez *et al.* 2001; Daggett *et al.,* 1993). Standard isolates for each class of metalaxyl resistance reaction were applied along with group of tested isolates.

Mitochondrial haplotype
Mycelia of *P. infestans* isolates collected from rye liquid medium were rinsed in sterile water and lyophilized. DNA was extracted using DNeasy Plant Mini Kit of Qiagen. Mitochondrial haplotype was detected according to the method described by Griffith and Shaw (1998). Two standard isolates of Ib mtDNA haplotype, representing the "old population", were kindly provided by Dr. Flier, The Netherlands.

Simple Sequence Repeats
In frame of Eucablight project *P. infestans* isolates were characterized with use of Simple Sequence Repeats (SSR) markers in Scottish Crop Research Institute, Invergowrie, Dundee, Great Britain. 56 isolates collected in 2005 from 17 locations, 28 isolates collected in 2004 from 7 sites and 4 isolates collected in 2000-2004 were tested with SSR markers. DNA was extracted either from fresh or from lyophilized *P. infestans* mycelium. PCR reactions were conducted with use of 12 SSR markers (Pi02, Pi89, Pi4B, G11, Pi04, Pi70, Pi56, Pi63, D13, Pi16, Pi33, Pi66). PCR primers were fluorescently labelled and particular SSR alleles were distinguished by the use of capillary electrophoresis (ABI Prism 3100 DNA Sequencer) and analyzed by GeneScan 3.7 NT and Genotyper 3.7 NT software package (PE Applied Biosystems) (Lees *et al.,* 2006).

Aggressiveness

Detached leaflets from susceptible to *P. infestans* cultivar Tarpan were used for evaluation of the aggressiveness of five isolates. The suspension was adjusted to 10^4 sporangia in 1ml. Fifty leaflets per isolate were inoculated by 10 l drop of suspension. The symptoms of infection were scored every 12 hours for the first three days and then every 24 hours, until 7 days. Following disease parameters were calculated: relative area under disease progress curve (rAUDPC), mean infection frequency (%), sporulation density (number of sporangia /1cm2 of infected area, counted for 10 leaflets) and latent period (days until sporulation appeared on 10% of leaflets).

Results

Mating type determination

In a group of 125 *P. infestans* isolates, collected in years 2005 and 2006, 83 were of A2 mating type and 42 of A1.

Virulence evaluation

All 11 virulence factors were found among isolates collected in Poland in 2005 and 2006 (Figure 1). Generally, virulence factors 5, 6 and 9 were relatively rare, but increase of virulence to gene R5 and R9 was observed for isolates collected in 2006. The frequencies of virulence against genes R1, R3, R4, R7, R10, R11 were high, above 88%. Complex races prevailed. For 72 isolates tested in 2005 and 97 tested in 2006, the average numbers of virulence factors per isolate were 7.4, and 8.0 respectively.

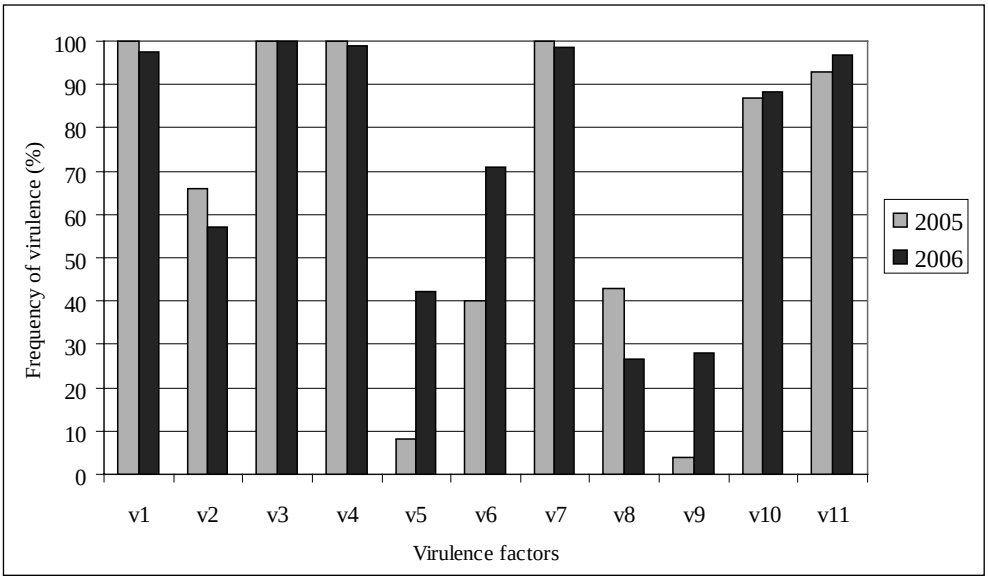

Figure 1. Virulence of P. infestans isolates collected in 2005 (n=72) and 2006 (n=97) in Poland.

Metalaxyl resistance

Out of 165 isolates tested, 73.3% were sensitive to metalaxyl, 19.3 were resistant and 7.3 % were intermediate. The isolates used as standards were classified to the resistance categories according to the expectations.

Mitochondrial haplotype
Two mitochondrial DNA haplotypes were detected, Ia and IIa, with predominance of Ia isolates in a group of 74 isolates collected mostly in 2005 (eight isolates were collected in years 1997-2004).

Simple Sequence Repeats
From two to 12 alleles for individual markers were determined using 10 SSR in a group of 88 *P. infestans* isolates. There were 55 different genotypes discriminated based on this analysis. The G11 marker allowed detecting 12 alleles and 24 genotypes among tested isolates.

Aggressiveness
Five *P. infestans* isolates differing in mating type, metalaxyl resistance, mitochondrial haplotype and genotype based on SSR analysis were assessed for their aggressiveness. Evaluated disease parameters are shown in Table 1.

Table 1. Components of aggressiveness of five different isolates of *P. infestans*

Name	Mean latent period (days)	Mean infection frequency (%)	rAUDPC	Sporulation density (sporangia x 103/1cm2)
MP 324	3.4	98	0.338	23.0
MP 618	4.0	100	0.336	34.0
MP 622	4.0	100	0.318	22.0
MP 650	4.0	100	0.284	8.0
MP 674	4.0	100	0.242	21.0
Average	3.9	99.6	0.303	21.6

Discussion
The increase of frequency of A2 mating type isolates was observed in Polish population. In the years 2005 and 2006 66% of isolates belonged to the A2 mating type. In a group of 217 isolates collected during five years (from 2001 to 2006) the ratio between A1 and A2 was close to 1 : 1, while in the previous five years, among 779 isolates, 72.6 % represented A1 mating type (Zarzycka *et al.,* 2002; Śliwka *et al.,* 2006).
The virulence of *P. infestans* isolates in Poland is complex. Most of virulence factors have been detected with a very high frequency but also the increasing presence of these factors of virulence (v5, v6, v9) which were in lowest frequency for years was observed. The Polish population of *P. infestans* is the most similar in frequency of virulence factors to Slovakian population and the less similar to the Irish population, in which factors 1, 2, 5, 6, 8, were found in very low frequency or factor 9 was not detected (www.eucablight.org). The average number of virulence factors per isolate was 8.0 in 2006 in Poland and it was higher than in other countries: Estonia (6.6), Norway (5.78), Finland (5.30) and France (4.8) (reviewed by Runno and Koppel, 2006).
Sensitive isolates collected from protected and unprotected fields prevailed in Polish population of *P. infestans*. The frequency of sensitive isolates was 73.3% for isolates collected in 2005 and 2006. Similar frequency of sensitive isolates (82.0%) was observed for isolates collected both from protected and unprotected fields in previous years by Śliwka *et al.* (2006). There were 17% of resistant isolates to metalaxyl in years 2005 and 2006, twice less than in studies conducted by Kapsa *et al.* (1999) and Kapsa (2001). From the isolates evaluated by Kapsa et al. (1999) 39.7% were resistant to metalaxyl, but these isolates were collected only from potato fields protected against late blight. In other countries (reviewed by Cooke *et al.*, 2003) the situation was similar, about 42% of isolates were resistant to metalaxyl in Scotland, 43% - in England and Wales in 1994, 56% - in Norway in 1996, 15-30% - in France between 1992 and 1996 and 48% in France and Switzerland in 1996 and 1997.
Two haplotypes of mitochondrial DNA were detected in a group of 74 isolates collected from 1997 to

2005. Ia predominated over IIa haplotype. Haplotypes Ib and IIb were not detected. The Ib haplotype represents "old" population of *P. infestans*, and 13 isolates of such haplotype were found among 63 Polish isolates collected in 1987-1991 (Gavino and Fry, 2002). The IIb haplotype might not been detected due to small sample size. The ratio among Ia and IIa haplotypes differs depending on the country. In some countries Ia haplotype dominated over IIa (Poland, England, Scotland, Wales, The Netherlands, France) but in some countries IIa dominated over Ia (Northern Ireland, Ireland, Finland, Hungary, Austria) (www.eucablight.org)

Analysis of 88 isolates revealed 55 different genotypes with use of 10 SSR markers, which showed high genetic diversity in the Polish population. The G11 marker allowed detecting 12 alleles and 24 genotypes among tested isolates.

The isolates which were selected for testing their aggressiveness differed for many other phenotypic and genotypic traits. The differences in disease parameters which were evaluated were rather small. They all were highly aggressive, what could be concluded based on the very high infection frequency, short latent period and abundant sporulation.

Conclusions

The Polish population of *P. infestans* comprise of isolates which are of complex races, in average composed of 9 virulence factors per one isolate. The sexual recombination can take place as the proportion of A1 to A2 mating type is near 1:1 for isolates collected during the last five years. Among 88 isolates 55 different genotypes were discriminated using 10 SSR markers, what shows high diversity of these isolates. Two mitochondrial haplotypes Ia and IIa were found among tested *P. infestans* isolates. Metalaxyl sensitive isolates predominates in Polish population of *P. infestans*. Five isolates selected for different phenotypic and genotypic traits were assessed for their aggressiveness and all were highly aggressive to potato leaves of susceptible cultivar Tarpan.

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Simulation of Potato Late Blight in the Netherlands: Validation of the BLIGHTSPACE Model Reveals Dichotomy in the Epidemiological Effects of Resistance Components.

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Summary
The epidemiological model BLIGHTSPACE is a spatiotemporal integro-difference equation model of the potato late blight pathosystem. To test and scrutinize the validity of model predictions, simulations were made and compared to independent data, collected in field trials on the spread of two genotypes of *Phytophthora infestans* in five potato cultivars in the Netherlands. Cultivar-isolate specific interactions were characterized in the model using three quantitative components of resistance: infection efficiency, lesion growth rate, and sporulation intensity. These were measured on potato leaflets in the laboratory. System and model were compared visually using disease progress curves, and numerically through a comparison of predicted and observed t_5 and t_{50} points (time in days until 5 and 50 % disease severity is reached respectively). For 80 % of the epidemics, performance criteria for both the t_5 and t_{50} points were met. Sensitivity analyses with the model revealed a dichotomy in the epidemiological effects of fitness parameters of *P. infestans*, providing two useful reference curves with which to formulate hypotheses regarding differences between observed and predicted epidemics.

Keywords
Phytophthora infestans, validation, resistance components, monocyclic, polycyclic.

Introduction
BLIGHTSPACE is a spatiotemporal/integro-difference equation model of the potato late blight pathosystem (*Phytophthora infestans* – *Solanum tuberosum*) that was originally developed and utilized to study the progress of epidemics in spatially heterogeneous mixtures of susceptible and resistant

host plants. The model was recently adapted to include the influence of the weather, host growth, fungicide use, long distance dispersal of spores and survival of spores during transportation. The model is intended as a research and educational tool and was designed for generating and testing hypotheses relating to epidemiological theory and for illustrating epidemiological principles. The novel contribution of BLIGHTSPACE is its ability to model spatial relationships in the potato late blight pathosystem and it has already been used to investigate the effects of different scales and patterns of host genotypes on the development of focal and general epidemics (Skelsey *et al.*, 2004). Here, we report tests of the quality of model predictions, in comparison to real measured epidemics in experimental field plots.

Theoretical framework and approach

Large field trials were conducted in 2002 and 2004 in Wageningen in the Netherlands. Five different potato cultivars with different levels of resistance against *P. infestans* were selected for use in this study: Agria (5½), Aziza (7½), Bintje (3), Remarka (6½) and Sante (4½). Foliar resistance ratings to potato late blight according to the Dutch National variety list are given in brackets. Two single isolates of *P. infestans* were used as inocula in the field trials, giving a total of 20 epidemics for model testing (5 cultivars x 2 inoculants x 2 years). Data from separate laboratory experiments were used to estimate model parameters.

The degree of confirmation of model predictions by observational data was first assessed graphically. Goodness of fit was then assessed numerically through a comparison of predicted and observed t_5 and t_{50} points (time in days until 5 and 50 % disease severity is reached respectively). A sensitivity analysis was conducted in order to improve understanding about the effect of model parameters, initial conditions and spatial context on the shape of disease progress curves. The results of these analyses were used to aid in the interpretation of discrepancies between model predictions and observations.

Results

Figure 1 shows a selection of observed and predicted epidemics. Based on a visual assessment, the disease progress curves generated by BLIGHTSPACE were a reasonably accurate fit of the observed epidemics in the field. Numerically, the model met predefined performance criteria in 80 % of the epidemics. The model was therefore able to translate measured resistance components, weather data and initial conditions into realistic disease progress curves for the majority of the data.

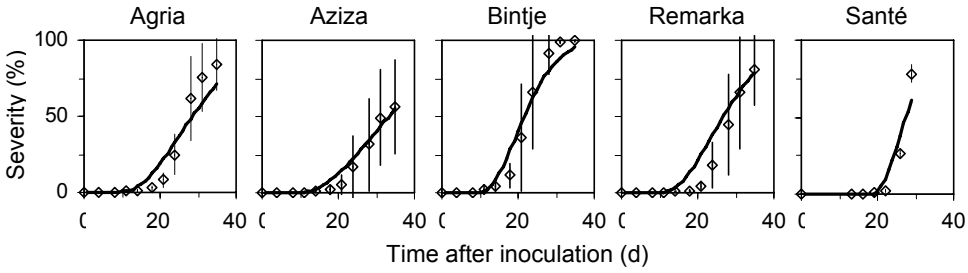

Figure 1. Observed (diamonds) and predicted (continuous line) disease progress curves of potato late blight epidemics under field conditions in Wageningen (NL) in 2002 and 2004. The simluated disease progress curves were obtained with the model BLIGHTSPACE. Vertical lines represent the standard deviation of the observed mean blight severity.

Figure 2 presents two extreme examples of a disease progress curve. The monocylic curve represents a *Phytophthora* strain with no local spore production. It is assumed that the initial inoculum came from an external source and that the polycyclic process of reproduction and establishment of new lesions is effectively shut off. This was achieved by setting any one of the model parameters pertaining to

the polycyclic process to zero, i.e. sporulation intensity (m^{-2}), deposition efficiency (-), or infection efficiency (-). Thus, the pure consequences of lesion expansion, or the monocyclic process, are realised, resulting in a gentle s-shaped curve with a short lag period that is very rounded in the terminal phase. Alternatively, if one would have a *Phytophthora* strain that has very little lesion expansion, then the polycyclic process - involving reproduction and establishment of new lesions - dominates the epidemic progress curve, as for a rust disease. To produce the polycyclic curve of Figure 2, lesion growth rates within the model were reduced to a minimal amount, and dominance of the polycyclic process increased by boosting sporulation. Thus, the epidemic was driven by the formation of new lesions as opposed to the growth of existing lesions. This near exponential process, plotted on a linear scale, yields a curve with a long lag period followed by a sudden 'explosion' towards 100 % infection. These two curve types can be thought of as the extreme end points of the range of shapes of disease progress curve that can be observed in the real world.

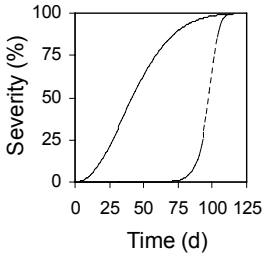

Figure 2. Dichotomy in the effect of resistance components on disease progress curves of potato late blight epidemics. Simulated disease progress curves were produced by the model BLIGHTSPACE. Complete dominance of the monocyclic process (dashed line) of lesion expansion is demonstrated by setting sporulation intensity (m^{-2}), deposition efficiency (-), or infection efficiency (-) to zero. Dominance of the polycyclic process (dotted line) of lesion propagation is demonstrated by reducing lesion growth rate ($m\ d^{-1}$) and increasing sporulation intensity, deposition efficiency or infection efficiency. Simulated plots were 4.5 x 4.5 m and isolated from external sources of inoculum with a 60 m border of non-crop area. Epidemics were initiated with 10 lesions / plant.

Figure 3 gives an illustration of using the model for ecological detective work (Hilborn & Mangel, 1997) leading to a diagnosis of events that may have contributed to shaping the outcome of an experiment. In the experiment, an inoculation was made with *P. infestans* on "date". A model simulation of this experiment, using nominal values for the parameters characterizing cultivar-isolate interaction, and taking into account weather influences, gave a substantial mismatch between the empirical and modeled disease progress curves (Figure 3A). The simulated disease progress curve was much steeper than the empirical disease progress curve, suggesting that the contribution of disease progress through formation of new lesions was overestimated in the simulation, while the contribution of expansion of existing lesions was underestimated. In a subsequent simulation, the infection chance was reduced by a factor 0.9 (-90%). As a result, the simulated and empirical disease progress curves came became much more similar. Then, we looked into the original records of the experiment and found that the fungicide Dithane (a.i. mancozeb) was used until two weeks before inoculation to prevent *P. infestans* from starting an epidemic prematurely. The fungicide appeared to have lingered until after the initial inoculation as the disease level was not above 0.003% across all cultivars, indicating low initial inoculation success and/or lack of significant disease increase, consistent with the change to model parameters that affected the 'improved' shape of simulated disease progress curve in Figure 3B. Evidently, this use of the model leads to hypotheses rather than firm conclusions. Taking into account this limitation, the truth-finding 'heuristic' value of model simulations is in our opinion substantial.

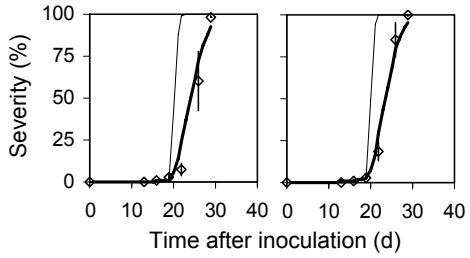

Figure 3. Observed (diamonds) and predicted disease progress curves of potato late blight epidemics under field conditions in the Netherlands in 2002 and 2004. Predicted disease progress curves were obtained with the model BLIGHTSPACE using values for model parameters and initial conditions that were estimated through experiments or observations (thin continuous line), or estimated using expert assessment (thick continuous line) through insights gained from the results of model sensitivity analyses.

Conclusions

Numerical predictions were in reasonably close agreement with the experimental data and predefined performance criteria were met in sixteen of the twenty epidemics. The model was therefore able to provide reasonably accurate estimates of the effect of weather, isolate, host resistance, initial conditions and spatial context on late blight epidemics. Some utility was found in classifying resistance components via their contribution to either the monocyclic or polycyclic epidemic process and separation of these two epidemic processes using resistance components provided two useful reference curves (Figure 3) with which to formulate hypotheses regarding differences between observed and predicted epidemics.

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Fungicide dose rates & cultivar resistance, results of five years of field experiments in the Netherlands

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Introduction

In 2003, the Dutch Umbrella Plan Phytophthora was launched. Within the Umbrella Plan, the Dutch grower organisation LTO, potato industry, potato trade and Wageningen–UR work together to achieve the common goal of 75% reduction of the environmental burden due to potato late blight control within 10 years.

One of the possibilities to reduce the fungicide input in a preventive control strategy is to use reduced dose rates of protectant fungicides on more resistant potato cultivars (Fry, 1975; Clayton & Shattock, 1995; Nærstad, 2002; Kessel *et al.*, 2004). This option was explored in a series of field experiments 2002 – 2006 in which several potato cultivars were protected with a range of Shirlan dose rates under high disease pressure. Protection of foliage as well as the protection of tubers were included in these experiments.

The aim of the project was to assess the posiblities of dose rate reduction based upon the resistance level of the cultivar. Calculated (minimum) dose rate for several cultivars under high diesease pressure are presented.

Materials and Methods

Foliar blight experiments

The possibilities to use reduced dose rates of protectant fungicides on more resistant potato cultivars was explored in the years 2002-2006. During 2002 -2004 experiments were set up to explore these possibilities for the protection of foliage (growth). 30 (2002) or 34 (2003 & 2004) varieties were protected with a range of Shirlan (fluazinam 500 g/l) dose rates (0%, 20%, 40%, 60%, 80%, 100% of the recommended (label) dose rate of 0.4 l/ha) under high disease pressure. Timing of the spray applications was based on PLANT-Plus recommendations except for the first three sprays which were applied at a weekly interval. Spreader rows (cv Nicola) within the field experiments were artificially inoculated with a mixture of 15 current *P. infestans* isolates. The isolates used were a random sample of isolates gathered during a survey in 2000 throughout the Netherlands. It is assumed that these 15 isolates are representative for the Dutch *P. infestans* population at the time.

Percentage infected foliage was assessed twice a week, from inoculation until haulm kill. Severity data on the epidemics occurring in 2002 and 2004 were analysed. The weather in 2003 was hot and dry resulting in non-representative low-level epidemic.

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Tuber blight experiments
In 2005-2006 the experiments were setup to determine the effect of reduced dose rate of Shirlan on tuber protection. 14 varieties were protected with a range of Shirlan dose rates (0%, 20%, 40%, 60%, 80%, 100% of the recommended (label) dose rate of 0.4 l/ha) under high disease pressure. Spraying time was based on disease pressure and the development of the epidemic except for the first sprays with Curzate M (mancozeb 68% + 4.5% cymoxanil) 2.5 kg/ha. which were applied at a weekly interval. Spreader rows (cv Nicola) within the field experiments were artificially inoculated with a mixture of 15 current *P. infestans* isolates. During the epidemic percentage infected foliage was assessed twice a week. Artificial rain (2005, 20 mm and in 2006 10 mm) was applied one day before desiccation of the foliage with Reglone (diquat dibromide 200 g/l).
Three weeks after desiccating the foliage the tubers were harvested (6 m^2/plot). Directly and three weeks after harvest percentage blighted tubers were assessed.

Analysis
The relative area under the disease progress curve (RAUDPC) was calculated based upon disease severity ratings. Disease parameters were calculated for each combination of variety and fluazinam dose rate.
Exponential curves (Equation 1) were fitted for each cultivar to establish the effect of dose rate on RAUDPC.

$Y_v = A_v + B_v.R^X_d$ (1)
Y_v = the RAUDPC of a given variety (v) at a given dose rate of fluazinam (d)
X_d = fluazinam dose rate
A_v, B_v and R_v are parameters of the fitted exponential curve of disease severity of the variety tested under different fungicide dose rates.

The effective dose rate of shirlan was calculated by comparing the exponential curves fitted based on RAUDPC at different dose rates of a given variety to the fitted exponential curve of the reference variety Bintje. Fitted curves were used to establish the effective dose rate of shirlan (Equation 2).
$Y_{ref} = Av + B_v.R^{Xv}$ (2)
Y_{ref} is the RAUDPC of the reference variety Bintje sprayed at a dose rate of 0.4 l/ha-1
X_v = effective fluazinam dose rate of variety tested
A_v, B_v and R_v are parameters of the fitted exponential curve of disease severity of the variety tested under different fungicide dose rates determinded using Equation 1.

To estimate the effect of dose rate reduction on tuber blight double exponential curves were fitted (Equation 3).

$Y_{vt =} B*(R^X) + C*(S^X)$ (3)
Y_{vt} = percentage tuberblight at dose rate X
B, C, R and S are regression parameters; in which 0<R < S < 1 and B+C represents percentage tuber blight without spraying
X = dose rate

To estimate the minimal dose rate to protect tubers, tuber blight incidence of Bintje sprayed at 0.4 l/ha was used as a reference in 2006. As a refenrce in 2005 a fixed value of 4 % was chosen since tuber blight incidence of Bintje was too high.

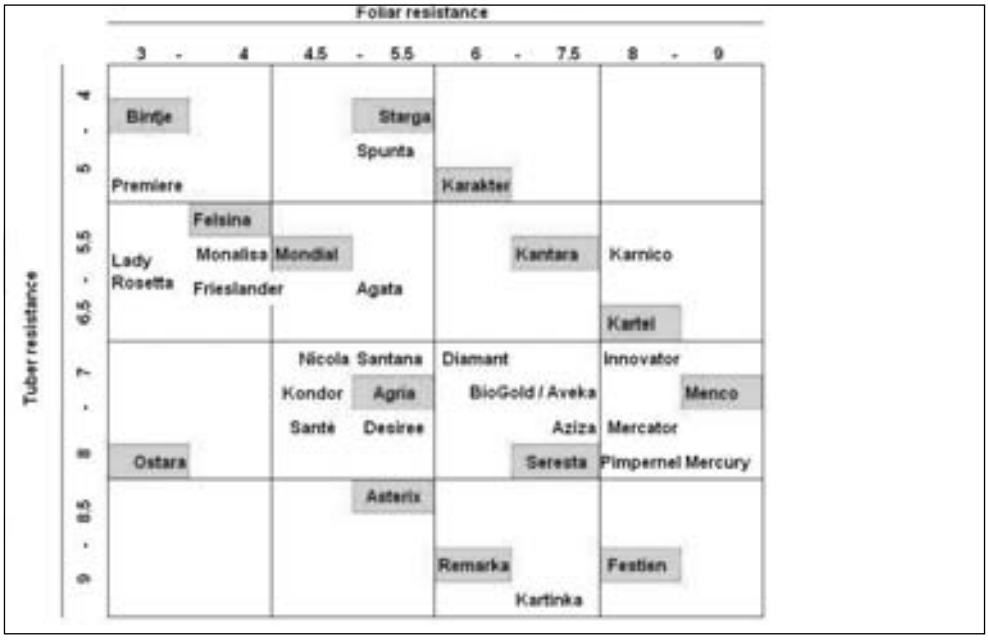

Figure 1. Potato varieties (with resistance level) used in the experiments. Varieties in green bars are also part of the tuber experiments.

Based on calculated dose rates varieties were rated in classes 1 to 4 for the posibility of dose rate reduction. Each variety was classified for foliar blight and tuber blight, seperately.

Statistics

The experiments were layed out as a split plot design. Dose rate treatments were allotted to the split stratum. Cultivars were randomized within the first stratum. The experiments consisted of three replicates each year. Spreader rows of cultivar Niocla were planted adjacent to each plot.

Statistical analysis was performed using Genstat 8 (Payne *et al.*, 2002). Least significant differences were calculated at a significance level of $\alpha=0.05$.

Multiple regression was performed to fitt curves to assess disease progress and to establish the effect of dose rate applied on disease progress parameters.

Results and discussion

In 2002 weather was very favourable for late bight and the epidemic developed rapidly.

As mentioned before the weather in 2003 was very hot en dry and epidemic did not develop very well. In 2004 the development of the epidemic was sufficient. Weather conditions were favorable for late blight in the second part of the season both in 2005 and 2006. Regular and heavy rainfall during the month of August in combination with the late blight epidemic ensured wash off of sporangia. Therefore disease pressure for tuber blight was high. A dose response effect of Shirlan on leaf blight was shown especially in 2004. Although it was more pronounced in varieties with a low level of resistance (cv. Bintje) compared to varieties with a higher level of resistance (Figure 2).

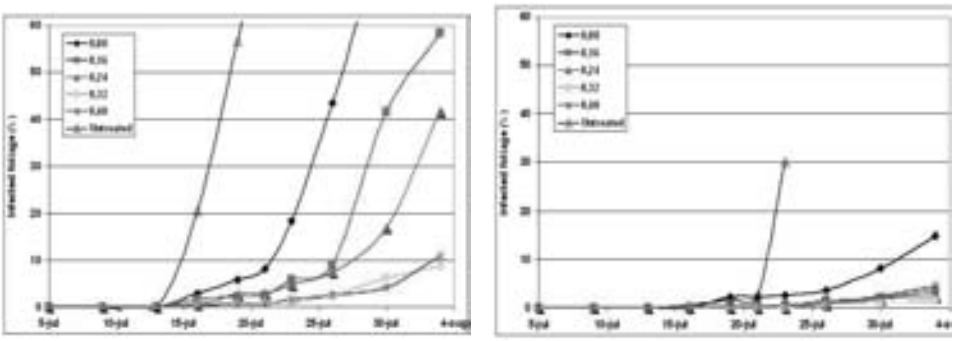

Figure 2. Dose response curves of fluazinam on the late blight susceptible cultivar Bintje (left) and the resistent cultivar Aziza (right).

Based on the dose response curves a suitable dose rate of fluazinam was calculated for each variety. In general potato varieties with a high level of resistance are classified in class 1 and varieties with a low level of resistance in class 4. However there are some varieties that differ from this statement. These varieties are Kantara (7) in class 1 and Kondor (4.5) in class 2. The varieties Kantara and Kondor have a relatively low level of resistance, according to the national list, compared to the class where they have been classified, according to our experiments. Erosion of late blight resistance occurs. The new introduced variety Biogold was rated a 9 for leaf blight resistance. However after introduction in agricultural practice compatible *P. infestans* strains were found. The leaf resistance rating was therefor adjusted to 7 for this variety. This illustrates the necessity of constant monitoring the resistance to late blight of cultivars. Resistance testing with modern isolates is therefore required. Re-evaluation of possibilities for dose rate reduction should be considered, regularly.
The experiments were conducted under high disease pressure. To calculate the acceptable dose rate for each variety, the efficacy of 0.4 l/ha Shirlan sprayed on Bintje was taken as a reference. In the Netherlands it is common practice that 0.3 l/ha of Shirlan is sprayed on Bintje and other varieties. So in practice (with low(er)) disease pressure even lower dose rates can be used without a large increase of the infection risk. This is also presented in Table 1. However, it is very important to spray before a critical period. Decision Support Systems can be very useful to determine the timing of the spray application. The possibility for using the level of cultivar resistance is illustrated in Figure 2.

Table 1. Dose rate classes for spray application of Shirlan calculated for 35 Dutch potato varieties.

Class 1: 0.1	Class 2: 0.2 (a)	Class 3: 0.3 (a)	Class 4: 0.4 (a)
Aziza (7.5) (b)	Diamant (6)	Felsina (3.5)	Agata (4)
Biogold (7)	Kondor (4.5)	Agria (5.5)	Asterix (5)
Festien (8)	Karnico (8)	Karakter (6)	Bintje (3)
Innovator (8)	Katinka (6.5)	Santé (4.5)	Frieslander (3.5)
Kantara (7)	Seresta (7)	Premiere (2.5)	Monalisa (4)
Kartel (8)	Aveka (7)	Santana (5)	Mondial (4.5)
Menco (9)	Pimpernel (8)	Starga (5.5)	Nicola (4.5)
Mercator (8)		Ostara (3.5)	Spunta (5)
Mercury (9)		Remarka (6.5)	Lady Rosetta (3)
			Desiree (5)

(a) At low disease pressure, dose rate can be decreased with maximum of 0.1 l/ha on top of corresponding reduction in the class of the variety.
(b) Between brackets the resistance level according to the national list 2007 is given (Anonymus, 2007)

Figure 2. Difference in level of infection in Biogold (7) and Agria (5.5), sprayed with the same dose rate of fluazinam.

Shirlan dose rate reduction for each variety seperately, were determined in experiments where development of foliage was present and tuber protection was no issue (first half of the growing season).
So to determine if these calculated reduced dose rates are adequate to control late blight in the second half of the growing season when tuber protection is important experiments were carried out then. Another argument to conduct experiments at the end of the season is that the foliar resistance does not correspond with the tuber resistance. So if resistance to tuber blight, is significantly lower than to leaf blight, continuation of spraying reduced dose rates in the latter part of the season increases the risks for tuber infection.

Based on the experiments a Shirlan dose rate for adequate tuber protection was calculated for each variety, seperately. Most of the varieties were classified in the same class for foliar and tuber protection. However, for some varieties with a relative low resistance to tuber blight the possibility to decrease the dose rate of fluazinam in the second half of the season is limited or not present. Some varieties allow dose rate reduction to protect the foliage, but reducing the dose rate for tuber protection is not possible. These varieties are Felsina, Kantara, Karakter, Ostara and Starga. Lowering the dose rate during the second half the season results in a higher level of tuber blight risk for these varieties.

Table 2. Dose rate classes of Shirlan for sufficient tuber protection of each variety.

Class 1: 0.1	Class 2: 0.2 (a)	Class 3: 0.3 (a)	Class 4: 0.4 (a)
Festien (**8** / 9)	Seresta (**7** / 8)	Agria (**5.5** / 7.5)	Asterix (**5** / 8.5)
Kartel (**8** / 6.5)		Remarka (**6.5** / 9)	Bintje (**3** / 4.5)
Menco (**9** / 7.5)			Mondial (**4.5** / 6)
			Felsina (**3.5** / 5.5)
			Kantara (**7** / 6)
			Karakter (**7** / 5)
			Ostara (**3.5** / 8)
			Starga (**5.5** / 4.5)

(a) At a low disease presure, dose rate can be decreased with a maxium 25%
*(b) Resistance ratings for **foliage** / tuber based on the National list (Anonymus 2007).*

General conclusions

- Application of Shirlan in reduced dose rates is feasible on more resistant varieties.
- Possibilities to reduce the dose rate of Shirlan are more feasible in the first half of the season than in the second part of the season for several varieties.
- If dose rate reductions are based on resistance ratings, reliable resistance ratings for both leaf and tuber blight are crucial.
- In the second half of the season tuber blight must be taken into consideration, which limits possibilities to reduce the dose rates for several but not all varieties.

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Early and Late Blight management in Italy

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Summary
Potato and tomato late blight is the most dangerous disease of solanaceus crops in Italy. Early blight rarely occurs on tomato crops. National potato and tomato production is given. Indications about the fungicides registered in Italy against both early and late blight are given along with the IPM guidelines and the list of the least toxic active ingredient farmers have to use. Moreover, information regarding the reduction of fungicide application in Emilia-Romagna region due to the use of IPI and MISP forecasting model, is shown.

Keywords
Potato, tomato, early blight, late blight, fungicides, IPM

Italian potato and tomato production
Italy is the most important country in Europe for the production of tomato, with particular reference to tomato for processing industry. The crop is grown in several regions of the country, but especially in Apulia, Emilia-Romagna and Sicily (Table 1). Potato is mainly grown in Sicily, Campania and Emilia-Romagna Regions (Table 2). The major competitors at international level are USA, Italy and Turkey, but new countries as Spain, China and Brazil are entering into the market.
Several diseases can affect tomato and potato plants, and lack of control of any one of them can result in serious reductions in yield and quality. Integrated pest management (IPM) is an approach to controlling pests and diseases by all available means, including the use of resistant cultivars. To date, resistances have been obtained toward several pests and diseases affecting tomato and potato, including the fungal pathogen *Alternaria solani* (Ellis & G. Martin) L.R. Jones & Grout; nevertheless, no resistance has been yet obtained toward late blight [*Phytophthora infestans* (Mont.) de Bary]. Nowadays, a number of fungicides are available to control tomato and potato late blight (Table 3). Yet, it is important their appropriate choice for the setting up of suitable IPM strategies. Moreover, for fungicides with a same mode of action it is necessary to limit the number of applications during the season for reducing the risk of acquiring resistance in the pathogen.

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Table 1 – Tomato production in Italy in 2006

	Ha	Tons/ha	Total yield (tons)
Italy	**90,823**	**57**	**5,156**
Puglia	25,760 (28.3%)	69	1,776
Emilia-Romagna	23,496 (26.0%)	63	1,494
Sicilia	11,280 (12.4%)	21	234
Lombardia	5,905 (6.5%)	58	342
Campania	5,365 (6.0%)	59	316
Calabria	3,852 (4.3%)	53	204
Toscana	2,342 (2.5%)	60	141
Lazio	2,000 (2.5%)	71	142

Source: ISTAT (http://www.istat.it/agricoltura/datiagri/coltivazioni/anno2006/06/)

Table 2 – Potato production in Italy in 2006

	Ha	Tons/ha	Total yield (tons)
Italy	**72,451**	**25**	**1,827**
Sicilia	11,358 (15.7%)	18	205
Campania	10,292 (14.2%)	33	344
Emilia-Romagna	7,018 (9.7%)	36	250
Puglia	6,088 (8.4%)	20	123
Toscana	5,453 (7.5%)	21	112
Abruzzo	4,404 (6.1%)	38	166
Veneto	3,549 (4.9%)	35	126
Sardegna	3,010 (4.2%)	17	51
Lazio	2,809 (3.8%)	25	71
Marche	2,003 (2.8%)	22	44
Piemonte	1,934 (2.7%)	25	49

Source: ISTAT (http://www.istat.it/agricoltura/datiagri/coltivazioni/anno2006/06/)

Table 3 – Fungicides authorised in Italy on potato and tomato against *P. infestans*

Potato	Tomato
Copper compounds	Copper compounds
Chlorothalonil	Chlorothalonil
Mancozeb	Mancozeb
Fluazinam	Methiram
Zoxamide*	Zoxamide*
Benalaxyl-M*	Benalaxyl-M*
Metalaxyl-M*	Metalaxyl-M*
Cymoxanil	Cymoxanil
Dimethomorph*	Dimethomorph*
Iprovalicarb*	Iprovalicarb*
Famoxadone*	Famoxadone*
Fenamidone*	Fenamidone*
Ciazofamide	Ciazofamide
	Azoxystrobin
	Pyraclostrobin*

** applied in mixture only*

Early and late blight Integrated production guidelines

To this aim, each Italian region annually issues guidelines for integrated production, according to EC Regulations 2200/96 and 1257/99, set up on the basis of specific territorial features and local production requirements. Such guidelines give also indications on the crop protection strategies, the correct use of plant protection products and the list of active ingredients for each considered crop, chosen on the basis of their low toxicity for human beings and environment. The farmers that intend to adopt the regional specifications can use only the plant protection products there listed. As an example, the 2007 specifications for the protection of tomato against late blight issued by the Apulia and Emilia-Romagna regions are respectively reported in Tables 4 and 5.

Table 4 - Specifications for the protection of tomato against late blight issued by the Apulia Region

PROTECTION CRITERIA	ACTIVE SUBSTANCES	NOTES AND RESTRICTIONS ON USE
Treatments must be started in the presence of climatic conditions favourable to the disease, employing non-systemic fungicides. With high levels of relative humidity and at the appearance of symptoms (max 3 days) fungicides capable to inhibit sporulation, or with curative activity and prolonged persistence are recommended.	Copper compounds (1) Dithianon Dodine Dimethomorph (2) Propamocarb (2) Iprovalicarb (3) Cymoxanil (3) Zoxamide (3) Mancozeb (4) Metiram (4) Famoxadone (5) Pyraclostrobin+metiram (5) Azoxystrobin (5) Fosetyl-Al Benalaxyl (6) Metalaxyl-M (6)	(1) It is preferably not to employ copper compounds during blossoming. (2) Max 2 applications per year. (3) Max 3 applications per year independently from the disease. (4) Fungicides to be used in alternation, with a maximum of 3 applications per year. (5) Fungicides to be used in alternation, with a maximum of 2 applications per year, independently from the disease. (6) Max 2 applications per year.

Table 5 - Specifications for the protection of tomato against late blight, issued by the Emilia-Romagna Region

PROTECTION CRITERIA	ACTIVE SUBSTANCES	NOTES AND RESTRICTIONS ON USE
Protection strategies to be started on the basis of information weekly issued by the Province Bulletins. Such Bulletins are drawn up taking into consideration the IPI forecasting model and the assessments carried out on warning fields. For the first treatments, it is preferable to use copper compounds that are effective also against bacterial diseases. In conditions of high levels of relative humidity systemic fungicides must be used. Fungicides with short pre-harvesting interval are to be used close to the harvesting time.	Copper compounds Dodine Metalaxyl-M (1) Benalaxyl (1) Benalaxyl-M (1)+Mancozeb (9) Dimethomorph (2) Cymoxanil (3) Azoxystrobin (4) (6) Pyraclostrobin (5) (6)+metiram (9) Fosetyl-Al Iprovalicarb (7) Zoxamide + Mancozeb (8) Mancozeb (9) Metiram (9)	(1) Max 3 applications per year. (2) Max 3 applications per year. (3) Max 3 applications per year. (4) Max 2 applications per year independently from the disease. (5) Max 3 applications per year independently from the disease. (6) Max 3 applications per year independently from the disease. (7) Max 3 applications per year. (8) Max 3 applications per year. (9) Max 3 applications per year independently from the disease; applications must be stopped 21 days before the harvest.

In Emilia-Romagna, Decision Support Systems have been developed and validated for several years and their use, from 1995 to 2005, allowed to reduce the number of sprays normally carried out on commercial plots. In particular, the forecasting model called IPI (Indice Potenziale Infettivo, Infection Potential Index) allowed a reduction of treatments between 15% and 65% during the period 1995-1999. The percentage of reduction of the number of sprays was 30-80% when IPI was integrated, on potato, with the model called MISP (Main Infection and Sporulation Period), during the period 1999-2005 (Bugiani *et al.*,1993, 1999, 2000). The information obtained with the IPI is the level of probability that infections do not occur at any considered moment. The probability is calculated on the basis of the daily data of temperature, rainfalls and relative humidity. The output of the IPI model is the moment for carrying out of the first treatment, whereas the output of the MISP model is represented by the indications on the dates for carrying out of treatments successive to the first one.
In Italy, epidemic pressure of late blight is usually low-medium during most of the crop growing season. Nevertheless, in particular conditions and/or areas, high levels of disease pressure can also occur. Under such conditions, field trials carried out in Northern and Southern Italy showed that the exclusive usage of copper compounds cannot ensure adequate protection levels while good effectiveness was obtained with several fungicides (chlorothalonil, cyazofamid, dimethomorph, fenamidone, fluazinam, iprovalicarb, metalaxyl-M, QoI, zoxamide) employed in different protection schedules (Berardi *et al.*, 2006; Dongiovanni *et al.*, 2006).

Specific experimentation aimed at evaluating the influence of irrigation management on disease development and, hence, at defining appropriate spray schedules is worthwhile to be conducted because of the lack of experimental data on this subject. The early blight on tomato is less severe than late blight, also because cultivar tolerant or resistant to the disease are available. Usually, specific sprays are not necessary, since most of the fungicides employed against late blight are also effective against early blight. Nevertheless, in particularly humid areas, one spray is to be carried out at the appearance of symptoms, followed by a second application 8-10 days after. On potato, the disease is quite rare and generally no treatments are needed, but copper compounds, difenoconazole and QoIs can be used in case of infections on young plants. In field trials carried out in Northern Italy QoIs proved to be among the most effective fungicides (data not published)

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Role of Fenamidone in the Management of Potato Early Blight

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Summary
Early blight, caused by *Alternaria solani*, is the most important foliar disease of potato in the Midwestern USA. Early blight is a chronic disease, endemic to all potato production areas in this region and foliar fungicides represent the most efficacious management tactic for this disease. Protectant fungicides alone, such as mancozeb and chlorothalonil, are generally inadequate to manage early blight. The QoI fungicide azoxystrobin was registered for use on potato in 1999 in the USA and provided excellent control of early blight until the development of resistance in *A. solani* due to the F129L mutation. Today, this mutation exists in every potato production area surveyed regardless of frequency of QoI fungicide use. In the presence of the F129L mutation in *A. solani*, all QoI fungicides are equivalent in their efficacy in managing early blight. Fenamidone has an advantage compared to other QoI fungicides in that it provides control of both early blight and late blight, caused by *Phytophthora infestans*.

Keywords
Early blight, *Alternaria solani*, Quinone-outside Inhibitor fungicides, strobilurins

Introduction
Early blight, caused by *Alternaria solani*, is a globally important foliar disease of potato. It is particularly important in the Midwestern USA, where much of the potatoes are produced under irrigation. This portion of the USA is characterized by frequent periods of dew which are important in disease development. *A. solani* is a diurnal pathogen, requiring alternating wet and dry conditions for the development of secondary conidia from lesions. Wet periods also occur during periods of rainfall or irrigation and conidia generally require 12 hrs for successful infection. *A. solani* is very aggressive on senescing foliage causing the disease to progress rapidly during the later stages of the growing season. As a direct result, early blight is usually more important as a production constraint in the central USA than late blight, caused by *Phytophthora infestans.*

Most potato cultivars grown in the USA are susceptible to early blight, although in varying degrees. Since genetic resistance to the disease is generally lacking, foliar fungicides are the most frequently utilized disease management strategy. Early blight is an endemic disease that tends to be chronic compared to the acute infections of late blight, which occurs sporadically. Therefore, fungicide programs implemented by the potato industry are most frequently directed at the management of early blight rather than late blight.

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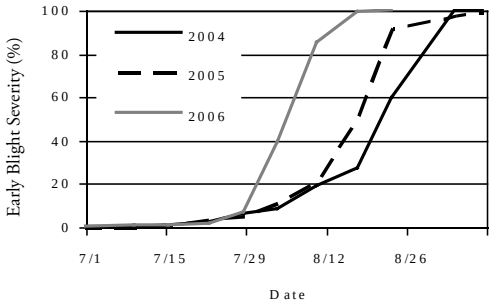

Figure 1. Disease progress curves for early blight development in non-treated fungicide plots in central Minnesota, 2004-2006.

In a number of areas of the USA, such as in the Pacific Northwest (Idaho, Oregon, Washington), early blight disease pressure is fairly low due to the arid conditions that predominate the region. In the East and Northeast (Pennsylvania, Maine, New Jersey), early blight pressure is low most years but occasionally severe under conducive environmental conditions, particularly in Pennsylvania. In these two potato production regions, regular applications of mancozeb (MCZ) or chlorothalonil (CHL) are generally sufficient to achieve economic control of the disease. However, in the central portions of the USA (North Dakota, Minnesota, Wisconsin, Nebraska, Texas, portions of Colorado) disease pressure is much higher for the reasons discussed above. Early blight disease progression varies from year to year but non-treated foliage is usually defoliated at some point during the growing season (Figure 1). High early blight disease pressure in most years means that potato growers must rely on foliar fungicides that are significantly more efficacious than MCZ or CHL.

Triphenyl tin hydroxide (TPTH, FRAC group 30) is a protectant fungicide used almost exclusively in the USA as a premium product providing enhanced early blight disease control at low use rates of 140 g a.i./ha. However, its toxicological profile makes it unattractive to most potato producers. In 1999, the first strobilurin-type QoI fungicide (FRAC group 11) was registered, azoxystrobin (AZS), which provided early blight control superior to TPTH. Given that AZS has a reduced-risk pesticide status from the Environmental Protection Agency, the potato industry immediately adopted this new chemistry for early blight disease management with great success (Stevenson & James, 1999). Subsequent to the registration of AZS, strobilurin fungicides trifloxystrobin (TFS) and pyraclostrobin (PRS) were registered for use on potato in 2001 and 2002, respectively. Non-strobilurin QoI fungicides famoxadone and fenamidone (FEN) were registered on potato in 2003 and 2004, respectively. As opposed to the strobilurin fungicides previously registered, these latter two QoI fungicides were initially marketed in potato for their enhanced control of late blight, despite the fact that they also have efficacy for early blight disease management.

Fungicide Resistance

The registration of AZS on potato had a significant impact on the potato industry, particularly in the Midwestern USA where early blight disease pressure is the highest. This strobilurin fungicide was so efficacious that in many research trials it was not unusual for early blight to be nearly absent in treated plots (Stevenson & James, 1999). As a result of this unusually high efficacy, irrigated potato producers used 4-6 applications of AZS alone per season, in alternation with MCZ or CHL, to control early blight. By 2001, our research group had observed a loss of efficacy with AZS and early blight, causing us to become concerned about fungicide resistance issues. Our approach was to first determine baseline sensitivities of AZS, TFS and PRS (Pasche *et al.*, 2004) using archived *A. solani* isolates retrieved from long-term storage (Holm, *et al.*, 2003). Subsequent to these studies, we developed baseline sensitivities to the QoI fungicides FAM and FEN (Pasche, *et al.*, 2005). Spore germination assays conducted on isolates recovered from potato plants after the registration of AZS demonstrated that *A. solani* isolates from 2000 and 2001 had reduced sensitivity to AZS, PRS and TFS (Pasche, *et al.*, 2004), and subsequently, to FAM and FEN (Pasche, *et al.*, 2005). These results

clearly demonstrated that *A. solani* had become less sensitive to the QoI chemistry in only two growing seasons (1999 and 2000), a remarkable discovery.

The reduced sensitivity to QoI fungicides was confirmed to be due to the F129L mutation (Pasche *et al.*, 2005). Perhaps most surprising to us was that the effect of the F129L mutation on the QoI fungicides was differential (Pasche, *et al.*, 2004; 2005). Spore germination assays demonstrated that the F129L mutation had the most profound effect on the strobilurin-type QoI fungicides AZS and PRS with resistance factors of approximately 10-15X while for TFS, FAM and FEN resistance factors were only 2-3X. The differential effect of the F129L mutation on in vitro activity of these QoI fungicides was also reflected in the loss of disease control. Early blight disease control provided by AZS and PRS on a mutant population was reduced by 40-50% compared to the wild type *A. solani* population (Figure 2A). Disease control provided by TFS, FAM and FEN was not significantly affected by the presence of the F129L mutation in *A. solani* (Figure 2).

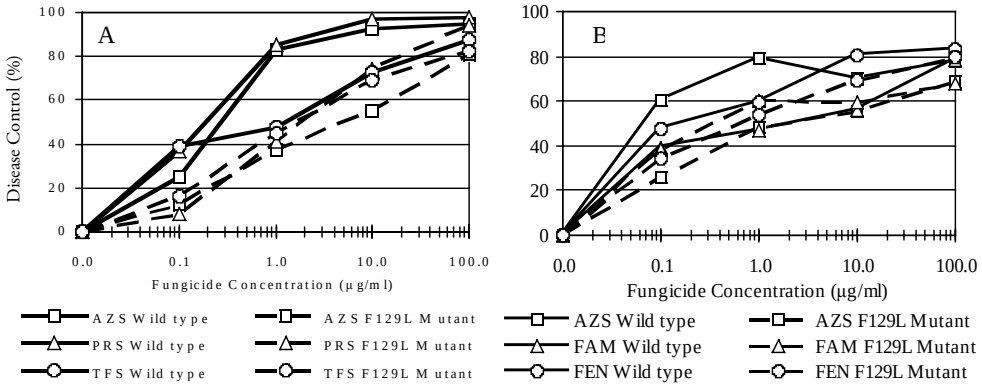

Figure 2. Effect of QoI fungicides on control of wild type and F129L mutant isolates of Alternaria solani at several concentrations of (A) azoxystrobin (AZS), pyraclostrobin (PRS), trifloxystrobin (TFS) and (B) AZS, famoxadone (FAM) and fenamidone (FEN). Trials were performed under growth chamber conditions using a 10-fold dose response curve for each fungicide.

The initial detection of reduced sensitivity to QoI fungicides in *A. solani* was in the state of Nebraska in 2000 (Pasche *et al.*, 2004). Since that initial detection, field reports from growers experiencing a loss of disease control became increasingly more widespread. Surveys conducted in 2002-2006 to detect the F129L mutation, using real-time PCR (Pasche *et al.*, 2005), demonstrated that the wild type *A. solani* population had been displaced in several Midwestern states (Table 1). Our analysis of the data, as well as examination of fungicide application records of potato growers, initially led us to believe that multiple exposures of the early blight pathogen to AZS over a 2-3 year period was largely responsible for the qualitative shift in QoI sensitivity of the fungal population. Most fields where the shift in QoI sensitivity had occurred had 12-16 AZS fungicides over 3 cropping seasons (Pasche, *et al.*, 2004). We found no evidence that AZS had been applied sequentially, indeed, all growers we studied had strict adherence to the EPA registration label at the time. However, more current survey data suggests that our hypothesis was incorrect, qualitative shifts in sensitivity to the QoI chemistry in *A. solani* does not require multiple exposures over a relatively short period of time. We have detected the F129L mutation in isolates obtained from collaborators in Colorado (94%), Idaho (15%), Oregon (60%), Washington (13%) and Wyoming (23%), areas where only 0-2 QoI fungicides per season have been applied since 1999. The F129L mutation in *A. solani* was detected in these states despite having very few samples to assay relative to the central potato production areas of the USA. It is apparent that

QoI fungicides such as AZS and PRS put tremendous selection pressure on the *A. solani* population, so much so that very few exposures are necessary to displace QoI-sensitive wild type isolates.

Materials and Methods

Effect of F129L on Early Blight Control. Field trials evaluating the efficacy of QoI fungicides were conducted in central North Dakota or Minnesota from 2000 to 2006. All trials were conducted in fields with overhead sprinkler irrigation and all agronomic practices performed on the field trials were consistent with those employed in the region. QoI fungicides AZS, PRS, TFS and FEN and the aniline-pyrimidine fungicide pyrimethanil (PYM) were evaluated for efficacy against the early blight pathogen *A. solani*. These fungicides were compared to non-treated controls as well as standard protectant fungicides CHL and MCZ. A typical foliar fungicide program for early blight control in this region includes ten applications of fungicide. In field trials presented here, QoI fungicides were applied five times during the growing season in alternation with CHL unless otherwise noted. The foliar fungicide trial in 2000 was conducted in the presence of a QoI sensitive/wild type *A. solani* population and used to illustrate the comparative disease control obtained through the use of strobilurin-type QoI fungicides and standard protectant fungicides CHL and MCZ. Field trials conducted after 2002 were predominated by the presence of the F129L mutation in the early blight fungus.

CHL, at a rate of 1190 g a.i./ha, was used as a standard protectant control treatment in every year the early blight disease control field trials were conducted. MCZ was used also as a standard protectant control treatment in 2000, 2004, 2005 and 2006 at a use rate of 1680 g a.i./ha. In 2000, the strobilurin-type QoI fungicides were used at label rates of 113 g a.i./ha for AZS and PRS and 105 g a.i./ha for TFS. In 2002 and 2003, AZS and PRS were applied at 113 and 226 g a.i./ha, while TFS was applied at 105 and 140 g a.i./ha. These treatments represent the lowest and highest labeled rate for each fungicide. In 2004-2006, FEN was applied at a rate of 200 g a.i./ha. In 2004 and 2005 it was applied three times in alternation with MCZ, while in 2006 it was applied twice in alternation with two applications of PYM and six applications of CHL. In addition to the two protectant fungicides, AZS (113 g a.i./ha) was used for comparison in three trials. All fungicides were applied with a field plot sprayer with a water volume of 560 L/ha delivered at 205 kPa.

All trials were performed as randomized complete block designs with four replications using potato cultivars susceptible to *A. solani* (Russet Burbank or Russet Norkotah). Percentage early blight severity was recorded weekly and continued until seven days after the final foliar fungicide application. Foliar disease severity was used to calculate the area under the disease progress curve (AUDPC). The relative area under the disease progress curve (RAUDPC) was calculated for each treatment of the replicated trials from each site-year by dividing AUDPC values by the total area of the graph and analyzed using analysis of variance (ANOVA).

Because of the need to develop fungicide programs for early blight management that best utilize all of the available chemistries, it is not possible to demonstrate efficacy for any specific chemistry in direct comparisons. Rather, data are shown from a number of field trials that compare QoI fungicides when these chemistries are alternated and/or tank-mixed with MCZ or CHL. Wherever possible, the number of specialty fungicides applied is the same or very similar and the timing or sequence of the specialty fungicides is also similar so that comparisons of efficacy can still be made in an appropriate manner. These data are derived from trials conducted by North Dakota State University (NDSU) performed in North Dakota or Minnesota, USA. It is important to note that the data that follows, unless otherwise indicated, were all generated in field plots conducted by NDSU under moderate

to high disease pressure in a potato production area where the F129L mutation dominates in the *A. solani* population. Early blight trials are conducted in a commercial potato production area and rely on natural infection by *A. solani*.

Results

The QoI fungicides represented a class of chemistry that provided superior control of early blight compared to standard protectant fungicides such as MCZ and CHL (Figure 3). During growing seasons with relatively low early blight disease pressure, significant differences among QoI fungicides and protectants such as CHL were not always evident (Figure 4). However, early blight severity was always significantly lower than the non-treated controls regardless of disease pressure (Figure 3, 4). In the presence of a QoI-sensitive/wild type *A. solani* population, AZS and PRS generally provided early blight control superior to that of TFS (Stevenson & James, 1999), however these differences were not always significant (Figure 3).

In the presence of a mutant *A. solani* population, isolates possessing the F129L mutation, QoI fungicides provide disease control comparable to MCZ or CHL (Figures 5-8).

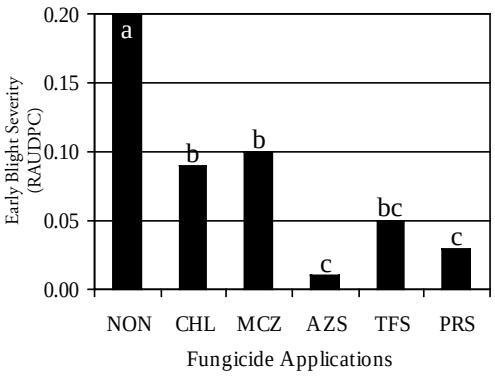

Figure 3. Comparison of early blight control using foliar fungicides in 2000. Relative area under the disease progress curve (RAUDPC) comparing non-treated (NON), chlorothalonil (CHL), mancozeb (MCZ), azoxystrobin (AZS), trifloxystrobin (TFS) and pyraclostrobin (PRS).

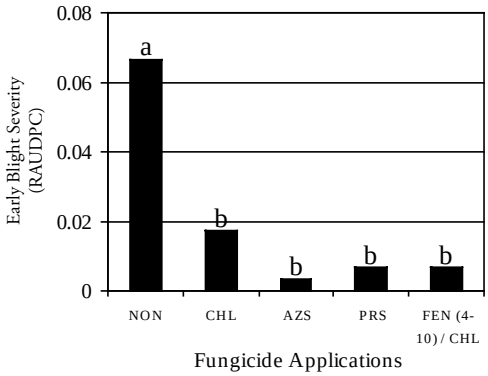

Figure 4. Comparison of early blight control using foliar fungicides in 2001. Timing and sequence of some fungicides denoted parenthetically. Relative area under the disease progress curve (RAUDPC) comparing non-treated (NON), chlorothalonil (CHL), azoxystrobin (AZS), pyraclostrobin (PRS) and fenamidone (FEN).

Extensive field studies by our research group on early blight disease control have demonstrated that *A. solani* isolates with the F129L mutation are not rate responsive (Figure 5). Studies using a 1x and 2x field rate of AZS, PRS and TFS did not affect early blight disease control in the same trial conducted over two years. Furthermore, it is apparent that the level of disease control provided by strobilurin QoI fungicides AZS and PRS, in the presence of an *A. solani* population dominated by the F129L mutation, was not significantly different than control provided by CHL. Since strobilurin QoI fungicides represent a premium-priced option for disease control relative to MCZ or CHL, we no longer recommend them for early blight management.

FEN has good efficacy against *A. solani* (Figure 6, 7, 8) and is always significantly better than the non-treated and when compared to a negative control fungicide such as fluazinam which was used in 2004 to prevent late blight infections (Figure 6). Depending on the level of early blight disease pressure,

two or three FEN applications alternated with MCZ or CHL will provide disease control equivalent to protectant fungicides alone (Figure 6, 7, 8). However, in other years, well timed applications of FEN have significantly improved disease control above that provided by MCZ alone (Figure 7). The use of FEN as the QoI in a fungicide program rotation is generally superior to AZS (Figure 6, 8), although these differences are not always significant (Figure 7).

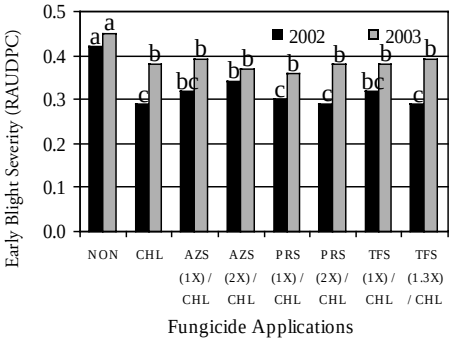

Figure 5. Effect of QoI fungicides azoxystrobin (AZS), pyraclostrobin (PRS) and trifloxystrobin (TFS) on a F129L mutant population of Alternaria solani over two growing seasons. QoI fungicides applied at normal field rates (105-113 g a.i. / ha) or 1.3-2.0X field

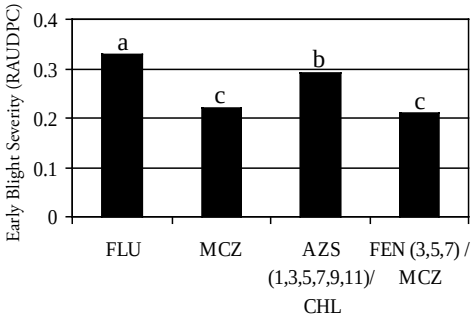

Figure 6. Comparison of early blight control using foliar fungicides in 2004. Timing and sequence of some fungicides denoted parenthetically. Relative area under the disease progress curve (RAUDPC) comparing fluazinam (FLU), azoxystrobin (AZS) alternated with (/) chlorothalonil (CHL), mancozeb (MCZ) and fenamidone (FEN) / MCZ.

