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Preface

EuroBlight Workshop Arras, France 3-6 May 2010

A European network of scientists and other specialists working on potato early and late blight meet every 18 months. The network combines two previous networks originating from European Concerted Actions and has 150 members.
- EU.NET.ICP: "European network for development of an integrated control strategy of potato late blight" (1996-2000). Coordinated by Huub Schepers
- EUCABLIGHT: "A potato late blight network for Europe"(2003-2006). Coordinated by Alison Lees.

The Twelfth Workshop was organised by Serge Duvauchelle, Ludovic Dubois (Ministère de l'Alimentation, l'Agriculture et de la Pèche, SDQPV), Didier Andrivon & Roselyne Corbière (INRA Rennes), Catherine Chatot (Germicopa SAS) and Marie-Pascale Latorse (BayerCropScience) in Arras, France from 3-6 May 2010.

Agrovision, BASF, Bayer, Belchim, Dow, DuPont, Germicopa, Gowan, Nordox, Nufarm and Syngenta sponsored the Workshop.

The Workshop was attended by 112 persons from 13 European countries, Russia, Algeria and United States of America. Representatives from all countries presented the late blight epidemic in 2009 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 is an increasing problem in Europe, also reports on this disease are included.

The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, PPO-Special Report no. 14. The Proceedings are also available on the internet www.euroblight.net.

Alison Lees, Scottish Crop Research Institute (UK)
Jens G. Hansen, Aarhus University (DK)
Huub Schepers, Wageningen University (NL)

For further information please contact the network secretariat where also additional copies of this Proceedings can be ordered.

Secretariat
PPO-AGV Lelystad
Att. H. Schepers
PO Box 430
NL-8200 AK Lelystad
The Netherlands
Telephone: + 31 (0) 320 291111
Telefax: + 31 (0) 320 230479
E-mail: huub.schepers@wur.nl
Internet: www.ppo.wur.nl

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PAPERS

Potato – a world production, a European business

MARTINE SCHWARTZMANN, UBIFRANCE[1]

[1] French agency for international business development
71 boulevard St Jacques – F-75014 Paris (France)

SUMMARY

Potatoes are grown on all continents, in around 130 countries in the world and under a great variety of climates. Third most important food crop in the world after rice and wheat, over a billion people worldwide eat it. But, as far as trade is concerned, Europe leads the world exchanges of table potatoes and potato seeds.

A WORLD PRODUCTION
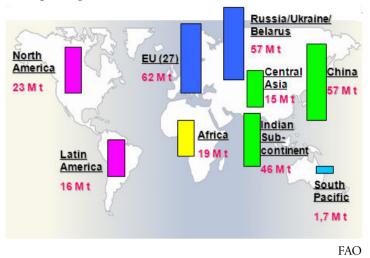
According to FAO (Food and Agricultural Organization), potato is currently grown on an estimated 18 million hectares, with a global production of 314 million tons (figures 2008). Asia and Europe are the two major potato growing areas.

1. Asia: 8.5 million ha, 131 million tons. China, the Indian subcontinent (Pakistan, Nepal and Bangladesh) and the countries of central and western Asia (southern CIS countries, Middle East) being the main growing zones.
2. Europe (as a geographical entity): over 6 million ha, 122 million tons. More than 2 million hectares are grown in the European Union, while 4 million hectares are cropped in the north-eastern zone including Russia, Ukraine and Belarus. While the EU represents only 34 % of the area, it represents 51 % of European crops. Respective shares of the Russia/Ukraine/Belarus zone are 62 % of area an only 47 % of crops.
3. Other potato growing zones: North and South America (1.5 million ha, 40 million tons in 2008, mainly industry crops), and Africa (2 million ha, 19 million tons, + 58 % since the beginning of the century, essentially in the sub-Saharan countries).

During the last 50 years, the growth of potato production is largely due to the extension of potato growing in developing countries: whereas developed countries represented 90 % of the world crop in the 60's, their share is nowadays less than 50 % (figures FAO/CIP). These countries registered a decline in potato consumption (potato is no longer a staple commodity but is included in a diversified diet), as well as a decline in the use for fodder (especially in Eastern Europe countries). On the contrary, in Asia, Africa or Latin America, potato is more and more regarded as an alternative source of food, income and employment: growth of consumption, interest in processed potatoes.

Potato growing worldwide

Russia/Ukraine/
Belarus

57 M t

North
America

23 M t

EU (27)

62 M t

China

57 M t

Central
Asia

15 M t

Indian
Sub-
continent

46 M t

Africa

19 M t

Latin
America

16 M t

South
Pacific

1,7 M t

FAO

According to the geographical and/or economic context, there are different types of potato growing:
- In Western Europe, North America, Australia, Japan potato crops are intensive and high yielding, thanks to the use of selected seeds (benefit of varietal innovation), quality inputs, advanced crop technology (including irrigation and storage);
- But, there is still a majority of countries, where potato is a traditional crop made by small family farms for subsistence: Eastern Europe, Asia, Andine countries, Africa. It means low yields, poor quality seeds, few phytosanitary treatments if any, no irrigation;
- In some cases, potato is a cash crop developed for export (new potatoes in the Mediterranean Basin) or for the supply of processing units.
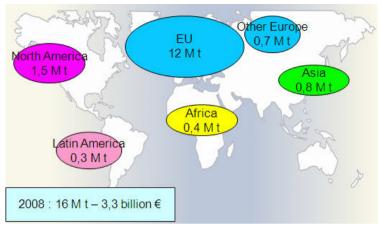
EUROPEAN LEADERSHIP ON INTERNATIONAL TRADE

As a whole, international trade for table potatoes (imports and exports of ware potatoes + new potatoes) concerned 16 million tons in 2008, worth a sales value of 3.3 billion EUR. Europe is the main trading area, with around 75 % in volume and value terms; the European Union making more than 90 % of this share.

The strength of the European potato business relies on:
- A controlled production, leading to a regular and constant supply of quality tubers to the markets,
- The supply of products adapted to modern distribution, as well as to the requirements of the processing industry,
- Post-harvest infrastructures and highly efficient logistics which enable the export to a wide range of countries: intra-EU, Eastern Europe, Mediterranean countries, Middle-East, Africa, America, Asia.

Most of non-European trade is regional: supply of the American processing units by Canadian potatoes or exchanges between neighbouring countries in South America, Africa or Asia. But we must also keep in mind, that production is most often self-consumed. Finally, a special mention to the exports of new potatoes from Egypt, Israel or Morocco to the European markets from January to April/May.

International trade

Exports: a total of 8 million tons worth 1.6 billion EUR in 2008. Europe represents 76 % of the exports in volume (75 % to the sole credit of the European Union) and nearly 70 % in value terms. France takes the lead of exporting countries, with 1.8 million tons in 2008, followed by Germany, the Netherlands, Belgium and Canada.

Imports: Belgium and the Netherlands are the main importing countries. For them, import is needed as a complementary supply for their local processing units, but also for their trading companies to re-export. Facing a decline of their own production, Spain and Italy are major European markets.

Imports		Exports	
Belgium	15 %	France	24 %
Netherlands	15 %	Germany	19 %
Spain	9 %	Netherlands	11 %
Germany	7 %	Belgium	9 %
Italy	6 %	Canada	7 %

Top five importing/exporting countries

POTATO SEEDS
According to FAO figures, the world production of potato seeds is estimated at 31 million tons in 2008. But most farmers still plant next season's crop using part of last season's crop, leading to the propagation of pests and diseases and poor yields. To improve the profitability of their crop, the growers need varieties with high yield potential and better resistance to pests and diseases.
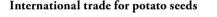
European breeders are world leaders for potato selection. In the European Union, the propagation area is estimated at 110 000 hectares, of which 87 % are located in the 15 Western Europe countries. The Netherlands take the lead with 36 000 ha, followed by Germany (16 000 ha), France and Great Britain (15 000 ha each). In Eastern Europe, production of potato seeds is found in the first place in Poland (5 000 ha), the Czech Republic (4 000 ha) and in Romania (2 000 ha).
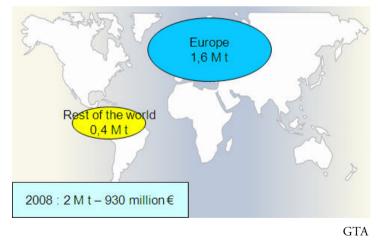
Potato seeds production in EU 27

AMI

In 2008, international trade of potato seeds represented 2 million tons, worth 930 million EUR. Europe takes the lion share with 80%.

International trade for potato seeds

GTA

Exports: 1.2 million tons, 521 million EUR. The Netherlands are absolute leaders (55 % of total volume), exporting to over a hundred countries, with major varieties like Spunta, Agata or Desiree. Its strength is based on an ample supply (production + re-export), an efficient marketing policy and reliable logistics. But intensive cropping and short rotations leads to growing problems of pests and diseases. France takes the 2nd place, exporting a wide range of varieties, bringing quality certification and technical support. Its main exporting zones are the south of Europe (Spain, Portugal, Italy and

Greece), North Africa (Tunisia, Algeria, Egypt, Morocco) and Middle East. The United Kingdom, Germany, Denmark and Belgium are the other major exporting countries in Europe, developing their own varieties on specific strategies and privileged marketing areas.

Imports: major importing countries are located around the Mediterranean Basin: Algeria, Egypt, Spain and Italy. Markets are growing in Eastern Europe, where there is a will to develop a professional sector, but also in North Africa, to come along with the development of a processing industry.

Top five importing/exporting countries

Imports		Exports	
Algeria	10 %	Netherlands	55 %
Spain	7 %	France	12 %
Egypt	7 %	UK	8 %
Germany	6 %	Canada	6 %
Italy	6 %	Germany	5 %

GTA

CONCLUSION - CHALLENGES FOR TOMORROW

The future of potato production and trade will depend on the ability of the sector to respond to the following challenges:
• The growth of the world population, which is a challenge of food security,
• The change in consumption patterns, with the growing demand worldwide for processed, ready-to-eat products,
• The climate changes which should bring motivation to adapt the product to new growing conditions (drought resistant varieties), and the need to find treatments filling the new environmental requirements,
• Resistance to pests and diseases, which is a current challenge,
• Developing storage to reduce the waste of production, regulate the marketing of the product and avoid low prices.

The development and control of Late Blight (*Phytophthora infestans)* in Europe in 2009

JENS GRØNBECH HANSEN (DENMARK)[1], BJÖRN ANDERSSON (SWEDEN),
RUAIRIDH BAIN & ALISON LEES (SCOTLAND), RICCARDO BUGIANO (ITALY),
FAYE RITCHIE (ENGLAND & WALES), LINDA BUCENA (LATVIA), STEVEN KILDEA
(IRELAND), 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 & RAGNHILD NÆRSTAD (NORWAY), JOZEFA KAPSA
(POLAND), MATI KOPPEL (ESTONIA), TOMKE MUSA (SWITZERLAND), ANTANAS
RONIS (LITHUANIA), HUUB SCHEPERS & KEES VOGELAAR (THE NETHERLANDS),
PIETER VANHAVERBEKE (BELGIUM)

[1] Aarhus University, Dept. of Agroecology and Environment, Research Centre Foulum,
PO- Box 50, 8830 Tjele, DK

INTRODUCTION

The EuroBlight late blight country profile was launched in 2007 to keep track of the development of late blight and its control in Europe in individual countries and over years. The profile information for 2006 was presented at the workshop in Bologna, Italy in May 2007. Information for 2007 and 2008 was presented at the workshop in Hamar, Norway in November 2008 and the results for 2009 were presented in Arras on the 4 May, 2010. This paper reports the development and control of late blight in Europe, 2009.

One important motivation for sharing data is that the results are analysed in a pan-European context. When data are available over several years it will be possible to analyse the data over years and across countries. This is especially interesting now that all countries in Europe have to adapt to the new EU pesticide package to be implemented by the end of 2013. Using the data we collect before and after 2013 might be used for impact assessment of this EU regulation. We will also use the data to stimulate to collaboration, harmonisation and coordination among countries.

Currently we are rebuilding the web version of this tool and for 2009, results was reported via word form and e-mail and then imported into the database.

METHODS

The country profiles have the following structure and content:

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 favourable - 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.

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.** In the country reports graphs for mating type distribution and virulence pathotypes are automatically included based on available data from the Eucablight database.

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

Use of DSS
- Write a short text (max 100 words) about use of DSS in the country.

The reports per country published below are the abstracts of the country reports taken directly from the database with only slight editing.

THE DEVELOPMENT AND CONTROL OF *PHYTOPHTHORA INFESTANS* IN EUROPE IN 2009

The abstracts of the country reports are provided by country in alphabetic order. General trends and observations on weather conditions, disease development etc. are discussed in the section of summary information. Information regarding "Date of first observation of late blight in covered or very early planted potatoes" and "Date when first infections were reported in more than five conventional, normally planted potato fields" for 2009 is shown for all European countries on maps in Fig. 1-2. The same data are combined into marker plots per year in Fig. 3 and 4. The weather based risk of late blight development in Europe is shown in Table 1 and Fig. 5. The level of tuber blight attack is given in Figure 6.

Belgium
The weather in the month of May was favourable for a swift emergence of the potato crop. For late blight on the other hand, conditions were less advantageous for development. Although diseased plants had been found on dump piles towards the end of April, a small amount of lesions in field crops was observed only after the first week of June. A rainy infection period from the 10th to the 15th of June, in combination with high levels of new growth, led to an increase of lesions during the third week of this month. Further spread however was again hampered by the sunny weather with high temperatures during the last decade of June. The month of July brought favourable growing weather: alternately rain with summery days, but without heat. Because the sequence of the disease cycles interfered nicely with the sequence of rainy days, late blight had the opportunity to develop strongly; attacks in field crops remained low however, because of good spraying conditions allowing for timely applications. From the beginning of August, weather turned into dry conditions and this lasted until the first week of October. Under these circumstances and spraying intervals until harvest increased to 12 – 14 days or more.

Czech Republic
Potato late blight had very favourable conditions for crop infection, epidemic spreading in foliage and tuber infection in 2009. Amounts of rainfall in most potato production regions were mostly above long-term normal between May and August. Weather progress was not quite typical for the disease (i.e. warm fronts with long-lasting cloudiness, relatively mild, but constant rainfall), but durable leaf wetting and favourable microclimate were provided by frequent and heavy rains, many

times of rainstorm character. The first crop infections in the potato production region occurred very early, already from the end of the second decade of June. Beginnings of epidemic and spreading of the disease in the foliage were recorded in the beginning of July. Intensive rainfall also supported tuber infection that already occurred in July, especially in very early and early varieties. Protection required intensive applications of fungicides and recovery of fungicide cover after stormy rainfalls. Considering tuber protection setting of term and way of vegetation ending was also decisive, especially for varieties susceptible to tuber blight. Potato late blight significantly influenced mainly yields, which reflected not only foliage infection, but also early tuber infection. The most favourable conditions for tuber infection were recorded in the second half of July. Therefore most infected tubers decayed in the soil, already before harvest and this displayed as yield losses; however, presence of infected tubers in the store was mostly mild. Markedly more appropriate situation for the disease was recorded in early potato production regions, where due to differential weather progress infection pressure was milder and conditions for tuber infection were absent.

Denmark
The total area with potatoes was 38.800 ha. About half of this area is grown for starch production. Planting was possible in first part of April due to warm weather. Soil temperature exceeded 8 °C on 7-10 April. The month of May was normal regarding temperature, but it was dry. The date of crop emergence was normal, 20-25 May, and only few (3) fields were found with indications of early attacks from oospores. This was during 3-10 June. Two of these attacks were on experimental stations. Widespread attacks in conventional fields were found only after 22 June. Early blight was a considerable problem in 2009. The levels of attacks were not very high but attacks were much more widespread in the country compared to normal. Early blight seems to be an increasing problem in DK, maybe due to warmer climate. Other indications on climate change are overwintering Colorado beetles in the south of Denmark, never happening before. Many days with optimal conditions for harvest in September resulted in good quality of stored potatoes. Tuber yield was relatively high as well as the starch content.

England & Wales
Early planting was delayed in January and February due to heavy rainfall and cold, wet weather. These conditions continued until mid-March, where conditions became more favourable and the majority of the crop was planted by mid-May. There were 152 confirmed incidents of late blight in GB in 2009, 100 of which were in England and Wales. Most reported outbreaks were in crops with 2 originating from outgrade piles and 13 from volunteers. Only 14% of outbreaks were reported in May, June and September, with the majority reported in July (40%) and August (46%). Growers used a wide range of products to control late blight. Most advisers were recommending intervals between fungicide applications to be no more than 7 days.

Estonia
The potatoes were planted in mid of May, which was dry and colder than usual. The potatoes emerged in normal time in the first decade of June. Dry and colder than normal weather delayed further potato development and did not favour the late blight in June. The first late blight outbreaks were recorded in home gardens where potatoes had been cultivated in the same soil for several years in July 8. These outbreaks had clear character of soil borne infection - lesions on lower leaves and many plants. Late blight infection in conventional fields was recorded in several places over the whole Estonia in July 15-20 after a rainy period in July 7-12 with 30-60 mm of precipitation. The time of first outbreaks was approximately one week later than normal of last ten years. Established late blight progressed extremely fast in the second half of July. The blight favourable warm, rainy and moist weather continued until the mid of September. Fungicides had to be applied at shortened

treatment intervals throughout the whole season to provide adequate blight control. Tuber blight was a problem for those growers who were unable to control the foliage blight in rainy conditions. Fast developing late blight caused serious yield reduction in organic production. Reasonable yield was harvested only from organic fields of most resistant varieties.

Finland
In 2009 weather in general in Finland was not conductive for blight development. The precipitation was relatively normal but most nights during June and July were too cold for blight development. There was only one two week risk period at the end of July and beginning of August and most blight epidemics were reported during that period. First blight attacks probably derived from oospores were reported in the middle of July, which is approximately 3 weeks later than usually during 2000s. In spite of low blight risk most farmers sprayed normally starting at the first half of July and spraying 4 – 6 times. In practice blight was not present in fields where fungicides were applied.

France
The season 2009 was marked by a very heterogeneous epidemic during the season. In general for France, the risk of blight in 2009 was lower than in 2007 and 2008. Periods with high risk for disease development were recorded during the season, but the use of the models such as MILEOS ® recognized these periods effectively and the control of blight was successful.

Germany
Planting of potatoes in Germany took place during end of March and mid of April. It is a normal planting date. Very warm conditions after planting resulted in an early emergence of potato plants (beginning of May= 4-14 days earlier than normal). The first outbreak of late blight in potatoes was in the mid of May in the early potato growing area. One week later we found late blight attacks in covered potato fields. In the second week of June late blight was observed in the southern potato growing region. The weather conditions for the development of late blight was low in May, low (northern part) to high (Southern part) in June, high to very high in July and moderate in August. The number of fungicide treatments was normal in 2009. All kind of products were used. The new product REVUS was registered and introduced this year. Very late in the season the Alternaria-fungicide SIGNUM was registered.

Republic of Ireland
Unfavourable weather conditions during April and early May delayed planting of most potato crops, with the subsequent planting continuing into early June. By mid June minor outbreaks of blight were recorded in crops, with foliar and stem blight reported. Following approximately two weeks of extreme disease pressure in early July more severe outbreaks were reported, particularly in the South-East and East of the country. Intensive fungicide programmes (tight spray intervals of seven days or less) were required to halt the spread of disease. A period of relatively dry weather at the latter end of July helped control the disease. Disease pressure during the month of August was again high, however the severity of disease was not as great as during the month of July. Although high levels of foliar disease were reported during the summer months, limited amounts of tuber blight have been observed. Unprecedented amounts of potato crops were left in the ground due to severe weather conditions from November through to late January (flooding followed by extreme frost).

Italy
Planting of potatoes in the north of Italy took place during half March and first week of April. Potato normally emerged starting from half of April onwards. Tomato is commonly transplanted from the last week of April until the second week of May. In the north of Italy late blight was not

very aggressive in 2009 on potato. Climate was not blight conducive in May, usually considered historically the month "at risk" due to the spring showers, since very few rainy period occurred in coincidence with susceptible crop. Rainy periods occurred at the end of April – first week of May only. Therefore the weather conditions for the development of late blight was low in May, moderate in June, low in July and moderate to medium in August and September. Blight occurred on outdoor tomato grown in the growing areas near the Adriatic Sea, in June due to infection events of the first week of June. Late blight on potato was easily controlled with the common disease control strategy due to the low disease pressure, while on tomato some control problems occurred during the season. No late blight occurred on tubers while some yield losses occurred on outdoor tomatoes. The number of fungicide treatments was normal in 2009. All kinds of products listed in the Integrated Production Guidelines were used (see table 1). In central and south of Italy, heavy attacks of late blight occurred in Central Italy (Abruzzo Region) due to a prolonged rainy period in the first two week of June. The disease infected the stem in majority of the commercial plots. In some cases damages reached 100% of yield although tuber blight has been rare. The prolonged rainy period made it difficult for farmers to enter into the field in time to protect the crop. Early blight does not cause problems on potato in North of Italy while some problems were registered in the central Italy during the end of the season. On tomatoes, early blight rarely occurred in the north of Italy. Among the product used to control it, QoI are the most used at the end of the season.

Latvia
Crop emergence was completed by the end of May. Cool weather conditions (average temperature of 8-10 ºC) delayed the crop growth and development of late blight, therefore the first warning of the development of late blight was received on the 26th of June when the temperature and humidity conditions were favourable for the development of the disease. Also the first warning of the development of *Alternaria solani* was received in this period. In June 95 mm of rainfall was recorded in western part of Latvia, but in the northern part - 135 mm of rainfall.

The first protective application of fungicide (systemic or translaminar + contact) was made before the infectious period. Temperature and humidity conditions were favourable for the development of both diseases. The second warning of the development of late blight was received on the 3th of July. The second protective application with fungicide (systemic or translaminar + contact) was made. The first symptoms of *Phytophthora infestans* were recorded on the 8th of July on unprotected crops. In July the infection pressure on unprotected crops was very high due to frequent precipitation and optimal temperatures. In July 136 mm of rainfall was recorded in western part of Latvia with average temperature of 15 – 19 ºC for the most of July. The following applications in July were made with translaminar + contact fungicides and in August - with contact fungicides, mostly with mancozeb and fluazinam.

Unprotected crops and those that were the most susceptible were totally killed in two weeks in the beginning of August. *Phytophthora infestans* and *Alternaria solani* progressed also in August. 101 mm of rainfall was recorded in northern part of Latvia in August and weather conditions were favourable for the development of tuber blight. The use of fungicides resulted in excellent control in all farms when the first protective application with systemic + contact fungicide was made at the end of June/ beginning of July. Control of late blight was very good to moderate in the 2009 season.

Lithuania
Unfavourable weather conditions during April delayed potato planting in many regions. In some places ware potatoes were planted until the middle of May while usually it ends in the first days of May. Throughout the country the specialist of State Plant Protection Service monitored situation in

the majority of crops including potato. First late blight symptoms by Service specialist were detected on 29 of June. After one-two week period the disease was detected in most regions. July and August were rainy with relatively high temperature and consequently late blight spread very fast. In central part of the country, first symptoms of late blight were detected in the middle of July and over 3 weeks its severity reached nearly 80.0% and after one more week – 100%. Late blight in this year affected both yield amount and tuber quality. Intensive fungicide treatments increased yield by 76 %.

The Netherlands
Planting of ware and starch potatoes in the Netherlands took place during the first decade of April. It is a normal planting date. Emergence of potato plants was normal, second half of May. The first outbreak of late blight in potatoes was at the end of May in starch growing area, probably caused by oospores after a few heavy rain showers in that region. In the first decade of June late blight was reported in more parts of the country. The weather conditions for the development of late blight in June were poor, so the disease pressure stayed at a low level. The first weeks of July precipitation was high all over the country and at the end of this month new outbreaks of late blight were reported in potato fields.

The second part of August and September were dry. So the disease pressure declined, especially in the southern part of the country. In this area there were problems to keep the level of tuber damage during harvest acceptable. In 2009 there were hardly any Alternaria problems reported. Due to the favourable growing conditions for potatoes the crop didn't seem to be vulnerable.

The number of fungicide treatments was normal to low in 2009. All kinds of products were used. The new products Infinito, Revus and Valbon are used more and more at the expense of Shirlan and Curzate M.

Northern Ireland
Some crops were planted early (March – early April) in good conditions, grew well and matured before blight pressure became severe. However, planting of the majority of crops was delayed by wet weather from mid-April-May and these then grew poorly. Rainfall in April and May was c. 150% of the 30-y average and although only 82% of the average in June, rainfall in both July and August was c. 180% of the 30-y average. The wet conditions made it difficult to maintain fungicide spray programmes and most crops had some blight by the end of the season, although relatively few were severely infected. Growers made use of a very wide range of fungicides, often in tank-mixes. Drier weather in September allowed harvesting in reasonable conditions. Yields were generally low because of the poor growing condition. However, relatively little tuber blight has been seen in store either because of good tuber protection by some products and also because blighted tubers rotted before harvest.

Norway
In May and June the weather was unfavourable for late blight and the first infections was a bit later than normal. In the main potato growing areas a long period of late blight favourable weather started in the first part of July and lasted until late August. During this period the precipitation was very high and that caused problems with late blight control. A lot of fields had some late blight attacks, but most farmers were able to spray their fields without getting heavy losses. More fields than normal got tuber blight and also pink rot was a big problem in 2009. Ranman and Revus were introduced in 2009 and more than 70 % of the treatments were carried out by these two products at the expense of Shirlan. The number of treatments was about the same as normal for the last years. Late blight was not found in the northern part of Norway in 2009.

Poland

The spring of 2009 was generally warm and relatively dry and resulted in early potato planting. Due to the spring weather conditions most of crops emerged in the period May 15-30. The first late blight infection was found only in two fields on May 10th and 26th. Disease did not spread out very much during May due to low disease pressure. Heavy rains in June and July were very favourable for late blight development. The most of outbreaks were observed in Poland between June 15th and July 15th. In that period disease pressure was very high. In several of the crops infections were found on small plants (BBCH 35-37) which indicated soil borne infections. In August the weather based risk of late blight development was moderate In September the disease pressure was low. In general the yield was good and there were little problems with tuber blight. Polish farmers started early with applications of fungicides. Average 2-6 sprays were carried out to control the disease. The most commonly used active ingredients were metalaxyl M, fluopicolid, fenamidon, cymoxanil. The first outbreak of early blight was detected on May 29th. In the most of the monitoring fields (26) early blight appeared in June and first days of July (21 crops). Only 4 infections were recorded at the end of July. Generally weather conditions in 2009 were moderately favourable for early blight development.

Russian Federation

According to our data and information, obtained from the regional Plant Protection Services of the European part of Russia, the severe late blight development (yield losses exceeded 20%) was observed in Kaliningrad and Vologda regions, the northern part of the Kirov region, and on the most part of the Komi Republic. The late blight development in other regions was rather weak or moderate. The first appearance of late blight symptoms was registered on July 6 (at the crop emergence phase) in the Komi Republic. In this day the disease was registered on many potato fields of this region. The most popular fungicides were Shirlan, Ridomil Gold MC, Acrobat MC, Penncoceb, Tanos, and Sectin Fenomen. The average number of fungicidal treatments in agricultural companies and private farms was 3; the maximum number was 10. Owners of small private gardens did not use any fungicides. In recent years some companies and farms used the Plant Plus (Dacom) and VNIIFBlight DSSs for the LB control. A high level of the early blight development was registered in the eastern part of the European Russia, mainly on potato fields, which were not treated with fungicides and have a shortage of nitrogen compounds.

Scotland

The 2009 weather in Scotland was less favourable for foliar blight development than that of 2008. There were fewer Smith Periods and the averages across seven Scottish sites in May, June, July, August and September were 0, 3.3, 2.3, 1.9 and 0.7 respectively. Sixty-two confirmed outbreaks were reported on the Potato Council-funded blight outbreak maps up until mid-September. The first outbreaks were not reported until July but the largest percentage was reported in that month. The progression of crop outbreaks was 56.5 % in July, 35.5 % more in August and 8.1 % in September. There were no outbreaks reported on dumps of potatoes but six outbreaks on volunteers (two in July and four in August). Most agronomists were recommending a maximum spray interval of 7 days unless there were extended periods of low risk. This was prompted by concerns regarding the change in the UK blight population to predominantly the more aggressive 13_A2 genotype.

Slovakia

Very early potatoes were planted in Southern part of country on the end of March. Very early potatoes were sprayed with fungicide only once or twice within vegetation period. Harvest of very early potatoes started earlier than the first observation of late blight was recorded. Ware and seed potatoes were planted in all other regions in the end of April and beginning of May 2009. Drought and warm weather lasted from April until the end of May. Lot of rainfalls and moderate temperature

was recorded in June. Good weather formed suitable conditions for late blight development in the end of June. The first manifestation of late blight infection was recorded on dumps: 29-06-2009, in the ware potato fields: 04-07-2009. Infection pressure decreased in July and progressively increased during August. Good conditions for late blight development on leaves were recorded in the end of August and in the beginning of September. Late blight infections on tubers did not overcome incidence in previous years.

Sweden
The spring was warm and dry in most of Sweden 2009 resulting in good conditions for planting. In 2009, the first blight report came 22 June from an uncovered field on the South coast. This is later than normal. Until mid July there were only sporadic reports of blight in south Sweden and mid Sweden. More widespread attacks in these areas were reported in early to mid July. All in all, 2009 can be considered as a year with relatively minor problems with late blight.

Switzerland
Compared to 2007 and 2008 with first late blight attacks on May 14 and May 19, the first late blight attack in 2009, was observed rather early, namely on 30 April on a covered potato field in the southern part of Switzerland (canton TI). There the weather based infection risk was very high during the last two weeks of April 2009. Mid of May, the weather conditions were very favourable for the development of late blight in many other regions of Switzerland. However, only one new attack was indicated until May 20. From May 28 until June 4, the so called "Bise", a cold and dry wind from the North, delayed the LB-epidemic. Then the weather changed and during the rest of June weather based infection risk was rather high. Several days with continuous main infection and sporulation periods (MISP) were registered for all weather stations. So, late blight epidemic started in all potato growing regions; but did not reach a high level, though the weather was still conducive during the rest of July. In summary, the spread of late blight started late and epidemic could spread during June and July, but the epidemic was weaker than during the two years before. Tuber blight was hardly found in 2009.

EARLY ATTACKS OF LATE BLIGHT

In **Denmark**, first attacks were found on 3 June in an experimental field in the North of Jutland with clear indications of oospores. A week later, 9 June, two conventional fields were recorded with attack probably also from oospores. Only after 22 June attacks were found widespread in the country due to a cold and dry June compared to normal. In **Norway**, the first attacks were generally relative late both in covered and the main crops in the main potato areas in the South. In the Trøndelag area in the Middle part of Norway the first attack was 22 of June (in covered crop), which is early for this region. Late blight was not found in Troms in Northern Norway in 2009. In **Sweden**, 2009, there were no reports of late blight from the early potato districts. It was earlier not uncommon to find blight infected fields originating from soil borne oospores in these areas in Mid May. During later years early attacks of late blight in the early potato have become rarer, mainly due to earlier removal of the fleece in combination with a fungicide application. In 2009, the first blight report came 22 June from an uncovered field on the South coast, which can be considered as 1 - 2 weeks later than normal. In **Finland**, the first late blight attack in conventional open field potato was reported on 12 July and the second on 20 July. The first report came from a potato field with long history of continuous potato growing and the second from an experimental field at Jokioinen used for oospore studies. Early attacks in 2009 were approximately 3 weeks later than usually during 2000s.

In **Estonia** first outbreaks on early potatoes were recorded during 8-10 July in allotment gardens where potatoes are cultivated without crop rotation. First symptoms were detected on lower leaves of plants on entire potato cultivation area. First outbreaks on conventional field were detected during 15-20 July several places all over Estonia. The outbreaks followed the prolonged rainy period of July 7-12 with 30-60 mm of rain. In **Latvia** the first symptoms of late blight were recorded on the 8th of July on unprotected crops. In July the infection pressure on unprotected crops was very high due to frequent precipitation and optimal temperatures. In Lithuania, first late blight symptoms by Service specialist were detected on 29 June. After one-two week period the disease was detected in most regions.

In **Russia**, the first appearance of late blight symptoms was registered on July 6 (at the crop emergence phase) in the Komi Republic. On this day the disease was registered on many potato fields of this region. In **Poland** crop protection services monitored 101 potato fields for the occurrence of late blight. Only two early outbreaks were recorded in crops during May (10 May in West region and 26 May in Southwest part of Poland). A few early outbreaks were recorded in potato fields (4) at the beginning of June. The first infections (6 fields) were reported in normally planted potato fields on 15th June, mainly in West region of the country. The most of late blight infections were observed in the second part of June and the first decade of July (in 68 fields). In **Slovakia,** the first manifestation of late blight infection was recorded on dumps on 29 June, in the ware potato fields on 4 July. Infection pressure decreased during July and progressively increased during August.

Following late planting of most crops in **Republic of Ireland**, the first serious outbreak of potato blight on normally planted crops was reported in early June on a seed crop. No further outbreaks were reported until late June. By mid July outbreaks were reported throughout the country, with severe outbreaks being reported in the South-East and East. In **Northern Ireland** blight was first seen on a dump in Co. Down on 1 June. The first field infection was found on 8 June on a single plant of the second early cultivar British Queen, grown near Broughshane, Co. Antrim. By 30 June, blight had been seen in a further six crops of second early or maincrops cultivars in Counties Antrim, Down and Londonderry; A total of 34 reports of blight had been received by the end of July from all potato-growing areas. In **Scotland**, the 2009 weather was less favourable for foliar blight development than that of 2008. The first outbreaks were not reported until July but the largest percentage was reported in that month. In **England and Wales**, there were few reports of early outbreaks of late blight with the majority reported during July and August.

As in the previous years, the first attacks were recorded on dump piles in **Belgium**. Throughout the early stages of the season however, it became obvious that the level of primary inoculum – both from dump piles or volunteers – was much lower than the previous years. It was believed that the exceptionally cold winter, and particularly the cold wave during the first decade of January, had decimated the potential sources of inoculum. In **the Netherlands**, the first attack was reported last week of May. This infection was found in a starch potato field after a few heavy rain showers. Oospores were most probably the source for this early attack. In 2009 there were very few reports of late blight on waste piles. Due to the unfavourable blight conditions during the early months of the growing season the disease pressure stayed low for a long time. So the first weeks of the season, it was fairly easy to control blight. In **Switzerland**, first attack was registered very early on 30 April in the southern part of Switzerland in a covered potato field. As this is a rather "isolated" region, this LB-attack was not so important for the other potato growing regions. On 20 May a second attack was indicated in the central plateau of Switzerland in normal planted potatoes. This year, even 4 of the 6 first registered LB-attacks were indicated as secondary infections. In Italy, Emilia Romagna, first outbreak both in commercial and organic potato growing was from 12 to 20 May. Disease development was very slow in May due to unfavourable weather conditions for disease development.
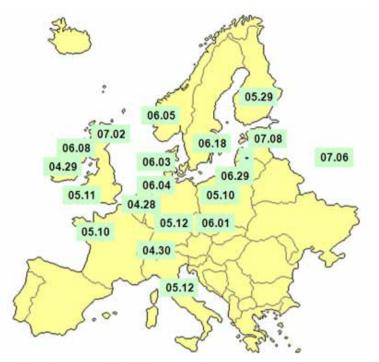
Figure 1. *Date of first observation of late blight in covered or very early planted potatoes, 2009*
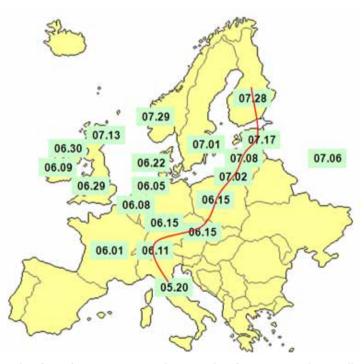
Figure 2. *Date when first infections were reported in more than five conventional, normally planted potato fields, 2009.*
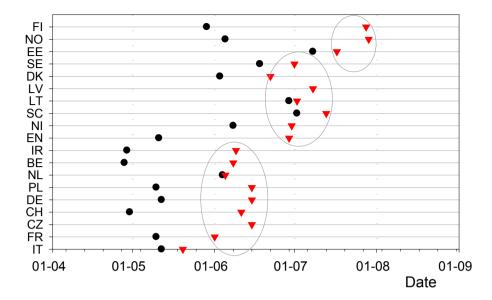
Figure 3. *Date of first observation of late blight in covered or very early planted potatoes (dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (triangles), 2009*
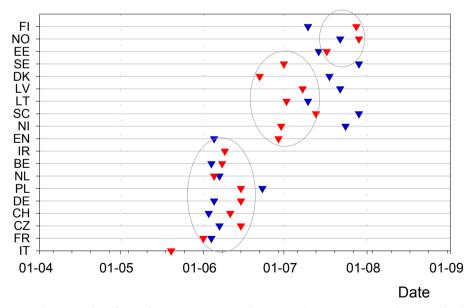
Figure 4. *Date when first infections were reported in more than 5 conventional, normally planted potato fields in 2009 (red triangles) compared to 2008 (blue triangles)*

WEATHER BASED RISK OF INFECTIONS AT SELECTED STATIONS, 2009

In the most recent country report it was concluded that there is a need for a harmonised European approach for calculating blight weather to replace the current method used in EuroBlight (Hansen *et al.*, 2009). With support from ENDURE, we have now created a freely available platform that allows users to test and compare weather-based sub-models for late blight development (Hansen *et al.* this proceedings). For the country report 2009, the risk of infections from *P. infestans* was calculated with the WURCP sub-model for critical periods and summarized as number of critical days per month at selected stations, Ranked according to season sum of critical days (Table 1). WURCP calculates critical periods, i.e. days with a very high risk of infection of the potato crop. WURCP assumes the presence of latently infected tissue in the surroundings of, but outside your field. For a critical period to occur, three sub-processes of the infection cycle have to be fully completed in sequence: formation of sporangia, dispersal of sporangia and infection. The algorithms calculating development rates for each of these processes are based on Crosier 1934.

Table 1. *The number of critical days for infection of P. infestans at selected stations in Europe, 2009, May, June, July, August & September.*

Location	May	June	July	August	September	Season sum
St Eloy (FR)	5	13	13	21	12	64
Tylstrup (DK)	0	5	17	14	10	46
Lindloh (DE)	4	5	15	16	6	46
Valthermond (NL)	2	4	12	15	9	42
Belfast (NI)	3	6	12	11	8	40
Reckenholz (CH)	7	5	6	9	13	40
Mathau (DE)	4	9	6	8	6	33
Dundee (SC)	1	5	8	14	3	31
Ås (NO)	0	0	9	13	3	25
Særheim (NO)	0	0	9	11	5	25
Payerne (CH)	4	2	3	4	12	25
Skara (SE)	0	3	15	5	0	23
Flakkebjerg (DK)	0	0	7	6	9	22
Bonin (PL)	0	1	8	3	7	19
Lelystad (NL)	1	3	5	3	3	15
Capofiume (IT)	2	2	0	0	6	10
Riposto (IT)	1	2	0	0	2	5
Average	2	3,8	8,5	9	6,7	30

The same data are presented on a map and the critical days are indicated per month with a coloured code: 0 days with infection risk per month = green (very low), 1 – 4 days = yellow (Low), 5-9 = light orange (moderate), 10 – 14 = dark orange (high) and 15 – 31= red (very high)

For Tylstrup, DK, the coloured code would be like this - each box representing a month from May - September:

| 0 | 5 | 17 | 14 | 10 |

Figure 5. *The weather-based risks of late blight development in Europe in 2009 at selected stations. Calculations are critical days for infection (WURCP) from 1 May to 30 September.*

The number of days (average for all stations) with infection risk was highest in August – increasing during the season: 2 days with infection risk in May, 3,8 days in June, 8,5 days in July, 9 days in August and 6,7 days in September. The station with the highest risk was Saint Eloy in Brittany in France. The lowest risk for the season as a whole was at the two Italian stations. A similar pattern was found for 2008 (not shown). The difference in blight risk was sometimes high for stations in the same country i.e. Tylstrup (46 infection risk days/season) and Flakkebjerg (22 days/ season) in Denmark and Valthermond (42 days /season) and Lelystad (15 days /season) in the Netherlands. A similar but less pronounced pattern was found for the 2008 season.

TUBER BLIGHT IN 2009

The level of tuber blight was low to medium in 2009, due to a combination of effective leaf blight control and favourable weather conditions during harvest (Fig. 6)

Figure 6. *The level of tuber blight attacks (low, medium or high) in 2009 compared to normal*

USE OF DECISION SUPPORT SYSTEMS

A full list of DSS in use in Europe and associated contact information is available at http://www,euroblight.net

In **Northern Ireland** growers and advisers can make use of DARD Blight-Net (http://www.ruralni.gov.uk/index/crops/potatoes/blight_net.htm), which is based on Risk Hours analogous to Smith

Periods and can also sign up to receive Blight Warnings by SMS. Warnings of Infection Periods are also given on the Blightline recorded phone message and via local radio. Growers can also access Blightwatch (http://www.blightwatch.co.uk) based on Smith Periods. DSS e.g. Plant-Plus are mainly used by pre-packing suppliers to supermarkets to provide justification for fungicide applications. In **Scotland** - in response to the population change most growers of conventional crops applied fungicide sprays at short, fixed intervals and therefore there was relatively little use of DSSs. The main blight risk model used in Scotland continues to be the Smith Period, but there is widespread speculation that the Smith Period criteria of temperature and relative humidity may no longer apply to the new genotypes of *P. infestans* that currently dominate the Scottish population. A new source of information on high-risk weather, called BlightCAST, was made available in 2009 by Syngenta Crop Protection. This continues to be available free on the internet to registered users. High-risk periods are forecast Smith Periods. Plant Plus and Forecast Extra are also available to subscribers. In **Italy** situation is different in each region. DSS are used in Emilia-Romagna region as a routine tool to better time the spray applications. Information of DSS elaboration, about the blight risk and the moment when to start spray to control late blight are used to prepare weekly IPM bulletins. Moreover, in some cases, warning is also send by SMS. IPI negative prognoses model and MISP are currently used. However in many Italian regions, no DSS are so far used. More than 2000 potato growers in **Belgium** receive advice on late blight control from one of the three warning services, depending on the region. A network of more than 70 automatic weather stations collects the necessary meteorological data. The disease models used are historically based on the Guntz-Divoux model, but have been adapted and modified in the course of the past 20 years based on field trials and observations, new pathogen data etc. In the region of Flanders, extensions and sub models (e.g. spore formation, spread and survival, spore germination, infection efficiency, lesion growth) have been added, leading to a much more quantitative disease model. Additionally, the model has been integrated with GIS software and linked with a late blight attacks monitoring service. Advices are updated several times per week and communicated via internet, e-mail, fax or post. A separate advice for organic growers is drafted. In **Norway** VIPS is a national system for plant protection forecasts including late blight on internet and is run in cooperation with Bioforsk and the advisory service. From 2009 a new late blight model developed by Ragnhild Nærstad et. al. was implemented. This model is run in addition to the Negative prognosis for timing the first sprays and the Førsund Model for timing the consecutive sprays. Late blight forecasts on VIPS are used by several farmers often via local advisors which "digest" the forecasts for local use. There are two decision support systems (PhytophthoraModel Weihenstephan, ISIP) for the control of late blight running in **Germany.** The informations of the DSS`s are also on the internet (www.krautfaeule.de; www.isip.de) The majority of the potato growers are directly informed by fax or e-mail. In many regions the state advisory service inform the farmers by telephone or fax. In **Switzerland** plot specific fungicide recommendations of PhytoPRE are used only by a small number (+/- 100) of farmers. But the two application offers (weather based infection risk and map with late blight attacks) is used very regularly (ca. 200'000 clicks/growing season). In addition PhytoPRE list of LB-attacks is weakly published in farmer's newspapers. A lot of farmers have learned due to PhytoPRE to mind the critical facts/periods of late blight.

ALTERNARIA REPORT, 2009

Early blight (*Alternaria spp.*) has not been considered as a problem in potato in **Norway** during at least the last 40 years. In 2009 classical symptoms of early blight was reported in the cultivar "Ramos" in Vestfold County, and *Alternaria solani* was isolated from these plants. In **Sweden**, the incidence of early blight in starch potato in Southern Sweden was high in the beginning of August

suggesting that the outbreaks of early blight started in the later part of July. In mid to late August a large proportion of the starch potato area was affected by early blight, even if treated with fungicides. July was drier than normal in southern Sweden, which enhanced the chances of development of early blight. In mid Sweden July and August was wet and the incidence of early blight was rather low. The incidence of early blight has increased during the past ten years, which could be an effect of reduction in use of fungicides based on mancozeb. In Sweden only two fungicides based on strobilurins are approved against *A. solani* in potato crops. Alternaria is not yet a problem in **Finland**, but individual lesions can easily be found at the end of season. Probably also a frequent use of mancozeb containing products against late blight keep levels of Alternaria low. Early blight was a considerable problem in 2009 in **Denmark**. The severities of attacks were not very high but much more widespread than normal. Early blight seems to be an increasing problem in DK, maybe due to a warmer climate.

In **Estonia**, the weather conditions were unfavourable for Alternaria, and infection took place only in a limited number of fields, where disease severity remained at low levels. In **Lithuania**, 2009, *Alternaria spp* was very rare. Only one fungicide Signum (active ingredients pyraclostrobin + boscalid) is registered specifically to control early blight. Dose rate of this product is 0,2 l/ha. In **Latvia**, The level of *Alternaria spp.* attacks was low. The first symptoms of early blight were observed at the beginning of July.

A number of early blight outbreaks were confirmed from the South-West and South-East of the **Republic of Ireland**. In most instances these were initially mistaken as late blight. The significance of these outbreaks is unknown. Early blight is not a problem on potatoes in **Northern Ireland**. Alternaria sp. is not considered to be a major disease of potatoes in **England and Wales**, although it was reported in 2009 on cv. Markies. First symptoms were reported to have appeared in mid- to late July.

In **Germany**, the outbreak of early blight was normal (2-4 weeks after the crop emergence). The start of the early blight epidemic depends on the cultivar, crop emergence (plant age), weather condition and inoculum. Therefore in some regions early blight has been a destructive disease and caused yield losses due to premature defoliation. Fungicide used to control early blight in Germany: Mancozeb-containing products, Ortiva (Azoxystrobin) and Signum (Boscalid + F500). There were very few reports of Alternaria attacks in **the Netherlands** 2009. At the end of the season a little infection was found in some susceptible varieties. In **Belgium**, in general, Alternaria outbreaks were only recorded late, or not at all; the level of attack was also very low, with the exception of a few cultivars e.g. Markies.

In **Poland** the first outbreak of early blight was detected on May 29th. In the most of the monitoring fields (26) early blight appeared in June and first days of July (21 crops). Only 4 infections were recorded at the end of July. Generally weather conditions in 2009 were moderately favourable for early blight development in Poland. Early blight is not a serious problem in the **Czech Republic**. More important occurrences were found in susceptible varieties in early potato production regions.

Early blight does not cause problems on potato in North of **Italy** while some problems were registered in the central Italy during the end of the season. On tomatoes, early blight rarely occurred in the north of Italy. Among the product used to control it, QoI are the most used at the end of the season.

DISCUSSION AND CONCLUSIONS

In 2009, very early attacks occurring in April were only reported in Belgium on dump piles and in Ireland and Switzerland in covered crops. The first serious outbreaks of potato blight on normally planted crops were reported in Belgium on 8 June, in Ireland on 9 June and in Switzerland on 11 June (Figure 2 and 3). In several countries (i.e. Finland Norway, England, Ireland, Belgium and Switzerland) there was a long gap between date of first attacks in early potatoes and the date when infections were reported in more than five conventionally, normally planted potato. The early attacks are found on dumps (Belgium, in covered crops (Ireland, Norway and Switzerland), in fields infected early from oospores Finland, Denmark, and the Netherlands) or in home gardens with potato after potato - oospores indications, in Estonia.

In Central – and Western Europe (zone 1) attacks in conventional fields were recorded in the first half of June, 14 days later, approximately 1 July in a zone from Great Britain, Denmark, Sweden, Latvia and Lithuania (zone 2) and finally mid until late July in Estonia, Norway and Finland (zone 3). Compared to the year 2008, the dates of attacks were the same for countries in zone 1 and 3, but earlier in 2009 than in 2008 for countries in zone 2 (figure 3). The date of first attack in conventional fields appears in a row (red line in Fig. 2) from Italy in the south of Europe (20 May) to Finland in the North (29 July). This might well represent the differences in the combined date of planting and rate of crop growth - earlier and faster in the south – and that late blight on average is found at the same growth stage shortly after row closing.

In the article about the comparison of blight weather sub-models, Hansen *et al.* (2010b) identified the year 2009 as a relatively low risk year compared to the years 2006-2009.

In most countries the chemical control strategies were reported to be effective in 2009 – despite rainy periods in July and August causing some problems. Problems with tuber blight in 2009 have been reported to be low in Central and West Europe and medium in some countries in the Nordic and Baltic countries (Fig. 6)

The survey and use of DSS show that most countries build their own DSSs based on known model components. The most widespread single DSS seems to be Plant-Plus from Dacom in the Netherlands.

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EuroBlight in a global context

GREG FORBES

International Potato Center, PO 1559 Lima 12, Peru

In the previous International Conference on Late Blight in Beijing, 2008[1], there was clear interest in greater coordination among late blight (LB) workers in the developing world. This was recognized as a general mechanism for improving LB research and for reducing the duplication of efforts that was evident from some of the presentations. It seems logical that this unfortunate situation where efforts are duplicated can occur if communication among researchers is limited.

In the previous EuroBlight meeting in Hamar, Norway, there was a stated interest in expanding EuroBlight technologies to other parts of the world; which, I believe, stemmed in large part from a realization of the benefits these technologies could provide. The idea of expanding EuroBlight's influence was also evident in an international meeting held in Bellagio, Italy in Nov, 2009[1]. The meeting was focused on a global solution to the LB problem and many Europeans were present, as were participants from developing countries. There was active discussion about the use of Euroblight services and technology in the developing world. Below, I discuss some of the mechanisms by which this globalization of the EuroBlight approach could occur.

A white paper was published as a result of the Bellagio meeting that identified five major areas where more work is needed to reduce the devastating effects of this disease (Table 1).

PATHOGEN MONITORING

One of the areas identified in Bellagio was that of pathogen monitoring; certainly one of the primary EuroBlight technologies that could be globalized is the package of data management tools for pathogen monitoring, particularly the data input tool Phytophthora.exe (Hansen 2007; Hansen *et al.*, 2008), the common pathogen database and the data processing tools on the Euroblight Web page (www.euroblight.net). This suite of technologies has taken an interesting turn recently and is actually being globalized, but not yet in the context of potato late blight. The technology attracted the attention of the FAO and the Borlaug Global Rust Initiative[2] and is being adapted for that endeavor. Thus, EuroBlight technology will be successfully expanded first for cereal rusts and hopefully later for LB.

There is a need to be clear about the benefits of pathogen monitoring in developing countries to support and orient the use of these technologies.

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1 *More information is available at the GILB web page:*
  https://research.cip.cgiar.org/confluence/display/GILBWEB/Home
2 *http://www.globalrust.org/traction/permalink/about2*

In the developing world, knowledge of the pathogen population might not be related so much to DSS as in Europe, where there are highly structured and sophisticated DSS, both from public and private sectors; knowledge of the pathogen population can be used to refine these DSS. However in the developing world, DSS of this type don't exist and probably won't for some time, at least for small-scale farmers. This is particularly true in the case of LB, which occurs in many highland areas in the tropics, which are topographically and climatically complex - environmental conditions are highly variable within small geographic areas.

Aside from DSS refinement, there are important potential benefits to global pathogen monitoring. I discuss a few here:

Explaining and anticipating change.
One good example of this is in sub-Saharan Africa. Several studies have documented that there is only one clonal population of *P. infestans* in the region, which is the US-1 "old" population. This population has been suspected of being less problematic for disease management than the new ones (see William E. Fry *et al.* 2009), which has been evidenced by the rapid displacement of US-1 by the new populations, and after displacement, an increase in fungicide needs and earlier disease initiation.

Interpreting research results.
Knowledge on the pathogen population may also help explain apparent discrepancies in results. Again for SSA, workers have been confused by the relative low numbers of fungicide applications used in SSA by farmers for disease control under conditions that would appear to be ideal for disease development - farmers often get away with 3-5 applications. This example of an apparent discrepancy is related to field-level disease management, but interpretation problems can also arise for large scale (GIS based) risk assessments, which use number of fungicide applications as an indicator variable (Sparks, Garrett, and G. A. Forbes 2009; Hijmans, G. A. Forbes, and Walker 2000). We have done a simulation exercise which showed that one would expect to use more fungicides to control disease in SSA than is actually being used by farmers (G. A. Forbes, Shtienberg, and Mizubuti 2009).

Anticipating durability of host resistance.
Another application of pathogen monitoring is in the study of the host-pathogen interaction. In the case where a cultivar is released as resistant (generally the case in a LB prone area), host monitoring can help explain any loss in resistance due to pathogen population change. By monitoring new cultivars, isolates can be collected from disease foci and compared for pathogenicity against isolates from other sources. This leads to clearer ideas about selection within the population. If this type of monitoring were done more often, we would currently have a much better idea of the durability of resistance and this would help us be more strategic in deployment of resistance.

Currently there are several efforts to produce resistant cultivars via genetic modification. At the same time, molecular tools are being developed to screen pathogen populations for effectors, even at the allele level. These data can also be used to make more accurate estimates of risk of pathogenicity against the new cultivars in the target pathogen population.

Fungicide resistance.
Surveys have shown that the phenylamide fungicide metalaxyl is still widely used in the developing world. At the same time, a few studies of the pathogen population (see for example Vega-Sanchez *et al.* 2000) have demonstrated that large portions of the pathogen population are resistant to metalaxyl in the laboratory. It is not known if this lab-based resistance is indicative of what is happening in the field. Since most systemic fungicides are combined with contact fungicides, it is not always easy for a farmer to see that the efficacy of the systemic component has been reduced.

Sexual reproduction and oospore production.

There are few clear examples of sexual reproduction occurring regularly (or even irregularly) in developing countries. Sexual reproduction is now occurring in some areas of Europe, although factors explaining this are not clear. Until conditions which lead to sexual reproduction are better known, we must assume that it could occur anywhere, should compatible isolates be brought into contact. The epidemiological effects of sexual reproduction are also unknown, but potential effects include increased genetic complexity from recombination and/or increased inoculum sources resulting from soil born oospores. Vigilance on this aspect of the *P. infestans* biology is warranted.

DATA MANAGEMENT

The suite of technologies developed in Euroblight for data management could be used to improve LB research in developing countries (William E. Fry *et al.* 2009). The number of studies on pathogen populations has increased in recent years in developing countries which demonstrates that there is an increasing number of laboratories that collect and maintain isolates and assess isolates using one or more of the published makers (D. E. L. Cooke and Lees 2004). In fact, pathogen population studies represented one area where the need for greater coordination was identified in the Beijing meeting mentioned above. One way to improve coordination and standardization would be to use the data input tool Phytophthora.exe developed in the Eucablight project[3]. Once data are entered in Phytophthora.exe they can be uploaded to a common database for comparison across locations. EuroBlight has also developed a database for surveillance data, as well as on-line analysis and graphing tools for weather based late blight sub-models (Hansen *et al.* this proceedings)

SOCIAL CAPITAL AND COLLABORATION

Use of the technologies described above in the developing world would promote the formation of networks of potato workers (plant pathologists, breeders and agronomists). It appears that a likely structure for these networks would be at the regional level: sub-Saharan Africa, Latin America, S E Asia. Broader networks involving potato researchers existed in the past, with PRAPACE in Africa, PRACIPA in Asia and PROINPA in Latin America. Although the networks were quite popular with the participants, donors tired of the concept, and all were eventually dissolved. There now seems to be renewed interest in the networks and there are potato breeding networks in both East Asia and Latin America, although to date they are only focused on breeding and cultivar development. Regional networks would seem to make particular sense for pathogen studies as this would contribute to the development of large common data bases that eventually would enhance capacity for pathogen monitoring across large geographic areas. Finally the development of a community of researchers through a network would provide opportunities for exchange of knowledge of methodologies and the greater synergy of working in a team. Given the regional and sometimes global nature of migrations of this pathogen, large scale pathogen monitoring makes sense.

It would appear logical to merge networking efforts for pathogen studies with existing potato breeding networks for several reasons. First, in many developing countries, potato pathologists and potato breeders work closely together and are frequently in the same organization. Second, as noted above, as more is learned about host pathogen interaction, and the role of pathogen diversity in the expression and durability of host resistance, the more it makes sense to monitor pathogen evolution with respect to resistance, particularly the apparition and establishment of pathogen genotypes that may overcome major resistance genes.

There may be a number of other ways in which the EuroBlight experience can help with coordination

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3 http://www.eucablight.org

in LB research in developing countries. For example, another area discussed in the Bellagio white paper is better information and knowledge management. EuroBlight's experience with their LB information/knowledge portal[4] can help orient efforts to do this for developing countries. Likewise, the now long-standing experience of organizing meetings and documenting the proceedings can also help provide "lessons learned" for similar efforts elsewhere.

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Table 1. *The 5 "Actions" identified in the late blight White Paper developed after the conference in Ballagio, Italy[5]*

Get resistant cultivars to farmers
Improve farmer disease management capacity
Know the enemy and develop a community of skilled pathogen monitors
Develop ecologically-based approaches to control late blight
Coordinate and monitor progress

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4   *http://www.euroblight.net*

5   *More information at the conference Web site: https://sites.google.com/site/bellagiolbnov2009/*

Chosen characteristics of Polish *Phytophthora infestans* isolates

Presentation

MARCIN CHMIELARZ, SYLWESTER SOBKOWIAK, RENATA LEBECKA,
JADWIGA ŚLIWKA

Plant Breeding and Acclimatization Institute – National Research Institute,
Platanowa 19, 05-831 Młochów, Poland

SUMMARY

Isolates of *P.infestans* collected in Poland from 2007 to 2009 were characterized by mating type, virulence, resistance to metalaxyl and mitochondrial DNA haplotype. In total 357 isolates were isolated, originating from different Polish regions. Majority of them were collected in 2009 when the late blight epidemic was very strong in Poland. Collected isolates were tested for mating type and of total 197 isolates 54 were of A1 mating type and 143 of A2 mating type. Two mitochondrial DNA haplotypes were detected in isolates - Ia and IIa, with Ia dominating – among 135 isolates tested 124 were of Ia mtDNA haplotype and 11 of IIa. In total 281 isolates were tested for metalaxyl resistance, and 211 were sensitive, 39 intermediate and 31 resistant. Virulence factors were frequent for genes *R1, R3, R4, R7, R10* and *R11*, moderately frequent for genes *R2*, *R6* and *R8,* and rare for *R5* and *R9.*

KEYWORDS

Phytophthora infestans, late blight, mating type, mitochondrial DNA haplotype, virulence, metalaxyl resistance

INTRODUCTION

Phytophthora infestans is the most destructive pathogen of potato and tomato worldwide, total losses in European Union are estimated to be around 1 billion Euro yearly – that include loss of crop, cost of fungicides (Haverkort, 2008). Chemical control of late blight is successful in high input potato production, but in Poland many small fields are left without any fungicide protection at all, and those can be totally destroyed by *P. infestans.* Before the 1980s worldwide population of late blight pathogen (with exception of those in central Mexico) consisted of only A1 mating type, which excluded sexual reproduction. Furthermore, population around the world was dominated by single clonal lineage – US-1. This situation was disrupted by migrations of A2 mating type and several clonal lineages from central Mexico, which occurred probably in the 1980s and early 1990s (Fry, 2008, Fry *et al.* 2009). Presence of both mating types made it possible for *P. infestans* to reproduce sexually and contributed greatly to genetic diversity of pathogen population worldwide and US-1 was replaced in many locations by new clonal lineages. Recently new clonal lineage called 13_A2 has increased its frequency in Europe, in years 2007-2009 it was found in many countries (United Kingdom, Netherlands, Germany, Switzerland, France, Poland), in United Kingdom it is dominating (www.eucablight.org, Cooke *et al.* 2010). In our institute we're monitoring late blight

population in Poland, testing its characters – both phenotypic and genetic ones. Information about the pathogen such as resistance to common fungicides, mating type distribution and virulence factors are required to find most effective strategy against late blight.

MATERIALS AND METHODS

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

Mating type determination
Mating type was determined by crossing tested isolate with A1 (MP 503) and A2 (US-8 isolate kindly supplied by W.Fry) isolates on ryeA agar medium with addition of β-sitosterol in concentration of 40 mg/l (Spielman *et al.*, 1990) and after incubation period of 10-14 days microscopic observation of oospore formation.

Virulence evaluation
Virulence was tested on 11 Black's differentials, each with single R gene (*R1-R11*), in detached leaflet assay (Zarzycka, 2001). Black's differential set was obtained from SASA, Edinburgh. Potato cultivars Sarpo Mira, Bzura and Biogold were used as additional differentials, together with wild potato species *S. ruiz-ceballosii* syn.*Solanum sparsipilum*(*rzc* 99-10/36) andapotato breeding line containing *Rpi-phu1* gene (04-IX-21).

Metalaxyl resistance
Resistance to metalaxyl was tested on agar plates with rye A medium, by measuring diameters of *P. infestans* cultures. Three variants of medium were made – without metalaxyl (control) and two with metalaxyl at final concentration of 5 and 100 mg/l (Metalaxyl-M, Syngenta Crop Protection). Isolates were classified as sensitive when diameters of culture on both 5 and 100 mg/l of metalaxyl were smaller than 40% of control. Intermediate isolates grew above 40% of control on 5 mg/l medium and below 40% of control on 100 mg/l medium. Resistant isolates achieved more than 40% of the control on both 5 and 100 mg/l medium (Bakonyi *et al.* 2002; Perez *et al.* 2001; Daggett *et al.* 1993). Standard isolates from each group of metalaxyl resistance were used along with tested isolates.

Mitochondrial haplotype
Selected isolates were grown of rye A liquid medium for 3-4 weeks, then the obtained mycelia were rinsed in sterile water, frozen and lyophilized. Using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) DNA was extracted, and then test for mitochondrial haplotype was done according to method described by Griffith and Shaw (1998).

RESULTS AND DISCUSSION

Mating type determination
197 *P. infestans* isolates from 2007 (36 isolates), 2008 (84 isolates) and 2009 (77 isolates) were tested for mating type. Relation of A1 to A2 in 2007 was 12 to 24 respectively, in 2008 it was 21 to 63 and in 2009 it was 23 to 67. In total 56 isolates represented A1 mating type and 154 represented A2 mating type.

Mitochondrial haplotype

Subset of 33 isolates from 2007, 50 isolates from 2008 and so far 52 isolates from 2009 were tested for mitochondrial haplotype. Among Polish *P. infestans* isolates two mitochondrial DNA haplotypes were detected – Ia and IIa. Results were as follows: among isolates from 2007 ratio of Ia haplotype to IIa was 28 to 5; among isolates from 2008 ratio was 49 to 1; among isolates from 2009 ratio was 47 to 5. In total 135 isolates were tested, 124 (92%) of them represented Ia haplotype and 11 (8%) represented IIa haplotype. Neither Ib nor IIb haplotypes were detected.

Virulence evaluation

All 11 virulence factors were observed among tested isolates (Figure 1). Virulence factors corresponding to genes *R1, R3, R4* and *R7* were found in above 95% of isolates, with exception of 2007 when percent of isolates virulent against *R1* was slightly below 80%. Virulence against *R10* and *R11* was also very common, ranging from 70% for *R10* in 2007 to over 90% for *R11* in 2008. Virulence factors against *R2*, *R6* and *R8* were found in between 20% and 50% of tested isolates. Virulence for *R5* and *R9* was rare, up to 10% of tested isolates with exception of *R9* in 2008 when it was slightly above 10% and *R5* in 2007 when it achieved just below 30% of tested isolates. Additional differentials used were cultivars Bzura, Sarpo Mira and Biogold, potato breeding line containing *Rpi-phu1* gene (marked as 04-IX-21) and one wild potato species *S. ruiz-ceballosii* syn. *S. sparsipilum*(marked as rzc 99-10/36). Number of isolates able to infect cultivar Bzura increased – in 2007 less than 30% of tested isolates were able to infect Bzura and in 2009 that ratio reached above 50%. In case of cultivar Sarpo Mira also increase of isolates able to infect was observed in 2009, from around 20% to around 35%. The biggest number of isolates infectious to cultivar Biogold was observed in 2008, when it reached just below 40%, in 2007 and 2009 that ratio was below 20%. Breeding line 04-IX-21 had very few infectious isolates, in every year number of virulent isolates was below 5%. In the case of wild potato species rzc 99-10/36 increase in number of infectious isolates was observed in 2009, but in was still below 10%.
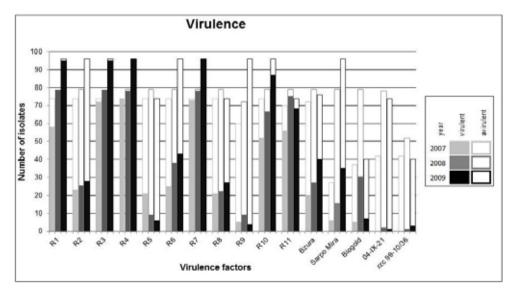
Figure 1. *Number of virulent and avirulent P. infestans isolates collected in 2007, 2008 and 2009 in Poland*

Metalaxyl resistance

In total 281 isolates were tested – 31 from 2007, 85 from 2008 and 165 from 2009. Results: in 2007: 5 resistant, 4 intermediate and 22 sensitive; in 2008: 6 resistant, 10 intermediate and 69 sensitive; in 2009: 20 resistant, 25 intermediate and 120 sensitive (Figure 2). In total 31 isolates were resistant, 39 intermediate and 211 sensitive, which in percentage is 11%, 14% and 75%, respectively.
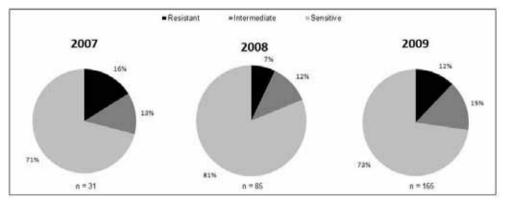
Figure 2.. *Resistance to metalaxyl of P. infestans isolates from 2007-2009*

DISCUSSION

Frequency of mating types of Polish *P. infestans* population has changed in last few years – in studies made in 2002-2004 A1 mating type was dominating with 61% against 39% of A2, higher frequency of A1 was also observed among isolates collected before 2002. Yet, from 2005 domination of A2 mating type has been observed – among isolates from 2005 and 2006 A2 appeared in 66% of tested isolates (Śliwka *et al*, 2006; Lebecka *et al*, 2007). And in years 2007-2009 this tendency continues with 73% of A2 mating type. Results from www.eucablight.org show that domination of A2 mating type occurred in most of countries for which data were published on this website. In Poland neighbouring countries distribution of mating types is variable – in Germany A2 dominated largely in 2007, in Slovakia data from 2003 shows about 60% of A2 mating type, but in 2004 only A1 mating type was detected. In Czech Republic results from 2003-2005 show distribution of mating types near to 1:1 (Mazakova *et al.,* 2006)

Virulence factors against *R1, R3, R4, R7, R10* and *R11* were found in most of tested isolates, which is very similar to results from 2005-2006 (Lebecka *et al*, 2007). Virulence factors against *R6* and *R8* also occurred with similar frequency as in previous studies, but against *R2* appearance was lower, in the case of *R5* and *R9* virulence was kept on low level. When isolates were tested against breeding line containing *Rpi-phu1* gene very small number appeared to be infective. This could happen because potatoes with this gene are rarely cultivated and therefore selection pressure on pathogen is relatively low. Another explanation could be that Avr factor corresponding to this gene is conservative and rarely undergoes changes, which would make resistance coming from *Rphi-phu1* gene durable and very useful for potato breeding. Increasing number of isolates able to infect cultivars Bzura and Sarpo Mira could be a problem for potato growers, especially that both those cultivars are supposed to be highly resistant to late blight (Haynes *et al.*, 1998; Hansen *et al.,* 2006). Infectivity of isolates against cultivar Biogold remains relatively low.

Results of virulence from Polish isolates are consistent with other European countries when comparing virulence factors against *R1, R3, R4, R7, R10* and *R11,* yet there are differences against rest of R genes from Black's differentials. In Belgium in years 2005-2008 virulence factors against

R2 and *R6* were found in above 50% of tested isolates and there were no isolates containing virulence factors against *R5, R8* and *R9.* In Denmark in data from 2003 virulence factors against *R6* were found in more than 50% of tested isolates, and no isolates containing virulence factors against *R9*. In Estonia according to data from 2004 to 2007 frequency of virulence factors are very similar to those in Poland, with exception of *R2* – in Estonia around 50% of isolates had virulence factors against this gene. In Finland virulence factors against *R2, R5, R6, R8* and *R9* are found very rarely, all below 10%. In France frequency of virulence is similar to those in Finland, but virulence against *R8* is higher – around 20%, and no isolates were tested against *R9,* therefore there is no information available. In Slovakia frequency of virulence against *R2* is above 50%, against *R8* around 60%, and against *R9* no virulent isolates were found (www.eucablight.org).

Resistance to metalaxyl was relatively low – 75% of isolates were sensitive, which is similar to results from 2005 and 2006 when 73,3% of isolates were sensitive, and to results from 2002 to 2004 when 82% of isolates were sensitive. Yet, lack of data concerning usage of fungicides on fields which were sources of isolates in years 2007 – 2009 prevents us from drawing conclusions. Low percentage of resistance to metalaxyl among Polish isolates was observed, compared to data from other European countries. For example in France resistant isolates dominated the population (www.eucablight.org). In Poland losses caused by late blight is estimated at 39,4%, mostly on fields with no chemical protection at all, which are still in majority in Poland (51,2-61%) (Kapsa, 2004) – therefore on those fields there is no pressure on pathogen to maintain resistance to fungicide – that could explain very low number of isolates resistant to metalaxyl in Polish population of *P. infestans*. Very similar frequency of resistance to metalaxyl was observed in Czech Republic, in years 2003-2005 81% of tested Czech isolates were sensitive to metalaxyl (Mazakova, unpublished data).

Polish population of late blight is heavily dominated by isolates with Ia mitochondrial haplotype with 92% of tested isolates representing this haplotype. Ia dominance also appeared in previous studies, when in group of 74 isolates from years 1997 to 2006 Ia haplotype was represented by 66 isolates (89%). Ratio of Ia haplotype dominance in Poland seem to be constant, and no Ib or IIb haplotypes were found. General tendency of Ia haplotype domination is common for most of Europe, with exception of Northern Ireland where IIa haplotype is more frequent (www.eucablight.org).

CONCLUSIONS

Polish population of *Phytophthora infestans* is complex and diverse. In most cases it's very similar to population in Europe – proportions of mating type, mitochondrial haplotype and most of virulence factors are very similar. Yet, the frequency of resistance to metalaxyl of Polish population differs from European populations.

ACKNOWLEDGEMENTS

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Monitoring the Dutch *Phytophthora infestans* population for virulence against new R-genes

MARIEKE FÖRCH[1], TRUDY VAN DEN BOSCH[1], PETRA VAN BEKKUM[1], BERT EVENHUIS[2], JACK VOSSEN[3] AND GEERT KESSEL[1].

[1]Wageningen University and Research Centre, Plant Research International, Biointeractions and Plant Health, P.O. Box 69, 6700 AB Wageningen, The Netherlands.
[2] Wageningen University and Research Centre, Applied Plant Research,
P.O. Box 430, 8200 AK Lelystad, The Netherlands.
[3] Wageningen University and Research Centre, Plant Research International, Plant breeding,
P.O. Box 16, 6700 AA Wageningen, The Netherlands.

SUMMARY

New possibilities offered by marker assisted breeding and GMO breeding have sparked renewed international efforts to breed for durable potato late blight resistance. *Phytophthora infestans* is however known for its adaptability, a trait confirmed by recent discoveries on the structure of the *P. infestans* genome. One of the possibilities to enhance the durability of newly introduced host resistance is to monitor the pathogen population for virulence to new *R* genes, prior to - and after their introduction. The late blight control strategy should be adapted accordingly.

The Dutch *P. infestans* population was monitored during the growing seasons 2006 – 2008. *P. infestans* isolates were collected from blighted production fields and from bait fields in which *R* gene containing potato clones were grown without fungicide protection.

A selection of the *P. infestans* isolates collected were characterized for virulence to a range of new *R* genes using a detached leaf bio-assay. Virulence for all single *R* genes tested was found. When we focus on *R* genes *Rpi-blb1* and *Rpi-blb2*, no virulence was found in 2006. One *Rpi-blb1* virulent isolate was found in 2007. Another 2007 isolate was found to be virulent to *Rpi-blb2*. Depending on the genetic background in which *Rpi-blb1* was placed 13 or 21 isolates were virulent in 2008. Depending on the genetic background in which *Rpi-blb2* was placed 4 or 11 isolates were virulent in 2008. One isolate was found to infect the stacked *Rpi-blb1* and *Rpi-blb2* resistance genes in a detached leaf assay.

From these findings it is recommended that monitoring systems should be part of future potato late blight control strategies. The resulting information on the dynamics of virulence within the local *P. infestans* population can then be used to enhance the durability of newly introduced host resistance.

KEYWORDS

Solanum tuberosum; potato; late blight; *Solanum bulbocastanum*

INTRODUCTION

Phytophthora infestans, the causal organism of potato and tomato late blight, is one of the most important and destructive diseases of potatoes. Frequent fungicide applications are necessary to grow modern high yielding potato cultivars which are mostly susceptible to potato late blight. The resulting high fungicide input has a negative effect on the economic feasibility of the crop and on the environment. Development and application of cultivars with a high level of resistance to potato late blight is therefore highly desirable.

In the past *R* genes derived from *Solanum demissum* were used for breeding potato late blight resistant potato cultivars, but resistance proved not to be durable since *P. infestans* was able to adapt and break these *R* genes in rapid succession (e.g. Van der Plank 1971, Turkensteen 1993).

At present, many different *Solanum* spp. are used in classical and molecular breeding programs aiming to produce durably resistant potato cultivars. *S. berthaultii, S. bulbocastanum, S. stoloniferum*, and many other *Solanum* spp. are part of these efforts.

However, recent discoveries on the *P. infestans* genome (Haas & Kamoun *et al.* 2009) combined with the past experience with *S. demissum R* genes make it highly likely that *P. infestans* is also able to adapt and quickly overcome also these new *R* genes. Additional, durability enhancing, measures such as stacking *R* genes and low input chemical control strategies specifically designed for resistant cultivars are explored by e.g. the DuRPh project (www.DuRPh.wur.nl). Monitoring of the local *Phytophthora infestans* population for new virulences, by e.g. screening for effector variation, allows early detection of adaptation within the *P. infestans* population which gives the possibility to adapt the control strategy to the new situation.

In this paper we summarize the results of monitoring the Dutch *P. infestans* population for virulence to new *R* genes from 2006 till 2008.

MATERIALS AND METHODS

Isolate collection and storage

Over the period 2006-2008 *P. infestans* isolates were obtained from infected leaf samples collected from two different sources: infected growers fields, dumps and volunteer potatoes from all over The Netherlands and from bait fields established at three locations (Lelystad, Valthermond and Vredepeel) in the Netherlands. Bait fields contained 50-100 different *Solanum* genotypes (susceptible and resistant cultivars, Black's *S. demissum* R1-R11 differential set, advanced breeding lines and wild *Solanum* spp). Fungicides were not applied on bait field, thus genotypes were subjected to local infection pressure. Infected plant parts were collected weekly and used to obtain *P. infestans* pure cultures. Severely infected plants were removed entirely from the bait fields. The isolates were included in the Plant Research International *P. infestans* culture collection and were stored as sporangial suspensions in DMSO in liquid nitrogen.

Isolate characterization

P. infestans isolates were genotypically characterized using twelve microsatellite markers (SSR) as described by Li *et al.* (2010). SSR data were used to assign the isolates to SSR groups. From the most prominent SSR groups at least 1 isolate was selected for the detached leaf virulence assays. The number of isolates tested was 18, 38 and 31 for 2006, 2007 and 2008 respectively. Isolates were characterized for virulence against a set of *R* genes using *Solanum* accessions (wild *Solanum* spp.) with a known *R* gene content and *R* gene containing genetically modified *Nicotiana benthamiana* and *S. tuberosum* cv Desiree material as *R* gene differentials. The replicated differential experiments contained two leaflets per differential x isolate combination per petri dish and two petri dishes per replicate experiment. Leaflets were inoculated by spraying them with a sporangial suspension of

20.000 sporangia per ml of the appropriate *P. infestans* isolate. Petri dishes were incubated at 15 °C and a 16h/8h light/dark regime. Severity, as measured by the leaf area covered by necrotic lesions (0 – 100%), and sporulation (0, 1 or 2 for "no sporulation", "low level sporulation" and "high level sporulation" respectively) was assessed visually after 1 week incubation.

RESULTS

Virulence patterns
894 *P. infestans* isolates originating from infected field crops, waste piles, volunteer potatoes or bait fields were collected during the 2006-2008 period. Of these isolates, 562 were SSR genotyped. The virulence spectrum was determined for a selection of isolates after SSR genotyping. Overall, it was shown that virulence for all *R* genes tested was found using the detached leaf-assay although some virulences were rare in the Dutch *P. infestans* population so far.

Rpi-blb1 and Rpi-blb2
As an example we focus on two of the most commonly used *R* genes, *Rpi-blb1* and *Rpi-blb2*. Both genes originate from *S. bulbocastanum* and were crossed into *S. tuberosum*. Virulence for both *Rpi-blb1* and *Rpi-blb2* was first observed in 2007. One isolate (NL07377) was able to overcome *Rpi-blb1* and another isolate (NL07434) was able to overcome *Rpi-blb2*. Isolate NL07337 originated from a RHO3-424 clone. Isolate NL07434 originated from a naturally infected potato clone with an ABPT background. Both isolates were collected in bait fields at Valthermond in the North East (starch potato area) of the Netherlands.
In 2008 more virulent isolates were found for the two *S. bulbocastanum* R genes. In total 13-21 isolates were found with the virulence for *Rpi-blb1*, depending on the genetic background of the R-gene differential used. In total 4-11 isolates were found with virulence for *Rpi-blb2*, also depending on the background of the R-gene differential. Thus an increase in virulence for both *Rpi-blb1* and *Rpi-blb2* was found during the survey period. Furthermore, one isolate was virulent to both *Rpi-blb1* and *Rpi-blb2* separately and was also shown to infect the stacked *Rpi-blb1* and *Rpi-blb2* genes. This strain was isolated from a *S. venturii* clone at Vredepeel in the South East of The Netherlands in 2008.

DISCUSSION

P. infestans virulence pattern
P. infestans was isolated from bait fields and farmers fields. To establish the virulence patterns of a selected number of isolates, bio-assays were carried out on R-gene containing *Solanum* genotypes.
In general, virulence for almost all *R* genes tested was found in the bait fields and virulence for all R genes tested was found in the detached leaf bio-assays and thus in the Dutch *P. infestans* population although some virulences were rare. In our bait fields and bioassays virulence was also found for both *Rpi-blb1* and *Rpi-blb2*, both alone (bait fields and detached leaf assays) and in combination (detached leaf assays only). These two R genes belong to a small group of intensively used R-genes in current breeding programmes.
Commercial cultivars containing *Rpi-blb1* are not grown in The Netherlands during the survey period. Commercial cultivars containing *Rpi-blb2* are rarely grown in The Netherlands during the survey period. Yet virulent strains to both R genes were found, suggesting that these "new" virulences were already present in the Dutch *P. infestans* gene pool or they were the result of on site mutations, in response to a low level selection pressure.

R gene break through

Cultivars with new *R* genes are introduced at present. These new introductions are especially beneficial to organic farming since this is the primary market for resistant cultivars at this point in time. In the past *Phytophthora infestans* has shown to adapt very quickly to *R* genes originating from *Solanum demissum* (Turkensteen, 1992). It is now known that effector genes, the natural target for R-genes, are localized in highly dynamic and expanded regions of the *P. infestans* genome (Haas *et al.*, 2009). Taking both aspects into account it is thus highly likely that *P. infestans* is also able to adapt to new, non *S. demissum, R* genes which are used in modern breeding programmes.

If we assume that *P. infestans* generates natural variation within the effector gene family, an R gene break through could occur as follows:

Imagine two potato fields close to each other. A susceptible cultivar is grown in field 1 and is heavily blighted. An unprotected (not sprayed with fungicides) resistant cultivar is grown in field 2. Theoretically, up to 4×10^{12} *P. infestans* spores can be produced per ha of potato. These billions of spores are produced on the infected (susceptible) cultivar, transported through the atmosphere and deposited (in part) on the neighboring fields (Skelsey *et al.*, 2008), including field 2. Even with a very low mutation frequency it is likely that the natural variation of effector genes results in a few individuals (sporangia) that are virulent on the R-gene "planted" in field 2. If the spores produced in field 1 are deposited on the unprotected resistant cultivar in field 2, virulent genotypes are selected, with a late blight epidemic and a broken R gene as the result.

R-gene deployment strategies

As a logical consequence of the above, we should look into measures capable of enhancing the durability of host plant resistance. For future growing systems it is therefore recommendable to use a combination of multiple (stacked) host resistance genes with a low input protective spray strategy to protect the investment into the creation of host plant resistance and to benefit rentability of the crop and the environment. Also, effort must be put in preventing situations where massive amounts of *P. infestans* spores are allowed to be produced in order to minimize the number of undesired mutations.

CONCLUSIONS

Virulence for new *R* genes (*Rpi-blb1* and *Rpi-blb2*) was found in the Dutch *P. infestans* population. Multigenic resistance is more durable than resistance based on single genes but it must be assumed R-gene based resistance can be broken by *P. infestans*. Additional control measures are therefore needed to significantly enhance the durability of host plant resistance. These additional measures aim to prevent development of large *P. infestans* populations and aim to prevent selection of virulent *P. infestans* genotypes on resistant cultivars by applying (low input) fungicide applications during periods with high infection pressure. The combination of these additional control measures with already established control methods should be able to minimize the risk for R gene break through and minimize the consequences of R-gene break through once it has occurred thus enhancing the durability of newly introduced host plant resistance.

ACKNOWLEDGEMENTS

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The phenotypic characteristics of Estonian populations of *Phytophthora infestans* from organic and conventional potato crops

E. RUNNO-PAURSON[1,2]

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences,
Kreutzwaldi 1, Tartu 51014, Estonia
[2]Department of Biochemistry and Plant Protection, Jõgeva Plant Breeding Institute,
Jõgeva alevik 48309, Estonia

SUMMARY

A total of 196 isolates of *Phytophthora infestans* were collected from conventional and organic productions in northern Estonia at several potato fields during 2004-2005. Most of the isolates were tested for mating type, virulence and metalaxyl resistance. In Estonia, 41% of the 175 isolates tested were A2 mating type. All 11 virulence factors were found among the tested isolates. The mean number of virulence factors per isolate was 6.6, with a very low frequency of virulence against resistance gene R9 (2%). The most common race was 1.3.4.7.10.11, representing altogether almost a half (49%) of the studied strains. The results indicated several differences between cropping systems in the population structure of *P. infestans*. Complex races were found to be typical for organic farms, and there was also a higher extent of A2 mating type in organic fields than in other potato productions. Resistance to metalaxyl was most common in large scale conventional fields. Such differences can have important implications for determining the optimal strategies in potato late blight management.

KEYWORDS

Phytophtora infestans, crop systems, mating type, metalaxyl resistance, virulence

INTRODUCTION

Potato late blight, caused by the oomycete *Phytophthora infestans*, is one of the most devastating diseases of potato worldwide. It is an ongoing threat to potato growers in temperate regions, requiring vigilance and often numerous applications of fungicide for effective control (Cooke *et al.,* 2003). In Estonia, it is not possible to achieve high yield with good quality in conventional potato production without using fungicides to control the late blight pathogen (Koppel, 1997). In organic fields, where mostly varieties with high resistance are used, yield loss may reach 50% (Runno-Paurson *et al.,* unpublished data). Copper based fungicides, which are used in organic production systems in Europe, are prohibited in Estonia.

In Estonia, the A2 mating type was first found in 1987. Data from 2002-2003 indicated the presence of both mating types at most study sites, suggesting the occurrence of sexual reproduction in Estonian populations (Runno-Paurson *et al.,* 2009). In such a situation, management of the new sexually reproducing populations is a challenge for conventional production and can be crucial for the economy of organic potato producers (Hannukkala & Lehtinen, 2005). The number of organic farms in Estonia has increased since the early 1990s, notably since 2002. About 10 percent of all cultivated land is used for potato production. However, organic farms in Estonia are varied; for example, many of them do not rotate crops and the seed potatoes they use are often not certified. More importantly, with the prohibition of fungicide use, organic farms have a higher risk of late blight epidemics and consequent yield loss than conventional fields.

The main objective of this study was to compare the population structure of *P. infestans* in organic and conventional productions in Estonia. It was postulated that *P. infestans* populations in organic production may differ in their resistance to fungicides or the diversity of certain phenotypic traits from those in conventional production. The results of this study can be compared with the populations in other regions of Estonia and other European countries to get a larger picture of the spatiotemporal variation in the population structure of this pathogen.

MATERIALS AND METHODS

In two consecutive years, 2004 and 2005, 196 isolates of *P. infestans* were collected from twelve potato fields (4 organic, 4 small scale conventional and 4 large scale conventional production) in northern Estonia. The small and large scale conventional farms sampled differed in their use of agrotechnical methods. In the small scale conventional farms, farmers used seed potatoes of uncertain quality and did not practise good crop rotation. Fungicides were applied only once per growing season. In the large scale conventional farms, farmers used high-quality certified potato seed, adhered to the recommended crop rotation and made at least 6-7 treatments against potato late blight per season. Copper based fungicides are not used in Estonian organic production.

Nine to twenty-three leaflets, each with a single lesion (one per plant) were collected in organic and small scale farms twice in each year: at the beginning of the outbreak and at the end of the growing season (an approximately equal number of isolates was taken early and late in the season). In the early stages of the outbreak, approximately 20-25% of leaf area of the infected plants and less than 10% of plants were infected with late blight. In the later stages, about 20-40% of the leaf area and more than 50% of the plants were infected. On the large scale farms, samples were collected at the beginning of the outbreak. The plants were selected by randomising the distance from field edges, and from each plant the blighted leaf was also randomly chosen, excluding those that had several or no lesions.

Isolations were carried out like described by Runno-Paurson *et al.,* 2009. Mating types were determined by the method described in Runno-Paurson *et al.,* 2009 and Runno-Paurson *et al.,* 2010. The resistance to metalaxyl of all 70 isolates was tested using a modification of the floating leaflet method (Hermansen *et al.,* 2000) as described in Runno-Paurson *et al.,* 2009. The specific virulence of each of the 196 isolates was determined using Black's differential set of potato genotypes containing resistance genes R1-R11 (Malcolmson & Black, 1966) (provided by Scottish Agricultural Science Agency). Laboratory procedures were performed as described in Runno-Paurson *et al.,* 2009.

RESULTS AND DISCUSSION

Mating type determination

Among the 175 tested isolates, 57% were A1 mating type, 41% were A2 mating type and 2% were self-fertile. Both A1 and A2 mating types were detected from 11 of the 12 fields. The proportion of the A2 mating type in the isolates sampled in 2004 was lower than those sampled in 2005 (28% resp. 54%). There were further differences between cropping systems, the proportion of A2 being highest in organic fields and lowest in large scale conventional fields (Figure 1).
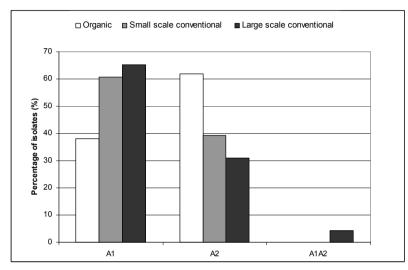
Figure 1. *Percentages of mating types among isolates of Phytophthora infestans from different cropping systems in Estonia (2004-2005).*

Nevertheless, the average proportion of A2 mating type found in this study (41%) was consistent with the results of a previous study conducted in Estonia (Runno-Paurson *et al.*, 2009; Runno-Paurson *et al.*, 2010). The presence of both mating types in the same field indicates the possibility of oospore production in potato foliage (Turkensteen *et al.*, 2000). In this study, both mating types were detected at nearly all sites (92% of studied fields), with a single exception of an organic field in 2004. This percentage is based on just twelve fields, but it is supported by previous studies, conducted in 2002-2003 and 2004-2007, based on 32 and 28 fields, respectively (Runno-Paurson *et al.*, 2009; Runno-Paurson *et al.*, 2010), where the two mating types co-occurred in 88% of the fields. Similar frequencies of co-occurrence of the mating types have been reported from Germany (Bouws and Finckh, 2007) where two mating types co-existed in 60-92% of the sites, and frequencies as high as 29-56 % have been found in Nordic countries (Lehtinen *et al.*, 2008). However, it is possible that the differences between studies arise from different numbers of isolates studied per field, rather than true differences in population composition, as the probability of detecting both mating types depends on sample size. This study did not support the previous findings that the co-occurrence of both mating types is more common in organic fields, as has been reported from Finland (Lehtinen *et al.*, 2007), southern Flevoland in the Netherlands (Zwankhuizen *et al.*, 2000) and Scotland (Cooke *et al.*, 2003).