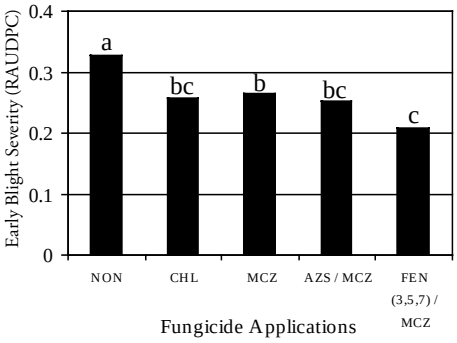

Figure 7. Comparison of early blight control using foliar fungicides in 2005. Timing and sequence of some fungicides denoted parenthetically. Relative area under the disease progress curve (RAUDPC) comparing non-treated (NON), chlorothalonil (CHL), mancozeb (MCZ), azoxystrobin (AZS) alternated with (/) MCZ and fenamidone (FEN) / MCZ.

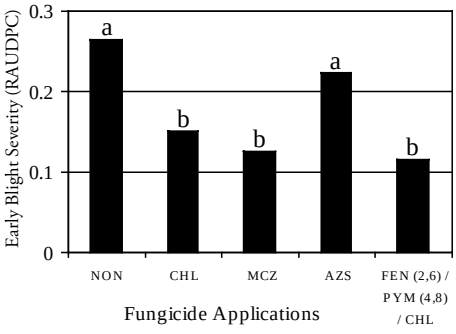

Figure 8. Comparison of early blight control using foliar fungicides in 2006. Timing and sequence of some fungicides denoted parenthetically. Relative area under the disease progress curve (RAUDPC) comparing non-treated (NON), chlorothalonil (CHL), mancozeb (MCZ), azoxystrobin (AZS), fenamidone (FEN) alternated with (/) pyrimethanil (PYM) and CHL.

Discussion

Early blight management continues to be a critical production issue in the USA. Fungicide programs are continually re-evaluated in order to provide a cost effective disease management program that delivers optimal disease control while preserving the fungicide chemistry from further resistance development. There are several key points that drive early blight fungicide program development in the USA:

1. Alternations and tank-mixes of premium fungicides with MCZ and CHL which are viewed by the potato industry as very cost effective.
2. Limiting the number of applications of fungicides possessing a single site mode of action, to preferably one and no more than two applications per season to minimize resistance development. This is considered a key attribute of any foliar fungicide program after the development of QoI reduced sensitivity.
3. The elimination or reduction of highly toxic fungicide chemistries, such as triphenyl tin hydroxide, from the fungicide program in favor of less toxic and preferably reduced risk fungicides.

As previously mentioned, the QoI fungicides were developed by agrochemical companies for different markets and there was a clear dichotomy as to the targeted pathogens. The strobilurin QoIs such as AZS, PRS and TFS were developed primarily as early blight fungicides. Although each of these fungicides has activity on the late blight pathogen, that activity is not generally regarded as being sufficiently better than MCZ or CHL alone to justify their expense. In contrast, the non-strobilurin fungicides FAM and FEN were developed primarily as late blight fungicides but also have activity against the early blight fungus. Much of the data from early development phases of FAM and FEN are from trials conducted primarily for late blight disease control. Data presented here indicate that FEN is also effective in managing early blight.

Conclusions

Early blight continues to be an extremely important foliar disease in the USA, in most years it is more important than late blight. The direct result is that potato growers in the central portion of the country use a base program of MCZ or CHL alternated with fungicides that are effective against *A. solani*. Unfortunately, due to the presence of the F129L mutation two of the most highly efficacious fungicides, AZS and PRS, have been rendered less effective in early blight disease control. This has, however, provided an opportunity for other QoI fungicides, those with improved efficacy against late blight, such as FEN, to gain entry into the early blight disease control market. QoI fungicides are an effective early blight management strategy when used in a program approach and alternated with standard protectants such as MCZ and CHL.

The development of reduced sensitivity in *A. solani* in response to exposure to strobilurins such as AZS and PRS occurred very rapidly in the USA. Although the qualitative shift was detected initially in those states where AZS was used extensively, the F129L mutation has since been detected in potato production areas that have used 0-2 QoI fungicides per season. From these data and observations, we hypothesize that mutant populations of *A. solani* will develop in Europe when exposed to QoI fungicides, particularly if AZS and PRS are used each season. We believe it is the high efficacy of these two strobilurin-type QoI fungicides places very high selection pressure on the early blight fungus. If this occurs, we predict that the potato industry will likely revert to QoI fungicides such as FEN that provide superior control of both late blight and early blight rather than using QoI fungicides such as AZS which lack significant efficacy to *P. infestans*.

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Integrated Control Strategy of potato late blight
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Evaluation of performance of Burkard trap in identification of Alternaria species infecting potato crops

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Summary
The causal agents of the early blight in Poland are two species of *Alternaria* genus. In the years 1997-2006 studies were conducted at the Plant Breeding and Acclimatization Institute of Bonin with the emphasis on: comparison of time of the occurrence and severity level of the early blight on potato crops in Poland and distribution of *A. alternata* and *A. solani* in collected population of *Alternaria* genus using Burkard trap to identification. Annual observations on disease appearance and time of first symptoms were performed in the years 1998-2006 on 500 potato crops in Poland. In that period early blight occurred at different level of incidence on over 86.0% of observed fields.
Microscopic analyzes revealed that 82.7% of spores were *A. alternata* and only 17.3% *A. solani*. There were some seasonal fluctuations in occurrence of both *Alternaria* species

Keywords
potato, disease, early blight, *Alternaria* genus, Burkard spore trap

Introduction

Early blight caused by two species of genus *Alternaria* occurs commonly worldwide on potato crops, particularly in regions with high temperature and alternating dry and high humidity periods. Early blight development results in premature dying of foliage and yield losses. The fungus was described for the first time in potato late in the 19th century (Ellis & Martin, 1882). Attacks on potato and tomato during the first three decades of the last century were described by Neergaard in 1945.
Harmfulness of early blight is estimated differently in various parts of the world. According to Bacanov (1970), Dorożkin (1972) by Reinoch (1974), the disease reduces yields up to 25%, locally 60%. According to Johnson *et al.* (1986) and Fry (1994), maximum documented yield reductions in USA are usually 20-30%. In Poland average losses are estimated for 10-32% (Kuczyńska, 1992), 6-45% (Kapsa & Osowski, 2004). In Polish climatic conditions high regional losses caused by early blight were recorded, however, most were related to cultivars with recognized susceptibility to this disease. Increasing yield losses due to early blight are observed in recent years that fungicide application scheduled by spore trapping or other methods is of economic importance. Protection of potato plants against early blight starts usually at first symptoms. First symptoms of disease might be missed due to abundant foliage

development. Hence, there is a necessity to develop a model of monitoring of early blight development and systems of forecasting that would allow determining more preciously time of first treatment.
The System of Forecasting Disease Epidemic (SPEC) has been operating in Poland since September 1st 2004. The SPEC was established by the Institute of Plant Genetics, Polish Academy of Science (IGR PAN) and the company DuPont in cooperation with research organisations. The SPEC is currently the largest system of monitoring stem canker (also termed "Blackleg"), the most destructive and spread out disease of oilseed rape (*Brassica napus*) – www.spec.edu.pl. The system is based on an evaluation of concentration of airborne ascospores of pathogenic fungi (*Leptosphaeria maculans* and *L. biglobosa*) by using volumetric Burkard Trap (Burkard Manufacturing Inc., United Kingdom and Lanzoni, Italy) – Figure 1.

The volumetric trap Burkard catches spores of different fungal species and also pollens. Based on that mechanism a research project on evaluation of the Burkard Trap in diagnosis of pathogens causing potato diseases and also possibilities of distinguishing *Alternaria* species, the casual agents of early blight was initiated at the Plant Breeding and Acclimatization Institute in Bonin. Due to unique shape and size of *Alternaria* spores their identification is not difficult.

Figure 1. Burkard Trap for sampling spores of plant pathogens (www.spec.edu.pl)

Material and Methods

In cooperation with the company DuPont in the growing season 2006 the tapes from the Burkard Trap set up at the Research Station of Cultivar Testing in Rarwino near Kamien Pomorski were evaluated for frequency occurrence of *Alternaria* species. The trap was placed about 40 m from potato field. The trap works continuously for period up to seven days. It sucks in air containing airborne particles such as fungal spores and pollens (10 m^3/h). Particles are caught on adhesive coated transparent plastic tape supported on a drum moving at 2 mm per hour. Each week the tape is removed from the drum and cut into section 48 mm long that responds to 24 hour period. Permanent slides are prepared from these sections for further microscopic studies (Jędryczka *et al.,* 2006). The microscopic identification allows to define what time and day the spores were present in the air.
Weekly cut sections were collected over 10-week period (from June 7th to August 15th). Each section was analyzed with the microscope for the presence of *A. solani* and *A. alternata.*

Results and discussion

In the years 1998-2006 in cooperation with the Plant Health and Seed Inspection Service an evaluation of early blight occurrence on potato crops was carried out throughout Poland. Annual observations on disease appearance and time of first symptoms were performed on 500 potato crops. In the period early blight occurred at different level of incidence on over 86.0% of observed fields (Tab. 1).

Table 1. Occurrence of early blight on potato crops in Poland

Year	Number of observed fields	Percentage of fields with early blight	EB appearance (mean number of DAP*)
1998	138	78,3	60
1999	93	88,0	65
2000	56	91.1	57
2001	50	96,0	61
2002	64	90,6	56
2003	34	85,0	63
2004	25	80,0	65
2005	21	86,0	54
2006	19	78,9	67
∑ / x	500	86,0	60.9

** DAP - days after planting*

Under Polish climatic conditions early blight disease occurs usually earlier than late blight. However, the important fact is that all observations were carried out on non irrigated fields and existing climatic conditions favored natural infection with the early blight.

The results from field trials from various countries on efficacy of selected fungicides in control of early blight on potatoes show high differentiation. It might be partially related to control technique and applied evaluation criteria but also contents of pathogen population occurring in different parts of the world including Europe as well.

Potato early blight appearing on Solanaceae is caused by two fungal species from Alternaria genus that belongs to a group of mitosporic fungi, class *Hyphomycetes*, order *Hyphomycetales* (Ainsworth and Bisby 1995 by Marcinkowska 1997). These two species are *A. solani* casual agent of early blight and *A. alternata* causal agent of brown leaf spots. Both species differ in some morphological features such as mycelium color and mycelium growth rate on the media, spore structures and temperature requirements (Tab. 2).

Table 2. Characteristic of two Alternaria species, the casual agents of potato early blight

Fungal species /synonyms	Host plants	Symptoms	Optimum temperature - ^{0}C*	Spore morphology**	Occurrence time
A .solani /*Macrosporium solani,* *A. porri* f.sp. *solani,* *A .dauci* f.sp. *solani*	plants from *Solanaceae,* except *Datura*	Early blight	intense sporulation: 26-28	Single spores formed on top of short conidiophores	First infection on senescent leaves
			spore germination and mycelium growth in infected tissue: 18-22		
A .alternata *A. tenuis* *Torula alternata*	about 40 plant species	Brown leaf spot	intense sporulation: 25-26	Spores are produced in chains of 2-10 from simple, sometimes branched, short or elongate conidiophores	Late occurrence at blooming
			spore germination and mycelium growth in infected tissue: 22-26		

** According to Dorozkin, Bel'skaja 1979*
*** According to Hooker 1990*

Spores of *A. solani* are usually borne singly. Average conidia have size estimated at 15-19 x 150-300 µm with 9-11 transverse septa. They are ellipsoid shape, tapering gradually to a long beak, occasionally branched. Spore color varies between pale to olive-brown. Spores of *A. alternata* are formed in chains. Conidia are smaller (20-65 x 9-18 µm) than spores of *A. solani*, without typical beak. Their size and shape may vary considerably (Hooker, 1990).

The casual agents of early blight are an example of typical necrotrophic organism i.e. a pathogen infecting weaker and older plants (Rotem, 1966). Potato plants infected with some viruses are more susceptible to the early blight infection (Hooker, 1990). This refers mainly to viruses PVY and PLRV (Dorożkin *et al.,* 1979) and PVX (Nagaich, Prased, 1971). Both species are very phytotoxic to plants as they produce the mycotoxins such as tenuazonic acid, alternariol, altenuene and tentoxin (Horoszkiewicz, 2007 – personal communication).

Quantity ratio of occurrence of both pathogens varies and is dependent upon climatic conditions. In the course of disease development the morphological symptoms are difficult to distinguish therefore they are evaluated jointly as the early blight.

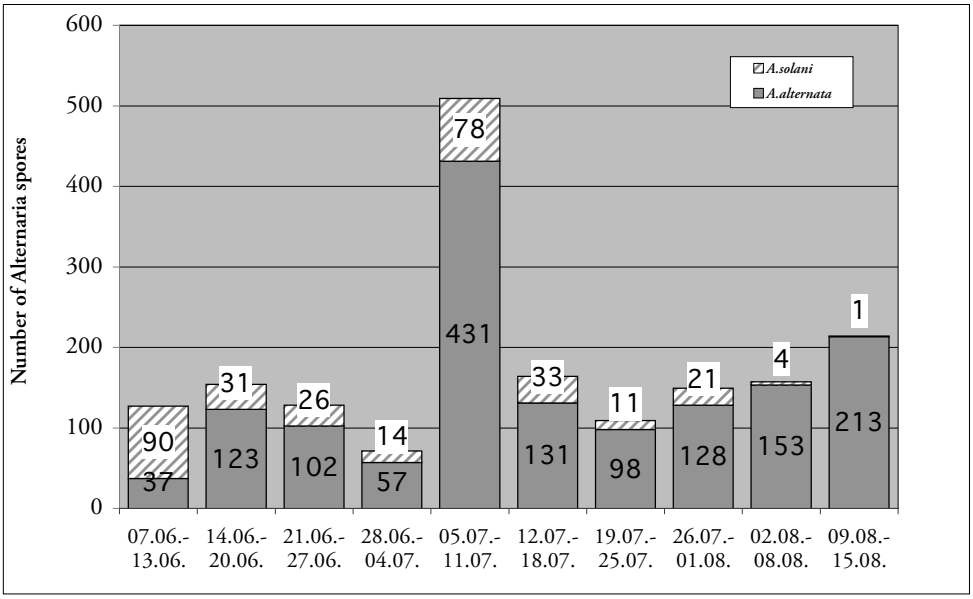

Figure 2. Number of spores of Alternaria species trapped on the Burkard Trap in 2006

Microscopic analyzes revealed that 82.7% of spores were *A. alternata* and only 17.3% *A. solani*. There were some seasonal fluctuations in occurrence of both Alternaria species (Figure 3).

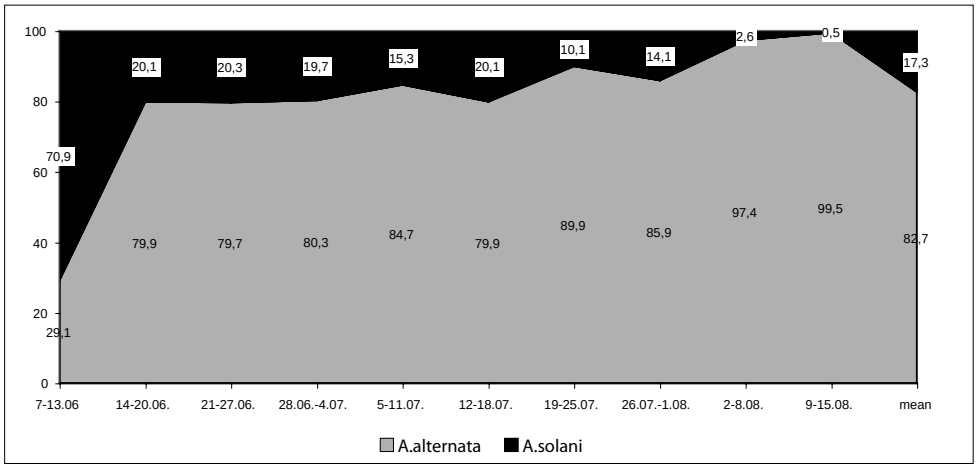

Figure 3. Percentage distribution of A. alternata and A. solani in collected population of Alternaria genus

A. solani was the most often present only in the first week of sampling (70.9%). Throughout the season the frequency of *A. solani* occurrence decreased and at the end of examined period (August 3-15) was 0.5%. These results conformed that *A. alternata* dominates during the growing season.
In 2007 the studies will be continued in five different locations to investigate a role and abundance of *A. alternata* in fungal population appearing of potato crops in other areas as well. In addition to the monitoring of *Alternaria* occurrence the meteorological data will be collected to examine a relation of particular fungal species and climatic conditions.

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Integrated Control Strategy of potato late blight
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Potato early blight in Germany *(Alternaria solani – Alternaria alternata)*

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Summary
Potato early blight occurs worldwide and is prevalent wherever potatoes are grown. Early blight of potato, caused by the fungi *Alternaria solani* and *Alternaria alternata*, can be found in all German potato growing areas. The disease is a risk to crop productivity in the field and results in significant yield losses. During the last years both pathogens became more and more important in the German potato production areas. An early blight research project at the Technische Universität München conducts a German wide survey accompanied by field frials concerning epidemics and fungicide strategies for the disease control. During the last six years the early blight epidemic was monitored in fungicide treated fields. The disease was found in all potato production areas at the beginning of July. In all years severe attacks were observed mainly in the eastern and southern parts of Germany. Fungicide treatments with Mancozeb results in a delay of the disease progress. Our results indicated that applicated dose rates of Mancozeb should be at least 1000g to reduce significantly early blight infections compared to the control plot.

Keywords
Alternaria solani, Alternaria alternata, early blight, fungicide strategy, chemical control

Introduction
Apart from the widespread potato disease late blight, early blight is causing increasing problems for German farmers in the last years. Since the last six years a monitoring has been accomplished in potatoes throughout Germany, which documents the occurrence of early blight in all German potato growing areas. Within the last years an increase in disease frequency was observed and disease has established as a relevant and destructive pathogen. Early blight became a more and more important disease in most of the potato growing areas.
The fungus survives the winter as conidia or mycelium on infected plant debris in soil or on seed (Rotem, 1994). At favourable conditions spores are formed and are either wind blown or splashed onto plant surfaces where infection occurs at first on lower leaves. On potatoes *Alternaria* causes big brown and necrotic lesions, clearly angular and restricted by the veines. Initial infections are most frequent on older leaflets. Lesions begin as dark brown to black spots, about 1 mm in diameter. The spots develop in a somewhat irregular shape and usually show concentric rings ("target spot") (van der

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Waals *et al.,* 2004). As disease progresses infected leaflets may turn yellow and either dry up or fall off. In some years stem infections can occur with symptoms similar to those on the leaves.

Alternaria solani belongs to the large-spored group within the genus, and produces simple, single-borne conidia that contain long and occasionally branched beaks (Ellis and Gibson, 1975). By contrast *Alternaria alternata* is one of the smaller-spored species, whose conidia are multiseptate, darkly pigmented and found as short chains borne on conidiophores, rather than single conidia (Petrunak and Christ, 1992).

In the field it is not possible to distinguish between necrotic spots caused by *Alternaria solani* or caused by *Alternaria alternata*.

Materials and methods

Samples with early blight infected leaves from 34 different areas throughout Germany were send to our laboratory (Figure 1). For incubation obained leaves were put in a petri dish containing wateragar (agar 15g and distilled water 1000ml). Usually, after three days of incubation sporulation was seen on the surface of the leaves. Single spore isolates were gained based on sporulating leaf tissues. Single spore isolates were cultivated and Alternaria species were distinguished on the base of the form of their conidia. By this way over 34 leaf samples from different locations were tested for their infestation with *Alternaria solani* and/or *Alternaria alternata*.

Figure 1: Isolates of Alternaria solani and Alternaria alternata were recovered from potato foliage submitted to our institute from various areas throughout Germany.

Figure 2: Early blight infected leaves were incubated in petri dishes containing wateragrar

Results

On the basis of leaf samples with known sort affiliation over 23 different varieties were checked during the investigation period between end of July (07/26) and mid of September (09/11).
One sample was infected only by *Alternaria alternata*. From the other samples both patogens (*Alternaria alternata* and *Alternaria solani*) could be isolated from the infected leaves.

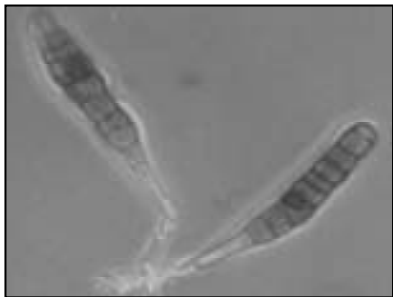

Figure 3: Alternaria solani belongs to the large-spored group within the genus, and produces simple, single-borne conidia.

Figure 4: Alternaria alternata is one of the smaller-spored species, whose conidia are multiseptate, darkly pigmented and found as short chains borne on conidiophores.

Chemical control

Many factors contribute to an effective control of early blight. Early blight disease is still primarly managed by the use of foliar fungicides. An increased early blight severity in Germany prompted us to study the efficacy of different dose rates of Mancozeb. The efficacy of various fungicide concentrations in the control of *Alternaria* leaf blight in potatoes was determined in two consecutive years (2004, 2005). In the field it is not possible to distinguish between necrotic spots on infected leaves caused by *A. solani* or caused by *A. alternata*. For this reason in field trials early blight was attributed to *Alternaria ssp.*
To prevent the occurrence of late blight (*Phytophthora infestans*) the complete trial was sprayed with Ranman (80 g a.i./ha cyazofamid) as coverspray. Treatments were carried out weekly. The active ingrediant Mancozeb was applicated at different fungicide concentrations. The content of Mancozeb varied between 500 g and 4000 g active ingredient of Mancozeb.

In both years first symptoms were observed on lower leaves three weeks after crop emergence. Although first early blight lesions were detected early in the growing season each year, disease severity remained at a low disease level (1-5%) until end of July, even in control (coversprayed) plots.
In the year 2004 in mid of August half of the canopy in the coversprayed plots was destroyed by early blight. During the next 14 days disease severity here increased dramatically until August 25th when over 95% of the foliage was affected.
In 2005 the onset of the early blight epidemic was later. As consequence the AUDPC value in the control was less than the AUDPC value reported for 2004. In the beginning of September the disease level was 20%. Two weeks later over 95% of the foliage was infected by *Alternaria*.
The overall effect of fungicide concentration on early blight severity, as expressed by AUDPC, was highly significant.

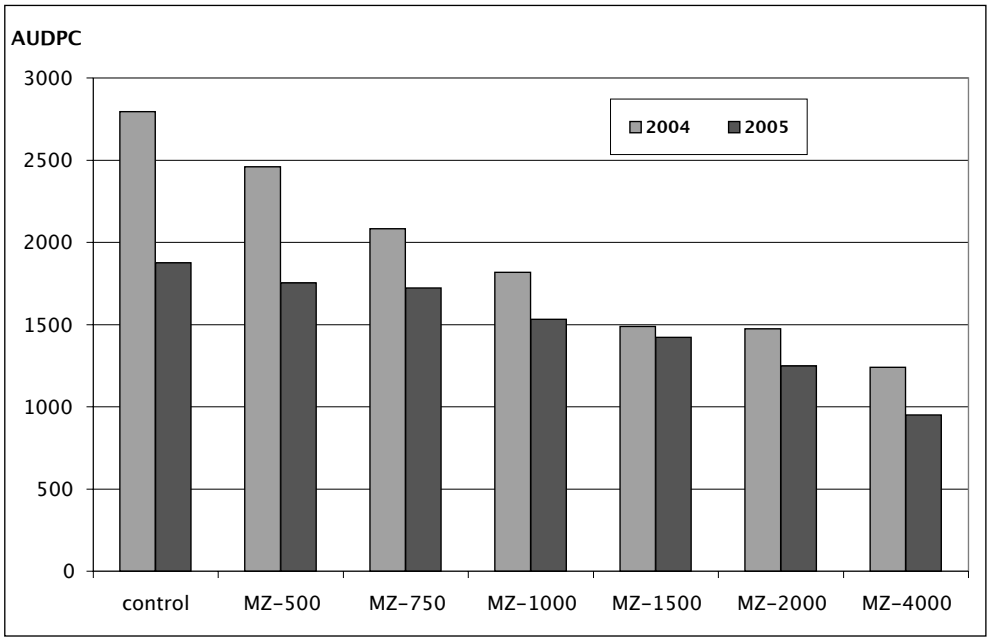

Figure 3: Early blight control at different fungicide concentrations with mancozeb tested at Kirchheim 2004 and 2005. Fungicide efficacy is expressed as area under the disease progress curve (AUDPC).

All applicated fungicide concentrations were able to reduce disease severity as compared to the against *Alternaria spp*. untreated control plot. However, Mancozeb at lower dose rates was less effective than at higher fungicide concentrations. Significant differences in disease severity among different fungicide concentrations were observed until the end of the season. Treatments against early blight showed that disease severity was the lowest at fungicide doses of 4000 g Mancozeb. In the year 2004 there was no further markable influence on disease progress at Mancozeb concentrations above 1500 g per hectare. Here the most effective results in disease suppression of early blight were shown in treatments with 1500 g doses of Mancozeb. This fungicide concentration of 1500 g active ingrediant of Mancozeb per hectare was defined as reference dose rate. Until the end of the season Mancozeb at the reference dose rate and higher significantly suppressed early blight infection levels below those of the untreated control.
In 2005 the mean AUDPC for the reference Mancozeb dose rate (1500 g mancozeb) was 1421. As in the year 2004 there was no significant difference among dose rate of 1500 g and 2000 g Mancozeb. In both years there was no significant difference between the control and the plots treated with 500 g Mancozeb. A reduction of 50% of the reference Mancozeb dose rate (1500 g) results in a significant higher AUDPC value.

Conclusions

The fungi *Alternaria solani* and *Alternaria alternata*, can be found in all German potato growing areas. Both pathogens are present in the field. This indicates that the efficacy of the early blight control by fungicide application in the field is a result of the efficacy against both pathogens. For a specific rating of the efficacy of diffent fungicides against *Alternaria solani* or *Alternaria alternata* a artifical inoculation is necessary.
Over both years in field trials early blight has been a destructive disease and caused yield losses due to premature defoliation. Mancozeb was effective as protectant fungicide for the control of early blight when applied as a preventative spray. Reduced efficacy was observed when mancozeb was used with less than the reference dose rate. Fungicide treatments with less then 1000 g mancozeb were not satisfying in disease control. The results indicate that the dose rates of mancozeb should be at least 1000g to reduce significantly early blight infections compared with the control plot.

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Early blight: influence of different varieties

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Summary
Potato early blight is a major disease of potatoes and other Solanaceae. In most potato growing areas in Germany early blight has established as a significant and destructive pathogen. The causal agents *Alternaria solani* and *Alternaria alternata* are becoming more and more important and are often greatly underestimated, although heavy infections can result in considerable yield losses. Integration of several factors (climate, nutrition, fungicides, variety) to prevent early blight disease can be effective. In addition to fungicidal disease suppression, cultivar resistance to early blight has become more important, although little research has been directed towards this area. In 2005 and 2006 several field trials were carried out in order to investigate disease susceptibility against early blight in several German potato varieties.
Early maturing varieties tend to be more susceptible than late maturing varieties (Johanson and Thurston, 1990). Early varieties showed a much greater and earlier increase in disease severity. Impact on yield was estimated at the end of the season. Yield loss was highest in late maturing varieties as early varieties showed only little influence.
More detail information about the influence of early blight disease susceptibility within different varieties could help to reduce the use of fungicide applications. In addition, early blight disease in highly affected potato growing areas could be better controlled through the cultivation of less susceptible varieties.

Keywords
Early blight, variety, yield loss, fungicide efficacy

Introduction

Early blight – an increasing problem
In many German potato growing areas early blight, caused by *Alternaria spp*., has established as a significant and destructive pathogen. On potatoes *Alternaria* causes large brown, necrotic lessions, which typically shows concentric rings (Rotem, 1994). As disease progresses whole leaves can be infected and drop off. Because *Alternaria* produce toxins that diffuse into host issues, it is not uncommon to see yellow halos around the brown spots (Laemmlen, 2004).
Recently an increase in disease frequency has been observed, with early blight becoming an important

disease in potatoes (Hausladen, 2004). Factors such as environment and plant physiology have a central influence on the disease progress of *Alternaria*. In addition, lower nitrogen fertilisation or varieties with higher leaf susceptibility are leading to a more rapid propagation.

Materials and methods

Field trials

Eight listed potato varieties were planted in plant plots in a randomised block design with four replications. Varieties were selected out of the three different maturing groups.

variety	maturity group	ware	starch
Karlena	early	X	
Marabell	early	X	
Agria	medium	X	
Solara	medium	X	
Albatros	medium		X
Maxilla	late		X
Logo	late		X
Kuras	late		X

Varieties were tested under four different treatments: 1) Control plots were completely untreated so that both early and late blight were able to establish. 2) Trials treated with Ranman (a.i. cyazofamid, 80 g a.i./ha) to prevent late blight. Ranman only protects against late blight infestation but has no influence on disease progress of early blight. 3) Trials treated with Mancozeb (1350 g a.i./ha). Mancozeb is the current standard early blight treatment, and has only protectant and antisporulant properties. Mancozeb was treated in combination with Ranman. 4) Trials treated with Azoxystrobin (125 g a.i./ha). Azoxystrobin is seen as the most potent active ingredient against early blight disease and has a broad spectrum of disease control with eradicant, protectant, translaminar and systemic properties (Bouwman, 2004). To get the maximum protection against late blight as well as early blight, trials were treated with Azoxystrobin in combination with Fluazinam (200 g a.i./ha). Fungicides were applied at the recommended dose. Mancozeb and Azoxystrobin were applied weekly from the beginning of first symptoms (July 2nd).

trial number	treatment	active ingredient
1	untreated	-
2	Ranman	80 gr
3	Ranman + Mancozeb	80 gr + 1350 gr
4	Fluazinam + Azoxystrobin	200 gr + 125 gr

Field trails were fertilised according to good agricultural practice. At the end of the season potatoes were harvested and tuber yield was determined. Tubers were sorted according to their size (<35mm, 35-55mm, >55 mm). Starch content was estimated out of each replication.

Rating

Over the season the expansion of the necrotic leaf area caused by early blight was observed weekly. In each of the four control plots 10 plants were checked for disease progress. For the ratings the potato plant was divided into three leaf levels (lower leaf level, medium leaf level and upper leaf level). From each of these levels one leaflet was chosen and the level of the necrotic leaf area was determined.

Results

First early blight symptoms were found in the field on July 2nd. There was no difference in disease occurence between the different varieties. Symptoms first appeared on older leaves at lower leaf levels. Disease severity stayed at a low level due to very hot temperatures and low humidity during July. The earliest and strongest increase in disease severity was shown during August in the early varieties Karlena and Marabell. Both varieties were killed by early blight at the end of August. In comparison, medium maturing varieties (Solara, Agria, Albatros) showed a slower build up in disease severity. Potatoes were killed by early blight about three weeks later than early cultivars. Disease progress was expressed as AUDPC and the area under the disease progress curve was calculated for all tested varieties. Strong differences between the varieties were found in the medium maturing group. Variety Albatros showed the lowest AUDPC value of all tested varieties. Increase in disease severity was the latest in the late maturing varieties (Logo, Maxilla, Kuras). Here varieties showed a delay in disease progress. As a result of their reduced symptoms these varieties had an average lower disease level.

Figure 1 shows the AUDPC values of all tested varieties in the Coverspray (Ranman) treatment. Ranman only protects against late blight infestation but has no influence on disease progress of early blight. The earlier the maturing group, the higher the AUDPC value, indicating that disease progress was much stronger in early varieties than late ones.

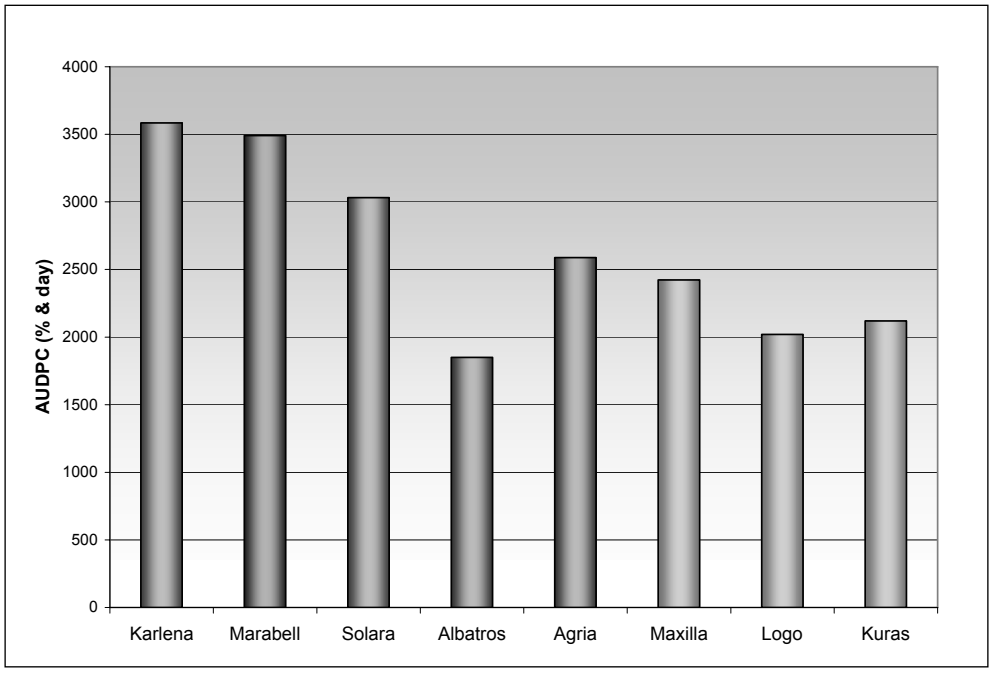

Figure 1. AUDPC values in the Coverspray treatment (Ranman)

Fungicide treatment

Medium maturing group

Trials treated with Coverspray (Ranman 80 g a.i./ha) expressed the highest disease level of all variants. Disease severity was reduced due to fungicide treatment with both Mancozeb and Azoxystrobin active ingredients. Figure 2 shows AUDPC values for different fungicide treatments against *Alternaria* in the medium maturing group. The lowest AUDPC values occurred in the treatments with Azoxystrobin, irrespective of the variety.

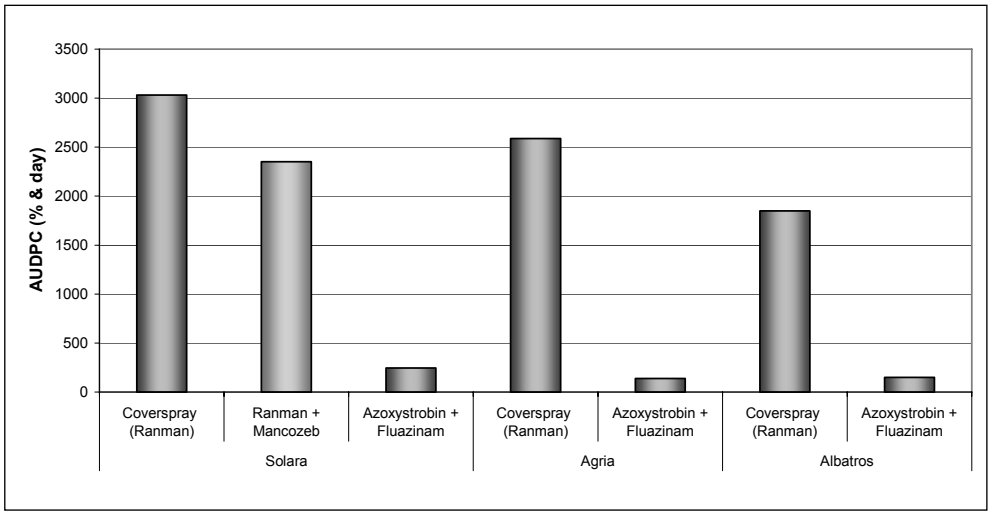

Figure 2. Reduction of AUDPC value through fungicide treatment in the medium maturing group.

Late maturing group

Similar results were obtained in the late maturing group. Highest AUDPC values were always obtained in the Coverspray plots. AUDPC values of the late varieties were lower compared to the medium maturing varieties. Use of Mancozeb reduced AUDPC values. However, as in the medium maturing group, the lowest AUDPC values were obtained in the treatments with the active ingredient Azoxystrobin (Figure 3).

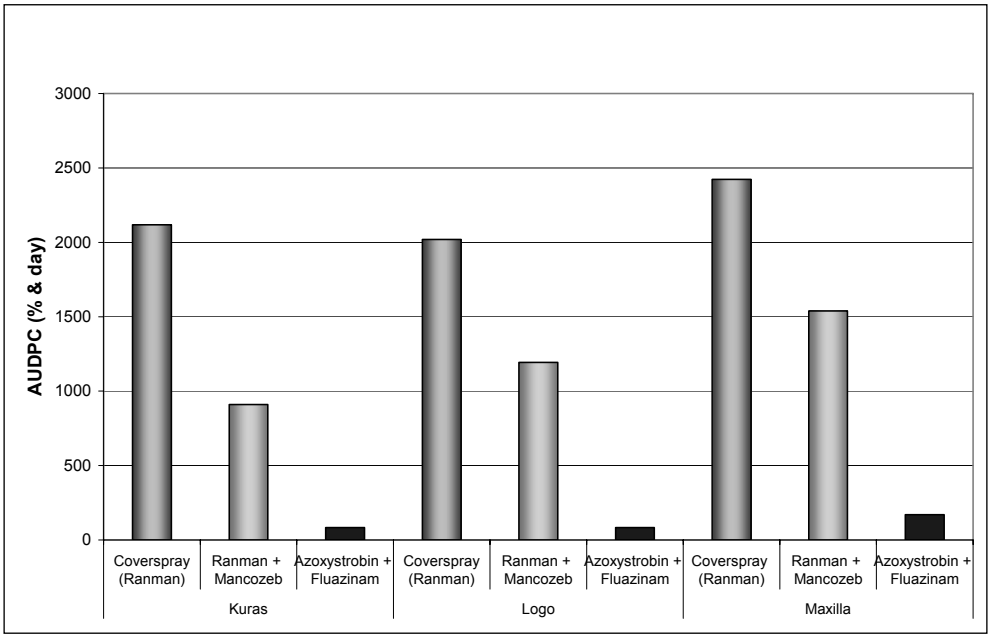

Figure 3. Reduction of AUDPC value through fungicide treatment in the late maturing group.

Tuber yield

Tuber yield, tuber size and starch content was estimated from each plot for each treatment. Harvested tubers were classified as < 35 mm, 35 to 55 mm and > 55 mm. Starch content was estimated 4 weeks after harvest.

Late maturing group
Tuber yield was increased in almost all varieties with active ingredients against early blight. Increase in tuber yield was found in all tested varieties of the late maturing group. In the late variety Kuras tuber yield was increased in both 35-55 mm and >55 mm categories. Total tuber yield was increased through the use of the active ingredient Azoxystrobin. Here, 20.7% more tubers were harvested in comparison to the Coverspray plots. In the treatment with the active ingredient Mancozeb tuber yield was only increased totally by 11.6% (Figure 4).

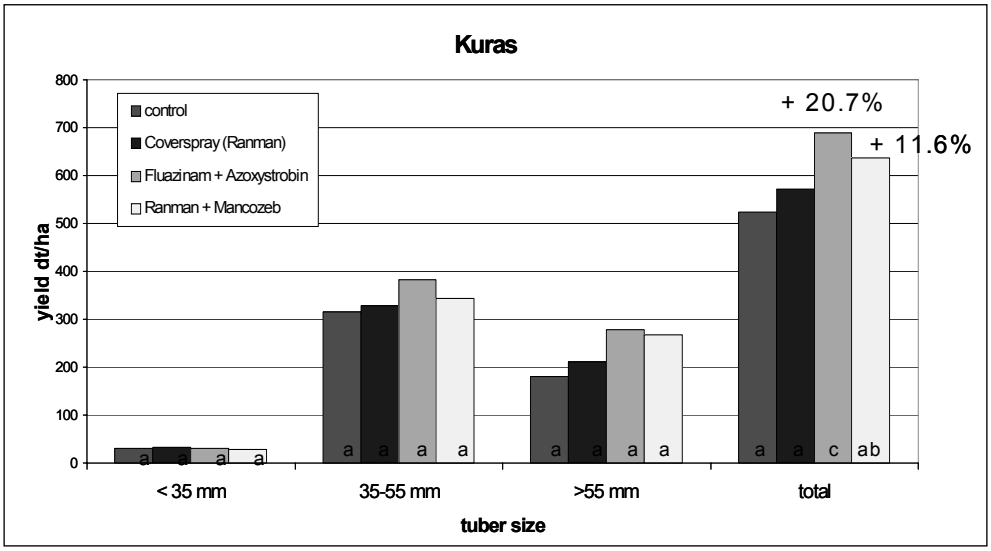

Figure 4. Tuber yield and size in variety Kuras (late maturing group)

Medium maturing group

In the medium variety Solara, tuber yield was increased by 14.4% when Azoxystrobin was used compared to the Coverspray plots (Figure 5). In the treatment with Mancozeb there was no increase in tuber yield. Potato plants treated with Mancozeb died from early blight two weeks later than in the Coverspray treatment as so there was no influence on tuber yield. In the treatment with Azoxystrobin, disease pressure stayed at a very low level until the end of the season. As assimilation time was greater, more tubers over 55 mm were harvested.

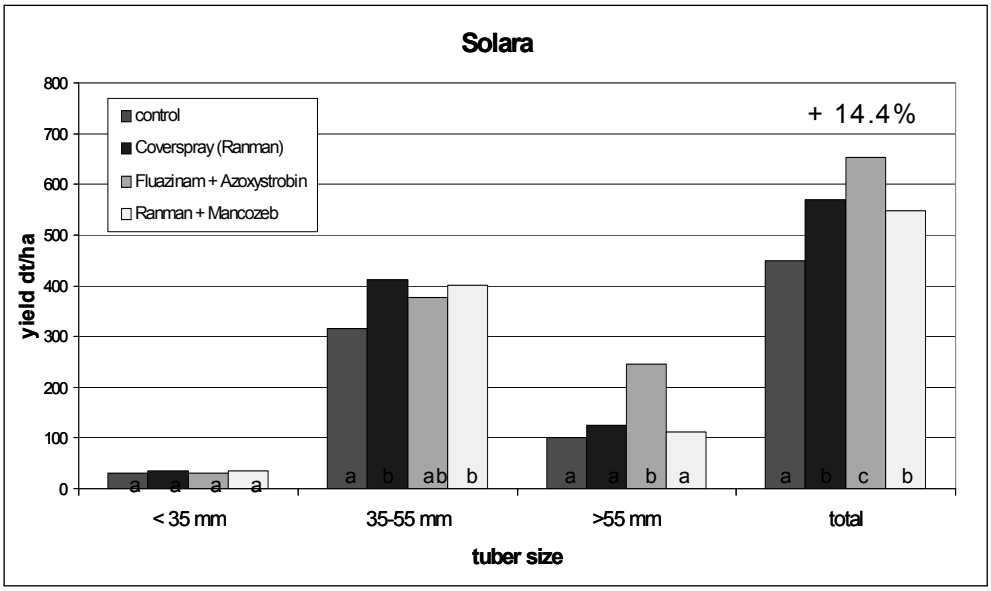

Figure 5. Tuber yield and size in variety Solara (medium maturing group)

Early maturing group

There was very little influence of early blight disease on tuber yield in the early maturing group. There was no significant difference between the Coverspray and the treatment with Azoxystrobin. Total tuber yield was only increased by 2.6% in the treatment with Azoxystrobin compared to the Coverspray. There was no influence on tuber yield in all size categories (Figure 6).

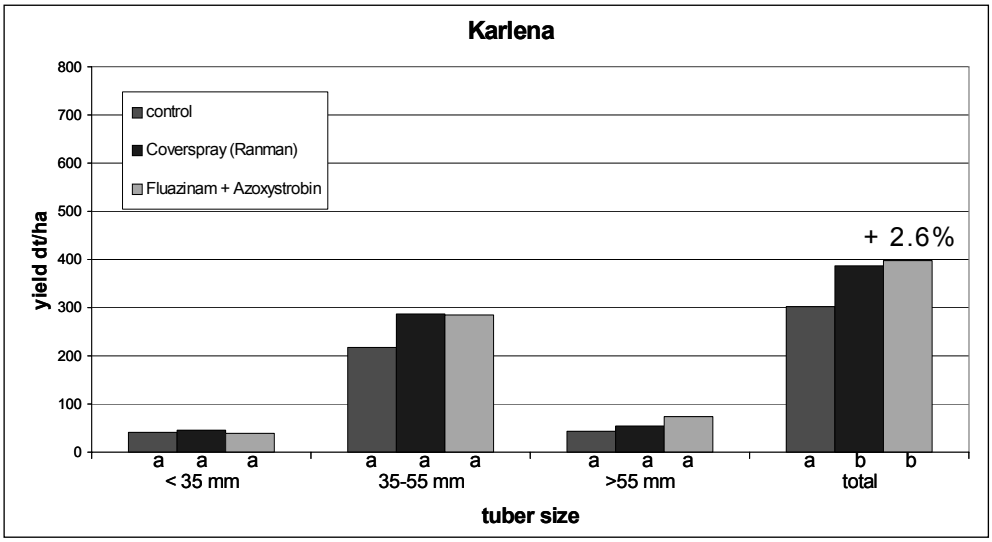

Figure 6. Tuber yield and size in variety Karlena (early maturing group)

Starch content

Starch content rose in all fungicide-treated varieties compared to the Coverspray treatment. As with the tuber yield results, starch content was influenced very little in the early maturing varieties. Starch content increased between 2.9% (Karlena) and 4.2% (Marabell). Unlike the early varieties, starch content increased the most in medium and late maturing varieties (Figure 7).

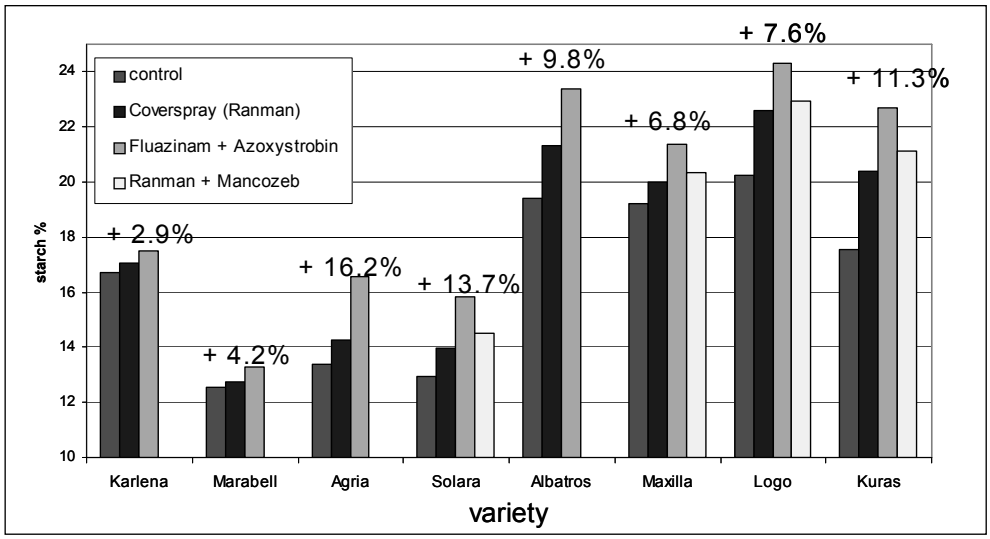

Figure 7. Starch content in all potato varieties with different treatments

All tested starch potatoes (Albatros, Maxilla, Logo, Kuras) showed a positive influence on starch yield with the use of Mancozeb or Azoxystrobin. Starch yield increased above average with the use of Azoxystrobin in combination with Fluazinam. Here starch yield was enhanced between 22.8% (Albatros) and 34.2% (Kuras) (Figure 8).
Treatments with Mancozeb were less effective than treatments with Azoxystrobin. The highest influence on starch yield was found in the variety Kuras where yield was increased by 15.7% with the use of Mancozeb. In all other tested varieties influence of Mancozeb on starch yield was very small: Logo (+1%), Maxilla (+7.4%).

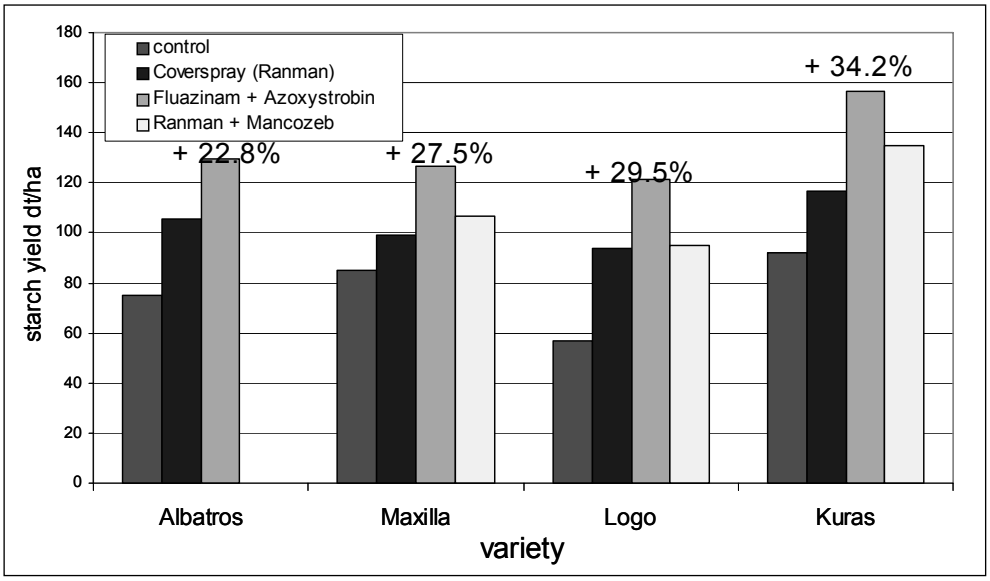

Figure 8. Starch yield in all four starch potato varieties

Discussion

The physiological causes of age-related resistance are not well known (Rotem, 1994).
Sources of genotype resistance to early blight in *S. tuberosum* are relatively rare. Varietal susceptibility which has been identified appears to show a correlation with the maturity of the variety. Early maturing varieties tend to be highly susceptible, whereas late maturing varieties tend to be more resistant. Disease progress curves for the eight tested varieties showed that early maturing varieties had the greatest disease severity in terms of percentage defoliation due to early blight compared to the later maturing varieties, throughout the growing season. The area under the disease progress curve (AUDPC) was significantly higher for the early varieties than for the later varieties. Considering a certain date of disease rating the earlier maturing the variety, the greater the early blight disease. The reason for the high correlation between resistance to early blight and maturity is not clear. Although field trials document this correlation between maturity and susceptibility, exceptions have also been found. We observed that the three medium maturing cultivars Solara, Agria and Albatros, which matured at approximately the same time, responded very differently to early blight infections. Although disease began at the same time in all cultivars, the rate of disease development differed among them. The variety Solara results in very high disease progress whilst the variety Albatros expressed the lowest AUPDC value of all tested varieties.
Disease pressure as well as disease progress was reduced considerably through the use of specific effective fungicides against early blight. Of the two active ingredients Mancozeb and Azoxystrobin,

Azoxystrobin suppressed early blight much better than Mancozeb. However, plant plots treated with Azoxystrobin were not free of early blight symptoms despite the effectiveness of this active ingredient. The use of potent fungicides against early blight disease could reduce severity effectively, keeping leaves healthy for a longer time and extending assimilation time.
Late maturing varieties benefitted from the longer assimilation time with significantly increased yield. In potato varieties of the early maturing group influence on tuber yield due to early blight was low. There was almost no difference between the Coverspray treatment and the early blight specific fungicides.
The highest increase in yield has been found in the medium to late maturing varieties in the treatments with Azoxystrobin. Early blight affects all economically significant factors such as tuber yield, tuber size and starch content. As a result of treatments with Azoxystrobin, starch yield increased significantly by 22% to 34%.

Conclusion

In Germany early blight is becoming more of a severe disease. Genotype resistance has been found between the different maturity groups. Early blight susceptibility appears to be highly correlated with cultivar maturity (Johanson and Thurston, 1990). Early maturing varieties were more susceptible, and late maturing varieties were more resistant. Genetic resistance to early blight in potato varieties may be of great importance in the future, although little research has been directed towards this area up to now. Less susceptible varieties will play a certain role in disease progress and disease severity.
Considering the specific fungicide impact differently good results could be obtained. The current fungicides are not able to ensure convincing disease control. Through the use of early blight specific fungicides like Azoxystrobin disease progress was slowed down. Tuber yield as well as starch content was significantly increased in treatments with Azoxystrobin in comparison to applications with Mancozeb.
Further investigations about the pathogen complex of early blight will provide more knowlege on the way to an effective disease control stategy.

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Assessing Late Blight Risk Under Polythene Covers in Jersey

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Summary
In 2006 & 2007 a comparison of late blight risk under polythene covers was made to risk above the polythene. The comparison was made in an early crop of Jersey Royal potatoes on the small island of Jersey from crop emergence in February to polythene removal in March/April. In both years the blight pressure under polythene was higher than above the crop but only by a small margin. When blight risk was low above the polythene it was also low below the polythene. Average temperatures above and below the polythene during the monitoring period in both years was mostly below the optimum for late blight development, which would explain why differences in risk were not as great as expected.

This study has shown that by monitoring late blight conditions under polythene it should be possible to improve the timing of polythene removal of the early crops in order to apply fungicides with curative activity.

Keywords
Late blight risk, Polythene, Jersey Royal,

Introduction
The cultivar Jersey Royal is the main variety of potato grown in Jersey, a small island close to northern France. It is grown mostly under polythene for the early market and exported to the UK in May and June. The earliest crops are planted on south facing slopes (Cotills) and are covered with plastic in January which is removed in March, a sequence of plantings will then be carried out across the island until March/April. A combination of a susceptible cultivar, maritime climate, potatoes grown in constant rotation and retained seed, makes blight control in Jersey a very challenging operation.

One company, Jersey Royal (Potato Marketing) controls most of the growing and marketing of the Jersey Royal, which accounts for 1800 ha of crop. JRPM have a network of 4 weather stations on the island covering an area of 15 x 8 km. (Figure 1). The company has been involved with blight forecasting since 2000, using the PLANT-Plus system (Hadders, 1996). During this time it was observed that there was a correlation between blight risk above polythene covers and outbreaks once polythene is removed in early spring (Hinds, 2004). A common assumption, however is that blight

risk is always high under polythene. To test this assumption a comparison was made of risk from under the polythene against open ground weather conditions. The aim of this exercise was to improve timing of polythene removal in order to give better protection against early blight outbreaks

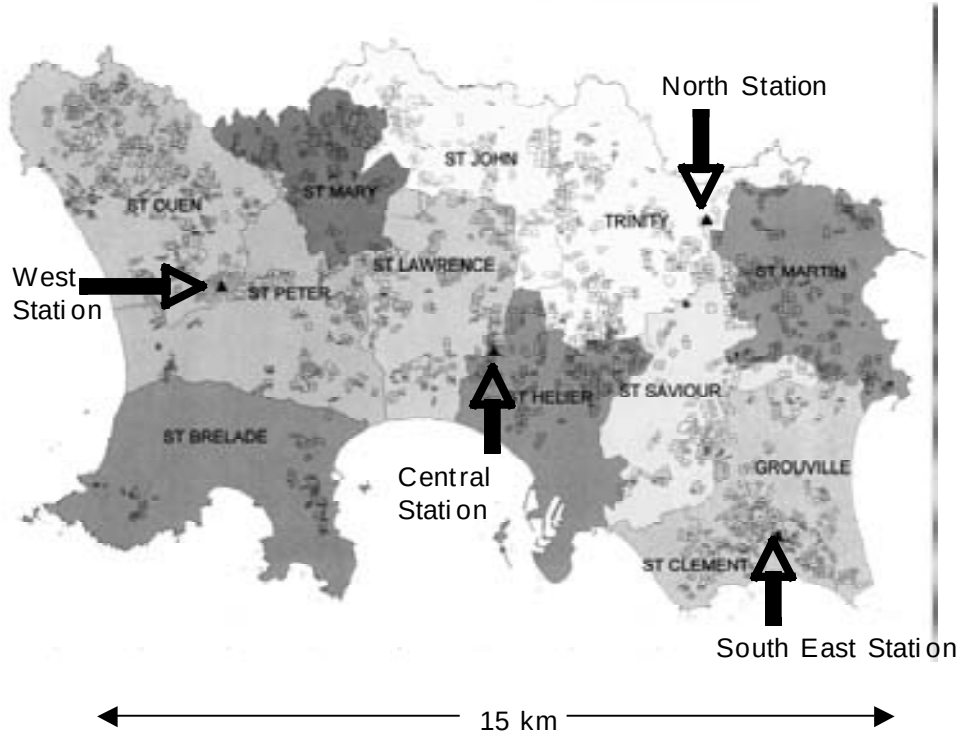

Figure 1

Material and Methods

In 2006 and 2007 seasons sensors for temperature and relative humidity were placed under polythene covers soon after planting (photo 1). From these sensors blight risk was calculated using the PLANT-Plus model under the polythene and compared to risk calculated from a nearby weather station with sensors above the polythene (photo 2).

The site chosen was in the South East called St Clements, an area where some of the earliest production occurs on the Island.

Results

In 2006 crop emergence under the polythene was relatively late on 22 February. Before polythene removal on 10 April (photo 3), three main blight risk events were recorded above and below the polythene in early March, late March and early April. Risk recorded under the polythene was higher than that recorded by the station above the polythene, but not by a large margin (Figure 2). When risk was low above the polythene it was also low below the polythene

In 2007 crop emergence was earlier on 2nd February and removal took place on 3rd March. A similar pattern occurred to 2006 with risk periods higher under polythene compared to the station above the polythene, but not by a significant amount (Figure 3).

Photo 1

Photo 2

Analysis of weather data from the sensors above and below the poly shows that average temperature and humidity under the polythene, as might be expected, was higher than that recorded above the polythene, however in 2007 average temperature difference was only 0.3ºC and humidity difference was just 2%. In both years the lowest temperatures recorded were below the polythene (table 1).