However, based on our results, differences in the A1/A2 ratio between cropping systems can be suggested, even though larger sample sizes are needed to explicitly prove this finding. For instance, in the organic fields, 62% of isolates were A2 mating type whereas in the large scale conventional

farm fields only 31% of isolates were A2 mating type. The possibly higher prevalence of A2 mating type, both mating types found from most fields, and no rotation may presume higher risk for sexual reproduction in the organic fields than in the other cropping systems. Organic fields were also more severely infected than conventional crops, even though less susceptible potato varieties were used. The main reason for this is probably the lack of fungicide use in organic fields; however, an additional risk factor may be an increased oospore production, which reduces the effect of crop rotation if it is not performed sufficiently frequently (Lehtinen *et al.*, 2007).

Resistance to metalaxyl
In total, 110 isolates were screened for resistance to metalaxyl. In the two years, 49% of the isolates were resistant to metalaxyl, 34% were intermediate and 17% were classified as sensitive. Of the metalaxyl resistant strains, 65% were A1 mating type, 30% were A2 mating type and 5% were self-fertile; however, the association between metalaxyl resistance and mating type was not significant.

Considerable differences between potato cropping systems were observed. In particular, in the large scale conventional fields, 66% of the tested isolates were resistant to metalxyl, while in the small scale farm fields 26% and in the organic fields only 14% of the isolates were resistant. There were no differences between years; however, when compared to the data collected in 2002-2003 (Runno-Paurson *et al.*, 2009) the prevalence of metalaxyl resistant isolates had increased from 30 to 49% .

Further differences between cropping systems were evident in the resistance of isolates to metalaxyl fungicides. Metalaxyl resistant isolates were found four times more often in the large scale conventional fields than in the organic fields. This difference could be explained by the use of metalaxyl products in the large scale conventional fields, even though no significant differences were detected between the large scale conventional fields treated and not treated with metalaxyl (statistics not shown).

Virulence
All known virulence factors (to overcome genes R1-R11) were found among tested isolates. Nearly all isolates were virulent on differentials with genotypes R1, R3, R4, R7, R10 and R11. Virulence factor 9 (1%) was rare and factors 5 (10%) and 8 (10%) were relatively rare. A difference in the prevalence of virulence factors 2, 5, 8, and 9 was observed between the two sampling years. The three rarest virulence factors in Estonia, R5, R8 and R9, only appeared in the large scale conventional fields, while virulence factors R2 and R6 with relatively low frequencies were more prevalent in the organic fields than in the other cropping systems (Figure 2).

Thirty-eight races were detected. The two most common races made up 70% of the isolates tested. The overall virulence complexity (average number of R-genes overcome) was 6.7. Virulence complexity was highest in the organic farms. Complex races predominated in the organic fields, but were less common in the small and the large scale conventional fields.

The Estonian population of *P. infestans* is most similar in the frequency of virulence factors to those described recently in Nordic countries (Hermansen *et al.*, 2000; Lehtinen *et al.*, 2007; Hermansen *et al.*, 2008), France and Switzerland (Leberton & Andrivon, 1998; Knapova & Gisi, 2002; Pilet *et al.*, 2005). The mean number of virulence factors found in Estonia (6.6) has remained at approximately the same level as in previous years (Runno-Paurson *et al.*, 2009; Runno-Paurson *et al.*, 2010); similar values were also found in Denmark (6.92) and Sweden (6.87) (Lehtinen *et al.*, 2008) in 2003.
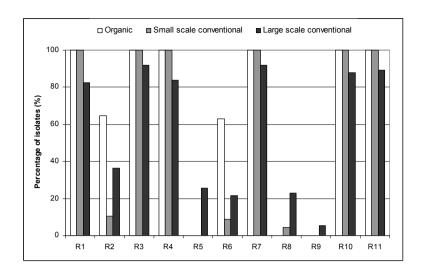
Figure 2. *Frequencies of specific compatibility (virulence) to potato R-genes in isolates of Phytophthora infestans among different crop productions in Estonia (2004-2005).*

Race diversity calculated by the normalized Shannon diversity index showed a much lower value (0.38) in this study compared to the very high diversity among isolates collected from Estonia in 2002 to 2003 (0.89, Runno-Paurson, *et al.*, 2009). As a comparison, in a sample of 432 isolates collected in 2004-2007, pathogen diversity was still relatively low (0.54) (Runno-Paurson *et al.*, 2010). Interestingly, even though lower values of diversity have been found in newer studies, the average virulence complexity was relatively high. The diversity index was much higher among isolates collected from large scale conventional fields. This result is particularly surprising because, unlike smaller farms, the large scale farms used certified potato seed tubers and practiced rotation. The reason for this may lie with the seed source used in those farms. Large scale farms grow potato varieties imported directly from western Europe, mostly from the Netherlands, where the local populations have highly complex virulence spectra (8-10 virulence factors per isolate) and the proportion of A2 is extremely high (Van Raaij *et al.*, 2008). Large quantities of seed potato are also imported from Germany and Denmark. The mean number of virulences per tested isolate was found to be 6.9 in Denmark and 6.2 in Germany, and the frequency of A2 mating type was over 50% in Denmark and 13-46% in Germany, with both mating types co-existing in 76% of the fields, on average (Bouws & Finckh, 2007; Lehtinen *et al.*, 2008). It is therefore likely that the higher diversity of the *P. infestans* populations in large scale farms is caused by mixing local genotypes with strains imported from other, highly diverse populations.

CONCLUSIONS

The results of this study clearly suggest that there may be cropping system-specific differences in the population structure of *P. infestans*, which most probably arise from different management practices in these systems. Such differences can likely lead to variation in the risk of yield loss. In contrast to the previous assumptions, several aspects of pathogen diversity, such as genotypic diversity, race complexity appeared to be highest in the large conventional fields. On the other hand, the proportion of the novel A2 mating type and virulence complexity were highest in the organic fields. The prevalence of metalaxyl resistance was also highest in the large conventional fields. Such differences should not be ignored by producers, and different precautions can be suggested

for managing different types of farms. In particular, conventional farmers may benefit from the use of other control methods beside metalaxyl fungicides to limit the spread of resistance in the pathogen population. The spatiotemporal variation observed in *P. infestans* population parameters across Europe may imply that managers also need to consider the regional situation to make optimal decisions. However, it would certainly be desirable to repeat these comparisons in further studies incorporating a larger number of fields to confirm more rigorously the differences between management practices. Importantly, the separate effects of crop rotation, chemical control, seed source and host resistance need to be addressed.

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Use of Geographic Information Systems (GIS) in Crop Protection Warning Service

BENNO KLEINHENZ AND THORSTEN ZEUNER

ZEPP – Central Institution for Decision Support Systems in Crop Protection, Rüdesheimerstr. 60-68, D-55545 Bad Kreuznach, Germany, E-mail: kleinhenz@zepp.info

SUMMARY

One of the important aims of the Governmental Crop Protection Services (GCPS) in Germany is to reduce spraying intensity and to guarantee an environmentally friendly and economical crop protection strategy. ZEPP is the central institution in Germany responsible for the development of methods in order to give an optimal control of plant diseases and pests. So far more than 40 met. data-based models were developed, most of which are introduced into practice. This study shows how to obtain results with higher accuracy for disease and pest simulation models by using Geographic Information Systems (GIS). The influence of elevation, slope and aspect on met. data were interpolated with GIS methods and the results were used as input for simulation models. The output of these models will be presented as spatial risk maps in which areas of maximum risk of the disease are displayed. The modern presentation methods of GIS will furthermore promote the use of the system by farmers.

KEYWORDS

Interpolation, GIS, temperature, relative humidity, risk maps, plant disease model

INTRODUCTION

During the last 40 years a number of weather based forecasting models have been developed for the control of plant diseases and pest attacks (KLEINHENZ AND JÖRG 2000). Several forecasting models have been established and introduced into practice to support the decisions in the control of diseases in Germany (KLUGE and GUTSCHE 1984; GUTSCHE 1999; GUTSCHE et al. 1999; KLEINHENZ and JÖRG 1999, KLEINHENZ and JÖRG 2000; ROSSBERG et al. 2001; HANSEN et al. 2002). In some agricultural areas, however, the distance between met. stations is more than 60 km. Thus forecasting models did not give satisfying results for plots located at such large distances to met. stations (ZEUNER 2007).

With the help of Geographic Information Systems (GIS) a plot specific classification of met. data will be calculated. To reach this aim, complex statistical interpolation methods are used (ZEUNER 2007). These calculated spatial input parameters for the current disease forecasting models should help to get satisfying forecasting results for areas between two or more distant met. stations. With the use of GIS, daily spatial risk maps will be created in which the spatial and the temporal process of first appearance

and regional development are documented. These risk maps may lead to a reduction of fungicide intensity and give best control.

In this study the new method to calculate the input parameters for forecasting models with GIS is validated on the first appearance of potato Late Blight. The models SIMBLIGHT1 and SIMPHYT1 predict the first appearance for Late Blight and are in practical use (KLEINHENZ *et al.* 2007). While SIMPHYT1 depends on a statistical approach forecast, the result of SIMBLIGHT1 basis on the current infection pressure and could be displayed in three classes (Figure 1). The results will be presented as spatial maps and graphs showing the Late Blight risk. Figure 1 and 2 show the difference between the current and the new risk maps in Germany as simulated by the model SIMBLIGHT1. SIMBLIGHT1 calculates infection risk of Late Blight is shown in three infection classes symbolized by different colours. Risk maps will be implemented into an internet application to provide a comfortable access to the system for farmers and advisers.

Figure 1: *Current SIMBLIGHT1 presentation. The prognosis results are shown at the met. station's location with coloured cloud symbols.*

Figure 2: *The new presentation of a spatial risk map for Late Blight as simulated by SIMBLIGHT1.*

MATERIAL AND METHODS

Workflow

The following steps have to be taken to reach the aim of building spatial risk maps:

    Step 1: data management
    Step 2: interpolation of met. data
    Step 3: calculation of the forecasting model using the results of the interpolation
    Step 4: display of the results as a risk map

Step 1 deals with data management. First hourly met. data are imported from a weather database which are necessary for the forecasting models SIMPHYT1, SIMPHYT3 and SIMBLIGHT1. Then a

geographic reference is providing to the met. data because the weather database is not a georeferenced. Thereafter that it is necessary to prepare the data base which is needed to characterise the met. data for interpolation. Step 2 is the main and the most difficult step. Different kinds of interpolation methods are compared to identify a method which gives the best results in interpolating met. data. Step 3 uses the interpolated met. data as input parameters to calculate the forecasting models (Figure 3). The last step is to connect the results to an internet application in which spatial information is displayed as a risk map of the first appearance and later on the daily infection risk of Late Blight for Germany.
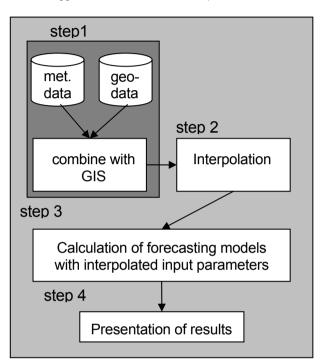
Figure 3: *Infrastructure to calculate risk maps.*

Data base
Met. data
The met. data are collected by 570 automatic met. stations all over Germany (Figure 1), operated by the German Meteorological Service (DWD) and by the GCPS. The stations are at least equipped with sensors for measuring temperature, relative humidity, precipitation and global radiation. All data are tested of plausibility and stored in a database called AGMEDAWIN (Keil and Kleinhenz 2007).

Geodata
A digital elevation model (dem) published by Behrens and Scholten (2002) was used to obtain all necessary information about the relief. The dem describes the landscape as a three-dimensional grid. It represents the earth's surface through digitally stored x, y, z values, where the x and y values specify the horizontal position and z-value of the vertical height of the grid cell (Bill 1999). With the help of mathematical, statistical methods, it is possible to calculate follow products from the dem. These derivatives are, for example, slope, slope direction, slope, or slope edges. The dem and various derivations from the dem provide as basis for the characterization of the meteorological parameters.

Spatial join

In order to store the results of interpolation, a grid was laid out over Germany. At present, the GCPS uses about 570 met. stations to represent agriculturally used area of Germany (some 200.000 km^2), that is on average one met. station per 350 km^2. With the new GIS method, a grid cell has a size of one km^2 and after interpolation is represented by a virtual met. station (Liebig and Mummenthey 2002).

Interpolation method

Two groups of methods have been tested to find the best interpolation method for met. data. First, there are the *deterministic interpolation methods,* e.g. Inverse Distance Weighted (IDW, "nearest neighbour method") and Spline Interpolation (SI) which are based on distance analyses. These methods were compared to *geostatistical interpolation methods* like Kriging and Multiple Regression (MR) which use mathematical and statistical procedures.

MR is an interpolation method that allows simultaneous testing and modelling of multiple independent variables. MR is a highly general and therefore very flexible data analysis system. It is used whenever a quantitative variable, the dependent variable, is to be studied as a function of, or in relation to, any factors of interest, i.e. the independent variables (Javis *et al.* 2002, Cohen *et al.* 2003). So parameters that have an influence on temperature and relative humidity, e.g. elevation, slope, aspect, can be tested simultaneously. MR uses matrix multiplication and only variables with a defined minimum influence will be included into the model. The result of MR is a formula (x = const + A_1*$const_1$ + A_2*$const_2$+ A_3*$const_3$+…+ A_x*const) which allows to calculate a parameter set for each grid cell from which independent variables are known (Javis 2002; Zeuner 2007, Mense-Stefan 2005).

RESULTS AND DISCUSSION

Interpolation of temperature and relative humidity

The first calculations with the four interpolation methods showed that deterministic interpolation methods were not suitable. IDW and SI have been rejected due to the fact that differences in elevation are not accounted for, because the elevation has been identified as a major factor for interpolation of considered meteorological parameters. Although producing similarly good results, the method Kriging was not able to produce as fast calculation times as compared to MR. So it was also rejected because the performance is also very important to produce daily risk maps in the internet. So method MR was chosen and the results are shown in the following.

To validate the results of the interpolation, 13 met. stations were not used in the interpolation process. After that the deviation between calculated values and measured data of these stations was compared. The period of time of this study was from January to August in the years 2003 to 2006. For all stations, MR was able to calculate results with highest accuracy (table 1). In all cases considered, the coefficient of determination (CoD) ranged between 96 and 99% for temperature and 92 and 96% for relative humidity. For all 13 met. stations, the mean deviation for temperature was less than 0.1°C and for relative humidity less than 0.6 % as calculated with MR. The absolute maximum and minimum for the temperature was less than 4.7°C and for the relative humidity less than 32.6 %. In addition, the data have been tested on significance between calculated and measured data using a t-test. The test indicated that for all stations the differences between the calculated and measured values were at random. The method MR gave plausible results, so this method was chosen to interpolate met. data to be used as input for the forecasting models.

Table 1: *Deviation between calculated values and measured data with MR*

year	2003	2004	2005	2006	2003	2004	2005	2006
parameter	temperature [°C]				relative humidity [%]			
CoD	96%	96%	99%	98%	94%	96%	95%	92%
mean deviation	0.0	0.0	0.0	0.1	0.3	0.1	0.1	-0.6
maximum	4.4	4.1	4.3	4.7	19.6	32.6	21.6	21.2
minimum	-3.8	-4.5	-4.5	-4.1	-18.9	-21.9	-22.8	-22.8
t-test	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant   n = 92.160 hours/year

Forecasting models
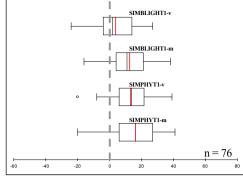
Results of the forecasting models SIMBLIGHT1 and SIMPHYT1 running on calculated met. data were validated against a set of field data collected between 2000 and 2007 in Germany. A model result was defined as correct when the date of the first appearance given by one of the models was earlier than the date of the first outbreak observed in field. In figures 4 to 8 the results of this study is displayed in box-whisker-plots. The results of the model with interpolated input are denoted with "a –v" and those with measured input with "a –m".

More than 90 % of all calculations have been classified as correct whereas in 2002 less than 60 % correct calculations were made. This is due to a high amount of precipitation during the spring and summer months in 2002. The various data sets yielded a similar percentage of correct results.

In all years, the mean deviation of the model results with –v were gave better results with respect to the first appearance of Late Blight found in field compared to results with –m. For example in the year 2001 the mean results of SIMBLIGHT1 -v showed a five to eight days higher accuracy than the calculations based on data measured by a distant met. station. In all other years the results for mean deviation were similar.
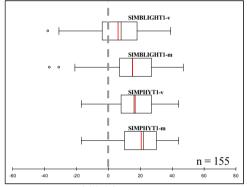
The largest differences between the minimum and maximum deviation (range) were shown by SIMBLIGHT1 in 2002. The range of SIMBLIGHT1-m exceeded that of SIMBLIGHT1-v by more than 30 days. In all other years and also with the model SIMPHYT1, the range of results with –v was 5 to 20 days less compared to –m. The results show that calculations based on interpolated data have a higher accuracy in comparison to field data for Late Blight because of their spatial index. Through this a detailed definition of the first treatment is possible, this effects in high efficiency.

To use other forecasting models, more meteorological input data play an important role. So it is necessary to analyse if MR is also able to calculate parameters such as soil temperature, leaf wetness or precipitation with high accuracy. With soil temperature this method seems to be successful. But for leaf wetness and precipitation it is not useful because of regional variations of precipitation especially in the summer months. For these parameters, other sources have to be found, so e.g. radar measurement of the DWD could be used to classify precipitation.

Figure 4: Box-Whisker-Plots of differences between the first appearances of late blight in the field and the model result of SIMBLIGHT1-v-m and SIMPHYT1-v-m in Germany in 2001.

Figure 5: Box-Whisker-Plots of differences between the first appearances of late blight in the field and the model result of SIMBLIGHT1-v-m and SIMPHYT1-v-m in Germany in 2002.

Figure 6: Box-Whisker-Plots of differences between the first appearances of late blight in the field and the model result of SIMBLIGHT1-v-m and SIMPHYT1-v-m in Germany in 2004.

Figure 7: Box-Whisker-Plots of differences between the first appearances of late blight in the field and the model result of SIMBLIGHT1-v-m and SIMPHYT1-v-m in Germany in 2005.

Figure 8: Box-Whisker-Plots of differences between the first appearances of late blight in the field and the model result of SIMBLIGHT1-v-m and SIMPHYT1-v-m in Germany in 2006.

CONCLUSIONS

By the combination of forecasting models for plant diseases and the analyses and interpolation methods based on GIS, a significant advance in advice to farmers can be realized. GIS methods will help to obtain more detailed calculations and results with higher accuracy and validity than before. Spatial maps will show hot spots of maximum risk which will make the results of forecasting models easier to understand and to interpret. This gets the decision support a step closer to the aim of a reduced pesticide use and an economical and environmental friendly crop protection strategy.

The results and methods of this study will initiate the introduction of risk maps in crop protection warning service. The internet platform www.isip.de is currently implementing a web GIS application to make use of the new methods. The new components will comply with all relevant standards (OGC, INSPIRE) to ensure interoperability with other geoservices. 'Improving decision support in plant production with GIS', this conference). GIS presentation methods will make DSS results easier to understand and will lead to a higher acceptance of warning systems by farmers.

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EuroBlight tool for the comparison of late blight sub-models - Status and perspectives

[1]HANSEN JG, [2]KESSEL G, [3]NÆRSTAD R, [2]SCHEPERS H, [4]NIELSEN BJ & [1]LASSEN P

[1]Aarhus University, Dept. of Agroecology and Environment, Denmark
[2]Wageningen University Research,
Plant Research International & Applied Plant Research, the Netherlands
[3]Norwegian Institute for Agricultural and Environmental Research, Norway
[4]Aarhus University, Dept. of Integrated Pest management, Denmark

SUMMARY

Partners from the EuroBlight network, with support from ENDURE, created a freely available platform that allows testing and comparing weather-based late blight models (www.euroblight.net). The platform contains extensive weather data: hourly data from many European Union countries, both north and south, between 2006 and 2009. It also contains seven different weather based late blight sub-models. Most recently, biological data for verification were uploaded from monitoring of field experiments and potato fields around Europe. The results from different models for disease risk or, infection risk give similar but by no means identical results. The tool is intended to improve the quality of existing sub-models and it will be used to analyse the weather based risk of late blight development in different regions of Europe and beyond.

KEYWORDS:

DSS, weather data, infection risk, EuroBlight, sub-models

INTRODUCTION

Weather based sub-models are key components in many decision support systems aiming at controlling pests and diseases and minimizing pesticide use. The evolving biology of pest and diseases in combination with changing climates calls for continuous testing and improvement of existing and new weather based sub-models. In the EU.NET.ICP project several DSS's were tested in field trials in Europe (Hansen *et al.*, 2002; Hansen *et al.,* 2001; Dowley *et al.*, 2005). It was concluded that: It was not possible to build one European DSS, it was difficult to compare whole systems in the trials and it was recommended to do comparisons on sub-model level. The ENDURE NoE supported the development of such a generic modelling platform now implemented on the EuroBlight research platform (www.euroblight.net) and introduced in this article.

METHODS

A generic modelling platform was developed for the comparison and test of weather based late blight sub-models. An interactive web page in www.euroblight.net allows users to select the location, (period of the) year and the sub-models for comparison (fig. 1). It is possible to select the number of sub-models to be included and also the RH Threshold (85, 88 or 90%) to be used in the model calculations. Finally it is possible to add biological data to the graphics if available. Primary weather data, model calculations for Infection pressure, WURCP and SMITH Criteria as calculated for Lelystad (NL), 2009 is given in fig. 1. For model description see Table 1.
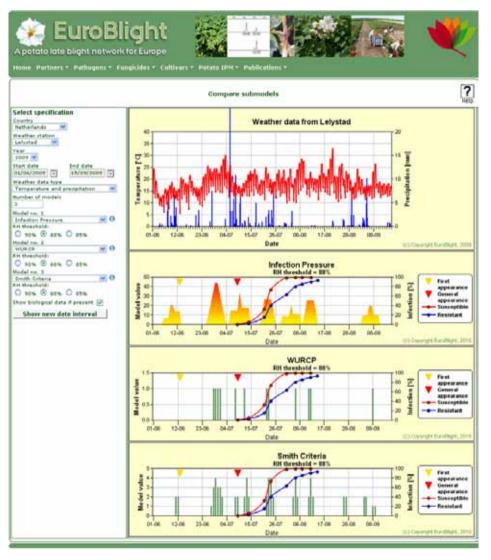
Figure 1. *Interactive Graphic Analysis Tool in EuroBlight. Left: Interactive selections of country, station, start and end date, number of models to show and settings of parameters i.e. RH threshold used in models. Right, from top: Hourly temperature and precipitation and the blight risk according to Infection pressure, WURCP and SMITH Criteria at Lelystad, 2009. The sub-models are described in Table 1.*

The following data were sampled:

Weather data: hourly data for temperature, relative humidity and rain from 15 EU countries, 2006-2009.

Sub-models output: PLANT-Plus disease risk, ProPhy disease pressure, Blight Management infection pressure, Blight Watch, Smith criteria, WURCP infection event, Contact Fungicide degradation. For PLANT Plus and ProPhy, the owner of these models, DACOM and AGROVISION, calculated results and only model outputs were stored in the database. All remaining models were reprogrammed on the platform.

Biological data: Surveillance data as "Date for first infection in early or covered potatoes" and "Date when 5 or more conventional fields were infected". Data for disease progress curves in susceptible and moderate resistant cultivars were collated from field experiments.
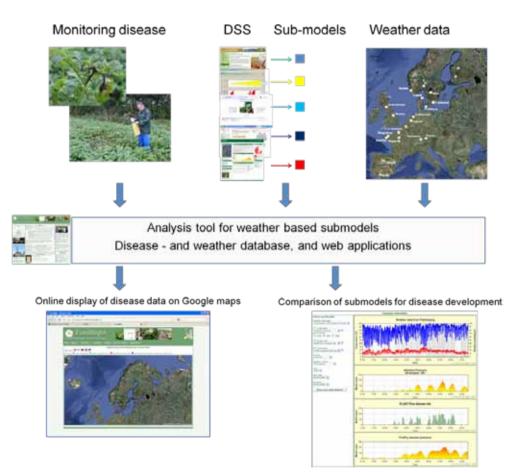
Figure 2. *Framework and dataflow on the blight risk sub-models test and development platform in EuroBlight. Three weather based sub-models are used for demonstration and calculations in this article and the methods are described briefly in Table 1. Other models and methods are presented on www.euroblight.net*

Table 1. *Description of sub-models presented and used in this article.*
Further information at www.euroblight.net.

Sub-model name	Description
WURCP **Contact:** Geert Kessel	WURCP calculates critical periods, i.e. days with a very high risk of infection of your crop. WURCP assumes the presence of latently infected tissue in the surroundings of, but outside your field. For a critical period to occur, three sub-processes of the infection cycle have to be fully completed in sequence: formation of sporangia, dispersal of sporangia and infection. The algorithms calculating development rates for each of these processes are based on Crosier 1934.
Infection pressure **Contact:** Jens G. Hansen	The infection pressure is a running sum of sporulation hours during a 5-day window including current date, 2-day weather forecast and two days of historic weather. Sporulation hours is defined as number of hours in periods of 10 or more hours when RH>88% and temperature at the same time is between 10°C and 24°C. HSPO is 5, if there is 10 consecutive hours of RH>88% and the temperature in 5 of those humid hours are above 10°C. During a high infection pressure it is expected that there is risk of both sporulation and infection. Infection pressure: < 20 is low; 20 - 40 moderate and > 40 is high.
Smith criteria **Contact:** Ian Barrie	A full Smith Period has occurred if, on each of 2 consecutive days: i) the minimum air temperature was at least 10 °C, and, ii) there were a minimum of 11 hours with a relative humidity of at least 90% .Within the calculation there is a provision for a 'near miss'. This occurs when the temperature criterion has been satisfied but the number of hours with a high relative humidity totalled only 10 hours on one or both days. Smith Criteria is calculated on a daily basis (10:00 previous day to 09:00 current day) and then calculated the running sum for two days (current day + previous day). This means that the Smith criteria index ranges between 1 and 4, corresponding to 1, one day near miss, 2, one day Smith or two days with a near miss, 3, a Smith day and a near miss; and finally 4, a full Smith.

Seventeen stations were used in the analysis of seasonal blight risk and the comparison of blight risk, 2006-2009 (Fig 3 and 4): St Eloy, FR, Tylstrup and Flakkebjerg, DK, Lindloh and Mathau, DE Valthermond and Lelystad, NL, Belfast, NI, Reckenholz and Payerne, CH, Dundee, SC, Ås and Særheim, NO, Skara, SE, Bonin, PL and Capofiume and Riposto, IT.

RESULTS
During this preliminary phase of analysing data we found some general results:

- Results from different models for disease risk, or, infection risk give similar but by no means identical results (not published). These differences will be analysed in a European context aiming at improving crop protection strategies in different regions of Europe.
- There was a good correspondence between calculation of blight weather with indices like infection pressure, WURCP and Smith Criteria and biological data sampled in surveillance networks and from field trials. Example: In 2008, late blight appeared one month earlier in the Central and Western part of Europe than in North and East Europe. This difference is very well indicated in the calculation of Infection pressure in the same regions of Europe (Figure 5).
- Comparing the years 2006-2009 and using WURCP as a blight risk indicator show, that 2007 was calculated with the highest and 2009 with the lowest seasonal blight risk as a mean of 17 selected weather stations in Europe (fig 3.)

- The year 2006 was characterized by low weather based risk of blight in the first part of season and a very high risk in August and September compared to the other years. The year 2007 was characterized by a relatively high risk in June and July and medium risk in August and September. "2007 was the worst year for foliar blight in Scotland for decades. One explanation for the severe blight in 2007 is the concentration of Smith Periods early in the growing season." (Hansen *et al.* 2009)(fig. 4)

More biological data are needed to allow a solid validation of the models as the core component behind spray decisions.
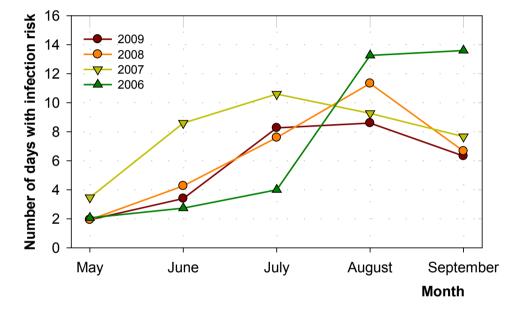
Figure 3. *The number of days with infection risk (according to WURCP) by month for 2006-2009, average of 17 weather stations in Europe.*
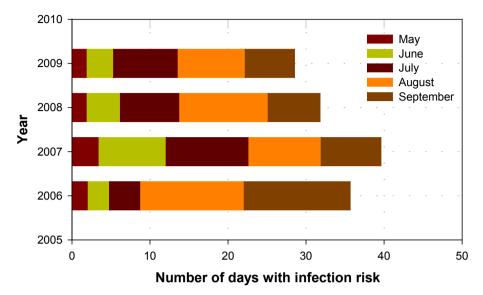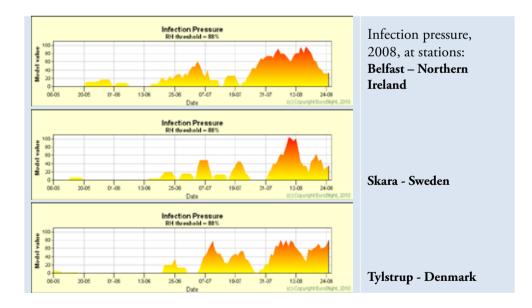
Figure 4. *The number of days with infection risk (according to WURCP) by year for 2006-2009, average of 17 weather stations in Europe.*

Infection pressure, 2008, at stations:
Belfast – Northern Ireland

Skara - Sweden

Tylstrup - Denmark
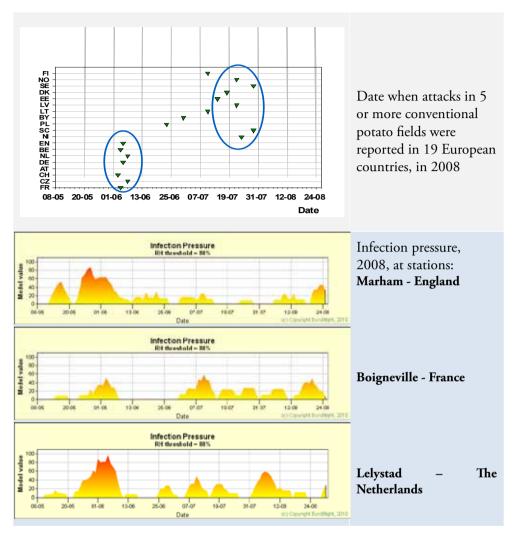
Figure 5. *Infection pressure at selected stations compared with biological data as "Date when 5 or more conventional fields were infected" at country level.*

DISCUSSION AND CONCLUSIONS

The new online test and development platform for blight models allows any user to compare for themselves the weather based sub-models currently available on the platform. By uploading biological data and weather data, and then comparing the output from all models available it is possible to identify the regional risk for late blight as well as which of the methods might be useful for use in a DSS adapted to regional conditions.

The next step will be to compare the sub-models in more detail and secondly to include biological data from more regions in Europe. More weather based sub-models will be added to the platform i.e. a tuber blight sub-model, and simple DSSs can be constructed using the models in the toolbox, which

is aimed at integrating and improving both existing and new DSS in Europe. Future possibilities include developing this platform for other pathosystems and using the platform to analyse the impact of climate change on late blight control in Europe.

We propose that calculations of blight weather from weather stations in 2-3- important potato regions per country should replace the one national figure for weather based blight risk used so far in the country reports (Hansen *et al.*, 2009).

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Opportunities for potato late blight DSS's in Argentina

G. KESSEL[1], M. HUARTE[2], F. LUCCA[2], M. SANTINI[3], C. RIJZEBOL[4], P. RAATJES[4], J. ROVERS[5], J. DEN BOER[5], AND H. SCHEPERS[6]

[1] Wageningen University and Research Centre, Plant Research International, Biointeractions and Plant Health, P.O. Box 69, 6700 AB Wageningen, The Netherlands.
[2] El Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Balcarce, Ruta Nacional 226, km 73.5, (7620) Balcarce, Argentina.
[3] Farm Frites, Av. Alexander Fleming 2238, B1606DAB, Munro, province of Buenos Aires, Argentina.
[4] Dacom B.V., P.O.Box 2243, NL-7801 CE, Emmen, the Netherlands.
[5] Farm Frites International B.V., Molendijk 108, 3227 CD, Oudenhoorn, the Netherlands.
[6] Wageningen University and Research Centre, Applied Plant Research, P.O. Box 430, 8200 AK Lelystad, The Netherlands.

KEYWORDS
Potato late blight, Argentina, disease management, decision support

INTRODUCTION
Continental Argentina stretches from just North of the Tropic of Capricorn to near the Antarctic Peninsula, resulting in a wide range of available climates. As the largest beef exporter in the world and the third largest producer of soybeans, Argentina has a very strong and significant agricultural sector. Almost 60% of Argentina's total exports are agriculture related. Currently, processing facilities are being updated to meet improved food quality and safety initiatives. Argentina has very good storage, distribution and logistic systems to facilitate the export oriented activities of the sector. Despite consumer hesitation surrounding genetically modified organisms, Argentina is a world leader in bio-technology. Soybeans, Argentina's most prominent commodity, are planted almost entirely with GMO seeds. In addition, approximately 90% of the corn acreage is grown using GMO seeds. At the other end of the spectrum, Argentina is also a strong producer of organic products, which are mostly destined for European markets.
Agriculture accounts for over 11% of Argentine GDP. Top agricultural field crops are soybeans, sunflower seeds, lemons, grapes, corn, tobacco, peanuts, tea and wheat. Top livestock production is in beef, poultry, dairy and some specialty meats such as llama, frog and iguana. Argentina's land base holds approximately 12% arable land, with less than 1% dedicated to permanent crops. Environmental concerns in Argentina include deforestation, soil degradation, desertification, and air and water pollution (1).

Potato is an important staple food and horticultural crop in Argentina. Although the potato area only covers 0,5% of the area covered by soybean, 0.08 mln ha versus 17 mln ha respectively, export of potato and potato products is growing whereas potato imports did not take place since 1984. The

total potato area of Argentina is around 77,000 ha with an average annual yield of around 35 t/ha. The processing industry transforms around 450,000 t of potatoes, of this quantity 70% is being exported. The technology level of potato production varies with the size of the growers potato area and the production goal. Potato production may reach high technology levels with large and medium size growers (from 150 ha up) producing potatoes for the processing industry although smaller growers producing potatoes for the fresh market still use a lot of hand labor, especially for harvest.
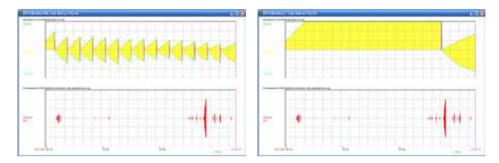
Fig 1. *Analysis of weather data from the 2009 - 2010 growing season in the Mendoza area assuming a weekly spray schedule (left panel) and a calculated optimal spray schedule (right panel). The lower half of the graphs indicate potato late blight infection risks whereas the upper half quantifies the unprotected foliage in the potato crop. Ideally, sprays are applied shortly before an infection event when a significant fraction of the foliage is unprotected.*

The main production areas can be found in the Provinces of Buenos Aires (Processing/Fresh, 26000 ha; November - March/May), Cordoba (Fresh/Processing, 36000 ha; August – November), Mendoza (Fresh/Processing, 7500 ha; November – March) and Tucumán (Fresh, 6000 ha June – October).

Bulk cold storage of ware potatoes is almost non existing considering that the country produces fresh potatoes all year round. Ware potatoes are therefore mostly stored on the fields for short periods prior to shipping for the market, with the exception of warm areas as Córdoba where some cold storage is used.

The Argentine potato sector may however benefit from the introduction of new technologies to boost yields and quality and to lower losses (1). The main crop protection problems are mostly shared between the growing areas and encompass thrips and related viral infections (Tomato Spotted Wilt Virus, TSWV), early blight (Alternaria spp.) and late blight (*Phytophthora infestans*). When possible and feasible, these threats are controlled with pesticide applications against the causal organisms. On the other hand, the Argentine potato sector benefits from absence of the Golden Nematode and an established commercial seed production system with post production quality control systems, in vitro multiplication and mini tuber production. Argentina has not imported seed for commercial production since 1984 with the exception of small quantities of new varieties.

Currently the first internet based decision support systems to control potato diseases have been introduced and the first farm managers are adapting from fixed (weekly) spray schedules to dynamic, DSS guided, spray schedules. Farm Frites and Dacom have introduced Dacom system in the Mendoza area and, following in the footsteps of an earlier DSS operational in the 1960's and 1970's, INTA and McCain are cooperating in the development of a potato late blight DSS for the South East region of the Buenos Aires Province (3, 4).

Mendoza's climate can be characterized as arid with Mediterranean influences. Average temperatures

for January (summer) are 32 °C during daytime, and 18.4 °C at night. In July (winter) average temperatures are 14.7 °C and 2.4 °C, day and night respectively. Mendoza's annual rainfall is only 223.2 mm. Intensive agriculture, including potato production, is made possible due to (continuous) irrigation. In contrast, Balcarce has a humid-temperate climate with an average annual temperature of 14°C and 890 mm average annual rainfall.

For the purpose of this paper, weather data from these two, climatologically very different, regions were analyzed to evaluate the opportunities and added value of decision support systems for potato late blight control in local potato production.
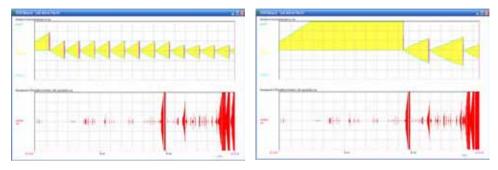
Fig 2. *Analysis of weather data from the 2007 - 2008 growing season in the Balcarce area assuming a weekly spray schedule (left panel) resulting in 13 applications and a calculated optimal spray schedule (right panel) resulting in 3 spray applications. The lower half of the graphs indicate potato late blight infection risks whereas the upper half quantifies the unprotected foliage in the potato crop.*
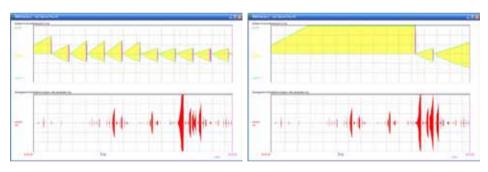
Fig 3. *Analysis of weather data from second half of the 2008 - 2009 growing season in the Balcarce area assuming a weekly spray schedule (left panel) and a calculated optimal spray schedule (right panel). The lower half of the graphs indicate potato late blight infection risks.*

RESULTS

Mendoza

Weather data were measured on farm during the 2009- 2010 growing season and analyzed for potato late blight infection risks using the Dacom system. As an approximation of the situation at San Fili farm, a weekly spray schedule (Figure 1, left panel) was assumed to provide reference data and an optimal spray schedule was generated by Dacom (Figure 1, right panel). The virtual weekly spray schedule for San Fili Farm resulted in 15 spray applications over the season whereas other growers in the same area would cover the season with around 10 applications. In contrast, the calculated (!)

optimal spray schedule suggests only one (1) spray application was needed. A difference of 9 - 14, potentially unnecessary, spray applications providing ample opportunity for DSS's to improve spray timing and efficacy while at the same time a reduction of production costs and emission to the environment can be achieved.

Balcarce

Weather data from the 2007 – 2008 (Figure 2) growing season and the second half of the 2008 – 2009 (Figure 3) growing season were also analyzed by Dacom for potato late blight infection risks. During the 2007 – 2008 growing season, a virtual weekly spray schedule would have resulted in 13 spray applications. Farmers in the Balcarce area generally cover the season with around 15 applications. The calculated (!) optimal spray schedule would have resulted in only three spray applications, a difference of 10, potentially unnecessary, spray applications. Also during the second half of the 2008 – 2009 growing season a weekly spray schedule would have resulted in around nine unnecessary spray applications: a weekly spray schedule during this period would have resulted in 11 applications whereas only 2 applications were necessary according to the Dacom system.

Despite the climatological differences between the Mendoza and the Balcarce area, the analysis of measured weather data gave similar results with respect to the potato late blight control strategy. In both areas, ample opportunities exist for application of DSS guided disease control, providing optimal spray timing, as related to the actual disease cycle and a reduction of the production costs and environmental side effects.

DISCUSSION

DSS's integrate available information on the (measured and forecasted) weather, the disease cycle, the crop and previous spray applications to generate an optimal spray schedule. Spray applications are matched with high risk periods thus providing optimal protection when necessary while at the same time the crop is left unprotected when infection risks are negligible. DSS's thus avoid unnecessary spray applications and provide an optimal timing for the remaining applications. The general result is an equal or improved control efficacy as compared to (nearly) weekly spray schedules, a reduction of the number of applications and thus a reduction of the production costs (e.g. Hansen *et al.*, 2002). Results from both, climatologically very different, regions in Argentina clearly illustrate the potential for introduction of DSS guided control strategies.

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Effect of spore density, cultivar resistance and *Phytophthora infestans* isolate on tuber blight under field conditions.

A. EVENHUIS, P.J. VAN BEKKUM, AND G.J.T. KESSEL

Plant Research International, Wageningen University and Research Centre.
PO Box 69, 6700 AB Wageningen, The Netherlands

Presentation

SUMMARY

Survival of *P. infestans* in soil was limited to 5 weeks at high inoculum density. With a ten fold dilution of the infection pressure survival of *P. infestans* in soil was limited to two weeks. Differences between isolates concerning survival in the soil were small. From these experiments we conclude that survival of *P. infestans* in soil depended on spore density rather than *P. infestans* isolate used.

Tuber blight infection rate depends on density of the sporangia in the soil, tuber blight resistance of the cultivar and to a lesser extent the *P. infestans* isolate used. Isolate of type EU 13 A2 was not more aggressive to tubers than IPO 428-2, regardless of the resistance level of the cultivar used. A mixture of isolates was found to be more aggressive than two single isolates tested on cultivar Bintje only. Thus measures to avoid infection of the soil with *P. infestans* sporangia lower the tuber blight infection risk, especially on the susceptible cultivar Bintje.

KEYWORDS

Solanum tuberosum; potato; late blight;

INTRODUCTION

Sporangia of *P. infestans* can be washed through the soil to the tubers (De Bary, 1863). Subsequently tubers can be readily infected with *P. infestans*. The number of spores washed to the soil depends on the late blight epidemic developing in the canopy and weather conditions, especially rainfall. Tuber blight incidence further depends on tuber blight resistance of the cultivar used. At present the *P. infestans* population in The Netherlands is dominated by isolates of the EU 13 A2 type. Isolates of type EU 13 A2 are considered more aggressive to potato foliage (Lees *et al.*, 2009) than isolates of the old population, but this does not necessary apply to tubers also. The aim of the experiments was to assess the effect of *P. infestans* isolates, cultivar resistance and spore density on tuber blight incidence, without the confounding effect of a late blight epidemic in the foliage.

MATERIALS AND METHODS

Treatments

Potato cultivars Bintje, Agria and Seresta were planted in spring 2009 on a heavy clay soil at Wageningen. Tuber blight resistance ratings according to the National list are 4.5, 7.5 and 8 respectively. To avoid late blight in the foliage the potato crop was sprayed regularly with a fungicide containing active ingredients cymoxanil + mancozeb. Desiccation of the crop took place at the end of August. Potato tubers were harvested by hand on September 21st.

Preparation spore suspension

Isolate IPO 428-2, an isolate of type EU 13 A2 and a mixture of 16 isolates were used to inoculate the experiments. The isolates were kept in liquid nitrogen storage until use. During the experiments the isolates were maintained alternating on detached leaves and slices of potato of cv. Bintje in a climate room at 15°C with a 16 hour photoperiod. Mycelium from slices were transferred to leaves on 1.5% water agar (WA) in a Petri dish with vents (Ø = 9 cm) in one week and parts of infected leaves were transferred under a slice in a Petri dish with vents (Ø = 9 cm) in the other week. Potato leaves were obtained from plants grown in a greenhouse at 18°C. To produce large amounts of sporangia wetted filter paper was placed in a large tray of 530x320x60 mm. A grid was placed on the wetted paper and wetted oases was placed at both sides of the tray. Detached leaves were put in the oases and inoculated with *P. infestans* isolates. The tray was covered with a transparent plastic bag and placed in the climate room. For each isolate treatment 16 trays with infected leaves were used.
Spore suspensions were made by washing off sporangia from infested leaves with cold tap water. Spore density of the suspension was determined with a coulter counter (Beckman Coulter, inc). The highest spore density (100%) was prepared, and subsequently a ten fold dilution was made (10%). The freshly made spore suspension (50 ml) was added directly to the top of the ridge, between the potato plants in the field. Each treatment consisted of 18 potato plants being inoculated, next to stem upon the top of the ridge.
The experiment was inoculated three times during the season on 15 July, 5 August and 9 September. Each time inoculation took place in plots which were not previously inoculated.

Survival of sporangia in the soil

Infectivity of the spores was measured in time. Soil samples were taken from the top of the ridge, where previously the soil was inoculated with *P. infestans*. A modified tuber-slice test as described by Lacey (1965) was used to estimate survival of sporangia. The trays were placed in a climate room at 15°C with an 16 hour photoperiod. After one day slices were cut in eight pieces (octants) and separated with a single edge razor blade and placed back in the climate room. Octants were examined six days later for infection by *P. infestans*. Octant infection rate was calculated by the dividing the number of infected octants with the total number of octants. If no infection of octants occurred for each replicate on two consecutive sample dates it was assumed that sporangia did not survive the treatment.

Tuber blight assessment

Tuber blight assessments were made shortly after harvest and after incubation for three weeks in a climate room at 15°C in the dark. Both number and weight of the potatoes were assessed. Infected tubers were removed at the first assessment. These tubers were counted and weighed. At the end of the incubation period tubers were washed and a second tuber blight assessment was made. The number and weight data from both assessments were combined and the percentage tuber blight was calculated.
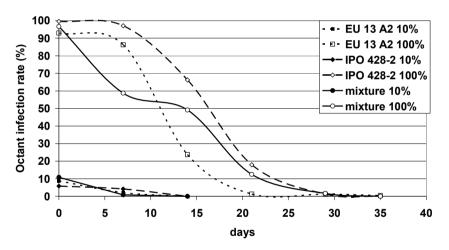
Statistical analyses

Three experiments were carried out. Each experiment was laid out as split plot design, with inoculation date and cultivar randomized as a complete block design. Within these plots two treatment factors, being the isolates and the inoculum density to be tested were randomized. Each experiment was carried out with three replicates. Each experiment was inoculated one time during the season. Inoculation dates were 15 July, 5 August and 9 September. Since differences between inoculation date (experiments) on tuber blight incidence and octant infection rate were marginal compared to the other treatment effects, data over the three experiments were pooled.

Analysis of Variance (ANOVA) was performed on tuber blight incidence based on weight, measured per experimental plot, using Genstat release 12.1 (Payne *et al.,* 2002).

RESULTS

Octant infection rates tended to approach zero in approximately 15 days at a low inoculum density (10%). At a high inoculum density (100%) octant infection was found up to five weeks after inoculation (Figure 1), depending on the inoculum source. Differences between isolates concerning survival in the soil, measured as octant infection rate, were small compared to density of sporangia applied.

Figure 1. *Octant infection rate depending on P. infestans isolate, spore density applied and time after inoculation (days).*

No foliar or stem blight occurred in the field during the experiment.

Average tuber blight incidence increased significantly from 0.5 to 1.5 (P<.001) when the inoculum density was increased from 10% to 100%. A significant (P <.001) cultivar and inoculum density interaction was observed (Table 1). On average the tuber blight incidence after inoculation with a mixture of isolates was 1.5% and was significantly (P=0.05) higher than after inoculation with the single isolates EU 13 A2 and IPO 428-2. Pair wise comparisons between treatments are given in Table 2.

Table 1. *Average tuber blight incidence after inoculation of the ridge with P. infestans isolates at two spore densities.*

	Cultivars					
Spore density	Agria		Bintje		Seresta	
10	0.9	. b . [1]	0.4	a b .	0.1	a . .
100	1.4	. b .	2.5	. . c	0.6	a b .

[1]: *Treatments followed by different characters differ significantly (P=0.05) from each other, based on angular transformation. Represented data are not transformed.*

Table 2. *Percentage tuber blight after inoculation of the ridge with P. infestans isolates at two spore densities.*

Isolate	Spore density	Cultivars					
		Agria		Bintje		Seresta	
EU 13 A2	10	0.7	a b c d . . . [1]	0.3	a b c	0.1	a . . . . . .
EU 13 A2	100	0.8	a b c d . . .	1.8	. . . . . f .	0.8	. b c d e f .
IPO428-2	10	0.7	a b c d e . .	0.0	a	0.2	a b . . . . .
IPO428-2	100	1.6	. . c d e f .	1.7	. . . . e f .	0.3	a b . . . . .
Mixture	10	1.4	. . . d e f .	1.1	. b c d e f .	0.2	a b . . . . .
Mixture	100	1.7	. . . d e f .	4.0	. . . . . . g	0.7	a b c d e . .

[1]: *Treatments followed by different characters differ significantly (P=0.05) from each other, based on angular transformation. Represented data are not transformed.*

DISCUSSION

Octant infection rate is a means to estimate survival of *P. infestans* in soil. Survival of *P. infestans* in soil was limited to 5 weeks maximum at high inoculum density in our experiments, which was in the range described by others (Murphy, 1922; Zan, 1962; Lacey 1965, Andrivon, 1994). Survival of *P. infestans* spores was 64 days in a previous experiment in a clay soil at Wageningen (Evenhuis *et al.*, 2006). In our experiments in 2009 inoculum was poured on to the soil whereas in 2004 the inoculum was placed in the soil in mesh bags. The survival in 2009 was possibly under estimated due to the inoculation method and subsequent sampling technique used.

Octant infection rate at the start of the experiment depended on inoculum density applied. Subsequently the duration of survival of *P. infestans* in the soil was influenced by the applied *P. infestans* inoculum density as well. The effect of *P. infestans* isolate used on spore survival was small. No differences in survival rate in soil of isolates of the US clonal lineages US-8 and US-11 were found (Porter & Johnson, 2007).

Tuber infection depends on several factors leading to the presence of *P. infestans* sporangia in the soil (Lapwood, 1977). On average tuber blight incidence was significantly higher after inoculation with a higher inoculum density, suggesting a quantitative relation between the presence of sporangia in the soil and tuber blight. Over all tuber blight incidence was low, possibly because a single inoculation has been carried out in each plot. Sporangia can be washed to the soil on several days, providing rain fall occurs during sporulation events, in agricultural practice, leading to a higher inoculum pressure.

P. infestans was inoculated on the ridge directly in our experiment. No late blight developed in the potato foliage. Thus the inoculum pressure applied was due to the inoculation of the potato ridge only. Thus treatments (isolate used and spore density applied) did not influence each other. Also the confounding effect of the resistance level of the cultivar to foliar blight was taken out of the equation. Furthermore the weather circumstances necessary for late blight development in

the foliage and wash down of spores from the foliage to the ridge was avoided. Water containing sporangia of *P. infestans* washed into the soil immediately, mainly through cracks in the clay soil. Further transport of the sporangia towards the tubers was not facilitated but depended upon the natural rain fall during the experiments. Thus the effect of inoculum density, isolate and cultivar resistance on tuber blight could be studied in a field situation.

Actual tuber blight infection, apart from the presence of sporangia, is dependent on soil moisture content and the resistance of the cultivar (Lapwood, 1977). Differences between isolates were less conspicuous. The isolate of type EU 13 A2, predominant in the Dutch *P. infestans* population (data not published), was not more aggressive in causing tuber blight than isolate IPO 428-2. IPO 428-2 was collected in 1992 and represents the old population, although this isolate is known to be aggressive towards tubers (Flier *et al.,* 2001). Isolate IPO 428-2 and the isolate of type EU 13 A2 were also included in this mixture of 16 isolates. The mixture of 16 isolates was more aggressive to tubers than both single isolates, when tested on cultivar Bintje. The total inoculum density applied in the mixture was the same as with the single isolates. Therefore one or more of the isolates in the mixture are responsible for the increased tuber blight incidence.

Differential interactions in tuber blight attack between potato cultivars and *P. infestans* isolates was found previously (Flier *et al.,* 2001). In our experiments no clear significant (P=0.062) differential interaction was found.

CONCLUSIONS

From these experiments we conclude that survival of *P. infestans* in soil depended on spore density rather than *P. infestans* isolates used.

Tuber blight infection rate depends on density of the sporangia in the soil, tuber blight resistance of the cultivar and to a lesser extent the *P. infestans* isolate used. The isolate of type EU 13 A2 was not more aggressive to tubers than isolate IPO 428-2 originating from 1992, regardless of the resistance level of the cultivar used. A mixture of isolates was found to be more aggressive than two single isolates tested on cultivar Bintje only. Measures to avoid infection of the soil with *P. infestans* sporangia lower the tuber blight infection risk, especially when the susceptible cultivar Bintje is grown.

ACKNOWLEDGEMENTS

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Post-Harvest Application of Phosphorous Acid for Control of *Phytophthora infestans* on Potatoes

STEVEN B. JOHNSON

University of Maine Cooperative Extension
P. O. Box 727, Presque Isle, Maine, USA, 04769

SUMMARY

Potato tuber rot from late blight causes economic loss and an effective post-harvest treatment to control this disease is needed. The purpose of this study was to further investigate the value of phosphorous acid at reduced application volumes in preventing tuber-to-tuber spread of *Phytophthora infestans* during the mechanical harvesting and tuber handling in a situation where the disease is present at harvest.

KEY WORDS:

Solanum tuberosum, late blight, potato storage

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary is a devastating potato tuber disease as well as foliar disease. Late blight regularly causes loss in many potato production systems (Stark and Love, 2003). Tuber loss owing to this pathogen with skinned or damaged tubers has long been established (Bonde and Schultz, 1949). Tuber-to-tuber spread is encouraged when tuber damage or skin damage occurs during the mechanical harvesting and tuber handling procedures (Lambert *et al.*, 1998). With late blight present in the field, storage losses can occur well beyond what would be expected based on the pathogen level in the field.

Phosphorous acid (phosphonate or phosphate) is the anionic metabolite of the systemic fungicide aluminum tris-O-ethyl phosphonate (fosetyl-Al) (Ouimette and Coffey, 1989). Phosphorous acid has systemic antifungal activity towards mycelial growth and is not a nutritional source (Fenn and Coffey, 1989). Phosphorous acid is effective in reducing oomycete-incited diseases (Forrester *et al.*, 1998). Reports on the effectiveness by phosphorous acid materials for late blight control have appeared. Most of the information is in-season applications and not post-harvest applications (Cooke and Little, 2001; Johnson *et al.*, 2004; Mayton and Fry, 2006). Phosphorous acid materials are now labeled for post-harvest use on potatoes (Johnson, 2007). Johnson (2007, 2008) previously discussed effectiveness of the applications with respect to the timing of applications following mechanical damage.

The purpose of this study was to investigate the value of phosphorous acid at reduced application volumes in preventing tuber-to-tuber spread of *P. infestans* during the mechanical harvesting and tuber handling in a situation where the disease is present at harvest.

MATERIALS AND METHODS

In 2010, potato tubers (cv. Katahdin) were selected to insure there was no existing late blight. The tubers were individually abraded. Abrasion was accomplished by holding a tuber against a belt sander operating at moderate speed. The abraded area encircled each tuber. The abrading completely removed the skin and exposed the tuber periderm. This mimicked skinning damage as well as more severe tuber damage. The abrading simulated damage which may occur during mechanical harvesting and tuber handling procedures and to facilitate transfer of pathogen from infected tubers to healthy tubers. The abrasion also provided ideal an infection site for the *P. infestans*.

Immediately following abrading, the tubers were dampened to improve infection conditions and then inoculated with *P. infestans* (US-8 genotype). Pathogen isolates were isolated from locally infected tubers onto water agar and then transferred to V-8 agar. Cultures of the pathogen were macerated to prepare inoculum. A titer of approximately 50,000 propagules per ml was used to inoculate the abraded tubers. Propagules consisted of both sporangia and mycelial fragments. Each abraded test tuber was atomized with approximately 1 ml of the pathogen solution.

Treatments consisted of phosphorous acid (Phostrol) at the rate of 378 ml per 907 kg of tubers applied at a volume of 1900 ml, 950 ml, and 475 ml per 907 kg, and an untreated control. The full rate of Phostrol was applied in full, half and quarter labeled application volumes to simulate coverage variability with reduced spray volumes. All treatments were applied 1 hour post inoculation. Treatment timing of 1 hour post inoculation was chosen as the potential waiting period between harvest and unloading in a commercial situation. In each case, experimental units consisting of 9.1 kg of abraded and inoculated tubers were arranged in a randomized complete block design, placed into a controlled atmosphere storage, and held at 13°C with a relative humidity greater than 95%.

The experimental units were removed and tubers individually peeled and evaluated for disease symptoms after three weeks of storage. Tubers were rated as either infected or not infected. Data were recorded on a percentage basis and analyzed untransformed with Fisher's LSD test. Data and analysis appear in table 1.

RESULTS AND DISCUSSION

With the high pathogen titer and warm storage conditions used in this study, presence or absence of rot was an effective disease rating. Tubers were either heavily diseased or not at all. Previously reported efforts document phosphorous acid materials provide complete control tuber-to-tuber late blight spread (Johnson 2007, 2008). However, less than complete control of *P. infestans* occurred at less than labeled rates. One quarter rate of phosphorous acid failed to provide complete control of tuber-to-tuber late blight spread. Uncertain was whether this result was a result of material effectiveness with reduced rates, inadequate coverage, or a combination of both.

This test was designed to simulate tuber-to-tuber late blight spread. The high inoculum titer and severe tuber damage under favorable conditions produced all diseased tubers or no diseased tubers with proper phosphorous application. Complete control under these conditions is significant and it can be postulated that other phosphorous acid materials would provide similar control.

Results from this study also show a less than complete control at a full rate of phosphorous acid applied at one quarter rate of the recommended application volume (table 1). Applications with less than half the recommended material or less than half the recommended application volume failed to provide complete control of tuber-to-tuber late blight spread. Concerns with reduced application volumes are as valid as previous concerns with reduced rates. Since most growers have a zero tolerance for late blight in storage, application volumes or phosphorous acid rates less than half should be avoided.

Table 1. *Severity of late blight on Katahdin potatoes after three weeks of storage with varied application volumes with Phostrol.*

Treatment	Application volume*	Late Blight (%)
Check	0	100.0
Phostrol**	475 ml	23.5
Phostrol**	950 ml	0.0
Phostrol**	1900 ml	0.0
LSD value at alpha = 0.05		6.1

*per 907 kg tubers, ** applied at the rate of 378 ml per 907 kg tubers*

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Initium®: a new fungicidal active ingredient for the control of Oomycetes

STEPHAN REIMANN[1], KLAAS JILDERDA[2], MATTHIAS GERBER[1],
VANESSA TEGGE[1], KRISTIN KLAPPACH[1]

[1]BASF SE, Agrocentre, 67117 Limburgerhof, Germany
[2]BASF Netherlands B.V., 6835 EA Arnhem

SUMMARY

Initium is an innovative fungicidal active ingredient developed by BASF. Initium is a mitochondrial respiration inhibitor and belongs to a new class of chemistry, the triazolo-pyrimidylamines. In numerous field trials performed in several climatic zones around the world, Initium has proven its high efficacy and its excellent crop safety. Initium is highly active against Oomycete pathogens, inhibiting zoospores in their formation and release, mobility and germination. In addition Initium inhibits the direct germination of zoosporangia. Initium has a high affinity for the epicuticular wax layers of plant surfaces. Initium redistributes under the influence of dew and due to this effect an increasing area can be protected. Because of this effect, Initium has also good protection on new growth. Taken together these characteristics lead to a premium preventive activity with long residual activity in the field. Initium containing products will be positioned in preventive spray applications against late blight in potatoes and against downy mildews in a wide range of speciality crops. Due to its excellent toxicological and ecotoxicological profile, Initium will be an important active ingredient for sustainable spray programs. Initium will only be sold in combination with other active ingredients. First registrations have been achieved in Romania, The Netherlands and The United Kingdom. The worldwide registration initiatives will continue from 2010 onwards and will ensure a coordinated setting of maximum residue limits and import tolerances.

INTRODUCTION

Late blight and downy mildews are devastating diseases of speciality crops world wide and play an important economic role in commercial food production. Economic losses by *Phytophthora infestans* in potatoes were estimated alone in developing countries by more than 2,7 Mrd. $ (CIP, Centro International de la Papa, Lima [Peru]). Initium is a fungicide developed by BASF with high activity against these Oomycete pathogens. Since its discovery in 2004, Initium has undergone detailed evaluation in laboratory tests and in extensive global field testing programmes. The aim of this paper is to give an overview of Initium's chemical and physical properties, mode of action and biological profile, field performance in potatoes, resistance management and safety profile.

CHEMICAL AND PHYSICAL PROPERTIES
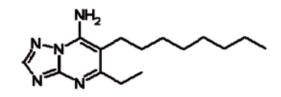
Figure 1: *Structural formula of Initium*

The following table shows the most important physical and chemical properties of the new active ingredient Initium:

Table 1: *Physical and chemical properties of Initium*

Common name:	Ametoctradin
CAS no:	865318-97-4
Chemical name (IUPAC):	[1,2,4] triazolo [1,5-*a*] pyrimidin-7 amine, 5-ethyl-6-octyl
Molecular formula:	$C_{15}H_{25}N_5$
Molecular weight:	275.4 g/mol
Vapour pressure:	2.1×10^{-10} PA at 20° C
Solubility (Water at 20° C Ph7):	0.15 mg/l
Partition coefficient	log P_{OW} = 4.4
Hydrolytical stability	Stable at pH 4-9
Photolytical stability	Slowly degraded by direct photolysis in neutral aqueous solution

MODE OF ACTION

Initium is a potent inhibitor of the mitochondrial respiratory chain in target pathogens. More specifically, Initium interferes with complex III, which is a membrane protein complex. By inhibiting complex III, Initium impairs the electron transport in the respiratory chain of the pathogen, thus making it unable to generate the energy required for keeping the organism alive. Research has demonstrated that Initium is not cross-resistant to phenylamides (e.g. metalaxyl), Qo inhibitors (e.g. strobilurins) and carboxylic acid amides (e.g. dimethomorph).

Initium is a highly effective inhibitor of zoospore formation, release, mobility and germination. In addition Initium inhibits the direct germination of zoosporangia. Microscopic observations show that Initium immediately stops the movement of zoospores and makes them burst within a few seconds at very low concentrations (ED_{50} = 0.021 ppm) (Figs. 2). Sporangia were examined as well under the microscope. They failed to germinate after treatments with Initium (ED_{50} = 0.08 ppm).

The efficacy of Initium against *Phytophthora infestans* during different stages of its life cycle was proven for very sensitive strains as well as for the more aggressive A2-mating-type populations, including Blue 13 strains from the UK. Initium's high activity against the different *Phytophthora* strains is important to ensure reliable control under different situations in the field with changing *Phytophthora* populations.

Due to the long acyl side chain of the Initium molecule, the AI exhibits a high log P_{ow} value of 4,4 and has a high affinity for the epicuticular wax layers of the plant epidermis.
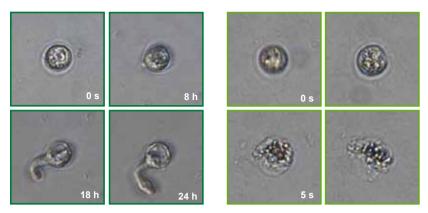
Figure 2: *Light microscope images of Phytophthora infestans zoospores. The untreated zoospore (left) remained intact and forms a germ tube. The Initium-treated zoospore (right) ruptured within 5 seconds and cytoplasm leaked from the spore.*

Through the adsorption of Initium to the wax layers and through the formation of AI depots on the plant surfaces, Initium forms a stable protective film on plants with a long-lasting efficacy against Oomycete pathogens (Fig. 3). Initium exhibits very good rainfastness, however, under the influence of moisture such as dew, a small but effective portion of the active ingredient is gradually redistributed from the protective film leading to a significant increase in protection. This effect ensures that growing leaves can be protected during the phase of active growth.
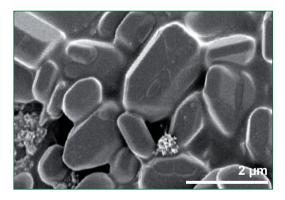
Figure 3. *Scanning electron microscopy image of Initium spray deposit on a tomato leaf. The particles of the active ingredient are bound to the surface like a film.*

Less than 10% of the applied active ingredient is taken up by the leaves after 1-7 days. The majority of the active ingredient remains on the leaf surface where it is adsorbed to the epicuticular wax layer. The vapour phase activity is minimal. These characteristics indicate that Initium is a non-systemic fungicide.

BIOLOGICAL PROFILE

Crop safety
Initium is characterised by excellent crop safety. At the recommended rates, no crop injury has been observed over several years of testing in a broad variety of crops.

Spectrum of activity
Initium controls all major Oomycete diseases, e.g. downy mildew caused by *Plasmopara viticola* in grapes, late blight caused by *Phytophthora infestans* in potatoes and tomatoes, and a broad range of downy mildews and late blights in vegetables (e.g. cucurbits, brassicas, onions, and lettuce).

The performance of Initium in potatoes was proven in several field trials on research stations in different countries. Summarized results are shown in the Table 2.

Table 2: *Control of Phytophthora infestans in potatoes in Spain, Germany, Brazil and Taiwan in 2004 – 2005 (orthogonal summary)*

Treatment[1]	Dose g a.i./ha	Mean % index of attack Leaves	Statistics[2]
Initium	300	11	C
Fluazinam	200	16	B
Untreated	-	78	A
Number of trials		8	

[1] *3-7 applications at BBCH 19-85 following a 5-11 day spray interval. Ave. of 8 trials.*
[2] *Statistical analysis: SNK-Test*

In the registered combinations products, Initium will be used at 200 to 300 g active ingredient per hectare.

RESISTANCE MANAGEMENT
Initium is not cross-resistant to Oomycete fungicide classes with confirmed field resistance (e.g. phenylamides, Qo inhibitors or carboxylic acid amides). Very important in potatoes and other crops attacked by *Phytophthora infestans* is that Initium is fully effective against metalaxyl-resistant *Phytophthora infestans* strains.

To ensure the long-term efficacy of Initium in all target crops, Initium will only be available in ready formulations combined with other fungicidal active ingredients of a different mode of action. To use the full activity of Initium, all applications should be done in a preventive manner following the recommendations on the product label.

TOXICOLOGICAL AND ECOTOXICOLOGICAL PROPERTIES
Initium displays an excellent toxicological profile. Acute mammalian toxicology studies indicate that Initium is not harmful after ingestion, dermal exposure or inhalation. It is also not irritating to eyes or skin and is not a sensitizer.

Furthermore Initium shows an excellent ecotoxicological profile. It is practically non toxic to birds, mammals, honeybees, earthworms and other soil macro-organisms, or to non-target soil micro-organisms and their ecosystem function. The use of Initium in accordance with good agricultural practice does not pose any risk to aquatic ecosystems. It is ideal for use in integrated crop management programmes.

CONCLUSIONS
Initium is a highly active preventive fungicide from a new chemical class for the use against Oomycete diseases. It has an excellent toxicological and ecotoxicological profile and is suitable for use in a range of speciality crops in integrated crop management programmes. BASF plans worldwide registrations for Initium. Initium will be marketed in ready-mixtures with other Oomycete active compounds to complement the spectrum of activity and to reduce selection pressure for resistance.

ACKNOWLEDGEMENTS
The authors would like to thank all colleagues who have contributed to the development of Initium.

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Initium® based products for the control of *Phytophthora infestans* in potatoes

STEPHAN REIMANN[1], KLAAS JILDERDA[2], MATTHIAS GERBER[1],
VANESSA TEGGE[1], KRISTIN KLAPPACH[1]

[1]BASF SE, Agrocentre, 67117 Limburgerhof, Germany
[2]BASF Netherland B.V., 6835 EA Arnhem

SUMMARY

Initium is an innovative fungicide belonging to a new class of chemistry, the triazolo-pyrimidylamines. In order to maintain this new class of chemistry and in order to ensure a sustainable use in different crops, Initium will be launched only in combination with other active ingredients. Initium containing products will be positioned in preventive spray applications against late blight (*Phytophthora infestans*) in potatoes and against downy mildews in a wide range of speciality crops.

In potatoes two Initium containing products will be launched in North Europe.
One product is the combination of Initium with the local systemic compound dimethomorph. This combination controls effectively all relevant development stages of *Phytophthora infestans*, causing potato late blight on leaves and tubers.
In the second new Initium containing product, Initium and Mancozeb were combined in a synergistic way to a premium preventive product. In addition to *Phytophthora infestans,* Initium+mancozeb provides a basic *Alternaria spp.* control due to the activity of mancozeb.

Many trials conducted by BASF, EuroBlight and official advisors in the European countries during the last years demonstrated an excellent performance of Initium+dimethomorph and Initium+mancozeb on *Phytophthora infestans* in potatoes at the same level of the best market standards.

First registrations of Initium containing products are available in Rumania, The Netherlands and The United Kingdom. The worldwide registration initiatives will continue from 2010 onwards and further registrations in important potato markets are expected.

INTRODUCTION

In all countries with relevant potato production *Phytophthora infestans* causes severe yield losses. Therefore *Phytophthora infestans* is deemed to be the most dangerous potato disease (Radtke & Rieckmann, 1990). For the control of *Phytophthora infestans* BASF has developed the active ingredient Initium. Initium belongs to a new chemical class called the triazolo-pyrimidylamines. More details about this new compound and its unique properties can be found in the paper "Initium: a new fungicidal active ingredient for the control of Oomycetes", available in this proceedings.

In order to avoid or slow down the development of resistance to Initium in target pathogens, and in order to ensure a sustainable use in all crops, Initium will only be launched in combinations with other compounds.

The aim of this paper is to give an overview about the product profiles and the mode of action. Furthermore the efficacy against leaf and tuber blight and the rainfastness characteristics of the products will be presented.

PRODUCT PROFILES

For the potato markets in North Europe, two Initium containing products will be launched.

Initium+dimethomorph is a combination product containing the contact fungicide Initium and the local systemic active ingredient dimethomorph. The product is formulated as a 525 g/l suspension concentrate (SC). The target rate against *Phytophthora infestans* in potatoes is 0.8 l/ha in a preventive application scheme. Early blight (*Alternaria* spp.) is not controlled by Initium+dimethomorph. First registrations of Initium+dimethomorph are available in Rumania (trade name: Orvego®), The Netherlands (trade name: Orvego®) and The United Kingdom (trade name: Resplend®, Zampro DM®). Further registrations are expected from 2010 onwards.

Initium+mancozeb is a combination product containing the contact fungicide Initium and the well known contact fungicide mancozeb. The product is formulated as a 560 g/kg water dispersible granule (WG). The target rate against *Phytophthora infestans* is 2.5 kg/ha in a preventive application scheme. At this target rate the mancozeb amount is high enough to ensure a basic control of *Alternaria* spp. The first registration of Initium+mancozeb is available in The United Kingdom (trade name: Decabane®). Further registrations are expected from 2010 onwards.

The product details are summarized in Table 1.

Table 1: *Product profiles of Initium+dimethomorph and Initium+mancozeb*

Active ingredients	Initium + dimethomorph	Initium + mancozeb
Mode of action	Complex III inhibitor + Inhibitor of phospholipids biosynthesis and interference with cell wall formation	Complex III inhibitor + Multi-site inhibitor
Formulation	300 + 225 g/l SC	80 + 480 g/kg WG
Target dose rate (potato)*	0.8 l/ha (= 240 + 180 gai/ha)	2.5 kg/ha (= 200 + 1200 gai/ha)
Target disease (potato)*	*Phytophthora infestans*	*Phytophthora infestans*, (*Alternaria spp.*)
First registration	2009 (RO) 2010 (NL, UK)	2010 (UK)
Further target crops*	Different vegetables, hops	Leek, Onions
Futher target diseases*	Late blight, downy mildews	Late blight, downy mildews

* General information; country-specific labels must be considered

MODE OF ACTION

The combination Initium+dimethomorph ensures that all infectious stages of the late blight life cycle can be controlled: By inhibiting complex III, Initium impairs the electron transport in the respiratory chain of the pathogen, thus making it unable to generate the energy required for keeping the organism alive. With this mode of action Initium is a highly effective inhibitor of zoospore formation and release as well as zoospore mobility and germination. For more details about the mode of action of Initium see the previous paper in this proceedings entitled "Initium: a new fungicidal active ingredient for the control of Oomycetes". Dimethomorph is a local systemic fungicide that inhibits the phospholipid biosynthesis and the cell wall synthesis of target pathogens. By inhibiting the formation of the Oomycete fungal cell wall, dimethomorph provides a good protectant and antisporulant activity.

In Initium+mancozeb the two contact fungicides are combined in a synergistic way. The Initium mode of action is ideally combined with the mode of action of mancozeb, which inactivates the sulfhydryl groups of amino acids and enzymes of fungal cells. This results in the disruption of lipid metabolism, respiration and production of ATP. As a non-systemic, multi-site fungicide mancozeb is able to provide an excellent efficacy at the early infectious stages of the *Phytophthora infestans* lifecycle.

The efficacy of Initium+dimethomorph and Initium+mancozeb at different development stages of *Phytophthora infestans* lifecycle are illustrated in Figure 1.
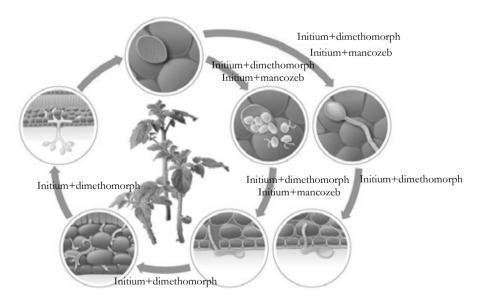
Figure 1: *Efficacy of Initium+dimethomorph and Initium+mancozeb at different stages of the Phytophthora infestans lifecycle.*

Initium+dimethomorph and Initium+mancozeb control effectively metalaxyl-resistant *Phytophthora* isolates, as neither Initium, mancozeb nor dimethomorph are cross-resistant to phenylamides (metalaxyl). Furthermore several lab and field studies indicate that Initium+dimethomorph and Initium+mancozeb provide reliable efficacy against A1 and A2 mating types of *Phytophthora infestans,* including the most aggressive strains, e.g. "Blue 13".

RESISTANCE MANAGEMENT

In order to ensure a sustainable use, Initium will only be sold in combination with other active ingredients. This decision ensures a built-in resistance management. Furthermore, the registered application number of Initium products will be limited. As routine activities, Initium+dimethomorph and Initium+mancozeb were already included in the past in existing resistance monitoring programs. These proactive measurements will ensure an efficient resistance management of Initium.

To use and maintain the full activity of Initium containing products it is nevertheless essential that all applications should be done in a preventive manner following the recommendations on the product label.

EFFICACY AGAINST *PHYTOPHTHORA INFESTANS* ON LEAVES

During the development phase of Initium+dimethomorph and Initium+mancozeb numerous lab and field studies were performed worldwide from 2004 onwards in order to evaluate the efficacy of Initium+dimethomorph and Initium+mancozeb against *Phytophthora infestans*.

Methods and Materials

The field trials were conducted in accordance to GEP and EPPO guidelines (EPPO PP 1/2, EPPO PP 1/181). Trials were designed with a randomized block design including 4 replications. Depending on the local conditions the untreated plots were either included as randomized plots in the field or as untreated boarder rows between the treated plots. The size of the blocks varied from 10 to 200 m². All trials were sprayed at the beginning of attack, either using special small plot tractor spray equipment or a knapsack sprayer. Treatments were applied in 150 - 600 liters water/ha. A visual assessment of the intensity of attack (in %) was made for each plot.

Figure 2 and 3 demonstrate the very good efficacy of Initium+dimethomorph and Initium+mancozeb in comparison to leading market standards. In this kind of preventive trials both Initium+dimethomorph and Initium+mancozeb are comparable or slightly better than the leading market standards. The standard deviations of Initium+dimethomorph and Initium+mancozeb are lower in comparison to those of the most reference products, indicating less variation in the performance of Initium+dimethomorph and Initium+mancozeb compared to the standards.
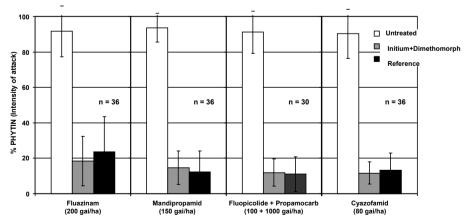
Figure 2: *Efficacy of Initium+dimethomorph against Phytophthora infestans in comparison to other active ingredients*
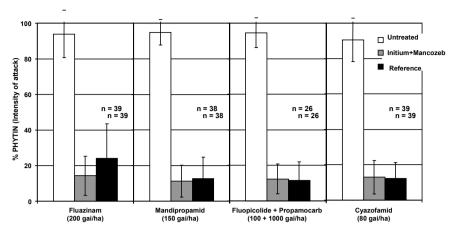
Figure 3: *Efficacy of Initium+mancozeb against Phytophthora infestans in comparison to other active ingredients*

EFFICACY AGAINST *PHYTOPHTHORA INFESTANS* ON TUBERS

Both BASF field trials and EuroBlight trials demonstrated that Initium+dimethomorph and Initium+mancozeb can effectively reduce the *Phytophthora infestans* attack on tubers. Looking closer to the activity of Initium and its combination partners, this effect was expected. Due to its good performance against *Phytophthora infestans* on leaves, Initium+dimethomorph and Initium+mancozeb reduce this important part of the inoculum source for *Phytophthora infestans* on tubers. Furthermore, Initium causes zoospore bursting and significantly fewer viable zoospores can therefore infect the tubers.

In 2010 additional trials will be conducted by the EuroBlight group and by BASF to create additional field data to further document the field performance of the new products.

RAINFASTNESS

Based on the high affinity of Initium for the wax layer of the leaf epidermis, it was assumed that Initium containing products have very good rainfastness properties. This assumption was confirmed in several lab and field trials on different crops.

One of these trials, a two factorial field trial conducted in 2008 by the BASF field research team in Limburgerhof is included in this paper. The trial was carried out according to the EuroBlight rainfastness protocol (Schepers *et al.*, 2007). It was located in Böhl in the Palatinate area in Germany and was conducted under GEP conditions. The variety Bintje was used as it is highly susceptible to *Phytophthora infestans*. Within the 4 replications the plots were randomly distributed. During the period of high late blight risk the trial was applied 4 times with an interval of 6-14 days. For all treatments five different irrigation intensities were tested after each application: no irrigation, 20 mm irrigation 1 hour after application, 20 mm irrigation 3 hours after application, 40 mm irrigation 1 hour after application and 40 mm irrigation 3 hours after application.

The results of this trial prove that both products, Initium+dimethomorph as well as Initium+mancozeb, have excellent rainfastness properties. Both products are significantly better than the rainfastness properties of fluopicolide + propamocarb and comparable or only slightly weaker than the rainfastness

properties of cyazofamid and mandipropamid.

Figure 4 illustrates the results of the assessment done 7 days after the last application.
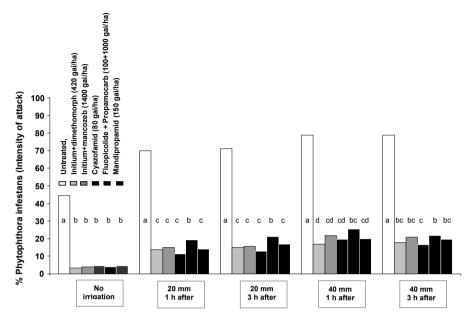
Figure 4: *Efficacy of Initium+dimethomorph and Initium+mancozeb against Phytophthora infestans in comparison to standard products after different intensities of rain (Statistical analysis: SNK-test after arcsin-transformation, α=0.05, statistics are within one rain level)*

CONCLUSIONS

Belonging to the new chemical class of triazolo-pyrimidylamines, Initium will ensure efficient *Phytophthora infestans* control even under high disease pressure and heterogeneous *Phytophthora* field populations. With the combination products Initium+dimethomorph and Initium+mancozeb, BASF has developed two products containing the new active ingredient Initium. Many trials conducted by BASF, EuroBlight and official advisors in the European countries demonstrated an excellent performance of Initium+dimethomorph and Initium+mancozeb on *Phytophthora infestans* at the same level of the best market standards. Worldwide registration initiatives ensure that Initium+dimethomorph and Initium+mancozeb will support potato growers in their efforts towards an efficient and sustainable disease management of *Phytophthora infestans*.

ACKNOWLEDGMENT

The authors would like to thank all colleagues who have contributed to the development of Initium.

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Infinito: protection of new growth from infection with

Phytophthora infestans

M-P. LATORSE[1], G. DUCLOS[1], S. RAUSCHER[1], C. RHONÉ[2], R. WANNINGEN[3] AND S. TAFFOREAU[1]

[1] Bayer S.A.S, research center la Dargoire , 14 impasse P. Baizet 69009 LYON, France
[2] Bayer S.A.S, 16 rue Jean-Marie Leclair, 69266 LYON CEDEX, France
[3] Bayer CropScience B.V., Energieweg 1, NL - 3641 RT Mijdrecht, The Netherlands

SUMMARY

This paper presents the results from field / laboratory combined tests carried out in France and in the Netherlands in 2009 in order to improve methodology and to benchmark the efficacy of INFINITO (fluopicolide + propamocarb-HCl) and other fungicides in protecting potato new growth against *Phytophthora infestans*. The same methodology as described in literature was implemented: fungicides were applied in the field then leaves or shoots were sampled and transferred to the laboratory for inoculation at different intervals after treatment. New growth development and leaf expansion were rapid in France (more than 5 new leaves formed within 12 days) and slower in the Netherlands (3-4 new leaves).

On expanding and newly formed leaves, the experiments showed that Infinito gave similar new growth protection to other new growth reference products; when growth rates correspond to typical European northern countries situations with 3- 4 new leaves developed within 10- 12 days.

KEYWORDS:

Phytophthora infestans, potato late blight, new growth protection, expanding leaves, newly formed leaves, Infinito, fluopicolide and propamocarb HCl

INTRODUCTION

In the cultivation of potatoes, the protection of new growth against potato late blight caused by *Phytophthora infestans* during the period of fast vegetative development is one of the most important challenges for potato growers. During the growing season when the new growth of the foliage is rapid: typically 3-4 new leaves develop within 10-12 days in North Europe. Sometimes, depending on local climatic conditions, more than 5 new leaves may develop, and parts of the foliage may not be protected against the disease.

Since 2004, methodologies have been discussed and developed to evaluate the performance of fungicides for the protection of new growth (PPO-special report N° 10, Jersey 2004, 157-160). As a

result, the EU Fungicide sub-group agreed to new definitions and experiments combined together field applications, laboratory inoculation and disease assessments. New growth is defined as "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 N° 11, Tallinn, 2005, 95-100). In addition, the following descriptions (used in the literature by several independent institutes and companies) have been used in this paper:

- Expanding leaves correspond to leaves present at the time of application without having reached their full size
- Newly formed leaves / completely new leaves are leaves which were not present at the time of application having developed later.
- Cut shoot: one stem separated from the plant, with several leaves.
- Cut leaf: one leaf separated from its stem, with its leaflets.
- Detached leaflet: leaflet separated from its leaf.
- Top (plant top): upper part of the one plant which consists in the terminal bud + one or several unfolded leaves.

The aim of the studies described in this paper was to evaluate the performances of Infinito for the protection of new growth of potatoes against late blight, in comparison with other commercial late blight fungicides in respect to new growth protection. In addition, some variations in the protocols mixing field and bioassay in the lab were tested.

MATERIALS AND METHODS

Two series of experiments were conducted in 2009. In France, two potato crops were planted at different dates to provide 2 different growth rates. In the Netherlands, trials were conducted at PPO with 2 consecutive fungicide applications in the field at BBCH 31-32. Assessments were done both in field and in laboratory to validate the relevance of the lab test.

Field trials
In France, 2 potato crops were planted with the variety "Bintje" at Bayer CropScience's research farm at Villefranche-sur-Saône (Rhône). One fungicide application was made with a conventional sprayer at a volume of 300 L water /ha. For each experimental treatment, one single large plot was sprayed. Sampling of leaves and shoots was carried out when the growth rate was 3-4 new leaves within 12 days, and also when more than 5 new leaves developed within 10 days. Selected shoots were tagged with coloured rings just before fungicide application to identify the "new growth" which consisted of the top plant and three expanding leaves (fig.1 and 2). Bioassays were done on detached shoots and leaves.

In the Netherlands, the field trial was carried out at PPO in Lelystad on the variety "Bintje" with 4 replicates (4 rows of 10 metres). Cover sprays of Dithane Neotech were applied prior to specific fungicide applications. Two consecutive sprays of test fungicides were done within 7 day interval according to good agricultural practices at 250 L water /ha. One artificial inoculation was performed in the field the day before the second application. To increase the success of the infection in the trial, artificial irrigation was provided by sprinklers (fig.3).

The fungicides used in the experiments and their dose rates are shown in Table 1.

Figures 1 & 2: *Field overview at the time of treatment and close-up of a tagged potato shoot (BCS farm location near Lyon, France)*

Figure 3: *overview of the trial design in Netherlands at the development stage of the crop on the day of the first application*

Table 1: *Dose rates of commercial fungicides applied in field trials in France and Netherlands*

Mode of action (distribution on/ in plant)	Fungicide (dose rate)	Active ingredient (dose rate)
Contact	Shirlan (0.4 L/ha)	fluazinam (200 g a.i./ha)
	Dithane neotech (2.0 kg/ha)	mancozeb (1500 g a.i. /ha)
	Ranman (0.2 L/ha + 0.15 L/ha adj.)	cyazofamid (80 g a.i. /ha)
Translaminar + Contact	Revus (0.6 L/ha)	mandipropamid (150 g a.i. /ha)
	Valbon (2.0 kg/ha)	benthiavalicarb + mancozeb (25 + 1400 g a.i. /ha)
Translaminar + Systemic	Infinito (1.2 L/ha)	fluopicolide + propamocarb HCl (75 + 750 g a.i /ha)
	Infinito (1.6 L/ha)	fluopicolide+ propamocarb HCl (100 + 1000 g a.i /ha)

Bioassays:

In the French trials, 20 shoots and leaves from 20 different potato plants were collected and transferred to the laboratory 0 to 12 days after the fungicide applications and subsequently artificially infected with a suspension of *P. infestans* sporangia calibrated at 40 000 sporangia per ml. After inoculation, cut shoots were incubated in a climatic chamber at 16°C and fog humidity. Cut shoots were placed in bottles containing a nutrition solution of Murashige and Skoog to prevent wilting (fig.4). Detached leaves were incubated on agar amended with kinetine 1% survival medium in Petri dishes (fig.5). Disease assessment was performed 5 days after inoculation on either detached leaves of expanding leaves in Petri dishes, or on entire plants including three expanding leaves and plant top.

In the Dutch test, the leaves were picked from the preventively sprayed plot 6 days after the first spraying, but 12 hours before artificial inoculation. The uppermost 3-4 leaf layers of ten plants were sampled and inoculated in the laboratory. The inoculated leaves were placed in a climatic room at 15°C and 98% Relative Humidity. Disease symptoms were assessed one week after inoculation.