Photo 3

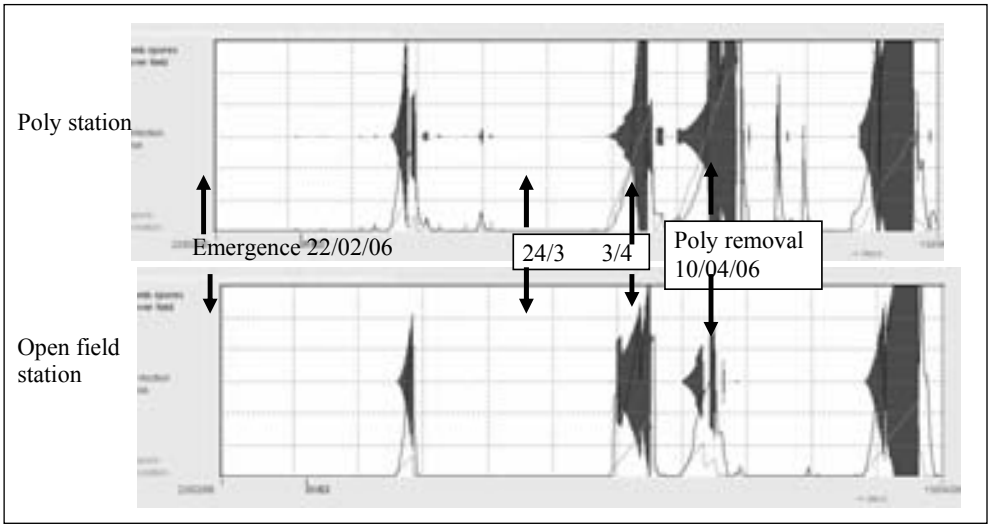

Figure 2 South East Blight Risk 2006 - a late season

Table 1 – Temperature and relative humidity records above and below polythene covers in spring 2006 & 2007

Year	System	T-Av	T-Max	T-Min	RH-Av
2006	Poly	7.8	26.6	-0.5	94
	Open	5.9	12.2	0.3	81
2007	Poly	8.3	13	2.5	88
	Open	8.6	11.9	2.8	86

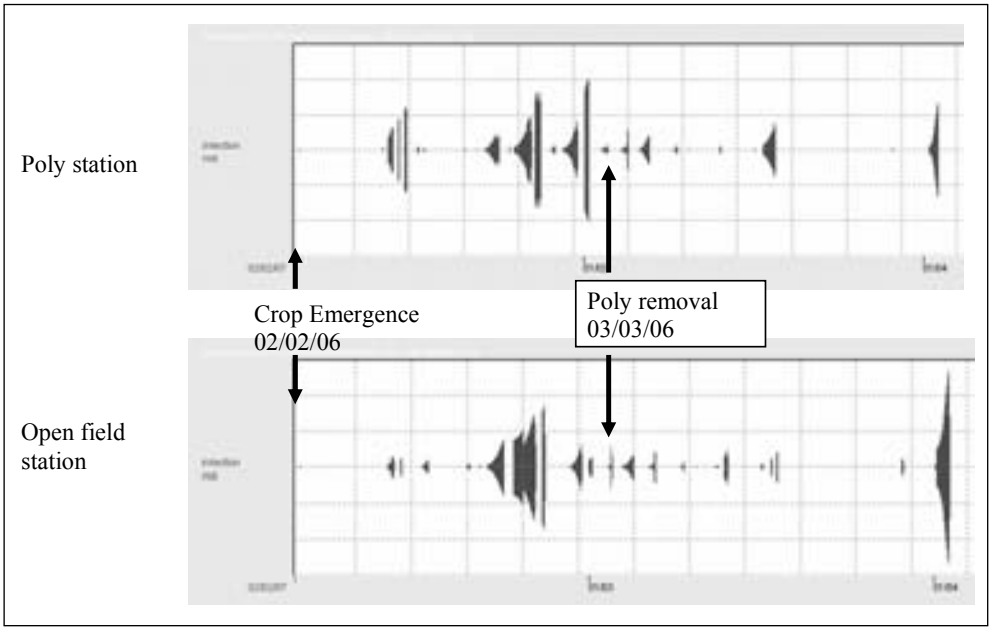

Figure 3 South East Blight Risk 2007 - an early season

Discussion

The surprise from this study has been the lack of any real difference between late blight risk under polythene compared to above the polythene. In Jersey the assumption, that blight risk is always high under polythene, is not necessarily true. The reason why this is so can be explained by low temperatures recorded in February and March when early crops are covered with polythene in Jersey. Although average temperatures during this period were higher under polythene than above, in both years this average was still well below 10 ºC a threshold below which late blight development is generally limited. Another factor is the crop leaf area produced by the variety Jersey Royal which is usually low. When polythene is removed crop ground cover is often less than 50%, therefore the amount of humidity created under the crop is potentially less than a crop of full cover. The results reflect this factor as differences between relative humidity under the polythene compared to above were not large, especially in 2007.

If this exercise were repeated in later crops when polythene covers are on in April and May, disease pressure under polythene could be greater, as it is likely average temperatures would be higher in this period. A different outcome could also be possible for crops grown under fleece (not used in Jersey) as crops are left covered for a much longer period than under polythene. A possibility in this situation would be to spray over fleece as there is evidence (Spits, 2002) that with some fungicides, application over covered crops can give some effectiveness in controlling blight.

From this study by monitoring blight conditions under polythene it should be possible to highlight periods when blight could develop under polythene. Timing of polythene removal should then be coincided with these periods in order to apply fungicides with curative activity. Because of the sequence of different planting dates monitoring would need to occur across the various planting periods (January-April) in order to time polythene removal with late blight risk for each crop.

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Studies of release and infectivity of *Phytophthora infestans* sporangia under field condition

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Summary
Influence of weather conditions on sporangia production, release, viability and infection of potato late blight (*Phytophthora infestans*) was investigated in Denmark, Norway and Finland using Burkard spore traps and exposure of trap plants in field plots. High amounts of sporangia were trapped after nights with long periods with high relative humidity. Sporangia produced in one humidity period were mainly released at the first humidity drop in the morning hours, but there were also some "delayed sporangia release". Local new infections occurred mainly during the morning hours when leaves were still wet. On many days sporangia did not survive until the afternoon and the results indicate that conditions for survival of sporangia and infection are major bottlenecks for the spread and development of the disease.

Introduction
The existing forecast and decision support systems used in the Nordic countries are based on data for temperature and humidity influence on *P. infestans* epidemiology obtained under controlled conditions from the 1930s and the 1950s (Crosier, 1934; Harrison, 1992). Only a few studies in recent time have tried to verify and update the "old" models (Hartill *et al.*, 1990; Harrison & Lowe, 1989; Fry & Mizubuti, 1998). It is generally agreed that sporangia are produced during wet periods overnight, are dispersed during drying in the morning and then cause infections when the leaves are again wetted by dew or rain (Fry & Mizubuti, 1998). Both temperature and relative humidity can affect the survival of airborne sporangia, but solar radiation is considered the main cause of death of dispersed *P. infestans* sporangia (Bashi *et al.*, 1982; Mizubuti *et al.*, 2000; Sunseri *et al.*, 2002). Similar conclusions have been drawn for other pathogens like *Peronospora destructor* and *Peronospora tabacina* (Bashi & Aylor, 1983) and *Bremia lectucae* (Wu *et al.*, 2000).
In earlier studies in Denmark viable sporangia were found in lesions nearly every day, and sporangia were caught in Burkard spore traps during periods when no risk of sporulation was calculated by the "Hours of SPOrulation sub-model (HSPO)" (Bay *et al.*, 2003). Similar results have been found in other studies (Bashi *et al.*, 1982). It is possible that formed sporangia are liberated over more than one day and still attached sporangia may be viable for several days (Bashi *et al.*, 1982; Hansen, 2002).

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On the other hand it has been questioned if the relatively low amounts of sporangia that (always) are caught above or close to the canopy in between sporulation events are alive or not (Ruckstuhl *et al.*, 1999; Hansen, 2002).
In 2006, conditions for sporulation, sporangia release, survival and infection were studied in semi-field trials in Denmark, Norway and Finland. Data from Burkard spore trap and trap plants exposed in the field during different periods of the day were used to test the hypothesis that (1) survival and infection is the major bottleneck for disease development under field conditions and (2) that infections happen during morning hours when sporangia release overlaps with few droplets still being left in the canopy. The most comprehensive dataset was obtained in Denmark from the national REFUKA project. More results are therefore provided from this source of data.

Materials and Methods
Experiments were carried out in 2006 at Research Centre Flakkebjerg, Denmark, at Bioforsk Plant Health and Plant Protection Division, Ås, Norway and at MTT, Agrifood Research Jokioinen, Finland.

Sporangia were caught with Burkard spore traps (air volume 14.4 m^3/day; www.burkard.co.uk). The number of sporangia was assessed as spores/m^3/hour and summarised to daily values, 24-24 hours. The spore traps were mounted in the middle of pure stands of cv. Bintje and Oleva (34 x 34 m) in Denmark and in a variety mixture in Norway.

Trap plants of cv. Bintje were placed close to the spore trap every day. In Finland there was no spore trap but trap plants were placed close to a variety mixture. Two sets of trap plants were exposed every day:

1. Trap plants exposed from 15–15 (next day). Dry incubation in growth cabins.
2. Trap plants exposed from 8-15. Wet incubation. Plants were misted with water before incubation in growth cabins to promote infections of sporangia on the leaves.

At 8 o'clock in the morning, the 15-15 trap plants will be wet from natural dew formation (at least on some days). At that same time, the 8-15 trap plants will be put out in the field – taken dry from the greenhouse. If infections happen during morning hours when sporangia release overlaps with few droplets still left in the canopy, new infections are expected on the 15-15 trap plants, but not on the 8-15 trap plants. The 8–15 trap plants are misted with water before taken to the growth cabins at 15:00. If sporangia are able to survive from dispersal in the morning hours until misting at 15:00, infections are also possible on the 8-15 trap plants.

Trap plants were exposed every day in Denmark from 26 June to 20 August, in Norway from 10 to 31 July and in Finland from 23 August to 7 September. All trap plants were incubated after exposure for 7 days in growth cabins at 18°C and assessed for attack of late blight as severity (%).

Mizubuti *et al.* (2000) showed that solar radiation plays an important role in sporangia viability, and they developed several models for standard germination rate – here we call it "survival rate of sporangia" (Y = 0.79exp-1.21* Global radiation [W/m^2] *0.0036). By combining the data for sporangia catchments with the model for survival rate this expresses the number of viable sporangia from this load of airborne sporangia:

Number of viable sporangia = Daily number of sporangia * (0.79exp-1.21* Global radiation [W/m²] *0.0036)

The 8-15 trap plants were put out dry at 8:00, and then misted with water at 15:00, just before incubation. If the sporangia survive until 15:00 they will give infections on these trap plants. Hence, there should be a better correlation between the infections in the 8-15 trap plants and the calculated number of viable sporangia than the daily number of sporangia.

Global radiation is for the hour from 11-12, Danish normal time. The daily numbers of sporangia were normalised using square root.

Outputs from the simulation model LB2004 (Andrade-Piedra, 2005) using a set of parameters for latency period, lesion growth rate and sporulation rate obtained in 2003 were compared with the biological data from the field trial plots. Establishment of new micro colonies according to LB2004 was expected to correlate with infections on the trap plants.

Results and discussion

Sporangia production

In all the Nordic countries, weather was very hot and dry during July (Schepers & Hansen, these proceedings and Figure 1).

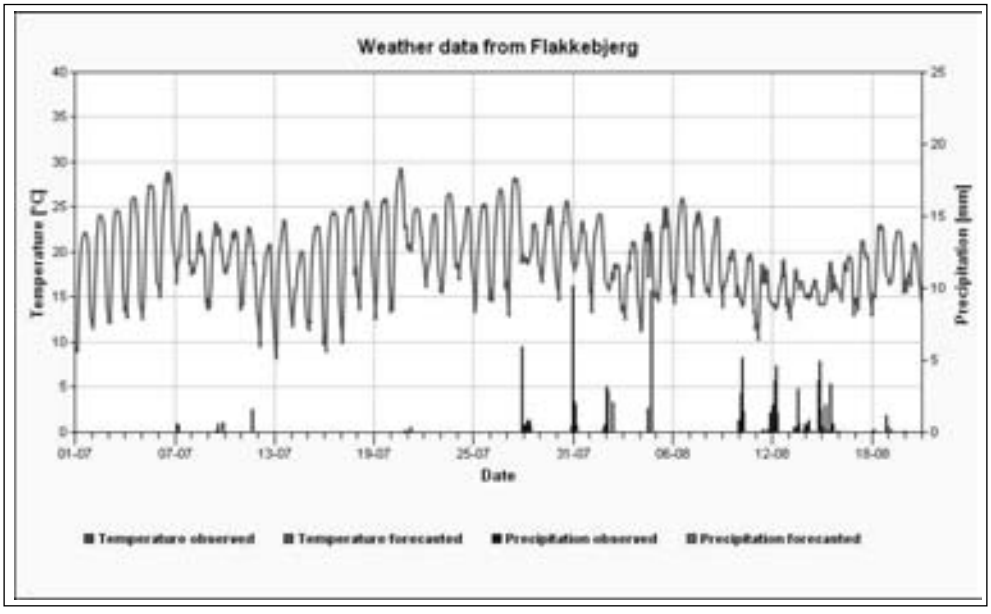


Figure 1. *Flakkebjerg, DK, 2006. Hourly temperature and precipitation from 1 July to 20 August, 2006 at Research Centre Flakkebjerg.*

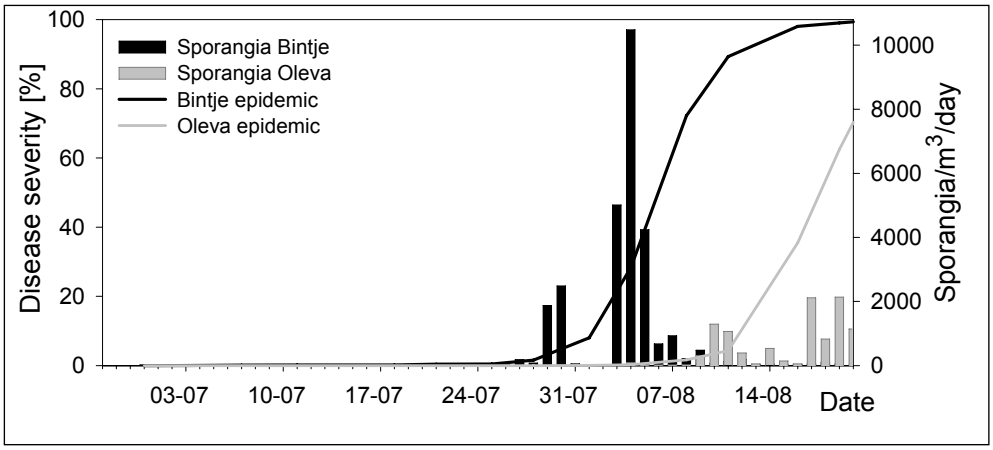
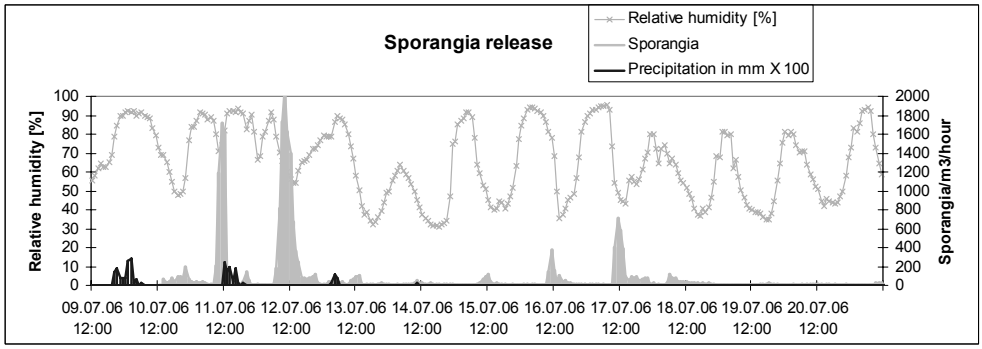


Figure 2. Flakkebjerg, DK, 2006. Disease development in Bintje (black line) and Oleva (grey line), and daily number of sporangia caught in Burkard spore trap mounted in Bintje plot (black bars) and Oleva plot (grey bars). Data from Research Centre Flakkebjerg, 2006. Related weather data are shown in Figure 1. The Bintje plot was artificially inoculated on 5 and 11 July. The Oleva plot was artificially inoculated on 11 July.

In ***Denmark*** the change into warm and wet weather in the last week of July started epidemic development in cv Bintje (ware potato, susceptible) and 10 days later in cv Oleva (starch potato, moderate susceptible) (Figs. 1 and 2). At Flakkebjerg viable sporangia were recorded in lesions of the potato plants during most of July. Low amounts of sporangia were recorded in the spore trap, but infections on trap plants were totally absent until the end of the month when the rain came (Figure 5). In ***Norway*** artificial inoculations started the disease development already in July. Significant amounts of sporangia were caught in the spore trap on 11, 12, 16 and 17 July. Accordingly, infections were recorded on the trap plants, but at only very low level on 16-17 July. On 11 July there were more infections on the 8-15 trap plants that were incubated wet than on the 15-15 trap plants that were incubated dry. This was a day with low global radiation, high relative humidity and some rain. However, on 12 July - the day with the highest sporangia release - there were fewer infections on the 8-15 trap plants that were incubated wet than on the 15–15 trap plants that were incubated dry. This indicates that most of the sporangia released that day did not survive until the afternoon, probably due to the deleterious effect of the high global radiation that day.



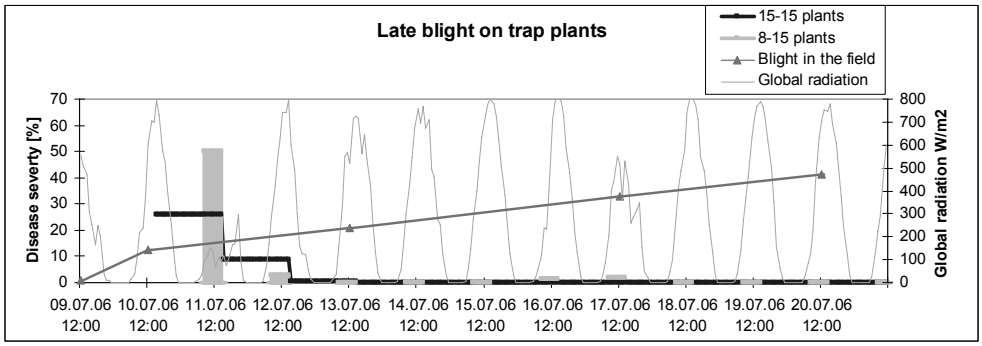


Figure 3. Ås, NO, 2006. Top: Sporangia in Burchard spore trap (sporangia/m³ air/hour) mounted in a variety mixture plot at Bioforsk Plant Health and Plant Protection Division, Ås, Norway and precipitation [mm x100] (Y2-axis). Relative humidity [%] at 2 m high on Y1-axis. Bottom: The per cent infection on exposed trap plants, 15-15 trap plants incubated dry (horizontal line) and 8-15 trap plants incubated wet (bar). Per cent attack of late blight in the field (line and scatter). Hourly global radiation [W/m²] (line)

In ***Finland*** a very blight favourable period was recorded during the period 25 August to 7 September. Both the 15-15 and the 8-15 trap plants were heavily infected on several days during this period. During a very favourable blight spell, the levels of infection were higher on the 8-15 trap plants compared to the 15–15 trap plants (Figure 4). The opposite was also recorded. The days with infection on trap plants corresponded well with calculation of the weather-based risk of sporulation (HSPO) during the previous or pre-previous night (Figure 4).

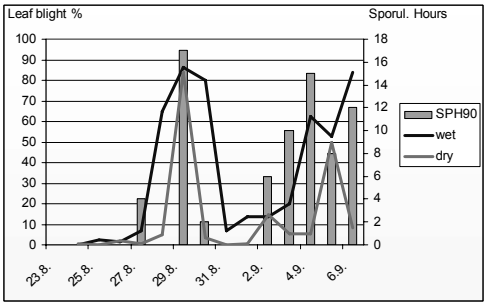


Figure 4. Jokioinen, FI, 2006. Per cent infections on exposed trap plants. Dry = 15-15 trap plants incubated dry, and Wet = 8-15 trap plants misted with water before incubation. SPH = HSPO risk index with use of relative humidity threshold of 90%.

Time of infection

Based on the results from trap plants and weather variables on hourly basis, the time of infection in the Danish experiment was evaluated visually based on graphs as given in Figure 5. The relative humidity exceeds 90% at 19:00 on 28 July and it stays high until next morning at 8:00 - a total of 13 hours. As all temperatures in the humid hours were higher than 10°C, the risk index according to the HSPO model is 13, indicating high risk of sporulation (see Hansen *et al.*, 2006 for definition of HSPO). Leaf wetness was recorded for 8½ hours (blue squares in Figure 5). A fast shift in humidity starts at 8:00. At 9:00, leaf wetness was at zero level. As leaf wetness is measured on a sensor fully exposed on the METOS weather station at 1½ m height, most probably some dew droplets remains in the canopy until 9:00-10:00 (Hansen, 1992; Hansen, 2002). This is time enough for germination by sporangia detached from the lesions and landing in droplets at approximately 8:00–9:00. The trap plants 15-15

were incubated dry, and after 7 days the disease severity was recorded to be 62.5%. The trap plants 8–15 were put out in the field at 8:00 with dry leaves. As germination requires free water, infections could only take place at 15:00 when these plants were misted with water before incubation. When no infections were found after 7 days of incubation, it is most likely that sporangia did not survive the prevailing weather conditions that morning.

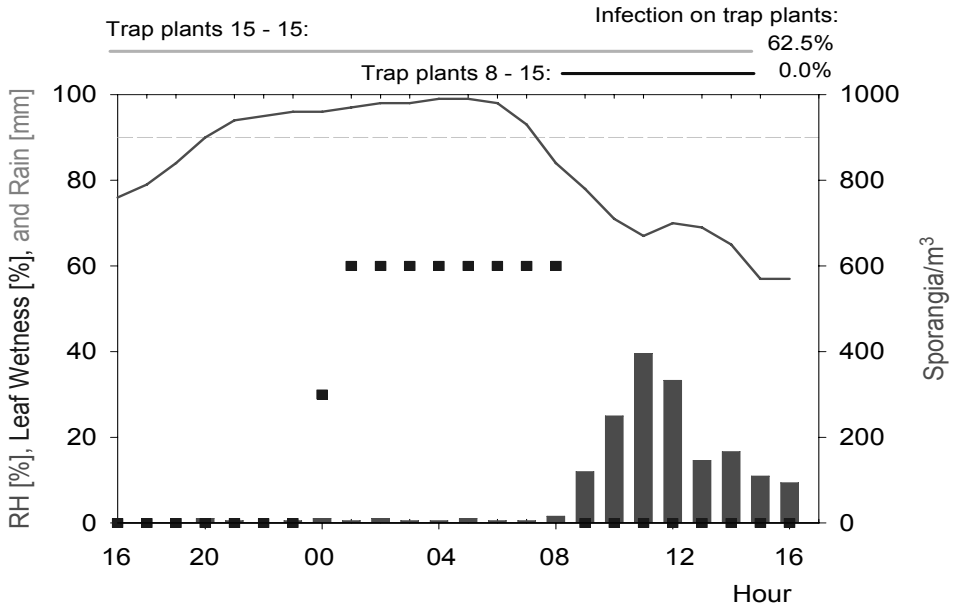


Figure 5. Flakkebjerg, DK, 2006. Infection on trap plants related to weather variables and number of aerial sporangia caught by a Burkard trap on hourly basis. X-axis: Hours from July 28 at 16:00 to July 29 at 16:00. Y1-axis: Relative humidity [%] (red line), Leaf wetness [minutes/hour] (Blue squares) and rain [mm] (grey vertical bars). Y2 axis: Sporangia caught in Burkard trap/m³/hour. Above the graph: Infection on trap plants as severity [%] on trap plants exposed from 15-15 (light blue, at top and long line) and on trap plants exposed from 8 – 15 (dark blue, lower and short line) The 8-15 trap plants were misted with water before incubated. The 15-15 trap plants were incubated dry. Rh, leaf wetness and rain was measured at 1.5m height, with a METOS weather station, placed approximately 500 from the plot (see text for further explanation).

For each day a graph was produced similar to Figure 5. In the Bintje plot, during the period 23 July to 4 August (12 days), on three days both sets of trap plants were attacked higher than 3% severity, on four days the trap plants 15-15 was attacked but not the 8-15 plants, on five days both sets of trap plants were not or only slightly attacked (<3%). A similar result was observed in the Oleva plot during an 18-day-period starting on 2 August. Infections on the 8-15 trap plants typically happened during cloudy weather conditions delaying the liberation of sporangia from the sporangiophores, or additionally including rain. Probably the sporangia survive until misting and incubation after midday. In Norway, Denmark and Finland it happened that infections occurred on the 8-15 plants but not on the 15-15. This may be due to situations when sporangia were released during morning hours, with no dew left, and therefore no infections on the 15-15 trap plants. If the weather is cloudy, then the sporangia may survive until the afternoon, and they may infect the trap plants 8-15 that are misted with water before incubation. On days with calm weather and no rapid drop in humidity due to

clouds and /or drizzle, sporangia stayed attached to the lesions until next time the humidity dropped rapidly. This phenomenon of delayed release has been described before (Bashi *et al.*, 1982; Schlenzig *et al.*, 1998; Hansen, 2002).

Survival of sporangia and simulation models

From the data obtained in this project, survival of the dispersed sporangia seems to be an important bottleneck for disease development. Mizubuti *et al.* (2000) showed that solar radiation plays an important role in sporangia viability. Only 1 h of direct exposure to sunlight decreased germination of sporangia by 95% and viability decreased within 15 min. On overcast days, germination of sporangia was not reduced substantially after 3 h of exposure. We applied one of the survival models developed by Mizubuti *et al.* (2000) to our data. This resulted in an improved correlation between sporangia catchments and infections on the 8-15 trap plants – considerably better than if only the raw sporangia catchments were used (Figure 6). The LB2004 simulation model simulated the late blight epidemic in Bintje very well (Figure 7) and the model simulated reasonably well the days when new infections happened on the trap plants, however not so well the magnitude of infection especially on the 15 – 15 trap plants in late July and early august (Figure 8). Extremely small amounts of sporangia (5-50/ day) were caught by the Burkard trap on 31 July, 1 and 2 August (see Figure 2). Heavy rain (17 and 9 mm) was recorded on 31 July and 2 August during the nights and combinations of delayed sporangia release and spread with rain splash might partly explain the inconsistency between the simulation of new infections and severity on the trap plants. The simulation model will be used for further studies of the obtained data as well as for further improvement of our DSSs on the Internet.

Implications for practice

These results have important implications for practice. A "risky day" provided by a weather-based DSS may be exceeded already at 9:00 in the morning, i.e. sporangia have germinated or even infected the plants and it may be too late to spray with a contact fungicide at 11:00. This means that reliable weather forecast data are important. Our data confirm that a mixture of sunshine and rain with suitable temperature are optimal conditions for sporangia formation, dispersal, survival and infection. The sporangia are probably released when the relative humidity in the boundary layer drops. During several days of calm, overcast or rainy weather conditions sporangia might build up in lesions without any liberation of the sporangia into the air at all. The first time the weather changes into more sunny or windy weather conditions this may trigger liberation of a huge amount of sporangia that were built up during the two to three previous days. In this way, many new infections may happen on days that were not characterised as a "blight weather day" according to for example Smith periods or sporulation hours (HSPO).

Survival models were applied to our data and resulted in an improved correlation between sporangia catchments and infections, especially on the 8-15 trap plants. However, for operational warning of late blight, sporangia catchments are not available. Next step will be to integrate the survival model with the HSPO model for sporulation. Those two together estimate the weather-based number of viable sporangia available. This model complex is driven by hourly data for temperature, relative humidity and global radiation – data that are available as forecast data.

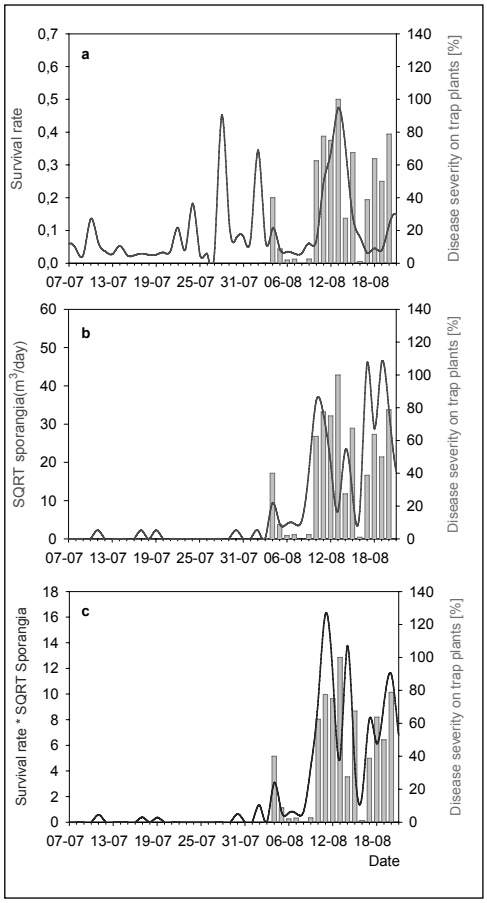


Figure 6.
a: Survival rate (red line) and disease severity on 8-15 trap plants placed in the Oleva plot, Flakkebjerg, DK, 2006.
b: Sporangia/m³/day caught in Burkard trap and disease severity as in a.
*c: Combining a and b - survival rate * sqrt sporangia/m³/day related to disease severity. This estimates the number of viable sporangia that can possibly infect the trap plants.*

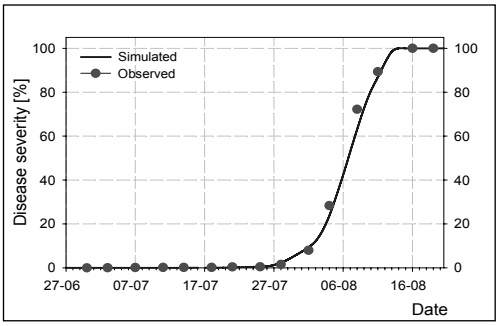


Figure 7. Simulated and observed disease severity in untreated Bintje plot at Research Centre Flakkebjerg, 2006. The plot was artificially inoculated on 28 June, 5 July and 11 July.

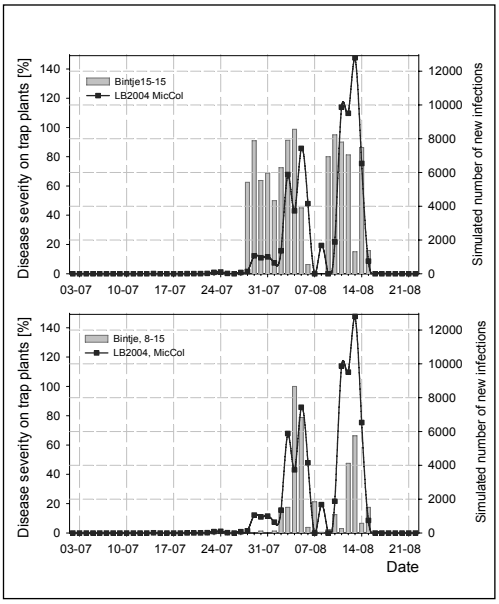


Figure 8. Disease severity on 15-15 trap plants (top) and 8-15 trap plants (bottom), Flakkebjerg, DK, 2006 compared with LB2004 simulation of new microcolonies. Trap plants were exposed in the centre of the field plot every day from 26 June to 15 August when severity reached 100%.

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Spread and Development of Late Blight Epidemics in Maine

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Summary
In Maine, potato late blight epidemics spread mainly from southeast to northwest. The degree of spread is influenced by the size and distribution of the initial inoculum. The host growth and fungicide applications have an influence on the spread dynamics. Reducing the risk of late blight losses can be achieved by specific late blight control targets, tactics and keys. These change through the season to reflect the changes in the host/pathogen interaction over the season. Presented are risk tables to quantify these variables and a proposed risk equation incorporating foci size and distance from the source of inoculum.

Keywords
Late blight, prediction, risk, *Phytophthora infestans*

Introduction
Potato late blight, caused by *Phytophthora infestans*, is one of the most destructive foliar diseases on potatoes and has been around over 150 years with much written on the disease over that time. In Maine, the potential for late blight to appear is predicted with severity values (Johnson, 2006; Krause *et al*., 1975). Severity values are based on hours of relative humidity above 90 percent and the average temperature during this period. Severity values accumulate when weather conditions are appropriate for the development of the pathogen. Once 18 severity values have accumulated from emergence, the first spray is recommended. Subsequent protective sprays are recommended based on additional severity value accumulation during the previous seven days (Johnson, 2006).

The historic focus on late blight has been on predicting the epidemic occurrence and scheduling fungicide applications. In effect, these are prediction and tracking of the epidemic. In Maine, efforts are being directed to prevention of a late blight epidemic as well as prediction and tracking of the epidemic. Spread of the late blight pathogen and subsequent infection and disease symptoms are contingent on appropriate conditions.

Late Blight Risk
During different periods of the growing season, late blight presents different risk potential and control activities target different aspects of the disease cycle. Late blight starts off presenting an extreme risk

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to foliage and a low risk to tubers. This goes to high, then to medium and finally to low foliage risk at the end of season. During the same period, the late blight risk to tubers goes from low to medium to high.

One focus of late blight prediction is risk. Late blight epidemic risk is a function of primary inoculum, disease distribution, secondary spread, and the effect of host growth. These are incorporated into a late blight risk table (tables 1 and 2). Included are damage potential (high, medium, low) to tubers and to foliage from late blight. If more than six late blight risk values have accumulated, the situation could be considered DIRE. If four to six late blight risk values accumulated, the situation could be considered RISKY. If less than three late blight risk values have accumulated, the situation could be considered OK.

Late Blight Control Target
Preseason and early in the season, the late blight control target is the primary inoculum. As the season progresses, the control target moves to reducing the rate of spread of the pathogen. If the pathogen spreads widely during the period of rapid plant growth the epidemic may no longer be controllable. Late in the season, the control target moves to not allowing the disease to reach maximum proportion.

Late Blight Control Tactic
Preseason, the late blight control tactic for reducing the primary inoculum is sanitation and keeping coverage on the new growth. This means timely and regular applications at an appropriate rate of material. As the season progresses, the late blight control tactic to reduce the rate of spread of the pathogen is to replace the eroded fungicide. Late in the season, the control tactic is tuber protection. This may include changing the protection material to target tuber protection or not allowing the disease to reach maximum proportion by early vine desiccation.

Late Blight Control Key
Early in the season, the late blight control key for keeping coverage on the new growth is timing of the protection material. Later in the season, the control key for replacing the eroded fungicide is timing and the rate of the protection material. As the season progresses, the control key for tuber protection is choice of the protection material. The late blight control target, tactic and key are listed in the tables 1 and 2.

Late Blight Prevention
One focus of prevention is primary inoculum. Effective prevention of a late blight epidemic starts with reducing primary inoculum to very low levels. The organism causing potato late blight overwinters in infected tubers, cull piles, and in infected volunteer plants. To address these issues, a seed screening program has been offered. Samples of seed are collected, incubated, and then evaluated for the visual presence of late blight infection. Discovery of late blight renders the seed unacceptable in the marketplace. Potato ground keepers, in the unusual year that they do occur, are chemically or mechanically removed. Maine has legislation dictating that cull piles be buried or covered by 10 June of each year. This law is aggressively enforced and reduces potential initial inoculum.

Late Blight Prediction
Size and distribution of late blight inoculum sources have a profound effect on the development of the epidemic. Local late blight epidemics develop differently depending on the size of the initial focus. Small-sized foci (fewer than 10 square meters) increase over several cycles affecting those plants near in proximity. Subsequent spread increases the size of the initial focus before distance dispersal

occurs (Hirst and Stedman, 1960). Medium-sized foci (between 10 and 40 square meters) rapidly increase in size and may soon distance disperse spores. Large-sized foci (more than 40 square meters) may distance disperse spores immediately. Small foci that are untreated or undiscovered can become medium-sized and eventually large-sized foci. The consequences for the area and the region are much more severe for untreated or undiscovered foci than known and treated foci. In treating late blight foci, the first decision to be made is whether to physically remove, by disking or other means, the portion of the field with late blight. This technique has been highly successful in the early and middle part of the growing season and should always be a consideration. The pathogen is already present and causing disease so the goal is containment. Removing the foci in a field in conjunction with chemical applications is far more successful than either alone. If seed-borne late blight is present in the field, moving up the stems and sporulating on the stems, there is no control. These plants and possibly a good portion of the field should be destroyed.

Late blight epidemics develop differently depending on the distribution of the initial foci. If the foci are distributed widely across a region, many small epidemics soon coalesce into a large-scale epidemic across the region. If the same number of foci are narrowly distributed in an area of the region, that area will develop a localized late blight epidemic. This localized late blight epidemic must then spread to the rest of the region before a large-scale epidemic occurs. If this first spread is early in the season and extensive, severe epidemics are to be expected. Distance from the source of inoculum has a role along with the size of the foci in late blight risk as proposed in table 3.

The direction from the inoculum source is as important as the magnitude of the distance from the inoculum source. Disease appearance follows the spore dispersal pattern which follows the wind patterns. In Maine, disease appearance and disease gradients appear from the south to the north (Figure 1). More specifically, spread occurs from the southeast to the northwest. The real threat and increased risk is associated with inoculum sources to the southeast. On rare occasions isolated spread has occurred from the southwest to the northeast. Any spread to the south or to the west is short range dispersal. The arrows on the chart indicating direction and magnitude show a show southeast to the northwest wind direction and greater than average wind speed the days after rainfall events. These correspond to observed late blight spread patterns one latent period later (Figure 2).

There is inherently more risk associated with late blight appearing while the plants are actively growing. Potato plants can double their leaf area in five days or less when growing rapidly and this could leave half the leaf area unprotected (Johnson, 2006). Rapid epidemic development can occur during this growth phase. As the plants approach harvest, limited epidemic movement occurs as the foliage tends to be less susceptible to the pathogen.

Conclusions

There is effectively a zero tolerance for late blight in the Maine potato production system so effort is directed at prevention of late blight epidemics as well as prediction and tracking of the epidemic. Initial late blight epidemics are affected by the levels of primary inoculum. Localized late blight epidemics are affected by the distribution of primary inoculum sources and early spread dynamics. The risk of late blight spread is associated with the distance and direction of known late blight outbreaks. The amount of spread is affected by host growth.

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Table 1. Risk table for early season late blight

≤ 15/06 – 31/06	01/07 –15/07
+ 1 each	+ 1 each
LB present in Region last season	LB present in Region last season
LB present in Area last season	LB present in Area last season
LB present on Farm last season	LB present on Farm last season
≥ 18 Severity Values met	≥ 18 Severity Values met
Cull piles in area	Cull piles in area
Seed was cut and held for ≥ 3 days	
Seed was not treated with MZ	
LB present in Region	LB present in Region
LB present in Area	LB present in Area
+ 2	+ 2
LB present in Field	LB present in Field
+ 1 per day per event	+1 per day per event
Weather forced longer spray	Weather forced longer spray
interval than recommended	interval than recommended
SW field capacity > 0.95 %	
3 days in a row	
Situation:	
DIRE >6	DIRE >6
RISKY 4-6	RISKY 4-6
OK ≤ 3	OK ≤ 3
Damage Potential for LB:	
Foliage: Extreme	High
Tuber: Low	Low
Control Target:	
Initial Inoculum	Rate of Spread
Control Tactic:	
Coverage of new growth	Coverage of new growth
Replacing Eroded Material	
Control Key:	
Timing	Timing/Rate

Table 2. Risk table for middle and late season late blight

16/07 – 15/08	16/08 – 31/08	01/09 – ≥ 15/09
+ 1 each	+ 1 each	+ 1 each
	Harvest ≥ 5% skinning	
LB present in Region	LB present in Region	LB present in Region
LB present in Area	LB present in Area	LB present in Area
+ 2	+ 2	+ 2
LB present in Field	LB present in Field	LB present in Field
+ 1 per day per event	+1 per day per event	+1 per day per event
Weather forced longer spray	Weather forced longer spray	Weather forced longer spray
interval than recommended	interval than recommended	interval than recommended
	SW field capacity > 0.95 %	Rain events > 0.75 inch
	3 days in a row	Vine kill < 10 days to harvest
		With LB in field
		SW field capacity > 0.95 %
		3 days in a row
		Vine kill < 14 days to harvest
		Mean soil temp < 54°F
Situation:		
DIRE >6	DIRE >6	DIRE >6
RISKY 4-6	RISKY 4-6	RISKY 4-6
OK ≤ 3	OK ≤ 3	OK ≤ 3
Damage Potential for LB:		
Foliage: Medium	Medium	Low
Tuber: Low	Medium	High
Control Target:		
Rate of Spread	Rate of Spread/	Maximum Disease
Maximum Disease		
Control Tactic:		
Replacing Eroded Material	Replacing Eroded Material	Protection of Tubers
Protection of Tubers		
Control Key:		
Rate	Rate/Material	Material

Table 3. Risk value based on late blight inoculum source distance and size

risk = $e^{-1.6*km}$ + source size factor $[(1-e^{-1.6*km})*(e^{-1.6*km})]$

Foci size	Source Size Factor
<10 m^2	-1
10 – 40 m^2	0
37 and 900 m^2	area/900
>900 m^2	+1

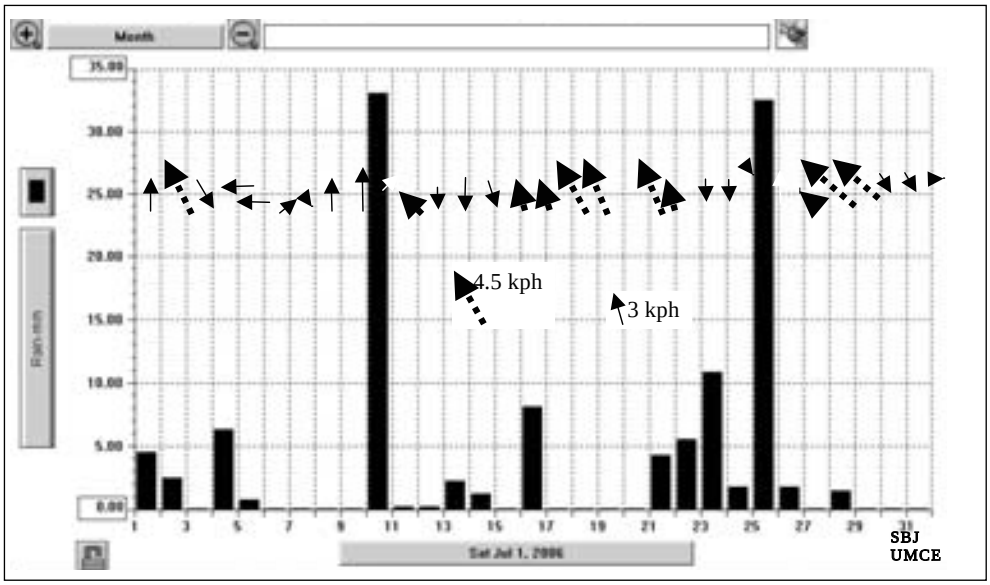

Figure 1. Rainfall, mean wind direction and magnitude for July, 2006 in the Littleton, Maine area

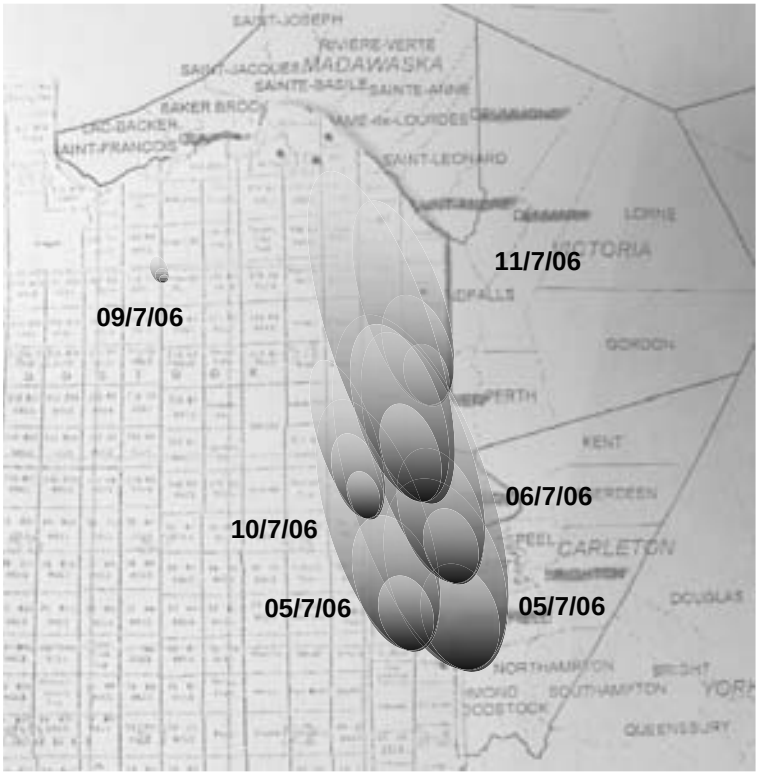

Figure 2. Observed late blight spread for July, 2006 with the darker areas epidemic foci.

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Multilocal field trials to test alternative products to reduce copper applications to control potato late blight in organic systems

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Summary
The main objective of those trials was to determine alternatives to massive copper utilization to control potato late blight (*Phytophthora infestans* (Mont.) de Bary) in organic systems.
To reach such a target, we first performed a screening of candidates products and additives under controlled conditions in the laboratory. Thereafter, the most promising products were tested in the field in 2006. Those trials were set up in three different sites, two sites in Belgium and one site in France. Herseaux (B) and Loos-en-Gohelle (F) are situated near by see level in an important potato culture basin with silty soil. Libramont is located at 500 m of altitude, far from any potato culture basin, with a sandy - loamy and stony soil.
The cultivar Ditta was used in Belgium while the cultivar Juliette was planted in France. Their resistance to foliage late blight is, respectively, medium and medium-high. In total 8 modalities were compared. The products were applied in accordance to the advice of the local late blight warning system. The control was sprayed, at each advice, with 3kg/ha of copper sulphate (Bordeaux mixture). We tested two additives to Bordeaux mixture, used at the 3kg/ha rate as well, the first one is a short chain amino-acid extract, used to enhance rainfastness, while the other one is an hydrogen peroxide stabilised with an organic molecule. This second product was used for its disinfectant effect added to the protection effect of copper sulphate. We also tested the efficiency of a formulation presenting a low copper concentration (Glutex CU 90 with 10% copper) and of an association between a potassium phosphite and a copper tallate (Solucuivre with 5% copper). Those two components were also evaluated separately. Finally, we tested a product containing rhamnolipid biosurfacant (Zonix) supposed to physically destroy the zoospore's membrane.
The 2006 climatic conditions were very particular. June and July were very dry while August was very wet with optimum late blight development conditions. The disease development was very slow during July and radically increased during August.

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The two additives tested didn't improve the efficiency of Bordeaux mixture. The association of potassium phosphite and copper tallate gave good results. Separately, both products gave good results excepted the copper tallate in the site of Libramont. The formulation with low copper concentration as the rhamnolipid biosurfacant allowed an as good crop protection as the Bordeaux mixture modality. Given the particular climatic conditions of 2006, those products have to be tested an additional year to confirm their efficiency.

Keywords
late blight, organic, pesticide, fungicide, phosphite, copper, rhamnolipids

Introduction
Recently, the European Union, in the regulation 2091/92, imposed to reduce the amount of copper application to control fungal diseases in organic production. It imposes to reduce the amount of copper metal to 6 kg per year and per hectare since January 2006. The point is that, today, there are no known effective alternatives to copper to control potato late blight in organic systems. So, the VETAB project, co-financed by the European Union in the INTERREG III Wallonie-France-Flandre program, aimed to explore the new control opportunities. To do so, promising products have been tested in field trials in three locations distributed in France and Belgium.

Material and Method
The products tested were the most promising identified in laboratory tests performed in 2006 (Dupuis *et al.*, 2007).

- Bordeaux mixture (3 kg/ha), widely used by the growers to control fungal diseases in organic farming (Tomlin, 2000), was used as positive reference. Its fungicidal activity on the spore is based on the accumulation of free copper ions in the cell till a toxic concentration and the formation of complexes, with sulfhydryl, carboxylic and hydroxyl groups, resulting in a non-specific denaturation of enzymes of the respiration chain (Schwinn *et al.*, 1991). It was tested at the dose of 3 kg/ha to correspond to the new EU prerogatives.
- Glutex CU 90 (4 l/ha) is a copper based product including an amount of 10% of copper.
- PK2 (2 l/ha) is a potassium phosphite. The efficacy of phosphite-based compounds on oomycete has been reported in the literature. Cohen and Coffey (1986) report the studies of Thizy *et al.* (1978) showing that various salts of phosphorous acid display activity against various oomycetes. The studies of Erwin and Ribeiro (1996) reported by Miller *et al.* (2006) confirmed that phosphites could be used to control 19 species of *Phytophthora*. Our previous studies in the laboratory (Dupuis *et al.*, 2007), proved that PK2 was as efficient as Bordeaux mixture to control late blight on inoculated potato detached leaves.
- Solucuivre (2 l/ha) is a copper tallate including an amount of 5% of copper, this product also presented an efficiency as good as Bordeaux mixture (Dupuis *et al.*, 2007).
- Ecoclearprox (3 l/ha) is an hydrogen peroxyde stabilised with organic molecule. Ecoclearprox wasn't efficient, alone, in the laboratory trials (Dupuis *et al.*, 2007). Nevertheless, we decided to test its association to Bordeaux mixture in the field to evaluate the efficiency of a combination of protection and disinfectant products.
- Zonix (0,5 l/ha) is a product containing rhamnolipids considered as biosurfactant that could explode the zoospores membranes. The Zonix didn't allow to reach a good level of protection in the laboratory trials (Dupuis *et al.*, 2007). Nevertheless, in other unpublished laboratory trials, Zonix tended to be efficient. So, we decided to test it in the field in 2006 to check those observations.
- Finally, the effect of Splinter (0,65 l/ha), a mixture of short amino-acids chain aiming to enhance product rainfastness, was tested in association with Bordeaux mixture.

Field trials were set up in 3 different locations, two in Belgium and one in France.
- In Loos-en-Gohelle, situated in the North department of France, near by see level, in an important potato culture basin with silty soil.
- In Herseaux, located in the Hainaut province of Belgium, 50 km, at the North-East of Loos-en-Gohelle, in the same potato culture basin.
- In Libramont, situated in the Luxembourg province of Belgium, 200 km at the East of Loos-en-Gohelle. Libramont is located at 500 m of altitude, far from any potato culture basin, with a sandy - loamy and stony soil.

Table 1: List of the products tested, in 2006, in different locations

Product	Loos-en-Gohelle	Herseaux	Libramont
Bordeaux mixture	X	X	X
Untreated	X		
Glutex CU 90	X	X	X
PK2	X	X	X
Solucuivre	X		X
PK2+Solucuivre	X	X	X
Zonix			X
Ecoclearprox + Bordeaux mixture		X	X
Splinter + Bordeaux mixture		X	X

The experimental scheme was a 4 fully randomized blocks device. The elementary unit included 60 plants. Blocks were separated with infecting rows planted with the late blight sensitive cultivar Bintje. The role of those rows was to homogenize the late blight infection distribution in the trial by placing each plot at the same distance of a strong source of potential infection. The cultivar Ditta was used in Belgian trials while the cultivar Juliette was planted in France. Their resistance to foliage late blight is, respectively, medium and medium-high according to the www.europotato.org website. These trials were managed under natural inoculation except in Libramont were artificial inoculation has been performed. Nevertheless, this inoculation has been performed in July and couldn't succeed due to the dry and hot weather. The crop was managed in accordance with organic farming rules. This means that the copper based products were applied according to a maximum amount of 6 kg native copper per hectare and per year. The products were applied in accordance to the advice of the local late blight warning system.

The 2006 climatic conditions were the same for the 3 regions. The month of May and August were very wet and the month of June and July were very dry. The wet conditions of May and August were particularly favourable to late blight development.

The observations were performed during the whole growing season and the notations began at first symptoms apparition. Eight observations were performed in Belgium (from 21 of June till 22 of August for Herseaux and from 11 of August till 5 of September for Libramont) while 5 observations were performed in Loos-en-Gohelle (from the 6 of July till the 3 of August). Each trial site used his proper notation scale. In Loos-en-Gohelle the French scale, ranged from 0 to 10, was used. In Herseaux, the Wageningen scale, ranged from 10 to 0, was used, while in Libramont, the Euroblightscale, ranged from 0 to 100, was used. All those scales are correlated to a percentage of foliage destruction by the disease.

Then, we calculated the relative stAUDPC (standard Area Under Disease Progression Curve divided by trial duration since first symptoms apparition). A two ways ANOVA, including the block (random factor, 4 levels) and the product (fix factor, 6 levels for Loos-en-Gohelle and Herseaux, and 8 levels for Libramont) factors was performed on the data of each location. Thereafter a multiple mean comparison objects was made using the Student-Newman-Keuls method (Dagnelie, 1975) to segregate the different objects.

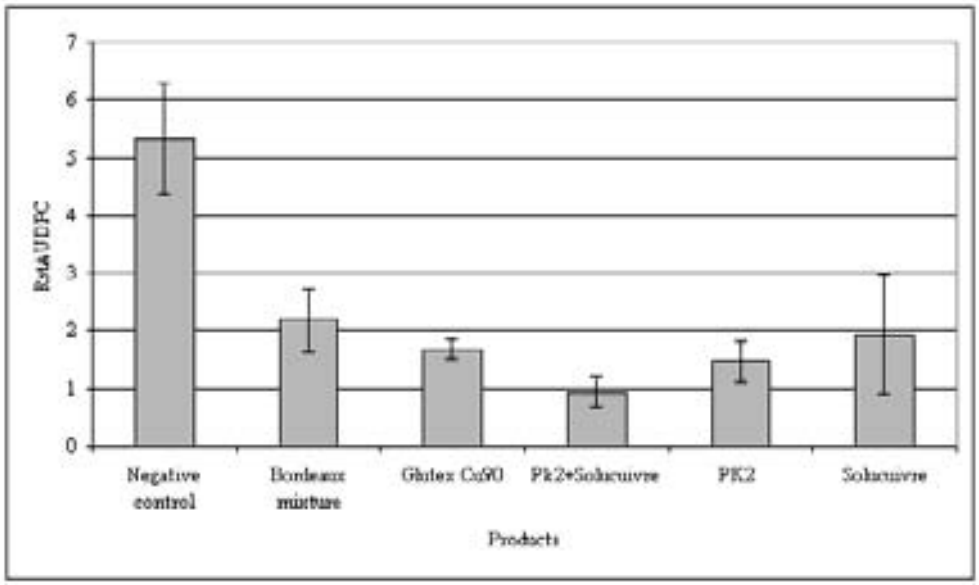

Figure 1: Products performances against late blight infection development quantified through the RstAUDPC index, in Loos-en-Gohelle's field trial.

Results

The two ways ANOVA allowed to identify significant differences among the products tested in Loos-en-Gohelle ($F(5,15) = 91.65$; $p < 0.001$). Using a multiple mean comparison, all the products were compared to the positive and negative controls: Bordeaux mixture and untreated object (Figure 1).

Glutex Cu 90 ($p = 0.787$), PK2 ($p = 0.482$), Solucuivre ($p = 0.987$) and PK2 + Solucuivre ($p = 0.056$) offered a protection level similar as or higher than Bordeaux mixture. We notice that the association of PK2 and Solucuivre present the lower infection values. Nevertheless, the efficiency of the association of both products couldn't be distinguished from the PK2 ($p = 0.749$) or the Solucuivre used alone ($p = 0.179$).

Loos-en-Gohelle was the only location were an untreated plot was installed. We observed that all the modalities tested significantly limit late blight disease development ($p < 0.001$).

The two ways ANOVA of Herseaux couldn't allowed to identify significant differences among the products tested ($F(5,15) = 1.37$; $p = 0.291$) (Figure 2).

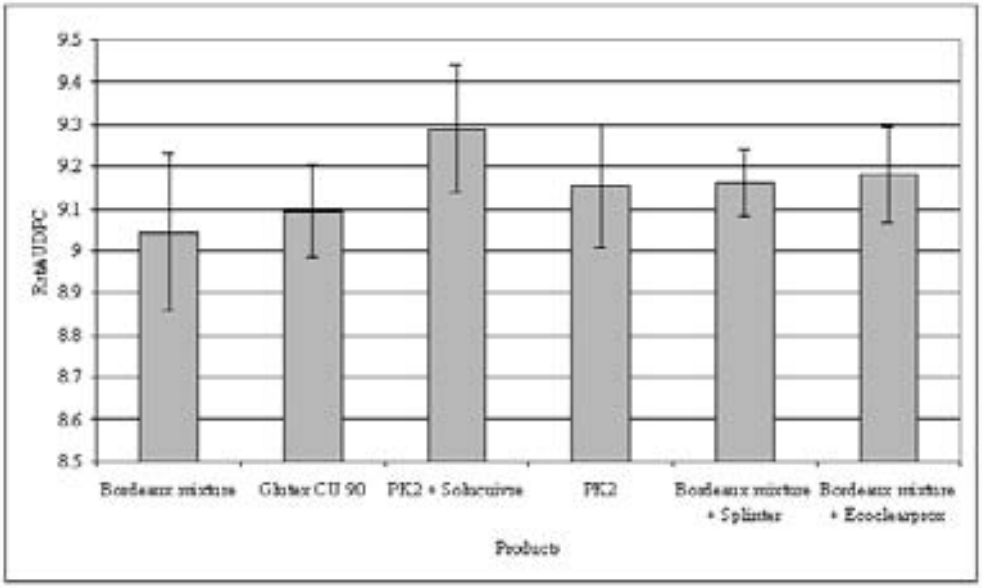

Figure 2: Products performances against late blight infection development quantified through the RstAUDPC index : Herseaux's field trial.

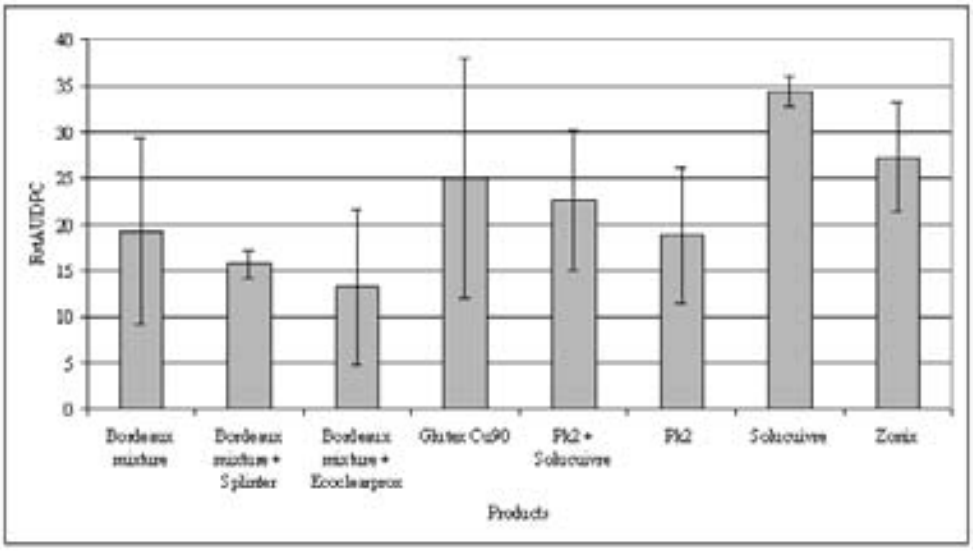

Figure 3: Products performances against late blight infection development quantified through the RstAUDPC index : Libramont's field trial.

Nevertheless, we didn't observe any significant difference between the different products tested and Bordeaux mixture ($p > 0.5$), excepted for Solucuivre treatment ($p = 0.058$).
Bordeaux mixture + Splinter ($p = 0.011$) and Bordeaux mixture + Ecoclearprox ($p = 0.003$) were significantly more efficient than Solucuivre.

Discussion and Conclusion

The late blight pressure was very high in 2006. The symptoms developed rapidly during August. In those conditions, it was necessary to repeat the treatments to renew foliage protection. It is possible that the products efficiency has been affected by the consecutives rain events. However, Loos-en-Gohelle results hasn't been affected by this phenomena as the observations ended at the beginning of August.

Glutex CU 90 presented, in the three trials, an efficiency similar to Bordeaux Mixture with a reduction of 30% of the amount of sprayed copper. Solucuivre was tested in two locations, this product allowed to reduce the copper amount by more than 80%. However, in one trial location, this product seemed to be less effective than Bordeaux mixture.

The two additives tested, Ecoclearprox and Splinter, didn't enhance significantly Bordeaux mixture efficiency. These results confirmed the laboratory results (Dupuis *et al.*, 2007).

PK2 and Zonix were the 2 copperless products presenting a level of protection close to the protection level of Bordeaux mixture. PK2 has been tested in three locations and confirmed its efficiency. It seems that the addition of Solucuivre to PK2 doesn't reinforce PK2 protection level. Zonix has only been tested in one location and couldn't be compared to an untreated control, the efficiency of this product has to be confirmed.

Acknowledgements

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Multi scale modeling of infection pressure from *Phytophthora infestans.*

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Summary
Aerial dispersal of inoculum is the primary means of movement for many plant diseases, but as influx of inoculum depends on a complex interplay of population biological, atmospheric and spore survival processes, it is difficult to predict. This research aims at building tools for such prediction. BLIGHTSPACE is a spatio-temporal model (parameterized for potato late blight) that has been developed and utilized to study the progress of epidemics in individual fields and networks of fields. Simulations were recently made and compared to independent data, collected in field trials on the spread of two genotypes of *Phytophthora infestans* in five potato cultivars in the Netherlands. In addition, two different atmospheric dispersion models were developed to provide long-range transport of spores within BLIGHTSPACE. Numerical results compared favorably with experimental data. A further sub-model for the survival of spores during long-range transportation has been added. Integration of these sub-models has produced an aerobiological 'add-on' for decision support systems and a multi-scale epidemic model for investigating various (spatial) strategies for the deployment of host resistance.