Figure 4: *Cut shoot next to inoculation and incubation in climatic chamber at 16°C*
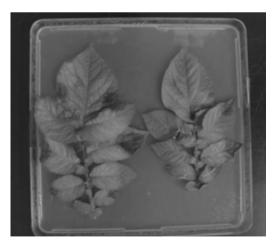
Figure 5: *Detached leaf test in Petri dishes*

RESULTS

Bayer CropScience trials, France 2009

In the trials a comparison of bioassay testing methods showed that protection of new growth on cut shoots was comparable to detached leaves (fig.6). However, efficacy results on whole cut shoots provided more reliable information than on the detached leaflets. In addition, this method was less time consuming. Therefore it is suggested that the whole cut shoot method is adopted for further testing of new growth protection.
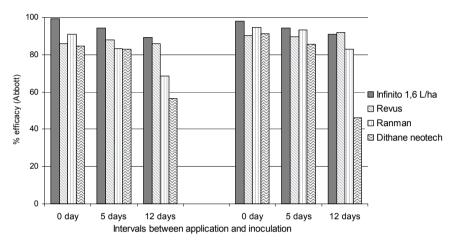
Figure 6: *Comparison of laboratory testing methods (detached leaves compared to cut shoots) on new growth protection collected from the field 3 hours, 5 days and 12 days after treatment*

The results from the trial carried out in low growth rate conditions (3-4 new leaves within 10-12 days) and in high growth rate conditions (more than 5 new leaves within 10 days) are presented in figures 7 and 8.

Without rain-washing between treatment and sampling / inoculation all the products tested, Infinito at 1.2 and 1.6 L/ha, mandipropamid (Revus) at 150 g a.i./ha, cyazofamid (Ranman) at 80 g a.i./ha + 0.15 L/ha adjuvant were equivalent, and gave 80% protection of expanding leaves already present at the time of treatment. The efficacy of mancozeb at 1500 g a.i./ha gradually declined to 50% control, 12 days after treatment. On new growth, corresponding to very small apical leaves at the time of treatment, all the products except mancozeb provided around 80% efficacy when the interval between treatment and inoculation was 7 days.

In the second trial carried out in rapid growth conditions, 5.4 mm rainfall occurred 4 days after treatment. Mancozeb was inactive in an assessment on leaves formed and developed after treatment when sampling was carried out 7 and 10 days after application. In these conditions cyazofamid also showed only a very small effect. Infinito at both dose rates and mandipropamid averaged less than 40% efficacy.
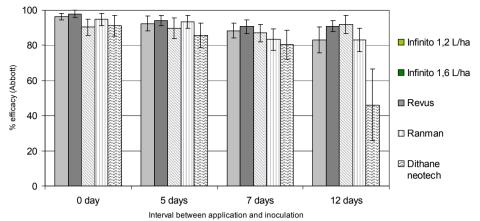
Figure 7: *Protection of expanding leaves in low potato growth rate conditions and no rainfall (field / lab cut shoots test)*
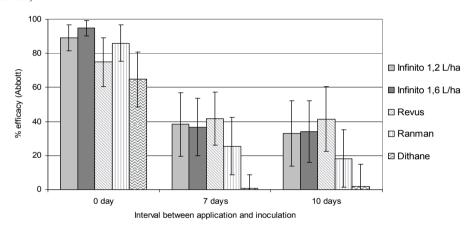
Figure 8: *Protection of newly grown leaves in high growth rate conditions with 5.4 mm rainfall 4 days after treatment (field / lab cut shoots test)*

The results from trials carried out in France demonstrated excellent protection of expanding leaves with Infinito and other fungicides except mancozeb. On newly formed leaves, all products were less effective and persistency was significantly reduced.

Trials carried out by PPO in the Netherlands, 2009

Results from field assessments, 9 days after artificial inoculation, showed the excellent efficacy of Infinito for the protection of new growth and plant tops corresponding to newly formed leaves (fig.9).
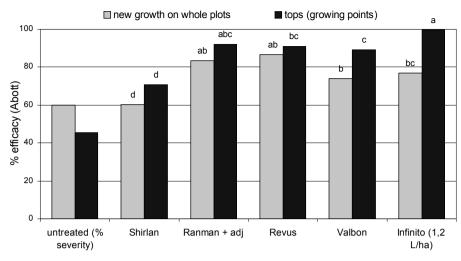
Figure 9: *New growth protection in field trial carried out in Netherland conditions (2009). Assessment 9 days after inoculation*

In the bioassay performed on expanding detached leaves collected 6 days after one preventive fungicide application, Infinito provided very good efficacy just following Revus, although differences were not significant. The protection offered by Valbon, Ranman + adjuvant and Shirlan were significantly inferior (fig.10).
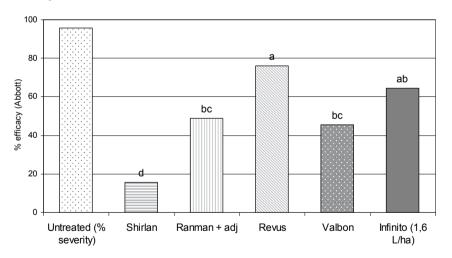
Figure 10: *New growth protection in field / bioassay tests on expanding detached leaf test carried out in the Netherlands (2009). Sampling 6 days after preventive fungicide application*

DISCUSSION-CONCLUSIONS

Consistent results were obtained with fungicides tested using different evaluation methods for new growth protection against potato late blight. Inoculations made on whole plants in the field or on cut shoots and expanding detached leaves in the laboratory demonstrated the performance of different fungicides with similar Euroblight rankings.

In practice, growth rates are often too low to make specific observations on newly formed leaves and/ or expanding leaves in the field. Therefore the combined methodologies with fungicide applications in the field followed by inoculation and disease assessment in the laboratory offer valuable tools to determine the protection of new growth by different fungicides.

Infinito demonstrated good protection of new growth in comparison to the best market standards. Overall, the performance of Infinito, Revus and Ranman was at the same level, while the contact fungicides Shirlan and Dithane were clearly less effective. Although all fungicides offered only moderate protection of new growth under very fast growing conditions, Infinito outperformed contact fungicides Ranman and Dithane

Results from 2009 trials confirm previous data and support the Infinito rating (++) for new growth effectiveness in the EuroBlight fungicide comparison table (= mandipropamid and cyazofamid)

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ACKNOWLEDGEMENTS

Evaluation of mandipropamid for the control of potato late blight in Northern Ireland

LOUISE R. COOKE[1,2] & GEORGE LITTLE[1]

[1]Applied Plant Science & Biometrics Division, Agri-Food & Biosciences Institute, (AFBI),
Newforge Lane, Belfast, BT9 5PX, UK
[2]School of Biological Sciences, Queen's University of Belfast, Newforge Lane, Belfast, BT9 5PX, UK

SUMMARY

Field trials in Northern Ireland over the years 2005-2009 compared potato late blight control by a standard fungicide programme consisting of two applications of metalaxyl-M + mancozeb followed by eight applications of fluazinam with programmes which included mandipropamid used either alone or tank-mixed with fluazinam. In 2005-2008, mandipropamid was applied after two applications of metalaxyl-M + mancozeb at various positions within the spray programmes. In 2009, mandipropamid was used early season in place of metalaxyl-M + mancozeb (sprays 1 and 2) and then again, after fluazinam, for sprays 4 and 5. 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), which included isolates of phenylamide-resistant A2 genotypes in 2008 and 2009.

Programmes including mandipropamid gave excellent foliage blight control which was consistently better than that achieved by the standard programme. In most years, plots receiving programmes including mandipropamid tank-mixed with fluazinam had less foliage blight than those receiving mandipropamid alone, although differences were not significant. Programmes containing mandipropamid tank-mixed with fluazinam tended to result in less tuber blight than both the standard programme and those containing mandipropamid alone and also achieved the greatest marketable yields. Used early season, mandipropamid proved an effective alternative to metalaxyl-M + mancozeb, suitable for use where phenylamide-resistant strains of *P. infestans* predominate. In regions such as Northern Ireland, where there is a high risk of tuber blight, mandipropamid should be used tank-mixed with fluazinam.

KEYWORDS

Phytophthora infestans, late blight control

INTRODUCTION

In Northern Ireland, weather conditions favour late blight, caused by the oomycete pathogen

Phytophthora infestans, in most years and the vast majority of potato cultivars planted have little or no blight resistance and therefore depend on fungicidal protection to survive blight attack. Crops generally receive about 10 fungicide applications, but may receive 15 or more in some years. Phenylamide-resistant strains of *P. infestans* were first identified in Northern Ireland in 1981 (Cooke, 1981). Their incidence increased during the 1980s and by 1988 they were detected in 90% of isolates, but in the early 1990s anti-resistance strategies were adopted and these proved effective up to 2006 (Cooke & Little, 2006). However, since 2007, new, aggressive phenylamide-resistant genotypes of *P. infestans*, notably 'Blue 13' (13_A2), have appeared and come to dominate the population (Kildea *et al.*, 2010), apparently as a consequence of their fitness rather than of selection by usage of phenylamide fungicides. This has increased the need for non-phenylamide fungicides with either systemic or translaminar activity.

The Syngenta fungicide, mandipropamid (Huggenberger & Knaufbeiter, 2007, 2008) belongs to the CAA (Carboxylic Acid Amide) fungicide group and was first registered in the UK in 2007 as 'Revus' for the control of potato late blight. It is specifically active against Oomycete pathogens and has a high affinity for the wax layer on leaf surfaces. After absorption into the wax layer, it gradually moves into the plant tissues so that it exhibits translaminar activity. To date, no strains of *P. infestans* resistant to CAA fungicides have been identified and mandipropamid is equally active against phenylamide-resistant and -sensitive strains of the pathogen.

As described previously (Cooke & Little, 2007), fungicide evaluation trials are conducted annually in Belfast, Northern Ireland under conditions of extreme blight pressure. The trials are planted with the maincrop cultivar Up-to-Date, which is very susceptible to both foliar and tuber blight, and are inoculated with recent Northern Ireland *P. infestans* isolates, collected for fungicide resistance and population studies. Here results of trials with mandipropamid formulations conducted between 2005 and 2009 are reported.

MATERIALS AND METHODS

Tubers cv. Up-to-Date were planted in May (or June in 2009) of each year (Table 1) at AFBI Headquarters, Newforge, Belfast, Northern Ireland in fully randomised blocks with five replicate plots per treatment. Each plot (2.8 x 3.0 m²) 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). In these rows, two leaves on every fourth plant were inoculated 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. In 2005-2007, the isolates used were all of the A1 mating type, but in 2008 and 2009, 25% of leaves were inoculated with phenylamide-resistant A2 genotypes including Blue 13. All isolates originated from Northern Ireland potato crops and were obtained within the last three years. When required, plots were misted after inoculation, usually for 2-3 h daily at dawn and dusk to encourage spread of blight.

Table 1. *Field trials for the control of potato blight, 2005-2009: dates of field operations*

Year	Planting date	Fungicide application dates		Inoculation	Desiccation	Harvest
		First	Last			
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
2007	2 May	20 June	21 August	3 July	28 August	12 September
2008	6 May	25 June	27 August	8, 21 July	29 August	29 September
2009	6 June	30 June	1 September	16 July	4, 9 September	30 September

Fungicide formulations were applied at manufacturers' recommended rates in *c.* 300 litres water/ha using a Cooper Pegler CP15 knapsack sprayer. The first applications were made before inoculation in the third or fourth week of June of each year (Table 1) and ten treatments were applied at 7-day intervals (as far as possible) until the end of 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, applied at 300 ml/ha, 2005-6 and 400 ml/ha, 2007-9), while the comparison programmes included mandipropamid (subsequently registered as 'Revus', Syngenta) either alone or tank-mixed with fluazinam. In 2009, fluopicolide + propamocarb HCl ('Infinito', Bayer) was included in one of the comparison programmes. Details of programmes and rates are shown in Table 2.

Table 2. *Fungicide programmes evaluated for the control of potato blight, 2005-2009*

Number of applications of active ingredients (g a.i./ha)[a]			Abbreviation[b]	Year				
Early season	Mid-season	Late season		'05	'06	'07	'08	'09
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x fluazinam (150)	3 x fluazinam (150)	F/S_L	√	√			
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x fluazinam (200)	3 x fluazinam (200)	F/S			√	√	√
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x mandipropamid (150)	3 x fluazinam (150)	F/R/S	√				
2 x metalaxyl-M + mancozeb (76 + 1216)	5 x mandipropamid (150)	3 x mandipropamid (150)	F/R		√			
2 x metalaxyl-M + mancozeb (76 + 1216)	2 x mandipropamid (150) 1 x fluazinam (200) 2 x mandipropamid (150)	3 x fluazinam (200)	F/R/S/R/S			√	√	
2 x metalaxyl-M + mancozeb (76 + 1216)	2 x mandipropamid + fluazinam (125 + 100) 1 x fluazinam (200) 2 x mandipropamid + fluazinam (125 + 100)	3 x fluazinam (200)	F/R+S/S			√	√	
2 x mandipropamid (150)	1 x fluazinam (200) 2 x mandipropamid (150) 2 x fluazinam (200)	3 x fluazinam (200)	R/S/R/S					√
2 x mandipropamid + fluazinam (125 + 100)	1 x fluazinam (200) 2 x mandipropamid + fluazinam (125 + 100) 2 x fluazinam (200)	3 x fluazinam (200)	R+S/S/R+S/S					√
2 x fluopicolide + propamocarb HCl (100 + 1000)	1 x fluazinam (200) 2 x fluopicolide + propamocarb HCl (100 + 1000) 2 x fluazinam (200)	3 x fluazinam (200)	I/S/I/S					√

[a] all applications were made at approx. 7-d intervals; [b] used in Figures 1 and 2, F = 'Fubol Gold WG'; S = 'Shirlan'; R = 'Revus'; I = 'Infinito'

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 (Anonymous, 1976) with added 0.01% and 10% categories. Plots were desiccated with diquat dibromide ('Reglone', Syngenta) in late August or early September within 7 days of the final fungicide application and tubers harvested

in September or October, at least two (and usually three) weeks after desiccation. 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 in late January-February, after which the final marketable yield was determined.

All data were subjected to analyses of variance with angular transformations of means used for percentage data. In each trial, the Area Under the Disease Progress Curve (AUDPC) was calculated from the untransformed percentage foliage blight for each plot and compared by analysis of variance.

RESULTS

Foliage blight development is shown in Figures 1 and 2. The data in Table 3 should be used for statistical comparisons.

Table 3. *Field trials for the control of potato blight, 2005-2009: final foliage blight, area under the foliar disease progress curve, tuber blight and yield assessments*

Year/Treatment[a]	Final foliar blight (% ang. trans.[b])	AUDPC[c]	Tuber blight (% ang. trans.[b] by number)	Total yield >35 mm (kg/plot)	Marketable yield (kg/plot)
2005					
F/S_L	25.9	254	7.1	59.4	53.8
F/R/S	11.1	40	7.9	62.5	56.1
L.S.D. (*P*<0.05)	7.52	135.7	2.90	n/a	n/a
2006					
F/S_L	32.2	236	21.7	44.6	35.7
F/R	24.1	143	31.3	42.0	27.4
L.S.D. (*P*<0.05)	5.65	95.5	n/a	3.16	6.41
2007					
F/S	32.8	471	8.0	52.3	49.2
F/R/S/R/S	14.2	120	13.8	53.4	47.6
F/R+S/S	10.6	57	9.4	55.2	51.7
L.S.D. (*P*<0.05)	9.71	292.1	4.37	n/a	n/a
2008					
F/S	27.6	130	27.1	46.6	33.2
F/R/S/R/S	12.7	30	26.6	47.5	34.2
F/R+S/S	10.5	19	24.5	47.1	35.4
L.S.D. (*P*<0.05)	10.82	102.0	n/a	n/a	n/a
2009					
F/S	20.1	45	7.6	35.5	33.0
R/S/R/S	14.0	18	4.5	35.9	34.0
R+S/S/R+S/S	10.2	4	7.6	41.0	38.4
I/S/I/S	20.7	120	6.7	38.2	35.8
L.S.D. (*P*<0.05)	4.60	58.0	n/a	n/a	n/a

[a] *treatments as defined in Table 1; F = 'Fubol Gold WG'; S = 'Shirlan'; R = 'Revus'; I = 'Infinito'*
[b] *% ang. trans. = angular transformed percentage data;* [c] *Area Under the Disease Progress Curve for foliage blight development*
n/a = not applicable; no significant effect of treatment (P>0.05)

2005 – 2006 TRIALS

In 2005 and 2006, the programmes in which metalaxyl-M + mancozeb was followed by mandipropamid (5 mandipropamid applications followed by 3 x fluazinam in 2005 and 8 mandipropamid applications in 2006) had significantly less foliage blight at the end of the season and lower AUDPC values than the standard programme (Table 3). In 2005, little tuber blight developed and there were no significant differences in terms of either total or marketable yield. In 2006, much more tuber blight developed and although differences in percentage tuber blight were not significant, the marketable yield from plots receiving the programme including mandipropamid was significantly lower than that from the standard; this was associated with losses from tuber blight.

2007 – 2008 TRIALS

In 2007 and 2008, programmes in which applications 3 and 4 and 6 and 7 of the standard programme were substituted with mandipropamid alone or mandipropamid tank-mixed with fluazinam were evaluated (Table 2). In both years, the two programmes including mandipropamid had significantly less foliage blight and lower AUDPC values than the standard (Table 3), with the programme including mandipropamid tank-mixed with fluazinam having the least infection. In 2007, although relatively little tuber blight developed, the programme including mandipropamid alone had significantly more blighted tubers than the standard or the programme containing mandipropamid tank-mixed with fluazinam.

In 2008, much more tuber rotting developed, but there were no significant differences between treatments in terms of tuber blight; assessments were complicated by the presence of many rots caused by pathogens other than *P. infestans*, particularly pink rot caused by *P. erythroseptica*, which developed after plots were waterlogged by very high rainfall during August. In neither 2007 nor 2008, were there significant differences between treatments in terms of yield, although in both years plots receiving the programme including mandipropamid tank-mixed with fluazinam had the greatest marketable yield.

2009 TRIAL

In 2009, the increasing incidence of phenylamide-resistant A2 strains of *P. infestans* prompted evaluation of options in which the two applications of metalaxyl-M + mancozeb at the start of the standard programme were replaced by two applications of either mandipropamid alone, mandipropamid tank-mixed with fluazinam or fluopicolide + propamocarb HCl. In each programme, these were followed by an application of fluazinam and then two more applications of the same products as were used at the start of the programme; all programmes were completed with five applications of fluazinam. Fluopicolide + propamocarb HCl was included at these positions in the spray programme at Syngenta's request to give a direct comparison with mandipropamid, although this is not when its use would normally be recommended. At the final assessments, the two programmes including mandipropamid had significantly less foliage blight than the standard or the programme containing fluopicolide + propamocarb HCl. The fluopicolide + propamocarb HCl programme had the largest AUDPC, which was significantly greater than those of the other three programmes. There were no significant effects of treatment on tuber blight or yield.
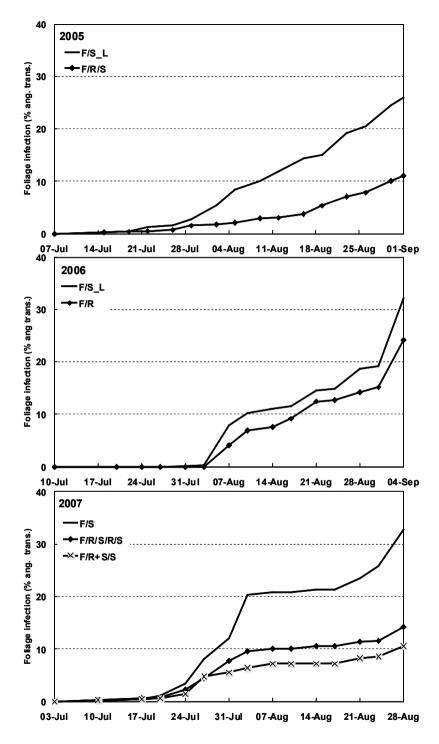
Figure 1. *Foliage blight development in field trials for the control of potato blight, 2005-2007 from the inoculation date until haulm destruction (for abbreviations, see Table 1)*
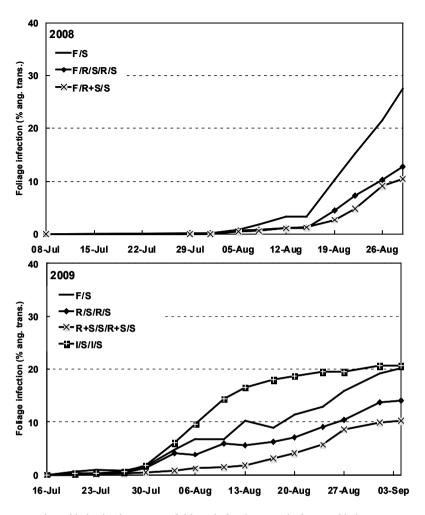
Figure 2. *Foliage blight development in field trials for the control of potato blight, 2008-2009 from the inoculation date until haulm destruction (for abbreviations, see Table 1)*

DISCUSSION

The difficulty of controlling potato late blight in Northern Ireland has been increased since 2005 by the arrival of fit A2 genotypes of *P. infestans* which are also phenylamide-resistant. Mandipropamid provides an alternative to metalaxyl-M, since, although not as systemic, it moves into plant tissue and helps to protect new foliage. In the trials reported here, mandipropamid gave excellent control of foliage blight and consistently out-performed the standard programme even in the trials from 2007 onwards in which fluazinam was applied at the 200 g a.i./ha rate. However, programmes including mandipropamid alone tended to be associated with more tuber blight than the standard programme, whereas programmes including mandipropamid tank-mixed with fluazinam were generally associated with less tuber blight. It was concluded that in regions of high rainfall such as Northern Ireland, where tuber blight is often a problem, mandipropamid should be used in a tank-mix with fluazinam to improve tuber protection.

Using the mandipropamid/fluazinam tank-mix at the start of the spray programme proved to be an effective alternative to metalaxyl-M + mancozeb. Fluopicolide + propamocarb HCl did not perform well when used at this position in the spray programme: previous trials have shown that this product is most effective when used mid-late season when its activity against tuber blight is particularly beneficial (Cooke & Little, 2007).

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Report of the fungicide sub-group meeting on 5 May 2010:

Discussion of potato blight fungicides,

their properties and ratings

RUAIRIDH A. BAIN

SAC, John Niven Building, Auchincruive Estate,
Ayr, Scotland KA6 5HW, UK

OBJECTIVES

The objectives of the sub-group meeting were:

- to discuss and approve new ratings for amisulbrom + mancozeb, initium + mancozeb and propamocarb + cymoxanil in the provisional table for late blight,
- to review the procedure for updating the fungicide tables with ratings calculated from trial results,
- to review the trial protocol for determining tuber blight ratings.

Before the discussion the following presentations were made to the subgroup.

Johnson SB	Post-Harvest Applications of Phosphorous Acid for Control of *Phytophthora infestans* on Potatoes
Edmonds J	Gowan and future developments with Zoxamide in Potato
Reimann S *et al.*	Initium - a new fungicide active ingredient for the control of Peronosporamycetes
Jilderda K, Reimann S & Tegge V	Initium based products for the control of *Phytophthora infestans*
Latorse M-P	Infinito: Protection of new growth from infection with *Phytophthora infestans*
Cooke LR & Little G	Evaluation of mandipropamid for the control of potato late blight in Northern Ireland
Desnouck J	The curative and eradicant activity of Proxanil
Schepers HTAM	Testing fungicides in the EuroBlight network

LATE BLIGHT

DISCUSSION AND AGREEMENTS REACHED

Approval of new ratings in the provisional table

The ratings proposed by the panel of experts in the seven countries for amisulbrom + mancozeb, initium + mancozeb and propamocarb + cymoxanil were agreed. The ratings are shown in Table 2 below.

Procedure for updating fungicide tables with ratings calculated from trial results

Products can be included for testing in the trials if they 1. don't already have a rating, in which case the rating is calculated from the results of six good trials or 2. already have a rating but the rating is re-calculated including the additional trial results.

Trial-based ratings in the main fungicide table on the Euroblight website will be updated after all of the sponsoring companies have approved the draft report covering that year's series of trials and the calculated ratings. It was agreed that comments on the draft report would be sought from the panel of experts in the seven countries at the same time as those from the sponsoring companies. Fungicide ratings based on trial results will therefore be updated in advance of Euroblight workshops except where there is a serious problem that requires the agreement of the Fungicide Subgroup. In this situation the problem will be resolved at the next meeting of the Fungicide Subgroup.

It was agreed, as an interim measure, that products would keep their calculated ratings for 7 years without the need for further trials. Seven years after a product was rated it will need to be included in three new trials. The seven-year period will be reconsidered at future meetings of the Fungicide Subgroup. If there is information that a fungicide has become, or is suspected of being, relatively less effective than its rating in the table due to changes in the *P. infestans* population, a statement to this effect will be included as a footnote to the fungicide table.

The procedures in 1.2 will from now on be included in the protocols for the leaf blight and tuber blight rating trials.

Changes to the plus ratings of fungicides can only be made at meetings of the Fungicide Subgroup.

Protocol for determining tuber blight ratings

In response to concerns that the incidence of tuber blight in all three trials in 2009 had been low, methods to encourage a higher incidence were discussed. It was agreed that blanket applications of fungicide could continue after the test fungicide treatments had started. These applications would dampen the foliar epidemic and therefore delay the separation of the disease progress curves for the test fungicides. Extending the use of the blanket fungicide could therefore allow more applications of the test fungicides, thereby extending the period of their application to later in the growing season when conditions are generally more favourable for zoospore release. In addition, an extended period of application for the test fungicides should result in more rainfall events to increase the number of tuber infection events.

The use of blanket sprays during the period that the test fungicides are applied will be left entirely to the discretion of the study director because he/she has the knowledge of prevailing local blight risk and experience of the variety used in the trial. Each week the study director will decide 1. whether to apply a blanket spray and 2. the dose rate.

After the subgroup meeting it was not sufficiently clear which fungicide(s) could be used for blanket applications. This was discussed further by those conducting tuber blight trials in 2010 and agreement reached that prior to the application of test fungicides the blanket spray can be either Dithane NT, or Curzate M where curative activity is required to prevent the foliar epidemic developing too quickly. However, only Dithane NT can be used from the time that the test fungicides applications are started. Also, the use of Curzate M as a blanket treatment should stop at least 7 days before the test treatments commence.

The timing and amount of irrigation will also be at the discretion of the study director.

Other agreements
It was agreed that at present trials to calculate ratings in the late blight fungicide tables should continue for leaf and tuber blight efficacy but should not be extended to include other characteristics of fungicides.

The subgroup agreed with the proposal made in Session 4 of the Arras workshop that Euroblight should prepare a common statement on the implementation of IPM within the EU with respect to control of late and early blight. It was agreed that a short (1 page), simple statement would be drafted and circulated among Euroblight members prior to submission to the EU.

There was agreement that the provisional late blight fungicide ratings table should be placed on the Euroblight website.

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. Table 1 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 given in all columns, except the one for leaf blight, are the opinion of the fungicides sub-group at the Arras blight workshop, 2010 and are based on field experiments and experience of the performance of products when used in commercial conditions. Ratings for leaf blight were calculated from the results of 13 Euroblight field trials during 2006-2009, and only compounds included in a minimum of six of these trials are rated for leaf blight. The scale for leaf blight is a 2-5 scale, to one decimal place. All other ratings are on a 0 to +++ scale, using (+) to indicate half marks. The ratings are intended as a guide only and will be amended in future if new information becomes available. Table 1 is available on the Euroblight website, www.euroblight.net/Fungicide/FungicideComparison.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 the consensus view of the fungicide sub-group and 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.

DEFINITIONS AND DISCLAIMER (REPRODUCED FROM THE TALLINN 2005 PROCEEDINGS)

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 that 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 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.

DISCLAIMER

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.

EARLY BLIGHT (*ALTERNARIA SOLANI* AND *ALTERNARIA ALTERNATA*)

There was agreement that the *Alternaria* fungicide tables should be placed on the Euroblight website. In the *Alternaria* tables one column to cover the efficacy of fungicides against both species was currently still appropriate.

At the very end of the subgroup meeting BASF requested that Signum (pyraclostrobin + boscalid) should be moved from the provisional *Alternaria* table to the main *Alternaria* table. This was not discussed at the Subgroup meeting. The panel of fungicide experts in the seven countries was consulted after the workshop on whether they could agree to Signum being moved from the provisional table to the main table. All agreed and it was therefore proposed to include Signum in the main table. This proposal was approved by the Fungicide Subgroup members (by e-mail).

Table 1. The effectiveness of fungicide products/co-formulations for the control of P. infestans based on the highest rate registered in Europe

| Product[1] | Effectiveness | | | | | Mode of Action | | Rainfastness | Mobility in the plant |
	Leaf Blight[2]	New growth	Stem blight	Tuber blight	Protectant	Curative	Anti-sporulant		
copper		?	+	+	+(+)	0	0	+	contact
dithiocarbamates[3]	2.0	?	+	0	++	0	0	+(+)	contact
chlorothalonil		?	(+)	0	++	0	0	++(+)	contact
cyazofamid	3.8	++	+	+++	+++	0	0	+++	contact
fluazinam	2.9	?	+	++(+)	+++	0	0	++(+)	contact
zoxamide+mancozeb	2.8	?	+[5]	++	+++	0	0	++(+)	contact+contact
famoxadone+cymoxanil		?	+(+)	N/A	++	++	+	++(+)	contact+translaminar
mandipropamid	4.0	++	+(+)	++[5]	+++	+[6]	+(+)	+++	translaminar+contact
benthiavalicarb+mancozeb	3.7	?	+(+)5	+(+)	+++	+(+)	+	++(+)	translaminar+contact
cymoxanil+mancozeb		?	+(+)	0	++	++	+	++	translaminar+contact
cymoxanil+metiram		?	+(+)	0	++	++	+	++	translaminar+contact
cymoxanil+copper		?	+(+)	0	++	++	+	++	translaminar+contact
dimethomorph+mancozeb	3.0	?	+(+)	++	++(+)	+	++	++(+)	translaminar+contact
fenamidone+mancozeb	2.6	?	+(+)5	++	++(+)	0	+(+)[5]	++	translaminar+contact
benalaxyl+mancozeb[4]		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+mancozeb[4]		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+fluazinam[4]		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
propamocarb-HCl+mancozeb		+(+)	++	++	++(+)	++	++	+++	systemic+contact
propamocarb-HCl+chlorothalonil	3.4	+(+)	++	++	++(+)	++	++	+++	systemic+contact
propamocarb-HCl+fenamidone	2.5	+(+)	++	++	++(+)	++	++	+++	systemic+translaminar
propamocarb-HCl+fluopicolide	3.8	++	++	+++	+++	++	++(+)	++(+)	systemic + translaminar

[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]Based on Euroblight field trials in 2006-2009. [3]Includes maneb, mancozeb, propineb and metiram.. [4]See text for comments on phenylamide resistance. [5]Based on limited data. [6]In some trials there were indications that the rating was +(+).

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.
The scale for leaf blight is a 2-5 scale (2 = least effective, 5= most effective).
Disclaimer : this is given in the text of this paper.

Table 2. *Provisional ratings[1] for the effectiveness of new fungicide products for the control of* **P. infestans** *in Europe. These ratings are the opinion of the Fungicides Sub-Group at the Arras blight workshop, 2010 and are based on field experiments and not experience in commercial potato production.*

| Product | Effectiveness | | | | Mode of Action | | | Rainfastness | Mobility in the plant |
	Leaf blight	New growth	Stem blight	Tuber blight	Protectant	Curative	Anti-sporulant		
amisulbrom + mancozeb	?	?	+	++(+)[2]	++(+)	0	?	+++	contact + contact
initium + mancozeb	3.6[3]	?[4]	?[4]	++	++(+)	0	0	+++	contact + contact
propamocarb + cymoxanil	?	?	?	?	+(+)	++(+)[5]	++(+)	?	systemic + translaminar

[1]*The ratings for individual products 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; an efficacy greater than ++(+) was observed in some trials.* [3]*Calculated from Euroblight trials* [4]*Observations from some field trials indicated that both new growth and stem blight efficacy were ++.* [5]*In some trials the curative activity was ++.*

Key to ratings: *0 = no effect; + = reasonable effect; ++ = good effect; +++ = very good effect; ? = no experience in trials and/or commercial*

Breeding for host resistance: the key to sustainable potato production

SIMON WHITE AND DAVID SHAW

Sárvári Research Trust, Henfaes Research Centre, Abergwyngregyn, Llanfairfechan, LL33 0LB, UK

SUMMARY

Phytophthora infestans (Mont.) de Bary, the causal agent of late-blight disease of potato, continues to be a major limiting factor to potato production worldwide. Field trials in 2009, inoculated with two highly aggressive and commonly occurring blight strains, Blue 13 and Pink 6, demonstrated that Sárpo Mira and Axona possess high levels of resistance to these strains. Resistance in many previously resistant cultivars was severely eroded when challenged with these new genotypes. A comparison of results from eighteen trial sites across Europe over the past six years shows that Sárpo Mira has maintained high levels of resistance to all blight populations. Durable resistance such as this should enable world potato production to become more sustainable.

KEYWORDS

Phytophthora infestans, late blight, host resistance, durable resistance.

INTRODUCTION

THE NEED FOR HOST RESISTANCE

Although late-blight disease can be effectively controlled in most situations it is becoming increasingly apparent that sole reliance upon fungicide application is not appropriate. The extremely wet summers in 2007/08 in Western Europe prevented routine applications to many crops on waterlogged soils. Even in "normal" years, control is proving more difficult as new pathogen populations are often highly resistant to the once widely used phenylamide group of fungicides. Increased public concern over residues in food and the large carbon footprint of intensive agriculture have led to increased pressure to reduce inputs at all stages of the food supply chain. Organic growers are still reliant upon preparations of copper for blight control but the use of copper in such systems is an anomaly and is being questioned more and more. Finally, many commonly used fungicides, including mancozeb, widely used in mixtures to prevent emergence of resistance, are scheduled for withdrawal under recent EU directives.

While it is clear from the above that host resistance is highly desirable in the developed world, it is in the developing world where the deployment of cultivars (cvs) with confirmed, broad-spectrum resistance would hugely increase crop yield. In many countries, growers risk crop failures when

chemical applications and advice are not affordable or unavailable. Late blight has devastating effects on yield in these circumstances (see Fig 1) and the use of host resistant cvs would go some way to alleviating this.

Potato yield, China

Yield(t/ha)

Virus

Clean seed

Late blight

National average
(14.5 t/ha)

Slide supplied by Pamela Anderson, DG, International Potato Centre, Peru
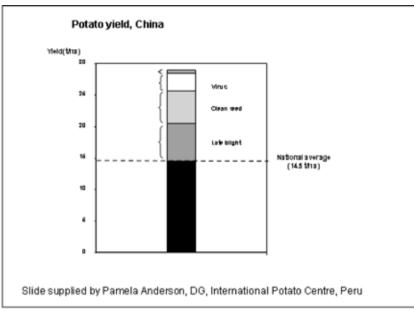
Figure 1: *potato yield in China showing the estimated effect of late blight on yield (courtesy CIP)*

SOURCES OF RESISTANCE

The first efforts to develop blight resistant cvs followed the devastating epidemics that led to the Irish Potato Famine in 1845 - 50 (Large, 1940; Salaman, 1949). These initial efforts resulted in cvs with partially successful field resistance, also known as quantitative or partial resistance (Wastie, 1991). The discovery of the major (R) genes in *Solanum demissum*, a wild species originating from Mexico, was seen by many as the "cure" for potato late blight (Reddick, 1934; Black, 1970) and became the focus of most breeding programmes during the first half of the 20th century. However, it was not long before strains emerged within pathogen populations that were able to overcome these R-genes (Malcolmson, 1969). Even the "pyramiding" of several R-genes into the same potato cv proved non-durable. The cv Pentland Dell, containing R1, R2 and R3, was initially immune to the U.K. population of *P. infestans*. Only four years after the start of commercial production, as the area planted increased, strains compatible with all three R-genes became common in the UK and control failed (Malcolmson, 1969).

As a result of the failure of available R-genes to provide durable resistance to late blight, breeders started to select for quantitative field resistance, either by selecting with races compatible with the R-genes in their material or by creating entirely R-gene free germplasm (Toxopeus, 1964). Black defined field resistance as the degree of resistance exhibited by a plant to all races of *P. infestans* to which it is not hypersensitive (Black, 1970). Black demonstrated that field resistance could be built up rapidly through hybridizations using appropriate breeding material (Black, 1970). Seedling selections had complex pedigrees tracing back to *S. demissum* but also included breeding material derived from *S. phureja* and *S. microdontum*.

Subsequent breeding programmes have utilized a wide range of wild *Solanum* spp., both as sources of major R-genes and also as part of field-resistance breeding strategies (Hawkes, 1990). Most recently, Rb genes from *S. bulbocastanum* have been used in both classical and transgenic breeding programmes and gene *Rpi-vnt1.1* from *S. venturii* has been identified and cloned (Foster *et al.*, 2009) with transformed plants of cv. Desiree containing this gene being field trialled in the UK for the first time this year. Whether or not these novel resistant lines prove durable remains to be seen.

A recent breakdown of resistance in many previously resistant cultivars has occurred in the last few years as new strains of the pathogen have become common in U.K. (Lees *et al.*, 2008). This paper aims to compare the resistance of two Sárpo cvs with that of several other resistant cvs to new strains of blight (Blue 13 and Pink 6) in field trials in 2009 and to examine the resistance of Sárpo Mira in other trials in Europe over the last few years.

MATERIALS AND METHODS

To assess the foliar resistance in a number of commercially available blight resistant cultivars as well as in Sárpo Mira and Axona, inoculated field trials were used.

Field trials in 2009 were carried out at two sites, Llanbedrgoch and Henfaes, both in North Wales. Both trials were planted in randomised 25-tuber plots and were triplicated at Llanbedrgoch and duplicated at Henfaes. Each trial contained Sárpo Mira and Axona as well as eight commercially available cvs, all purporting to have high resistance to late blight (NIAB blight rating >5 on a 1-9 score of resistance (NIAB potato variety guide, 2007). Susceptible and resistant Eucablight standard cvs, Bintje and Robijn respectively were included.

The Llanbedrgoch trial was inoculated with an isolate of strain Blue 13 (SSR genotype 13_A2) and the trial at Henfaes with an isolate of strain Pink 6 (SSR genotype 6_A1), these two genotypes representing the predominant strains within the UK population (Cooke *et al.*, 2008). The central plant of each plot (plant 3 in row 3) was sprayed with 4×10^4 sporangia in suspension on 13th July. Inoculated plants were bagged in plastic overnight to maintain leaf wetness and promote rapid infection. No spreader rows were used in these trials.

Scoring of the percentage of foliar late-blight in both trials was according to Cox & Large (1960). Observations were made at 3-5 day intervals. Relative Area Under Disease Progression Curve (rAUDPC) values for all cvs in each trial were calculated (Fry, 1978).

Both trials were managed under conventional agronomic practices and had received similar rates of NPK fertilizer prior to planting. Weeds were controlled with a pre-emergence application of Defy (prosulfocarb) at 5 l/ha.

RESULTS

2009 FIELD TRIALS

Llanbedrgoch, North Wales inoculated with strain Blue 13.
Highly blight-conducive conditions allowed rapid establishment and progression of the disease through plots of susceptible cultivars (cvs). Bintje, the Eucablight susceptible standard variety, had reached 90% foliar infection thirteen days post inoculation. Non-Sárpo cvs showed low levels of resistance whereas Sárpo Mira and Axona showed a slow-blighting phenotype with Robijn showing intermediate resistance (Fig. 2).
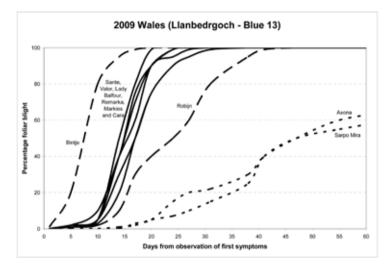
Figure 2. *Progression of foliar blight at Llanbedrgoch, North Wales in 2009 inoculated with strain Blue 13. Disease progression curves are shown for Eucablight standard cvs, Bintje (susceptible) and Robijn (resistant) and six commercially available blight-resistant cvs, all with an official NIAB foliar blight rating of five or above (source: NIAB potato variety guide 2007) and the Sárpo cvs Axona and Sárpo Mira.*
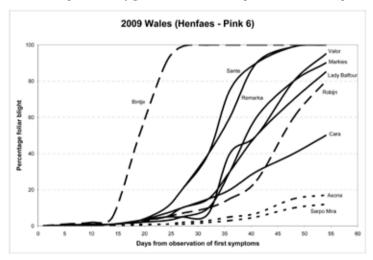
Fig. 3. *Progression of foliar blight at Henfaes Research Centre, North Wales in 2009 inoculated with strain Pink 6. Disease progression curves are shown for Eucablight standard cvs, Bintje (susceptible) and Robijn (resistant) and six commercially available blight-resistant cvs all with an official NIAB foliar blight rating of five or above (source: NIAB potato variety guide 2007) and the Sárpo cvs Axona and Sárpo Mira. Henfaes Research Centre, North Wales, inoculated with strain Pink 6.*

Similarly, conducive weather conditions enabled rapid establishment of blight. However, the subsequent progression was much slower than in the Llanbedrgoch trial (Fig. 3). Bintje reached 90% foliar infection twenty five days post inoculation, almost twice as long as it took when inoculated with strain Blue 13. Non-Sárpo cvs showed a range of rates of slow blighting and Sárpo Mira and Axona clearly showed higher levels of foliar resistance.

DURABILITY OF RESISTANCE OF SÁRPO MIRA

Sárpo Mira has been included in our field trials in Wales and Cornwall since 2004. Foliar resistance scores for this cv are shown in Table 1, calculated via the Eucablight website (www.eucablight.org) or from unpublished data of the Sárvári Research Trust.

Table 1. *rAUDPC values and 1-9 scores (where available) for Sárpo Mira, 2004-09.*

Year	Location	SSR Genotype (where known)	rAUDPC	1-9 score
2004	Wales (Llanbedrgoch)	Unknown A1	0.00	
2006	Wales (Llanbedrgoch)	Unknown	0.00	
2007	Cornwall (Duchy College)	13_A2	0.00	9.0
2007	Wales (Henfaes Research Centre))	13_A2	0.06	9.1
2007	Wales (Llanbedrgoch)	13_A2	0.06	
2008	Wales (Llanbedrgoch)	13_A2	0.25	8.1
2009	Wales (Henfaes Research Centre)	6_A1	0.02	8.6
2009	Wales (Llanbedrgoch)	13_A2	0.31	9.1

It is clear that even when challenged by isolates of strain Blue 13, Sárpo Mira expressed high or very high resistance.

Sárpo Mira has also been included in trials in many other countries in Europe over this period and has therefore been exposed to a wide range of strains *of P. infestans*. Scores for foliage blight calculated via www.eucablight.com (Fig. 5) are consistently high and rarely drop below 8.
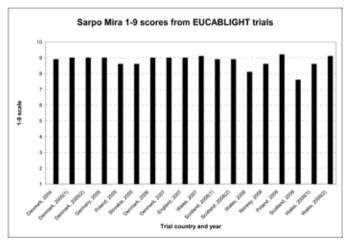
Fig. 5 *Eucablight 1-9 scores for Sárpo Mira from trial sites across Europe, 2004-09. Denmark (1) represents the Vejle site; (2) Vestsjaelland site, Scotland (1) and (2) are both sites in South Ayrshire and Wales (1) and (2) correspond to Llanbedrgoch and Henfaes respectively.*

Resistance scores, as represented by rAUDPC, for Sárpo Mira, Lady Balfour and Bintje from 2004 until 2009 (Fig. 6) show the increase in susceptibility in Lady Balfour from 2007 to close to that of Bintje where strain Blue 13 was introduced to the trials or was suspected to be present. Comparative scores for Sárpo Mira also increased but remained usefully resistant. It should be pointed out that susceptibility of Lady Balfour was high in the trial in 2004 even before strain Blue 13 was detected in North Wales.
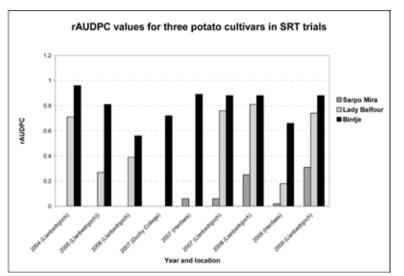
Fig. 6. *rAUDPC values for cvs Sárpo Mira, Lady Balfour and the susceptible standard, Bintje from trials over several years. Lady Balfour was not included in trials at Duchy College, Cornwall or at Henfaes in 2007. Sárpo Mira was not included at Llanbedrgoch in 2005. Sárpo Mira had an rAUDPC value of 0 in the trials at Llanbedrgoch in 2004 and 2006 and in the trial at Duchy College, Cornwall in 2007.*
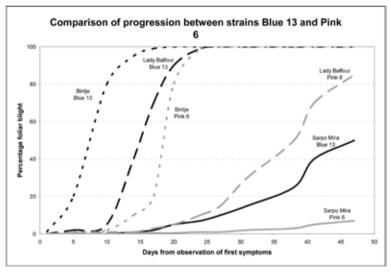
Fig.4. *Disease progress curves for three cultivars inoculated with the Blue 13 and Pink 6 strains from the trials in 2009.*

DISCUSSION

The slower disease progressions with one isolate of strain Pink 6 compared with that of one of strain Blue 13 may have been partly due to environmental factors. The trial site with strain Pink 6 had a lower rainfall and was more exposed to prevailing winds which undoubtedly reduced leaf wetness relative to that at the Blue 13 site. Also, there is a possibility that the isolate of strain Pink 6 used to inoculate the trial had a lower aggressiveness than other isolates of Pink 6 or that it had lost some of its pathogenicity due to a period in pure culture.

In the Henfaes trial inoculated with Pink 6, the resistant cvs showed various degrees of slow blighting compared to the Bintje control. Sárpo Mira and Axona were clearly the most slow blighting cvs (Fig 3). In contrast, with strain Blue 13, Sante, Valor, Lady Balfour, Markies and Cara showed some resistance until day 10 after first symptoms appeared, then progressed as rapidly as Bintje. Axona and Sárpo Mira still showed a distinct slow-blighting phenotype, albeit not as slow as with Pink 6 and Eucablight resistant control retains an intermediate slow-blighting phenotype (Fig. 2).

In conclusion, our trials show that the partial resistance of several popular cvs is eroded by strain Blue 13 which is now common and widespread in U.K. and NW Europe.
While Sárpo Mira and Axona were more susceptible to Blue 13, they have continued to retain a useful slow-blighting phenotype under extremely high blight pressure. The resistance of Sárpo Mira may be mainly due to a gene or genes within an R-gene cluster on chromosome 11 (Jadwiga Śliwka and Iga Tomczynska, personal communication). So far, this resistance has proved durable to new strains of *P. infestans*, including strain Blue 13. But if Sárpo Mira and Axona come to be grown on a large scale, there is a danger that compatible virulence genotypes may become selected in the pathogen population.

Now that new populations of *P infestans* with increased ability to overcome certain resistance genes have become common in NW Europe, the published official 1 – 9 scores used by growers to select varieties need to be updated. Table 2 compares the foliar blight scores published in Great Britain by National Institute of Agricultural Botany (NIAB) for a range of resistant cvs and the values calculated via Eucablight for our trials in 2008 and 2009

Table 2. *1-9 scores for a range of commercially available blight resistant cultivars (9=resistant, 1=susceptible). Values from field trials were calculated by Eucablight from Sárvári Research Trust field trial data.*

Cultivar	NIAB score	2008 (Blue 13)	2009 (Blue 13)	2009 (Pink 6)
Sárpo Mira	9	8.1	9.1	8.6
Axona	7	6.3	8.7	8.4
Lady Balfour	8	3.4	4.7	6.3
Markies	7	4.3	5.2	6.3
Orla	8	3.7	4.4	3.8
Sante	7	3.1	4.3	3.8
Cara	6	3.5	5.3	7.0
Valor	5	3.5	4.8	4.9

CONCLUSIONS

Sárpo Mira and Axona continue to show high resistance to all blight populations they have been exposed to, both in the UK and Europe over the past ten years as new aggressive strains have evolved. How durable this resistance is cannot be predicted.

Any future breeding, whether classical or transgenic, must be aware of the lessons of history when attempting to produce durable resistance to late-blight in potato cultivars. Previously resistant cvs have been overcome quickly and the ability of *P. infestans* to evolve is now potentially greater with the presence of both mating types in most countries where blight is a problem. Fry (2008) states that "any strategy for mitigating pathogenicity needs to be based on a knowledgeable respect for the powerful plasticity of this organism". This "powerful plasticity" remains as much of a challenge as ever.

ACKNOWLEDGEMENTS

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Phenotypic and genotypic characteristics of Algerian isolates of *Phytophthora infestans*

ROSELYNE CORBIERE[1], FATMA ZOHRA REKAD[2], AMINA GALFOUT[3],
DIDIER ANDRIVON[1] & ZOUAOUI BOUZNAD[3]

[1]INRA, UMR Inra - Agrocampus Ouest - Université Rennes
I - 1099 BiO3P, BP 35327, 35653 LE RHEU, France
[2]Abdelhamid Ibn Badis University – Site 1 – Route de Belahcel –
BP 300 - MOSTAGANEM – Algeria
[3]Ecole Nationale Supérieure Agronomique (ENSA) – 16200 El Harrach - ALGIERS - Algeria

SUMMARY

Late blight is one of the most important biotic constraints to potato production in Algeria. Characterization of Algerian *P. infestans* isolates collected on potato and tomato during 2007 to 2009 was performed for mating type, metalaxyl sensitivity, virulence on a set of potato *R*-gene differentials, *in vitro* mycelial growth on agar medium at five temperatures, aggressiveness on leaflets of four potato cultivars and genotypic diversity with 11 microsatellite loci. All isolates from potato but two were A2 mating type, and most of them overcame all 11 *R*-specific genes from the international differential host set; all A2 isolates were metalaxyl resistant. The two isolates collected from tomato were A1 and metalaxyl sensitive. The presence of both mating types in *P infestans* in Algeria raises the possibility of the formation of oospores. Mycelial growth was fastest at 19°C for all the isolates, but A1 isolates from potato and tomato grew significantly faster than A2 isolates at 27°C. No aggressiveness differences were noticed between A1 and A2 isolates, but the relative rankings of the isolates changed according to the cultivar and to the component (lesion area, sporangia production) measured. Cvs. Bintje and Spunta (the dominant cv. in Algeria) were highly susceptible. By contrast, the sporulation of the isolates was relatively low on the moderately susceptible cvs Désirée and Atlas. SSR markers revealed a large genotypic diversity and distinct genetic groups, according to mating types and host plants. Phenotypic and genotypic traits suggest that the A2 Algerian population of *P. infestans*, collected from potato, could be closely related to Western European populations.

INTRODUCTION

Late blight is one of the main biotic constraints to potato production in Algeria. A nation-wide crop failure due to late blight was observed in 2007, and regional outbreaks are annually noticed on both potato and tomato crops in Western and Central Algeria. In these major potato production areas, the climate is favourable for *Phytophthora infestans* and severe late blight epidemics occur during spring and autumn, as two main potato crops are grown during a calendar year. Furthermore, in some areas, potato and tomato are grown in close proximity to each other, and volunteers and refuse piles are often observed in or close to the fields. These factors lead to the potential for late blight and aerial inoculum at any time of the year. The severity of blight epidemics is further fuelled by a number of additional causes, such as incorrect spray programs (no preventive application and excessive use of phenylamine fungicides), overhead irrigation, absence of crop rotation, and the widespread use of susceptible cultivars, such as Spunta (the dominant cv. in Algeria). New *P. infestans* populations, and/or presence of oospores in soil, making possible early attacks and the survival of the pathogen outside its host, over several years, may also worsen the severity of late blight epidemics. Algeria annually imports 100 000 tons of potato seeds, primarily from the Netherlands (58%), France (16%) and Denmark (13%), and part of these are multiplied locally in the spring for the autumn potato crop. Latently infected seed tubers are thus an important potential source of primary inoculum for late blight epidemics, and European *P. infestans* isolates might have been introduced in Algeria through such seed tubers. Except a study on a small number of Algerian isolates (Beninal *et al.*, 2009), very little is known about the population characteristics of *P. infestans* in Algeria. However, information on the pathogen population structure is a prerequisite for understanding the epidemiology of the disease and for selecting durable disease resistance sources for crop breeding. Therefore, we intended to further characterize Algerian *P. infestans* isolates collected on potato and tomato during 2007 to 2009, and compared them with some French isolates sampled on potato at the same period. We studied phenotypic and genotypic traits to determine : (i) the mating type ratio in the studied population ; (ii) the population level of metalaxyl sensitivity ; (iii) which *R*-genes are these Algerian isolates able to overcome; (iv) the effect of temperature on *in vitro* mycelial growth on agar medium ; (v) if these isolates differ from each other with respect to aggressiveness on four potato cultivars ; (vi) their genotypic diversity with microsatellite markers.

MATERIALS AND METHODS

Phytophthora infestans isolates

A total of 36 isolates was sampled in 2007, 2008 and 2009 from naturally infected potato and tomato crops in Western and Central wilayas (Algerian regions) between Algiers and Tlemcen (distant from about 450 km) (Table 1). Some French isolates, collected in 2008, were added for comparison with Algerian populations. Infected leaves or stems were collected from independent plants from different cultivars. Single-lesion isolates were obtained by placing 1 cm² pieces of infected tissue on tuber slices of a susceptible potato cultivar. Pure axenic cultures were then obtained by transferring small pieces of mycelium growing on the upper side of the potato slice on pea agar medium, and subsequently maintained in darkness by serial transfers on pea agar medium.

Mating type determination

The mating-type of each isolate was determined by individually pairing them on pea agar with known A1 and A2 testers. After 10-14 days incubation in darkness at 15°C, the presence or absence of oospores was recorded under a microscope.

Effect of temperature on *in vitro* mycelial growth

Radial growth of mycelium on agar medium was used to assess behavior of the isolates at several temperatures. Mycelial growth was compared at five constant temperatures (11, 15, 19, 21 and 27°C) for nine Algerian isolates (2 from tomato and 7 from potato) and two French isolates (1 A1, 08-P13-02 and 1 A2, 08-P13-08). Agar plugs (8 mm diameter) were cut from the edge of 2-week old cultures and placed in the center of Petri dishes (90 mm diameter), containing pea agar medium. All Petri dishes were prepared on one day with the same medium. There were three replicate dishes per isolate and temperature. Cultures were placed together in a plastic box and incubated in darkness at each temperature. After seven days, the size of each colony was measured along two perpendicular directions. After the diameter of the mycelium plug was substracted, the two measurements were averaged. The experiment was carried on for one further week at the two extreme temperatures (11°C and 27°C), as cultures had not reached the edge of dishes after 7 days, and the colony diameters were measured again as described above. Data were subjected to analysis of variance (ANOVA) using SAS statistical software. Whenever significant effects were detected, means were compared using the Student-Newman and Keuls test.

Pathogenicity tests

Potato plant material

Plants from potato genotypes were grown from seed tubers in pots filled with 1:1:1 sand-peat-compost mixture, in a glasshouse regulated at 15-20°C. They were watered with a nutrient solution (Hakaphos; NPK 15/10/15) once a week. Leaflets were collected for experiments on 6-8 week-old plants.

Virulence patterns were determined using Black's differential set of potato clones, each having one of the *R1-R11* pathotype-specific resistance genes, and Bintje as susceptible cultivar. This set was originally provided by the Scottish Agricultural Science Agency (SASA, Edinburgh, UK) and seed tubers were multiplied by INRA (UMR APBV, Ploudaniel, France). Recent work has shown that the *R3* differential (CEBECO-4642-1) actually contains two closely linked genes, *R3a* and *R3b* (Huang *et al*., 2004). Virulence to each of these could not be assessed separately, due to the lack of available differential hosts with only one of these two genes. Therefore, virulence to *R3* was regarded as a single factor.

Aggressiveness was quantified on four potato cultivars: Bintje (highly susceptible to late blight in Europe and not cultivated in Maghreb), Spunta (susceptible and dominant in Algeria), Désirée and Atlas (moderately susceptible with partial non-specific resistance, and grown in Algeria).

Inoculum preparation

Each isolate was multiplied separately on detached cv. Bintje leaflets. Leaflets were stored abaxial side up on the lids of inverted Petri dishes containing 1% water agar. They were infected by deposing a 20µL drop of suspension of *P. infestans* sporangia collected by flooding a 3-week-old culture with 5-6 mL sterile distilled water and gently scraping the colony surface to remove sporangia. Prior to inoculation, sporangial suspensions were chilled at 4 °C for at least two hours to promote zoospore release. Dishes containing the inoculated leaflets were deposited in clear plastic boxes, in an illuminated incubator. After seven days of incubation in humid chambers under controlled conditions (15°C/18°C night/day temperatures, 16h daylight), the sporangia produced on infected leaflets were collected in sterile water ; the resulting suspensions were adjusted to 5 x 10^4 sporangia mL^{-1}, chilled at 4°C for two hours, and used for pathogenicity experiments.

Metalaxyl resistance test

The sensitivity to metalaxyl of 28 Algerian isolates and 5 French isolates was assessed in a floating leaf disk bioassay as previously described (Beninal *et al*., 2009). Sensitivity was tested with metalaxyl

(Ridomil 25 WP, Novartis experimental compound) at concentrations of 10 and 100 mg L^{-1}. Isolates sporulating on the disks floating on water containing 100 mg L^{-1} metalaxyl were rated as resistant, those on 10 mg L^{-1} were rated as intermediate and those that sporulated only on water were rated as sensitive.

Virulence phenotype determination

A total of 19 Algerian isolates collected in 2007 and 2008 (2 from tomato, 17 from potato) were tested and compared to 7 French isolates (4 A1 and 3 A2). Each leaflet was placed abaxial face up on a moist filter paper in a clear plastic dish and inoculated by depositing a 20 µL drop of the sporangial suspension on each side of the midrib. Two leaflets per isolate and differential host were inoculated. After incubation as described above, each inoculation site was scored for the presence or absence of a sporulating lesion and interaction was considered compatible if sporangiophores were visible.

Aggressiveness quantification

Aggressiveness was performed with 13 isolates : 2 Algerian isolates from tomato, 7 Algerian isolates from potato and 4 French potato isolates tested for comparison (2 A1 named 08-P15-02 and 08-P43-01, and 2 A2 named 08-P13-11 and 08-PON01-01). Experiment was conducted on four potato cultivars, Bintje, Spunta, Désirée and Atlas, chosen according to their level of susceptibility to late blight and grown in Algeria, except cv. Bintje, used as reference. Six leaflets were inoculated for each isolate-cultivar combination. Each leaflet was placed abaxial face up on the lids of inverted Petri dishes containing 10 g L^{-1} water agar (two leaflets per dish), and inoculated by depositing a 20 µL drop of sporangial suspension (about 1 000 sporangia) at the leaflet center. Infected leaflets were incubated for six days as described before. Lesion area (LA, in cm^2) was measured with a image analyser and the Histolab software (Microvision Instruments, Evry, France). Each leaflet was washed in 10 mL saline buffer (Isoton II), and sporangia were counted with a Beckman Coulter Z2 counter (Villepinte, France) to determine sporangia production per lesion (SP). The spore capacity (SC) was then calculated as the mean number of sporangia produced per cm² of lesion. Data were subjected to analyses of variance using the general linear models (GLM) procedure of the SAS statistical software. Whenever significant effects were detected, means were compared using the Student-Newman and Keuls test.

DNA extraction and microsatellite amplification

Eighteen Algerian isolates (2 from tomato and 16 from potato) were grown separately in pea broth, previously autoclaved for 20 min at 120°C. After three weeks of incubation at 15°C, mycelium was washed three times in sterile water, and lyophilized. DNA was extracted using the Blood and Tissue 96 kit (Qiagen) according to the manufacturer's instructions, and stored at -20°C. Alleles at 11 polymorphic microsatellite loci - Pi4B, Pi4G and PiG11 developed by Knapova and Gisi (2002); and Pi02, Pi89, Pi04, Pi16, Pi33, Pi56, Pi63 and Pi70 developed by Lees *et al.* (2006) - were amplified in Polymerase Chain Reactions (PCR) performed in a 12,5 µL volume containing between 20 and 200 ng of DNA of *P. infestans*, 2.5 µL of 10X PCR Buffer (Promega), 0.3 mM of each dNTP, 2 mM of MgCl$_2$ and 0,5 U of Taq DNA polymerase (GoTaq, Promega). Concentrations of forward and reverse primers followed Eucablight protocols for SSR analysis of *P. infestans* (SCRI, Scottish Crop Research Institute, UK). In order to detect simultaneously the alleles at several loci, primers were labeled with fluorescent dyes and pooled into four panels : 1) Pi02, Pi89 and Pi4B, 2) PiG11, Pi04, Pi70; Pi56 and Pi63, 3) Pi16 and Pi33, and 4) Pi4G. PCRs were performed under the following conditions: the PCR started with a cycle of 5 min at 94°C, followed by 30 cycles of 20 s at 94°C, 25 s at 58°C, 30 s at 72°C, and finished with an elongation cycle of 5 min at 72°C. PCR products were added to deionized formamide loading buffer, and samples were loaded into an ABI Prism DNA sequencer run according to manufacturer's instructions (Applied Biosystems). DNA fragments were

automatically sized with the GeneMapper™ 3.5 software. Allele sizes were calibrated to the allele sizes of reference isolates and with SSR allele information sheet, kindly provided by D.E.L. Cooke (SCRI).

RESULTS

The A2 mating type is prevalent on potato, in Algeria
Of the 36 isolates collected between 2007 and 2009, 34 were from potato and 2 from tomato. All isolates from potato but two (GH-2007 collected in 2007 and AG7 in 2009) were of the A2 mating type. The two isolates sampled from tomato (ITC and AD) in 2008 were of the A1 mating-type (Table 1).

All A2 isolates from potato are resistant to metalaxyl
All 25 Algerian A2 isolates tested proved to be metalaxyl resistant, while the two A1 isolates from tomato were metalaxyl sensitive (Table 1). The A1 Algerian isolate from potato (GH-2007) had intermediate sensitivity, whereas the 4 French A1 isolates from potato were sensitive.

The virulence phenotypes are highly complex
The virulence spectrum in the Algerian isolates was highly complex, except for the A1 isolate from potato, GH-2007 (Table 1). The majority of the A2 isolates from Algeria overcame all 11 *R*-specific genes of the differential set, as did those from France sampled at the same period. Four A2 isolates, collected in 2007, were not virulent to *R9*, but they overcame all the other specific resistance genes. This pathotype was not found in 2008. The two A1 isolates from tomato also had complex profiles, but they were different from each other: ITC was avirulent to *R2*, while AD was avirulent to *R9*, as some A2 isolates from potato. The A1 isolate GH-2007 from potato showed the least complex pathotype, with 7 virulence factors; this pathotype (1.3.4.7.8.10.11) was very common in French A1 isolates (Corbière *et al.*, 2010).

Table 1. *Origin, mating-type, metalaxyl sensitivity and virulence profiles of P. infestans Algerian isolates collected from 2007 to 2009 and of French isolates.*

Isolate name	Date of isolation	Location (wilaya)	Cultivar	Mating type (MT)	Metalaxyl sensitivity *	Virulence profile on 11 R specific genes (from R1 to R11)
Algerian isolates from potato						
Z0	5/05/2007	Ain Defla	Spunta	A2	R	virulent to 11 genes
Z1	5/05/2007	Ain Defla	Martina	A2	R	**avirulent to *R9***
Z3	5/05/2007	Mostaganem	Spunta	A2	R	virulent to 11 genes
Z5	5/05/2007	Mostaganem	Spunta	A2	R	virulent to 11 genes
Z12	10/05/2007	Oran	-	A2	R	**avirulent to *R9***
Z13	10/05/2007	Tiaret	Atlas	A2	R	**avirulent to *R9***
Z18	10/05/2007	Tiaret	Atlas	A2	R	virulent to 11 genes
Z21	10/05/2007	Chlef	Désirée	A2	R	**avirulent to *R9***
GH- 2007	10/11/2007	Boutlelis (Oran)	Spunta	**A1**	I	**1 3 4 7 8 10 11**
G33-2008	10/03/2008	Boutlelis (Oran)	Spunta	A2	R	virulent to 11 genes
SABL	10/03/2008	Mazagran (Mostaganem)	Safrane	A2	R	virulent to 11 genes
ABD	23/03/2008	Mostaganem	Spunta	A2	R	virulent to 11 genes
AT	02/03/2008	Chentouf (Ain Temouchent)	Spunta	A2	R	virulent to 11 genes

Isolate name	Date of isolation	Location (wilaya)	Cultivar	Mating type (MT)	Metalaxyl sensitivity *	Virulence profile on 11 R specific genes (from R1 to R11)
TLE	15/03/2008	Honaine (Tlemcen)	Spunta	A2	R	virulent to 11 genes
SBA	19/01/2008	Tabia (Sidi Bel Abbès)	Spunta	A2	R	virulent to 11 genes
G28	2008	-	-	A2	-	-
Z22	24/01/2008	Algiers (CNCC)	Atlas	A2	R	-
Z30	13/05/2008	Zerralda (Algiers)	Atlas	A2	R	-
Z31	13/05/2008	Boumerdes	Timate	A2	R	-
Z32	15/05/2008	Staoueli (Algiers)	-	A2	R	virulent to 11 genes
Z33	15/05/2008	Tipaza	Kondor	A2	R	virulent to 11 genes
ST5	17/07/2008	Ain Defla	Spunta	A2	R	-
ST6	17/07/2008	Ain Defla	Spunta	A2	R	-
ST7	17/07/2008	Khemis Meliana (Ain Defla)	-	A2	R	-
ST8	17/07/2008	Khemis Meliana	-	A2	R	-
ST9	25/07/2008	Algiers	-	A2	R	-
ST10	25/07/2008	Algiers	-	A2	R	-
AG1	2009	Algiers (INA)	Spunta	A2	-	-
AG2	05/05/2009	Ain Defla	Spunta	A2	-	-
AG3	20/05/2009	Mostaganem	Spunta	A2	-	-
AG4	20/05/2009	Mostaganem	Atlas	A2	-	-
AG5	20/05/2009	Mostaganem	Amila	A2	-	-
AG6	20/05/2009	Mostaganem	Désirée	A2	-	-
AG7	2009	Oued Smar (Algiers)	Fabula	**A1**	-	-
Algerian isolates from		***tomato***				
ITC	24/02/2008	Hassi Bounif (Oran)	Zahra	**A1**	**S**	**avirulent to *R2***
AD	11/03/2008	El Abadia (Ain Defla)	Actana	**A1**	**S**	**avirulent to *R9***
French isolates from		***potato***				
08-P13-02	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 7 10 11**
08-P13-08	23/06/2008	Ploudaniel (29)	Bintje	A2	-	-
08-P13-05	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 5 6 7 8 10 11**
08-P13-01	23/06/2008	Ploudaniel (29)	Bintje	A2	R	virulent to 11 genes
08-P15-02	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 5 6 7 10 11**
08-P43-01	23/07/2008	Plougar (29)	Atlas	**A1**	S	**1 3 4 6 7 8 10 11**
08-P13-11	23/06/2008	Ploudaniel (29)	Bintje	A2	-	virulent to 11 genes
08PON01-01	25/06/2008	Pluméliau (56)	Atlas	A2	-	virulent to 11 genes

* S : sensitive ; I : intermediate ; R : resistant.

- : not tested

Algerian and French isolates do not differ for *in vitro* mycelial growth

Radial growth of mycelium was fastest at 19°C for all 11 isolates tested, and was significantly slower at 11°C and 27°C Figure 1 (part A, A2 isolates and part B, A1 isolates). Variance analysis showed significant differences among temperatures (F = 521,92, *P*<0.0001), isolates (F = 46,91, *P*<0.0001) and interactions between temperatures and isolates (F = 8,75, *P*<0.0001). The isolates could be classified into three distinct groups according to their mean growth. The first group was composed of 2 A1 isolates from potato, the second one of 7 A2 isolates and 1 A1 isolate from tomato (AD), and the third group of 1 A1 isolate from tomato (ITC). Growth of A2 isolates from Algeria and France was not different.
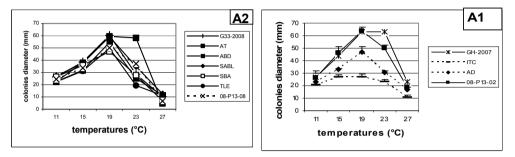
Figure 1. *In vitro mycelial growth of 11 P. infestans isolates cultivated on pea agar medium and incubated in darkness during 7 days, at 5 constant temperatures : 11, 15, 19, 23 and 27°C. Each number is the mean value of three replicate culture plates per treatment. A : colonie diameter of A2 mating type isolates (6 Algerian isolates and 1 French isolate, 08-P13-08) ; B : colonie diameter of A1 mating type isolates (in dotted lines : 2 isolates from tomato and in fulled lines : 2 isolates from potato).*

The experiment was carried on for a second week at 11°C and 27°C, as colonies have not reached edge of dishes, and radial growth of mycelium of the 11 isolates for 14 days is presented in Figure 2 (part A, 11°C and part B, 27°C). Variance analysis revealed significant differences among temperatures (F = 480,92, *P*<0.0001), mating types (F = 37,97, *P*<0.0001) and interactions between temperatures and mating-types (F = 18,56, *P*<0.0001). Growth of the isolates was twice as fast at 11°C than at 27°C. At 27°C, A1 isolates grew significantly faster than A2 isolates; the A1 isolate ITC from tomato had the highest colony diameter at that temperature. After 14 days of incubation at 27°C, there was no difference between A1 isolates according to their hosts of origin, potato or tomato.
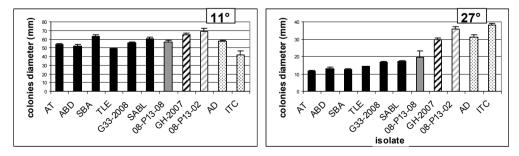
Figure 2. *In vitro mycelial growth of 11 P. infestans isolates cultivated on pea agar medium and incubated in darkness during 14 days, at 2 constant temperatures, A : 11°C and B : 27°C. Dotted columns : A1 Algerian isolates from tomato ; shaded columns : A1 isolates from potato ; fulled columns : A2 isolates from potato (in black : Algerian isolates ; in grey : French isolate). Each number is the mean value of three replicate culture plates per treatment.*

No large differences are noticed between aggressiveness on potato of A1 and A2 isolates

Infection experiments showed highly significant effects of cultivars and isolates when the three aggressiveness components were analysed for 13 isolates on 4 potato cultivars. Lesion areas (LA) ranged from 6 cm^2 for isolate ITC on cv. Désirée to more than 11 cm^2 for isolates SABL and 08-PON01-01 on cv. Bintje. Spores production per lesion (SP) ranged from 48000 sporangia for isolate GH-2007 on cv. Désirée to more than 350 000 sporangia for isolates AD and 08-PON01-01 on cv. Bintje (Figure 3). Sporulation capacity (SC) was also highly variable, ranging from 6 500 sp/cm^2 for isolate GH-2007 on cv. Désirée to more than 36 000 sp/cm^2 for isolates AD and AT on cv. Bintje. Isolate ITC showed the lowest LA on all cultivars, except on cv. Spunta; it also had the smallest radial growth of mycelium, at 15°C and 19°C after 7 days of incubation on pea agar medium.

ANOVA performed for LA data showed highly significant differences for cultivars (F = 22,45, $P<0.0001$) and isolates (F = 17,06, $P<0.0001$), but a non significant C x I interaction (F = 1,34, $P = 0,1$). Isolates were fully aggressive to cv. Bintje for LA. Mean LA values on cv. Spunta were not significantly different to those on cvs Désirée and Atlas. ANOVA of SP values also revealed significant effects for cultivars (F = 75,94, $P<0.0001$), isolates (F = 3,04, $P = 0.0005$) and cultivar x isolate interaction (F = 2,06, $P = 0,0006$), while variance analysis of SC data indicated high significance for cultivars (F = 42,67, $P<0.0001$), isolates (F = 4,08, $P<0.0001$) and C x I differential interaction (F = 2,21, $P = 0.0002$). Among the four potato cultivars, Desirée and Atlas presented the lowest SP and SC values. On these two cultivars, isolates produced on average two times less sporangia than on cv. Bintje (SP mean values of 12,6.10^4 and 13,7.10^4 sporangia versus 27,6.10^4 sporangia, respectively). However, the relative rankings of isolates changed according to the cultivar (Figure 3) and to the component. Nevertheless, and quite interestingly, all Algerian isolates were highly aggressive on cv. Bintje and were adapted to this cultivar, although it is not grown in Algeria. There was no significant difference between isolates according to their mating type or to their country of origin.
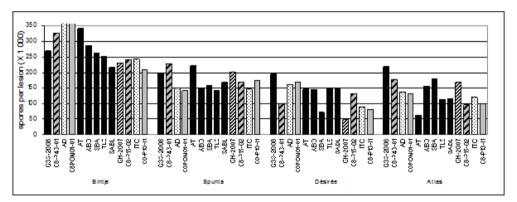
Figure 3. *Spore production (number of sporangia per lesion) of 13 P. infestans isolates on 4 potato cultivars : Bintje, Spunta, Désirée and Atlas. Data were obtained in a detached-leaflet assay, after 6 days of incubation at 15°C/18°C night/day temperatures, 16h daylight. Dotted columns : 2 A1 Algerian isolates from tomato ; shaded columns : 3 A1 isolates from potato (1 from Algeria and 2 from France) ; fulled columns : A2 isolates from potato (in black : 6 Algerian isolates ; in grey : 2 French isolate). Each number is the mean value of six replicate leaflets per treatment.*

SSR markers reveal different *P. infestans* populations on potato and tomato

The genotypes of 18 Algerian isolates (2 from tomato and 16 from potato) were explored using microsatellite markers. A total of 32 alleles were detected over the 11 microsatellite loci, with two to five alleles per locus (Table 2).

Table 2. *Single sequence repeat multilocus genotypes detected with 10 microsatellite markers, in 18 Algerian isolates of P. infestans from potato and tomato.*

Isolate name	MT	Plant *	Pi02	Pi89	Pi4B	G11	Pi04	Pi70	Pi56	Pi63	Pi16	Pi33
						Alleles detected with 10 SSR markers						
AD	**A1**	**T**	**162**/164	179/**181**	**213/217**	**162/162**	**160/168**	192/**195**	174/**174**	**148**/157	176/178	203/203
ITC	**A1**	**T**	**162**/164	179/**181**	**213/217**	**162/162**	**160/168**	192/**195**	174/**174**	**148**/157	176/178	203/203
GH 2007	**A1**	P	**152**/162	179/179	**217/217**	**156/156**	166/170	192/**195**	174/176	-	176/178	**200/200**
Z33	A2	P	**162**/162	179/**181**	**217/217**	**140/140**	166/170	192/192	**176**/176	151/157	176/178	203/**206**
G28	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	**200/200**
G33	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	**200/200**
AT	A2	P	160/162	179/179	205/**205**	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z13	A2	P	**162**/162	179/179	205/**205**	154/160	166/170	192/192	174/176	151/157	**178**/178	203/203
Z18	A2	P	-	179/179	-	154/160	166/170	192/192	174/176	151/157	**178**/178	203/203
Z12	A2	P	**162**/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	**178**/178	203/203
Z32	A2	P	**162**/162	179/179	-	**160**/160	166/170	192/192	174/176	151/157	**178**/178	203/203
Z21	A2	P	-	179/179	-	**160**/160	166/170	192/192	174/176	-	**178**/178	203/203
TLE	A2	P	**162**/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z3	A2	P	**162**/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
SABL	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z0	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z1	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
ABD	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203

*Plant * : T = tomato ; P = potato ; - : no data*

Of 11 SSR markers tested, all primers showed polymorphism, except Pi4G marker (two alleles were revealed, 159 and 161 bp and data were missing for 5 isolates). The 18 *P. infestans* isolates genotyped split into 10 unique multi-locus genotypes (MLGs), five of which being represented by a single isolate (GH2007, AT, Z12, Z13, Z33) and one of them detected in four A2 isolates (Z0, Z1, SABL, ABD). Five of these 10 MLGs were not present in French populations collected in 2006 to 2008 on potato (Corbière *et al.*, 2010). That concerned the three A1 isolates sampled on potato (GH-2007) and on tomato (AD and ITC), and four A2 isolates (Z12, Z21, Z32 and Z33) collected on potato in 2007 and 2008 in different wilayas. The remaining 5 MLGs, present within French populations, all corresponded to A2 isolates. Although the number of isolates was limited, the 8 MLGs corresponding to A2 isolates showed a limited genotypic diversity (polymorphism at 1-3 of the 11 loci), except that corresponding to isolate Z33. Among A1 isolates, two genotypic groups were distinguished according to their host of origin. The two isolates AD and ITC from tomato belonged to the same MLG, which differed markedly from that of isolate GH-2007 from potato. Moreover, the MLG of isolates AD and ITC presented alleles relatively rare in Europe, *e.g.* allele 164 at locus Pi02, and alleles 160 and 168 at locus Pi04 (SSR allele information sheet, D.E.L. Cooke, SCRI, UK).

DISCUSSION AND CONCLUSIONS

Monitoring of *P. infestans* in the Western and Central coastal regions of Algeria showed that the A2 mating type is present at a high frequency in potato crops in Algeria, although some A1 isolates were also detected. In contrast, only A1 mating-type isolates were isolated from the few tomato samples that could be analysed. The frequency of A2 isolates is similar to that found in Western European populations on potato where, since 2005, a dramatic shift in mating type was noted (Cooke *et al.*, 2010 ; Corbière *et al.*, 2010). In Maghreb, the presence of the A2 mating type was also reported in

Presentation

Moroccan populations (Hammi, 2003; Andrivon *et al*., 2007), and at a lower frequency, in Tunisian population (Hamada and Harbaoui, 2010). In Algeria, further work needs to be performed to obtain a more comprehensive sampling, and thus better evaluate the respective frequencies of the two mating types. However, the presence of both A1 and A2 isolates within *P infestans* populations in Algeria raises the possibility of sexual reproduction and the generation of oospores.

All the Algerian A2 isolates tested were metalaxyl resistant. The continued use of foliar application of metalaxyl on potatoes in Algeria, in spite of resistance, may have contributed to the predominance of the metalaxyl-resistant isolates on this crop, by a high selection pressure. Nevertheless, within Western European and Maghrebian populations, a strong association between metalaxyl resistance and A2 mating type has also been noticed (Hammi, 2003; Cooke *et al*., 2010; Corbière *et al*., 2010; Hamada and Harbaoui, 2010; Kildea *et al*., 2010). The A1 isolate recovered from potato in Algeria (GH-2007) was identified with intermediate sensitivity. According to Klarfeld *et al*. (2009), isolates with intermediate resistance to metalaxyl could suggest an oospore origin. Isolate GH-2007 with intermediate sensitivity and a unique MLG could thus be a recombinant isolate. Continuous blight monitoring in potato and tomato production regions must therefore be carried on to identify any trend towards sexual reproduction. On tomato, the two A1 isolates exhibited in contrast metalaxyl sensitivity. The absence of metalaxyl resistance in tomato isolates has also been found in the Netherlands, South Africa and Morocco where high levels of resistance have been found on potato (Mc Leod *et al*., 2001; Hammi, 2003). This result might be explained by host preference of *P. infestans* isolates, although all isolates are pathogenic on potato.

The interaction between climate changes and thermal adaptation of *P. infestans* may have profound effects for the future of potato production in Algeria. Our results on mycelia growth at different temperatures are consistent with early work which found that growth on medium was the most rapid at 20-21°C, with a minimum temperature of 2-3°C and an upper temperature limit close to 30°C (Crosier, 1934 *in* Harrison, 1992). They thus do not support the idea that *P. infestans* isolates from Algeria and France show a different pattern of temperature adaptation than earlier populations, or from current French populations of the pathogen. However, the behavior of the isolates from potato was slighly different according to their mating type; mycelial growth of A1 isolates was higher than those of A2 isolates, especially at 27°C, after two incubation weeks. This observation suggests that A1 isolates could be more tolerant to high temperatures than A2 isolates, but this needs confirmation. These data should however be supplemented by additional results from *in-planta* experiments, since Harrisson (1992) demonstrated that temperature and polygenic resistance to blight interact to determine hyphal growth in leaflets.

Investigating pathotype composition provides information that is especially important in breeding for crop resistance. This study confirms the presence of highly complex pathotypes of A2 *P. infestans* isolates in Algeria, as reported previously (Beninal *et al*., 2009). These results are also consistent with data from French populations, where 75% of the A2 isolates, collected in 2007 and 2008, had these two virulence profiles (Corbière *et al*., 2010). Our data showed that the virulence spectrum of the Algerian A1 isolate from potato differs from those of A2 isolates, and corresponds to a pathotype which is prevalent in many European countries (Lehtinen *et al*., 2008; Hannukkala *et al*., 2009; Chmielarz *et al*., 2010; Corbière *et al*., 2010). By contrast, on tomato, A1 isolates presented highly complex profiles with 10 virulence factors. It is unclear at this point whether *P. infestans* isolates from tomato have host-specific virulence profiles, and it should be considered in future research. Based on our results, pathotypes in Algeria are not associated with potato cultivars or with regions. The isolates that were virulent on all 11 *R* differentials were collected from different cultivars (Spunta, Atlas and Kondor), in several wilayas between Algiers and Tlemcen. Since most cultivars grown

in Algeria (or sampled in this study) have no or few *R* genes, it is likely that random mutation plays an important role in the diversification of pathotypes. Moreover, *P. infestans* seems highly mobile through airborne sporangia or infected tubers and could migrate on hundreds of kilometers (Montarry *et al.*, 2010).

An increase in aggressiveness has often been postulated to be linked to the appearance of the A2 mating type isolates in *P. infestans*. Here, no significant differences between A1 and A2 isolates, except for isolate ITC from tomato, were noticed. This result is consistent with a previous report on French populations, where A1 isolates were slightly more aggressive than A2 isolates on cv. Bintje (Corbière *et al.*, 2009). Some of the isolates differed strikingly in aggressiveness on the four cultivars tested, so pathogenic fitness on one potato cultivar was not related to pathogenic fitness on the other potato cultivars. Therefore, using several cultivars with different levels of pathotype-nonspecific resistance is desirable, as it increases the value of the results (Lehtinen *et al.*, 2009). In the same way, the present work confirms, as reported by several authors, different levels of pathogenic fitness across isolates. It is therefore important to evaluate resistance levels of potato genotypes with different isolates of *P. infestans*.

Our results showed a large variation in sporangial production on the four potato cultivars. Spore production is an important component when assessing pathogen aggressiveness, because, in a polycyclic disease such as late blight, secondary cycles and new lesion formation are determined to a large extent by the amount of spores produced. This component has also important implications for late blight management regarding the use of decision support systems (DSS). According to this aggressiveness component, the classical behavior of the cultivars was confirmed. Cv. Bintje was the most susceptible to Algerian isolates; cv. Spunta, dominant in Algeria, was also fully susceptible, while cvs Désirée and Atlas exhibited moderate susceptibility. In Moroccan *P. infestans* population, isolates also produced significantly fewer sporangia on cv. Désirée compared with cv. Spunta (Hammi, 2003). We did not notice local adaptation of Algerian isolates to cv. Désirée in comparison with cv. Bintje, although cv. Désirée is grown for a number of years in Algeria and cv. Bintje is not cultivated. Algerian and French isolates did not show significant aggressiveness differences. Then, as with others factors analysed in the work, *P. infestans* populations of these two countries seem presented large similarities. On the other hand, because of the small sample size, it was not possible to study regional aggressiveness differences in *P. infestans* populations within wilayas separated by hundreds of kilometres, or within types of potato production (cultivated on spring and autumn or grown from locally produced and imported seeds).

In our experimental conditions, AD isolate from tomato was highly pathogenic on potato and this result did not indicate evidence for host adaptation. However it is practically impossible to know how many generations this isolate has spent on the host it was isolated from. This tomato isolate might have been on potato in former generations and still possess high fitness on potato. It would thus be premature to conclude before more studies are conducted to evaluate host preference and quantitative aggressiveness of isolates collected on potato and tomato, on both hosts, and with larger sample sizes. Indeed, Hammi (2003) showed differencies between potato and tomato isolates when he tested the ability of Moroccan isolates to infect leaves of both hosts. According to this author, isolates from potato equally attacked both potato and tomato ; in contrast, isolates from tomato were highly pathogenic on tomato cv. Daniela , but less aggressive on potato cvs Spunta, Désirée, Nicola and Kondor.

Algerian *P. infestans* isolates, collected from potato, proved to be highly aggressive, complex pathotypes. This emphasizes the need to incorporate diverse sources of resistance into breeding programs and to focus on non-specific resistance. Indeed, specific *R* genes, *e. g. R9*, is now overcome although it has never been introduced into commercial cultivars. The plasticity of *P. infestans* genome will reduce the efficacy of breeding resistance based simply on the accumulation of *R*-genes.

In contrast, cv. Sarpo Mira has proved to possess high partial blight-resistance, with no apparent changes in resistance level in recent cultivar trials (Lees *et al*., 2009 ; Chmielarz *et al*., 2010 ; Galfout *et al*., 2010).

Finally, molecular markers were used to assess genomic variations, which are not affected by host or environmental factors that influence the expression of phenotypes. SSR markers revealed three distinct genetic groups according to mating types and host plants : one is composed with two A1 isolates from tomato; a second one with one A1 isolate from potato and a third one with A2 isolates from potato. The predominant A2 genotype, characterized in our study with four isolates, had a multilocus genotype close to the genotype identified by D.E.L. Cooke as genotype 13 or "Blue 13", but D13 marker missed in our analysis. The other 10 A2 isolates, except Z33, deviated from the predominant pattern with variation at one, two or three loci. Such minor changes could be attributed to mutation or mitotic recombination within clonal lineages. The origin of A2 isolate Z33 has to be explored to know whether it could be a recombinant isolate, as it is suspected for isolate GH-2007. In Great Britain, Ireland and France, the clonal genotype 13 is now dominant within A2 isolates (Cooke *et al*., 2010; Corbière *et al*., 2010; Kildea *et al*., 2010). It is possible that this clonal A2 MLG will have higher pathogenic fitness than other genotypes and then spread in potato crops of many regions. In cold winter regions, as in Nordic European countries, the *P. infestans* populations have been shown to be genetically diverse and it is suggested that this variation is maintained by sexual reproduction, as both mating types were present in 29-56% of the fields (Widmark *et al*., 2007; Lehtinen *et al*., 2009). Due to an effective clonal propagation and spread, a highly pathogenic *P. infestans* genotype would have the ability to respond quickly to selective pressure, and successful isolates can, over short periods, become dominant in the pathogen population. However, in Algeria as in France, none of the factors (metalaxyl resistance, effect of temperature on mycelial growth, virulence, aggressiveness) could apparently explain the invasion of potato crops by A2 mating type isolates of *P. infestans*.

Although only a limited number of tomato crops were sampled, the SSR analysis suggests that the population of *P. infestans* on tomato is genotypically distinct from that on potato. Reports on the host specificity of *P. infestans* populations on tomato and potato vary among regions. In some locations, *P. infestans* populations that infected both tomato and potato could not be distinguished by neutral genetic markers (Chen *et al*., 2008). In contrast, the same genetic markers have shown that distinct genotypes are associated with different hosts. The current study revealed that the two isolates collected from tomato are genotypically identical with the markers used, but that they are really different in their phenotypic characters. Moreover, despite the clonal structure of A2 *P. infestans* isolates, there was also a lack of association between genotypic and phenotypic traits of the isolates. This result is not unexpected because molecular neutral markers are not necessary linked to phenotypic markers. Further research is warranted to investigate the genetic diversity of Algerian isolates with a wider range of isolates and SSR markers, as new sets are now available (Guo *et al*., 2009, Cooke *et al*., 2010). A comparative study of Algerian *P. infestans* population structure with European populations, *e.g.* from the Netherlands, France and Denmark from where large amounts of seed tubers are imported, could help to understand origin and diversity of Algerian isolates.

In conclusion, this study provides important data to understand the population diversity and pathogenicity fitness of *P. infestans* in Algeria. Such data might be helpful in supplying information to breeders for Algerian markets, extension specialists and farmers to make rational decisions regarding late blight control.

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Changes within the Irish potato late blight population

[1]S KILDEA, LR COOKE, L QUINN, G LITTLE, C ARMSTRONG, [1]F HUTTON, [1]LJ
DOWLEY & [1]D GRIFFIN

[1]Teagasc Crops Research Centre, Oak Park, Carlow, Ireland; [2]Applied Plant Science & Biometrics
Division, AFBI, Newforge Lane, Belfast, Northern Ireland, BT9 5PX, UK

SUMMARY

As part of an all-Ireland late blight initiative the 2008 and 2009 Irish *Phytophthora infestans*
populations have been characterised both phenotypically and genotypically. Over both seasons
a collection of 659 single lesion *P. infestans* isolates was established. To characterise these isolates
they were subject to mating type tests, phenylamide sensitivity assessments and SSR analysis. In
both seasons the presence of the A2 mating type 'Blue 13' was confirmed, with increasing numbers
of the strain detected in 2009. Their detection was predominantly in the eastern counties, with
limited numbers detected in the north-west and none detected in the south-east. The presence and
rapid spread of 'Blue-13' has confirmed that the Irish late blight is currently undergoing a dramatic
change.