Keywords
Phytophthora infestans, spores, dispersal, survival.

Introduction
The spread of pathogen inoculum to uninfected hosts is critical to the spatio-temporal development of plant disease epidemics. Improved computer simulation of spore transport in heterogeneous landscapes could lead to an increased understanding of the epidemiology of many aerially transmitted

diseases. An increased understanding could in turn lead to new plant disease management strategies that rely more on information and less on insurance sprays. It is the long term aim of our research to develop and use a multiple scale epidemiological model for potato late blight to investigate (in a spatial context) operational and strategic issues pertaining to disease management. An enhanced understanding of the spatial aspects of epidemic development at the field and regional scales could lead to the identification of new strategies for the regional management of potato late blight.

Simulating potato late blight on plants and in fields

BLIGHTSPACE is a spatially explicit, age-structured, integro-difference equation model that was developed to simulate general (blanket) and focal (developing from a point source) epidemics of *P. infestans*, and to explore the effect of heterogeneous genotype mixtures on the development of disease. The model simulates the life cycle of the pathogen, the growth of the potato host plant, environmentally dependent host-pathogen interactions, fungicide applications and the temporal and spatial development of general and focal late blight epidemics for various scales and patterns of host genotypes and with various different dispersal kernels (within field transport). Thus, the novel contribution of BLIGHTSPACE is its ability to model spatial relationships in the potato late blight pathosystem and it has already been used to investigate the effects of different scales and patterns of host genotypes on the development of focal and general epidemics (Skelsey *et al.*, 2004). Observed data for validation of this model came from field trials with five potato cultivars in the Dutch location of Wageningen in 2002 and 2004. Epidemics were initiated using two different isolates. The number of replications was three. These data had not been previously used for estimating model parameters. Predefined performance criteria were met in 80 % of the epidemics, demonstrating that the model is able to translate measured resistance components, weather data and initial conditions into realistic disease progress curves. Two examples of observed and predicted epidemics are given in Fig. 1.

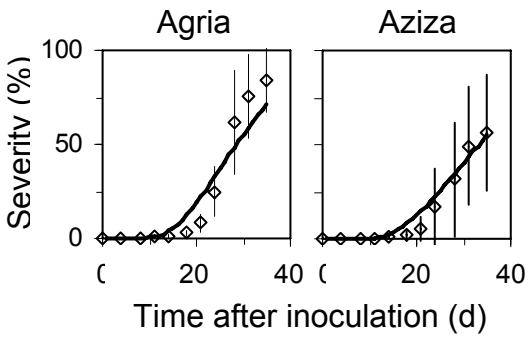

Figure. 1. Observed (diamonds) and predicted (continuous line) disease progress curves of potato late blight epidemics under field conditions in Wageningen (NL) in 2002. The simluated disease progress curves were obtained with the model BLIGHTSPACE. Vertical lines represent the standard deviation of the observed mean blight severity.

Simulating potato late blight in heterogeneous landscapes

BLIGHTSPACE can also be used to generate larger landscapes that contain networks of host fields, where each field undergoes local epidemic and host development as described in the previous section. In such a virtual environment, connection of fields through the dispersal of spores necessitates the use of a long distance spore transport model as the simple, probabilistic dispersal kernels used for within field transport are no longer suitable at the larger, regional scale. Development of such a model requires an amalgamation of knowledge on the life-cycle of the disease, atmospheric physics, and the interaction of the spore with the environment. Before model construction could begin, a very fundamental question had to be addressed – how many spores are a threat to a potato crop? 'Folk wisdom' maintains that a single spore is all that is required for an epidemic to take place and as no dispersal model is accurate to the level of single particles, it became important to find out the range of spore inputs of importance to the pathosystem. BLIGHTSPACE was used to determine the sensitivity of the late blight pathosystem to spore inputs; the yield (t(DM)/ha) response of potato

crops to spore inputs was investigated under the influence of variety properties and various fungicide management regimes and with 10 different years of meteorological data as input. Approximately 5,000 epidemics were simulated; the results indicating that contrary to folk wisdom, some resistant scenarios were able to tolerate fairly high levels of spore influx, suggesting that there was scope for simulating long distance dispersal of spores with resistant cultivars but not for some of the highly susceptible varieties which were extremely sensitive to spore input. Fig. 2 shows the yield response for a resistant cultivar under four different fungicide management regimes:

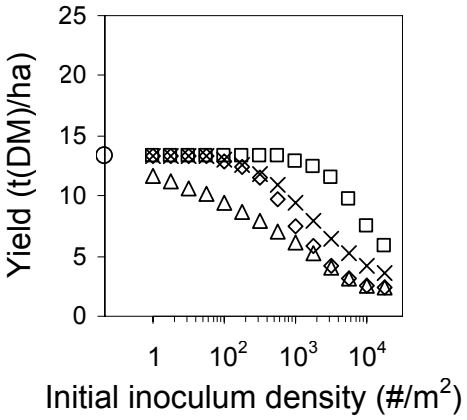

Figure. 2. Relationship between final yield and initial inoculum density in simulated potato late blight epidemics in a late/susceptible cultivar. Four fungicide regimes are compared: □ = adaptive, × = 7 day fixed schedule, ◊= adaptive with the first application missed, and Δ = no applications. ○ = final yield in a crop with no disease. Each data point represents an average over 10 years of meteorological input data.

Two different models for the dispersal of spores from a low-level release were developed. The first was a numerical 'quasi-Gaussian' plume dispersal and deposition model. It offers advantages over other Gaussian plume models for spore dispersal as it offers a more physically realistic representation of vertical diffusion. The second was a fully analytical Gaussian plume dispersal and deposition model which has the advantage of modest computing requirements. Both models were tested by calculating expected spore concentrations and assessing the goodness-of-fit with experimental data, where spore concentrations were measured above a potato crop at up to 100 m from a point source of *Lycopodium clavatum* spores during 10 minute release sessions (Spijkerboer *et al.*, 2002). Fig. 3 shows that numerical results compared very favorably with experimental data for the quasi-Gaussian model, and to a lesser degree for the fully analytical plume model:

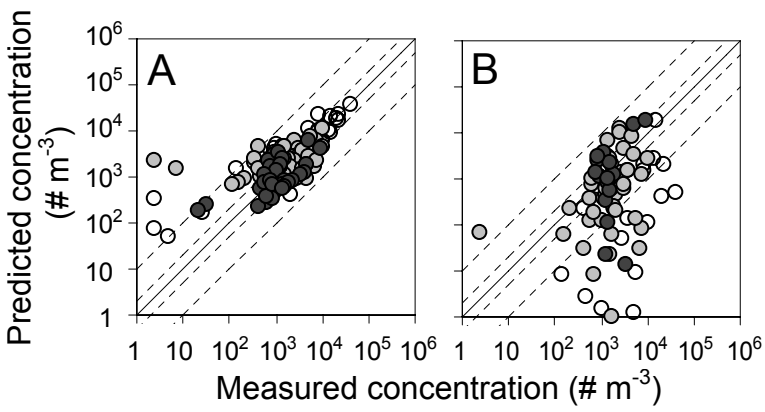

Figure. 3. Predicted versus measured spore concentrations. The outer dashed lines mark the limits of a prediction error of factor 10, the inner dashed lines a prediction error of factor 2, and the solid line is a 1 to 1 line. Data points are color-coded on a grey scale representing increasing distance from the point of spore release. Panel A shows results for the quasi-Gaussian plume model and panel B the analytical Gaussian plume model.

Spore survival

Solar irradiance, temperature and relative humidity are the key weather variables that influence the survival of *P. infestans*. In a recent study, solar irradiance was highlighted as the major factor responsible for reductions in sporangia viability (Mizubuti *et al*., 1999). The results of this study were used to create a simple model for spore survival during transportation.

Integration

Two different integrated versions of the aforementioned submodels were created. In the first, the numerical quasi-Gaussian plume model is used in conjunction with the spore survival model and various elements of BLIGHTSPACE to produce a simulation model that modifies the spray recommendations of standard decision support systems according to aerobiological aspects of the pathosystem. Field experiments are currently underway to test the efficacy of this model in increasing spray intervals. In the second, more computer intensive version, the analytical dispersion and spore survival models are fully integrated within BLIGHTSPACE to produce a multi-scale epidemic model for potato late blight in heterogeneous landscapes. This model is currently being used to develop new spatiotemporal strategies for the deployment of host resistance at field and landscape scales. This is becoming a particularly important area of research given recent advances in plant breeding.

Conclusions

The minimal modeling approach adopted in the development of these models means that they can be used to generate and test hypotheses about the epidemiology of plant diseases. Translation of new scientific knowledge into practical management strategies for the regional management of potato late blight is currently underway.

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Using a forecasting system to develop integrated pest management strategies for control of late blight in southern Chile.

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Summary
Potatoes are an important crop to Chile and are a vital part of the agriculture and economy. The major disease in Chile is late blight, caused by *Phytophthora infestans,* which spreads fast and attacks vast areas if weather conditions are favorable. The disease can affect plants at any growing stage depending on inoculum and weather. An integrated disease management plan for disease control considers the knowledge of the genetic characteristics of the pathogen population, the relative susceptibility of the host and the proper timing of chemical controls based on weather conditions favorable for the disease. Since 2003, the Agricultural Research Institute of Chile (INIA), associated with public and private institutions, has had in place the Project FIA-PI-C-2003-1-A-17 entitled "Use of forecasting for developing strategies of integrated management of potato late blight in southern Chile". The main objective of this study is to implement an integrated pest management for late blight based on a disease forecasting system. During the years of 2003-04 and 2004-05 250 *P. infestans* isolates were collected from lesions on potato plants and tubers in the southern Chile. The population was analyzed for mating type, sensitivity to metalaxyl, virulence, allozyme genotypes and DNA polymorphisms (SSR). Thirty one cultivars and selected advanced clones were test for relative susceptibility to late blight in three different locations. At the same time three forecasting models were evaluated, calibrated and validated for late blight management: Blitecast, Negfry and Dacom Plant Plus. The Blitecast model was calibrated and used to evaluate chemical control strategies and the interaction with cultivar susceptibility and agronomic management in 2006-07 season. All isolates collected were the A1 mating type, most were sensitive to metalaxyl with an EC50 of less than 3 ppm, and highly complex in pathotypes. According to the isozyme and SSR patterns, 7 and 12 genotypes were distinguished during the collecting seasons 2003-04 and 2004-05, respectively. However, for both seasons, one

genotype was predominate with a frequency of 67% and 54%. The potato cultivars Pehuenche, Amadeus and the clones R89063-84 and R 89063-59 showed good late blight resistance. Under the weather conditions of the crop season 2003-04 to 2005-06, Blitecast predicted late blight most accurately, warning of favorable disease conditions just before the first symptoms appeared. By using the Blitecast model it was possible to decrease the amount of fungicide applications by 40% and 15% for late blight control in dry land and irrigated crops, respectively, with susceptible cultivars.

Keywords
Late blight, *Phytophthora infestans*, forecasting, integrated pest management

Introduction

The potato crop in Chile is the third most important crop producing close to 1.3 million tons on 80,000 ha. Potatoes have imortant economic and social value, since production requires more than 91,000 potato producers with more than 5 million man-days of work, and a market value estimated in about US$300 million. The main production areas in the south are the Araucania and Los Lagos regions, producing about 62% of the national total. Potatoes are a basic food in the Chilean population, with consumption near to 54 kg per capita, contributing 94 calories and 3.5 g of protein to the daily diet. Diseases are one of the most limiting factors for growing potatoes, significantly reducing yield and quality. Pesticides are extensively used to control fungal diseases, insect attacks, and nematodes. Pesticides are expensive and represent risks for human health and threat to the environment. Integrated pest management (IPM) is considered the best approach to control pests and diseases in potato crops. Late blight is the most important disease affecting potatoes and is well distributed in the world. It spreads fast and attacks vast areas if the weather conditions are favorable (Secor, 2003). Late blight can affect plants at any growing stage, and when infection occurs early, can cause losses of 100%. Late blight affects all parts of the potato plant including leaves, stems, and tubers. The first symptoms appear in the lower leaves, as small dark-green water-soaked spots. Under high humidity conditions the spots expand quickly becoming irregular blighted areas, and if favorable weather conditions prevail, the entire plant is may die. If dry weather conditions are present following infections, the disease stops but will remain latent until the conditions again become favorable for disease. Brown purple lesions on stems may be formed by direct infection or via infected petioles. Tuber lesions are irregular and depressed and have a brown-purple color, and internal tissues have a brick-red coloration (Acuña and Torres, 2000). Epidemics of late blight depends on weather conditions during the growing season. *P. infestans* is well adapted to relative humidity near 100% and temperatures between 15 and 25° C. The zoospores need 12 hrs of free water to germinate and penetrate, and once infection occurs the disease spreads rapidly at 21°C (Agrios, 1997). *Phythophthora infestans* (Mont.) De Bary, the causal agent of late blight, like the potato, has been able to adapt to different climates and latitudes (Garlick *et al.*, 2002). New biotypes have arisen in the last decade making the control more difficult (Fry and Goodwin, 1997). *P. infestans* is heterothallic, with two mating types, A1 and A2. The A1 type has been predominant worldwide, and the A2 type was reported only in Mexico until late 80's. At the end of the 20th century *P. infestans* migrated from Mexico increasing the genetic diversity of populations of this pathogen in most of the continents. The first new genotypes were detected in Europe, then in South America, Africa and Asia and finally USA and Canada. The A2 type has become predominant and more aggressive (Stevenson *et al.*, 2001). In South America the group A2 has been reported in Argentina, Bolivia, Brazil, Ecuador, and Uruguay (Adler *et al.*, 2002; Crissman and Lizárraga, 1999). The first reports of disease caused by *P. infestans* in Chile are from the 1950's and it is thought that it originally came from Argentina (Anónimo, 1951). This had a great impact on the potato crop because most varieties cultivated in that period have almost disappeared, as actually happened to the red potato variety Coraila. Since that time, few, if any studies have been done to characterize the

late blight population in Chile. Fernandez (1979) studied *P. infestans* virulence in southern Chile populations, describing complex pathotypes able to infect five plant differentials. Riveros *et al.,* (2003) reported that isolates collected in northern Chile during 2001-2003 belonged to A1 mating type and US1 genotype, with several pathotypes of low aggressiveness. All isolates were highly resistant to, mefenoxam (> 300 ppm), presumably due to the continuous use of Ridomil (Secor, 2003).

Integrated Control

The main subjects to be considered in an integrated management program are prevention, use of healthy seed, avoiding the use of seed from areas affected by main diseases, elimination of volunteers and alternate host plants; cultural techniques such a crop rotations, surveillance for timely detection and elimination, the use of disease forecasting to determine when and where a disease becomes important; and finally, efficient use of fungicides. Implementation of an integrated control system involves several considerations such as ecology of the planting area, genetics of the pathogen population and economical considerations. All these factors influence the way a crop is managed. Economical factors are the most important for efficient control of a disease, especially in developing countries, because in these countries both the small scale farming (low yields) and the large scale farming (high yields) coexist (Mizubuti and Forbes, 2002). For example, in small scale farming, farmers do not control late blight or they use fungicides incorrectly. On the other hand, developed countries use pesticides to a great extent, and the important consideration is appropriate use, including knowledge of the effects, mechanisms of control, and more importantly, timing and frequency related to cultivar resistance, weather conditions, and the presence or not of initial inoculum (Schepers, 2002). Incorrect use of fungicides may cause serious economical, community, and environmental problems. In developing countries, investigations in epidemiology must optimize the use of fungicides without compromising profit and strong training in integrated management must be implemented. (Mizubuti and Forbes 2002)

Forecasting allows better disease control and more efficient use of fungicides (Secor, 2003). The forecast computer systems widely used are based on studies done by Hyre (1954) and Wallin (1962). These systems use precipitation data, temperatures (max. and min.) and the first signs of the disease. Forecasting methods have been improved by using software capable of predicting conditions that allow disease development and providing recommendations for control (Krause *et al.,* 1975; Stevenson, 1997). Integrated pest management (IPM) programs widely use forecasting components for more precise disease management, reduction of pesticides, increased food quality, reduced impact on the environment, improved knowledge of diseases and increased crop profit (Bimsteine and Turka, 2002; Fry *et al.,* 2002; Myint *et al.,* 2001; Chow and Bernard, 1999; Sedegui *et al.,* 1999; Johnson *et al.,* 1998; Stevenson, 1994).

Because weather conditions in southern Chile are highly variable, efficiency of late blight control is difficult. Fungicides are often applied in excess or efficacy is reduced because of the prolonged adverse weather. Under these conditions, resistance to fungicides can develop. Moreover, the A2 mating type is present in neighboring countries, and can enter Chile (Adler *et al.*, 2002). The use of forecasting may be a useful technique to manage this potential problem.

Since 2003, The Agricultural Research Institute of Chile (INIA), in association with public and private institutions, has had in place the Project FIA-PI-C-2003-1-A-17 "Use of forecasting for developing strategies of integrated management of potato late blight in southern Chile" . The main objective of this study is to implement an integrated management strategy for late blight based on a forecasting system. This project objectives are: to characterize the *P. infestans* populations in the area, to evaluate the relative resistance of the commercial potato cultivars and selected advanced clones from the INIA program to late blight, to implement a weather network in the Araucania and Los Lagos regions of Chile, covering some of the main potato production areas, and to test, calibrate and validate late blight forecasting models.

Materials and Methods

P. infestans characterization

During the period of 2003-04 and 2004-05, 250 *P. infestans* isolates were collected from lesions on potato plants and tubers in the Araucania (parallel 39°S) and Los Lagos (parallel 43°S) regions of Chile. A piece of leaftlet or tuber with symptoms was placed between two potato slices of the susceptible cultivar Bintje and incubated at 18°C for 9 to 14 days. After incubation, four pieces of Bintje infected tissue were transferred to a Petri plate containing either CV8 or rye B agar medium, both amended with antibiotics (Forbes, 1997). The isolates were incubated for four to seven days at 18°C in darkness. *P. infestans* isolates were transferred to CV8 or Rye B media and maintained at 18°C in darkness for further work.

Because the A2 mating type has not been found in Chile, a copy of each isolate was sent to G. Secor, North Dakota State University for mating type determination. The test was conducted by placing an agar plug containing mycelium on the edge of two rye B agar plates pairing with a similar size agar plug of known A1 and A2 isolates from NDSU *P. infestans* collection. After 15 days at 15°C, the plates were examined for oospore production (Tooley *et al.,* 1989; Miller *et al.,* 1998; Dorrance *et al.,* 1999).

In vitro metalaxyl sensitivity was assessed by comparing radial growth of *P. infestans* on Rye B media amended with five different concentrations of metalaxyl (0, 0.1, 1.0, 10, 100 µ/ml) to growth on metalaxyl free Rye B medium (Deahl, 1993). The test for each isolate and metalaxyl concentration was performed by placing a five mm plug from a 10 day old colony in the center of a nine cm Petri dish containing the amended medium. After 10 days of incubation at 18°C in the dark, two perpendicular measurements of colony diameter were taken for each plate. The percentage of relative growth on amended media versus control was scored and the EC50 was calculated as described by Miller *et al.* (1998).

Virulence assay was conducted for 214 isolates by inoculating detached leaflets of a differential set of plants with the 11 known major R genes for resistance. Craigs Royal cultivar was used for Race 0. Differentials, originally from the Scottish Crop Research Institute, Scotland, were obtained from NDSU. Race determination was based on compatible host-pathogen reactions seven days after inoculation with a 2 x10^4 zoospores/ml (Miller *et al.,* 1998). Lesions were read on a scale 0=no symptoms, 1= hypersensitive reaction, 2= necrosis without sporulation, and 3= necrosis and a sporulating lesion.

Each isolate was characterized for their respective of GPI and PEP isozyme pattern according to CIP (2001) protocol using potato starch gels. DNA polymorphism among isolates was established using the SSR Pi02, Pi04, Pi16, Pi26, Pi33, Pi56, Pi66, and Pi70. Primer sequences and PCR protocol for SSR were courtesy of Dr. David Cooke from the SCRI. Amplified PCR products were separated by standard DNA sequencing PAGE and silver staining method was used to visualize the DNA fragments.

Relative resistance to late blight

During the crop seasons 2003-04 to 2006-07, trials were carried out at three locations in southern Chile: Carahue in the Araucanía Región (UTM 18 Datum SAD 69 E 0643192 N 5716262) and Osorno (UTM 18 Datum SAD 69 E 0663882 N 5512594) and Castro, Chiloe Island (UTM 18 Datum SAD 69 E 0611319 N 5320286), both in the Los Lagos Region. The experiment was set as a randomized complete block design with 4 replications. The experimental unit was 45 plants distributed in 3 rows. Thirty-one potato cultivars and selected advanced clones were tested. During the season the plants were scored for late blight incidence and severity, estimating the percentage of total leaf area affected by the disease and calculating the Area Under the Disease Progress Curve (AUDPC) (Forbes and Korva, 1994).

Forecasting systems

The weather network for late blight was composed of ten evaluation sites: Carahue, Puerto Saavedra, Teodoro Schmidt, Pillanlelbun and Vilcun in the Araucania region and Rapaco, Osorno, Purranque,

Los Muermos and Castro in the Los Lagos region. The weather stations were connected via modem by mobile cellular phones using CDMA protocol.
Three Models for late blight forecasting were evaluated: Blitecast, Negfry and Dacom Plant Plus Online ((Hyre (1954); Smith (1956); Wallin (1962); Ullrich and Schrodter, (1966); Krause *et al.,* (1975); Fry *et al.,* (1983); Forsund (1983); Winstel (1993); Hansen *et al.* (1995), Dacom Plant Plus (2003)), plus a calendar scheduled application and an untreated control. Experimental plots to evaluate and calibrate the models were established at the above ten sites during three seasons, 2003-06. During the 2006-07 season, an experiment was performed to evaluate the Blitecast model under irrigated and non-irrigated plots using two potato cultivars: Desiree and Yagana-INIA. The Blitecast model was modified two ways for first spray alert alarms: Alarm 1: alarm notice at 18 accumulated severity values using 80% relative humidity, and Alarm 2: alarm notice at 15 accumulated severity values using 80% relative humidity and decreasing one severity value in the matrix relating severity values and rain favorable days (Krause *et al.,* 1975). The treatments included seven fungicide applications strategies sprayed according to Alarm 1, Alarm 2, the calendar schedule and two untreated controls. The experimental unit and the evaluation of the plants during the season were as described previously.

Results and Discussion

P. infestans characterization
Of the 250 *P. infestans* isolates collected and used to characterize the *P. infestans* population from the Araucania and Los Lagos regions of southern Chile during the crop seasons of 2003-2004 and 2004-2005, All isolates were the A1 mating type. The majority of the isolates were sensitive to metalaxyl (less than 3 ppm of EC50). Less than 1% of the isolates showed resistance to metalaxyl with EC50 values of 27.7 and 100 ppm (Table 1, Figure 1).
Virulence testing showed a population with 69 different pathotypes dominated by R10,11; R1,4,5,10,11 and R1,5,10,11. Some isolates were complex and able to infect differentials with nine R genes. However, most of them having two, four or five virulence genes (Table 2). The most frequent genes R found were 10 and 11 with 93.9 and 97.2% of the population, respectively (Figure 2).
According to the isozyme and SSR patterns, seven and 12 genotypes were distinguished during the collecting seasons 2003-04 and 2004-05, respectively (Table 3). For both seasons one genotype was predominate with 67% and 54% frequency The predominate genotypepattern for both years for Gpi, Pep, Pi02, Pi16, Pi70, Pi26, Pi33, Pi56, Pi66, Pi04 was86/100, 78 /100, 161/161, 172/170, 191/194, 171/174, 203/203, 178/178, 155/152, 172/163, respectively. NOTE: Pi's 33,56,66 and 04 and their respective patterns are missing from Table 3. Among the genotypes, most polymorphisms were detected at Pi02 and Pi16 loci. Almost no differences were detected for the Gpi, Pi70, Pi26, Pi33, Pi56, Pi66 and Pi04 loci. The exception was one genotype (Nº15 in Table 3) that only was detected during the second season, which showed differences in five loci. The dominate genotype (Nº1) presented a relative genetic frequency of 67% and is probably a new introduction from an outside region. Future steps in this research will be to compare this local population to other worldwide databases information, using standard probes like RG57, RFLP patterns, and mitochondrial haplotypes.

Table 1. In vitro resistance to Metalaxyl of *Phytophthora infestans* isolates from Araucania and Los Lagos Region in Chile during the season 2003-04 and 2004-05.

Growth on 10 µg/l media relative to unamended media	Isolates (%)		Metalaxyl resistance
	Season 2003-04 (n=98)	Season 2004-05 (n=152)	
0-10	23.4	43.4	Sensitive
10 to 60	76.5	55.9	Intermediate
>60	0	0.7	Resistant

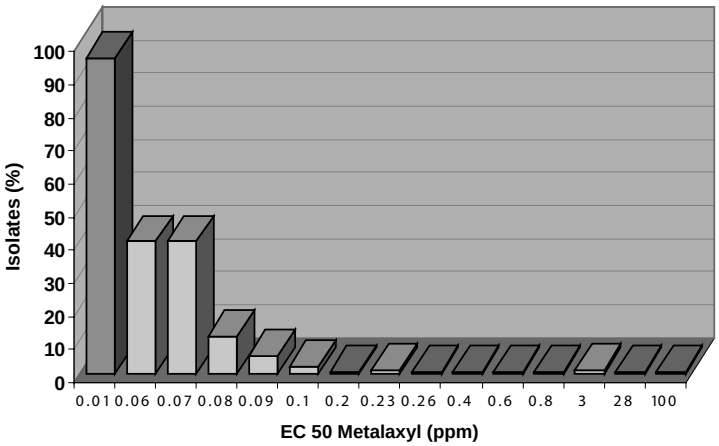

Figure 1- Sensitivity of Phytophthora infestans isolates from Araucania and Los Lagos Regions in Chile to metalaxyl during the 2003-04 to 2004-05 seasons as measured by EC50 values.

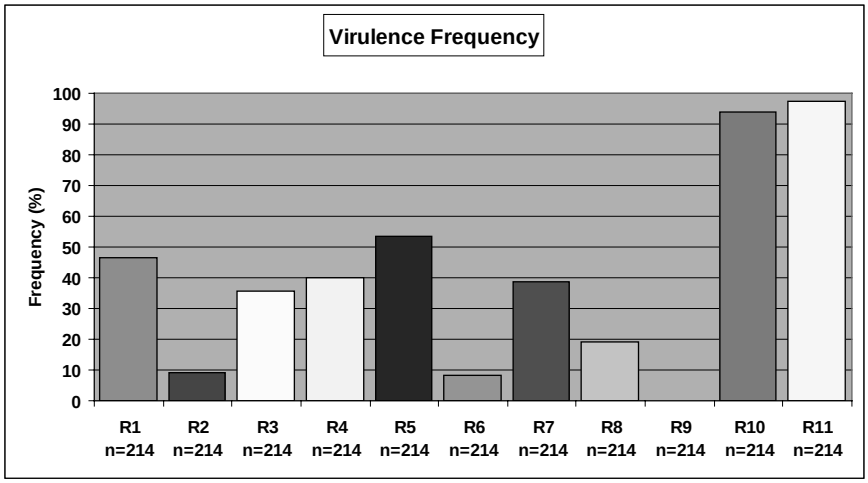

Figure 2. Virulence frequency of Phytophthora infestans populations from Araucania and Los Lagos Region in Chile during the 2003-04 to 2004-05 seasons.

Table 2. Frequency of virulence genes in the *Phytophthora infestans* population from Araucania and Los Lagos Regions in Chile during the 2003-04 to 2004-05 seasons.

Number of isolates	Virulence genes in the isolate
3	1
42	2
25	3
42	4
42	5
33	6
12	7
11	8
4	9

Table 3. Genotypes of *P. infestans* from South Chile identified by isozyme and SSR patterns.

Nº	Genotypes						Relative Genetic	Frequency Season of collection	
	Gpi	Pep	Pi02	Pi16	Pi70	Pi26	distance	2003-04	2004-05
1	86 /100	78 /100	161/161	172/170	191/194	171/174	0.00	67	54
2	86 /100	78 /100	161/157	172/170	191/194	171/174	0.08	15	6
4	86 /100	78 /100	161/161	173/171	191/194	171/174	0.08	3	4
5	86 /100	78 /100	161/161	170/168	191/194	171/174	0.08	2	0
6	86 /100	78 /78	161/161	172/170	191/194	171/174	0.08	1	0
7	86 /100	100/100	161/161	172/170	191/194	171/174	0.08	1	1
8	86 /100	78 /100	CP	172/170	191/194	171/174	0.17	1	3
9	86 /100	78 /100	162/158	173/171	191/194	171/174	0.33	0	1
10	86 /100	78 /100	CP	173/171	191/194	171/174	0.33	0	1
11	86 /100	78 /100	162/159	173/171	191/194	171/174	0.25	0	3
12	86 /100	78 /100	162/158	172/170	191/194	171/174	0.17	0	3
13	86 /100	78 /100	162/159	172/170	191/194	171/174	0.17	0	10
14	86 /100	78 /100	162/162	173/171	191/194	171/174	0.33	0	13
15	100/100	82 /111	163/150	172/170	197/194	181/178	0.67	0	1

For each isozyme and SSR loci, the two alleles for relative migration or size (pb) are indicated, respectively. PC indicates complex pattern.

Relative resistance to late blight

From an economic and environmental point of view it is desirable to use cultivars with genetic resistance to late blight. Most breeding programs around the world have huge programs to achieve this, but the task has been difficult because of the capability of the pathogen population to overcome the host resistance, and because the complexity of potato genetics and breeding. The Agricultural Research Institute of Chile (INIA), has developed a successful breeding program in Chile beginning in the 1980's releasing cultivars as Pehuenche, Ona, Karu, Pukará, and Yagana, as well some advanced selections evaluated in this project.

Figure 3 shows the evaluations for late blight resistance of 31 cultivars and clones from various origins in the last crop season, 2006-07. Most of the cultivars are susceptible to late blight, but under the same conditions they have different degrees of susceptibility. Similar trials have been conducted during the last four seasons in three different locations and disease severity has been variable each year because of differing environmental conditions. This past season, conditions were very favorable for late blight , therefore, cultivars developed discriminatory symptoms. However, every year, the cultivars

Amadeus and Pehuenche and clones as R89063-84 and R 89063-59 have shown good resistance to late blight.

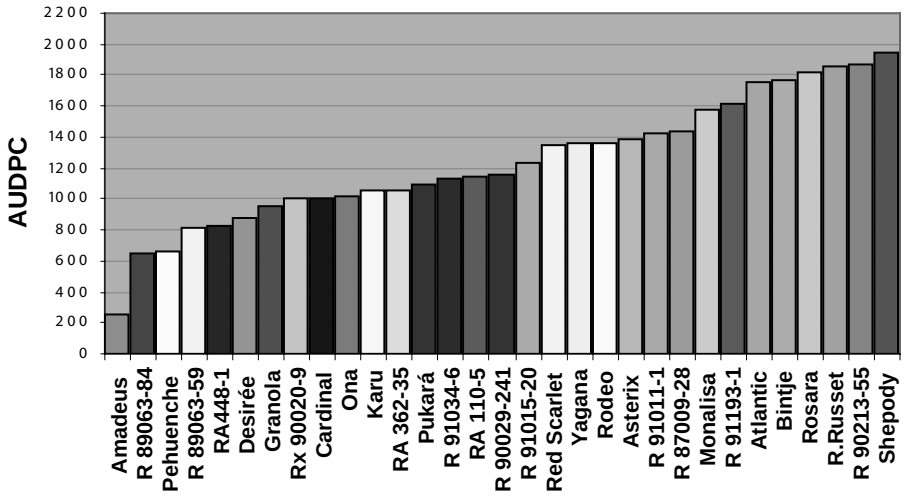

Figure 3. Relative resistance to late blight of 31 potato cultivars and advanced selections grown in the Osorno, Los Lagos region, Chile. 2006-07. AUDPC=Area Under the Disease Progress Curve. ANDEVA p=0.0001; LSD= 271.52, p=0.05.

Forecasting systems

During this project, three different forecasting models, Blitecast, Negfry and Dacom Plant Plus, were evaluated for late blight in ten different locations. Under the weather conditions of the crop seasons 2003-04 to 2005-06, Blitecast predicted late blight more accurately, warning of disease conditions just before the first symptoms appeared. On the other hand, Negfry and Dacom Plant Plus recorded, on average, two or three more alarm periods than Blitecast. The results of an experimental plot near Los Muermos during the 2005-06 season using only mancozeb and chlorothalonil based fungicides are shown in Figure 4 and Table 4. The data indicate that late blight symptoms appeared late in the season and developed very fast, and all the chemical treatments significantly reduce foliar damage compared to the untreated control. Because of these results and considering that the weather stations are located outside of the potato crop, for the next season it was decided to use only Blitecast, but with modifications in the relative humidity threshold for the accumulation of severity values. At the same time, 2006-2007 season , different chemical strategies were used in combination with late blight forecasting. Figures 5 and 6 show part of the results of this experiment. These Figures show the high level of attack of late blight during the season (untreated control) and the differences in susceptibility to the disease of Yagana and Desiree under the same conditions. It also shows higher disease severity in the irrigated treatment versus non-irrigated conditions.

Forecasting based on alarm 1 recommended four fungicide sprays while alarm 2 recommended six, while the calendar schedule recommended seven applications. Comparing the control level of late blight to applications under the calendar schedule, alarm 1 and alarm 2, it is concluded that alarm 1 under non-irrigated conditions resulted in control similar to the calendar schedule, but, under irrigated conditions, alarm 2 resulted in better control than alarm 1 and similar to calendar schedule. By using the Blitecast model it was possible to decrease the number of fungicide applications by 40% and 15% for late blight control in non-irrigated and irrigated crops respectively. In order to choose

an adequate late blight chemical strategy using a forecasting system it is also important to consider cultivar susceptibility, mode of action of the fungicide and agronomic management of the crop.

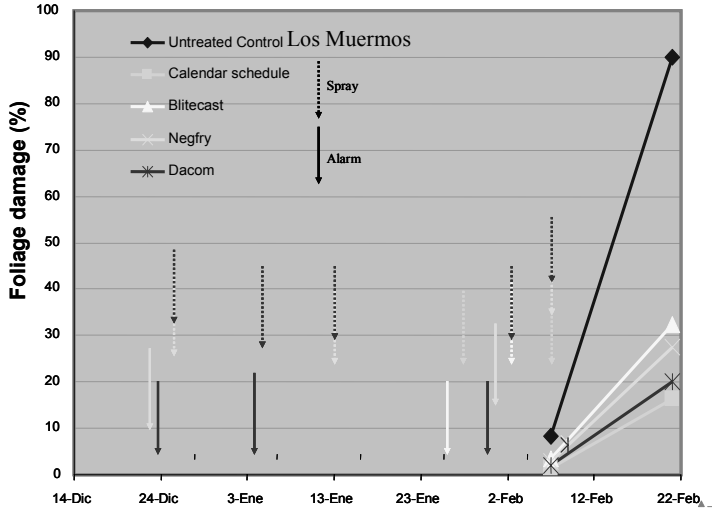

Figure 4. Late blight incidence and severity on potato plants cv Desireé, managed under different forecasting systems. Los Muermos, Los Lagos region, Chile. 2005-06.

Colored arrow indicated alarm (↓) and spray (⇣) of the forecast model shown in the same color. Chemical applications were done only with mancozeb or chlorothalonil based fungicides.

Table 4. Late blight incidence and severity on potato plants cv Desireé, managed under different forecasting systems. Los Muermos, Los Lagos region, Chile. 2005-06.

Treatments		Foliar damage (%) Evaluation date				AUDPC	
		07-feb-06		21-feb-06			
1	Untreated control	8.6	d	90.0	d	982.9	a
2	Calendar schedule	0.2	a	16.2	a	177.8	d
3	Blitecast	4.4	c	32.5	c	358.2	b
4	Negfry	1.3	b	27.5	bc	302.4	bc
5	Dacom	0.1	a	20.0	ab	220.9	cd
	Cv	1.94		6.49		14.24	
	Probability	0.0001		0.0001		0.0001	

Numbers in a column followed by the same letter are not significantly different at p=0.05.

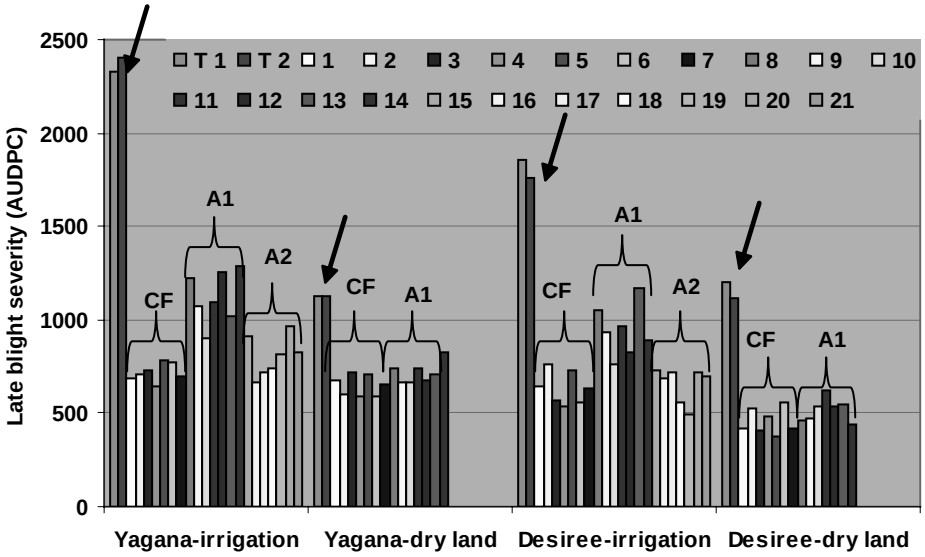

Figure 5. Late blight symptoms in potato plants cv Yagana and Desirée under irrigation and non-irrigated conditons treated-with different chemical and forecasting strategies. Osorno, Los Lagos Region, Chile. 2006-07.

Arrows show the untreated control (↓), CF: chemical application under calendar schedule (7 sprays), A1 chemical application under alarm 1 (4 sprays), A2: chemical application under alarm 2 (6 sprays). Seven different chemical strategies were tested. Treatments 1 to 7 are the different fungicide strategies sprayed under calendar schedule. Treatments 8 to 14 are the different fungicide strategies sprayed under alarm 1. Treatments 15 to 21 are the different fungicide strategies sprayed under alarm 2. Yagana-irrigation: ANDEVA p=0.0001; LSD= 252.81, p=0.05. Yagana-dry land: ANDEVA p=0.0001; LSD=144.96, p=0.05. Desiree-irrigation: ANDEVA p=0.0001; LSD= 256.59, p=0.05. Desiree-dry land: ANDEVA p=0.0001; LSD= 160.38, p=0.05.

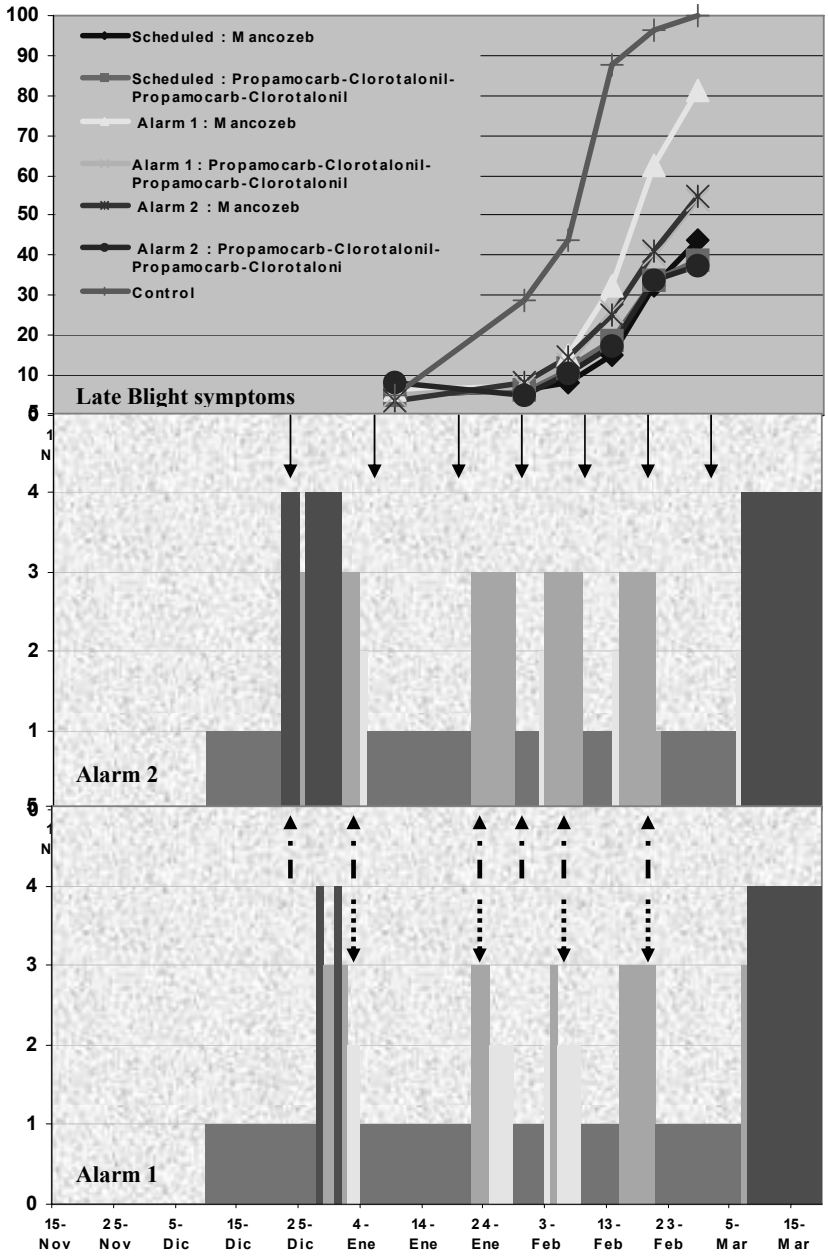

Figure 6. Alarms and late blight symptoms in potato plants cv Yagana under irrigation spray with two differents chemical and forecasting strategies for fungicide application. Osorno, Los Lagos region, Chile. 2006-07.

Arrows show the chemical application under calendar schedule (↓), Alarm 1(⇣) and Alarm (2 (↓). Alarm Figures show the forecasting for late blight: green: no alarm, Yellow: warning, Orange: a 7-10 day spray schedule is recommended, Red: a 5-7 day spray schedule is recommended.

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Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

A simple DSS for control of potato late blight

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Summary
To help Estonian potato growers in scheduling fungicide treatments in late blight control we have developed a simple Web-based potato late blight warning system giving advice on regional base. Late blight warning system is bringing together information on climatic data, variety resistance and fungicide properties. The system consists of maps providing data on timing of fungicide application and information for choice of fungicides and management of variety resistance in an easily understandable way. The simply understandable nature and reliable late blight control have been the major objectives in development of the DSS system.

Keywords
Potato, late blight, DSS

Introduction
According to recommendations of pesticides producing companies' late blight control has to be started at the row closing and has to continue with regular intervals of 7-10 days until the end of growing season. Such recommendations do not consider the yearly variation of climatic conditions and resistance of potato varieties what influence the timing of the first fungicide application and scheduling of following fungicide treatments. Numerous PC- and Web-based decision support programs for control of potato late blight have been worked out in several countries to help growers in making right decisions (Disease Model Database, 2001). Unfortunately many of them have relatively limited use. Necessity for real time climatic data and sophisticated nature of computer programs are major limiting factors for their widespread use by ordinary farmers

Decision support program NegFry (Hansen, 1993) programmed to use real time climatic data from Hardi Metpole or Metos Compact weather stations has been successfully used in Baltic countries (Hansen *et al.*, 2002; Koppel *et al.*, 2003). Following the recommendations of NegFry program have provided good, economic and reliable late blight control in experimental trials carried out in Estonia. Several Estonian potato growers have got interested in use of similar decision support system, but availability of real time local weather data and/or sophistication of the use of the program have turned to be major limiting factors for the use of NegFry. Use of wide range of fungicides by the farmers, complications arising from farm size and problems connected with extremely unfavorable weather conditions are additional limiting factors of widespread use of NegFry. NegFry is designed only for

use of certain fungicides with 7 days treatment interval, what excludes the use of several fungicides with longer treatment intervals. Current Estonian farmers have often potato fields located rather far from each other, what complicates the exact fulfillment of NegFry recommendations for fungicide treatments. NegFry has no means for case, when the needed treatment has delayed by for example by extreme weather circumstances.

To overcome the listed problems and to help Estonian potato growers in scheduling fungicide treatments in late blight control we have developed a simple Web-based potato late blight warning system giving advice on regional base.

Program description

The simply understandable nature and reliable late blight control have been the major objectives in development of the DSS system. Developed late blight warning system is bringing together information on climatic data, variety resistance and fungicide properties and provides disease control advice in regional level. The system consists of maps providing data on timing of fungicide application and tables with information for right choice of fungicides and management of variety resistance in an easily understandable way. It does not give fixed advice for any concrete field, but the users have to bring the information together and make the final decision by themselves. By taking the users into the decision making process we hope to increase the credibility of the DSS system among them.

Climate effect

The climatic data obtained from network of seven Metos Compact automatic weather stations are used for calculation of risk values of late blight outbreaks with NegFry programme. Climatic data obtained in 23 meteorological stations of Estonian Institute of Meteorology and Hydrology and the daily precipitation maps based in data of 61 stations (www.emhi.ee) are used for extrapolation of the calculated late blight risk into the wider area. The weather forecast for next four days is obtained from the international program HIRLAM - HIgh Resolution Limited Area Model (www.hirlam.org). Based on the calculations and extrapolations of late blight risk an advice for timing of first fungicide treatment is visualized in form of colored map of Estonia in homepage of Jõgeva Plant Breeding Institute (www.sordiaretus.ee) by simple three color system: green – no risk; yellow – risk is approaching, red- high risk, time for fungicide application. Such advice enables to avoid too early start of fungicide applications and to save amount of used fungicides in years of late establishment of infection or guarantees timely application and reliable blight control in years of early epidemics.

NegFry program is used also for timing of subsequent fungicide treatments. The information is visualized in another map by the same three color system: green – use intervals recommended by fungicide manufacturer, red – the interval should by shortened for one day, yellow – the interval could be prolonged for one day. We do not recommend more prolonged intervals of fungicide treatments as conditions suppressing late blight could be favorable for early blight.

Variety resistance

Majority of potato varieties cultivated in Estonia have been assessed in foliage resistance assessment trials at Jõgeva PBI in frames of EUCABLIGHT project (Colon *et al.*, 2005). Area under the disease progress curves (AUDPC) of tested varieties is transformed into 1-9 point scale by formula worked out by Hansen *et al.* (2005). The variety resistance is recommended to use in following way: varieties with resistance score 4-5 points should be treated according to intervals recommended by fungicide manufacturer, in varieties with resistance score 2-3 the treatment interval should be shortened for one day, in varieties with resistance score 6-7 the treatment interval could be prolonged for one day.

Table 1. The resistance classification and recommendations for treatment intervals of most widespread potato varieties

Resistance score	2	3	4	5	6	7
Treatment interval	Shorten 1 day		Normal		Extend 1 day	
Varieties	Aminca	Berber	Asterix	Agria	Escort	Ando
	Arielle	Bintje	Ditta	Ants	Granola	Anti
	Princess	Carlita	Fontane	Evita	Juku	Kuras
	Sinora	Courage	Milva	Fresco	Oleva	Robijn
	Velox	Eerseling	Sante	Maret	Piret	Sarme
		Folva	Satina	Picasso	Raja	
		Impala	Sava	Remarka		
		Latona	Van Gogh	Vigri		
		Platina	Victoria			
		Secura				

Such recommendations for adjustment of treatment intervals are based on experiences from numerous fungicide trials in varieties of different resistance levels throughout several years. The information on variety resistance is provided in DSS system in separate table. As the variety resistance does not change during the growing season, the user can take the correction value once and use it easily throughout the fungicide application period.

The corrections based on weather conditions and variety resistance should be summarized together making the total change of treatment interval from –2 (more susceptible varieties in blight favorable conditions) to +2 days (less susceptible varieties in blight unfavorable conditions). Depending on registered interval of fungicide treatment the recommended interval could be from 5 (fungicide Dithane in susceptible cultivars at blight favorable weather) to 14 days (fungicides Ridomil Gold and Tattoo in less susceptible cultivars at blight unfavorable weather).

Choice of fungicides

Comprehensive characterizations of potato late blight fungicides are agreed by fungicide sub-group meetings in EU.NET.ICP workshop (Bradshaw, 2006). Information in effectiveness and mode of action in control of foliage blight, tuber blight and early blight caused by *Alternaria* ssp. is basis for using of fungicides at different climatic and epidemic conditions and at different stages of plant development. In addition to technical characterization of late blight fungicides, the warning system provides baselines and recommendations for selection of most effective and reliable fungicides according to field conditions (Table 2). The recommendation for fungicide selection is provided together with advice for start of first fungicide application or for treatment interval, so the user can get together the information on timing and choice fungicides.

Table 2. Selection of fungicides according to climatic and epidemic conditions and stage of plant development.

Climatic, epidemic and field conditions	Most effective fungicides
Beginning of infection, normal or unvafourable conditions for late blight	Dithane, Shirlan, Electis, Acrobat Plus
Beginning of infection, favourable conditions for late blight, risk for being late with the first treatment	Ridomil Gold, Tattoo, Glory
Active growth ot potato plants before the flowering	Ridomil Gold, Tattoo, Glory
Period of intensive infection and spread of late blight	Shirlan, Ridomil Gold, Electis
Rainy period	Ranman, Ridomil Gold, Tattoo, Glory
Prolonged dry period suppressing late blight, but favourable for early blight	Electis, Dithane, Glory, Sereno, Tanos
Last treatments to avoid tuber blight infection	Ranman, Shirlan

A simple web-based decision support system was first time in use in 2006. The year was characterized with extremely late outbreak of potato late blight (end of August) what did not provide enough data for testing the reliability of the new system. Testing of the updated system is continued in 2007.

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Tracking Late Blight in the Field

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Keywords
Potato Late Blight, SSR genotype, host resistance, selection

Introduction
Differences in aggressiveness and fitness of potato and tomato adapted strains of *P. infestans* are well documented, but adaptation within potato germplasm itself has not been studied intensively and the rate, mechanisms and significance of such adaptations are not known. The extent to which cultivar-specific adaptation influences disease in the field is also largely unknown. The development of co-dominant SSR molecular markers (Lees *et al.,* 2006) has facilitated epidemiological studies to monitor or 'track' isolates with distinct genotypes throughout the growth of a crop. The effects of management practices, such as host resistance and chemical control on the predominance of particular isolates throughout an epidemic can therefore be studied, as these examples show.

Materials and Methods
A field trial was carried out simultaneously at 2 sites in Scotland, one on the East (irrigated) and one on the West coast (non-irrigated). Five cultivars, representing a range of foliage blight resistance but with as few known R-genes as possible, were planted: Bintje (S), Desiree (I), Teena (I), Pimpernel (R), Stirling (R). The trial was designed as a split-plot design with the 8 main plots consisting of 2 fungicide treatments (+/- metalaxyl) replicated 4 times. Sub-plots consisted four-plant plots of each cultivar fully randomised within the plot. Plants of cv. King Edward were inoculated with mixed inoculum of 4 isolates of *P.infestans* (C1-C4) that had previously been characterised for relative aggressiveness in glasshouse tests (no significant difference), sensitivity to metalaxyl, virulence and genotype (Table 1). These were then used as infector plants within spreader rows to infect trial plots. Lesions were sampled from each plant at 4 sampling dates (2 before and 2 after metalaxyl application) during the epidemic and disease scores made. Each isolate sampled was characterised using SSR markers and identified as C1-C4. An analysis of the effect of host on isolate frequency was made.

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Table 1. Isolate characterisation

Isolate	Pi02 alleles	Pi33 alleles	Pi26 alleles	Race	Metalaxyl sensitivity
C1	152/162	203/203	177/179/185	1,2,4	I
C2	162/164	203/206	179/181/183/187	1,3,4,7	S
C3	162/162	203/203	179/181/183/187	1,3,4,7,10	R
C4	162/162	203/203	177/181	1,2,3,4,6,7	R

Results and discussion

Application of the fungicide metalaxyl had the expected result, with the largest effect on reducing the frequency of the sensitive isolate C2 at both sites (Figure 1).

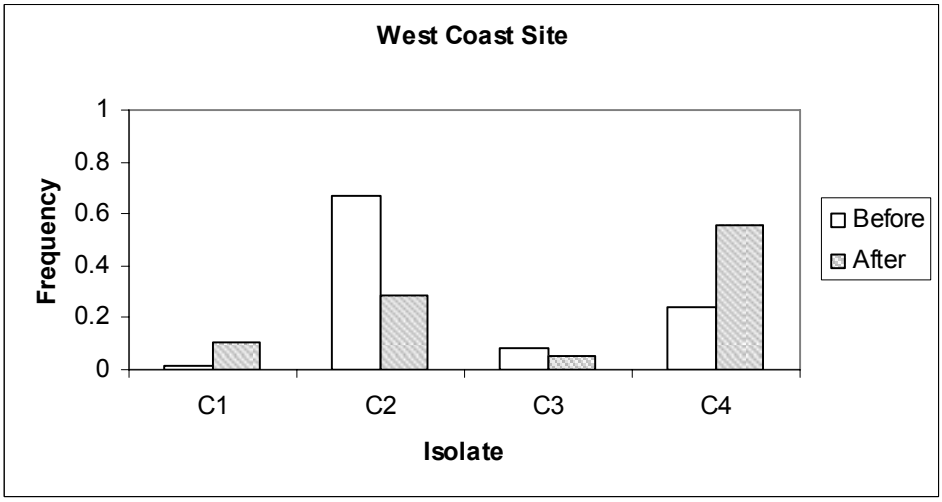


Figure 1. Frequency of isolates C1-C4 in the field before and after application of metalaxyl

Overall disease rating was consistent with expected host resistance (Figure 2). Isolates C1 and C3 were present at lower frequencies across trial sites and cultivars, reflecting a reduction in aggressiveness and fitness. Isolate C2 was the dominant isolate on all cultivars, apart from cultivar Stirling, at both trial sites over all sampling dates. An example of this is given in Figure 3 where a comparison between cultivars Desiree and Stirling shows that isolate C2 is much less frequent on cv. Stirling compared to cv. Desiree throughout the epidemic. Isolate C4 out-competes isolate C2 on the foliage blight resistant cultivar Stirling. In addition, Figure 4 shows that C4 is apparently better adapted to cv. Stirling compared to other cultivars at both sites and at all sampling dates

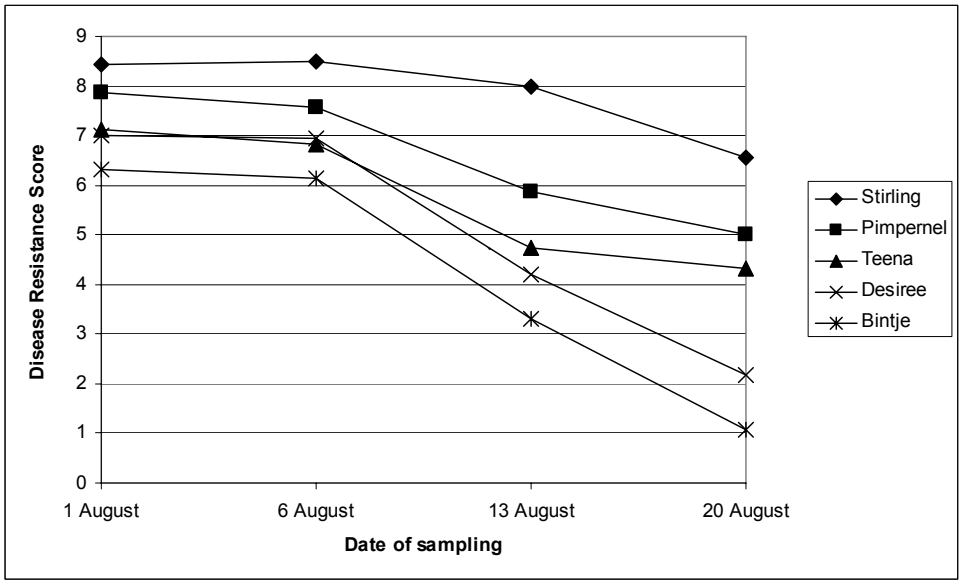
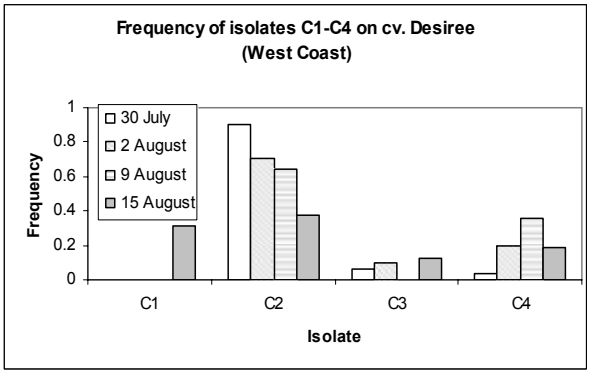


Figure 2. Disease Resistance score (1-9 scale) of cultivars inoculated with isolates C1-C4 at 4 scoring dates.



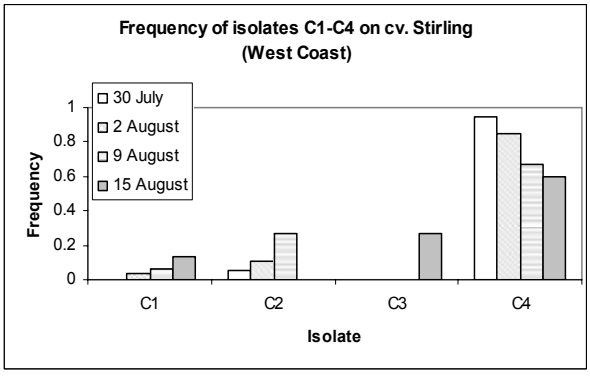
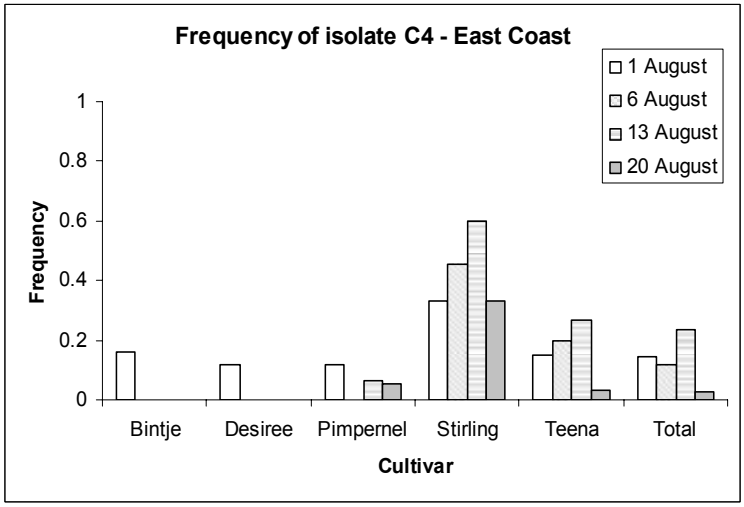


Figure 3. Comparison of frequency of isolates C1-C4 on cultivars Desiree and Stirling at 4 sampling date



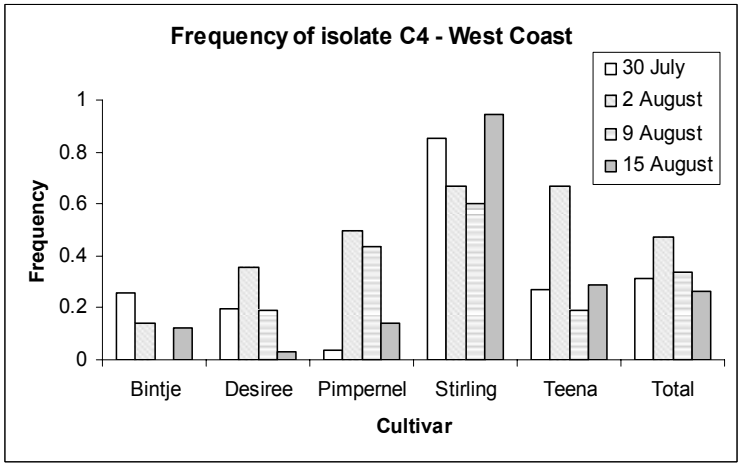


Figure 4. Frequency of isolate C4 at 4 sampling dates on 5 cultivars grown at 2 sites

SSR markers are a useful tool for 'tracking' distinct isolates of *P.infestans* to investigate the effect of management practices on adaptation of the pathogen population under field conditions. In these trials it was demonstrated that fitness and aggressiveness of the 4 isolates differed markedly, even in the in the absence of any effect of known host R-genes. For example, there was a clear selection for isolate C4 on the resistant cultivar Stirling. Strong pathogen competition and host selection can drive population change under agricultural conditions.

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Activation of late blight resistance in potato foliage and tubers by the Russian fungicide Aluphyt containing phosphorous acid

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Summary
Fungicide Aluphyt, developed by Russian company "Agropromchimsnab", belongs to the group of phosphonates and activates defense reactions in potato plants infected with *Phytophthora infestans* in addition to its anti–oomycetic direct action.

Keywords
Phytophthora infestans, potato, chemical control, phosphorous acid

Introduction
Aluphyt (Agropromchimsnab) is registered in the National list of recommended pesticides and permitted for the treatment of grape vines against *Plasmopara viticola*, cucumber against *Pseudoperonospora spp.*, and potato against *Phytophthora infestans*. Based on our results obtained, the direct fungicide action of Aluphyt on the late blight of potato and tomato is more effective than of Aliette (Rhone-Poulenc), which also belongs to the group of phosphonates. The acting substance of Aliette, aluminium fosetyl, is partially converted into phosphorous acid (H_3PO_3) in plants (Dolan and Coffey, 1988). Phosphorous acid appeared to be more active form against oomycetes, as compared with the aluminium salt (Gisi, 2002). Aluphyt represents a mixture of phosphorous acid, aluminium tris-phosphonate, and copper sulfite (2%).
It was also shown that phosphonates activate some defense reactions in treated and infected plants in addition to their anti-oomycetic potential (Mustafa and Djakov, 1980; Langcake, 1981; Gisi, 2002). Phosphonates appear to cause changes in the lipid and cell wall composition of pathogens that might reduce their virulence (Dustin *et al.*, 1990). The sensitivity of oomycetes to aluminium fosetyl can be different in various pathogen race-plant cultivar combinations (Bashan *et al.*, 1990).

Materials and Methods

We carried out three series of experiments with Aluphyt.

Experiment 1. We used the Black's set of 21 R-gene differential potato genotypes, grown in a greenhouses (Malcolmson and Black, 1966) and isolates of four races of *P. infestans*, collected in different regions of Russia (1.2.3.4.5.7.9.10.11, 1.2.3.4.7.8.9.10.11, 1.2.3.4.6.7.8.9.10.11, 1.2.3.4.7.9.10.11). Plants of each genotype were divided into three groups.

The first and second groups of plants were sprayed with Aluphyt in a concentration of 0.01 and 1.0 μg/ml, respectively, and the third group was sprayed with water (control). Earlier it has been found that Aluphyt directly affects the mycelium growth and zoospore germination if its concentration exceeds 10 μg/ml.

The leaflets of compared plants were inoculated with the pathogen races 6 h after the leaflet detachment. One droplet of inoculum (20 μl), containing about 15000 sporangia/ml was placed on the abaxial surface of each leaflet. Inoculated leaflets were incubated in a moist chamber at RH 98% at 20°C. The assessment was carried out after 6 days of incubation. The positive reaction represented a complete necrosis and sporulation on the leaflet surface.

Experiment 2. This experiment was carried out in both field and laboratory conditions. Thirty potato seed tubers cv. Sante in each variant were sprayed either with various concentrations of Aluphyt (25, 50, and 100 ml/ton) or with water (control) before their planting. For such treatment we used low-volume sprayers. The treated tubers were planted in field experimental plots. After shoot appearance we analyzed 30 detached leaves for each variant of treatment every 7-10 days during vegetation period. The detached leaves were sprayed with the sporangial suspension at concentration 15000 sporangia/ml and incubated in dark at 20°C and RH 98% for 24 h. The leaf petioles were put in water. After 6 days of incubation we calculated the number of the late blight necroses per each leaf (Figure 1).

Figure 1. Necrotic lesions on the potato leaves from tubers untreated and treated with Aluphyt

Experiment 3. This experiment was carried out in the field. We designed the experiment as a randomized block with four replicated plots. Each plot consisted of four drills, totally containing 168 plants. We applied the triple treatment with Aluphyt (30, 40, and 50 days after the planting) according to the accepted recommendations (3 l Aluphyt in 300 l water per ha). In control variant we applied triple treatment with water.

We dug out potato tubers from one drill of each plot 3, 10, and 30 days after the last treatment. The blocks (0.7 x 0.5 x 3.0 cm) were cut from ten largest tubers of the drill tested. The top of each block was dipped into the thin layer of the sporangial suspension (15000 sporangia/ml). The inoculated blocks were incubated in dark at 20°C/18°C (day/night) and RH 98% for 7-8 days. The area and depth of *P. infestans* lesion/necrosis of each block and its coverage with the air mycelium was determined (Figure 2).

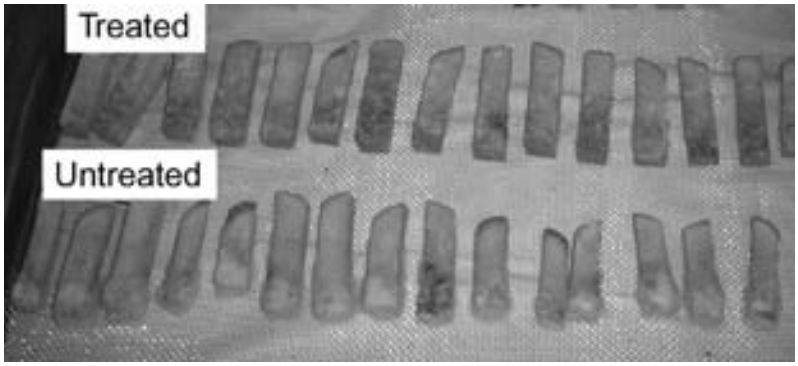

Figure 2. Inoculated tuber blocks from plants untreated and treated with Aluphyt.

Results and discussion

All three experiments demonstrated a significant protection effect of the Aluphyt application for both potato leaves and tubers against LB.

Only one race (1.2.3.4.7.9.10.11) among four complex races, used for the inoculation of the leaflets of Black's differential plants, treated with Aluphyt, demonstrated the reaction, similar to the untreated control (Table 1). Isolates of other races (1.2.3.4.5.7.9.10.11, 1.2.3.4.5.7.8.9.10.11, and 1.2.3.4.5.6.7.8.9.10.11) caused a reaction typical for the less complex races. Some virulence genes (5, 6, and 8) were not recognized after the Aluphyt treatment. These data might evidence that the small doses of phosphorous acid induce enhanced resistance of potato plants to the LB as well as reduce *P. infestans* virulence. The similar enhanced resistance was described for *Plasmopara viticola* (Derks and Creasy, 1989) and *Phytophthora palmivora* infections after the treatment with aluminium salt of phosphorous acid (Dustin *et al.*, 1990).

Table 1. The effect of Aluphyt on race–specific resistance of potato to *P. infestans*

Races	Type of reaction on Black differential set		
	Untreated plants	Aluphyt (0.01 ppm)	Aluphyt (1 ppm)
1.2.3.4.5.7.9.10.11	R1,R2,R3,R4,R5,R7,R9,R10,R11	R1,R2,R3,R4,R7,R9,R10,R11 (R5)*	R1,R2,R3,R4,R7,R9,R10,R11 (R5)*
1.2.3.4.7.8.9.10.11	R1,R2,R3,R4,R7,R8,R9,R10,R11	R1,R2,R3,R4, R10,R11 (R7,R8,R9)*	R1,R2,R3,R4,R7,R9,R10,R11 (R8)*
1.2.3.4.6.7.8.9.10.11	R1,R2,R3,R4,R6,R7,R8,R9,R10,R11	R1,R3,R4,R7,R9,R10,R11 (R2,R6)*	R1,R2,R3,R4,R7,R9,R10,R11 (R6)*
1.2.3.4.7.9.10.11	R1,R2,R3,R4,R7,R10,R11	R1,R2,R3,R4,R7,R10,R11	R1,R2,R3,R4,R7,R10,R11

** Necrotic lesions without sporulation*

The pre-planting treatment of tubers with Aluphyt inhibits the late blight on the plants grown from these tubers. Our experiments showed that the number of necroses on leaves inoculated with *P. infestans* was significantly reduced in comparison with plants grown from untreated tubers (Figure 3). This effect of tuber treatment was observed up to the flowering stage of potato plants. Additional experiments are necessary to prove the hypothesis that in the course of the experiment 2 Aluphyt induced potato defense reactions.

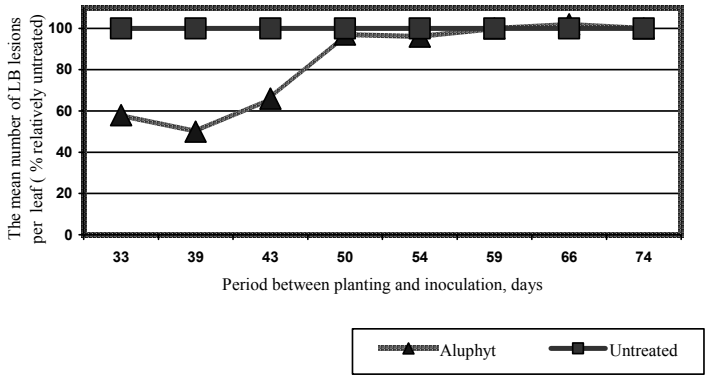

Figure 3. The effect of pre-planting treatment of potato tubers with Aluphyt on susceptibility of potato leaves to P. infestans infection.

Phosphorous acid is easily taken up by roots and translocated via floem to the aerial parts of a plant (Gisi, 2002). Therefore, it is possible that Aluphyt has the direct anti-oomycetic activity of phosphorous acid and enhances the plant resistance.

The data, presented in Figure 4 and 5, show that the 3 times repeated treatment of potato plants with Aluphyt essentially suppresses the *P. infestans* development in the tubers of treated plants. The effect of such treatment becomes noticeable 3 days after last treatment. The size of necrotic lesions on the potato blocks from the treated plants, was smaller, than on untreated ones; these lesions were shallow; many of them (16–20%) did not show any air mycelium. Such shallow lesions and poor air mycelium are usually registered in tubers of highly LB-resistant cultivars. On the contrary, blocks cut from the tubers of untreated plants had deep lesions and abundant air mycelium on their surface.

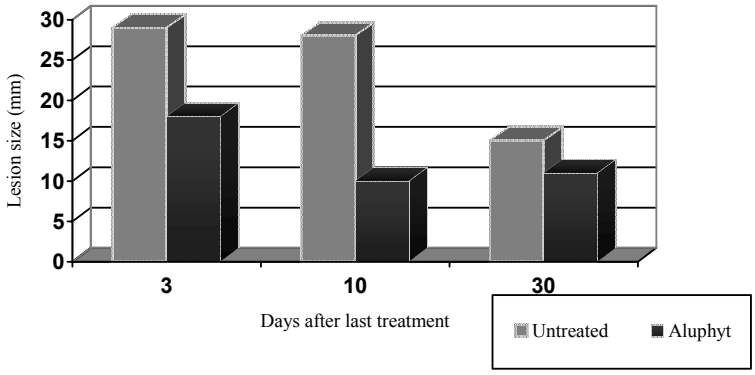

Figure 4. The effect of potato foliage treatment with Aluphyt on tuber susceptibility to P. infestans

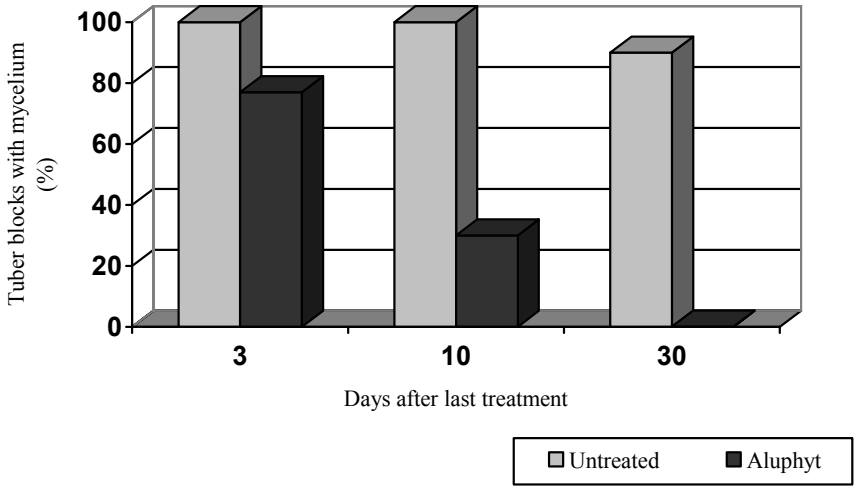

Figure 5. The effect of potato foliage treatment with Aluphyt on the level of the air mycelium coverage of tuber blocks

The difference between the tuber susceptibility of treated and untreated plants increased within 10 days from the last fungicide application. Only 31% of inoculated potato blocks, taken during this period from the treated plants, showed the air mycelium, whereas 100% blocks from untreated potato blocks were covered by the profuse mycelium.

Tuber tissues from the treated plants became almost completely resistant to the LB pathogen during the harvesting period (the harvesting was carried out 30 days after the last treatment) and showed only small shallow lesions without any air mycelium.

The presence of the fungicide in the tuber samples was not detected at harvest. Either Aluphyt was absent in tubers, or the fungicide content was below the detectable level. We suggest that the tuber resistance to LB is caused rather by the induction of plant defense mechanisms, than by a direct fungicidal action.

Acknowledgements

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Tenth Workshop of an European Network for development of an
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Prevalence and Significance of the F129L mutation in *Alternaria solani* from the United States

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Summary
Alternaria solani isolates collected over a five year period from 2002 to 2006 were evaluated for the presence of the F129L mutation. Overall, 96.5% of these isolates were determined to have reduced sensitivity to QoI fungicides and/or to contain the F129L mutation. The detection of these isolates in areas where conditions are less conducive for the pathogen and where there is less selection pressure by QoI fungicides suggests that the mutation is stable. Field trials were performed in central North Dakota in 2000 when the *A. solani* population was dominated by wild type isolates as well as in 2002 and 2003 when F129L mutant isolates dominated. These trials support in vitro and greenhouse results indicating that the F129L mutation has affected the field performance of strobilurin-type QoI fungicides. Overall, field trial results suggest that strobilurin-type fungicides no longer provide improved disease control over standard protectant fungicides such as chlorothalonil and mancozeb.

Keywords
Potato early blight, *Solanum tuberosum*, fungicide sensitivity

Introduction
Early blight, caused by *Alternaria solani,* is the most important foliar disease of potato in the Midwestern USA due to heavy inoculum pressure and favorable conditions for the spread and development of the pathogen. Standard protectant fungicides such as chlorothalonil and mancozeb are generally inadequate alone in controlling this disease; therefore, registration of azoxystrobin was important for improved early blight disease control. Reduced sensitivity to azoxystrobin was documented in isolates of *Alternaria solani* collected from Nebraska in 2000, and became widespread in other Midwestern states in subsequent years (Pasche *et al.*, 2004). Little is known about the prevalence of isolates with reduced sensitivity to QoI fungicides outside the central portions of the USA.
Reduced sensitivity observed in *A. solani* has been attributed to the action of the F129L mutation, the substitution of phenylalanine with leucine at position 129 (Pasche *et al.*, 2004, 2005). The G143A mutation, which is responsible for QoI resistance in many other fungi, has been demonstrated to provide cross-resistance among QoI fungicides (Kim *et al.*, 2003), while the F129L mutation has been shown to have a differential effect on fungal sensitivity to QoI fungicides (Kim *et al.*, 2003; Pasche *et al.* 2004, 2005; Vincelli and Dixon, 2002). The objectives of this research were to determine the prevalence of the F129L mutation among *A. solani* isolates collected from commercial potato fields

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across the USA from 2002 through 2006 and to determine the effect the F129L mutation on disease control of strobilurin-type QoI fungicides via replicated field trials conducted in 2000, 2002 and 2003.

Materials and Methods

Fungicide sensitivity evaluations and detection of the F129L mutation. From 2002 to 2006, leaves with early blight lesions were collected randomly from across the USA. This five year survey included samples from 11 potato producing states (Table 1). Isolations to recover *A. solani* and concomitant culture purification and storage were as previously described (Pasche, *et al.* 2004). The presumptive presence of the F129L mutation was determined via EC_{50} values generated in vitro or by real-time PCR. Both methods were performed as previously described (Pasche *et al.* 2004, 2005).

Field evaluation of early blight fungicides. Field trials evaluating the efficacy of QoI fungicides were conducted in central North Dakota in 2000, 2002 and 2003. All trials were conducted in fields with overhead sprinkler irrigation. In field trials presented here, QoI fungicides were applied five times during the growing season in alternation with five applications of chlorothalonil. The foliar fungicide trial in 2000 was conducted in the presence of a QoI wild type *A. solani* population and field trials conducted in 2002 and 2003 were predominated by the presence of the F129L mutation in the early blight fungus.

Chlorothalonil and mancozeb, at a use rate of 1190 g a.i./ha and 1680 g a.i./ha, respectively were used as standard protectant control treatments in the early blight disease control field trials. Azoxystrobin and pyraclostrobin were applied at 113 and 226 g a.i./ha, while trifloxystrobin was applied at 105 and 140 g a.i./ha. These treatments represent the lowest and highest labeled rate for each fungicide. Only the low rate was applied in 2000. Percentage early blight severity was recorded at approximately seven day intervals from the onset of disease development to the end of the season. Foliar disease severity was used to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977) and then relative area under the disease progress curve (RAUDPC).

Table 1. Number of and percentage F129L mutant isolates of *Alternaria solani* collected from across the United States from 2002 to 2006.

State[a]	2002[b]		2003		2004		2005		2006		2002-2006	
	Total[c]	% Mutant[d]	Total	% Mutant	Total	% Mutant	Total	% Mutant	Total	% Mutant	Total	% Mutant
Nebraska	240	98.8	247	100.0	758	99.0	46	100.0	9	88.9	1300	99.3
Minnesota	-	-	-	-	767	96.7	136	91.9	309	92.2	1212	95.7
North Dakota	181	100.0	225	91.2	230	98.7	66	97.0	58	62.1	760	93.8
Wisconsin	-	-	-	-	-	-	258	96.1	138	89.1	396	93.7
Michgan	-	-	11	100.0	148	94.1	55	98.2	-	-	214	96.7
Texas	-	-	1	100.0	60	100.0	131	98.5	-	-	192	99.0
Colorado	-	-	9	100.0	-	-	20	100.0	78	91.0	107	93.5
Idaho	-	-	-	-	-	-	26	15.4	-	-	26	15.4
Wyoming	-	-	-	-	-	-	13	23.1	-	-	13	23.1
Oregon	-	-	-	-	-	-	-	-	10	60.0	10	60.0
Washington	-	-	-	-	-	-	8	12.5	-	-	8	12.5
Total	421	99.3	493	95.9	1963	98.4	759	91.4	602	87.9	4238	96.5

a State from which isolates were originally collected.

b Year isolate was collected.

c Total number of isolates examined for a given time period.

d Percentage of isolates determined to contain the F129L mutation for a given time period.

Results and Conclusions

Fungicide sensitivity evaluations and detection of the F129L mutation. Between 2002 and 2006, 4238 *A. solani* isolates were collected from 11 major potato producing states across the USA and 96.5% of isolates were determined to have reduced sensitivity to azoxystrobin, dominating the *A. solani* population in potato production areas of the USA (Table 1). In the central portion of the USA the frequency of reduced sensitive/F129L mutant *A. solani* isolates generally ranged from 88-100% in each year of the survey. The exception to this is in North Dakota where only 62% of isolates collected in 2006 were determined to contain the F129L mutation. Among isolates of *A. solani* collected only in 2005 from the Western USA, frequency of the F129L mutant populations were much lower, generally ranging from 12-60%, indicating that the mutation is present in *A. solani* populations with little exposure to QoI fungicides.

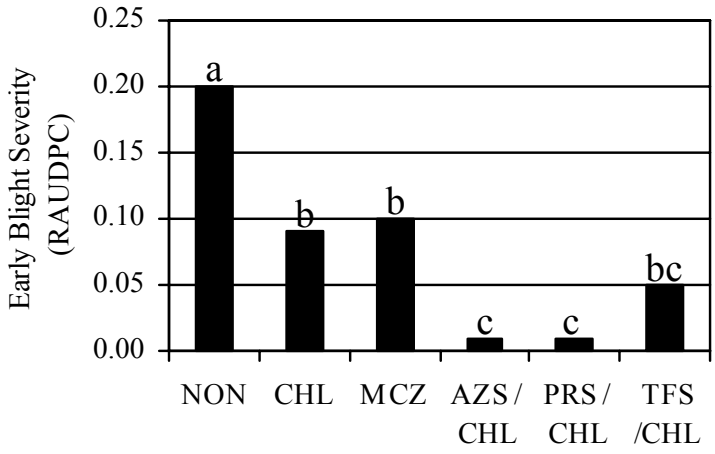

Figure 1. Early blight disease severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2000 using cultivar Russet Burbank. A total of ten foliar fungicide applications were performed during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); five applications each of azoxystrobin (AZS); trifloxystrobin (TFS); and pyraclostrobin (PRS). All strobilurins were alternated (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

Field evaluation of strobilurin-type QoI fungicides. Results from field trials performed in 2000 in central North Dakota, in the presence of a QoI wild type *A. solani* population, confirmed that these fungicides represented a class of chemistry that provided control of early blight significantly superior to standard protectant fungicides chlorothalonil and mancozeb (Figure 1). Azoxystrobin and pyraclostrobin provided early blight control superior to that of trifloxystrobin, although not significantly so. Field trials conducted in central North Dakota in 2002 and 2003 demonstrate that early blight disease in plots treated with azoxystrobin, pyraclostrobin and trifloxystrobin were not significantly different from plots treated chlorothalonil and mancozeb alone ($P < .0001$), illustrating that strobilurin-type QoI fungicides no longer provide superior early blight disease control in the field (Figure 2). Furthermore, increasing application rates of the QoI fungicides did not have any affect on the level of disease control provided by these fungicides. In light of these results, new fungicide chemistries are needed to manage *A. solani*.

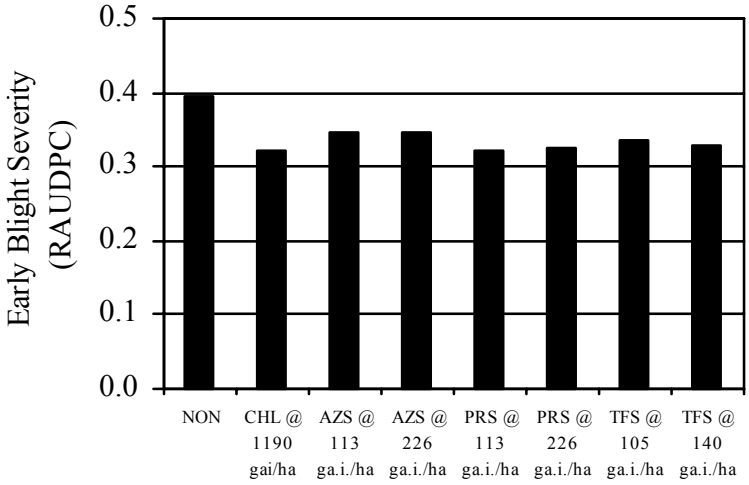

Figure 2. Early blight disease severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2002 and 2003 using cultivar Russet Norkotah. Data were combined and represent means of the data set from both years. A total of ten foliar fungicide applications were performed during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); five applications each of azoxystrobin (AZS) at 113 or 226 g a.i./ha; pyraclostrobin (PRS) at 113 or 226 g a.i./ha; and trifloxystrobin (TFS) at 105 or 140 g a.i./ha. All QoI fungicides were alternated (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test (P < 0.05).

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Effect of adjuvants on the efficiency of dimethomorph plus mancozeb (Acrobat 2 kg/ha) on the control of late blight in potato

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Summary
Industrial adjuvants were tested in combination with dimethomorph plus mancozeb (Acrobat 2 kg/ha) in the field to investigate their efficacy on foliar late blight caused by *Phytophthora infestans*. The tested adjuvant fungicide treatments for late blight control were applied 5 times at 7-day intervals. The effect of the adjuvant fungicide treatments on epidemic development, tuber rot and blight incidence and tuber yields were determined. Last summer late blight development was arrested in July due to high temperatures and lasting drought. August was characterized by rather low temperatures and high rainfall. These weather conditions were very favourable for the development of late blight. Due to the heat waves of June and July the foliage started to die already in August. Because of that no incidence of foliage blight was scored. No significant differences in yield were observed for the different treatments applied. The addition of an adjuvant had a clearly positive effect on the tuber yield. In the control 12,7 % infected tubers were observed. The mean tuber infection of plots sprayed with the Acrobat-adjuvant combinations was 7,4 %.

Keywords
Potato, late blight, *Phytophthora infestans*, fungicide adjuvant combination efficacy of adjuvants on fungicide efficiency

Introduction
An adjuvant is broadly defined as any substance added to the spray tank, separate from the pesticide formulation, that will improve the performance of the pesticide or the physical properties of the spray mixture, or both. The right adjuvant may reduce or even eliminate spray application problems, thereby improving overall pesticide efficacy. Adjuvants are designed to perform specific functions, including wetting, spreading, sticking, penetrating, reducing evaporation, reducing volatilization, buffering and dispersing. No single adjuvant can perform all these functions, but different compatible

adjuvants often can be combined to perform multiple functions simultaneously.
Within the scope of a research project financed by the Institute for the Promotion of Innovation by Science and Technology in Flanders a range of industrial adjuvants were screened for possible application in agriculture. They were tested in different model systems. One model system was potato-*Phytophthora infestans* since potato late blight remains one of the most serious constraints to potato production world wide. To control *P. infestans* and to protect the potato crop, potato plants are sprayed preventively with fungicides. Therefore, successful production of healthy potato crops relies on repeated applications of several fungicides during the potato growing season. A good rainfastness, spreading and sticking of a fungicide are important characteristics to improve the efficacy of a fungicide.

The objective of this study was to investigate the efficacy of Acrobat (dimethomorph plus mancozeb) in combination with adjuvants to control late blight during the growing season. Dimethomorph is a translaminar fungicide while mancozeb is a contact fungicide.

Material & Methods

Field trial
A field experiment was carried out on the experimental farm of the 'University College Ghent' at Bottelare during the growing season 2006. The first two treatments were the same for the different objects (mancozeb 1 kg/ha). Later on the different adjuvants were compared in a spray system based on 7-day intervals to test their effect on the efficiency of dimethomorph plus mancozeb (Acrobat). Therefore an artificial inoculation was done when the test period was started. The experiment was set up with the variety 'Bintje'. Treatments were carried out with a AKZO sprayer to 3 m wide and 10 m long plots. The spray boom was equipped with TeeJet nozzles (Teejet XR 11003 VK: 300 l water/ha or XR 110015 VK: 150 l water/ha) spaced 50 cm apart. The water volume was always 300 l/ha with the exception of Magic Sticker for which a water volume of 150 l/ha was used. The tested adjuvants and the applied doses are summarized in table 1.

Table 1: Adjuvants tested and applied dose

Adjuvant		Dose
Actirob B	methyloleate	500 ml/ha
Magic Sticker	styrene acrylate copolymerxypropoxy polyether	500 ml/ha
FullStop	styrene acrylate polymer	250 ml/ha
G850	fatty amido alkyl betaine	500 ml/ha
Softanol EP7025	alkyloxypolyethylene oxyethanol	0.10%
Softanol 70	alkyloxypolyethylene oxyethanol	0.10%
AE 5	vetalcohol ethoxylate	0.10%
Famee 5	methylester ethoxylate	0.10%
Zipper	trisiloxane ethoxylated propoxylated ethoxy-propoxy polyether	100 ml/ha
TB5031	block copolymer	0.10%
Purasolv BL	n-butyllactate	0.50%
P-25--12010	inuline starch derivative	0.10%
P01	Sunoco	1 l/ha
BC02		500 ml/ha

The experimental design was a completely randomised block design with four replicates. The adjuvants fungicide treatments were randomised within the blocks.

Following crop husbandry measures were taken: planting date of certified seed potatoes: 28 April 2006; row distance: 0.68 m; fertilisation: in spring 18 ton digested dung, 120 kg/ha N, 100 kg/ha P_2O_5

and 160 kg/ha K_2O. A second fraction of N 148 kg/ha was done 60 days after planting. Herbicide treatment: linuron + pendimethalin + prosulfocarb: 750 g + 800 g + 3,2 kg/ha (Luxan Linuron 1,5 l/ha + Stomp 2 l/ha + Defi 4 l/ha); control of Colorado potato beetle: lambda-cyhalothrin: 7,5 g/ha (Karate 75 ml/ha).
Diquat 600 g/ha (3 l/ha Reglone, Zeneca) was used to dessicate leaves and stems on 13 September.

Inoculum production and foliage inoculation
A mixture of 2 isolates of *P. infestans* was used for artificial infection. Inoculum was produced by the following procedure: sporangia were washed from sporulating lesions on detached leaflets of the susceptible potato cultivar 'Bintje' by rinsing the lesions with chilled distilled water + 0.01 % Tween and adjusted to 10^4 sporangia per ml using a Bürker counting chamber. To release zoospores, the resulting sporangial suspension was chilled for 1.5 h at 6 °C prior to inoculation. Plants of the infection drills between the experimental blocks were inoculated by spraying ~ 2620 sporangia/plant on 14 July in the late afternoon. In total 54 plants were infected with *P. infestans*. Before inoculation and 15 h after inoculation, the plants were sprayed with water to create optimal humidity conditions for infection. Due to high temperatures with daily average temperatures above 25 °C and low humidity the *P. infestans* infection was not successful. The plants were inoculated again in the second half of August. August was characterized by rather low temperatures and high rainfall. Those weather conditions favoured the development of *Phytophthora* infections all over the plots.

Disease estimates
To measure the intensity of foliage blight caused by *P. infestans* the foliar blight assessment key of the Blight Workshop in Tallinn was used: 0.0 % blight: no disease observed; 0.1 %: more than 1 lesion in a plot of 100 plants; 0,2 %: up to 25 lesions in a plot of 100 plants; 0,3 %: up to 50 lesions in a plot of 100 plants; 0,4 %: up to 75 lesions in a plot of 100 plants; 0,5 %: up to 100 lesions in a plot of 100 plants or 1 lesion per plant; 0,6 %: 2 lesions per plant in a plot of 100 plants; 0,7 %: 4 lesions per plant in a plot of 100 plants; 0,8 %: 6 lesions per plant in a plot of 100 plants; 0,9 %: 8 lesions per plant in a plot of 100 plants; 1 %: 10 lesions per plant in a plot of 100 plants; 5 %: 1 lesion per compound leaf or 50 lesions per plant in a plot of 100 plants; 10 %: 2 lesions per compound leaf or 100 lesions per plant in a plot of 100 plants; 25 %: nearly every leaflet with blight lesions, but plants retain normal form, plants may smell of blight, 75 % of plot leaf area remains green; 50 %: about 50 % of leaf area destroyed by blight; 75 %: about 75 % of leaf area destroyed by blight; 95 %: only a few leaves on plants, but stems green; 100 %: all leaves dead, stems dead or dying.
The overall amount of percentage blight was assessed at regular intervals for all the plots.

Data were analysed by performing analysis of variance (SAS 2.0). The One-sample Kolmogorov-Smirnov test was used to analyse the normal distribution of the obtained results. The Tukey test was used to compare treatment means when data were normally distributed, otherwise the Kruskall-Wallis test was performed..

Harvest
Tubers were harvested mechanically. Two rows over a distance of 8 m were harvested from the centre of each plot. All tubers were washed, weighed after grading and assessed for blight within 8 days after harvest. Washed tubers were examined visually for the presence or absence of lesions symptomatic of late blight. Furthermore, infected tubers were cut longitudinally to confirm the presence of dry brown corky rot in the tuber beneath the lesion, a symptom typical of late blight tuber infection. The diagnosis of tuber blight was further confirmed by observing sporangia production after incubating tubers with characteristic lesions in plastic containers containing moist paper towels. The amount of

blighted tubers was defined as the rotten tubers (but due to the secondary bacterial rot no characteristic blight symptoms could be observed) plus the tubers visually clearly infected by *P. infestans*.

Results & Discussion

The growing season 2006 was characterized by high temperatures and almost no rain in June and July. Due to these weather conditions the experimental plots were not infected starting from the infection drills. The period following the artificial inoculation was characterized by high temperatures and low humidity: half June 31,6 mm of rain was fallen and up to 17 July 1,4 mm, while between 18 June and 17 July the mean temperature fluctuated between 14,6 and 25,2 °C and during 14 days the maximum temperature was higher then 25 °C. In August the weather was cloudy, rather cold with a lot of rain. These weather conditions were very favourable for late blight. Therefore, the trials were twice inoculated in August, but the *Phytophthora* infection only developped in the second half of August. Only at the end of the growing season *P. infestans* was spread homogenously over the experimental fields. But at that moment the incidence of foliage blight could not properly be scored due to the early die off of the potato crops. Due to the heat waves of June and July the spray schedule was interrupted between 13 July and 18 August. Tubers were harvest and the occurrence of tuber blight was investigated.

No significant differences in yield were observed for the different treatments applied (Figure 1). The yield of the untreated plot was 35,0 ton/ha and the yield for Acrobat (dimethomorph + mancozeb) was 44,1 ton/ha. For the treatments of Acrobat in combination with an adjuvant the yield fluctuated between 45,6 and 50,8 ton/ha and the mean yield of all treatments with adjuvant was 47,6 ton/ha. Acrobat combined with Actirob B had the lowest yield: 41,0 ton/ha. The addition of an adjuvant had in general a clearly positive effect on the tuber yield: a mean increase of 3,5 ton/ha was obtained.

In the control plots 12,7 % infected tubers were observed (Figure 2). The plots sprayed with Acrobat (dimethomorph + mancozeb) had a tuber incidence of 9,3 %. The mean tuber infection of plots sprayed with the Acrobat-adjuvant combinations was 7,4 %: the percentage diseased tubers fluctuated between 3,9 and 10,9 %. The adjuvants FullStop and softanol EP7025 in combination with Acrobat (dimethomorph + mancozeb) did not improve the tuber protection. The adjuvants TB5031 and BC02 had a distinctly positive effect on tuber protection: only an infection of 4,0 % was observed against 9,3 % diseased tubers for Acrobat (dimethomorph + mancozeb) without adjuvant.

Conclusions

The growing season 2006 was characterized by high temperatures and almost no rain in June and July. In August the weather was cloudy, rather cold and we received a lot of rain. These weather conditions were very favourable for the development of late blight. Due to the heat waves of June and July the foliage started to die in August and as a consequence no foliage observations could be carried out. Nevertheless, the effect of the tested adjuvants on the efficiency of Acrobat (dimethomorph plus mancozeb) on tuber yield and tuber blight was investigated. From this field trial can be concluded that the tesed adjuvants, with the exception of Actirob B, had a positive effect on the tuber yield in combination with Acrobat (dimethomorph plus mancozeb). In the control 12,7 % infected tubers were observed. The mean tuber infection of plots sprayed with the Acrobat-adjuvant combinations was 7,4 %.

Acknowledgements

This field trial was part of a research project financed by the Institute for the Promotion of Innovation by Science and Technology in Flanders.

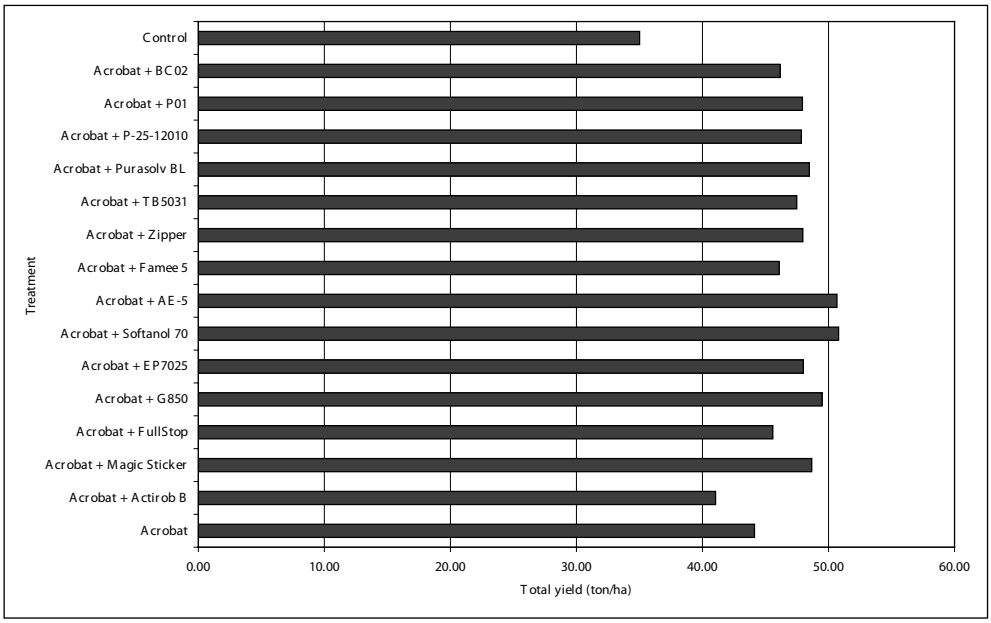

Figure 1: Influence of the fungicide-adjuvant combinations applied on tuber yield of 'Bintje' during the growing season 2006.

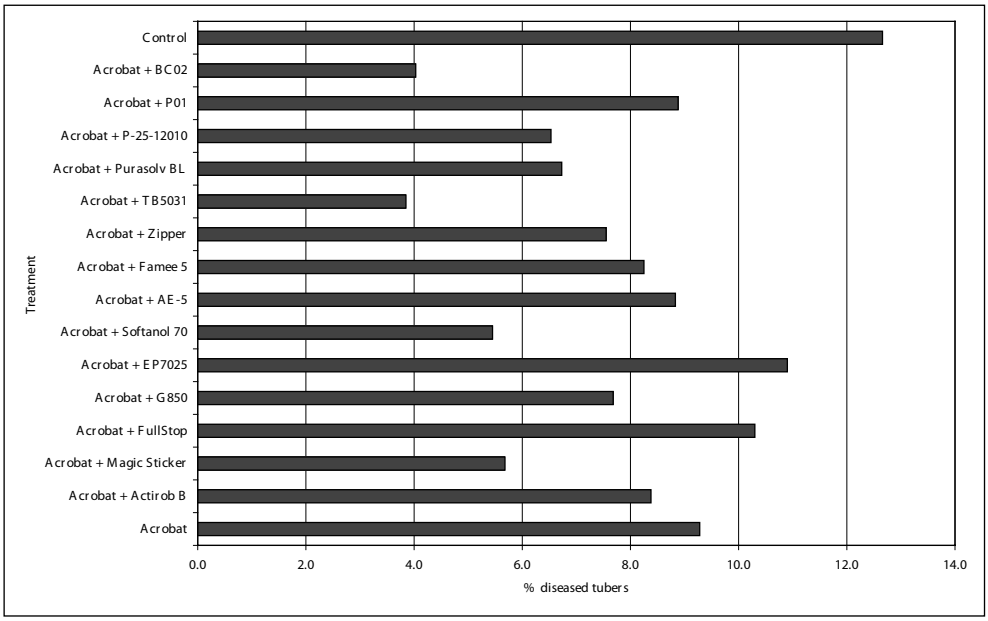

Figure 2. Influence of the fungicide-adjuvant combinations applied on tuber blight in 'Bintje' during the growing season 2006.

Tenth Workshop of an European Network for development of an
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Study on the STOP effect of fungicide combinations to control late blight in potato

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Summary
The effectiveness of different fungicide combinations to control late blight and the STOP effect of these fungicide combinations for the control of foliar and tuber blight under high infection pressure was investigated. Last summer late blight development was arrested in July due to high temperatures and lasting drought. In August the weather was cloudy, rather cold and a lot of rain. These weather conditions were very favourable for late blight. Due to the heat waves of June and July the foliage started to die already in August and *P. infestans* developped very fast in the second part of August. Because of that no incidence of foliage blight was scored. No significant differences in yield were observed for the different treatments applied. The combination cyazofamid + heptamethyltrisiloxaan (Ranman) + propamocarb + chlorothalonil (Tattoo C) had the highest yield. The percent diseased tubers fluctuated between 14,9 and 45,1 % for the different treatments tested. The amount of blighted tubers was lowest for fluazinam (Shirlan) + cymoxanil +chlorothalonil (Mixanil) and fluazinam (Shirlan) + benthiavalicarb + mancozeb (Valbon).

Keywords
Potato, late blight, *Phytophthora infestans*, fungicide combinations efficacy, STOP effect

Introduction
Potato late blight, caused by *Phytophthora infestans*, remains one of the most serious constraints to potato production world wide. Succesful production of healthy potato crops relies on repeated applications of several fungicides during the potato growing season. The reasons why late blight pressure is so high in Belgium are numerous: crop rotation is quite narrow and the intensity of growing potatoes is high which influences the infection pressure of *P. infestans*, the potato varieties used have, in general, a high susceptibility to potato blight on foliage and tubers and weather conditions favourable for *P. infestans*, a moderate sea climate, are frequently present in Belgium. Furthermore, the use of tin based fungicides were prohibited since 2005 in Belgium. These tin based contact fungicides were characterized by a good rainfastness and antisporulant activity. Blight-conducive weather allows a rapid progression of the disease on plots of susceptible varieties. Therefore, fungicide combinations with protectant and curative and/or antisporulant mode of action were used in the blight management.

This paper describes the effectiveness of different fungicide combinations to control late blight and the STOP effect of these fungicide combinations for the control of foliar and tuber blight under high infection pressure.

Materials & Methods

Field trial

A field experiment was carried out on the experimental farm of the 'University College Ghent' at Bottelare during the growing season 2006. The first three treatments were the same for the different objects (mancozeb 1 kg/ha). The fungicide treatments were conducted at 7-day intervals. The tested fungicide combinations were applied 3 times at 3-day interval. Finally, all objects were 2 times sprayed by fluazinam (200 g/ha) and 2 times by cyazofamid + heptamethyltrisiloxaan (80 g/ha + 126,9 g/ha). The different fungicide treatments and the applied doses are summarized in table 1. Cyazofamid + heptamethyltrisiloxaan (Ranman) and fluazinam (Shirlan) are contact fungicides, dimethomorph + mancozeb (Acrobat), benthiavalicarb + mancozeb, cymoxanil + chlorothalonil (Mixanil) are translaminar + contact fungicides and propamocarb + chlorothalonil (Tattoo C) is a systemic + contact fungicide.

Table 1: Applied fungicide treatments.

Treatment	Control	Object 1	Object 2	Object 3	Object 4	Object 5	Object 6	Object 7	Object 8	PCA
1	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb
2	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb
3	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb	mancozeb
4	untreated	Ranman + Valbon	Ranman + Acrobat	Ranman + Mixanil	Ranman + Tattoo C	Shirlan + Valbon	Shirlan + Acrobat	Shirlan + Mixanil	Shirlan + Tattoo C	Tattoo C
5	untreated	Ranman + Valbon	Ranman + Acrobat	Ranman + Mixanil	Ranman + Tattoo C	Shirlan + Valbon	Shirlan + Acrobat	Shirlan + Mixanil	Shirlan + Tattoo C	Acrobat
6	untreated	Ranman + Valbon	Ranman +Acrobat	Ranman + Mixanil	Ranman + Tattoo C	Shirlan + Valbon	Shirlan + Acrobat	Shirlan + Mixanil	Shirlan + Tattoo C	Acrobat
7	untreated	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan
8	untreated	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan	Shirlan
9	untreated	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman
10	untreated	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman	Ranman

Doses used: mancozeb: 1 kg/ha
Ranman: 0.2 l/ha + 0.15 l/ha
Valbon: 1.6 kg/ha
Acrobat 2.5 kg/ha
Mixanil: 2 kg/ha
Tattoo C: 2.7 l/ha
Shirlan: 0.4 l/ha

The field trial was carried out as a completely randomised block design with four replicates and with a plot size of 36 m^2. The experiment was set up with the variety 'Bintje'. Treatments were carried out with a AKZO sprayer. The spray boom was equipped with TeeJet nozzles (Teejet XR 11003 VK) spaced 50 cm apart. The water volume was always 300 l/ha.

Following crop husbandry measures were taken: planting date of certified seed potatoes: 28 April 2006; row distance: 0.68 m; fertilisation: in spring 18 ton digested dung, 120 kg/ha N, 100 kg/ha P_2O_5 and 160 kg/ha K_2O. A second fraction of N 148 kg/ha was done 60 days after planing. Herbicide treatment: linuron + pendimethalin + prosulfocarb: 750 g + 800 g + 3,2 kg/ha (Luxan Linuron 1,5 l/ha + Stomp 2 l/ha + Defi 4 l/ha); control of Colorado potato beetle: lambda-cyhalothrin: 7,5 g/ha (Karate 75 ml/ha).
Diquat 600 g/ha (3 l/ha Reglone, Zeneca) was used to dessicate leaves and stems on 7 September.

Inoculum production and foliage inoculation

A mixture of 2 isolates of *P. infestans* was used for artificial infection. Inoculum was produced by the following procedure: sporangia were washed from sporulating lesions on detached leaflets of the susceptible potato cultivar 'Bintje' by rinsing the lesions with chilled distilled water + 0.01 % Tween and adjusted to 10^4 sporangia per ml using a Bürker counting chamber. To release zoospores, the resulting sporangial suspension was chilled for 1.5 h at 6 °C prior to inoculation. From bloom plants of the mid rows (4 plant/row) of each experimental plot were inoculated by spraying ~ 9352

sporangia/plant on 14 July in the late afternoon. Before inoculation and 15 h after inoculation, the plants were sprayed with water to create optimal humidity conditions for infection. Due to high temperatures with daily average temperatures above 25 °C and low humidity the *P. infestans* infection was not very successful.

Disease estimates
To measure the intensity of foliage blight caused by *P. infestans* the foliar blight assessment key of the Blight Workshop in Tallinn was used: 0.0 % blight: no disease observed; 0.1 %: more than 1 lesion in a plot of 100 plants; 0,2 %: up to 25 lesions in a plot of 100 plants; 0,3 %: up to 50 lesions in a plot of 100 plants; 0,4 %: up to 75 lesions in a plot of 100 plants; 0,5 %: up to 100 lesions in a plot of 100 plants or 1 lesion per plant; 0,6 %: 2 lesions per plant in a plot of 100 plants; 0,7 %: 4 lesions per plant in a plot of 100 plants; 0,8 %: 6 lesions per plant in a plot of 100 plants; 0,9 %: 8 lesions per plant in a plot of 100 plants; 1 %: 10 lesions per plant in a plot of 100 plants; 5 %: 1 lesion per compound leaf or 50 lesions per plant in a plot of 100 plants; 10 %: 2 lesions per compound leaf or 100 lesions per plant in a plot of 100 plants; 25 %: nearly every leaflet with blight lesions, but plants retain normal form, plants may smell of blight, 75 % of plot leaf area remains green; 50 %: about 50 % of leaf area destroyed by blight; 75 %: about 75 % of leaf area destroyed by blight; 95 %: only a few leaves on plants, but stems green; 100 %: all leaves dead, stems dead or dying.
The overall amount of percentage blight was assessed at regular intervals for the middle and outer rows of all the plots separatly.

Data were analysed by performing analysis of variance (SAS 2.0). The One-sample Kolmogorov-Smirnov test was used to analyse the normal distribution of the obtained results. The Tukey test was used to compare treatment means when data were normally distributed, otherwise the Kruskall-Wallis test was performed..

Harvest
Tubers were harvested mechanically. Two rows over a distance of 10 m were harvested from the centre of each plot. All tubers were washed, weighed after grading and assessed for blight within 8 days after harvest. Washed tubers were examined visually for the presence or absence of lesions symptomatic of late blight. Furthermore, infected tubers were cut longitudinally to confirm the presence of dry brown corky rot in the tuber beneath the lesion, a symptom typical of late blight tuber infection. The diagnosis of tuber blight was further confirmed by observing sporangia production after incubating tubers with characteristic lesions in plastic containers containing moist paper towels. The amount of blighted tubers was defined as the rotten tubers (but due to the secondary bacterial rot no characteristic blight symptoms could be observed) plus the tubers visually clearly infected by *P. infestans*.

Results and Discussion
The growing season 2006 was characterized by high temperatures and almost no rain in June and July. Due to these weather conditions the *Phytophthora* infection did not develop in the experimental plots since the period following the artificial inoculation was characterized by high temperatures and low humidity: half June 31,6 mm of rain was fallen and up to 17 July 1,4 mm, while between 18 June and 17 July the mean temperature fluctuated between 14,6 and 25,2 °C and during 14 days the maximum temperature was higher then 25 °C. In August the trial was twice inoculated, but the *Phytophthora* infection only developped in the second half of August. Only at the end of the growing season *P. infestans* was spread homogenously over the experimental fields. But at that moment the incidence of foliage blight could not properly be scored due to the early die off of the potato crops. Due to the heat waves of June and July the fungicide combinations were sprayed the last week of August and the first week of September. Tubers were harvest and the occurrence of tuber blight was investigated.

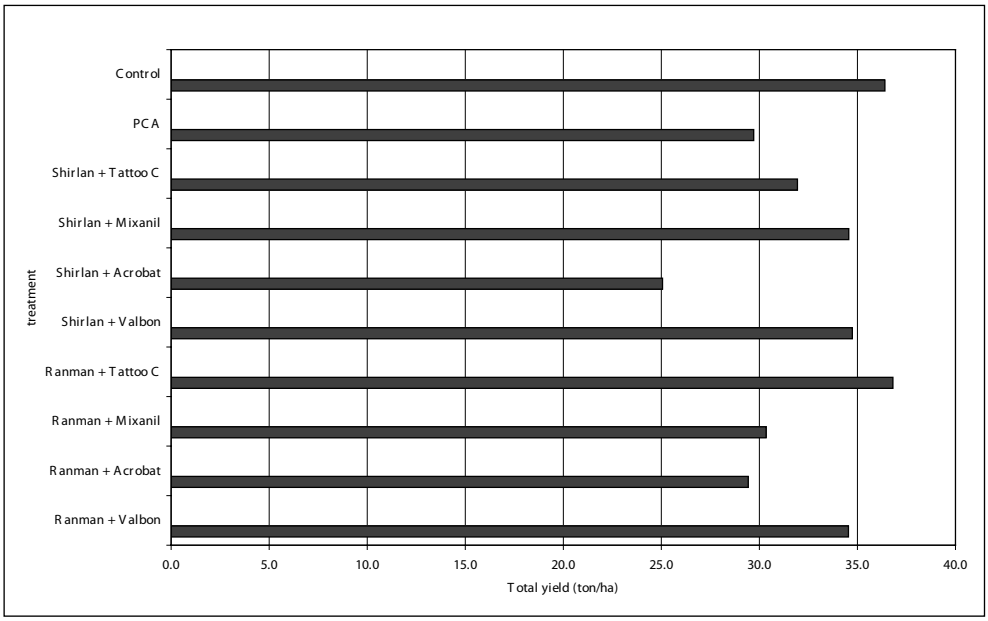

Figure 1: Influence of the fungicide combinations applied on tuber yield of 'Bintje' during the growing season 2006.

No significant differences in yield were observed for the different treatments applied (Figure 1). The mean yield of all treatments was 32,3 ton/ha and the combination cyazofamid + heptamethyltrisiloxaan (Ranman) + propamocarb + chlorothalonil (Tattoo C) had the highest yield: 36,8 on/ha. By comparison with the tested fungicide combinations the yield of the control was rather high: 36,4 ton/ha. These observations could be explained by the fact that the trial plot led under the down pours of August which led to bad growing conditions for the potatoes. This also led to a high variation coefficient for the yield: 18,09. The treatments with dimethomorph + mancozeb (Acrobat) were characterized by a lower yield: plot sprayed according to the advice of the decision support system (PCA) (2x Acrobat): 2937 ton/ha, Ranman + Acrobat: 29,4 ton/ha and Shirlan + Acrobat: 26,1 ton/ha. Also the treatments with fluazinam (Shirlan) + benthiavalicarb + mancozeb (Valbon), fluazinam (Shirlan) + cymoxanil +chlorothalonil (Mixanil) and cyazofamid + heptamethyltrisiloxaan (Ranman) + benthiavalicarb + mancozeb (Valbon) had a good yield: respectively 34,7, 34,6 and 34,5 ton/ha.

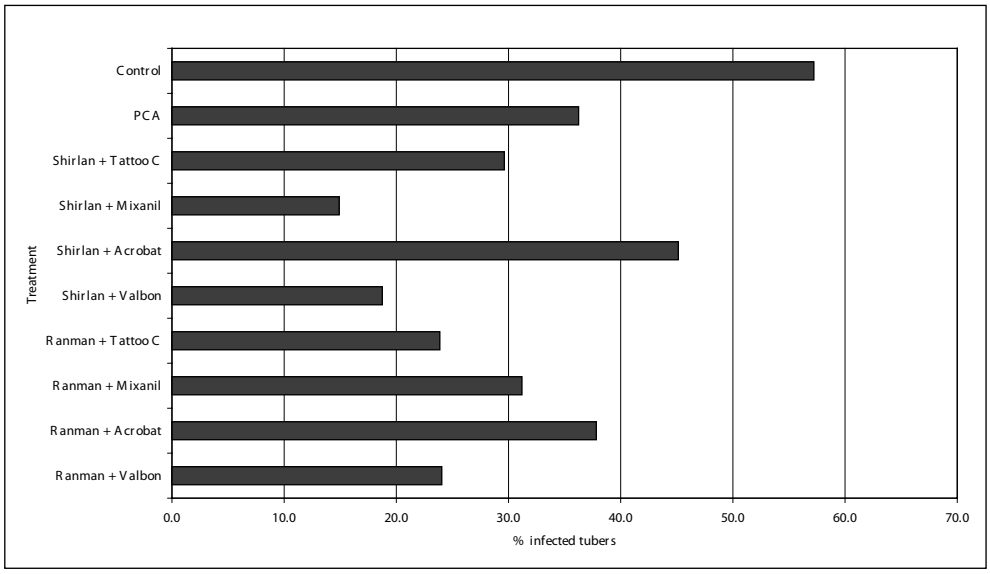

Figure 2: Influence of the fungicide combinations applied on tuber blight in 'Bintje' during the growing season 2006.

The percent diseased tubers fluctuated between 14,9 and 45,1 % for the different treatments tested (Figure 2). In the control 57,2 % infected tubers were observed. This observation can be explained by the intense rainfall of August. The amount of blighted tubers was lowest for fluazinam (Shirlan) + cymoxanil + chlorothalonil (Mixanil) and fluazinam (Shirlan) + benthiavalicarb + mancozeb (Valbon): 14,9 and 18,8 % respectively. The plots treated with dimethomorph + mancozeb (Acrobat) had the highest percent of diseased tubers: 36,3 % for the plot sprayed according to the advice of the decision support system (PCA) (2x Acrobat), 37,8 % Ranman + Acrobat and 45,1 % for Shirlan + Acrobat.

Conclusions

The growing season 2006 was characterized by high temperatures and almost no rain in June and July. In August the weather was cloudy, rather cold with a lot of rain. These weather conditions were very favourable for the development of late blight. Due to the heat waves of June and July the foliage started to die already in August and as a consequence no foliage observations could be carried out. Nevertheless, the effect of the tested fungicide combinations on tuber yield and tuber blight was investigated. From this field trial under no optimal growth conditions can be concluded that highest yield was obtained with cyazofamid + heptamethyltrisiloxaan (Ranman) + propamocarb + chlorothalonil (Tattoo C). Tuber blight was lowest for fluazinam (Shirlan) + cymoxanil +chlorothalonil (Mixanil) fluazinam (Shirlan) + benthiavalicarb + mancozeb (Valbon). The plots sprayed with dimethomorph + mancozeb (Acrobat) had a lower yield and higher percentage of infected tubers.

Acknowledgements

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Monitoring virulence and mating type of *Phytophthora infestans* in the Netherlands in 2004 and 2005.

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Introduction

In the Netherlands and elsewhere in the world, *Phytophthora infestans* is a major constraint to potato cultivation. Daily grower attention is required to prevent crops from being infected and destroyed. Annually, an average of 10 – 15 fungicide applications are needed to control potato late blight in the Netherlands. The annual cost of control amount to approximately 130M€ or 20% of the farm gate turn over (KWIN 2006).

It is expected that new, highly resistant, potato varieties are going to play a major role in future potato late blight control strategies and the effort to reduce the fungicide input in potato cultivation. However, *P. infestans* has proven to be highly efficient in adapting to restraints to population development such as newly introduced host plant resistance. So far, the lifespan of resistance genes was therefore generally short.

Introduction of combinations of resistance genes will, at least theoretically, slow down adaptation of the *P. infestans* population towards virulence. Continuous monitoring of the virulence spectrum, towards old and new R-genes is however necessary. A thorough knowledge of developments concerning the virulence spectrum provides additional opportunities to exploit R- gene resistance in a more durable way.

The goal of the work described in this paper was to collect and characterize *P. infestans* isolates in the Netherlands in 2004 and 2005 to gain insight in developments in the virulence spectrum of the Dutch *P. infestans* population.

Materials & Methods

Bait fields, containing old and new resistant material, were established at three locations, Lelystad, Valthermond and Vredepeel, in the Netherlands in 2004 and 2005. Black's R-gene differential set R0 – R11 (Black *et al.,* 1953; Malcomson & Black, 1966) and genotypes or cultivars provided by breeding companies were planted in 6 plants plots per genotype on all three locations. The plots were not treated with fungicides. Infection was monitored on a weekly basis, infected plant material was collected and occurring *P. infestans* was cultured. *P. infestans* isolates were subsequently stored in liquid nitrogen and characterised. A representative number of isolates per location, solanum genotype and year were characterized for AFLP-fingerprint (primer combination E21/M16), mating-type and virulence spectrum. 52 isolates obtained in 2004 and 62 isolates obtained in 2005 were characterized.

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The mating type test was initiated by co-inoculating the isolate to be tested and a A1 or A2 tester strain on pea-agar. Isolate IPO98014 was used as the A1 tester strain whereas isolate IPO655-2A was used as the A2 tester train. Formation of oospores was checked microscopically.
The virulence spectrum of the isolates was determined using Black's R-gene differential set (R0 – R11), by spray inoculating the lower side of two detached leaflets per genotype with a sporangial suspension (1 x 10^4 sporangia per ml). Inoculated leaflets were incubated in petri dishes containing 1.5 % water agar in a climate chamber at 15°C and 16 hours light per day as described by Flier and Turkensteen (1999). Following one week of incubation, necrosis and sporulation were quantified visually with the aid of a stereo microscope. Compatibility was assumed if at least 5 % of a leaflet was necrotic and sporulation could be detected. Two replicate experiments were carried out.

Results

AFLP
AFLP results are given in Table 1. The level of polymorphism within the 6 *P. infestans* populations is shown in terms of heterozygosity and the number of polymorphic loci. Fourteen and twenty eight loci (out of 78) were polymorphic in 2004 and 2005 respectively. Seven and ten groups of identical isolates were distinguished in 2004 and 2005 respectively. Genetic distances between the 6 *P. infestans* populations are shown in Figure 1.

Table 1. Analysis of genetic variability of *P. infestans* isolates based on AFLP results. Fourteen and twenty eight loci (out of 78) were polymorphic in 2004 and 2005 respectively.

year	Location	Population	# isolates	Average heterozygosity	# polymorphic loci	% polymorphic loci
2004	Lelystad	1	13	0	0	0
	Vredepeel	2	17	0.07	12	16
	Valthermond	3	19	0	0	0
2005	Lelystad	4	23	0.02	8	10
	Vredepeel	5	24	0.04	12	15
	Valthermond	6	19	0.10	23	29
2004	All locations	1,2,3	49	0.07	14	18
2005	All locations	4,5,6	66	0.08	28	35

Mating type
Table 2 gives an overview of the results of the mating type determination of the 2004 and 2005 isolates on all three locations.