KEYWORDS

Phytophthora infestans, Ireland, A2 mating type, metalaxyl resistance

INTRODUCTION

Following the introduction of the A2 mating type into Ireland in early the 1980s (O'Sullivan &
Dowley, 1991; Cooke *et al*, 1995) and the establishment of the 'new' *P. infestans* population that
had become prevalent in Europe during the previous years (Tooley *et al.,* 1993) the Irish population
became dominated by clonal lineages of a limited number of *P. infestans* genotypes (Carlisle *et al.,*
2001; Griffin *et al.,* 2002). During the same period the frequency of phenylamide resistance has
fluctuated depending on the usage of the fungicide. During the period 2000-2007 a decrease in
resistance was detected in the population within the Republic of Ireland despite the continued usage
of the fungicide (LJ Dowley, *personal communication*).

In Great Britain a similar *P. infestans* population structure was observed up until 2005 when the
genotype 13_A2, also referred to as 'Blue 13' was first detected (Day *et al.,* 2004; Cooke *et al.,*
2007). Since then 13_A2 has rapidly spread throughout Great Britain, where it now dominates
the *P. infestans* population (Lees *et al.,* 2009). In 2007 it as first detected in Northern Ireland
(Cook *et al.,* 2009). With increased aggressiveness and virulence reported on commonly cultivated
commercial cultivars (Lees *et al.,* 2009; White & Shaw, 2009) and the lack of an apparent fitness
penalty previously associated with phenylamide resistance, determining its presence and/or

prevalence throughout both the Republic of Ireland and Northern Ireland is essential to ensure the best disease control strategies can be implemented.

MATERIALS & METHODS
Collection, isolation and storage of Phytophthora infestans
Surveys of the Irish *P. infestans* population were carried out during the 2008 and 2009 growing seasons. Blighted potato leaf material was collected from mainly commercial crops by members of the seed certification within the Irish Department of Agriculture Fisheries and Food (DAFF), the Northern Irish Department of Agriculture and Rural Development (DARD) Potato Inspection Service and Teagasc potato advisors. Once received the blighted material was incubated in a moist environment for approximately 24 hrs to promote sporulation. To establish single lesion isolates sporulating mycelium was transferred to antibiotic pea agar (riampicin 50 mg/l) or antibiotic rye agar (rifampicin 25 mg/l and natamycin 25 mg/l). Once pure cultures were established they were maintained on rye agar either as plates or slants.

Mating type, metalaxyl sensitivity and SSR determination
The mating type of each isolate was determined on unamended carrot agar with known reference isolates of the A1 or A2 mating types and as described by Cooke *et al.* (2009). The sensitivity of the isolates to the fungicide metalaxyl was determined using a floating disk assay as described by Cooke (1986). Isolates were deemed sensitive if showing sporulation only on the untreated disks, intermediate if showing sporulation on the untreated and 2 mg/l metalaxyl amended disks only, and resistant if sporulating on all three treatments (0, 2 and 100 mg/ metalaxyl).

The isolates were genotyped by SSR analysis using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) and in accordance to the protocol developed by EUCABLIGHT. DNA from each isolate was extracted from freeze dried mycelia of 14 day old cultures grown in either unamended liquid pea broth or on unamended pea agar. Post-PCR processing analysis was performed on an Applied Biosystems 3130*xl* genetic analyzer and the subsequent DNA fragments were sized automatically using the Applied Biosystems Genemapper* software, version 4.0. Genotypes were determined by comparing fragment sizes with isolates previous genotyped (kindly supplied by David Cooke, SCRI).

RESULTS
In 2008 234 single lesion *P. infestans* isolates were established from 55 samples collected from throughout Ireland (203 isolates from the Republic of Ireland; 31 from Northern Ireland). Weather conditions in 2009 were extremely favourable for the spread of *P. infestans* and 425 single lesion isolates (266 from Republic of Ireland; 159 Northern Ireland) were established from 93 samples.

In 2008 and 2009 the A2 mating type was found in almost an identical frequency in both the Republic of Ireland (25% in 2008 and 50% in 2009) and Northern Ireland (23% in 2008 and 56% in 2009). In both seasons the A2 isolates were found predominantly in the Eastern counties (Fig 1.). In both seasons metalaxyl resistant isolates dominated the populations in both the Republic of Ireland (62% in 2008; 52% in 2009) and Northern Ireland (54% in 2008; 68% in 2009). All A2 isolates tested were metalaxyl resistant. These isolates did not appear to suffer a fitness penalty as they were detected in crops in early June 2009.

SSR analysis of a collection of the A1 mating type isolates confirmed the presence within the Irish *P. infestans* population of the genotypes 8_A1, 5_A1, 12_A1 and 6_A1 (commonly referred to as 'Pink 6'). All A2 mating type isolates genotyped to date were confirmed as 13_A2 (commonly referred to as 'Blue 13').

DISCUSSION

The results presented indicate the Irish *P. infestans* population is currently undergoing a dramatic change. Since its first detection in Northern Ireland in 2007, the 'Blue-13' genotype has spread at a rapid pace similar to that observed in Great Britain (Lees *et al.,* 2009). The apparent east-west divide present with the country in relation to the presence of 'Blue-13' is surprising. Why such a divide exists, and why it has been maintained over the two years of sampling justify further investigation.

Possible problems associated with the presence of 'Blue-13' in the Irish *P. infestans* can be viewed in both the immediate and medium-term time scales. The fact that these isolates do not appear to suffer fitness penalties previously associated with phenylamide resistance reduces the potential of these fungicides in fungicide control regimes. In the medium-term the presence of A1 and A2 mating type isolates within the population provides the potential for the sexual recombination of *P. infestans* and the associated risks.

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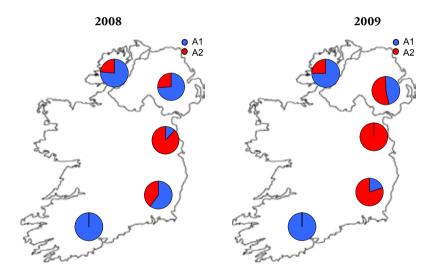
Fig 1. *Frequency of the A2 mating type within the main potato growing regions of Ireland and Northern Ireland in 2008 and 2009.*

Genotypic variation of *Phytophthora infestans* within and between fields in the Nordic countries

L. GRÖNBERG[1], B. ANDERSSON[1], N. HÖGBERG[1], A.-K. WIDMARK[1] & J. YUEN[1]

[1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (SLU), Uppsala, PO Box 7026, SE-75007, Uppsala, Sweden

Presentation

SUMMARY

In this study, we have investigated the population structure of *Phytophthora infestans* in Denmark, Finland, Norway and Sweden. We tested the hypothesis: (i) Sexual reproduction of *P. infestans* is common in the Nordic countries and (ii) no predominant clonal lineages of *P. infestans* exist in the region.

Isolates of *P. infestans* were collected using stratified sampling with country, field and disease foci as the different sampling strata. The sampling was done in both conventional and organic potato fields during 2008. Only single lesion leaflets, randomly chosen from different plants in different disease foci in each field were collected. The leaflets were dried and later DNA was extracted using a CTAB-based protocol. Seven microsatellite markers were used to determine the genotypic variation in the sampled material.

A stratified sampling approach makes it possible to calculate the level and distribution of genetic/genotypic in the different sampling levels. This will give an indication if and to what extent sexual recombination is contributing to the population structure. It will also enable studies on the spread of the pathogen within and between fields. The results show a high genotypic variation in the Nordic countries since most of the genotypes were found only once and very few clones were found among the collected isolates. The major part of the genotypic variation was observed within the fields, with little differentiation between the fields.

The results strongly suggest that sexual reproduction of *P. infestans* does occur frequently in the Nordic countries and that oospores are an important inoculum source.

KEYWORDS

Phytophthora infestans, SSR, population, oospores

Presentation

Öko-SIMPHYT (= Organic-SIMPHYT): A forecasting system for specific scheduling of copper fungicides against late blight

BEATE TSCHÖPE[1], BENNO KLEINHENZ[1], SVEN KEIL[2] & MICHAEL ZELLNER[2]

[1] Central Institution for Decision Support Systems in Crop Protection, Germany
[2] Bavarian State Research Centre for Agriculture, Germany

SUMMARY

During a 4-year project a fungicide strategy against late blight in organic potatoes based on the use of copper was developed resulting in the decision support system (DSS) Öko-SIMPHYT. Öko-SIMPHYT is based on SIMPHYT1 model which recommends first treatment and SIMPHYT3 model which recommends treatment interval and application rate. The aim of Öko-SIMPHYT is to reduce the number of treatments and the application rate. In phases with very low disease pressure calculated by SIMPHYT3 a break of the spraying schedule is recommended. In phases with high disease pressure the aim of the DSS is to achieve best antifungal activity based on the maximum allowed application rate (3 kg/ha copper). 49 nationwide demonstration trials were carried out to validate Öko-SIMPHYT. By timing the treatment interval and adjusting the application rate with the help of the decision support system Öko-SIMPHYT it was possible to get results comparable to standardized weekly applications, applying less copper. In certain cases it was possible to save up to 1000g/ha of copper. On average 0.6 applications were saved and the reduction of copper was 535g/ha.

KEYWORDS

Phytophthora infestans, Decision support system, SIMPHYT, simulation model, infection pressure, copper

INTRODUCTION

In organic potato farming the control of late blight disease is a problem because a range of highly effective fungicides does not exist. So it is very important to use every preventive method to delay the outbreak of late blight. Such preventive methods are choice of location and variety, pregermination, nutrient supply and the use of plant resistance improvers. Moreover the use of protective copper fungicides is allowed. The maximum allowable application rate is 6 kg/ha Cu given by EU Organic Regulation. The German Grower's associations allow maximal 3 kg/ha with special approval (Bioland, Naturland) or prohibit the use of copper (Demeter). On the market there are different copper fungicides with following active ingredients: copper hydroxide, copper octanoate and copper oxychloride. In conventional farming the simulation models SIMPHYT1 and SIMPHYT3 are successfully used for years (Gutsche, 1999; Kleinhenz and Jörg, 2000). Based

on these experiences, models were developed to the conditions of organic farming and the DSS was named Öko-SIMPHYT.

MATERIAL AND METHODS

Different application strategies were examined by the Bavarian State Research Centre for Agriculture (LfL) and the Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI). Based on the experiments the DSS Öko-SIMPHYT was developed. It is based on the use of copper hydroxide and a maximum application rate of 3000 g copper per ha and year. Copper hydroxide showed the best results concerning rainfastness.

For testing the DSS, Öko-SIMPHYT was programmed by the Information System for Integrated Plant Production (ISIP) in the internet portal www.isip.de.

Öko-SIMPHYT-Input

To run Öko-SIMPHYT some plot-specific information like field name, variety and date of emergence are needed. Moreover a weather station representative for the site has to be chosen from a list. For each model run the input parameters potato growth and rainfall have to be considered since last application. They influence the treatment interval and have to be updated at times. The model requires additionally hourly measurements of air temperature and relative humidity (2m) and sums of precipitation. Figure 1 shows the input form of Öko-SIMPHYT.

Öko-SIMPHYT-Output

SIMPHYT1 predicts the date for the expected start of the late blight epidemic (first outbreak). The date is calculated with a predictive time span of 8 days. The first application should be done within this period.

After the treatment start is predicted, the calculation continues with SIMPHYT3. Based on the variable input data and a calculated weather dependent infection pressure, SIMPHYT3 delivers three results: Treatment interval in days based on last application, recommended application rate and the possibility of an interruption of copper treatments. The infection pressure is grouped into 5 classes from very low to very high. From this infection pressure, recommendations of treatment interval and application rate can be derived (Figure 2). A very low infection pressure corresponds with a treatment interval of 12 days and an application rate of 250 g copper per ha and a very high infection pressure means a short treatment interval of 4 days and a corresponding application rate of 750 g per ha. Dependent on the individual input data of potato growth and rainfall since last application, the treatment interval is calculated. So the maximum treatment interval is 13 days and minimum 4 days.

Moreover a daily Phytophthora efficiency value is calculated by SIMPHYT3. The value ranges from 0 to 1 and gives information about how favourable the day was for late blight infections. If there are 7 consecutive days with Phytophthora-efficiency-value = 0 Öko-SIMPHYT recommends an interruption of copper treatments until two consecutive days with pev > 0 appear.

Figure 1: *Input form of the DSS Öko-SIMPHYT*

Figure 2: *Öko-SIMPHYT scheme*

Öko-SIMPHYT-Trials

Validation of Öko-SIMPHYT was done by 49 nationwide trials carried out from 2006 to 2009 in Germany, 17 of which had 4 trial elements, 20 with 3 trial elements and 12 on-Farm-trials. Table 1 shows the different elements. The trials were designed as a block system with four replications. The disease incidence and severity was examined weekly (Tschöpe, B. *et al.*, 2008).

Table 1: *Trial variants for Öko-SIMPHYT validation*

No. element	Description
1	Untreated control
2	500 g/ha Cu, weekly
3	Variable rate and interval (Öko-SIMPHYT)
4	500 g/ha Cu, variable interval (Öko-SIMPHYT)

RESULTS AND DISCUSSION

Validation of SIMPHYT1

For validation of SIMPHYT1 the hit rate of correct forecasts was calculated. Therefore the difference between recommendation and first outbreak of late blight was evaluated. A forecast was rated as correct if the predicted date of outbreak was before the first observations in the field. During the four years (2006-2009) a hit rate of 72% correct forecasts was achieved on average.

Moreover the data were validated with an additional model to predict first appearance of late blight called SIMBLIGHT1 (Kleinhenz *et al.*, 2007). This model includes soil moisture and crop prevalence. In average the model achieved a hit rate of 81%, so it fits better than SIMPHYT1 but some forecast were considerable to early.
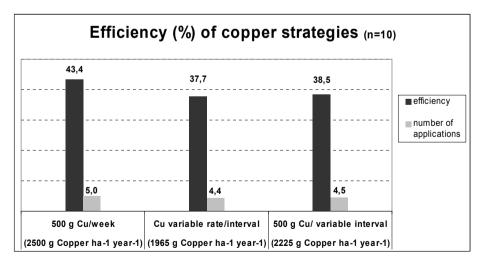
Figure 3: *Efficiency (%) of copper strategies, n= 10*

Validation of SIMPHYT3

For validation of SIMPHYT3 the disease severity curves of the variants were converted into an AUDPC. Then the efficiency of the copper applications was calculated by comparing the AUDPC of the copper treated trial elements to the untreated control element. Figure 3 shows the results of 10 trials with 4 elements in the years 2006-2009. Using Öko-SIMPHYT it was possible to get an efficiency of about 40% which is comparable to standardized weekly applications. On 5 trial sites Öko-SIMPHYT saved up to 1500g/ha copper. On average the number of applications was reduced by 0.6 and the reduction of copper was about 535g/ha.

CONCLUSIONS

The forecasting system Öko-SIMPHYT serves as an important tool for site specific scheduling of copper fungicides against potato late blight. Based on the daily calculated infection pressure the spray interval was adjusted. An optimized control of late blight is possible and the use of copper fungicides can be minimized. The DSS is available for farmers and extension officers via the internet on the homepage www.isip.de

ACKNOWLEDGEMENTS

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Report of subgroup epidemiology and decision support systems

GEERT J.T. KESSEL[1] AND JENS GRØNBECH HANSEN[2]

[1] Wageningen University and Research Centre, Plant Research International, Biointeractions and Plant Health, P.O. Box 69, 6700 AB Wageningen, The Netherlands.
[2] Dept. of Agroecology and Environment, Faculty of Agricultural Sciences, Aarhus University, Blichers Allé 20, P.O.Box 50, DK-8830 Tjele, Denmark.

DEVELOPMENTS DECISION SUPPORT MODELS

In France, the DSS's Mildi-LIS and MilPV merged to form a new DSS for advisors and potato growers: Mileos. Furthermore, DSS's for organic production in France (Fredon) and Germany (Öko-SIMPHYT) were developed to help scheduling copper applications within the national and local regulations for application of copper.

The EuroBlight platform saw the birth of an application which allows comparison of the performance of core algorithms for potato late blight DSS for a range of years and locations (weather). EuroBlight now also provides a list of DSS's, contact persons etc. available in Europe.

Over the past period, the use of GIS and spatial interpolation of weather data for use in DSS systems was explored in Germany with good results. Spatial interpolation was used to increase the spatial resolution of weather data by "adding virtual weather stations" up to a density of 1 (virtual) station per km^2. Geographic data such as elevation, aspect and slope were used to improve the accuracy of interpolated virtual weather data. The high resolution weather data obtained were then used to improve the quality of disease management.

General knowledge gaps with respect to the current models were identified and included:

- Phenotypic data to update models are not available
- First infection: models assume inoculum comes from tubers. Include oospores? 8 – 10% tuber infection in conventional production, more in organic production. Situation seems to be stable and is more or less under control with the current systems.
- Tuber infection: Most systems only address foliar problems; specific advice to prevent tuber infection is not available.
- Cultivar resistance is treated differently by the different systems and used to e.g. adapt spray intervals and or fungicide dose rates. Reliable experimental data are difficult to obtain. On top of that there are concerns on the stability of resistance as related to the adaptive abilities of *P. infestans*.

EUROBLIGHT IN A GLOBAL CONTEXT

Globally, the use of DSS's for potato late blight management is increasing with large areas of potato managed by VNIIFBlight in Russia. In China, china-blight (http://wwwchina-blight.net/) is providing information on potato late blight management, general weather based disease forecasts (adjusted MISP) and a simple DSS for farmer use (CIP derived Questionnaire).

Furthermore, the way EuroBlight operates, its pathogen and host databases, control strategies and the fungicide table has attracted global attention. Research groups in Asia and Latin America aim to start up "Asia Blight" and "Latin Blight", EuroBlight's spin off partners in Asia and Latin America.

EU PESTICIDE PACKAGE AND DSS's

The introduction of the EU pesticide package will bring about changes in European agriculture, with each member state implementing a national action plan and adoption of Integrated Pest Management (IPM).

DSS's can play an important role in optimization and justification of pesticide applications while keeping track of inputs at the same time. DSS's should thus be promoted as a tool enabling a responsible implementation of the EU directive while at the same time providing growers with the most up to date and effective control strategy. It was recommended to communicate this information to inform EU policy makers on the potential contribution of DSS's to implementation of the EU pesticide package.

Occurrence of *Alternaria solani* in Sweden and its sensitivity to strobilurins

EVA BLIXT & BJÖRN ANDERSSON

Swedish University of Agricultural Sciences, Dept. of Forest Mycology and Pathology,
P.O. Box 7026. SE 750 07 Uppsala, Sweden

SUMMARY

Potato leaves showing symptoms similar to early blight were collected three times during August - September 2009 in Sweden. Using diagnostic PCR methods *A. solani* was identified in 56% of the 432 samples and *A. alternata* in one single sample. In some of the sampled fields the incidence of early blight was high during September despite one or two applications of strobilurin fungicides. DNA extracted from the samples with confirmed *A. solani* was sequenced in order to determine whether the loss of effect was due to fungicide resistance. None of the *A. solani* sequences showed substitutions at any of the three amino acid positions associated with resistance to strobilurins.

KEYWORDS

Alternaria solani, *A. alternata*, early blight, fungicide sensitivity, strobilurin

INTRODUCTION

The plant pathogenic fungi *Alternaria solani* and *A. alternata* cause early blight and brown spot on potato, respectively. During the last decade the number of reports of early blight has increased in the south-eastern part of Sweden, especially in starch potato crops. Both *A. solani* and *A. alternata* were detected in field trials during 2005 and 2006, but *A. solani* was more frequent (Andersson & Wiik, 2008). A survey of the two *Alternaria*-species performed in Germany showed that both species could be found simultaneously in a field and that they occurred in all areas where potato is grown (Hausladen & Leiminger, 2007). Mancozeb, a common substance in many fungicides used against late blight on potato (*Phytophthora infestans*), has been reported to have an effect on *Alternaria*-species. In Sweden, concerns of the carcinogenic effects and neural tubes defects linked to mancozeb (Belpoggi *et al*., 2002; Nordby *et al*., 2005) has led to far reaching restrictions in the use of this fungicide. As a consequence, the use of mancozeb has drastically declined in Sweden, which may have been one cause of the increase in incidence of early blight. Strobilurins have so far shown efficient control of early blight in Sweden and has helped achieving high potato yields (Andersson & Wiik, 2008). However, in parts of the USA the use of strobilurins no longer gives the desired effect against *Alternaria*-species (Luo *et al*., 2007; Pasche *et al*., 2005; Rosenzweig *et al*. 2008). In the American population of *A. solani,* three different nucleotide substitutions leading to strobilurin resistance have been found. These substitutions are located in the gene encoding cytochrome *b* in

the amino acid position 129 (F129L). In California, isolates of *A. alternata* infecting pistachio and almonds have a corresponding substitution at position 143, (G143A). This substitution results in a higher degree of resistance compared to F129L.

In Sweden, potato growers are experiencing increasing problems with early blight. There are reports of fields treated with strobilurins where severe attacks of early blight have occurred. The aim of this project was to determine the causal agent of the lesions similar to early blight collected in south-eastern Sweden during 2009 using diagnostic PCR. The occurrence of strobilurin resistance was analysed in the sampled material by sequencing the gene encoding cytochrome *b* in order to identify any relevant amino acid substitutions.

MATERIALS AND METHODS

Identification of *Alternaria solani* and *A. alternata*

Plant material
Sampling was performed at six locations three times during 2009 (early August, late August and in mid September). Twenty-four potato leaves showing early blight symptoms were collected at each location and at each sample time, resulting in 432 samples in total. If no lesions were found at a particular sampling spot, a symptomless leaf was collected instead. The six sampling locations were commercial starch potato fields located in the south-eastern parts of Sweden, five of which were cv. Kuras while one was cv. Kardal. Four of the fields with cv. Kuras were treated twice with strobilurins, either during the second or the fourth week of July.

DNA extraction and identification of causal agent of the lesions
A small leaf piece containing one lesion was cut from each leaflet and was washed twice in sterile distilled water. From each lesion three discs, 2 mm in diameter, were cut from the edge of the lesion containing both healthy and necrotic tissue. The discs were homogenised with five glass beads (3mm ø) in a 2 ml micro centrifuge tube in a Precellys homogeniser. DNA was extracted using a CTAB protocol. The two species were identified using species specific PCR primers developed by Rosenzweig *et al.* (2008) for *A. solani* and Zur *et al*. (2002) for *A. alternata*. Both species and a non template control were included each run. A DNA fragment of the gene encoding cytochrome *b* was amplified and sequenced using a newly developed forward primer (unpublished) and the 143 reverse primer developed by Rosenzweig *et al*. (2008). The PCR-product included the amino acid positions 129, 137 & 143.

RESULTS AND DISCUSSION
The disease incidence increased during the season and in September the majority of the lesions were confirmed to be caused by *A. solani*. The proportion of samples identified as *A. solani* in each field varied between 45% and 81% over the season for cv. Kuras while cv. Kardal had a total incidence of 22%. One sample containing *A. alternata* was identified from the second collection in one of the cv. Kuras fields. All of the 242 samples with confirmed *A. solani* had the wild type version of the gene encoding cytochrome *b* suggesting that the Swedish population of *A. solani* is still sensitive to strobilurins.

CONCLUSIONS

This study showed that early blight in south-eastern Sweden is caused by *A. solani*. *A. alternata* was rarely found on leaves with early blight symptoms. No amino acid substitutions associated with resistance to strobilurins was found in the genome, indicating that these compounds still are effective against the Swedish population of *A. solani*. However, further and continuous investigations must be performed in order to monitor the risk of loss of sensitivity towards strobilurins.

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Presentation

Will the real Alternaria stand up please

Experiences with Alternaria-like diseases on potatoes during the 2009 growing season in The Netherlands

L.J. TURKENSTEEN, J. SPOELDER, A. MULDER

Hilbrands Laboratory for Soil-borne Pests and Diseases, Kampsweg 27, 9418 PD, Wijster

INTRODUCTION

During the period June 25 till September 18, 2009, a survey was made on causes of Alternaria-like lesions present on potato leaves. This survey was part of a project called Development of Digital Detection and Diagnosis Service (short name DDDD-Project) financed by the Provincial Authority of Drenthe and European Regional Development Fund (ERDF). The aim of the project is to link images of disease symptoms in the field to a databank of causal agents and corresponding symptoms and sequently to select a cause. Then to the farmer an advice is to be formulated how to manage the problem concerned. As a first crop to deal with, potato was chosen and as a first target disease Alternaria-like lesions.

MATERIAL AND METHODS

Target organisms.

A first target organism is *Alternaria solani*. However, from prior research (see for example the Mimi-case, this article), it was learned that in Alternaria-like lesions as well as in lesions of *Phytophthora infestans*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, two organisms are commonly found to be present. It concerns *Alternaria alternata* and *Cladosporium cladosporioides*. Among the three named organisms, *A. solani* is a true pathogen whereas *A. alternata* is a weakly pathogenic organism capable to affect wounded foliar tissues of potato (Pits *et al.,* 2005). *C. cladosporioides* is an extremely common saprophyte.

Collection.

Cooperators were requested to collect potato leaflets with symptoms, which to their opinion were typical for *A. solani*. For the collection of leaflets with symptoms, Petri-dishes with water agar amended with 250 mg streptomycin per l were made available to cooperating institutions. After receiving samples by mail or by handing over, both lesions and agar were examined on the presence of spores of the target organisms with the help of a binocular loupe. Then as far as available, for a minimum of three leaflets of each sample three lesions per leaflet were excised and placed on water agar amended with 250 mg/l streptomycin. The three lesions per leaflet were placed in a single petri-dish. After 3 and 9 days, lesions and agar were examined on the presence of the target organisms as well as for other organisms showing up. The three target organisms show a strong tendency

to profusely sporulate both on the lesion tissues as well as on mycelium entering the water agar connected to the excised tissues.

RESULTS

Results are presented in Tables 1, 2 and 3 and Figure 1. In total 112 samples were received, which yielded 768 excised lesions incubated on water agar. The first sample yielding *A. solani* was obtained on July 21. Before that date, already 22 'Alternaria' samples had been received, which yielded 286 excised lesions void of *A. solani*. From that date onwards another 90 samples were received, which yielded 48 samples and corresponding 219 excised lesions with *A. solani* and 42 samples and corresponding 263 excised lesions void of *A. solani* (Table 1).

Table 1. *Results on presence of A. solani in Alternaria-like lesions in samples received during the growing season of 2009 from June 25 till September 18.*

Sample information	June 25 till July 21	July 21 till September 4	September 4 till 18	June 25 till September 18
Samples received	**22**	**59**	**31**	**112**
yielding *A. solani*	0	19	29	48
without *A. solani*	22	40	2	64
Lesions excised	**286**	**301**	**181**	**768**
yielding *A. solani*	0	68	151	219
without *A. solani*	286	233	30	549

For the period of June 25 till July 21, *A. alternata* was found to be present in 33.9% of the excised lesions. In that period there was no *A. solani* found.

A first conclusion is that in 123 or 66% of the lesions, which were very similar to lesions of Alternaria, both *A. alternata* and *A. solani* were absent (Table 2).

Table 2. *Occurrence of A. solani and A. alternata in Alternaria-like lesions sampled for incubation during the growing season of 2009.*

Period	*A.solani*	*A.alternata*	*A. solani* and *A. alternata*	Lesions void of *Alternaria* spp.	All lesions
June 25 till July 21	0	67	0	123	186
July 21 till Sept. 4	50	215	18	88	401
Sept. 4 till Sept. 18	70	19	81	24	181
Total period	120	301	99	261	768

For the growing season of 2009, the presence of *A. alternata* in lesions occurred according to an average infection rate of 43.8% (Table 3). To be able to do so and if the average size of lesions is supposedly 1 cm^2 then there must be at least one active propagule per 1 cm^2/0.44= 2.28 cm^2 to achieve the encountered percentage of invaded lesions. If *A. alternata* was acting as a pathogen and infecting independently of *A. solani* to form lesions, there should be at least one lesion with *A. alternata* at any 2.28 cm^2 leaf surface of any leaflet. This was not the case. In fact, *A. alternata* is not an organism that is strongly pathogenic to potato foliage, but is much more an extremely successful invader of necrotic lesions due to any agent to cause those.

Table 3. *Incidence of lesions with A. alternata with or without A. solani and C. cladosporioides and the corresponding Chi square test.*

Presence of *A. alternata* in lesions with *A. solani* and with *C. cladosporioides*	Frequency	
(Pairs of *A. alternata* and *A. solani*) / (all lesions with *A. solani*)	0.4393	
(Pairs of *A. alternata* and *C. cladosporioides*) / (*all lesions with C. cladosporioides*)	0.4345	
(All *A. alternata* isolates) / (All isolates)	0.4394	
Average frequency of *A. alternata*	**0.4377**	
Chi square test based on average frequency of *A. alternata*	**Expected**	**Encountered**
Group 1: *A. alternata* and *A. solani* together	93.67	94
Group 2: *A. alternata* and *C. cladosporioides* together	129.93	126
Group 3: *A. alternata* versus all lesions	393.50	395
Chi square test for groups 1, 2 and 3: P = 0.9931		
Chi square test for groups 1 and 2: P = 0.9285		

For the period of July 21 till September 4, *A. alternata* was present in 59.1% and *A. solani* was present in 17% of the lesions excised. For that period still 114 or 28.4% of Alternaria-like lesions were found to be void of both *A. solani* and *A. alternata*.

For the period of September 4 till September 18, *A. solani* was commonly found in the field. From 181 lesions collected during that period, 151 lesions yielded *A. solani* and 100 lesions yielded *A. alternata*. In 91 of these lesions, *A. solani* and *A. alternata* occurred together. From 181 lesions collected during this period only 24 lesions or 13.3% were found to be void from both Alternaria species.

In samples obtained from the field *A. solani* gradually increased from 0% in the first period to 17.0% in the second one and to 83.4% in the third period (Table 2, column *A. solani*).

In general, it can be said that in the Netherlands, *A. solani* came late during 2009. At the end of the growing season, most of the Alternaria-like lesions contained *A. solani*, but also to a high percentage *A. alternata*. It should however be mentioned here that those crops, which yielded so many lesions void of *A. solani* and *A. alternata*, had already acutely died off in early August; this without any further increase in the number of lesions.
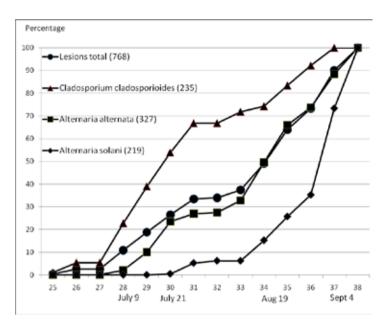
Figure 1. *Proportional increase in percentages of the three target organisms and number of lesions laid out on water agar during the period of June 25 till September 18, 2009. On the x-axis numbers of weeks are presented as well as specific dates mentioned in the text. The 50% line presents when 50% of the lesions concerned were collected.*

Regarding the whole testing period there were considerable numbers of Alternaria-like lesions void of both *A. solani* and *A. alternata* (Photo 2). It means there is symptom development in farmers' fields leading to Alternaria-like lesions for which the presence of either *A. solani* or *A. alternata* is not needed. The key question is what the origin of this symptom development may be. An interesting candidate is ozone stress associated with boron deficiency (See The Mimi case).

The Mimi case

In 2007 two rows of the very early Scottish potato variety Mimi were grown in the Noordoost Polder in the neighbourhood of Marknesse. On the 23rd of June, the first and third author were called in as the cultivar was battered by early blight. Indeed, the crop showed relatively large Alternaria-like lesions with diameters ranking between 4 and 15 mm with the typical Alternaria-like concentric rings (Photo 1). However, at that period nowhere in the Netherlands *A. solani* had been reported. Leaflets with lesions were

Photo 1. *Alternaria-like lesions on leaflets of cv. Mimi void of Alternaria spp.*

collected in Petri-dishes with water agar. Lesions concerned were internally and externally studied microscopically. However, no fungal hyphae were to be seen either on or inside the lesions. Thirty lesions were laid out on water agar. Of those, a single lesion yielded an isolate of *C. cladosporioides*; all other lesions did not show any development of fungi. On the 29th of June a second visit was paid to this field. Lesion's size had increased considerably with diameters increases of more than

100% and many lesions had coalesced to form large areas of affected necrotic tissues. Again leaflets were collected as described before. This time, all 30 lesions yielded *C. cladosporioides*. *A. alternata* was found to be present in a single lesion only. Due to foregoing weather conditions and based on experiences in the laboratory, the disease was claimed by the two authors to be due to ozone stress and not because of attack by any pathogen.

However, in addition to the development of Alternaria-like lesions, cultivar Mimi showed typical symptoms of boron deficiency such as: thickened and inflexible leaflets, coalescence of the top leaflet with one or two of the two closest lateral leaflets, typical damage of the leaflet borders due to failing of meristematic activity, and shortening of the petioles and stems. The cultivar is reputed for its shallow and weak root system, which curbs its possibilities to obtain boron from the soil, as this element is in a very low concentration directly available to the plant. This is even the case for soils, which in general are not boron deficient.

Relation between ozone stress and boron

What could be the relationship between boron deficiency and development of ozone stress associated with foliar lesions imitating early blight? Well, apart from being involved with plant growth and especially with the growing points and other meristematic tissues, the micro-element boron is involved in the assimilation process of plants and especially so in preventing super oxidation through formation of radicals as a consequence of the most critical process of transforming H_2O and CO_2 with the help of sun light into sugars. If boron is short in supply, super oxidation leads to a rapid disintegration of affected foliar tissues at sunny days (Mulder and Turkensteen, 2008). Ozone, which is also a super oxidant, is produced out of NO_2 and alkanes under the influence of sunlight. Both compounds originate from industrial processes as well as traffic. It enters leaflets through stomata and affects cells of inside tissues. In case of insufficient availability of boron, this reaction is very difficult to stop and especially so on sunny days. There is quite a difference between cultivars. Mainly cultivars with weak root systems are affected as for such varieties, it is most critical to obtain sufficient boron to prevent super oxidation.

State of crops with (Pseudo-)Alternaria-like lesions

Crops concerned showed some typical symptoms pointing at acute deficiency of boron as cause, as there is the sudden halt of stem growth, growth and development of new leaves and formation of micro-rosettes in the highest axils. Furthermore, the highest leaves showed symptoms of acute manganese deficiency associated with the typical olive green colour, loss of luster and the presence of tiny speckles along major veins. In addition on these same leaves symptoms of

Photo 2. *Close-up of Alternaria-like lesions on a leaflet due to ozone toxicity*

ozone stress developed, leading to lesions increasing in size due to the formation of new concentric zones (Mulder and Turkensteen, 2008; Photo 2).

DISCUSSION AND CONCLUSIONS

Concerning the date 21st of July, *A. solani* came about relatively late during the growing season. Thirty percent of the samples yielding *A. solani* were obtained during August, the other 70% between September 4 till 18. The question is whether this was the common moment for *A. solani* to occur or that this late occurrence was specific for the season of 2009. It should be considered that a

relative great part of the increase of *A. solani* is due to the fact that the crops with symptoms due to ozone stress had already died off.

During most of the season *A. alternata* was found to be present. The first isolate of *A. alternata* was obtained from leaflets collected on the 9th of July. However, the first Alternaria like lesions came in on June 24, most of which yielded *C. cladosporioides*. Averaged over all isolates, it was found to colonize 43% of all lesions independently whether it concerned lesions with or without *A. solani* and with or without lesions due to ozone stress (Table 3). *A. alternata* showed towards the end of the season a relatively strong affinity to *A. solani*, as in the period of September 4 till 18, it was found within 51.9 % of the lesions of *A. solani*, whereas in the period July 21 till September 4, it was found within 26.5% of the lesions only. The higher incidence later in the season is most likely due to the much higher frequency and hence much shorter distance between lesions of *A. solani* and the invader *A. alternata*.

C. cladosporioides was found to have invaded 38.4% of the lesions studied. It was present from the beginning, but became less frequently seen at the end of the season, which is probably due to the fact that lesions associated with *A. solani*, which were the most common ones observed at the end of the season, did apparently not present a favourable substrate to support invasion by *C. cladosporioides*. It was found with 40.5% of lesions with *A. alternata* but only with 10,5% of the lesions associated with *A. solani*.

From the 768 lesions laid out on water agar, 71.8% was void of *A. solani*, 48.0% was void of *A. alternata* and 34.0% was void of both *A. alternata* and *A. solani*. Nevertheless, all these lesions looked very similar to those of early blight. Considering the large group of lesions void of Alternaria spp., there must be another cause for the development of such lesions than *A. solani* and *A. alternata*. Taking into consideration the particular state of the affected crops in 2009, boron deficiency may be part of it. In addition, ozone stress may be a second player. The combination of boron deficiency and ozone stress may be the cause for the development of early blight like lesions. (See Mimi case, this article).

A. alternata is not as successful as *A. solani* to form lesions in the open. Only in 6% of the lesions with *A. alternata*, it was found as the single organism present. If we consider *A. alternata* not as an invader but instead to infect healthy foliar tissues to form lesions, and lesions of *A. solani* and *A. alternata* to be formed at random, than there should not be more than 6% of the lesions co-infected by the two organisms at the maximum. Considering that at the time of sampling about 10% of the foliar surface was covered by lesions, it could be in fact only 0.6%. This value is far from the found value of 94%. Like earlier in the season lesions yielding *A. alternata* only may be caused by other causes as well.

FINAL CONCLUSIONS

During the growing season of 2009 and especially so in June and July, many lesions were formed, which looked very similar to lesions caused by *A. solani*, but were void of *A. solani* and *A. alternata* (Photo 2).

The most probable cause for the early blight like lesions in June and July is ozone stress in combination with shortage of boron, and especially so with varieties respectively crops with shallow roots or weekly developed root systems.

It must be concluded that *A. alternata* is a very successful invader of necrotic lesions, but has a very poor capability if any to infect green foliage in the field (Spits *et al.*, 2005).

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Population genetics – consequences on early blight disease

JUERGEN LEIMINGER[1], GUENTHER BAHNWEG[2] & HANS HAUSLADEN[1]

[1] Technische Universität München, Center of Food and Life Science Weihenstephan,
Chair of Phytopathology,
Emil Ramann-Str. 2, D –85350 Freising-Weihenstephan, Germany,
[2] Institute of Biochemical Plant Pathology, Helmholtz Zentrum München,
German Research Center for Environmental Health, 85764 Neuherberg, Germany

SUMMARY

Members of the genus *Alternaria* are found in a wide variety of habitats worldwide. As causal agents of early blight disease *Alternaria solani* and *Alternaria alternata* are important pathogens in potato representing a risk to crop production. Due to premature defoliation, early blight epidemics can cause major yield losses. Recent investigations have been carried out in order to achieve more detailed information about disease progress and early blight control. Epidemiologic studies showed that both species can lead to necrotic symptoms. Up to now, field observations do not ensure positive proof for the differentiation of necrotic spots either caused by *A. solani* or *A. alternata*. By the use of specific primers, both pathogens could be isolated and quantified out of leaf samples. Therefore, PCR-based methods enabled a clear and simple differentiation of both pathogens. Little is known about pathogenic specialization and the generation of fungicide resistant isolates. Therefore, genetic analyses of plant pathogen populations are important for understanding epidemiology and host-pathogen interactions. This work deals with the analysis of intra-specific diversity of different *A. solani* isolates. By the use of RAPD markers spatial genetic diversity within and among *A. solani* isolates from different fields in Southern Germany were investigated. Our results show that the fungus is highly divers. This indicates that the fungus has a high potential to adapt to different environments, potato cultivars or even to build up fungicide resistance.

KEYWORDS:
Early blight, species differentiation, PCR, genetic diversity, RAPD

INTRODUCTION

Species of the genus *Alternaria* are very common and abundant. *Alternaria* species in general, including *A. solani* and *A. alternata* have a world-wide geographic distribution (Rotem, 1994). The genus *Alternaria* includes saprophytic and pathogenic fungi. Due to its high adaptability, early blight has the potential to become a serious threat for potato cultivation in Germany. Apart from the widespread potato disease late blight, early blight is causing increasing problems (Hausladen, 2006). During the last years, early blight became more and more important in German potato

growing areas. Recognized primarily as a foliage pathogen, epidemics occur when the weather is warm and dry with short periods of high moisture. Both species infect the plant by conidia which are either wind blown or splashed onto plant surfaces. Visual analysis of the symptoms does not allow to distinguish if the necrotic spot is caused by *A. solani* or *A. alternata*. One objective of this study was thus to investigate the occurrence and distribution of both species (*A. solani* and *A. alternata*) on early blight infected leaves. For this, PCR-based methods have been used for specific molecular diagnostics (Bahnweg *et al.*, 1998). Beyond morphological characteristics, PCR enables a clear and simple differentiation of both pathogens, which can be used irrespective of symptoms or typical sporulation. *Alternaria* species have no known sexual stage, which precludes genetic analyses other than parasexual. *Alternaria* pathogens should consist of many asexual lineages evolving independently with little or no genetic exchange (Peever *et al.,* 1999). Variation in neutral genetic markers (RAPD) should accumulate independently in each lineage due to mutation. Molecular analysis may demonstrate the existence of diversity in these asexual pathogens. In fungal systems, RAPD-PCR has been widely applied for the characterisation of species and isolates. RAPD analysis was used to examine the levels of genetic variation of *A. solani* isolates within and among different geographical origins. The amount and distribution of genetic variation within populations gives an indication of disease evolution (Adachi *et al.,* 1993). An improved understanding of the genetic basis of species specificity has important basic and applied implications. Genetic analyses of pathogen populations are important for understanding epidemiology and host-pathogen interactions, as well as for the development of disease control strategies (Aradhya *et al.*, 2001; Morris *et al.*, 2000). The aim of this study was to estimate the amount of intra-specific variation within 45 isolates of *A. solani* from different locations in Bavaria and Germany and to assess the potential of RAPD-PCR as a diagnostic tool to distinguish between morphologically similar or identical isolates of one species.

MATERIALS AND METHODS

EARLY BLIGHT MONITORING
A monitoring programme has been accomplished in naturally infected potato fields throughout Germany in order to document the distribution and occurrence of early blight in German potato growing areas. Early blight-infected leaves were obtained during this monitoring programme since 2005. Collected leaf samples were investigated for the occurrence of *A. solani* and *A. alternata* according to morphological characteristics. Up to now, investigations on disease incidence of early blight in Germany are only based on single observations by farmers or advisory services. The realization of this early blight monitoring enabled an outline of the distribution as well as the local occurrence of both *Alternaria* species in German potato growing areas.

ISOLATE COLLECTION
Infected leaflets were sampled at random and one isolate was taken per collected leaflet. Leaf pieces bearing a single lesion were cut from infected leaves and surface sterilized. Leaf cuts were transferred to petri dishes and incubated under UV-light. Isolates were obtained by single spore isolation and prepared for long-term storage. *A. solani* cultures were identified on the basis of morphological characteristics and spore size. Isolates of *A. solani*, 17 in 2006 and 20 in 2008, were collected from one potato field located in Weihenstephan. In addition in 2008 eight isolates have been collected from different potato fields in Bavaria and Germany (locations: Atting, Ehetal, Geltolfing, Straßmoos, Thonstetten, Uelzen).

QUANTITATIVE PCR ANALYSIS
The amplification of specific DNA-segments allowed the characterization of both species *A. solani*

and *A. alternata* out of small amounts of fungal DNA. Quantitative PCR (qPCR) enabled an overview about the distribution and quantitative amount of both species within infected leaves. Leaf sampling started 8 weeks after crop emergence and were carried out until vine death. Out of each repetition, ten potato leaves were collected every second week and stored in liquid nitrogen immediately. Specimens were homogenised with a mortar in liquid nitrogen and were used for further analysis. By the use of quantitative analysis, early blight development in the leaf could be investigated in the course of the potato vegetation cycle.