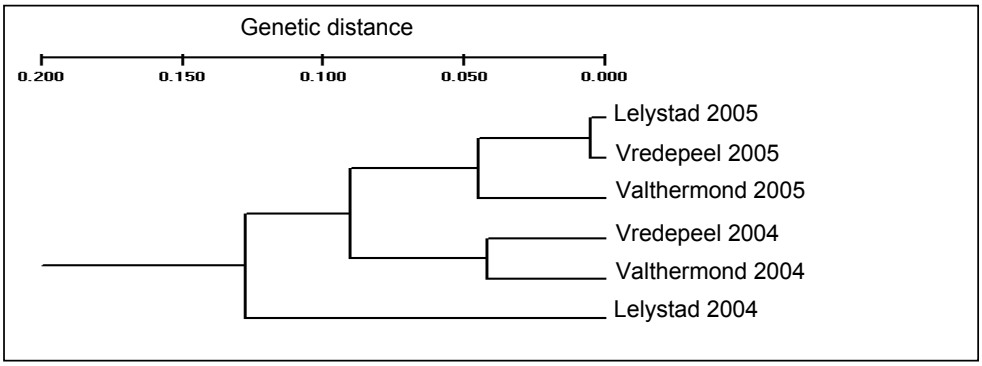

Figure 1. Dendrogram of the P. infestans population for years and locations.

Table 2. Mating type of the bait field isolates collected in 2004 and 2005 in Lelystad, Valthermond and Vredepeel.

	2004			2005		
Mating type	Lelystad	Vredepeel	Valthermond	Lelystad	Vredepeel	Valthermond
A1	28	1	0	1	8	21
A2	0	37	37	35	45	16
% A2	0	97	100	97	85	43

On all locations and in both years, one of both mating types was dominant except in Valthermond in 2005 where both mating types occurred in similar numbers (Table 3).

Table 3. *P. infestans* mating types per *Solanum* genotype occurring in bait fields in two years and three locations.

Genotype	Vredepeel		Lelystad		Valthermond	
	2004	2005	2004	2005	2004	2005
R 0	A2	A2	A1	A2	-	A1
R 1	A2	A2	A1	A2	A2	A1
R 2	A2	A2	A1	A2	A2	A2
R 3	A2	A2	A1	A2	A2	A1
R 4	A2	A2	A1	A2	A2	A1
R 5	A2	A2	*	A2	A2	A2
R 6	A2	A2	A1	A2	A2	A1 & A2
R 7	A2	A2	A1	A2	A2	A1
R 8	A2	A1	*	*	*	A1
R 9	A2	A1	A1	*	*	*
R 10	A2	A2	A1	A1	A2	A1
R 11	A1 & A2	A2	A1	A2	A2	A1
AM 66-42	A2	A2	A1	A2	A2	A1 & A2
Axona	-	A2	-	A2	-	A2
Biogold	A2	A2	A1	A2	A2	*
CMK-MCD1	A2	A2	*	A2	A2	A1
HZPC-02	A2	*	*	*	A2	*
HZPC-04	A2	A2	*	*	A2	*
HZPC-05	A2	A2	A1	A2	A2	A1 & A2
KA-0001	-	A2	-	A2	-	A2
KA-0002	-	A2	-	A2	-	A2
KA-95-0140	A2	A2	*	*	A2	*
Sarpo Mira	-	A2	-	*	-	*
Spirit	-	A2	-	A2	-	A1
VR -92-501	-	A2	-	A2	-	A2

–: Genotype not included in this experiment.
**: P. infestans not found in this genotype.*

Virulence

All *P. infestans* isolates displayed highly complex virulence spectra with an average of 8 to 10 virulence factors for Black's differentials R1 – R11 per isolate (Table 4). Figure 2 shows the frequency of individual virulence factors in Dutch *P. infestans* isolates in 2004 and 2005 at AFLP group level.

Table 4. Average number of virulence factors for R1 - R11 per isolate per year observed on the different locations. The maximum number of virulence factors is 11.

Location	2004	2005
Vredepeel	9.0	9.9
Lelystad	8.7	8.9
Valthermond	8.7	7.9

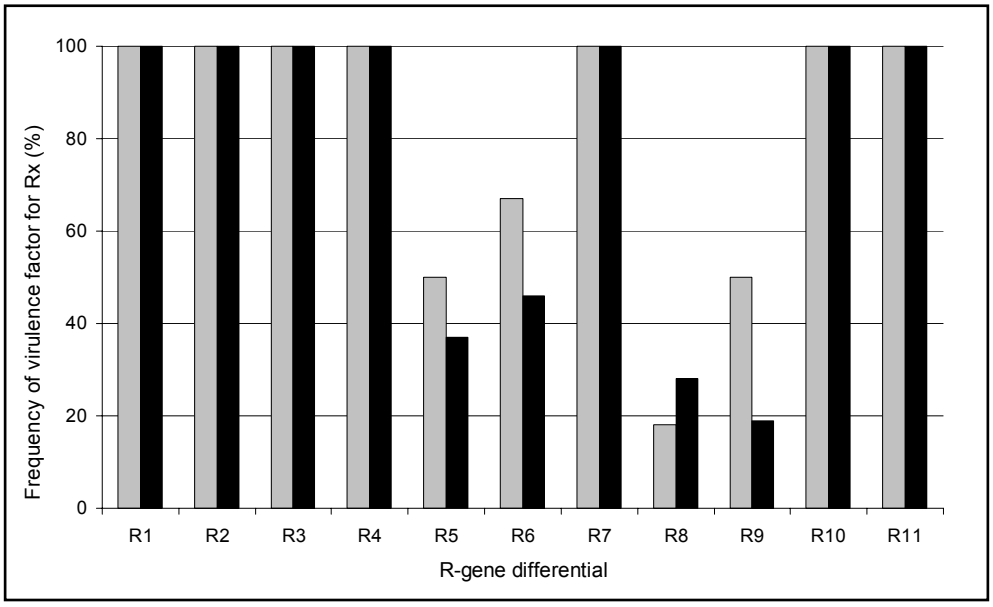

Figure 2. Frequency of virulence factors for Black's R1 – R11 in Dutch P. infestans isolates from 2004 (■) and 2005 (■)at AFLP group level.

Discussion & Conclusions

The aim of the work described in this paper was to collect and characterize *P. infestans* isolates in the Netherlands in 2004 and 2005 to gain insight in development of the virulence spectrum of the Dutch *P. infestans* population. For this purpose bait fields containing Black's differentials and resistant breeding material were established in 2004 and 2005 at Valthermond, Lelystad and Vredepeel. Naturally occurring *P. infestans* was removed, cultured and characterized for AFLP pattern, mating type and virulence spectrum.

P. infestans was found on - and cultured from all genotypes included in the experiments indicating that, at least low level, virulence is present for all R-genes included in these experiments. With respect to virulence to Blacks R-gene differentials R1 – R11, highly complex virulence spectra were common. On average, *P. infestans* isolates contained 8 – 10 virulence factors including at least virulence for R1, R2, R3, R4, R7, R10 & R11. Considering the development of the virulence spectrum in the Dutch *P. infestans* population, virulence factors seem to accumulate without a pronounced effect on pathogenic fitness.

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Development of spray strategies to control late blight, 2003-2006.

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Introduction

Late blight is the most devastating disease of potato in The Netherlands. The new and more aggressive *P. infestans* population requires even more alertness than in the past. The potato crop is usually sprayed between 8 and 14 times. Timing and choice of fungicide are key factors to control late blight successfully. An adverse effect of spraying is the environmental burden by emission of the fungicides to the environment. Experiments were set up to compose strategies to improve late blight control. Besides the main objective of effectiveness, the environmental effects of the sprayings, the risk and the costs were evaluated. The possibility to reduce the dose rate of Shirlan on more resistant cultivars was especially implemented in 2006. Spray strategies were tested by Applied Plant Research at different locations throughout the Netherlands, during four years. The locations were chosen considering the climatic conditions, purpose of potato cultivation, disease pressure and type of soil. During and after the experiments farmers were informed at the internet site www.kennisakker.nl.

Materials & Methods

To compose a spraying strategy the characteristics of the fungicides have to be adjusted to the growing stage of the potatoes and external circumstances as weather conditions and disease pressure. Therefore the season was divided into two growing stages presented in Figure 1.

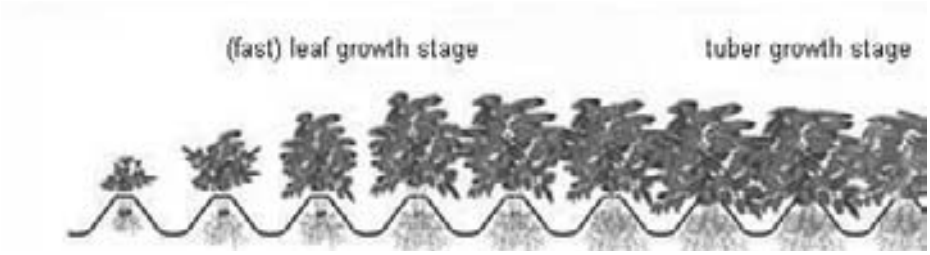

Figure 1. The growing season split up in two growing stages; the (fast) leaf growth stage and the tuber growth stage.

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Fungicides, which have the capacity to protect newly grown leaves, were used in the early stage of the growing season. Fungicides with tuber protecting characteristics were used in the second part of the growing season. The experiments were carried out at 5 locations (Table 1), during 2003 - 2005.

Table 1. Design of the experiments carried out from 2003 until 2005.

location	cultivar	leaf resistance	tuber resistance	purpose	Soil type
Lelystad (Flevoland)	Agria	5.5	7.5	Ware	clay
Valthermond (Drenthe)	Karakter	6.0	5.0	Starch	peat
Kollumerwaard (Friesland)	Asterix	5.0	8.5	Ware	clay
Westmaas (Zuid-Holland)	Agria	5.5	7.5	Ware	clay
Wijnandsrade (Limburg)	Lady Olympia	3.0	4.5	Ware	peaty soil

The spraying strategies were carried out as presented in Tables 2 and 3. Dose rates used were usually the recommended dose rates.

Table 2. Spraying strategies carried out at Lelystad, Kollumerwaard, Wijnandsrade and Westmaas; 2003 - 2005.

strategy	(fast) leaf growth			Tuber filling	
A	According to good agricultural practise				
B	Spraying with Shirlan				
C	Shirlan	Tanos (3x)	Ranman (3x)	Shirlan	Ranman (3x)
D	Shirlan	Fubol Gold (2x)	Curzate M	Shirlan	Ranman (3x)

Table 3. Spraying strategies carried out at Valthermond; 2003 - 2005.

strategy	(fast) leaf growth			Tuber filling	
A	According to good agricultural practise				
B	Spraying with Shirlan				
C	Curzate M	Curzate M	Curzate M	Curzate M	Curzate M
D	Dithane NT	Dithane NT	Dithane NT	Dithane NT	Dithane NT

Spray strategies were linked to cultivar resistance in 2006. Results of experiments lowering the dose rate according to the degree of cultivar resistance were used. The dose rate of Shirlan used was lower when highly resistant cultivars were grown. The experiments were carried out at Lelystad, Valthermond and Westmaas. Spraying strategies using local cultivars were carried out as presented in Tables 4 and 5.

Table 4. Spraying strategies carried out at Lelystad and Westmaas; 2006.

strategy	Cultivar	(fast) leaf growth	Tuber filling	
A	Bintje	0.4 l/ha Shirlan	0.4 l/ha Shirlan	
B	Agria	0.3 l/ha Shirlan	0.4 l/ha Shirlan	
C	Innovator	0.2 l/ha Shirlan	0.3 l/ha Shirlan	
D	Agria	Curzate M	0.4 l/ha Shirlan	
E	Agria	Valbon	Sereno	Ranman 3x

Table 5. Spraying strategies carried out at Valthermond; 2006.

strategy	Cultivar	(fast) leaf growth	Tuber filling
A	Seresta	As practise	
B	Karakter	0.4 l/ha Shirlan	0.4 l/ha Shirlan
C	Seresta	0.2 l/ha Shirlan	0.3 l/ha Shirlan
D	Festien	0.1 l/ha Shirlan	0.2 l/ha Shirlan
E	Seresta	Dithane NT	Dithane NT
F	Seresta	Curzate M	Curzate M

The number of spray applications, the number of spraying points, the amount of active ingredients used and the effect of fungicide sprays on the environment was established. Spraying points were calculated by multiplying the number of spray applications with the average relative dose rate compared to the full dose rate as permitted under Dutch legislation. Fungicides are rated for their effect on the environment (air, soil water, surface water and soil). Environmental effect of each strategy was established by adding up the effect of each separate spray application on each of the different aspects of the environment. Disease development was assessed weekly. Yield and tuber blight were established at the end of the season.
Data were analyzed using Genstat 9th edition.

Results

2003
The summer of 2003 was very warm and dry. Late blight was not found. Under these circumstances all strategies were very effective.

2004 and 2005
In both summers of 2004 and 2005 the weather conditions were favourable for the development of late blight. In these years there were no differences in effectiveness on leaf blight between the strategies applied. Tuber blight occurred in 2004 only. Table 6 shows the percentage tuber blight found after applying different late blight control strategies. Tuber blight incidence was not significantly different between the different spray strategies except for Valthermond.

Table 6. Effect op spray strategies on tuber blight incidence in 2004.

Spray strategy	% tuber blight at different sites				
	Lelystad	Kollumerwaard	Westmaas	Wijnandsrade	Valthermond
A	1.5	0	< 0.1	0	3.9 a
B	< 0.1	0	< 0.1	0	1.9 ab
C	0	0	0	0	0 b
D	< 0.1	0	< 0.1	0	2.4 ab

2006
The summer of 2006 (July) was very warm and dry and August was very wet. The wet August resulted in a high disease pressure during almost the whole of the month August. Infection was limited to several leaves per plot only, despite the high disease pressure in August 2006. This indicates that the strategies to control late blight were effective. When cultivars with a certain level of resistance were grown, the dose rate of Shirlan could be lowered accordingly.

Experiments showed that when in the foliage late blight lesions were observed and there is regularly rainfall, tuber protecting fungicides had to be sprayed.

The effect of spray applications in 2006 on the environment are presented in Table 7. The requirement to water organisms was not met in any of the strategies.

Table 7. The spraying strategies and their environmental impact in the experiment in 2006 at Lelystad.

strategy	number of sprayings	dose rate of the sprayings	spraying points	kg/ha active ingredient	exposure to the air	exposure to water organuisms
quality objective					0.7	0
Strategy A Bintje	14	14*1	14	2.8	0.89	100
Strategy B Agria	14	4*0.75 / 10*1	13	2.6	0.84	100
Strategy C Innovator	14	4*0.5 / 10*0.75	9.5	1.9	0.62	100
Strategy D Agria	16	16*1	16	9	0.78	62
Strategy E Agria	14	14*1	14	9.3	0.25	71

Discussion and conclusions

Spray strategies had no effect on yield. Probably because no difference in late blight control was found between the control strategies applied. Yield differences were found in 2006. However differences in yield were associated with the cultivar used and did not depend on the spray strategy (data not shown).

The past 4 years in which the experiments were carried out showed that the timing of the spraying moment is very important. The spraying moment depends primarily on the weather conditions, the disease pressure and the time since the last spray was carried out. On the whole all strategies gave an acceptable control of late blight under circumstances found in the agricultural practice. Some singinificant tuber blight was found only at Valthermond in 2004.

Dose rate reduction based on the resistance level of the cultivar proved to be possible. Timing of the fungicide application however is crucial. Lowering of the dose rate saves the farmer money.

Depending upon the growth stage a suitable fungicide has to be sprayed. For instance when it is supposed that the disease pressure originates from latently infected tubers which are expressed by stem infection, cymoxanil containing fungicides or Fubol Gold are advised to spray. The type of fungicide is also crucial when leaf blight is observed and (heavy and/or long lasting) rainfall is predicted. In that case it is necessary to spray the crop with tuber protecting fungicides. An example of that was shown in 2004 at Valthermond (Table 6).

Due to yearly adjustments of the spray strategies the environmental burden decreased during the research period. This was partly caused by exploiting the possibility of dose rate reduction in resistant cultivars. Also the effect of mancozeb on the environment was re-evaluated. The effect on air, soil water and soil was less when strategies based on fluazinam were used compared to the other strategies. Fluazinam has some effect on life in the surface water however.

In 2007 experiments will continue and will be carried out comparable to the 2006 experiment at 7 locations. The aim is to show farmers the possibilities of exploiting cultivar resistance by lowering the dose rate of the fungicide applied. Also the effect of spray strategies on early blight will be investigated.

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Efficiency of three fungicides in leaf disc assays against *Phytophthora infestans* isolates from fields with different late blight management

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Summary
A total of 48 isolates of *Phytophthora infestans* were collected from two sites in Hungary which had been treated with fungicides. Sensitivity of the isolates was tested against three fungicides, cymoxanil, azoxystrobin and dimethomorph in leaf disc assays. A comparison among the EC_{50} values of the isolates using leaf discs of tomato and the number of fungicide application on the fields did not show significant differences in either case of cymoxanil and azoxystrobin, but less sensitive isolates were present when the crop was treated with dimethomorph. Regardless of the number of treatments with a particular fungicide, the distribution of EC_{50} values for that fungicide was not significantly different at the two sites sampled. Our work has estimated the baseline sensitivity to cymoxanil and azoxystrobin of two populations of *P. infestans* in Hungary.

Keywords
Cymoxanil, azoxystrobin, dimethomorph, Hungary, fungicide sensitivity.

Introduction
Phytophthora infestans, the causal agent of potato and tomato late blight is one of the most destructive pathogen in agriculture, fungicide application is still the main method of control in Hungary. The risk of fungicide resistance/tolerance in the pathogen population increases with the increased number

of treatments with a specific active ingredient. Insensitivity of *P. infestans* to phenylamides has been well known (*e. g.* Dowley and O' Sullivan, 1981) and has been reported from Hungary (Bakonyi and Érsek, 1997a; 1997b). Other systemic fungicides such as cymoxanil, dimethomorph and azoxystrobin, all with specific target site, are commonly used in mixtures with protectant fungicides in potato late blight management by Hungarian growers. Therefore, our aim was to test the sensitivity of field isolates of *P. infestans* from potato to these active ingredients.

Materials and methods

Blighted leaves and tubers were collected at different dates at two locations about 200 km apart. Fields were managed according to the farmers's practice. Isolates from Nagykálló originated from an experimental field divided into several small plots, each treated with the same contact and one of the two systemic fungicides, dimethomorph or azoxystrobin, plus a non-treated control plot. Isolates from Solt originated from 3 fields treated with the same chemicals, except for the last spray of copper + cymoxanil or fluazinam (Table 1). All fungicide doses were determined according to the instructions of the official licence; if a range of concentrations was given, the highest permitted dose was applied with the exception of azoxystrobin (Szabadi, 2006).

EC_{50} values of isolates were assessed in three replicates using a dilution series of 100, 10, 1, 0.1 and 0 mg active ingredient/L for azoxystrobin (Quadris) and of 1000, 100, 10, 1 and 0 mg a. i./ L for cymoxanil (Curzate 50 WP) and dimethomorph (pure active ingredient). The youngest fully expanded leaves of 6-week-old tomato plants (cv. Zömök) were used for inoculation. Plants were grown in the greenhouse and had 5–6 fully expanded leaves at this stage. Fungicide treatment was applied prior to the inoculation by means of submerging the plant material into fungicide solution for 10 minutes. Leaf disks were then dried and covered evenly on the abaxial side with a suspension of 10^4–10^6 sporangia/ml using a laboratory hand mist sprayer. The inoculated plant material was incubated at 14–16 °C for 7 days in moist chambers.

EC_{50} values for the three fungicides were computed after logarithmic transformation of the inhibitory effects calculated with Abbott's formula.

The whole range of EC_{50} values was divided into several groups with an equal interval and the frequency of isolates for each group was determined. An independence test was used to check the correlation between the number of fungicide treatments and the distribution of EC_{50} values. The sampling locations were also compared in a homogeneity test in order to ascertain if the distributions of $lg_{(EC50)}$ values of the isolates differ significantly.

The crop in Solt was sprayed once with famoxadone, another QoI fungicide. Although famoxadone and azoxystrobin have different binding modes to the cytochrome bc_1 complex in the mitochondrial transpiration system (Jordan *et al.,* 1999), high risk of cross resistance has been shown between them (e. g. Steinfeld *et al.,* 2001; Fernández-Ortuño *et al.,* 2006). Famoxadone and azoxystrobin treatment were therefore considered interchangeable in this analysis.

Table 1. Spraying programme on the sampling sites until blighted blight samples were collected from the fields.

	Nagykálló		Solt	
Date of treatment	Fungicide (active ingredient)	Dose	Fungicide (active ingredient)	Dose
6 June			Tanos 50 DF (cymoxanil+famoxadone)	0.4 kg/ha1
13 June			Kupfer Fusilan WG (copper+cymoxanil)	2.7 kg/ha
19 June	Cuproxat FW (copper)	5.0 L/ha2		
20 June			Altima (fluazinam)	0.4 L/ha
30 June	Cuproxat FW (copper)	5.0 L/ha		
7 July			Acrobat MZ & Kupfer Fusilan WG (dimethomorph+mancozeb & copper+cymoxanil)	2.0 + 2.7 kg/ha
14 July	Cuproxat FW (copper)	5.0 L/ha	Forum R (dimethomorph+copper)	3.5 kg/ha
	Manzate 75 DF (mancozeb)	1.3 kg/ha		
24 July	Cuproxat FW (copper)	5.0 L/ha		
	Manzate 75 DF (mancozeb)	1.3 kg/ha		
	Acrobat MZ (dimethomorph+mancozeb)	2.0 kg/ha		
	Amistar (azoxystrobin)	0.8 L/ha		
28 July			Acrobat MZ (dimethomorph+mancozeb)	2.0 kg/ha
9 August			Kupfer Fusilan WG (copper+cymoxanil)	2.7 kg/ha
10 August			Altima (fluazinam)	0.4 L/ha
11 August	Cuproxat FW (copper)	5.0 L/ha		
	Manzate 75 DF (mancozeb)	1.3 kg/ha		
	Acrobat MZ (dimethomorph+mancozeb)	2.0 kg/ha		
	Amistar (azoxystrobin)	0.8 L/ha		
24 August	Cuproxat (copper)	5.0 L/ha		
	Manzate 75 DF (mancozeb)	1.3 kg/ha		
	Acrobat MZ (dimethomorph+mancozeb)	2.0 kg/ha		
	Amistar (azoxystrobin)	0.8 L/ha		

[1]*Fungicides in Solt were applied in a 300 L/ha solution/suspension except the first treatment with Tanos (100 L/ha).*
[2]*Fungicides in Nagykálló were applied in a 400L/ha solution/suspension.*

Results

Twenty nine isolates were collected from Solt and 19 isolates from Nagykálló. The EC_{50} values of individual isolates collected in Solt and Nagykálló are summarised in Table 2. and Table 3., respectively. In general all isolates were susceptible to the fungicides tested in this study. The population of *P. infestans* was most susceptible to azoxystrobin. Cymoxanil performed less effectively whereas isolates were most resistant to dimethomorph, although the EC_{50} values for this fungicide were also low, not affecting efficiency of plant protection in practice.

In this study all the isolates were susceptible to cymoxanil. Nearly half of the isolates had EC_{50} values lower than 0.31 mg a. i./L [10 of 19 isolates in Nagykálló (52%) and 14 of 29 in Solt (48%)]. Twelve and 17 isolates were treated 3 and 4 times with this fungicide but 19 were not treated at all. These three groups of isolates did not show significant difference in their EC_{50} values in an independence test ($x^2 = 10.321$, $P = 0.05$).

Azoxystrobin was the most effective of the three fungicides. Average EC_{50} values for this chemical were 0.139 mg a. i./L and 0.088 mg a. i./L in Nagykálló and Solt, respectively. Although all isolates except two were from crops at both sites which had not been treated with azoxystrobin, the crops were sprayed with another QoI fungicide in Solt. After this single treatment with famoxadone the resistance level of the population of *P. infestans* did not increase, because the EC_{50} values of the isolates were not different from the untreated ones in an independence test (x^2 = 15.630, P = 0.05).

Isolates of *P. infestans* were also sensitive to dimethomorph having an average EC_{50} values of 0.893 mg a. i./L and 1.239 mg a. i./L for Nagykálló and Solt, respectively. The isolates were treated either three times or were not treated at all with dimethomorph except one isolate from Nagykálló which had two dimethomorph treatments. Excluding this single isolate from the analysis the average of the EC_{50} values of isolates with three treatments or without it did not differ significantly at P=0.05 (t = 1.228) therefore the application of dimethomorph did not increase the resistance/tolerance of isolates of *P. infestans* against this fungicide.

Populations of the late blight pathogen at the locations were also compared. The distribution of EC_{50} values at the two sampling sites were homogenous for cymoxanil and azoxystrobin (x^2 = 7.143 and x^2 = 7.892, P = 0.05), respectively (Figure 1. and 2.). In contrast to this result the distribution of EC_{50} values for dimethomorph were significantly different in Nagykálló and Solt (x^2 = 15.327, P = 0.05) (Figure 3.).

Table 2. EC_{50} values of isolates from Solt tested in a leaf disc assay for three fungicides.

Fungicide	Number of treatments	EC50
Cymoxanil	3	2.000; 0.520; 0.480; 0.410; 0.360; 0.310 (7)[2]
Cymoxanil	4	3.400; 2.000; 1.000; 0.630; 0.490; 0.460 (2); 0.360; 0.340 (2); 0.310 (7)
Azoxystrobin[1]	0	0.350; 0.250; 0.220 (2); 0.210 (2); 0.140; 0.100; 0.070; 0.068; 0.050; 0.048; 0.047; 0.045; 0.041 (3); 0.040; 0.038; 0.036 (3); 0.035 (3); 0.031 (4)
Dimethomorph	3	6.500; 3.200 (3); 2.000; 1.900; 1.700; 1.500; 1.400; 1.300; 0.870; 0.750; 0.520; 0.480; 0.410; 0.400 (4); 0.390; 0.380; 0.350; 0.340 (2); 0.330 (2); 0.320 (3)

[1] The whole crop in Solt was treated once with famoxadone of the same cross-resistance group.
[2] Figures in brackets refer to the number of isolates with equal EC_{50} value.

Table 3. EC_{50} values of isolates from Nagykálló tested in a leaf disc assay for three fungicides.

Fungicide	Number of treatments	EC50
Cymoxanil	0	1.800; 1.500; 0.500 (2)[1]; 0.480; 0.440 (2); 0.400; 0.340; 0.310 (7); 0.300 (3)
Azoxystrobin	0	0.440 (2); 0.310; 0.300; 0.240; 0.150; 0.120; 0.075; 0.073 (2); 0.064; 0.049; 0.042; 0.036; 0.034; 0.033; 0.031
Azoxystrobin	3	0.072; 0.056
Dimethomorph	0	2.400; 1.800; 0.850; 0.800 (3); 0.760; 0.630; 0.520; 0.510; 0.420 (2); 0.360; 0.300; 0.270; 0.210
Dimethomorph	2	4.500
Dimethomorph	3	2.300; 0.300

[1] Figures in brackets refer to the number of isolates with equal EC50 value.

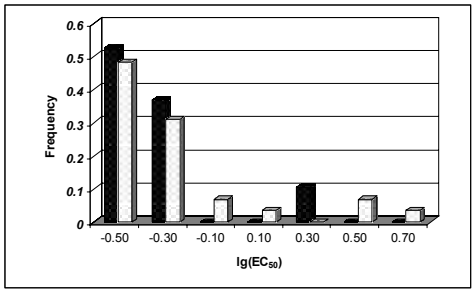

Figure 1. Distribution of EC50 values for cymoxanil in Nagykálló (black bars), and Solt (white bars).

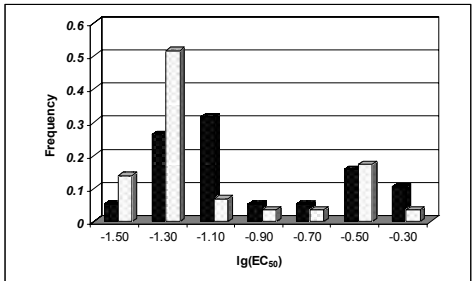

Figure 2. Distribution of EC50 values for azoxystrobin in Nagykálló (black bars), and Solt (white bars).

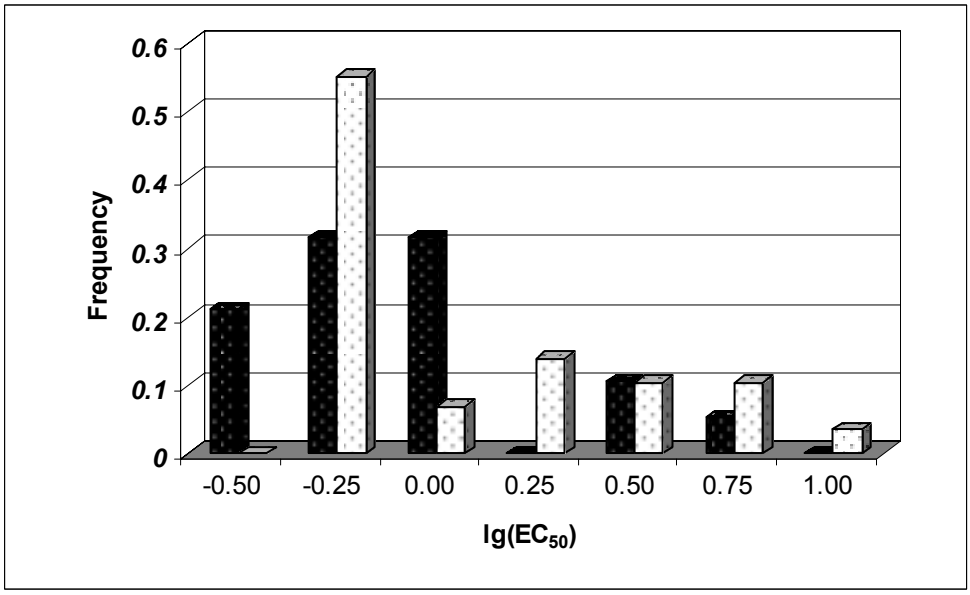

Figure 3. Distribution of EC50 values for dimethomorph in Nagykálló (black bars), and Solt (white bars).

Discussion

Two small areas of infected potato crop were intensively sampled. Of the three fungicides tested azoxystrobin and cymoxanil gave similar results. Potato late blight was unaffected by these chemicals in our experiment. Isolates of *P. infestans* did not decrease in growth rate on leaf discs and EC_{50} values were not different regardless of the number of fungicide application in the field. However, rapid emergence of resistance in fields of various crops where strobilurins are used is well-known (Bartlett *et al.,* 2002), therefore long-term predictions cannot be given from this limited amount of data.

The distribution of the EC_{50} values for azoxystrobin and cymoxanil was also similar at two distant locations. Being 200 km far from each other these two sampling sites represent two subpopulations of *P. infestans* in Hungary.

Despite this relatively great distance between the sampling sites the good fit in the resistance/tolerance distribution of isolates means that it may be close to the baseline sensitivity to the Hungarian population of late blight pathogen to cymoxanil. The presence of cross resistance between azoxystrobin and famoxadone requires isolates to be tested for famoxadone as well as for azoxystrobin to get an insight to the baseline sensitivity to strobilurins in Hungary.

There is one important difference between cymoxanil and azoxystrobin. On the basis of the resistance threshold (EC_{50} > 5 mg a. i./L, Power *et al.,* 1995) isolates of *P. infestans* resistant to cymoxanil have already been found in Hungary (data not shown). In spite of the fact that resistance to strobilurins can emerge rapidly due to point mutations in the mitochondrial genome (Bartlett *et al.,* 2002) resistance to azoxystrobin has not been found so far, although a resistance threshold has not been established to this chemical in Hungary. The limited amount of strobilurin application, which is in accordance with the proposition of the Fungicide Resistance Action Committee, did not have a strong selective effect. If the mode of application does not change, strobilurin fungicides may remain a useful fungicide for late blight control in the future.

As for azoxystrobin, no resistance threshold has been established for dimethomorph, the less effective of the three fungicides in our study. Dimethomorph had the highest EC_{50} values, but this fungicide still worked well in the practice. In contrast to cymoxanil and azoxystrobin, dimethomoph affected the EC_{50} values of *P. infestans*. Isolates originating from treated crop were more resistant to this fungicide than those from untreated crop. This slight difference became apparent only when one isolate from a crop which had been treated twice, were taken into account in the independence test. Otherwise there was no difference in the EC_{50} values according to the number of treatments. However, more work with a greater sample size is needed in order to decide whether the increased number of dimethomorph applications selects for decreased sensitivity of *P. infestans* isolates in field conditions.

When the two sampling sites were compared there was a difference, although very slight, between isolates from Solt and Nagykálló. When the EC_{50} values were distributed into fewer but wider intervals in the independence test this difference was within the statistical error, but it became clearly visible when EC_{50} values of the isolates were partitioned into finer concentration ranges *i. e.* the difference between $lg(EC_{50})$ of two neighbouring categories was less than 1 (Figure 2.). The reason of this discrepancy has not yet been clarified. More work is necessary to evaluate whether this slight difference between the sampling sites has biological reasons or may be due to statistical error.

Conclusions

Our results give a preliminary assessment of fungicide sensitivity of the population of late blight pathogen in Hungary. Although a more detailed survey is warranted, because there were hints of a decrease of sensitivity to the most frequently used fungicides in the *P. infestans* populations, azoxystrobin, cymoxanil and even dimethomorph are expected to remain effective in late blight control in the near future in Hungary.

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Steady progression in using potato late blight DSS-MilPV in Brittany (France): a safe tool for farmers to comply with EU agroecological regulations

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Keywords
Potato, late blight, DSS-MilPV, EU agroecological regulations.

Introduction
Due to seed certification requirements, potato seed production implies highly qualified and technically up-dated operators. Potato foliage has to be protected against major pests, blight and viruses, namely. Still too often, late blight control relies on a seven-day routine treatment. But, due to ecological concerns, these practices should be progressively replaced by more integrated control measures. Late blight risk monitoring coupled with accurate meteorological data have been implemented and followed by seed growers for the past years in western Brittany.
The recent development of DSS-MilPV as a personal computer-based facility has been tested in 2006 by a larger group of seed potato growers (20). New met stations have been deployed allowing more accurate blight risk assessment and the model recently calibrated with potato cv levels of blight resistance offer a new challenge for an integrated management of chemical input. Seed professional organizations such as Bretagne Plants and Germicopa, federated with Plant Protection Service and FEREDEC have implemented this action.

Materials & Methods
MilPV-DSS : MilPV is designed according epidemiological models Guntz-Divoux & Milsol (Figure1). Input variables are climatic and sanitary environmental data. Chemicals (A.I.) and most potato cv's resistance level data have also been integrated (Figure2). Action threshold (treat or not) is according met data into models, *P. infestans* pressure and cultivar susceptibility. On a daily basis, growers up-date their own data (crop growth status, local *Pi* pressure, irrigation, chemical treatment etc…) via an Internet connection.

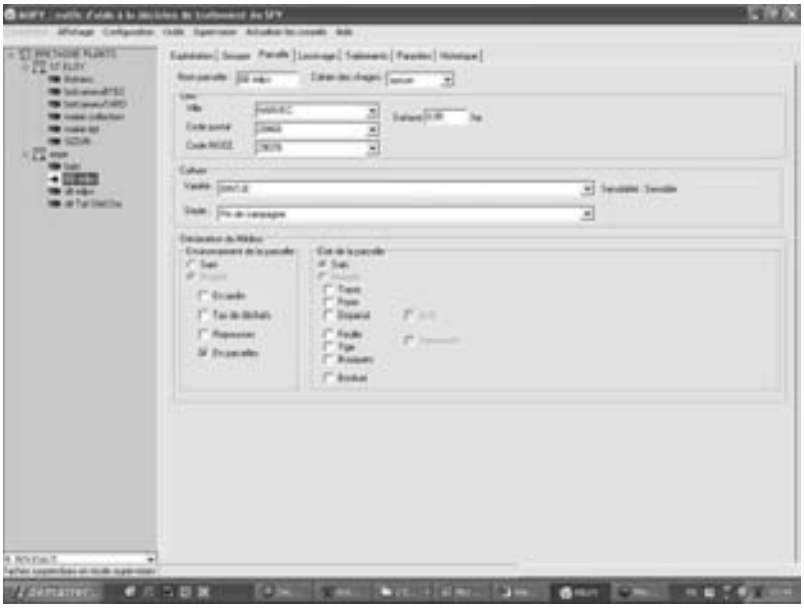

Figure 1. MilPV Input : Personnal data (field description, met station, potato cultivar and level/type of Pi resistance, list of applied chemicals, nearby volunteers, allotment gardens, waste pile) are recorded into MilPV. Any met (mist, rainfall) and/or agronomical (irrigation) events are also recorded.

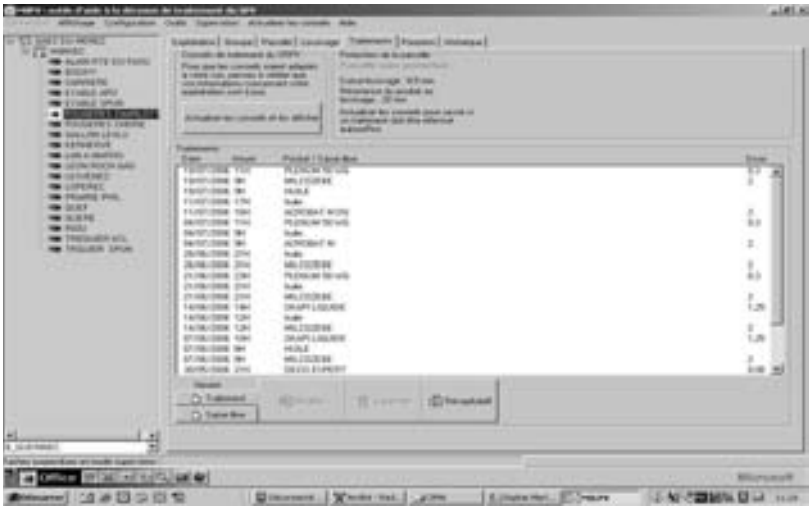

Figure 2. MilPV Input 'Cultivar List' : In a given area where MilPV is to be used, mostly grown cultivars are made available for farmers to choose and integrate in their personal set of data.

Climatic data : 4 automatic met stations, located amid seed production areas, record 4-hourly temperature, RH and rainfall, from April 1rst until end July. On a 24h-basis, met data are validated by the agro-met centre of FEREDEC Midi-Pyrénées.

Seed growers : In 2006, 20 seed growers were connected individually to MilPV server, monitoring all together a total of 100 ha in the 2 major seed production areas of Brittany. Each farmer could monitor up to 15-20 different fields/cultivars per farm.
Official *P. infestans* field monitoring network : Tracking any out break in a given environment is crucial for the quality of DSS'output and treatment recommendations. Consequently, field scouting has to be organised thoroughly. From mid-April until end-July, many different field watchers are active in all potato growing areas ; they not only visually scout for blight outbreak but they also collect diseased material for isolation and subsequent observation by specialized lab (LRPV Loos). Results of this exhaustive monitoring are weekly mailed to all seed potato growers, whether they are connected to MILPV or not.

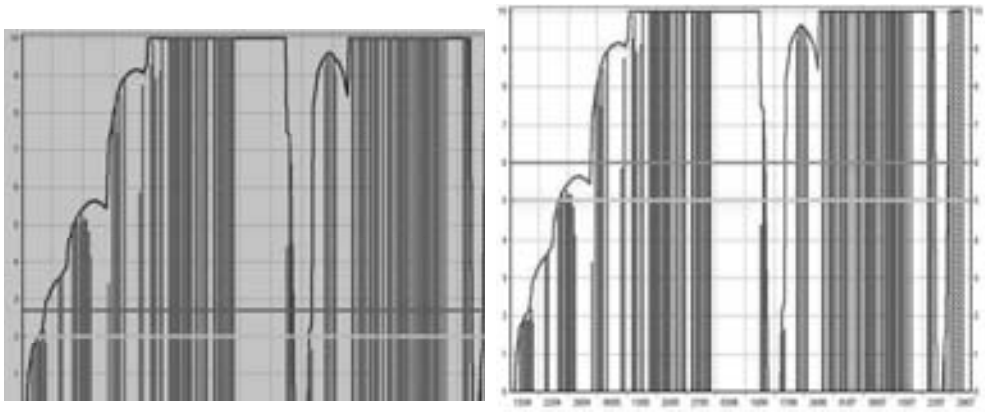

Figure 3. MilPV Output : P. infestans Risk Curvs, the above line is the threshold limit for treatment, on the left, for susceptible cv's (level 2.75), on the right, higher level (level 6) for resistant one.

Results
MilPV Outputs : After updating climatic data, the model retrieves instantly risk curves (Figure3) for every field/cv ; *P. infestans* risk threshold levels depend also on cv's resistance level. During the growing period, MILPV gives daily *P. infestans* risk and recommends a chemical treatment –or not, with a suggested choice of active ingredients. The grower is the final judge for action.

End-of-campaign Data : For a given field, all biological events and technical operations can be traced down, ie disease or pest outbreaks, chemical treatments (what and when), irrigation, haulm destruction and harvest ; these data are fully saved and retrievable when necessary.

Table 1. *P. infestans* population analysis: Isolation and biological characterization for material collected in 3 of the Brittany counties.

	Département	22	29	56	Région Bretagne
	Nb de parcelles	4	17	3	24
	Nb d' isolats	4	26	12	42
Détermination Sexuelle	A1	100%	96%	75%	90%
	A2	0%	4%	25%	10%
Test de Résistance au Métalaxyl - M	sensible	25%	31%	75%	43%
	résistante	75%	69%	25%	57%

General Info : Besides daily and field-specific info issued by the personal DSS's, seed farmers are informed, by postal mail or fax or e-mail, of the regional sanitary situation via the Info Fax sheet (Figure4).

In 2006, through intensive scouting 42 *P. infestans* isolates have been analysed (Tab.1). Almost 90 % of the isolates are from the A1 sexual type but 50 % are found to be metalaxyl-resistant under lab conditions.

Figure 4. Info fax Sheet : Weekly technical bulletins with sanitary info(blight, aphids….) sent to all potato seed farmers.

Conclusions & Perspectives

In 2006, MilPV has been used by 20 seed potato growers in Brittany. At the first place, the tool helps the operator to chemically treat according *P. infestans* actual risk in a given agroecological environment and EU agricultural regulations. It generates and saves all agricultural actions done on any individual field, contributing to the endless need for traceability.

Being a tool in a constant dynamics of improvement (fate of any predictive model), the two biological variables, ie host (potato cv) and pathogen (*P. infestans* pop), calibrated in the model are under constant watch over and the use of the DSS is the best mean of improvement. Through *P.infestans* accurate monitoring, it is also an indirect control for efficacy of phytosanitary products.

Perspectives for 2007 are 1-) adding new met stations where potato fields are mostly concentrated and 2-) convincing new users to join the action.

Acknowledgement to farmers for their failless confidence and motivation.

Tenth Workshop of an European Network for development of an
Integrated Control Strategy of potato late blight
Bologna (Italy), 2007

Potato late blight in Lithuania

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Summary
Potato late blight caused by *Phytophthora infestans* is one of the most destructive potato diseases worldwide. This disease causes serious losses in Lithuania, too. Having analyzed the articles and reports previously published by other researchers the authors of this paper tried to link global warming issues with an increasing late blight problem in Lithuania. Also, we attempted to find out if there are any signs of diseases and pets which have not been known before or their influence has been insignificant so far. After reviewing previously done works and analyzing weather data we have concluded that late blight does not appear earlier in the potato growing season. This is primarily due to the lack of rain at the end of spring and first months of the summer. Other reasons are that potato production area has dwindled by half over a 10-year period. Currently potato production is concentrated in the farms practicing intensive crop protection. All these facts reduce the possibility of early occurrence of late blight. But high temperatures and drought in the first months of potato growing season favours the occurrence of early blight (*Alternaria solani)* and Colorado beetle (*Leptinotarsa decemlineata*).

Keywords
Phytophthora infestans, late blight, climate change

Introduction
Late blight of potato (*Solanum tuberosum*), caused by the oomycete pathogen (*Phytophthora infestans* Mont. de Bary) is considered to be the most devastating disease of potato worldwide. This disease causes serious losses in Lithuania, too (Stuogienė, 1987; Valskytė *et al.*, 2003; Ronis and Tamošiūnas, 2005).
Late blight is a disease driven primary by weather conditions. Climatic observations in Finland in the 20th century and especially since 1980 show increasingly warm springs (April and May), slightly warmer summers (June to August), and reduced diurnal range of temperatures. In Scandinavia and Finland, the reduced range is related to increased cloudiness and to the strengthening of western air flow (Hannukkala *et al.*, 2007). In Lithuania, climate has been warming since the 18th century and, as projected by forecast climate models, by the middle of the 21st century it will have warmed up by 1.5 – 1.7 °C (Bukantis, 2001). These climate trends mean more favourable weather for potatoes in the early growing season, but also more conducive to late blight.

Changes in pathogen population, migration of new pathogenic genotypes and especially global climate change increased awareness about much severe attacks of currently known diseases and/or possible introduction of a new ones (Boland *et al.*, 2004; Platt, 2006). Number of published works in other crops as well in potato already proved that severe diseases attacks are the result of global warming (Chakraborty *et al.*, 2000; Rosenzweig *et al.*, 2000; Hannukkala *et al.*, 2007).
Some authors note that global warming will have significant effect on the insect word (Hansen, 2005). In the spring of 2007, we observed 2 – 3 Colorado potato beetles (*Leptinotarsa decemlineata*) per plant shortly after potato emergence. The same situation was observed in the neighbouring Latvia. By previous studies it is determined that heavy attacks of Colorado beetles are primarily linked to mild winters and hot springs (Šurkus, 1995).
The aim of our research was to evaluate the effects of global warming on the occurrence of potato diseases in Lithuania over the past decade.

Materials and methods
In this study we observed and analyzed the articles and reports that had been previously published by other authors. Also, the amount of rainfall of the sites under study is presented. Statistics about the acreage of potato crop are available online (www.stat.gov.lt).
The results of the first late blight outbreaks in Dotnuva (central part of Lithuania) and Elmininkai (approx. 100 km from Dotnuva to the Northeast) are taken from fungicide efficacy, potato cultivar and other trials carried out by the different projects. Also, the specialists of the State Plant Protection Service assess for the first late blight outbreaks across the country annually. They data are available online (www.vaat.lt). Booklets about the situation with pests and diseases in the country are published annually.

Results
The first late blight onset is usually recorded by the specialists of the State Plant Protection Service (*Figure 1*). The specialists look for the first disease outbreaks throughout the country, and the first disease symptoms are usually spotted in small scale gardens. Such places are usually one of the primary disease focuses (Plant protection …1998-2006). Late blight was detected earlier at the Elmininkai research station than in Dotnuva.

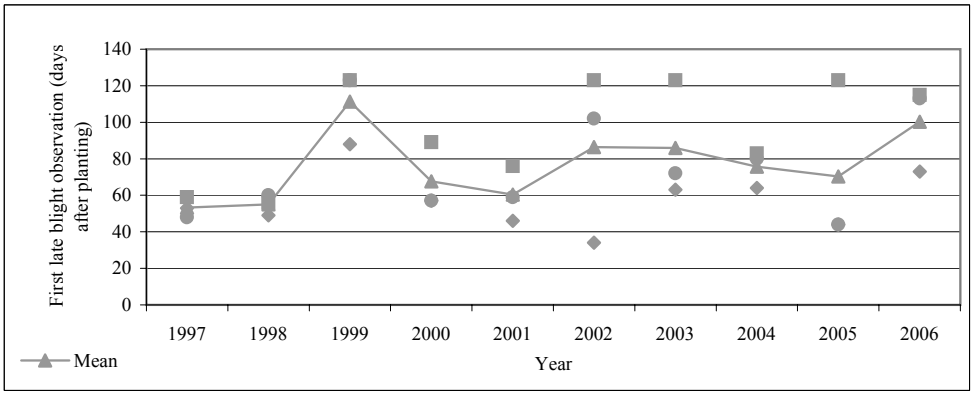

Figure 1. First late blight symptoms after potato planting (1 May) in small scale gardens (♦), Elmininkai (●) and Dotnuva (■).

At the Elmininkai research station potato late blight occurrence and damage to the crop varied from small to serious losses. In 1998, the disease occurred early (at the end of June) and a 4- time fungicide application program was not sufficient to retain healthy plants. In the next season, late blight occurred at the very end of potato growing, but another disease – early blight, caused the biggest losses over this ten year period. Early blight occurred in the fields in the 2006 season too, but in the second half of August heavier rain favoured the occurrence of late blight. In the year 2000, it was observed that late blight significantly dispersed on the haulm of potato. Earlier, haulm damage was not so obvious at this site. Also heavy haulm damage caused the rotting of tubers in the soil before harvested. That season was memorable, because due to the late blight the potato yield was the lowest over the century. In 2002, due to high temperature and drought potato foliage was healthy but the yield was not high. In Dotnuva site, late blight occurrence and damage to the crop varied like in Elmininkai. In this area, late blight usually was recorded about 56 – 89 days after potato planting, *i.e.*, from the end of June to the end of July. In four years out of ten, the disease was spotted at the very end of potato growing. Late blight is a disease driven primary by weather conditions. In some cases the disease appears early in the season but causes relatively small damage to the crop and vice versa. For instance in 2006, the first symptoms of late blight were detected on 23 August in Dotnuva. After 10 days unprotected potato foliage was completely destroyed. Yield losses due to the disease were about 75 percent in the cultivar Fasan (unpublished data).

In 1997 and 1998, late blight was spotted very early in three different places within two weeks. This is due to the heavy rain which occurred in the first months of the potato growing season. In June of 1997 rainfall amounted to 149.5 percent from mean value and in May of 1998 to 150.2 percent in Dotnuva site. Also, the year 1998 was very favourable for the disease outbreak because the amount of rainfall in July amounted to about 275 percent from the mean value.

Discussion

Hannukkala (2007) determined that the outbreaks of the epidemics begin 2 – 4 weeks earlier in Finland. This is due to the climate change and newly emerged populations of *Phytophthora infestans* which are more aggressive on potato than the old clonal lineage (Carlisle *et al.*, 2002). No research on the population and mating type structure of late blight pathogen in Lithuania has been carried out yet.

Volunteer potatoes which are common in warmer regions (Zwankhuizen *et al.*, 1998) became a problem in our country as well. It was observed that mild Lithuanian winters could favour overwintering of volunteer potatoes for two seasons. Our observations also suggest that obviously warmer winters and earlier springs favour better overwintering of late blight pathogens. Volunteer potatoes could be not only foci of late blight outbreak, but also an early "pasture" for Colorado beetle.

According to the data presented in *Figure 1*, the disease outbreak in Lithuania tends to occur later, presumably because of reduced area devoted for potato production (*Figure 2*). However, more potato is grown on intensively-managed farms using plant protection and other measures in order to prevent pest damage.

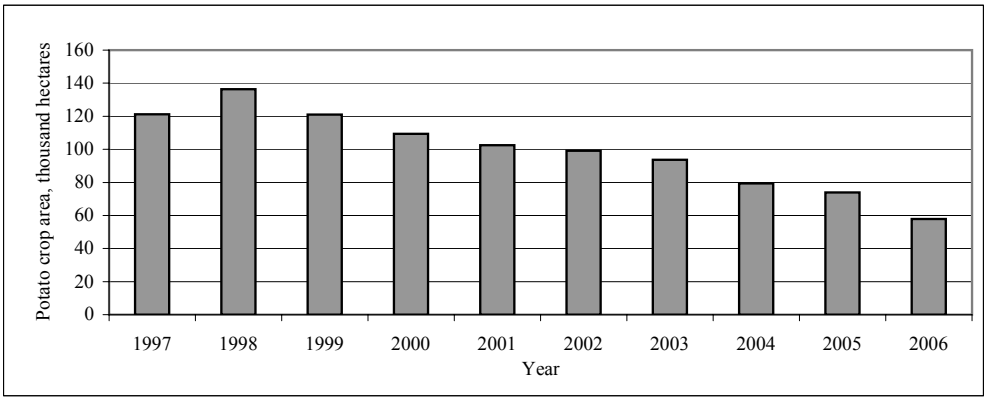

Figure 2. Potato crop area (thousand hectares) in 1997 – 2006.

In non irrigated plots potato growing is under risk. Dry years are not favourable for the late blight, but other disease – early blight (*Alternaria solani*) and lack of moisture in the soil could be detrimental for potato. In *Figs. 3* and *4* it is shown that only in two out of 10 years the amount of rainfall was above the average at Dotnuva site, whereas at Elmininkai site rainfall in six years was above the average. Sufficient amount of rainfall led to earlier occurrence of late blight.

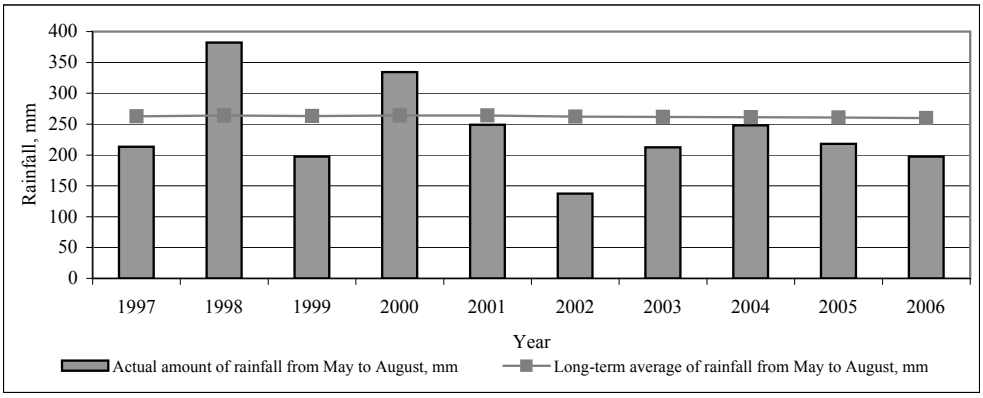

Figure 3. Actual and mean values of rainfall from May to August in central part of Lithuania (Dotnuva) over 1997 – 2006. Source: Dotnuva weather station

In some cases serious attacks of late blight are the consequences of using modern potato cultivars. Tubers of these cultivars grow much faster, which leads to cracking of the soil surface. Exposed tubers can be infected by zoospores of late blight if rain occurrs.

In previous studies researchers discussed more about potato diseases such as late blight, rhizoctonia cancer (*Rhizoctonia solani*), common scab (*Streptomyces scabies*), black leg (*Erwinia carotovora*) and virus diseases (Šurkus and Valskytė, 1998). Nowadays, they are increasingly concerned with early blight disease (Ronis and Tamošiūnas, 2005).

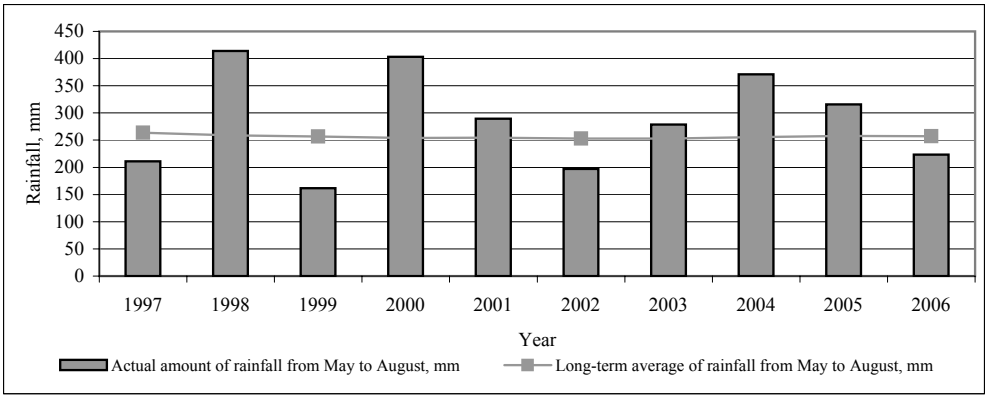

Figure 4. Actual and mean values of rainfall from May to August in Elmininkai research station during 1997 – 2006. Source: Elmininkai weather station.

Conclusions

Late blight does not occur earlier in the season because: (i) climate change causes hot springs and summers without sufficient rainfall, (ii) more potato is grown on intensively-managed farms and, (iii) reduced potato acreage.

Hot summers occur every two or three seasons. This leads to emergence of a new potato disease – early blight (*Alternaria solani*). This disease did not cause any significant losses a decade or more ago. During 1999, 2002 and 2006 early blight caused considerable losses in some places.

In order to avoid serious yield and quality losses potato breeders should develop new cultivars which would be more resistant to both early and late blights.

Potato growers have to use appropriate crop rotation, certificated seed, irrigate fields and use chemical protection.

Acknowledgements. The authors gratefully acknowledge the financial support from the Lithuanian Ministry of Agriculture in connection with this project (Registration No. M-07010).

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Bologna (Italy), 2007

Integrated Production of Solanaceous Crops In Emilia Romagna Region, (Italy): How To Promote The Reduction of Fungicide Input In The Field

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Summary
In Emilia-Romagna, tomato and potato crops are cultivated and protected from early and late blight epidemics following the Integrated Production Guidelines based on the application of European regulation 2078/92 measure A1, 1257/99 measure 2F, and regional law n.28/98. With these programmes, farmers willing to follow integrated production guidelines, are financed both directly and indirectly. Integrated late blight control guidelines for potato and tomato are given. Rules defining the choice of active ingredients allowed to control early and late blight are given along with the list of least toxic fungicides, farmers have to use following the regional IPM programmes.

Keywords
IPM, solanaceus crops, late blight, early blight, European regulations.

Introduction
Tomato and potato are the solanaceous crops mostly grown in the region with 47 and 12 % regional surface grown with vegetables (64.000 ha) respectively. In the early '90 in Emilia-Romagna IPM guidelines for the most important vegetable crops were set up with the aim to reduce the environmental impact, protect farmer and consumer's health, and promote guaranteed quality production along with farmer's income. Integrated Production guidelines provide informations both on pest and disease control strategy other cultural practices such as irrigation, soil and post-harvest management. In Italy, the integrated production programmes considerably improved with the application of the measure A1 of the EC Regulation 2078/92 and the measure 2F of the EC Regulation 1257/99.
At present, the Integrated Production Guidelines are applied on 32 vegetables covering 49% of the regional agricultural surface (31.913 ha). Tomato is the most extensively crop grown following IPM guidelines (25.087 ha which means roughly 88% of the tomato grown in the region). Potato crop grown following IPM guidelines is 1.800 ha (26% of the potato grown in the region) because the

crop is not included in the UE reg. n.2200/96, one of the measures Emilia-Romagna region uses to promote the Integrated Production (Figure1).

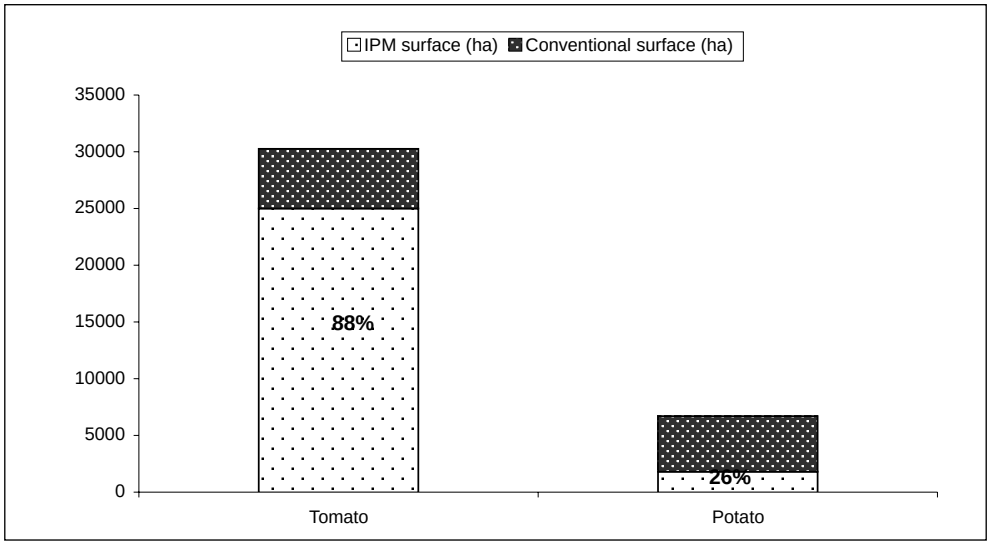

Figure 1 Incidence of IPM programmes on solanaceous crops

Measures used to promote integrated production of the time

In Emilia-Romagna region, the Integrated production pest and disease control programmes were promoted using several measures with the aim to finance farmers directly and indirectly. With the application of the measure A1 EC Reg. 2078/92 until 2003 and then with that of measure 2F EC Reg. 1257/99, farmers willing to apply the integrated production guidelines (IPG) for pest, disease and weed control strategies, irigation, crop rotation, fertilization and atomizer check. On the contrary, with the application of the EC Reg. 2200/96 and the regional law 28/98, financing were given to Farmer's Associations who, in turn, had to provide farmers with technical assistance in order to make farmers apply the IPG. Finally, with the regional law 28/98, farmers applying IPG were allowed to commercialize their productions with a regional "Controlled Quality" label (tab.1).

Tabel 1 - Measures used to promote integrated production over the years in Emilia-Romagna Region

Measures	Benefit for farmers
Reg. CE 2078/92	Financial support
Reg. 2200/96 (OCM)	Qualified technicians
L.R n. 28/98	Research, Experimentation, Supervisors, Technical supports Qualified technicians
Reg. CE 1257/99	Financial support
L.R. n. 28/99	"Controlled Quality" label

Procedure for technical guidelines definition

IPM guidelines are defined and up-dated by financing specific research and experimental projects. On the bases of the results of such projects, the Plant Protection Service each year discuss with producer's associations the up-dating of the IPM guidelines. The new guidelines are then presented at the Minister of Agriculture and evaluated by a National Committee, made up of plant pathologist experts, for the approval.

Tabel 2 – IPM Guidelines used in Emilia-Romagna region to control early and late blight on tomato (A) and potato (B)

A

Disease	Criteria for disease control	Active Ingredients	Spray Limitations
Phytophthora infestans	**Start sprays on the bases of IPM bulletin** The bulletin considers information provided by forecasting model and field surveys on unsprayed plots After the crop emergence copper compounds should be applied. With rapid crop growth and wet climate, systemic fungicides should be preferred, while close to the harvest fungicides with a short safety time should be applied.	Copper compounds Dodine Metalaxyl-M (1) Benalaxil (1) (Benalaxyl M(1)+Mancozeb)(9) Dimetomorph (2) Cymoxanil (3) Azoxystrobin (4) (6) (Pyraclostrobin (5)(6) + Metiram (9)) Fosetyl-Al Iprovalicarb (7) Zoxamide-Mancozeb (8) Mancozeb (9) Metiram (9)	(1) max 3 applications/year with phenylamide (2) max 3 applications/year (3) max 3 applications/year (4) max 2 applications/year independently by disease (5) max 3 applications/year independently from the disease (6) max 3 applications/year independently from the disease (7) max 3 applications/year (8) max 3 applications/year (9) max 3 applications/year independently from the disease Stop spraying 21 days before harvest
Alternaria solani *Alternaria alternata*	<u>Agronomic measures:</u> - use healthy seed - long crop rotations - avoid waterlogging and limit the irrigation <u>Chemicals</u> : - usually specific sprays are not necessary in that those carried out against late blight are able to control early blight as well - in case of heavy attacks and in humid areas two sprays when first symptoms occur with 8-10 days interval are recommended	Copper compounds Azoxystrobin (1) (3) (Pyraclostrobin (2)(3) + Metiram (4)) Difenconazolo (5)	(1) max 2 applications/year independently from the disease (2) max 3 applications/year independently from the disease (3) max 3 applications/year independently from the disease (4) max 3 applications/year independently from the disease Stop spraying 21 days before harvest (5) max 3 applications/year

B

Disease	Criteria for disease control	Active Ingredients	Spray Limitations
Phytophthora infestans	**Agronomic measures:** - use of healthy seeds - use of unsusceptible varieties - elimination of volunteer potato plants - long rotations - avoid exceeding nitrogen fertilisation - avoid dense stands Chemicals: - first sprays when climatic conditions are favourable for infections or on the basis of forecasting model - following sprays should be carried out with 6-10 days interval on the bases of fungicide persistence or on forecasting model	Copper compounds Dodine Fosetyl Al+Cu oxychl. Fluazinam Cymoxanil (1) Metalaxyl-M (2) Benalaxyl (2) (Benalaxyl M(2)+ Mancozeb)(7) Dimetomorph (3) Iprovalicarb (5) Zoxamide-Mancozeb (6)(7) Mancozeb (7)	(1) max 3 applications/year (2) max 3 applications/year with fenilamides and with Xi formulations only. (3) max 3 applications/year (5) max 3 applications/year (6) max 3 applications/year (7) max 3 applications/year Stop spraying 21 days before harvest
Alternaria solani Alternaria alternata	Agronomic measures - long rotations - use of healthy seeds Chemicals: - Specific sprays are needed only on infected young plants, as fungicides used against late blight are able to control the early blight as well.	Copper compounds	

Criteria for limiting pesticide use

The content of IPG is coherent with the general criteria defined by E.U. and recognized by Minister of Agricultural and Regional Governments. Decision n. 96/3864 of U.E. STAR Commitee on 31/12/96 (tab.2). In this respect, for each crop a disease strategy using a limited number and kind of fungicides aiming to protect the environment and the consumer's health, was elaborated. The use of forecasting model (in particular those already validated in Italy for potato and tomato late blight) is a useful tool to optimize the chemical application in the field in that it provides information about the need to spray and when to do it correctly. As far as the choice of the fungicide is concerned, for every active ingredient, direct and chronic toxicity, environmental impact and residue amount that can remain on the edible product, are evaluated using the following criteria:

- **Efficacy**
- **Toxicology**
  - Risk sentences on the product's label: formulations with risk sentences R40, R48, R68, R61, R62, R63 should be avoided or limited;
  - Toxic marks on the label: formulations classified as T, T+ e Xn should be avoided or limited;
- **Environment**
  - evaluation of the negative effects on non-target organism**,** selectivity for beneficials
  - waterflows, soil and persistence in the environment
  - limiting the risk of occurrence of pathogen population resistant to fungicide
- **Residues on treated crops**
  - evaluation of risk, for some active ingredients, to leave high residues on the edible product.

Organisation system of integrated pest management in Emilia Romagna

Emilia-Romagna Region has long been very active to invest resources to support the extension activity in the field. The aim is to promote organizations able to provide farmers with technical information about the disease control strategy and the most suitable product to use.

For the potato and tomato late blight control, the warning service of the Regional Plant Protection Service provides information about the risk of blight using the IPI and MISP forecasting model (Bugiani *et al.,* 1997;1999). From February to October the diffusion of such information is given by using several means (from internet to sms). Moreover, such information is also diffused by the regional information system. Blight situation is discussed during weekly meetings both at regional and then at provincial level with all the field technicians operating in the regional potato and tomato growing areas.

During the provincial meeting, bulletins regarding the blight situation (either by field scouting and the use of forecasting model), information about the need to spray, and the fungicides allowed by the Integrated production guidelines, are issued.

Such activity is supervised at regional level by the Plant protection service. Integrated Production Bulletins are also diffused by internet to the farmers.

Since 1995, in order to rationalize the fungicide application in the potato and tomato growing areas of Emilia-Romagna region, the negative prognosis forecasting models I.P.I. (Infection Potential Index) and MISP (Main Infection and Sporulation Period) have being used within the regional warning service. On potato, from 1995 to 1999, the use of IPI forecasting model alone allowed farmers to averagely reduce from 10 to 60 % of fungicide applications. When IPI model has been used in combination with MISP model, the spray reduction increased up to more than 75% (Figure2).

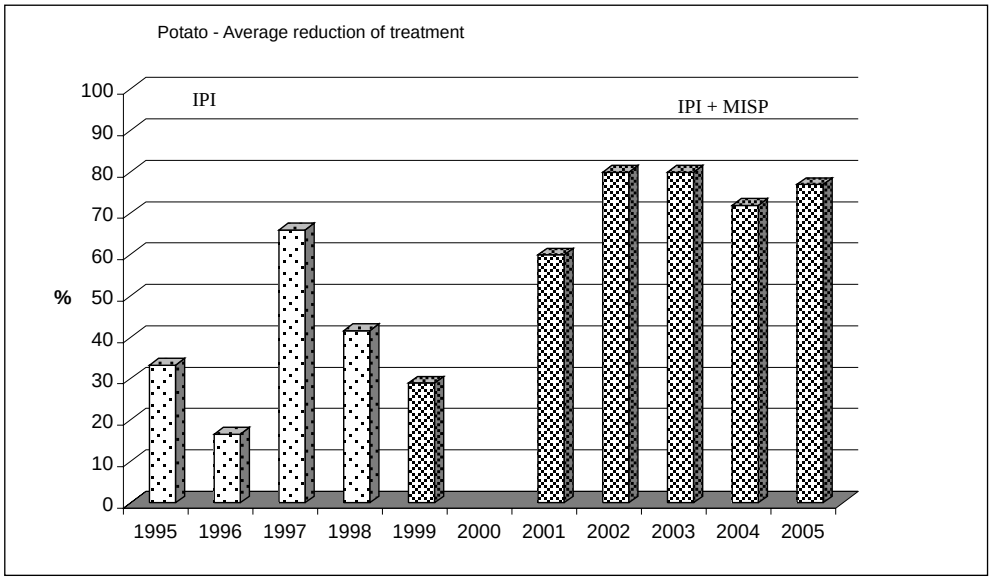

Figure 2. Average percentage reduction of the number of fungicide applications on potato from 1995 to 2005.

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Control of Tuber Blight with Fungicides in Great Britain

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Summary
Between 2003 and 2006, newly introduced fungicides were compared with established products for the control of foliar and tuber blight at ADAS Rosemaund, near Hereford and at SAC, Auchincruive Estate, Ayrshire. At each site the fungicides were applied to King Edward with foliar and tuber blight resistance ratings of 3 and 4 respectively. The fungicides were evaluated as components of season-long spray programmes and were applied from canopy stable stage through to haulm desiccation. The treatment ranking order for tuber blight control was broadly similar and consistent across sites and years. The exception to this was the effectiveness of the Invader spray programme at the ADAS site in 2004 compared with 2005. The average rankings over the five trials using a mean of both measures of tuber infection were Ranman TP (1.4), Shirlan (2.6), Electis (2.9), Invader (3.5), Sonata (4.2) and Curzate M (5.8). In 2006, the relative effectiveness of Ranman TP, Shirlan and Curzate M at Rosemaund matched the results in 2003 to 2005. However, at Auchincruive this was not the case because tuber blight control was confounded by the control of foliar blight. The results obtained for Infinito, Valbon and Shirlan (0.4 l/ha rate) should be regarded as preliminary because they are from two trials only.

Keywords
Late blight, *Phytophthora infestans*, tuber blight, fungicides

Introduction
Fungicides will continue to be used routinely for blight control in conventional potato production at least for the foreseeable future because of the lack of robustness, perceived or real, in existing blight forecasting systems. Intervals between sprays are usually no longer than 14 days reducing to 7 days, sometimes 5 days, depending on blight risk and crop growth stage. Maintaining short spray intervals in high-risk conditions is essential and in these situations the interval between fungicide applications is often as important as product choice. In the UK, official surveys have shown that, on average, between six and 15 fungicide applications (mean 11) are made in years of severe disease pressure (Bradshaw, *et al.,* 2000).

A number of new fungicides have been registered and approved for use in the UK during the last few years. These are C50, Consento, Electis, Epok, Infinito, Sonata, Ranman TP, Tanos and Valbon. As a result of the re-evaluation of registration data required for Annex 1 listing under Council Directive 91/414 EEC, the approval for a number of blight fungicides has been revoked and these are no longer available to UK and EU potato growers. This may be either due to commercial considerations and the cost of providing new data packages, or the safety profile does not meet modern standards. The revocation of the fentin-based products was considered to be a major loss to the industry as they were regarded as having an important role in the control of tuber blight.

Between 2003 and 2005, the British Potato Council funded ADAS and SAC to evaluate the then newer fungicides Electis, Ranman and Sonata compared with three of the more established fungicides, Curzate M, Invader and Shirlan. The work formed part of the BPC's Fight Against Blight campaign (Bradshaw *et al.,* 2004) with the aim of contributing to the understanding and development of disease management strategies. One of the objectives was to examine the control of tuber blight. In 2006, the effect of Infinito, Valbon and the 0.4 l/ha rate of Shirlan were also evaluated.

Materials & Methods

At ADAS Rosemaund, Herefordshire, England and SAC, Auchincruive Estate, Ayrshire, Scotland, fungicide sprays were applied to plots of the blight susceptible maincrop cultivar King Edward (foliar and tuber resistance ratings of 3 and 4 respectively). The treatments were replicated in a randomised complete block design and the plots were four rows wide and 6 to 9 m in length. To maximise the chances of a successful disease challenge, unsprayed guard areas surrounding the experiments were inoculated with several isolates of the blight pathogen and development of the epidemic was encouraged by using overhead mist irrigation. At Rosemaund, fungicide treatments were applied in 250 litres of water per hectare using an Oxford Precision Sprayer operating at 2.5 Bar through 110° flat fan nozzles. The spray booms were mounted on a Growmobile mechanised sprayer that allowed up to eight different treatments to be applied in one pass whilst maintaining a constant forward speed (Turley *et al.*, 1995). At Auchincruive, fungicides were applied in 200 litres of water per ha using a tractor-mounted, modified AZO compressed air sprayer operating at 3 Bar, to give a medium/fine spray quality using Lurmark F03-110 nozzles.

In 2003-2005, all spray programmes started with three applications of propamocarb+mancozeb (992+1200 g a.i./ha as Tattoo: Bayer CropScience) to protect the plots during rapid haulm growth. The different fungicide treatments were then applied at 7 to 10 day intervals depending on blight risk from application four until haulm desiccation. The fungicides tested were:- fluazinam (150 g a.i./ha as Shirlan 500 SC; Syngenta Crop Protection), cymoxanil+mancozeb (90+1360 g a.i./ha as Curzate M 68; DuPont), dimethomorph+mancozeb (150+1334 g a.i./ha as Invader WG; BASF plc), cyazofamid (80 g a.i./ha as Ranman TP; Belchim Crop Protection) zoxamide+mancozeb (150+1200 g a.i./ha as Electis; Dow AgroSciences) and fenamidone+ mancozeb (150+750 g a.i./ha as Sonata; Bayer CropScience).

In 2006, spray programmes also started with three applications of Tattoo but the fungicides subsequently tested were fluazinam (150 and 200 g a.i./ha as Shirlan 500 SC), cymoxanil+mancozeb (90+1360 g a.i./ha as Rhapsody: DuPont), fluopicolide+propamocarb HCl (100+1000 g a.i./ha as Infinito: Bayer CropScience), benthiavalicarb+mancozeb (28+1120 g a.i./ha as Valbon + ZinZan 0.15 l/ha : Certis) and cyazofamid (as Ranman TP). For some fungicides the label restriction on the number of permitted applications was overridden in the trials to allow a robust and scientific evaluation of their efficacy against foliar and tuber blight.

Although not reported in detail here, foliar blight was assessed regularly during the epidemic as the percentage of leaf area destroyed using a modified version of the keys Anon. (1976) and Large (1952). The incidence of tuber infection was assessed on a sub-sample of 100 marketable tubers (>35 mm) taken from each plot at harvest. Visual assessments were made after washing tubers either at harvest or following a period of ambient storage in dry conditions to allow the expression of latent symptoms.

Results

The incidence of tuber blight infection following the different spray programmes is expressed as the percentage infected tubers by weight and by number in Tables 1 and 2 respectively. In four of the five trials conducted between 2003-2005 there were significant differences in the percentage by weight and number of infected tubers (*P* ranged from <0.001 to 0.042) The treatment ranking order was broadly similar and consistent across sites and years. The exception to this was the effectiveness of the Invader spray programme at the ADAS site in 2004 compared with 2005. The average rankings over the five trials using a mean of both measures of tuber infection were Ranman TP (1.4), Shirlan (2.6), Electis (2.9), Invader (3.5), Sonata (4.2) and Curzate M (5.8). In most of these trials we consider that the control of tuber infection was a direct effect.

In 2006 the relative effectiveness of Ranman TP, Shirlan and Curzate M at Rosemaund matched the results in 2003 to 2005. However, at Auchincruive this was not the case because tuber blight control was confounded by the control of foliar blight. Unlike in 2004 there was little relationship between the ranking orders for the fungicides at the two trial sites in 2006.

Table 1. Effect of fungicide spray programmes on the percentage by weight of blighted tubers at SAC, Auchincruive and ADAS Rosemaund 2003-2006 (and their ranking at each site).

	SAC, Auchincruive				ADAS Rosemaund			Mean* Ranking
Fungicide spray programme	2003	2004	2005	2006	2004	2005	2006	
Tattoo/Curzate M	2.1 (6)	13.7 (6)	4.2 (6)	4.2 (3)	24.8 (5)	5.6 (6)	3.6 (6)	5.8
Tattoo/Electis	0.2 (1)	11.4 (4)	2.2 (3)	NT	11.9 (2)	4.3 (5)	NT	3.0
Tattoo/Infinito	NT	NT	NT	1.0 (1)	NT	NT	1.8 (5)	
Tattoo/Invader	0.9 (5)	9.5 (3)	2.2 (3)	NT	27.2 (6)	1.5 (1)	NT	3.6
Tattoo/Ranman TP	0.3 (3)	5.0 (1)	1.2 (1)	1.0 (1)	6.8 (1)	2.4 (2)	0 (1)	1.6
Tattoo/Shirlan (0.3)	0.2 (1)	7.5 (2)	2.0 (2)	7.5 (5)	12.4 (3)	3.7 (4)	0.4 (3)	2.4
Tattoo/Shirlan (0.4)	NT	NT	NT	4.4 (4)	NT	NT	0.2 (2)	
Tattoo/Sonata	0.5 (4)	12.6 (5)	3.6 (5)	NT	16.1 (4)	2.9 (3)	NT	4.2
Tattoo/Valbon + ZinZan	NT	NT	NT	12.4 (6)	NT	NT	0.7 (4)	
F pr.	0.517	<0.001	0.011	<0.001	<0.001	0.042	<0.001	
Df	51	48	45	50	51	45	54	
LSD (5%)	1.50	5.56	6.99	4.57	13.37	4.53	1.77	

*NT = Not Tested *Excludes 2006*

Table 2. Effect of fungicide spray programmes on the **percentage by number** of blighted tubers at SAC, Auchincruive and ADAS Rosemaund 2003-2006 (and their ranking at each site).

	SAC, Auchincruive				ADAS Rosemaund			Mean* Ranking
Fungicide spray programme	2003	2004	2005	2006	2004	2005	2006	
Tattoo/Curzate M	2.3 (6)	15.4 (6)	3.9 (6)	5.4 (4)	25.0 (5)	5.6 (6)	3.3 (6)	5.8
Tattoo/Electis	0.3 (1)	12.0 (4)	2.0 (2)	NT	10.2 (2)	4.3 (5)	NT	2.8
Tattoo/Infinito	NT	NT	NT	0.5 (1)	NT	NT	0.3 (2)	
Tattoo/Invader	1.3 (5)	10.9 (3)	2.0 (2)	NT	27.2 (6)	1.5 (1)	NT	3.4
Tattoo/Ranman TP	0.3 (1)	5.5 (1)	1.0 (1)	1.3 (2)	5.0 (1)	2.4 (2)	0 (1)	1.2
Tattoo/Shirlan (0.3)	0.3 (1)	7.6 (2)	2.3 (4)	8.5 (5)	13.0 (3)	3.5 (4)	0.3 (2)	2.8
Tattoo/Shirlan (0.4)	NT	NT	NT	4.5 (3)	NT	NT	0.3 (2)	
Tattoo/Sonata	0.5 (4)	13.0 (5)	3.4 (5)	NT	17.3 (4)	2.9 (3)	NT	4.2
Tattoo/Valbon + ZinZan	NT	NT	NT	13.2 (6)	NT	NT	0.8 (5)	
F pr.	0.262	<0.001	0.006	<0.001	<0.001	0.010	<0.001	
Df	51	48	45	50	51	45	54	
LSD (5%)	1.43	5.53	7.03	4.75	12.47	3.572	1.278	

NT = Not Tested **Excludes 2006*

Discussion

These results clearly demonstrate that there are effective 'fentin' replacements for the control of tuber blight. Good control of tuber blight was consistently given by several treatments and the effectiveness of the different fungicides in controlling tuber blight was generally consistent across sites and years. Using the average incidence of tuber blight across five trials, the ranking order of the six fungicides tested between 2003-2005 (with the most effective first) was Ranman, Shirlan, Electis, Invader, Sonata and Curzate M. Although the results from Rosemaund in 2006 reflected a similar trend, the results for Infinito, Valbon and Shirlan (0.4 l/ha) should be regarded as preliminary because they are from two trials only, both of which were carried out in the same growing season.

During the period of this investigation, high levels of tuber blight were recorded. In 2004 at Auchincruive, the foliar epidemic was particularly severe, due to exceptionally high rainfall during one 3-day period, and control of tuber blight was confounded by the control of foliar infection. In contrast, at Rosemaund in the same year the control of tuber blight by fungicides was considered to be through an effect on the tuber infection process. There was a good correlation between the tuber blight results at both Rosemaund and Auchincruive in 2004 (r=0.90, *P*=0.019), even though the two sites differed in weather conditions and disease pressure. Whilst the indirect effect of fungicides on tuber blight was demonstrated in the Auchincruive experiment, the direct effect was tested at Rosemaund (Table 3).

Table 3. Comparison of fungicides for the control of foliar and tuber blight (by weight) at Rosemaund and Auchincruive in 2004 (mean percentage infection)

	Rosemaund		Auchincruive	
Fungicide treatments	% Foliar blight 6 Sept*	%Tuber infection	% Foliar blight 5 Sept*	%Tuber infection
Untreated	99.8	25.0	99.7	20.9
Tattoo/Curzate M	2.5	24.8	78.1	13.7
Tattoo/Electis	3.3	11.9	46.9	11.4
Tattoo/Invader	3.4	27.2	70.0	9.5
Tattoo/Ranman	3.9	6.8	21.9	5.0
Tattoo/Shirlan	2.8	12.4	46.3	7.5
Tattoo/Sonata	2.7	16.1	80.0	12.6
F pr.	0.33	<0.001	<0.001	<0.001
LSD (5%)	NS	13.37	17.07	5.56

*NS=not statistically significant (P>0.05): *Untreated controls excluded from the analyses*

In 2005 both sites showed that high incidences of tuber blight can occur where there is little obvious foliar blight This is almost certainly a function of the pace and duration of the foliar blight epidemic where a relatively 'slow blighting' epidemic extends the period that tubers are exposed to inoculum. As in 2004, this provides a strong Knowledge Transfer message demonstrating the importance of maintaining fungicide protection until the crop has completely senesced or has died following desiccation.

Acknowledgements
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Strategies to reduce copper amounts in organic potato production

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Keywords
Late blight, stem blight, seed treatment, tuber infection

Introduction
Potato late blight (*Phytophthora infestans*) is still an unsolved problem in organic farming. Currently the disease can only be controlled by copper fungicides. The project is aiming to reduce the application of copper by introduction of the new blight forecasting system ÖKO-SIMPHYT. To control secondary leaf infections, strategies should be elaborated to achieve best efficacy with reduced copper amounts. Therefore copper amounts and application intervals should be adjusted to the infection pressure. Primary stem infections should be reduced by copper seed treatment in order to postpone the beginning of the blight epidemic as well as the start of spraying.

Materials und Methods
Secondary leaf infections with late blight were controlled by several copper strategies. In field trials different copper amounts were sprayed with variable application intervals adjusted to infection pressure. Bit-parallel copper amounts and spraying intervals were adjusted variable on the infection pressure. The potato varieties Ditta and Nicola were used. Infection pressure was calculated with the new blight forecasting system ÖKO-SIMPHYT.

To ensure the appearance of primary stem infections, artificially infected tubers (varieties Agria and Quarta inoculated with zoospores) were planted in field trials. Subsequently seed tubers were treated with different application methods and copper fungicides. After emergence every week visible primary stem infections were measured and also PCR detection of latent stem infections was conducted. Finally the daughter tubers were analysed for tuber blight by PCR.

Results
In 2005 and 2006 late blight infection pressure was very low and disease appearance was late. Under these conditions the new blight forecasting system ÖKO-SIMPHYT reliably predicted the start of spraying and disease progression. In field trials all copper strategies achieved reduced secondary leaf infections with late blight, whereas between different copper amounts no significant differences were

assessed. An efficient control of late blight with reduced copper amounts without yield losses was possible (Figure 1).

A copper seed treatment significantly reduced primary stem infections of potato plants (Figure 2). In 2005 seed treatment resulted in reduced secondary leaf infections. Thus, a delay of the blight epidemic as well as start of spraying were possible. Furthermore a copper seed treatment reduced tuber blight infections of daughter tubers (Figure 3). The PCR detection of daughter tubers showed a decreased latent tuber infection with *Phytophthora infestans*.

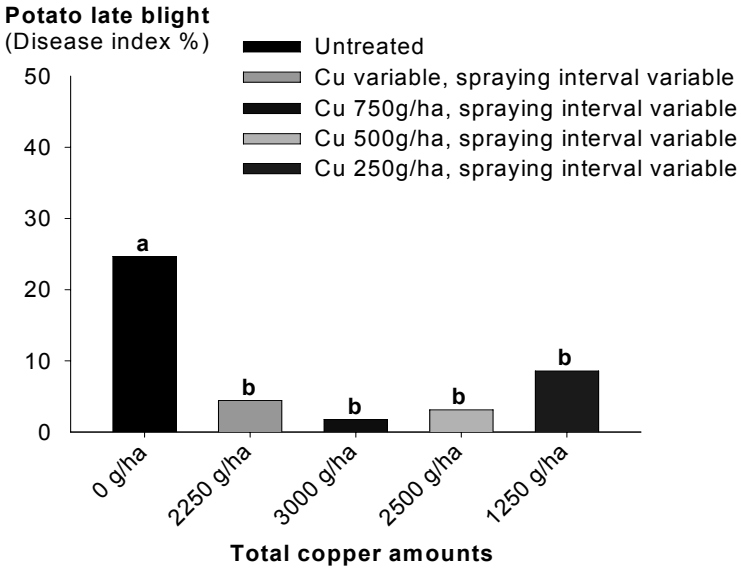

Figure.1: Effect of different copper application strategies on potato late blight

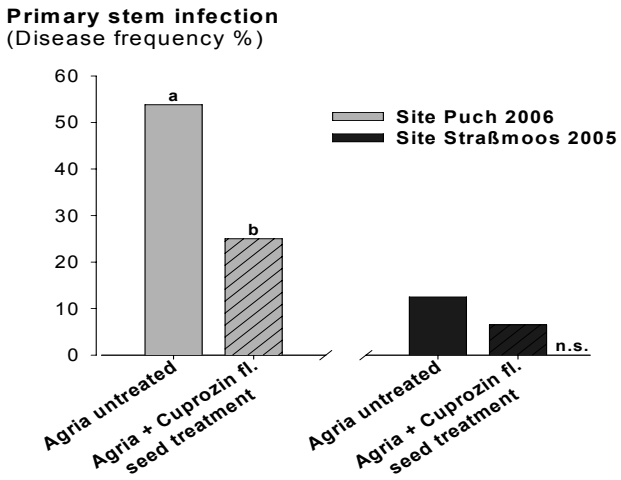

Figure 2: Effect of copper seed treatment on primary stem infection (48g/t Cu)

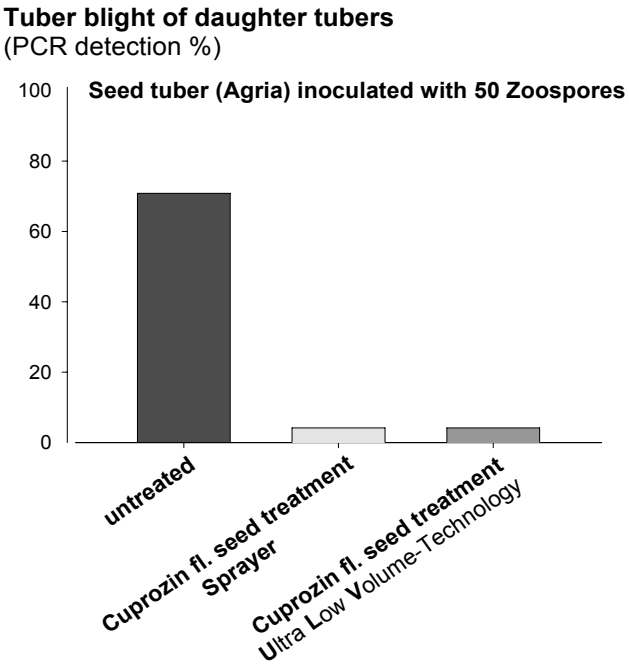

Figure 3: Effect of copper seed treatment on tuber blight of daughter tubers

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Late blight resistance of potato breeding clones and response to ASM treatment

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Summary
The suitability to organic farming of 18 Italian breeding clones of potato was assessed testing their resistance to late blight (*Phytophthora infestans* (Mont.) de Bary) under controlled conditions and field. Some of them were also tested for their response to acibenzolar-S-methyl (ASM) a chemical inducer of systemic acquired resistance (SAR) to investigate the relationship between genetic resistance levels and SAR response.
One breeding clone showing good resistance level in greenhouse was found very tolerant to late blight in field, but altogether, a partial correspondence between results obtained under controlled condition and in the field has been observed.
Inducing resistance treatment enhanced chitinase and glucanase accumulation during the early phases of pathogen infection suggesting an involving of SAR mechanism.

Keywords
Phytophthora infestans, potato, SAR, genetic resistance, breeding

Introduction
Phytophthora infestans (Mont.) de Bary causes late blight on a range of solanaceous plant species. The most frequently management strategy against this disease consists on repeated fungicide applications (Pilet *et al.*, 2005). In organic farming cupric compounds are among the few chemicals allowed and their use is strictly regulated (Reg. CEE n.2092/91, Reg. CE n. 473/02). Therefore genetic resistance becomes a very important factor to obtain acceptable potato yield in terms of quantity and quality.
Eighteen Italian breeding clones of potato were assessed for resistance to late blight under controlled condition and field. Some of them were also tested for their response to acibenzolar-S-methyl (ASM) a chemical inducer of systemic acquired resistance (SAR) to investigate the relationship between genetic resistance levels and SAR response.

Materials and methods
A *P. infestans* isolate phenotypically characterized for mating type (A1) and avirulence genes (R1, R2, R3, R4, R6, R7, R8, R 10, R11) was used for plant and tuber inoculation in experiments under

control conditions. The isolate was maintained on potato tuber slices and was inoculated as sporangia suspension obtained by washing the tuber slice with distilled water.

Foliage assay

Inoculation was done on 60 days old plants with 10 ml per plant of a water suspension of sporangia ($1x10^4$ sporangia ml^{-1}, 3 replicates). Potatoes were maintained in greenhouse at 18°C and 90% R.H. Disease severity was expressed as AUDPC according to Forbes *et al.*, 2005 (tab. 1). Experiment was repeated once.

Table 1. Late blight resistance of tested clones under controlled conditions and field. (n.p.= not present; AUDPC resistance scale: 0-9= very high, 10-99= high, 100-249= medium, 250-349= low, >350= very low; disease incidence scale: 0-24= very high; 25-49= high; 50-69= medium; 70-80= low; >80 very low).

cv or clone	Foliage resistance		Tuber	Field resistance		
	AUDPC ± s.d.	resistance level	Dis. Inc. (%)	Dis. Inc. (%) 2006	Dis. Inc. (%) 2007	field emerge
ISCI 4F 88	471,67 ± 9,90	very low	93,75	np	50	early
Primura	408,00 ± 105,36		100,00	np	np	
ISCI 2101	383,33 ± 14,14		50,00	np	np	
CS 99-4-20	359,67 ± 12,73		87,50	np	95	early
Q 115	350,00 ± 11,31		31,25	np	25	late
MN 1503 R2	338,33 ± 3,30	low	75,00	0	50	early
MN 1511	295,00 ± 7,07		75,00	80	75	early
CS 99-2-8	270,83 ± 81,32		93,75	0	75	late
MN 2-1577	260,00 ± 9,90		68,75	0	75	late
CS 99-6-5	257,50 ± 60,10		87,50	60	75	early
ISCI 87/3	246,67 ± 2,83	medium	93,75	np	np	early
Ditta	245,00 ± 7,07		51,39	0	95	late
MN 1404-05	242,50 ± 2,83		75,00	0	100	late
MN 3-1500	239,16 ± 5,66		37,50	0	75	early
ISCI 2/9-99	225,00 ± 7,07		50,00	0	100	early
MN 3-1469	210,83 ± 67,18		93,75	0	50	late
CS 99-11-28	210,00 ± 66,00		93,75	0	10	early
MN 1501 R5	203,33 ± 4,24		59,76	60	95	early
CS 99-6-3	93,33 ± 4,24	high	75,00	np	50	early
Kuras	6,67 ± 1,41	very high	0,00	np	np	
Terragold	0,00		0,00	np	np	
Signum	0,00		0,00	np	np	

Tuber assay

Four tubers per clone were tested following the shallow wounds method proposed by James *et al.*, 2003. The incidence of infection, as number of inoculation sites with symptoms, was recorded for each tuber and express in percentage (tab.1). Experiment was repeated once.

Field trials

Experiments were carried out in 2006 and 2007 at Siracusa (Sicily, Italy) under natural pathogen *inoculum* without chemical or biological treatment. Clones were sown on the middle of February in a randomised block experimental design with 4 replicates. Late blight symptoms were recorded at the end of April as percentage of disease incidence. Susceptible and resistant cultivars were included as controls (tab. 1).

ASM treatment and SAR response

Thirty days old plants of three clones and two cultivars showing different resistance levels were treated with ASM (30 mg l^{-1} a.i., 5 ml per plant, 3 replicates) 6 days before pathogen inoculation. Water treated plants served as control. Treated and untreated leaf samples were collected 3 days before and 1, 4, 7 days after inoculation and stored at -80°C. Total proteins were extracted from sample and quantified by Bradford method (Bradford, 1976). Chitinases (PR3) and β–glucanase (PR2) which are two accepted markers of SAR (van Loon *et al.*, 2006) were detected following an agarose plate assay (Bargabus *et al.*, 2004). Thirty µg of protein (3 replicates) were put into wells on the agarose gels, incubated at 37°C over night and stained with calcofluor white (Sigma). Finally the enzymatic activity was quantified under UV by Quantity One software (Biorad).

Results and discussion

One breeding clone (CS 99-6-3) out of 18 tested showed high resistance level on foliage assay, similarly to the most resistant cultivars, but only medium resistance in field and low resistance in tuber assay (tab. 1). Moreover 7 clones behaved in a similar way than cv Ditta (medium to high resistance).

The performance of Q115 and MN 3-1500 was similar to the cultivars used as resistant control in the tuber shallow wounds test, and, altogether, new clones did not express combined foliage and tuber resistance as shown by the best cultivars (tab. 1).

In the field trials during 2006 the pathogen pressure was very low, causing symptoms on some clones only. In 2007, on the contrary, under high pathogen *inoculum* pressure, one clone (CS 99-11-28) with good resistance levels in greenhouse test was found very tolerant to *P. infestans* (tab. 1). Two clones showed high (Q 115) or medium (MN 3-1469) resistance level in field but their score was probably positively influenced by the late time of emergence (tab. 1).

Regarding resistance induced by ASM, in most cases, the plants showed an accumulation of chitinase (fig. 1a) and-glucanase (fig. 1b) higher than the untreated control during the early phases of pathogen infection, while later the differences were no more significant (data not shown). ASM seems to induce an early pathogen recognition and a faster plant defence activation in comparison to untreated plants.

No differences in enzyme accumulation (PR 3 and PR 2) were detected between genotypes with different resistance levels (fig. 1 and tab. 1) These data confirm the results obtained for other species under the challenge of a different pathogen (Faize *et al.*, 2004).

Conclusions

Our results did not highlight significant correlation between tuber and foliage resistance of the same genotype as reported in literature, but they suggested that preliminary greenhouse screenings could partially predict field resistance levels helping breeder's work. Therefore a further breeding goal will be to select genotypes combining high foliage and tuber resistance.

Moreover new experiments will be carried out to understand if the early activation of plant defence responses following ASM treatment could really confer an enhanced resistance to late blight.

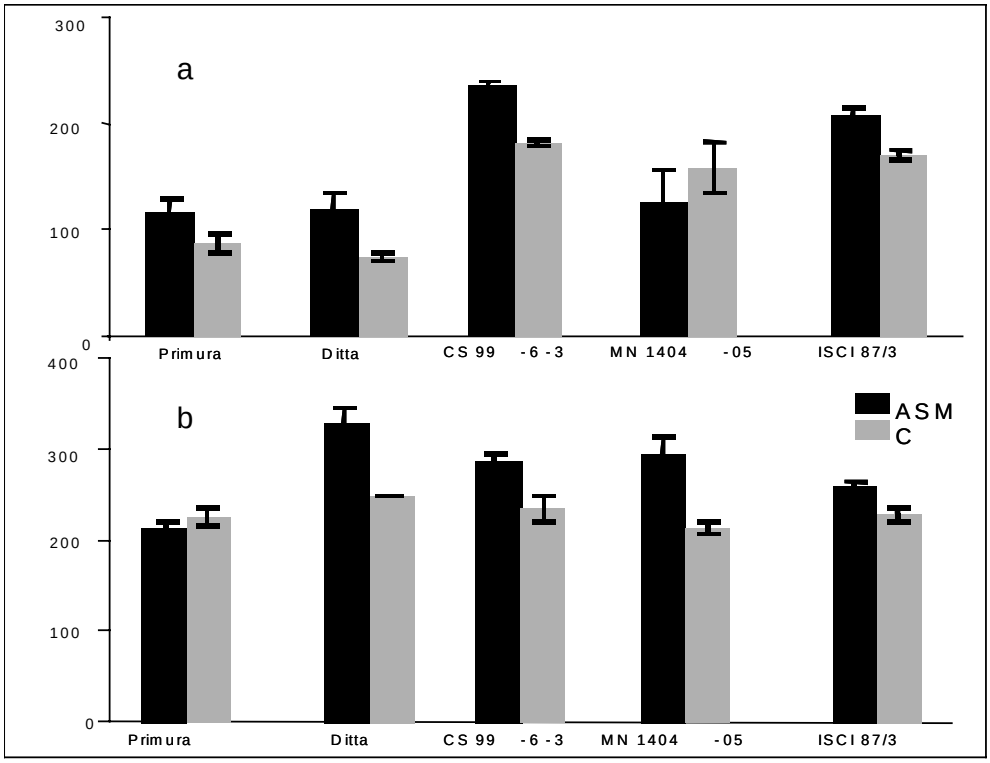

Figure 1. Chitinase (a) and β-glucanase (b) activity of protein extracts of potato 1 day after P. infestans inoculation. Histograms represent the mean value of halo area in agarose gels (3 replicates, ± s.e.; C = water treated control).

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Tenth Workshop of an European Network for development of an
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Activity of biostimulants towards *Phytophthora infestans* on tomato

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Summary
Several biostimulants were tested in greenhouse and laboratory for efficacy on late blight caused by *Phytophthora infestans.* The greenhouse trials were carried out on tomato plants to assess the effectiveness of Kendal and Pom-PK and to test the activity of their components. The laboratory assays were carried out to verify the probable direct activity on *P. infestans* of the biostimulants effective *in vivo*.
In greenhouse Kendal and Pom-PK confirmed their good activity against tomato late blight whereas most components applied separately were not effective. Only oligosaccarides and glutathione whether singly applied or in mixture showed any efficacy towards *P. infestans.*
The laboratory tests demonstrated a fungistatic activity of Kendal, Pom-PK and oligosaccarides on *P. infestans.*

Keywords
Phytophthora infestans, Kendal, Pom-PK, oligosaccarides, glutathione, tomato

Introduction
Induced resistance is an alternative way to control fungal diseases and products able to promote it include some fertilizers with various compositions (mineral, vegetal and animal) also called biostimulants.
Previous studies showed that two products based on various vegetal, animal and mineral components (Kendal and Pom-PK) utilised in Italy as promoters of growth were also able to protect tomato against late blight (*Phytophthora infestans*) (Portillo *et al.*, 2006).
The aim of this study was to confirm the effectiveness of Kendal and Pom-PK on *P. infestans* on tomato and to understand the activity against the disease of some components reported on the labels of the two biostimulants.

Materials & Methods
The study was carried out in greenhouse on tomato plants and in laboratory on artificial medium.

Greenhouse plant experiments

The aim of these experiments was to test the effectiveness towards *P. infestans* of Kendal and Pom-PK and of some commercial formulates based on their components (*Ascophillum nodosum*, blood meal, fluid borlande) all utilised at the label rate. Furthermore, the pure components (glutathione and oligosaccarides), used at a dose corresponding to that of formulated products, and the two known defence stimulators (fosetyl-Al and acibenzolar-S-methyl) were assayed (Table 1). The tests were performed on tomato plants (cv Marmande) grown in pots in greenhouse under controlled conditions (20-25°C, RH 60-80%).

Four-five tomato plants were used for each treatment. Sprays were conducted with a manual nebulizer at different times before or after inoculation of *P. infestans.* The plants inoculated by sporangia suspension (3.5 x 10^4 sporangia/ml) were kept in a humid chamber for 24 hours. After the appearance of disease symptoms on untreated plants, the percentage of infected leaf surface was evaluated. Data were processed by analysis of variance and Duncan test ($p< 0.05$).

Table 1 – Tested products

a- Commercial biostimulants

Name	Composition	Rate ml or g /100L	Label crops
Kendal	Oligosaccarides, glutatione, vegetal extracts, blood meal, fluid borlande, N 3.5%, K2O 15.5%, C3%	300-400 ml	Fruit crops, Vegetables, Ornamentals
Pom-PK	Amino acids, oligosaccarides, peptides, vitamins, flavonoides, macro&micro elements, seaweed extract (Ascophillum nodosum)	300-400 ml	Tomato
Bio-Active	Blood meal	300 ml	Fruit crops, Vegetables, ornamentals
Bio-saral	seaweed extract (Ascophillum nodosum)	250 g	
Alga-Vital	seaweed extract (Ascophillum nodosum)	150 g	
Kinaktin	Microelements (B 0.7%, Mn (EDTA) 0.4%, Zn 0.75%)	70 ml	
Vitaflow	Fluid borlande	100 g	

b - Pure components

Glutathione	Glutamic acid, Cysteine, Glycine	50 g	
Oligosaccarides		50 g	

c – Comparison products

Aliette	Fosetyl-Al 80%	250 g	
Bion	Acibenzolar-S-methyl	5 g	

In vitro experiments

The aim of these assays was to verify the probable direct activity on *P. infestans* of the biostimulants that were effective *in vivo*.

P. infestans was inoculated on V8 medium (250 mL V8 Campbell Grocery; 2.7 g $CaCO_3$ Fluka; 15 g Agar Grade BBL, distilled water up to 1.0 Litre) amended with the products at field dose and mycelial growth was evaluated after 7 days of incubation at 20°C and 12 hours of photoperiod, measuring and averaging the diameters of colonies. The treated *P. infestans* colonies were then transferred on unamended V8 medium and the mycelial growth was again assessed after 7 days.

Results

Greenhouse plant experiments

Kendal and Pom-PK applied at different times before inoculation showed good preventative effectiveness against *P. infestans* (Table 2). The activity of Kendal was found to be significantly higher with application between 4 days – 3 hours before inoculation, while for Pom-PK the disease control was better with applications between 2 days – 3 hours.

The different components of Kendal or Pom-PK applied 2 days before inoculation (*A. nodosum*, blood meal, fluid borlande) did not appear effective (Table 3). Only pure oligosaccarides and glutathione, whether applied alone or mixed (at half rate), showed an activity lower than that of Kendal, Pom-PK and standard product fosetyl-Al.

Oligosaccarides, glutathione and their mixture applied at different times before inoculation, showed a significant reduction of the disease severity at all preventive timings (5 days – 3 hours) but not as low as achieved with the two fertilizers Kendal and Pom-PK (Table 4). An activity similar to that of Kendal and Pom-PK was shown by the well known SAR inducer, acibenzolar-S-methyl.

In the test with curative application (1 day after inoculation), Kendal appeared effective (as good as fosetyl-Al) but not Pom-PK, and only oligosaccarides (also in mixture with glutathione) showed some activity against *P. infestans* (Table 5).

Table 2 – Activity on *P. infestans* of Kendal and Pom-PK (rate 400 ml/100L) applied at different times before inoculation

Days before inoculation	% infected leaf surface	
	Kendal	Pom-PK
7	14.4 b	56.3 b
6	8.8 c	55.0 b
5	8.2 c	32.5 c
4	2.4 d	20 d
2	0.2 d	8.3 e
1	2.6d	5 e
3 hours	1.8 d	2.3 e
Untreated	91 a	94.5 a

Table 3 – Activity on *P. infestans* of Kendal and Pom-PK in comparison with some of their components (formulate or pure): application 2 days before inoculation

Treatment	Rate / 100L	% of diseased leaf area	
		3 days after inoculation	6 days after inoculation
Kendal	300 ml	2 d	19.2 ef
Pom-PK	300 ml	5 d	25 e
Aliette (fosetyl-Al)	250 g	4 d	15 f
Bio-Active (blood meal)	300 ml	62 a	98.5 ab
Bio-Saral (Ascophillum nodosum)	250 g	63 a	99.2 ab
Alga-Vital (Ascophillum nodosum)	100 g	62 a	100 a
Kinaktin (microelements)	70 ml	63.6 a	97.6 ab
Vitaflow (fluid borlande)	150 g	65 a	98 ab
Oligosaccarides	50 g	21,6 a	85.4 c
Glutathione	50 g	34 b	94 b
Oligosaccarides + Glutathione	25 + 25 g	18.2 c	78.4 d
Untreated	-	65 a	100 a

Table 4 – Activity on *P. infestans* of oligosaccarides and glutathione in comparison with Kendal, Pom-PK and Bion: application at different times before inoculation

Treatment	Rate/100L	Days between application and inoculation	% of diseased leaf area
Kendal	300 ml	5	18.5
		3	7
		0	2.5
Pom-PK	300 ml	5	15
		3	7.2
		0	2
Bion (acibenzolar-S-methyl)	5 g	5	20.8
		3	13.3
		0	8.6
Oligosaccarides	50 g	5	36.8
		3	38.8
		0	35
Glutathione	50 g	5	39
		3	40.2
		0	38.8
Oligosaccarides + Glutathione	25 + 25 g	5	30.3
		3	32.7
		0	33.4
Untreated	-	------	67.8

Table 5 – Post-infection activity (1 day after inoculation) on *P. infestans* of oligosaccarides and glutathione in comparison with Kendal, Pom-PK and fosethyl-Al

Treatment	Rate / 100L	% of diseased leaf area	
		3 days after inoculation	6 days after inoculation
Kendal	300 ml	3 d	11.8 c
Pom-PK	300 ml	40 ab	69.8 a
Aliette (fosetyl-Al)	250 g	5.3 d	12.6 c
Oligosaccarides	50 g	35.2 b	70 a
Glutathione	50 g	43.8 a	72.6 a
Oligosaccarides + Glutathione	25 + 25 g	30.4 c	65.1 b
Untreated	-	45 a	73 a

In vitro experiments

Kendal, Pom-PK, oligosaccarides and oligosaccarides + glutathione (at half rate) showed a direct activity against mycelial growth of *P. infestans* as well as fosetyl-Al but this effect disappeared after transferring the mycelial disk on unamended V8 medium (Table 6). No activity was shown by glutathione, which on the contrary seemed to stimulate the mycelial growth of *P. infestans*.

Table 6 – Activity *in vitro* of some biostimulants in comparison with fosetyl-Al

Treatment	Rate / 100L	Mycelial growth (mm) after 7 days on amended medium	Mycelial growth (mm) 7 days after transferring to unamended medium
Kendal	300 ml	13	53
Pom-PK	300 ml	17	7
Aliette (fosetyl-Al)	200 g	11	5
Oligosaccarides	50 g	2	7
Glutathione	50 g	77	-
Oligosaccarides + Glutathione	25 + 25 g	13	66
Untreated	-	61	72

Discussion and conclusions

Kendal and Pom-PK are examples of biostimulants with an interesting efficacy on tomato *P. infestans.*

The greenhouse results of the present study confirm the good activity of these biostimulants against *P. infestans* and they also show that most of their components applied separately (blood meal, fluid borlande, brown seaweed) are not effective against *P. infestans* on tomato. Only oligosaccarides and glutathione whether applied singly or in mixture (at half rate) show efficacy against late blight but not as good as that of Kendal and Pom-PK.

The laboratory tests demonstrate the fungistatic activity of Kendal, Pom-PK and oligosaccarides on *P. infestans*. The lack of *in vitro* activity of glutathione, considering its preventive effectiveness *in vivo*, could indirectly show the capacity of this product to promote host plant resistance. This hypothesis can only be confirmed by biochemical studies.

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Baseline sensitivity of *Phytophthora infestans* lifecycle components to NC–224 20SC (Amisulbrom 200 g/l)

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Summary
The aim of this study was to determine EC_{50} values of NC-224 20SC (active ingredient amisulbrom (ISO proposed), a new fungicide introduced by Nissan Chemicals Industries, Ltd.) for four stages in the life cycle of *Phytophthora infestans*. The four stages selected were the release of zoospores, motility of zoospores, germination of cystospores and the formation of oospores in planta. The EC_{50} of NC-224 20 SC for zoospore release, motility of zoospores and germination of cystospores was found to be 0.016 ppm, 0.0002 ppm and 0.061 ppm. Oospore formation also responds sensitive to exposure to NC–224 20SC. Both, the total number of oospores and the number of viable oospores formed are reduced. The EC_{50} value for the fraction of viable oospores formed was determined to be 35% of the recommended dose rate.

Keywords
Phytophthora infestans, Amisulbrom, NC-224 20SC, zoospore release, zoospore motility, cystospore germination, oospore formation.

Introduction

Amisulbrom is a new fungicide introduced by Nissan Chemicals Industries, Ltd. Currently, the company is introducing 'NC-224 20SC', a suspension concentrate containing 200g a.i./L amisulbrom, for use on potatoes for the control of late blight caused by *Phytophthora infestans*.

The aim of this study was to determine EC_{50} values of NC-224 20SC for four stages in the life cycle of *Phytophthora infestans*, the causal organism of late blight on potato and tomato. The four selected stages in the *P. infestans* life cycle were: 1) the release of zoospores, 2) motility of zoospores, 3) germination of cystospores and 4) the formation of oospores in planta. The EC_{50} value is defined as the NC-224 20SC concentration or dose rate at which 50% inhibition of the process studied is obtained relative to the control treatment. This study was carried out for Nissan Chemical Industries, Ltd.

The life cycle of *P. infestans* can be separated into an asexual cycle and a sexual cycle. The asexual cycle is completed many times during the potato growing season. Sporangia are formed and dispersed and germinate directly or indirectly. Direct germination results in formation of a germ tube and potentially infection. Indirect germination results in formation of motile zoospores. When the zoospores loose their flagellae, they become cystospores which germinate and infect through a germ tube.
The sexual cycle is completed only once per growing season. Oospores are formed in host tissue infected by both, the A1 and A2 mating type.

Materials and methods
Two types of experiments were carried out: an in vitro experiment aimed to determine the EC_{50} of NC-224 20SC for the release of zoospores and germination of cystospores in vitro. Furthermore, this experiment aimed to approximate the EC_{50} of NC-224 20SC for zoospore motility in vitro. The second experiment was an in planta experiment to determine the EC_{50} of NC-224 20SC for oospore formation in potato foliage.

In vitro experiment
Sporangia in suspension were exposed to a dilution series of NC-224 20SC introduced at different times during indirect germination. A sporangial suspension (50000 sporangia/ml) of *P. infestans* isolate IPO82001 was obtained by rinsing sporulating potato leaves (c.v. Bintje) in tap water of 4°C. NC-224 20SC was added to aliquots of this suspension at three points in time during zoospore release and germination such that a dilution series for each of the three target processes was created at final concentrations of: 0, 0.001, 0.01, 0.03, 0.1 and 1.0 ppm. The points in time were:
1) From the start (targeting zoospore release)
2) After 2 hours (targeting zoospore motility)
3) After 4 hours (targeting cystospore germination)

Zoospore release (% sporangia releasing zoospores) was determined after 2 hours incubation in the presence of NC-224 20SC at 10°C by microscopically assessing approximately 100 zoosporangia for the release of zoospores (sporangia are empty when zoospores have been released).
Zoospore motility was assessed after 45 minutes incubation at 10°C in the presence of NC-224 20SC and classified as follows:
+: zoospore motility is not inhibited as compared to the control treatment.
+/-: zoospores motility is partially inhibited as compared to the control treatment,
-: zoospore motility is completely inhibited as compared to the control treatment.
In addition it was found possible to count motile and non-motile zoospores. Zoospore motility was therefore also determined by microscopically assessing approximately 100 zoospores for their motility (motile or non-motile).
For cystospore germination, the zoospore suspension was plated on 1.5% water agar plates 15 minutes after adding NC-224 20SC. Germination was assessed after a total of 20 hours incubation in the presence of NC-224 20SC at 10°C by microscopically assessing 100 cystospores for germination.

Statistically, this experiment was set up as completely randomized experiments with 6 concentrations of NC-224 20SC, 3 response variables (zoospore release, zoospore motility and cystospore germination) and 3 replicates.

In planta experiment
Potted potato plants (c.v. Bintje, 8 weeks old) were spray inoculated with a 1:1 mixture of *P. infestans* A1/A2 (isolates Kartel and US8 respectively). Symptoms were allowed to develop for eight

days before treatment with NC-224 20SC, at 5 different dose rates equivalent to 100 g a.i./ha (full rate), 50 g a.i./ha (½ rate), 25 g a.i./ha (¼ rate) , 12.5 g a.i./ha (1/8 rate), 0 g/ha and Tattoo C (2.7 l/ha as commercially available).
Disease severity was assessed 0, 8 and 14 days post inoculation. In addition fourteen days post inoculation, 10 leaflets with multiple lesions were picked from each plant and incubated on water agar at 10°C in a climate chamber for 3 weeks to allow completion of oospore formation. Following this incubation, oospores were extracted from the remaining tissue using the methods described by van Bekkum and Kessel elsewhere in this volume, stained using tetrazolium bromide (Sigma M-2128) and quantified, including a differentiation between live and dead oospores (Jiang and Erwin, 1990).
Statistically, this experiment was set up as a completely randomized experiment including 5 concentrations of NC-224 20SC, 1 response variable (number of viable oospores) and 4 replicates (plants).

Statistical analysis
In vitro experiment:
To allow for a logistic regression analysis, NC-224 20SC concentrations were ^{10}Log transformed and data on zoospore release, zoospore motility and cystospore germination were scaled such that the control treatment (0 ppm NC-224 20SC) represented 100%. A generalized linear model (GLM) for binomially distributed data using the logit function as link function was fitted to these data using Genstat (seventh edition). The EC_{50} value was calculated from the fitted sigmoid curve.

From results it is obvious that zoospore motility is highly sensitive to even the lowest concentration of NC-224 20 SC included in this experiment. From the effect of the dilution series NC-224 20SC on the fraction of motile zoospores it can be derived that the EC_{50} of NC224 20 SC is located in the 0 – 0.001 ppm range. For the statistical analysis this meant the concentration range of NC224 20 SC incorporated in this experiment was not ideally suited to accurately estimate the EC_{50} for zoospore motility.

Oospore formation:
The fraction viable oospores was analyzed through logistic regression analysis using the original (untransformed) NC-224 20SC dose rates and the fraction of viable oospores scaled such that the control treatment (0 ppm NC-224 20SC) represented 100%. A generalized linear model (GLM) for binomially distributed data using the logit function as link function was fitted to these data using Genstat (seventh edition). A comparison of means to test for differences between individual treatment was carried out using a Least Significant Difference (LSD) test at p = 0.05. The EC_{50} value was calculated from the fitted sigmoid curve.

Results
Zoospore release
The sigmoid function, resulting from the logistic regression analysis, describing the effects of a dilution series of NC-224 20SC on the release of zoospores is given in equation 1 and Figure 1.

$$Y = \frac{e^{(-5.567 - 3.122\ X)}}{(1 - e^{(-5.567 - 3.122\ X)})}$$ *Equation 1*

In equation 1, Y represents the proportion of empty sporangia (zoospores released) and X the ^{10}Log of the NC-224 20SC concentration (ppm). This relationship is plotted in Figure 1. The EC_{50} of NC-224 20 SC for zoospore release calculated from this relationship is 0.016 ppm with a standard error of 0.003 ppm.

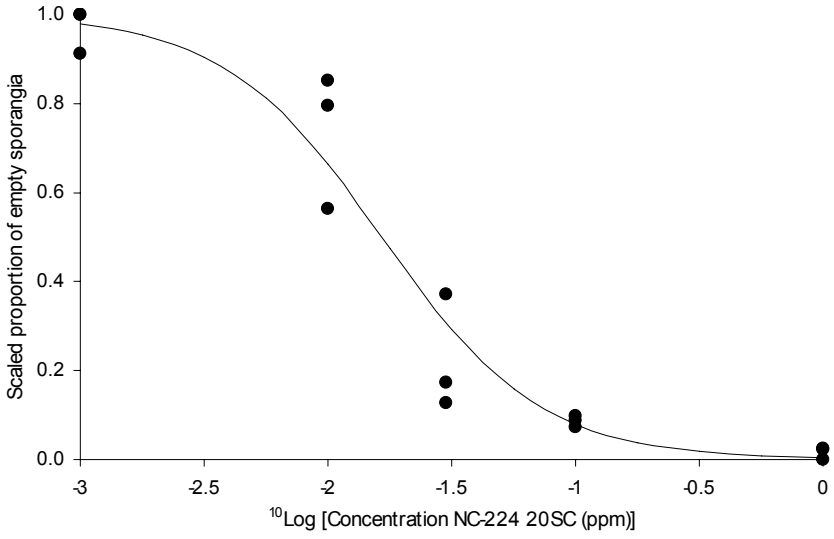


Figure 1. Observations (●) and fitted sigmoid curve (solid line, described by equation 1) describing the relationship between the scaled fraction of empty sporangia (zoospores released) and the ^{10}Log of the concentration of NC-224 20SC (ppm).

Zoospore motility

The sigmoid function, resulting from logistic regression analysis, describing the effects of a dilution series of NC-224 20SC on zoospore motility is given in equation 2 and Figure 2.

$$Y = \frac{e^{(-8.180 - 2.237\ X)}}{(1 - e^{(-8.180 - 2.237\ X)})} \qquad \textit{Equation 2}$$

In equation 2, Y represents the proportion of motile zoospores and X the ^{10}Log of the NC-224 20SC concentration (ppm). This relationship is plotted in Figure 2. Although the EC_{50} of NC-224 20 SC for zoospore release cannot be estimated reliably because it falls outside the range covered by the dilution series used in the experiment, it was calculated to be 0.0002 ppm (0.2 ppb) with a standard error of 0.0001 ppm.

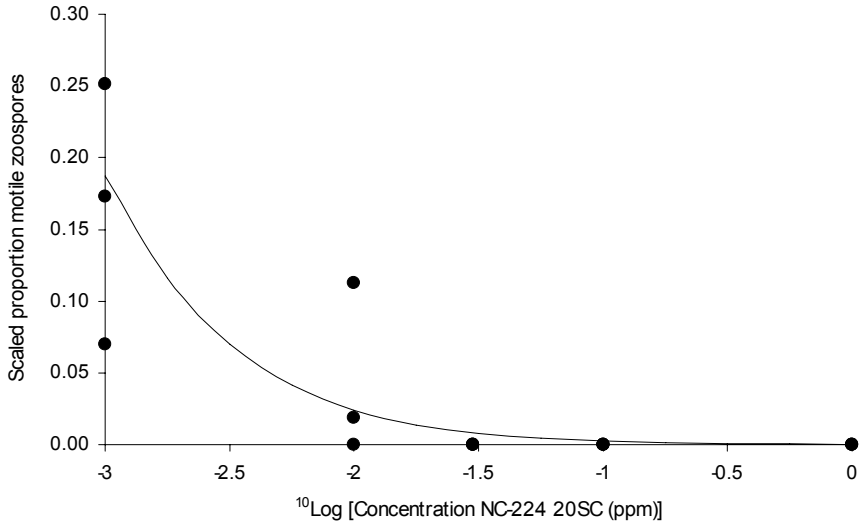


Figure 2. Observations (●) and fitted sigmoid curve (solid line, described by equation 2) describing the relationship between the scaled fraction of motile zoospores and the ^{10}Log of the concentration of NC224 20SC (ppm).

Cystospore germination

The sigmoid function, resulting from logistic regression analysis, describing the effects of a dilution series of NC-224 20SC on cystospore germination is given in equation 3 and Figure 3.

$$Y = \frac{e^{(-2.992 - 2.458\ X)}}{(1 - e^{(-2.992 - 2.458\ X)})} \qquad \textit{Equation 3}$$

In equation 3, Y represents the proportion of germinated cystospores and X the ^{10}Log of the NC-224 20SC concentration (ppm). This relationship is plotted in Figure 3 together with the observations. The EC_{50} of NC-224 20 SC for cystospore germination calculated from this relationship is 0.061 ppm with a standard error of 0.014 ppm.

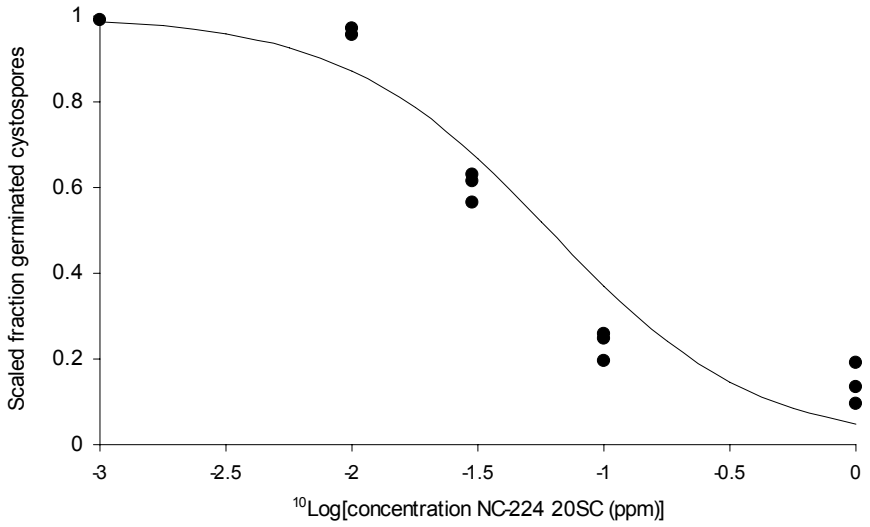


Figure 3. Observations (●) and fitted sigmoid curve (solid line, described by equation 3) describing the relationship between the scaled fraction of germinated cystospores and the ^{10}Log of the concentration of NC-224 20SC (ppm).

Oospore formation in planta

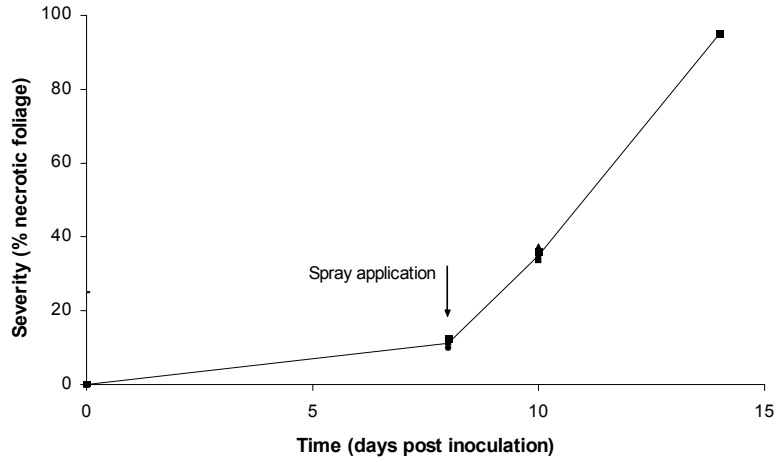
Oospore formation responds sensitive to exposure to NC–224 20SC. Results on the fraction of viable oospores are given in Table 1. Both, the total number of oospores and the number of viable oospores formed are reduced. The EC_{50} value for the fraction of viable oospores was determined to be 35% of the recommended dose rate. NC–224 20SC performed at least equal to Tattoo C, which was included as a reference treatment.

Table 1. Effect of spray treatments using different dose rates of NC-224 20SC, Tattoo C and a water control on the fraction of vital oospores formed in the leaves of infected potato plants. Averages are followed by the standard error of the mean in brackets.

Treatment	Dose rate (% of recommended)	Average fraction viable oospores[1]	Scaled average fraction viable oospores (relative to control treatment)
NC-224 20SC	100%	0.078 a	0.327 (0.111)
Tattoo C [2]	100%	0.093 a	n.d.
NC-224 20SC	50%	0.106 ab	0.448 (0.126)
NC-224 20SC	25%	0.148 ab	0.624 (0.178)
NC-224 20SC	12.5%	0.121 ab	0.540 (0.105)
Control	0	0.237 b	1

1 Statistical analysis was done on original counts in 50µl aliquots of the purified oospore suspension.
2: Tattoo C was applied at 2.7 l/ha as commercially available in the Netherlands.

A



B

Figure 4. A: Development of foliar infection following inoculation at time = 0. Spray applications were carried out eight days after inoculation. B: Potato plants (c.v. Bintje) from the oospore experiment 8 days after inoculation and just prior to treatment with fungicides.

Discussion

In vitro and in vivo experiments were carried out to determine EC_{50} values of NC-224 20SC to four stages in the life cycle of *P. infestans*, the causal organism of potato and tomato late blight. EC_{50} values of NC-224 20 SC for zoospore release, zoospore motility and cystospore germination were determined using logistic regression analysis and found to be 0.016 ppm, 0.0002 ppm and 0.014 ppm respectively.

Oospore formation also responds sensitive to exposure to NC-224 20SC. Both, the total number of oospores and the number of viable oospores formed are reduced by at least 50% and 75% respectively. The EC_{50} value for the fraction of viable oospores was determined to be 35% of the recommended dose rate. When compared to the standard treatments included in the experiment, NC-224 20SC reduces both, the fraction of viable oospores and the total number of oospores formed and performs equally well as Tattoo C. In earlier work, Tattoo C was found to be one of the most effective fungicides

against oospore formation in vivo, currently available on the Dutch market (Kessel *et al.,* 2002).

Overall, NC-224 20SC was found to be very effective against the stages of the *P. infestans* life cycle tested. The sensitivity of sporangia, zoospores and cystospores to NC-224 20SC give reason to believe that this compound could be developed into a fungicide highly effective against spores of *P. infestans.* Protection against foliar and tuber infection are thus likely to be two of the strong points of this new fungicide.

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Extraction from plant tissue and germination in soil of *Phytophthora infestans* oospores

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Summary
Existing methods were modified and optimized to extract oospores of *Phytophthora infestans* from small (1-5 leaflets) and larger quantities (10-35 gram of dry weight) of potato leaf tissue. Recovery of extraction of oospores from small quantities was 96.7%. Extracted oospores were used to study germination of oospores in soil as influenced by time, soil type, soil moisture and soil temperature. Oospores were mixed with quartz sand as an inert medium to facilitate simple recovery. After incubation oospores were recovered and germination and viability were determined. Viability was determined using tetrazolium bromide. Oospore viability was not affected by any of the treatments during the duration of the experiment. Germination in soil, quantified as the formation of primary sporangia was found to be a slow process making quantitative analysis difficult. Trends for effects of soil moisture, temperature and incubation period were observed. The soil types included in the experiments did not have a statistically significant effect on oospore germination.
For practical potato late blight management purposes on oospore infested soils, it must be assumed that oospore germination is possible throughout most of the growing season. This results in a continuous presence of sporangia in the soil as an extra soil borne source of inoculum. This additional soil borne source of inoculum within the crop must be taken into account for effective potato late blight management.

Keywords
Late blight, potato, epidemiology, viability, temperature, soil moisture.

Introduction

In the Netherlands, surveys in 2000, 2001, 2002, 2003, 2004 and 2005 carried out within the framework of the growers (LTO Nederland) initiative "MasterPlan Phytophthora" and the Umbrella Plan Phytophthora indicate that oospores are becoming an increasingly important source of inoculum, especially in the North Eastern starch potato growing area (Turkensteen *et al.*, 2000; Kessel *et al.*, 2001). Consequently, contemporary management strategies for controlling potato late blight may have to be adapted to minimise the impact of oospores on late blight epidemiology.
Conventional potato late blight management basically applies protective fungicides when the protection level of the crop is low and an infection event is predicted. At least theoretically, this should

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protect the crop from infection events originating from airborne inoculum produced outside the crop. Infection events originating from a soil borne source of inoculum inside the crop, such as oospores, may well occur under a different range of climatic conditions. Quantitative insight into oospore germination, as related to soil type and abiotic conditions, would help to predict critical periods with respect to oospore related infections. This knowledge would aid further refinement of potato late blight management strategies and decision support systems. Quantitatively however, germination of *P. infestans* oospores is poorly understood and the techniques available to quantitatively study oospore germination are not adapted for use in soil with the potato - *P. infestans* pathosystem.
The purpose of the present study was to adapt and optimise available methods for extraction of oospores from various plant tissues for use with the *P. infestans* - potato pathosystem. Furthermore we aimed to develop experimental techniques allowing incubation of oospores in soil followed by simple recovery and quantification of germinated oospores from soil. The final objective was to quantitatively study viability and germination of *P. infestans* oospores as influenced by time, soil type, temperature and soil moisture.

Materials and methods

Isolate selection and culturing

To optimise oospore production, nine combinations of A1 and A2 parental strains were tested for their capacity to produce large quantities of oospores in leaflets of potato cultivar Bintje. Three *P. infestans* A1 isolates (SN001a, IPO99001 and IPO98014) were mated to three *P. infestans* A2 isolates (US8, PIC96002 and IPO82001) in all nine possible combinations. Details on the isolates are given in Table 1.
Sporangial suspensions of all *P. infestans* isolates were prepared in tap water from potato leaflets, cultivar Bintje, showing abundant sporulation and adjusted to a concentration of $1x10^4$ sporangia/ml. Mixed A1/A2 sporangial suspensions of all nine parental combinations were prepared by mixing the appropriate sporangial suspensions in a 1:1 ratio. Detached potato leaflets of cultivar Bintje were placed in 9 cm Petri dishes containing 1% water agar (WA), one leaflet per Petri dish, lower side up. Three Petri dishes were included for each parental combination. Leaflets were spray inoculated, using a spraying nozzle at a pressure of 0.5 kg m^{-2}, covering the leaflets with tiny droplets of the appropriate sporangial suspension. Petri dishes containing inoculated leaflets were placed in plastic trays lined with wet filter paper. Trays were placed in transparent polyethylene bags and incubated for at least 10 days using an incubation regime of 24h at 15°C in the dark followed by 24 hours at 15°C including a light period of 16h (12 Wm^{-2}), followed by 11°C and a light period of 16h/day (12 Wm^{-2}) for the rest of the incubation period. During incubation, leaflets were regularly sprayed with tap water to prevent dehydration.
Following incubation, oospore densities in the inoculated leaflets were determined using a microscope at 10 x 10 magnification. Four leaf areas of 1 mm^2 for each of three leaflets per parental combination were examined.

Extraction of oospores from leaf tissue.

The oospore extraction protocol used was modified from a protocol described by Van der Gaag & Frinking (1996) for extraction of *Peronospora viciae* oospores from pea tissue. The total leaf area of small samples (1 – 5 leaflets) was determined using an interactive digitizer (Minimop, Kontron, Oberkochen, Germany). Leaflets were homogenised in 5 ml crushed ice and 5 ml tap water (4°C) using an Ultra Turrax mixer (T25 basic, IKA Labortechnik, Germany,) at 24000 rpm for 90 seconds. The resulting suspension was cooled to 4°C and homogenised a second time at 24000 rpm for 90 seconds. Cellulase (C8001, Duchefa, Haarlem, The Netherlands) and macerase (M8002, Duchefa, Haarlem, The Netherlands) were added to a final concentration of 0.5 mg ml^{-1} each to degrade

leaf tissue. Suspensions were incubated on an orbital shaker (SM25, Edmund Bühler, Tübbingen, Germany) at room temperature for 2 h at 100 rpm followed by sonication (Branson 2510, Branson ultrasonics corporation, Danbury, USA) for 2 x 5 minutes. Following sonication, samples were again incubated on the orbital shaker at room temperature for 2 days at 100 rpm. The resulting suspensions were washed on a set of sieves (75 and 20 µm), using tap water, to remove enzymes and particles smaller than 20 µm. The residue on the 20 µm sieve was transferred to a 50 ml centrifuge tube and spun down for 3 minutes at 5000 g. The volume was reduced to 5 ml by removing supernatant. The oospore concentration in the remaining suspension was determined using a microscope at 10 x 10 magnification and a Fuchs-Rosenthal haemocytometer. The resulting suspensions can be air dried and stored at room temperature for future use in experiments as a highly concentrated source of *P. infestans* oospores.

For larger quantities of leaf tissue (10–35 g dry weight) the procedure described above was modified as follows: Stems were removed. The remaining leaflets were weighed and washed in water to remove sand. Washed samples were homogenized using a blender (Waring commercial, model 38BL40) in 50 ml crushed ice and 100 ml tap water (4°C) for 60 seconds at low speed followed by 30 seconds at high speed. The resulting suspensions were transferred to 500 ml Duran bottles and treated with cellulase and macerase as described above. Suspensions were then washed on a 250 µm, 125 µm, 75 µm and 20 µm sieve set. Residue on the 20 µm sieve was transferred to a 500 ml Duran bottle and left to settle over night before reducing the volume to 100 ml.

The recovery of the extraction protocol for small leaf samples was determined for three leaf samples of 1380 mm^2, 815 mm^2 and 1403 mm^2 respectively. Residue fractions on the 75 µm sieve, on the 20 µm sieve (oospore yield) and the residue passing the 20 µm sieve were collected separately and checked for presence of oospores.

Table 1. Characteristics of *Phytophthora infestans* isolates used in the oospore production experiment.

P. infestans isolate	Isolated from	Country	Year of collection	Mating type	Haplo type[1]
SN001A	Black nightshade *(Solanum nigrum)*	The Netherlands	1999	A1	IA
IPO99001	Commercial starch potato crop	The Netherlands	1998	A1	IA
IPO98014	Commercial starch potato crop	The Netherlands	1998	A1	IA
US8	Commercial potato crop	USA		A2	IA
PIC96002	Commercial potato crop	Mexico	1996	A2	IA
IPO82001	Commercial potato crop	Belgium	1982	A2	IA

1: Mitochondrial Haplotype (Griffith and Shaw, 1998)

Oospore viability and germination

Oospore viability and germination was quantitatively monitored in a replicated experiment in which *P. infestans* oospores were incubated for three weeks in four types of soil at four constant temperatures and two levels of soil moisture. Bulk samples of a sandy soil, clay soil, a peaty soil and quartz sand, representing the three dominant soil types of the major Dutch potato growing areas and a reference soil respectively, were adjusted to field capacity (pF = 2). Characteristics of these soils are given in Table 2. Eight plastic containers (11x7.5x4.5 cm) were filled with each of these soils, resulting in a total of 32 containers. Saturated soils were produced by adding water to four out of the eight containers per soil type until a thin layer of water was permanently present on top of the soil.

Air-dried oospore-containing leaf residue from a bulk sample, produced as described above, was re-suspended in tap water, thoroughly mixed and adjusted to a concentration of $5x10^4$ oospores per ml. A volume of 10 ml oospore suspension was added to 200 g of dry sieved sterile quartz sand only containing particles > 125 µm, and thoroughly mixed. Approximately 1.5 g of this oospore

containing quartz sand was placed in each of 192 small polyester gauze bags (10 x 5 cm, 15 µm mesh, Lampe technical textiles b.v. Sneek, The Netherlands). Six bags were buried in each container after which the containers were covered with aluminium foil which was taped to the containers to prevent dehydration. Containers were incubated for three weeks in the dark at 5°C, 10°C, 15°C or 20°C. One container was used for each combination of soil type, soil moisture and incubation temperature. Initial oospore viability and germination was assessed from the oospore containing quartz sand bulk sample. During incubation, two randomly chosen gauze bags were removed from each of the containers after one, two and three weeks. Oospore viability and germination in these samples was determined using the following method:

Oospore containing quartz sand from the gauze bags was transferred to 5 ml water in a 50 ml centrifuge tube and carefully inverted ten times. The suspension was left to settle for approximately 10 seconds before the supernatant was transferred to a 10 ml centrifuge tube. 5 ml of tap water was added to the pellet in the 50 ml centrifuge tube and the procedure was repeated. The combined supernatant was spun down at 1500 g for 3 minutes. The resulting supernatant was carefully discarded until only 1 ml was left in the centrifuge tube. This residue, including the oospores, was transferred to a 1.5 ml eppendorf vial. Oospore viability was determined using tetrazolium bromide (MTT, Sigma M-2128) according to Jiang & Erwin (1990). A volume of 150 µl 0.1% MTT in 0.01 M phosphate buffer (pH=6.2) was added to each oospore-containing eppendorf vial which was then incubated for 2 days at 35°C in the dark. Following incubation, viability and germination were quantified in 50 µl aliquots using a microscope at 10x10 magnification. Viability was assessed using the colour reactions described by Erwin & Ribeiro (1996). Oospores were considered germinated when a germ tube and sporangium was attached to the oospore. At least 60 oospores were examined per aliquot.

Table 2. Soil characteristics for the soil types used in the oospore viability and germination experiment.

	Sandy soil	Clay	Peaty soil	Quartz sand
pH-KCL[1]	7.3	7.1	4.8	6.3
Organic matter[2] (%)	3.0	3.4	20.2	0.5
CaCO3 (%)	0.6	2.2	0.1	0.1
Silt 0 – 16 (%)	8.6	64.1	6.9	0.1
Total sand 16 – 2000 (%)	87.8	30.4	72.8	99.3

1: - log (H+) in suspension
2: g/100g dry matter

Data analysis

Oospore viability and germination were determined as percentages (counts with a known maximum) and are therefore expected to follow a binomial variance function. The experimental design was a split-plot design where temperature was randomized over incubators, soil and moisture were randomized over containers and the sampling time was randomized over de bags within the container. This resulted in different levels of variances for the different treatments. For oospore viability the percentages measured were in the range 20-80%. Therefore it's reasonable to adopt a normal distribution and an analysis of variance was performed taking the split-plot design into account.

Oospore germination resulted in very low percentages (range 0-1%) of germination. In a GLMM-model (Generalized Linear Mixed Model) the binomial distribution and the different levels of variance of the data can be taken into account simultaneously. The Wald test can be used to test the significance of the treatment model term as it is added into the model. Although some results were obtained, the IRREML-procedure (Iterated Re-weighted REsidual Maximum Likelihood model) did not converge for the germination data due to the many zero's and very low percentages in the data. Therefore, an analysis of variance (ANOVA) was done on the germination data as well, leaving the treatments with only zero-data (all data obtained at 5°C and all data obtained after 1 week incubation) out of

the analysis to avoid a very small mean square (MS)-residual. The results of this analysis agreed with the Wald test of the IRREML analysis. Statistical analyses were performed using GenStat release 8.1 (GenStat, 2005).

Results

Selection of isolates and production of oospores

Five out of nine parental crosses (US8 x SN001A, US8 x IPO98014, PIC96002 x SN001A, IPO82001 x IPO99001 and IPO82001 x IPO98014) tested did not produce oospores in leaflets of cultivar Bintje . Crosses IPO99001 x US8, IPO99001 x PIC96002, and SN001a x IPO82001 produced oospores in one out of three inoculated leaflets. Cross IPO98014 x PIC96002 proved to be the most reliable with oospores in 2 out of 3 leaflets. In addition, the latter cross produced the highest oospore densities in potato leaf tissue.

P. infestans isolates IPO98014 and PIC96002 were therefore selected to produce oospores for the survival experiment using the methods described above.

Extraction of oospores from potato leaf tissue.

The oospore extraction procedure for small quantities of leaf tissue was found to be efficient. In the recovery experiment, 1338, 413 and 2063 oospores were found in the residues left behind on the 75 µm sieve for the 1380 mm^2, 815 mm^2 and 1403 mm^2 leaf samples respectively. The residues on the 20 µm sieve yielded 49500, 22690 and 38365 oospores respectively. The residue fractions passing the 20 µm sieve did not contain oospores for any of the samples. Average recovery was thus calculated as 96.7%. The recovery of oospores in large tissue samples could not be investigated. High numbers of oospores were recovered on the 20 µm sieve but the large amount of debris trapped on the 75 µm sieve made it impossible to reliably quantify oospores on the 75 µm sieve. The high dilution factor necessary to discern oospores from remaining leaf debris proved too high to reliably detect oospores. However, it can be safely assumed that the loss of oospores is significantly higher than for the extraction method described for small quantities leaf tissue. Consequently, this extraction method is only useful to extract large quantities of oospores in a production step for storage and/or later experimentation.

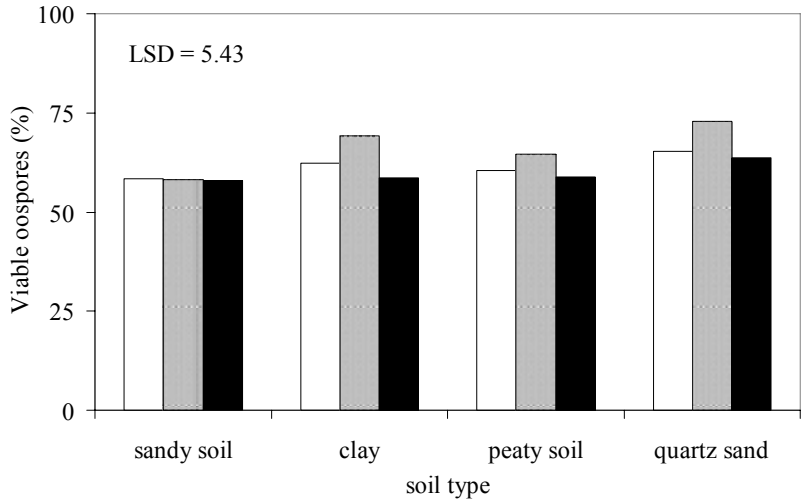

Figure 1. Effect of the incubation period (one week: white bars, two weeks: hatched bars and three weeks: black bars) on the viability of P. infestans oospores during incubation in three different soil types: a sandy soil, clay, a peaty soil and quartz sand, representing the three dominant soil types of the major Dutch potato growing areas and a reference soil.

Oospore viability and germination

The average initial oospore viability at the start of the experiments, as determined from the bulk samples, was 65%. Oospore viability was analysed using ANOVA. Significant effects for "incubation period" and the "soil x moisture" interaction were found. Temperature treatments incorporated in the current experiments did not significantly affect oospore viability. The effect of incubation period on oospore viability during this experiment is, although statistically significant, quantitatively negligible. Oospore viability remained more or less constant during the experiment (Figure 1). With respect to the "soil x moisture" interaction, generally, oospore viability is lower in saturated soils than in soils at field capacity for all three soils representing the Dutch potato growing areas. The reverse is true for quartz sand, causing a statistical interaction between the effects for soil and moisture (Figure 2).

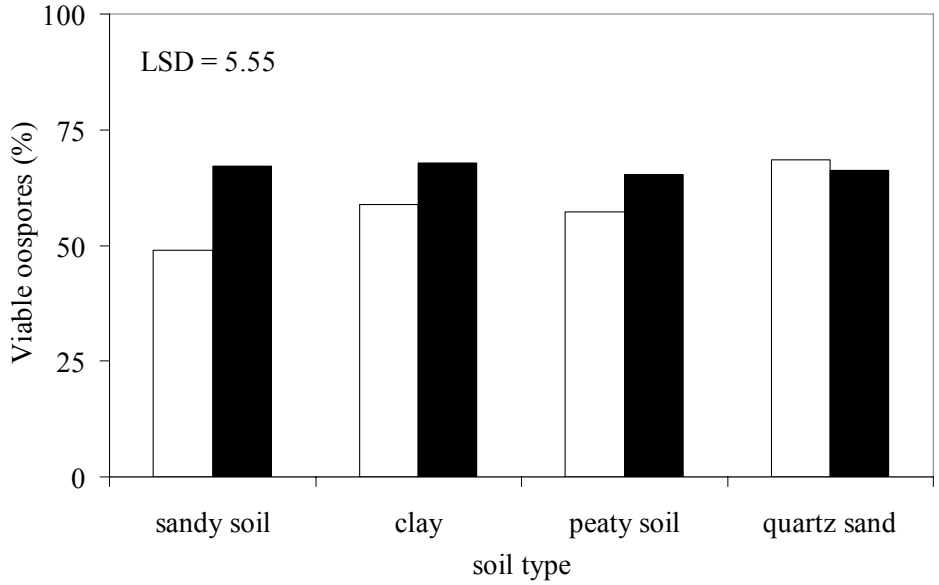

Figure 2. Effect of soil type and soil moisture level on viability of P. infestans oospores. Oospores were incubated in a sandy soil, clay, a peaty soil and quartz sand under saturated conditions (white bars) or at field capacity (black bars). Viability was determined using tetrazolium bromide.

No germinated oospores were found at the start of the experiments. Oospore germination was analysed using ANOVA. Germination did not occur in any of the treatments at 5°C or during the first week of incubation. To avoid very small MS-residues, ANOVA was performed excluding data from these two treatments. Three significant two-way interactions involving temperature, moisture and incubation time, and one, nearly significant, three-way interaction between these factors was found. Soil type was the only factor not significantly affecting oospore germination. In general, oospores germinate slowly, with percentages lower than 1% after three weeks incubation (Figure 3, Figure 4 & Figure 5). This makes it difficult to draw conclusions, despite the fact that statistical significance was reached for three two-way factorial interactions. Based on these significant two-way interactions, some trends may be discerned:

Although soil type did not significantly affect oospore germination, average germination in the peaty soil (0.111 %) was highest followed by quartz sand (0.098%), the sandy soil (0.058%) and clay soil (0.037%).

With respect to the temperature x moisture interaction, germination levels seem to increase with increasing temperatures for soils at field capacity but less so for saturated soils causing the interaction between the two factors (Figure 3).

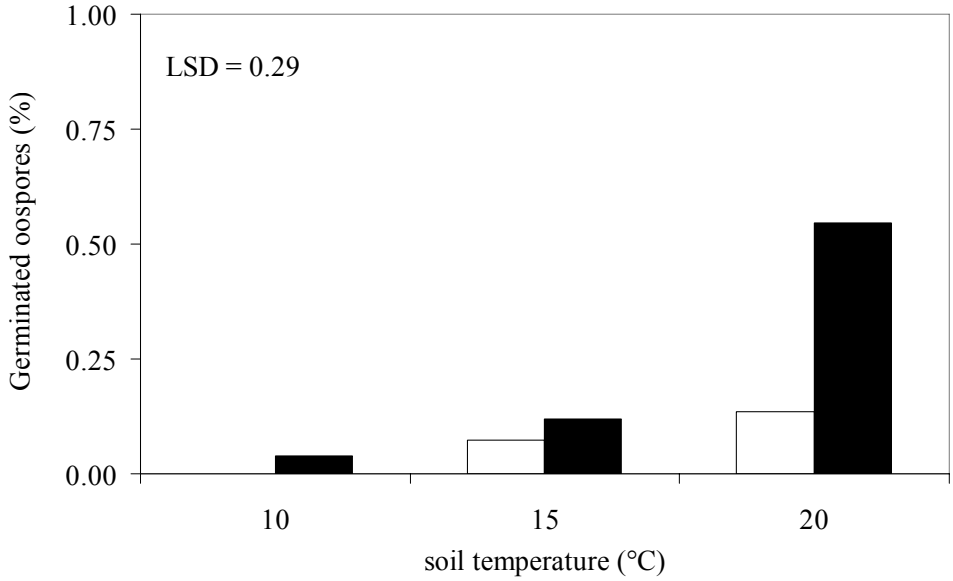

Figure 3. Effect of temperature and soil moisture level (white bars for saturated soils, black bars for soils at field capacity) on germination of P infestans oospore

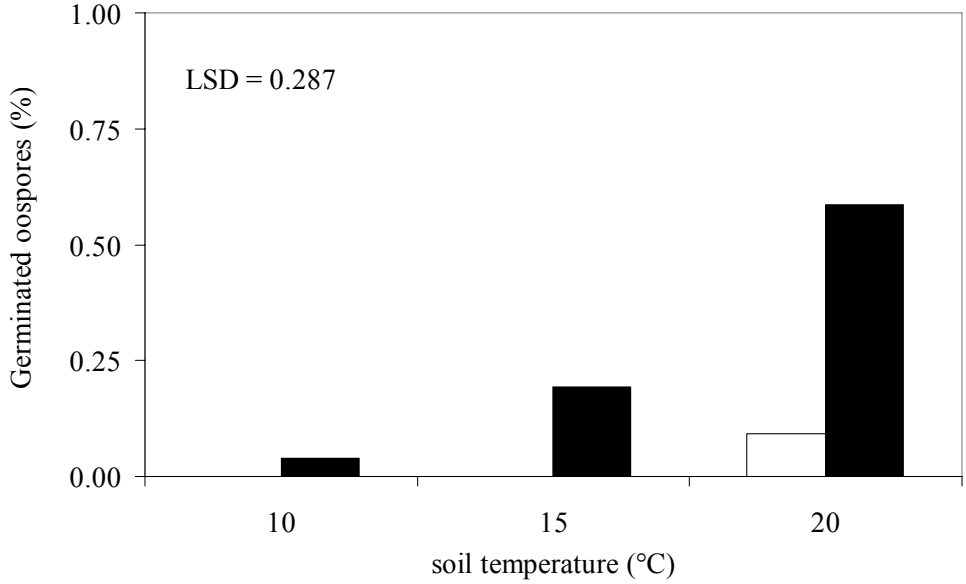

Figure 4. Effect of temperature and incubation period (two weeks: white bars and three weeks: black bars) on germination of P. infestans oospores. No germination was found at 5°C and after 1 week of incubation at all temperatures and soil types. Formation of a primary sporangium was used as the criterion for germination.

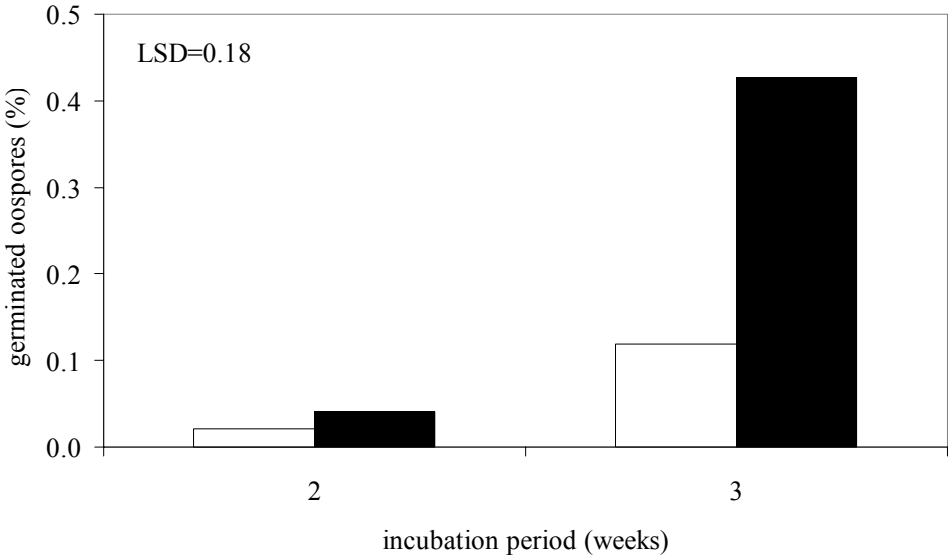

Figure 5. Effect of incubation period and soil moisture level (white bars for saturated soils, black bars for soils at field capacity) on germination of P. infestans oospores.

From Figure 3 and Figure 4, it can be concluded that the oospore germination rate increases with temperature. However, only at the highest temperature (20°C), germination is found already after two weeks incubation. At 10°C and 15 °C it took at least three weeks before oospore germination was detected. This contrast is likely to cause the factorial temperature x incubation period interaction. A similar explanation can be given for the incubation period x soil moisture interaction (Figure 5). In general, the oospore germination rate is higher at field capacity than under saturated conditions. This effect is however much less pronounced after two weeks incubation than after three weeks incubation causing the statistical interaction between both factors.

Discussion

Since the 1990's, evidence has been mounting that new introductions of *P. infestans* into Western Europe included both the A1 and A2 mating type. As a consequence, the Western European potato industry now faces a new *P. infestans* population which is reproducing sexually and is more aggressive than the old population (Drenth *et al.,* 1994; Drenth *et al.*, 1995; Flier & Turkensteen, 1999). For practical control purposes this implies that: 1) epidemic progress is faster and 2): oospores have to be taken into account as an additional, soil borne, source of (primary) inoculum. To adapt current control strategies to the presence of oospores as an extra soil borne source of inoculum, the population dynamics of soil borne oospore populations and factors driving survival and germination have to be studied. The work described in this paper was carried out with a dual purpose: to adapt and optimize methods facilitating quantitative research into *P. infestans* oospore ecology and to study *P. infestans* oospore germination as influenced by time, soil type, temperature and soil moisture.

As reported earlier by e.g. Cohen (2000) and Flier (2001), current results confirm that not all *P. infestans* A1 – A2 combinations produce oospores. Four out of nine A1 – A2 combinations tested produced oospores in potato leaflets but only one A1 – A2 combination (IPO98014 x PIC96002)

resulted in high oospore densities in two out of three crosses. Similar to the present findings, Flier (2001) also found that isolates differ in their average capability to form oospores when engaged in compatible matings. In both studies, specific parental combinations produced more oospores than other parental combinations. Cohen (1997) demonstrated that availability of free water is necessary for oospore production and that oospores were formed abundantly when leaflets were floating on water. In our experiment the availability of water was less than used by Cohen (1997) and may have been less than required for optimal oospore production, possibly explaining the relatively low oospore densities in the current experiment.

Both methods described to extract *P. infestans* oospores from small and larger leaf samples basically concentrate the available oospores in as little remaining leaf tissue as possible. For this purpose the leaf sample is comminuted, enzymatically degraded and sieved to remove as much of the leaf tissue as possible whilst at the same time retaining as many of the oospores as possible. The extraction procedure developed for small leaf samples is more time consuming than the procedure developed for large leaf samples but results in cleaner samples for microscopical oospore quantification.
Protocols were adapted from original protocols to extract *Peronospora viciae* oospores from pea (*Pisum sativum*, L.) by Van der Gaag & Frinking (1996). Major differences between the pea and potato extraction protocol for leaf material include two 90 sec. blending steps at 24000 rpm instead of one 5 min. step at an undefined high speed in the original protocol and a much longer enzymatic degradation step of over two days instead of 2 hours in the original protocol. These adaptations were done to accommodate for *P. infestans* oospore sensitivity to temperatures > 25°C (Drenth *et al.*, 1995) and for the relatively hard potato leaf tissue as compared to pea leaf tissue. Furthermore, surface sterilization of the plant tissue and antibiotics were not employed in the germination assays to mimic survival and (inhibition of) germination under natural conditions as much as possible.
Vice versa, adaptation of extraction techniques for use with other pathosystems is possible by adaptation of the duration of blending and enzymatic degradation. However, the temperature of the sample during blending has to be monitored and stabilized as much as possible since temperature directly influences oospore survival and germination (e.g. Ribeiro 1983; Drenth *et al.*, 1995; Fay & Fry, 1997). Thus, the above protocol was recently optimized to extract *Plasmopara viticola* oospores from grape leaves (van Bekkum, unpublished results).

Recovery of oospores from quartz sand containing only particles > 125 µm as described was found to be a simple but very effective method to quickly recover oospores from incubated gauze bags. This method potentially has a wide range of applications including experiments to shed light on factors stimulating or slowing down oospore germination by adding treatments with altered chemical (pH, CaCO3) or biological (antagonists) properties of the otherwise inert quartz sand.
Oospore viability and germination was monitored during three weeks in a replicated factorial experiment including four constant temperatures, four soil types and two levels of soil moisture. Oospores were classified as germinated when a germ tube and sporangium were present. Presence of a germ tube by itself (Van der Gaag & Frinking, 1996) was not deemed sufficient because biologically, germination is not functionally completed when a germ tube is formed and practically, it was difficult to discriminate between adhering remaining leaf tissue and oospore germ tubes.
Oospore viability was not very much affected by any of the treatments during the experiment (Figure 1 and 2) as can be expected for soil borne long term survival structures. The average percentage viable oospores remained around 65% although viability in saturated soils seemed to be somewhat reduced. Apart from an effect on viability itself, incubation in water may have changed permeability of the oospore cell wall affecting the uptake of water and MTT and thus influencing the result of the viability test (Sutherland & Cohen, 1983).

Germination of oospores was found to be a slow process influenced by soil temperature and soil moisture. The germination rate increased with increasing temperatures (within the temperature range included) and too much available water (soil saturation) slowed germination down. The only factor not (statistically significant) affecting germination was soil type. The low percentages of germination found in the experiment make it however difficult to draw solid conclusions. Observations on germination in the experiments all fall within the 0 – 1% range. Purpose designed experiments could shed more light on some of the effects found in the current experiments but they are likely to be even more labour intensive.

When oospores are present in the soil, as a result of *P. infestans* infection of (a) previous crop(s), it can be assumed that during the growing season, apart from prolonged periods of hot and dry weather, oospores germinate continuously at a very low rate. Thus, newly formed sporangia are continuously being released to the soil at a low rate. The sporangial density in the soil thus depends on the oospore density in the soil, the oospore germination rate and the survival of sporangia under these conditions. In laboratory experiments sporangia have been reported to survive in unsterilized soil for periods up to 11 weeks (Lacey, 1965). Therefore, when oospores are expected, or known to be present in the soil, it has to be assumed that sporangia are also present in the soil during large parts of the growing season. Infections from this soil borne source of inoculum are therefore to be expected during each wet period fulfilling the requirements for puddle formation and/or splash dispersal together with circumstances favouring direct- or indirect germination of the sporangia. The foliage therefore has to be optimally protected by fungicides during each critical period with even less margin for error or delay than in the situation without oospores being present in the soil. When oospores, and thus sporangia, are in the soil during harvest, we should even consider the possibility of infection of tubers from this soil borne source of inoculum.

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Tenth Workshop of an European Network for development of an
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Monitoring Primary Sources of Inoculum of *Phytophthora infestans* in The Netherlands 1999 - 2005

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Summary
In the period 1999 till 2005, a project on monitoring early outbreaks of late blight in Dutch potato fields was performed in the context of the so-called Master Plan Phytophthora (MP) and Umbrella Plan Phytophthora.
In the course of eight years, 184 fields with primary foci were surveyed and the farmers concerned were interviewed according to a question list. In total 2075 isolates of *Phytophthora infestans* were collected and stored in liquid nitrogen. Mating type, haplotype and AFLP fingerprints were determined. Based on all information gathered, a classification concerning the origin of initial inoculum and timing of infections was made.
Regulations issued to cover potato pile culls reduced the impact of cull piles on early infections considerably but cull piles continue to be one of the important initial inoculum sources. It was learned that early during the growing season, and already within the first week of emergence, potato crops may attract infections of *P. infestans*. More than 80% of the fields visited were not protected when the first infections occurred, despite warnings for relevant critical periods for late blight infection.
The occurrence of very small numbers per acreage of isolated, heavily affected single plants resulting into large foci around mid June could be assigned to latently infected seed potatoes. Depending on the region, either latently infested seed potatoes and/or oospores proved to be the most important inoculum sources.

Introduction
A range of initial sources of inoculum is reputedly held responsible for primary and early outbreaks of potato late blight. Infected tubers (cull piles and volunteer potatoes), latently infected seed tubers and oospores are distinguished as primary sources of inoculum. In the frame of minimizing the use of fungicides to control late blight, but at the same time effectively controlling the disease with the help of disease forecasting systems, it was concluded that more regional information was needed on sources of initial inoculum and date of primary infections occurring in farmers' fields.
Fostered by the so-called Master Plan Phytophthora and Umbrella Plan Phytophthora, a cooperative

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project of farmer organizations (LTO, HPA), scientific institutions (PPO, PRI) private organizations (Extension services, Pesticide firms, DSS builders) and The Dutch Ministry of Agriculture, Nature and Food Quality was performed in the period from 1999 till 2005. This article gives an overview of the results obtained during these surveys.

Materials and Methods

Four Dutch potato-growing regions were selected:

1. A seed and ware potato-growing region in the South West, growing potatoes on clay soil.
2. A similar region in the North West.
3. A mainly ware potato-growing region in the South East on sandy soils.
4. A mainly starch potato-growing region in the North East on sandy and reclaimed peat soils.

Fields with early development of late blight were reported by extension services of private companies (Cebeco Agrochemie, Profyto, Nestlé, Syngenta, HLB and De Landbouw Voorlichting), providers of Decision Support Systems (Dacom Plant Service, Prolion) and farmers. Experts visited the reported fields with the consent of the farmer. Evaluation of the primary focus was based on several criteria:

1. Number of foci encountered
2. Focus size (area plus number of lesions)
3. Infection place, specifically affected leaf layer or layers
4. Infected plant parts (leaf, stem, seed tuber)
5. Classification of lesions based on their development and size (relative age), and determination of the number of classes
6. Number of leaf layers of diseased and healthy stems
7. Length of diseased and healthy stems
8. Crop history (date of planting, weed control, emergence, etc)
9. Spray schedule and fungicides applied
10. Weather data and specifically periods critical for infection using Plant Plus
11. Assessment of the age of the classes of lesions based on information under points 3 to 10
12. Crop rotation
13. Presence of volunteer potato plants in the foregoing three years and in the field examined
14. Presence of *Phytophthora* tuber rot during storage or in the seed lot

The farmer concerned was interviewed to obtain information over the abovementioned points. Infected plant material was sampled and *P. infestans* isolated. Isolates were stored in liquid nitrogen and genetically characterized by determining:

- Mating type (A1, A2)
- Haplotype
- AFLP- fingerprint patterns

The genetic information of the isolates was used to back-up assessment of primary inoculum sources. Primary inoculum sources distinguished are:

- (Latently) infected seed potatoes
- Oospores
- Sources nearby and far away
- Unknown

In case of a nearby source, the actual primary source was found (cull pile; neighbouring field, volunteers, etc) and/or the disease dissemination pattern in the filed indicated a nearby source. In case of a far

away source no potential primary source was found and the disease dissemination pattern in the field suggested a far away source. Else if no original source could be identified the origin was classified as unknown. This event was commonly associated with a single focus in a large region.

Results and discussion

In the period of 1999 till 2005, 184 primary and early foci of *P. infestans* were visited and their most likely origin investigated (Mulder & Turkensteen, 1999; Baarlen & Raatjes, 2001; Turkensteen, 2003; Turkensteen *et al.*, 2004, Turkensteen *et al.,* 2005; Evenhuis *et al.*, 2006). Table 1 shows the relative importance of the various primary inoculum sources of late blight for the four selected regions during the surveying period. During this period 2075 isolates of *P. infestans* were sampled and stored in liquid nitrogen.

Information obtained on primary inoculum sources was used to optimize the late blight control strategy. Different strategies have to be used to control each particular primary inoculum source and their effects. Flyers explaining the consequences for the late blight control strategy were disseminated to the farmers through Master Plan Phytophthora (LTO-Nederland, 2005 & 2006).

Table 1. Relative importance of primary inoculum sources of late blight in four regions of The Netherlands during the survey period 1999 – 2005.

Region	Source of primary infection					Number of fields examined
	Seed	Oospores	Far away	Nearby	Unknown	
North East	37	32	13	12	6	90
South East	24	9	46	12	9	33
North West	26	0	40	30	4	27
South West	53	0	32	15	0	34
Weighed average[a]	36	17	27	15	5	184

[a] *Percentage of fields infected by late blight originating from the primary inoculum source indicated in the heading (n=184).*

First spray Prevention of primary late blight development is an essential part of an effective control programme. Hence, eradication of primary inoculum sources is the first step. Furthermore the crop has to be protected from primary infections by spraying in good time before critical periods occur. From 42 fields in our survey sufficient data on spray applications were available. A large percentage (83%) of the visited fields (n=42) with early foci was not sprayed at all at the time of the first relevant critical period for late blight for the crop concerned. With respect to these fields, 39% should already have been sprayed as early as during the first week at the start of crop emergence. In 47 % of these cases, late blight development was initiated by oospores, the remaining part was mostly due to infection originating from sources outside the potato field.

Cull piles Cull piles are long known as a primary inoculum source of *P. infestans* (Hänni, 1949). Cull piles were found to serve as a primary source of inoculum in 74% (n=19) of the infested potato fields during a survey from 1994 till 1996 in South Flevoland (Zwankhuizen *et al.,* 1998). Since regulations (HPA) to cover cull piles have been enforced, the numbers of cull piles carrying late blight infected plants decreased initially. However, the last three years (2005-2007) the number of uncovered cull piles stabilized at a non-acceptable level (LTO-Nederland, 2005 & 2006; NAK-Agro personal communication). The first reports of occurrences of late blight in the season were usually from cull piles, during the years of our surveys. It indicates that cull piles continued to act as an important early source of primary inoculum, although to a lower level. It implies that regulations to cover cull piles

have to be enforced even more stringently. Nevertheless, with the reduction of uncovered cull piles, which in most years are the earliest inoculum sources, the other inoculum sources, (latently) infected seed and oospores, became more notable.

Cultivation under cover Cultivation of early potatoes under plastic cover is vulnerable to late blight infection. Soil and canopy under cover remain longer moist than in the open field. The moist conditions and the higher temperatures early in the season favour late blight infection and development. Usually covered crops are not sprayed to control late blight, before the cover is removed. Considering the earliness of this type of crop, these fields form primary inoculum sources for late blight to surrounding potato fields. An early and heavy epidemic in such crops occurred both in 1999 (Mulder & Turkensteen, 1999; Hadders, 2003) and 2005 (Evenhuis *et al.*, 2006) going along with large areas with widely isolated, single infections that took place around the time the cover was removed. To control late blight, these crops should be sprayed even when fields are still covered. Preliminary experiments showed effectiveness of over cover sprays (Spits & Bus, 2003). It is assumed that the initial infection sources are diseased or latently infected tubers. Oospore initiated epidemics were not found with these type of cultivation, but may be feasible as well.

Latently infected seed Every year, single heavily infected plants were found in the season around mid June, which for an initial source is relatively late in the growing season. Symptoms usually occurred on most if not all stems of these plants. After the stage of infected stems, the pathogen spread to the leaves. Under conditions favourable to late blight, heavy sporulation occurred leading to large foci of 100 to 1000 m^2. These foci were characterized by the presence of only one or two age-class-lesions in full-grown crops. Each of the original single diseased plants started off from a diseased mother tuber, which again originated from a latently infected seed tuber. These findings concerning the acting and timing of *P. infestans* through latent infected seed tubers were supported by results from Germany, which showed that the pathogen could be latently present in tubers in storage, and in sprouts and stems formed on these tubers in storage and in the field (Adler, 2001). If these plants were raised under moist conditions they showed similar late blight symptoms (Adler, 2001) as found in the field during our survey.

Infection from latently infected seeds occurred in each region. If circumstances were favourable for tuber infection in the previous year, a relative high percentage of latently infected seed tubers might be present. However expression of late blight in spring depends on weather circumstances during May and June. Infection risks from seed tubers increase when the soil is moist. A period of 5 to 7 days of moist soil between emergence and closing of the canopy favours expression of late blight originating form (latent) tuber infection (Adler, 2001; Bässler *et al.,* 2002). Usually stem lesions developed. Fungicides with a curative or systemic mode of action showed to be effective to prevent the stem blight, when sprayed at due time (Kalkdijk *et al.*, 2005).

Oospores During a survey in 2000, oospores were encountered in 78%, 50%, 30% and 15% of two lesion single leaflets samples collected from regular crops in North East, South East, Central and South West of the Netherlands, respectively (Flier *et al.*, 2004). On unprotected potato plants oospores can be formed abundantly, especially on volunteer potato plants (Kessel *et al.*, 2002; Förch *et al.*, 2004). To prevent oospore formation, volunteer potato plants should be destroyed. Oospores can survive up to four years in a sandy soil, whereas survival is limited to three years in a clay soil (Turkensteen *et al.*, 2000).
The North East of the Netherlands is characterized by growing potatoes for starch production. To save

costs occasionally sprays at the end of the season are left out. Further crop rotation schemes are narrow and soils are sandy. All these circumstances favour oospore formation and consequently infection through oospores.
Infections originating from oospores were mainly found on sandy soils, and were most common in the North East of the Netherlands. In case of oospore infections, large numbers of plants synchronously attract single or a few primary infections on infested fields. At the same time neighbouring potato fields with a same protection history may be totally free of late blight. From three of such fields with a recently emerged crop, AFLP-fingerprints were made from four isolates per field in 2005. The fact that for each of the fields, the four isolates from the same field had different AFLP patterns is in agreement with what to expect from an epidemic initiated through oospores, since every oospore has a unique AFLP-pattern. A mix of genotypes of both mating types also indicated that oospores might be the most logical source of infection, as was found in Sweden (Widmark *et al.*, 2007).
Oospores based infections frequently occur after heavy rainfall. Sometimes even in the first week of crop emergence, infection of the canopy occurred. Contrary to popular believe, a spray to control late blight might be necessary when weather circumstances are critical during or shortly after crop emergence, and especially so in case oospore are present in the field. Furthermore, spray application schemes should be followed through until the haulm is killed.

Main conclusions

- Foci of late blight yielded useful information on moments of primary and secondary infections, inoculum sources and importance of (failures in) timing of fungicide spraying.
- As many as 83% (n=42) of the fields with an early outbreak of late blight were not treated with fungicides prior to the initial infections, indicating the importance of proper timing of the first application. Decision support systems can assist the grower to do so.
- Reports on first infections often concerned diseased plants on potato cull piles in spite of regulations to cover cull piles. These regulations have to be implemented even more strictly.
- The importance of latently infected potato tubers in the onset of epidemics was revealed. It is a relatively late acting source of initial inoculum. Nevertheless it formed an important source of primary inoculum in all regions and especially so for the ware and seed growing regions on clay soils. Strict potato late blight control in seed potato crops to prevent infection of the next season seed is therefore imperative.
- Expression of infection from (seed) tuber to plant is strongly dependent on weather conditions. When plant infection from infected seed tubers is to be expected spray application with a (local) systemic fungicide in the early season is recommended.
- Oospore based infections are found on sandy soils mainly and most frequently in the starch potato growing area in the North East of The Netherlands. Oospore based infections may already occur within the first week of above ground crop development at emerging.
- Potato late blight should be strictly controlled during the whole growing season to avoid both tuber infection and oospore formation.
- Volunteer potato plants should be destroyed to prevent a boost of the late blight epidemic and to avoid mass production of oospores.

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Curative control of *P. infestans* in potatoes

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Summary
In August 2006 the *P. infestans* pressure was high in the Netherlands. The incidence of the *P. infestans* infections of potato fields in the Netherlands varied a lot. In a field trial, several fungicides and additives were tested on their curative action in an infected crop. Two sprays with mineral oil added to Mancozeb/cymoxanil improved the curative action against *P. infestans* as compared to mancozeb/cymoxanil applications. Propamocarb/chloorthalonil performed less but still better than mancozeb/cymoxanil applications. Adding fluazinam to mancozeb/cymoxanil improved also the efficacy, while the additional product ATS in a tank mix with mancozeb/cymoxanil showed no curative control as compared to the mancozeb/cymoxanil applications

Keywords
P. infestans, curative control, mancozeb/cymoxanil, fluazinam, mineral oil, Ammoniumthiosulphate.

Introduction
In August 2006 many potato fields were infected with *P. infestans*. The incidence of the infection varied a lot. Figure 1 shows the *P. infestans* pressure periods in 2006 in Wijster.

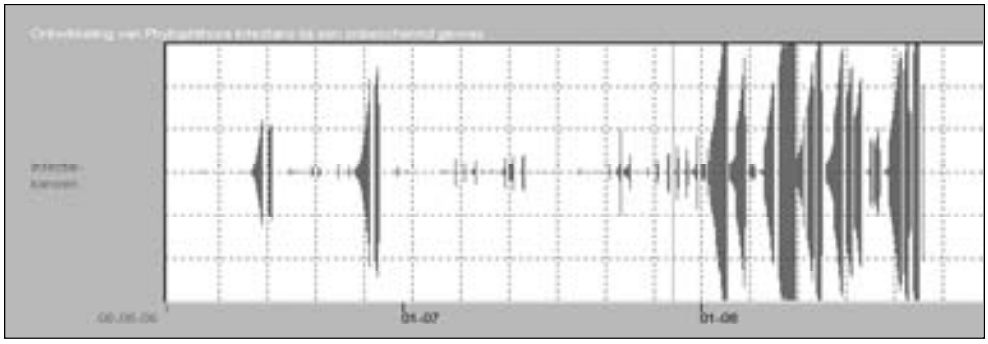

Figure 1: P. infestans pressure periods in 2006 according to the Dacom advice system.

Infested potato crops are normally treated with metalaxyl or propamocarb/chloorthalonil, though metalaxyl is risky because resistant *P. infestans* strains can be involved. Also fungicides are added to

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the regular spraying with for instance mancozeb/cymoxanil to improve their curative properties. HLB carried out a field trial to establish the effectiveness of some additives to regular fungicide sprayings.

Materials and methods

The field trial was carried out in an infected potato crop, cv. Amyla. The initial infection with *P. infestans* was around 5% and was mainly found in the upper leaf layers. The curative treatments were applied at a five days interval on the 18th and 23rd of August 2006 in three replicates. The treatments are presented in table below.

	Active ingredient	Concentration	Dose rate (/ha)
1	propamocarb/chloorthalonil	375/375 g/l	2.7 l
2	mancozeb/cymoxanil	68%/4.5%	2.5 kg
3	mancozeb/cymoxanil ammoniumthiosulphate	68%/4.5% 12%NH4-N+26%S	2.5 kg 15.0 l
4	mancozeb/cymoxanil fluazinam	68%/4.5% 500 g/l	2.0 kg 0.3 l
5	mancozeb/cymoxanil mineral oil	68%/4.5% 800 g/l	2.5 kg 4.0 l
6	fluazinam	500 g/l	0.4 l

After these two curative treatments, all plots were sprayed once with fluazinam 0.4 l/ha on 29 August 2006. The applications has been carried out according normal farm procedures.

The infection with *P. infestans* (% of the leaf area infected) was assessed weekly from beginning of the trial. The vitality (# sporangia per cm² lesion) was determined on the 25th of August 2006 (2 days after the second curative treatment).

Results

Curative sprays with fluazinam 0.4 l/ha or mancozeb/cymoxanil 2.5 kg/ha in a infected crop, showed an increase of 65% (Figure 2). Comparing two standard curative control treatments in an infected crop, propamocarb/chloorthalonil 2.7 l/ha performed a little better (increase 40%) as compared to the tank mix mancozeb/cymoxanil 2.0 kg/ha + fluazinam 0.3 l/ha (increase 50%).

Adding mineral oil 4.0 l/ha to mancozeb/cymoxanil 2.5 kg/ha improved the efficacy (increase 30%). The efficacy of this tank mix with mineral oil is significantly better (AUDPC, Figure 3) as compared to the other treatments.

Adding ATS 15 l/ha to mancozeb/cymoxanil 2.0 kg/ha did not improve the efficacy (increase up to 65%).

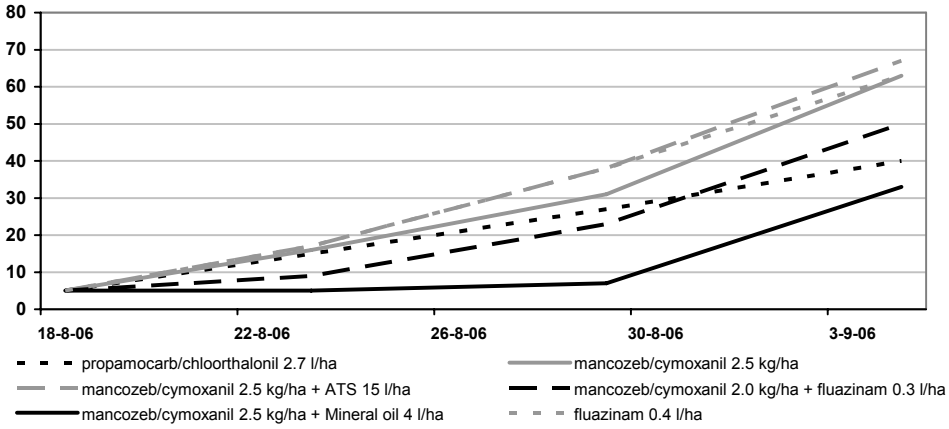

Figure 2: Disease progress Curve of P. infestans

Regarding the vitality of the lesions (Figure 3), the lesions after two sprayings with the tank mix mancozeb/cymoxanil 2.5 kg/ha + mineral oil 4.0 l/ha were less vital as compared to the other treatments. It seems in this trial that a lower vitality of the lesions lead to a better control of *P. infestans* (AUDPC is lower).

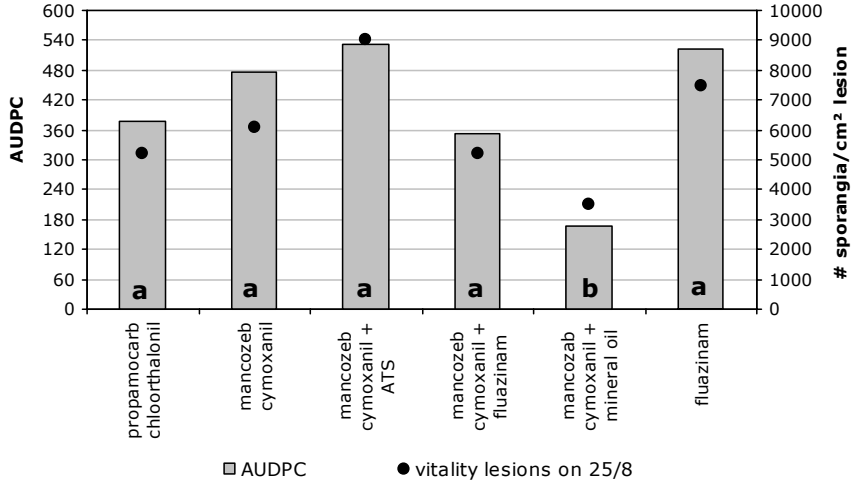

Figure 3: AUDPC P. infestans (period 18 August – 4 September 2006) and the vitality of lesions on 25 August 2006 after two curative treatments. For AUDPC, bars with the same letter do not significantly differ (P=.1, LSD).

Discussion

In this trial, the *P. infestans* infections increased throughout all the plots. It is assumed that the curative action of mancozeb/cymoxanil is one day. In this trial, two treatments with mancozeb/cymoxanil showed an increase of 60% of the infections. The efficacy of two treatments with propamocarb/chloorthalonil and mancozeb/cymoxanil + fluazinam performed better. The vitality of the lesions of the above mentioned treatments was almost comparable namely between 5.300 and 6.000 sporangia/cm^2 lesion. In relation to this, it is remarkable to note that the treatments propamocarb/chloorthalonil

and mancozeb/cymoxanil + fluazinam have a lower AUDPC than the treatment mancozeb/cymoxanil. This might be the consequence of a better curative action.

Adding mineral oil to mancozeb/cymoxanil treatment made the treatment more effective in this trial. This effect is probably caused by the low vitality of the lesions (3.500 sporangia/cm^2 lesion). The effect of mineral oil application can be explained by:
1: the wax layer becomes permeable so that the fungicide can penetrate easier,
2: the fungicides remains in the wax layer and remains therefore effective for a longer period.
The two points mentioned above might a valid explanation for the improved efficacy of the additive mineral oil to mancozeb/cymoxanil. In this trial 4.0 l/ha was used, maybe a lower dose rate will also do.

Another additive product to control *P. infestans* is ATS. ATS stands for Ammoniumthiosulphate, a foliar fertilizer agent (12% NH_4-N + 26% S). It is mainly used to slow down aging of the potato plants. It has been noticed that in 2005, under hot and dry conditions, this application controlled *P. infestans* when applied in combination with mancozeb/cymoxanil. The same application in 2006 was not effective. The circumstances during application time were different in 2006, namely cold and rainy. It seems that in 2005 the addition of ATS to the fungicide application had an effect on *P. infestans*. This might be caused by:
1: the salt concentration on the leaf might be so high that the fungus can not grow anymore,
2; ATS contains certain elements that might have a negative impact on the development of the fungus (i.e. sulphur or boron).
The reason why in 2005 the ATS addition was more effective then in 2006 is probably the different weather conditions at application time.

Conclusions
Based on the results, the following conclusions can be made:
- Additives can increase the efficacy of standard chemical fungicide applications, like this year when the mineral oil 4 l/ha was added to mancozeb/cymoxanil 2.5 kg/ha.
- Adding ATS 15 l/ha to mancozeb/cymoxanil 2.5 kg/ha did not improve the curative action in 2006.
- Propamocarb/chloorthalonil 2.7 l/ha and mancozeb/cymoxanil 2.0 kg/ha + fluazinam 0.3 l/ha showed a comparable curative control. Mancozeb/cymoxanil 2.5 kg/ha and fluazinam 0.4 l/ha performed less.

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