PCR-BASED GENETIC DISCRIMINATION

The RAPD technique relies on the presence of priming sites for a single primer on the genome in an inverted orientation. No prior knowledge of the genome to be analysed is required. For the RAPD technique, random 10 bp OP (Operon Technologies, Alameda, California) oligonucleotides primers were used to produce amplified DNA fragments. RAPD markers have been demonstrated to provide a quantitative assessment of genetic relationships and similarities of genotypes. Preliminary tests were performed with 5 complete sets of OP primers. Random primers yielding amplicon patterns discriminating between isolates or groups were used for further investigations. Only bands that could be clearly distinguished as present or absent were scored. Similarity coefficients between all pairs of isolates were calculated by the Sokal & Michener coefficient (simx/y = (a+d)/(a+b+c+d)). Data of all pairwise comparisons of isolates are presented as a two-way similarity matrix, which was then used to perform an average linkage cluster analysis.

RESULTS AND DISCUSSION

EARLY BLIGHT MONITORING

Over several years, *Alternaria* species were monitored and isolated out of leaf necrosis. Early blight is a destructive disease, which can cause premature defoliation of the potato plant. Morphological analysis and pathogen specific investigations showed that early blight pathogens are present in almost every potato growing area in Germany. Within the monitored years, the frequency in the occurrence of both *Alternaria* species showed differences. In 2006 *A. solani* and *A. alternata* could be isolated from almost every infected leaf sample. In the following years, mainly *A. alternata* was isolated from the first samples. In 2007 and 2008 *A. solani* was isolated out of 55 to 60% of the investigated leaf samples (Table 1). In both years, *A. solani* was missing in almost every leaf sample until mid/end of July. Weather conditions may influence the development of both fungal species and favour the development of *A. alternata* in the beginning of the potato growing period. According to Viskonti and Chelkowski (1992) weather conditions may influence the development of both species in a different way.

Table 1: *Isolation frequency of A.solani and A. alternata out of infected leaf sample*

species / Year	A. solani	A.alternata
2006	95%	98%
2007	60%	96%
2008	55%	100%

Our results show that only by regarding symptoms it is not possible to distinguish if the necrotic spot is caused by *A. solani* or *A. alternata*. Although necrotic symptoms can be truly defined as early blight symptoms there are obvious differences in the occurrence of both species. Morphological or PCR investigations should be used in order to obtain a clear differentiation and distribution of both *Alternaria* species.

QUANTITATIVE PCR ANALYSIS

Quantitative real-time PCR has been carried out over several years (2003 to 2005). Beyond morphological analysis, PCR-based methods allowed the verification of species-specific DNA fragments. Optical disease ratings only gave an idea about disease distribution and disease epidemic in the field. However, quantitative real-time PCR enabled a specific assessment of *A. solani* and *A. alternata* and could be also used to quantify the amount of fungal DNA in infected plants. Specific primers for the detection and identification of both *Alternaria* species were highly sensitive and showed a clear differentiation between both *Alternaria* species.

Figure 1 and 2 show the amount of fungal DNA, which could be verified for *A. solani* and *A. alternata* during the vegetation periods 2003 and 2005. In all investigated years, both species could be detected out of analysed leaf samples (data 2004 not shown). However, species showed a different seasonal distribution as well as different levels of DNA amounts. Early blight species were first attested at the beginning of June out of sampled leaves within all years (June 16th (2003), June 7th (2004) and June 2nd (2005)), thus showing that *Alternaria* pathogens could be detected quite early in the stage of the potato development. In general, fungal DNA amounts stayed at a very low level at the beginning and increased till the end of the season. In comparison of the years, the distribution of both species was not equal. In 2003, *A. alternata* developed much faster than *A. solani*. First observations of *A. solani* were made before the end of July. Until the end of the season, DNA levels of *A. alternata* were almost twice as much as of *A. solani*. In 2004, both species were distributed much more equal just from the beginning of the observations (data not shown). 2005 showed opposite results, as DNA amounts of *A. alternata* stayed at a very low level. Only fungal DNA of *A. solani* was detected with increasing levels until the end of the season.
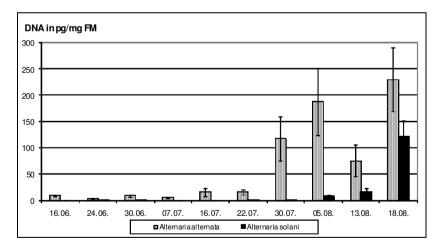
Figure 1: *Amounts of fungal DNA of A. alternata and A. solani out of sampled leaves in Weihenstephan 2003*
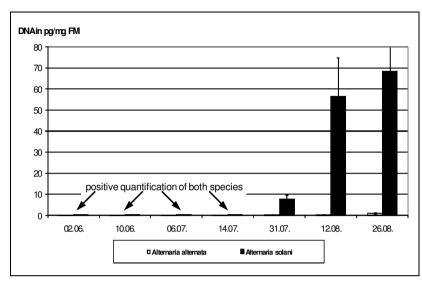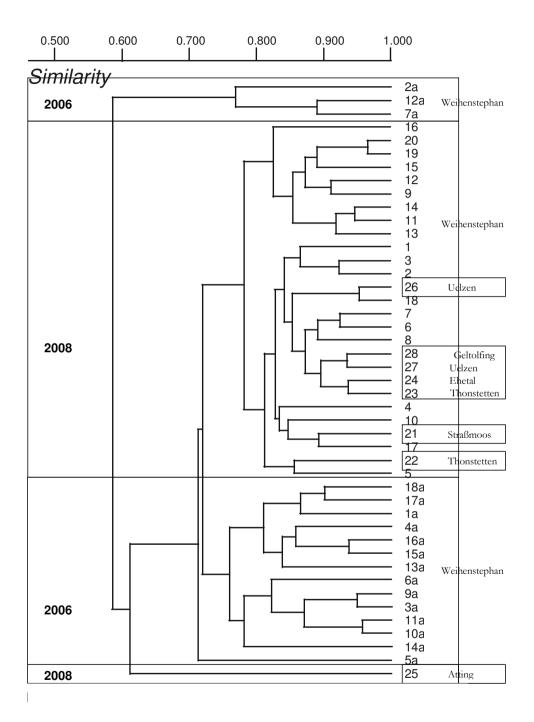
Figure 2: *Amounts of fungal DNA of A. alternata and A. solani out of sampled leaves in Weihenstephan 2005*

Our results show, that already small amounts of fungal DNA can be detected in leaves, often without any visible leaf necrosis. Therefore, molecular analysis enables a quick and safe proof of primary infections. According to the high primer specificity, which has been tested at more than 80 fungal diseases of potatoes and other crops, *A. solani* and *A. alternata* can be distinguished efficiently. According to Viskonti and Chelkowski (1992), weather conditions play a role for the development of both *Alternaria* species, which may explain the different distribution of species in comparison of the years. Our results show, that both species could be detected together in all years at different levels, indicating that both species are involved in early blight disease and could be seen as a "pathogen complex".

PCR-BASED GENETIC DISCRIMINATION

To further discriminate *A. solani* isolates, a RAPD analysis was performed. The highly polymorphic nature of RAPD markers makes them especially useful for the differentiation of clonal lineages of fungi that reproduce asexually. Ten primers were chosen based on a preliminary screening of primers. A total of 90 different amplicons were generated by the 10 different RAPD primers. Figure 3 shows the cluster analysis of all RAPD profiles. Cluster analysis of all RAPD profiles revealed high genetic diversity, not only at different locations of Bavaria (isolates 22-28) but also in samples of the same field (1a-18a (2006), 1-20 (2008)). Isolates from geographically distant fields were equally genetically divergent when compared to isolates sampled from one single field. A clustering according to geographic origin was not apparent. High differences in genetic diversity were detected between two sampling years, although isolates were collected from the same location. Weir *et al.* (1998) inferred from this high heterogeneity the possibility of pathogenic specialization. The mechanisms available for genetic change in *A. solani* are still largely unknown. Van der Waals *et al.*, (2004) and Weir *et al.*, (1998) hypothesized that evolutionary processes like mutation, selection, and gene flow may have influenced *A. solani* populations. The incidence of causal recombination might be the cause for the high diversity within isolates. Another cause for genetic variation may be natural mutation or large population size. The large population size of *Alternaria* species makes it more likely that new mutants with higher fitness will emerge and be able to multiply within the infected host. New alleles introduced in the population increase the chances of breakdown of resistance genes. However,

some of the investigated isolates, which were sampled from widely separated regions, pointed out only little genetic variation (isolates 23, 24, 27 and 28), indicating similar, if not identical isolates. These results indicate, that the various geographical subpopulations are not genetically isolated. Our results show high diversity within isolates of *A. solani*, which suggests that mechanisms for recombination and production of novel genotypes are available. How these factors may influence the population dynamics and evolution of the fungus demand further attention.

SUMMARY AND OUTLOOK

Enhanced investigations about population diversity help to determine the genetic structure of pathogen populations. This knowledge of genetic structure offers insights into the future evolutionary potential of pathogen populations. Our results indicate the presence of surprising genetic heterogeneity within the populations of *A. solani* which must be kept in mind when designing protective measures for agriculture. This may prove useful to optimize the management of fungicides to maximize their useful life expectancy and minimize the losses that result from reduced efficacy of these control methods. Understanding the genetic diversity of *A. solani* on potato will thus aid in future disease management strategies of early blight.

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Molecular Analysis of Alternaria Populations Early Blight Causal Agents in Potato Plants

M.P. LATORSE[1], F. SCHMITT[1], S. PEYRARD[1], S. VELOSO[1] AND R. BEFFA[1]

[1] Bayer S.A.S, centre de recherche de la Dargoire, 14 impasse P. Baizet 69009 LYON, France

SUMMARY

Early blight of potato can be caused by the two fungi *Alternaria solani* and *Alternaria alternata*, with significant impact observed on crop productivity in the field from the last past years in Europe. However, it is impossible to determine visually if the necrotic symptoms are caused by one or the other species. On the other hand, the differentiation of the two species based on the observation of the morphology of the spores is not considered as fully reliable because this spore diagnostic first means that the fungi remain alive despite of the collect conditions (treatments and drying situations) in the field. Secondly, *Alternaria alternata* differentiates spores much faster than *Alternaria solani* when isolated on artificial medium in Petri dish introducing a bias in this morphological diagnostic when both are present on the same sample.

Finally the efficacy of fungicides has been demonstrated dependent on the fungal species especially for the QoI fungicides. In fact, the use during these last years of QoI fungicides has shown the development of resistance due to the respective F129L and G143A mutations according the species in the genes encoding the cytochrome bc1, Qo site of the respiration complex III.

To improve the diagnostic of both *Alternaria* species and the detection of the point mutations conferring the resistance to the respiration complex III inhibitors, Bayer SAS has developed molecular tools to support *Alternaria* sp. monitoring and appropriate fungicide recommendation in the field.

The fine analysis of the sequences of the genes encoding the cytochrome bc1 reveals the presence of not yet described sequences in the introns of several *Alternaria* isolates. The presence of these particular sequences will be discussed in term of *Cyt bc1* gene organization, fungal population evolution, and horizontal gene transfer.

KEYWORDS:

Potato, early blight, *Alternaria solani*, *Alternaria alternata*, molecular analysis, qPCR

INTRODUCTION

When Early blight in US is reported to be mainly due to *Alternaria solani,* the agents responsible of Early blight in European countries are belonging to the two species *Alternaria solani* and *Alternaria alternata*. The occurrence of the disease was correlated to significant yield losses even if the economic input is difficult to evaluate. Based on the disease frequency monitoring, Early blight was mentioned to become more and more important in the last years in Germany and in Poland for example. In regards of more and more specific fungicides against Late blight reaching the market, of the reduction of the mancozeb rates, and of the potential climatic changes favourable for *Alternaria sp.*

in potato growing area, it could be expected that problems related to *Alternaria* sp. infection could continue to increase in the future in Europe. On potato, both *Alternaria* sp. cause necrotic lesions which are definitively not possible to be distinguished between spots caused by *Alternaria solani* or *Alternaria alternata*. The biological spore diagnostic after subculture on an artificial medium is time consuming and not fully reliable since *Alternaria alternata* is able to produce spores more rapidly and easily than *Alternaria solani* in such conditions. Therefore in a mixed population of spores, mainly *A. alternata* can be detected.

The widely used fungicides designed as Qo inhibitors (QoIs) inhibit mitochondrial respiration by binding to the Qo site of the cytochrome *bc1* enzyme complex. This blocks the electron transfer process in the respiration pathway leading to the death of the treated fungi (1). Resistance to QoI is often conferred by single amino acid exchanges in cytochrome *bc1*, either in position 143 where a glycine is replaced by an Alanine (G143A; 2), or in position 129 for which a phenylalanine is substituted by a leucine (F129L; 3, 4). Cytochrome *bc1* gene structure has been studied in several fungal species and the position of the intron close to the codon 143 seems to protect the sequence from possible mutations at this site in some species (5).

These limitations and the advances which occurred this last years in molecular phyto-diagnostic (6) drive us to establish methods based on Polymerase Chain Reaction (PCR) to quantitatively assess the presence of each *Alternaria* species and the cytochrome *bc1* gene appeared to be a good candidate to quantify at the same time the fungal species and the point mutations conferring resistance to Qo inhibitors.

MATERIALS AND METHODS

Alternaria sp. strains
Alternaria solani and *Alternaria alternata* strains were provided respectively by N.C. Gudmestad and J.S. Pasche for US references and collected randomly from across Europe fields respectively in Germany, Netherlands and Poland during the 2007 and 2008 monitoring campaigns (table 1).

Table 1: *Alternaria samples collected from across Europe in 2007 and 2008*
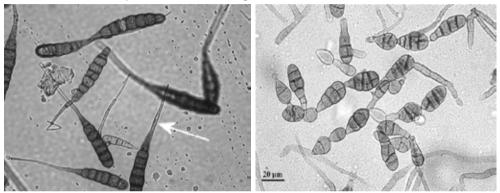
Sample code	Expectation/ Field symptõms	Conidia diagnostic
07GE051 A	?	No spores
07NL020	?	No spores
07NL024	solani	alternata
07NL029	solani	alternata
07PL008	?	alternata
07PL009	?	alternata
07PL010		alternata
07PL011		alternata
07PL059A		alternata
07PL059B	?	solani
07PL060B	?	alternata
08GE028	solani	alternata
08GE029	alternata	alternata
08BE041	solani	solani
08GE029	alternata	alternata

Mycelium and spore morphology study

The fungi are readily cultured on artificial media such as V-8 juice agar (7). The observation was done under a binocular magnifier to distinguish the size and morphology of conidia (Fig.1 & 2). *A. solani* has conidia (15-19 X 150-300 µm) with 9-11 transverse septa and few if any longitudinal septa. Spores are usually borne singly. Spores of *A. alternata* (20-63 X9-18 µm) are smaller than those of *A. solani*, are formed in chain and lack the typical long beak

Figure 1&2: *Long conidia (15-19 X 150-300 µm) of A. solani with a typical long beak and smaller conidia of A. alternata (20-63 X9-18 µm) in chain without long beak*

In vitro cultivation of fungal strains and mycelium preparation

Purified strains of *Alternaria solani* and *Alternaria alternata* were maintained in Petri dishes on Potato Dextrose Agar (PDA) medium at 21°C. 100 ml of liquid Potato Dextrose Broth (PDB) medium are inoculated with 10 agar plugs (5x5mm) infected with mycelium and cultivated at 21°C for 72h under agitation at 110 rpm. Mycelium is collected on 100µm nylon mesh, blended for 30 sec at maximum speed in a Warring Blendor in 50ml of PDB. 5 ml of this solution is used to inoculate 100 ml of PDB and further cultivated for 48h at 21°C under agitation 110 at rpm. Mycelium is then collected by centrifugation 30 min at 3200g, frozen in liquid nitrogen and lyophilized for 24h and stored at -80°C.

DNA preparation

 For the preparation of fungal strains genomic DNA, 30mg of lyophilized mycelium was used as a starting material. For plant infected by fungi, ten leaf disks of 10 mm diameter were frozen in liquid nitrogen and further lyophilized. Plant material was grinded 2 x 1min at 30Hz in a RetschMM301 mixer mill. DNA was extracted using QiaPreP miniprep (Qiagen) according to the manufacturer protocol with the following modifications: the mix is vortexed for 5 min after the addition of 250 µl P1 buffer, lysis is allowed for 5 min, let sit 5 min after addition of PE buffer. Elution from column is performed by adding 25µl of hot water (70°C), let sit 1 min and centrifuge to collect eluted DNA. Extracted DNA quantity was measured by OD260nm using a Nanodrop spectrophotometer (Thermo Scientific).

Sequencing of CytB gene

Genes were amplified by PCR from genomic DNA using species specific primers based on published sequences (5). For *A. solani* the forward primer was AS5F 5'-AGAACTCTAGTATGAACTATTGG and the reverse primer Asint143a_R 5'-CACAGTGGCTATGTGCTTGG leading to a theoretical 2216bp amplicon. Amplification was performed using Advantage2PCR system (Clontech) with 1

min at 95°C, 30 cycles of 30 sec at 95°C, 1min at 60°C, 3 min at 68°C and 13 min at 68°C for elongation. For *A. alternata* the forward primer was DTRcytb2: 5'-CTAGTATGAACTATTGGTAC and the reverse primer was DTRcytb2Rmodified 5'-GGAGCAAAAGATATTCTTTC leading to theoretical 338 bp amplicon. Amplification was performed using Phusion high fidelity enzyme (Finnzyme) with 1 min at 98°C, 30 cycles of 30 sec at 98°C, 30 sec at 57°C, 30 sec at 72°C and 5 min at 72°C for elongation. Resulting PCR fragments were cloned using pGEM-T easy for *A. solani* (Promega) or pCR4Blunt Topo (Invitrogen) for *A. alternata* and transformed into E coli Top10 by the heat shock procedure. Positive cloned were send for sequencing at MWG Biotech using M13 universal forward or reverse primers and SP-sol-1868Reverse 5'- TGGTGGAAAAGGCAGGGTAT for *A. solani* or SP-alt-1302Reverse 5'- CGAGCTTATTGTTGGTATTACTCACTCA for *A. alternata*.

Quantitative PCR
Primers were designed using the Primer Express software and all the Q-PCR experiments were performed in an ABI Prism7900HT cycler (Applied Biosystems). For each species to be analyzed, 4 PCR primer sets were designed. Primers concentrations were optimized, primer specificity was tested on the non targeted *Alternaria* species, and primer set amplification efficacy was calculated. The following primers set were selected: for *A. solani* forward primer is SP-sol-1658F (5'-GTAGAGTATGTTGAATACTCTAACCAGACAA) and reverse primer is SP-sol-1759R (5'-ATGTTAAGAATTTGTCCTGAACAGTTT) respectively used at 900nM and 50nM in the reaction mix; for *A. alternata* forward primer is SP-alt-153F (5'-CTTATGAGTGCTATACCTTGAGTAGGTCA) and reverse primer is SP-alt-376R (5'-TCTCCGTCTATCAATCCTGCTAAA), respectively used at 300nM and 50nM in the final reaction mix. The Q-PCR reaction mixture contained 10µl Master mix sybergreen (AppliedBiosystem), the corresponding primers, 5µl of diluted DNA and water to 20 µl. The Q-PCR cycle was the following: 2 min at 50°C, 10 min at 95°C followed by 50 cycles of 15 sec at 95°C and 1 min at 60°C, and ended by 15 sec at 95°C, 15 sec at 60°C, 15 sec at 95°C.

Quantification of Alternaria species present on leaf infected material
Serial dilutions at 1/10; 1/100 and 1/1000 of the extracted DNA were amplified by Q-PCR. Dilutions of *A. alternata* and *A. solani* purified DNA between 10^{-2} to 10^{-7} µg/µl were used to plot standard curves. Leaf disks artificially infected by *A. solani* or *A. alternata* were mixed together in various proportions to determine standard curves of amplification. Primers efficacies were calculated with the SDS software (Applied biosystems). The fungal presence detection limit was set to Ct equivalent to non infected plant samples. Differences of Ct (cycle threshold) were used to determine the relative proportion of *A. alternata* and *A. solani* in the samples.

RESULTS

Cytochrome bc1 gene structure and species specific sequences
Primers as previously described (5) were used to amplify the corresponding sequence of the cytochrome *bc1* gene of five European strains of *Alternaria alternata*. After having sequenced the amplicons, it appeared that a novel intron was found downstream of the position 143. The codons related to the amino acids 129 and 143 remained localized on the same exon (Fig. 3). A similar approach was done on four European and 2 American strains of *Alternaria solani* (Fig. 4). The gene structure of two European strains was similar to that previously described (5). The sequences obtained for the two American strains and the other two European strains showed that the first intron differed from the one known. The sequence comparison showed 88% homology with the

corresponding intron of *Pyrenophora teres*. In addition the novel intron is localized between the codon encoding the amino acid 129 and that encoding the amino acid 143. In addition the size of the exons was modified.
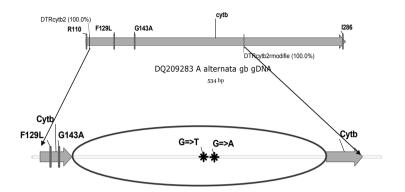
Figure 3: *A .alternata cytochrome bc1 gene structure. The upper part corresponds to the known structure (5), the lower part corresponds to those found in this study with a novel intron surrounded in red.*
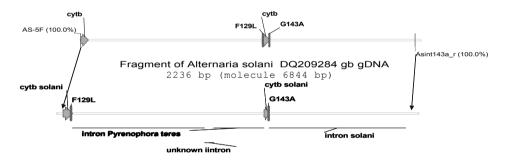
Figure 4: *A. solani cytochrome bc1 gene structure. The upper part corresponds to the known structure (5) found in two strains, the lower part corresponds to those found in this study with a novel intron with a significant homology to that found in P. teres.*

Quantitative PCR and quantification of Alternaria species present on infected leaves

For each fungal species four PCR primer sets were designed (cf. Material and Methods). After the optimization of their concentrations, primer specificity was assessed on the non targeted *Alternaria* species as well as the amplification efficacy of each couple of primers. DNA prepared from the mycelium was used. This allowed the selection of the final sets of forward and reverse primers for respectively *A. alternata* and *A. solani* as described in Materials and Methods.

DNA from potato leaves infected with either one or the other fungal species was then used to validate the selected primer sets. As control, DNA from non infected leaves was isolated. Using the primers specific for *A. alternata* a Ct of 31 was found whereas a Ct of 37 was observed with the *A. solani* specific primers. This showed that the plant DNA was not significantly interfering in the quantification of fungal DNA. Then 10 infected leaf discs infected with either *A. solani* or *A. alternata* were mixed in different ratio from respectively 10/0, 9/1, 8/2 to 0/10 (Table 2). Quantitative PCR on isolated DNA was performed to determine the ratio of each fungal species. The more accurate results (Table 2) were obtained using a dilution 1/10 of the DNA template.

Table 2*: % of DNA of each fungal species found by qPCR in different ratio of infected leaf tissues*

Number of infected leaf discs		% of DNA of each species	
A. alternata	A. solani	A. alternata	A. solani
0	10	0	100
1	9	30	70
2	8	56	44
3	7	65	35
4	6	72	28
5	5	82	18
6	4	86	14
7	3	88	12
8	2	91	9
9	1	95	5
10	0	100	0

The data obtained showed that the selected primers are specific to detect the DNA of each fungal species in extracts from infected leaf tissues. Only *A. alternata* was detected in the leaf samples not infected with *A. solani* and vice versa. Whereas it is not a linear relationship, when the proportion of leaf tissues infected with a given fungal species is increasing, the proportion of DNA corresponding to this species was also increasing. This led us to analyze samples harvested in the fields and compare the qPCR data obtained with the data obtained by biological analysis of the spore phenotype(s) and the field data observations (Table 3).

Table 3: *comparison of the fungal species characterization from the field symptoms, the spore phenotype analysis in the laboratory and the qPCR molecular diagnostic*

Sample code	Expectation/ Field symptôms	Conidia diagnostic	Molecular diagnostic % A. alternata - % A. solani	
07GE051 A	?	No spores	50% alt	50% sol
07NL020	?	No spores	93% alt	10% sol
07NL024	solani	alternata	100% alt	
07NL029	solani	alternata	100% alt	
07PL008	?	alternata	50% alt	50% sol
07PL009	?	alternata	85%alt	15%s ol
07PL010		alternata	100% alt	
07PL011		alternata	100% alt	
07PL059A		alternata	100% alt	
07PL059B	?	solani	14%alt	86% sol
07PL060B	?	alternata	80%alt	20% sol
08GE028	solani	alternata		100% sol
08GE029	alternata	alternata	95% alt	5% sol
08BE041	solani	solani		100% sol
08GE029	alternata	alternata	95% alt	5% sol

On the 15 representative samples analyzed, only 6 were characterized in the fields as having "typical" symptoms of *A. alternata* or *A. solani*. Two samples suspected as *A. solani* were identified as *A. alternata* only. Two were confirmed by qPCR as *A. solani* infected when 2 others were found a mixture *A. alternata* and *A. solani*.

In the laboratory, isolation of the fungi to be cultured on an artificial medium was successful for 13 samples on the 15. For each sample, the fungal species was determined by analysing the phenotype(s) of the spores. The qPCR analysis confirmed the data for 6 samples only. For the other 6 samples, whereas one species was clearly identified by biological analysis, the qPCR data clearly showed that a mixture between *A. solani* and *A. alternata* was present.

CONCLUSION AND PERSPECTIVE

Molecular diagnostic methods are necessary to improve the detection of plant pathogens (6). Our data showed that it is possible to set up a molecular based PCR diagnostic method using primers designed from the cytochrome *bc1* gene to determine the presence of *A. alternata* and *A. solani* in infected potato leaf tissues. The non linear relationships found by mixing known ratio of infected leaf tissues can be explained by the fact that the two fungal species showed different time course and ability to infect and multiply in the leaves. Nevertheless the technology appears to be robust, fast and reliable. Using the adequate primers, either by performing a second PCR or by establishing a multiplex approach, both the species and the mutation G143A and F129L could be assessed. With the evolution of the PCR technology, *e.g.* the geneXpert technology (8), it is possible to foresee applications at the field level.

The finding of the presence of an intron in the cytochrome *bc1* gene of *A. solani* closely related to the sequence of the intron found in *P.teres* suggests that a transfer of genes between the two species could have occurred. Whereas the relevance of Horizontal Gene Transfer (HGT) in eukaryotes is still under discussion, examples have been described in some species like *Candida parapsilosis* (9) and gene transfer between prokaryotes and fungi seems to occur relatively frequently as it has been recently reviewed (10). This suggests that exchange of gene between fungi is also possible and indeed it has been described (10, 11). What is the consequence of this exchange of genetic material on the evolution of the recipient species or on its pathogenic behaviour or its fitness? Are differences related to HGT between fungal clades? The development of molecular diagnostic will offer the possibility to find more of such events which will provide biological tools to study in more details and to better understand the evolution of fungal population.

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Control of potato late blight using a dose model to adjust fungicide input according to infection risk

NIELSEN[1], BENT J; BØDKER[2], LARS & HANSEN[3], JENS G

[1]Aarhus University, Department of Integrated Pest management, Denmark
[2] Knowledge Centre for Agriculture, Denmark
[3]Aarhus University, Department of Agroecology and Environment, Denmark

SUMMARY

Spraying against potato late blight prior to periods with a risk of infection results in a very effective control. The 2009 trials (two field trials x two varieties x 3 locations) shows that it is possible to reduce the application of effective fungicides such as Revus and Ranman by up to 30% by adjusting the dose in relation to the infection pressure of potato late blight. The risk dependent dose model is developed for potato late blight, but the 2009 trials show that control of early blight should also be included in the overall strategy.

KEYWORDS

Potato late blight, *Phytophthora infestans*, DSS, dose model, reduced fungicide dose

INTRODUCTION

The most effective control of potato late blight is through preventive treatments before infection. In periods with a high risk of infection it is therefore important to use a full dose of the best fungicides. By contrast, in periods with a low risk of infection it is possible to reduce the applications. By optimising the spray applications according to need there is thus a possibility of cheaper disease control in potatoes while still having an effective control. A risk dependent dose model (RDDM) has been developed based on the idea that the fungicide input could be adjusted to the actual need (Nielsen, 2004). In 2009 trials were carried out using RDDM to determine the actual dose and to investigate whether a safe and economically profitable control could be achieved by adjusting the dose according to need in comparison with other models.

METHODS

In 2009 experiments were carried out according to two trial designs in which spraying against potato late blight was managed according to the infection pressure of potato late blight using a dose model.

A) Field trials with different dose models

The trials were carried out in the susceptible variety Folva and the moderately resistant variety Kuras at three sites (Flakkebjerg, Sunds and Dronninglund). The trial design includes 1) routine treatment with Dithane NT (*mancozeb*) 2 kg/ha; 2) routine treatment with Ranman (*cyazofamid*) 0.2 l/ha; 3) spraying with Ranman according to a Stepwise Increase Dose Model (SIDM) in which the dose is increased during the season dependent on the incidence of late blight from a 25% dose if late blight does not occur in Denmark to a full dose if late blight is found in the field; 4) spraying according to the Risk Dependent Dose Model (RDDM) in which the current dose depends on how close to the potato field late blight has been observed, the calculated infection pressure (www.planteinfo.dk) and the resistance level of the cultivar (Hansen *et al.*, 2002). Early in the season when potato late blight has not been observed in Denmark and when the calculated infection pressure is low, no spraying will be recommended according to RDDM. Later, when late blight has been observed in the area, the dosage will increase depending on the infection level; 5) spraying according to the Dutch program Plant Plus (www.dacom.nl). On the basis of a calculated risk of spore dispersal and infection as well as plant growth the most suited fungicides will be recommended. Plant Plus uses a full dose of the fungicides, but the spray interval may vary depending on the conditions. The trials are supported by the Danish Potato Duty Foundation, KAF.
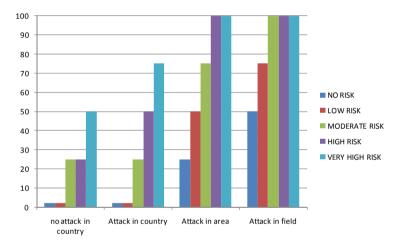
Figure 1. *Example of calculated dose (% of standard dose, 100% is full label dose) in the risk dependent dose model (RDDM) in the moderately resistant variety Kuras depending on occurrence of late blight in the country/ area and the local infection pressure (measured as sum of sporulation hours, HSPO. Low risk HSPO 1-19, moderate risk HSPO 20-39, high risk HSPO 40-60, very high risk HSPO >60, see www.planteinfo.dk for more explanation)*

B) Field trials with reduced inputs of effective fungicides

The trials were carried out in the susceptible variety Ditta and moderately resistant starch variety Kuras, and in the trial design spraying alternated between 2 x Revus (*mandipropamid*) and 2 x Ranman throughout the season. The dosage of either Revus or Ranman was adjusted according to either a fixed scheme or fixed doses using the model RDDM. The trial included the following treatments: 1) untreated, 2) Dithane NT 2 kg/ha, 3) Revus or Ranman at 1/3 dose, 4) Revus or Ranman at 1/2 dose, 5) Revus or Ranman at a full label dose (0,6 l/ha and 0,2 l/ha respectively) and 6) in which the dose of either Revus or Ranman was decided by RDDM from 25% of the full dose at low infection pressure to the full dose at high infection pressure.

The trials were carried out in collaboration between the Faculty of Agricultural Sciences, Aarhus University and Knowledge Centre for Agriculture

DISCUSSION

In the Risk Dependent Dose Model (RDDM) the current dose depends on how close to the potato field late blight has been observed, the calculated infection pressure (www.planteinfo.dk) and the resistance level of the cultivar. An example of actual dose for the moderately resistant variety Kuras is shown in Fig. 1. Early in the season when potato late blight has not been observed in Denmark and when the calculated infection pressure is low, no spraying will be recommended according to RDDM. Later, when late blight has been observed in the area, the dosage will increase up to full dose depending on the infection level.

Variety Folva   Variety Kuras
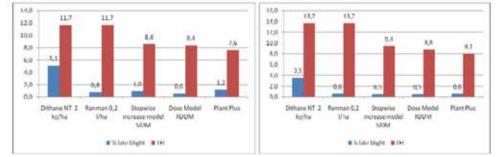
Figure 2. *% attack of potato late blight in the different model treatments (blue column) and the amount of fungicide applied (red columns, expressed as treatment frequency index, TFI). Trial A: Spraying at approx. 7-day intervals with Dithane NT 2 kg/ha, Ranman + additive 0.2 l/ha+ 0.15 l/ha; Stepwise Increase Dose Model (SIDM) using Ranman from dosage 25% to 100% in case of late blight occurrence; Risk Dependent Dose model (RDDM) where the dosage is dependent on late blight in the area, infection pressure and cultivar resistance; Plant Plus, see explanation in the text. Average of three trials (Flakkebjerg, Sunds and Dronninglund), 2009. Left figure Folva, right figure Kuras.*

It appears from Fig. 2 (trial A) that almost the same level of control of potato late blight was achieved with the different models as with the routine treatment with Ranman and better than after the routine treatment with Dithane NT but with approx. 30% less fungicide application. For both models (SIDM and RDDM) the reduction in fungicide consumption was obtained through reduction of the applied dose at the beginning of the season. Plant Plus used full doses but at longer intervals (only 7-8 treatments) with a TFI (number of sprayings with standard dose) of 7.6 and 8.1 in Folva and Kuras, respectively. There were some attacks of early blight *(Alternaria solani/A. alternata)* in the cultivar Kuras in the trial at Sunds. As Ranman has no effect on early blight, a severe attack developed late in the season in the treatments that included Ranman. There is no statistical difference in the harvested yield for the different treatments.

Variety Ditta   Variety Kuras
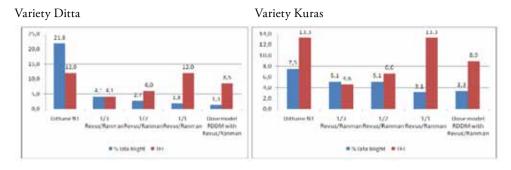
Figure 3. *% attack of potato late blight in the different model treatments (blue column) and the amount of fungicide applied (red columns, expressed as treatment frequency index, TFI). Trial B: Spraying at approx. 7-day intervals with 2 x Revus and 2 x Ranman in sequence throughout the season. Dose of applied fungicide is: 1/3 dose Revus or Ranman; ½ dose of Revus or Ranman, full dose (0.6 l Revus and 0.2 l Ranman) In the plot with dose model the actual dosage of Revus or Ranman is decided by the Risk Dependent Dose Model (RDDM). Average of three trials in the variety Ditta (left) and Kuras (right) at Flakkebjerg, Sunds and Dronninglund, 2009.*

It appears from Fig. 3 (trial B) that by using models (RDDM) in which the dose of Revus or Ranman is regulated by the infection pressure a level of control is achieved that is on a level with a full dose but with approx. 30% less fungicide consumption. There were attacks of early blight in the variety Kuras at both Flakkebjerg and Sunds, but only at Sunds did the attacks develop in the treatments sprayed with Revus or Ranman. When the trials are taken together, the yield and the quality have been maintained through use of the dose model despite a reduction in TFI (Nielsen *et al.*, 2010). The work with the dose models will be continued including further development of the sub model for calculating the infection pressure (Nielsen *et al.*, 2007, Nielsen *et al.*, 2008)

CONCLUSION

Spraying against potato late blight prior to periods with a risk of infection results in a very reliable and effective control. In 2009 the attacks of potato late blight occurred relatively late and did not develop until the end of July. Under these conditions the trials with reduced doses show that it is possible to reduce the application of effective fungicides such as Revus and Ranman by up to 30% by adjusting the dose in relation to the resistance of the potato cultivar and the infection pressure of potato late blight. The dose model is developed for late blight, but the 2009 trials show that control of early blight should also be included in the overall strategy.

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Reduced fungicide input in late blight control (REDUCE 2007-2011) –Preliminary results from 2007 to 2009

NÆRSTAD R[1], LE V H[1] & HERMANSEN A[1]

[1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research,
Plant Health and Plant Protection Division. Høgskoleveien 7, N-1432 Ås, Norway.

SUMMARY

Preliminary results from potato late blight experiments in Norway during 2007 to 2009 are presented. Field trials with fungicide doses adjusted to host resistance and late blight risk showed that it is possible to reduce the fungicide input to about 40 to 60% of the recommended dose in a cultivar with high resistance to late blight. Pot trials with silt and light clay soil and different irrigation regimes showed that most of the inoculum is not washed down into the soil, but remains in the top soil. Trials with tubers harvested at different maturity levels and inoculated with late blight spores showed no clear difference in susceptibility to tuber blight between the maturity levels. Field trials harvested at four successive dates where one half of each sample were dipped in chlorine showed that most of the tuber infections happened at harvest and not during the growing season.

KEYWORDS:
Phytophthora infestans, resistance, fungicide, tuber blight

INTRODUCTION

Potato late blight caused by *Phytophthora infestans* is an important disease in Norway. In average the potato area is sprayed 5.6 times to protect against late blight (Sætre *et al.* 2006). Some of the potato cultivars grown in Norway have moderate to high resistance to late blight in the haulm and tubers. It is possible to exploit host resistance to reduce the use of fungicides to control potato late blight (Nærstad *et al.* 2007), however this is not a normal practice in Norway. Reduced fungicide input in late blight control is a part of the project 'Reduced pesticide loads and risks in cropping systems' (REDUCE 2007-2011). In this project one of the goals is to develop models and best management practices to facilitate a good late blight control with minimal fungicide input. Preliminary results from experiments from 2007 to 2009 are presented.

MATERIALS AND METHODS

Exploiting host resistance in the haulm to reduce the fungicide input.

Field trials with inoculated spreader rows were conducted at two locations (Rygge and Solør) in 2008 and 2009. The field trials were designed as block trials with three replications with spreader rows on both sides of each block. The spreader rows consisted of an unprotected late blight susceptible cultivar (Mandel) inoculated with a spore suspension of *P. infestans* in the first half of July. Three potato cultivars with different resistance to late blight in the haulm and moderate resistance to late blight in the tubers were tested in combination with different fungicide doses. The cultivars used were Asterix (3 - 7) (resistance in the haulm and tubers on a scale from 1 to 9 where 9 is the most resistant), Saturna (5 - 6) and Peik (7 – 7). The fungicide Shirlan (fluazinam 500g/l) was applied preventively at the same times for all the treatments. Timing of the fungicide applications were done according to a new late blight model at www.vips-landbruk.no (Nærstad *et al.* 2009). The protection period of each application was 5-7 days, 5 days protection period if there were 4 or more blight risk days since last application. The three different treatments were: 1) untreated control, 2) full dose of the fungicide and 3) adjusted fungicide dose according to host resistance and late blight risk.

Table 1. *Rules used to adjust the fungicide dose to host resistance and late blight risk.*

Cultivar (resistance to late blight in the haulm, scale 1-9, 9 is the most resistant)	Number of blight favorable days (today and tomorrow) at www.vips-landbruk.no	Consecutive days with more than 1 mm rain the next 5 days at www.yr.no	Dose (% of recommended dose)
Asterix (3)	1	0-1	75
		2 or more	88
	2	0-1	100
		2 or more	100
Saturna (5)	1	0-1	50
		2 or more	75
	2	0-1	75
		2 or more	100
Peik (7)	1	0-1	25
		2 or more	50
	2	0-1	50
		2 or more	75

Wash down of spores into soil

Pot experiment with two different soil types (silt and light clay) and three different irrigation regimes (5, 10 and 15 mm of water) were conducted in 2007 and 2008. The soils were inoculated with two different inoculum doses, low inoculum dose (20 ml of 3.75 spores/ml) and high inoculum dose (20 ml of 37500 spores/ml) which were sprayed on the top of 1.5 liter pots with soil. The experiment had 3 replicates and negative controls. Soil samples were taken from the top surface and from 4 cm depth from each pot and tested with a bioassay (Lacey 1965). In the bioassay each soil sample was mixed with water and distributed on the top of 10 potato slices (approx 7 mm thick). These 10 potato slices and a negative control, without soil, were incubated in the dark at 16°C on a metal grid in a humid box. The potato slices were cut into octants after one day of incubation. The frequency of octants with *P. infestans* sporangiophores were recorded after 7 additional days of incubation at 16°C in the dark.

Tuber blight resistance at different maturity

In 2008 and 2009 three cultivars with different level of tuber resistance were grown in a field in an area of Norway with low blight risk. Tubers were harvested every second week from the beginning of August to the middle of September. Each time 60 plants of each cultivar were harvested. One to two days after harvest the tubers were washed and used for the experiment. For each cultivar the harvested tubers were divided into ten experimental units. Five units were artificially wounded by rolling the tubers over a group of 10 pins, 1 mm high, 1 cm apart, pointing upwards from a wooden base. The tubers from each unit were put into a plastic bag and spray inoculated with 20 ml of a spore suspension (10000 spores/ml in 2008, and 5000 spores/ml in 2009). Before inoculation the spore suspensions were chilled for 2 hours at 4°C to induce zoospore release. Four independently made spore suspensions were used to infect each of the units of both wounded and unwounded tubers. The last two units, one wounded and one unwounded were used as non inoculated controls. The tubers were mixed in closed plastic bags for half a minute and incubated at 16°C in the dark for one day. The bags were cut open to let the tubers dry and incubation of the tubers was continued for three weeks further at 16°C in the dark. Then the number of tubers with blight was recorded.

Tuber blight field trials

Field trials were conducted at two locations in 2008 and 2009. The fields were artificially inoculated by spraying all the plants with a spore suspension in the first half of July. The field trials had three replications and had a split plot design, with or without irrigation at critical dates on the whole plots and three cultivars with different level of resistance to tuber blight on the subplots. The critical dates were defined as days with a lot of inoculum produced in the haulm (sum of attached and released viable spores calculated according to New Late Blight Model (Nærstad unpublished data)) and no rain according to the weather forecast from The Norwegian Meteorological Institute. Each trial was irrigated on a critical day with 5 mm of water on one or two occasions in the season. Tubers were harvested four times (from ten plants per plot), approximately every second week from the end of July/beginning of August to September. The tubers from each plot were harvested into a net and kept dry. In 2009 the sample from each plot were split in two nets. One of the two nets was dipped in a chlorine solution (1% NaOCl) immediately after harvest. The number of blighted tubers was recorded after dry incubation in the dark at 16°C for 3-4 weeks.

RESULTS AND DISCUSSION

Exploiting host resistance in the haulm to reduce the fungicide input.

The weather was very blight favorable in August 2008 and in the end of July and beginning of August in 2009. In the unprotected control plots late blight killed the haulm in the end of August at both locations in 2008. In 2009 the haulm was also totally killed at Rygge in the end of August but some green leaves remained at the end of the season at Solør. Some blight developed in the plots treated with fungicide, but not significantly more in the treatments with fungicide dose adjusted to host resistance and late blight risk than in treatments with full fungicide dose. In these four field trials it was possible to reduce the fungicide use with 6-19% for the cultivar with low resistance (Asterix), 17% - 36% for the cultivar with medium resistance (Saturna) and 42%-61% for the cultivar with high level of resistance (Peik). These results are affected both by the rules used for adjusting the doses and also the actual weather conditions during the experimental period. However, the experimental results confirm earlier data (Nærstad *et al.* 2007) that there is a potential of reducing the fungicide input by exploiting the host resistance.

Wash down of spores in soil

The surface soil inoculated with high inoculums dose gave almost 100 % infections of the octants after 5 and 10 mm of irrigation and about 96% infection at 15 mm of irrigation. The surface soil with low inoculum dose gave approximately 10%, 8% and 2% infection at 5, 10 and 15 mm of irrigation respectively. A very small proportion of the inoculum was washed down to 4 cm depth, and there was a tendency that more spores were washed down at high amounts of irrigation water. Soil samples taken at 4 cm depth from soil with high inoculum level gave approximately 12%, 9% and 15% infection at 5, 10 and 15 mm of irrigation respectively. Samples from 4 cm depth in the soil with low inoculum dose gave no infections in the bioassay. There were no significant differences between data from the two soil types in this experiment.

Tuber blight resistance at different maturity

There was no clear trend in the effect of maturity on the tuber blight infections. In 2008 almost all the wounded tubers of Kerrs Pink became infected, and approx. 90 to 95% of the unwounded tubers became infected. About 85 to 95 % of the wounded Saturna tubers were blighted and about 60 to 90% of the unwounded tubers were infected. The wounded Troll tubers got approx. 75 to 90 % blighted tubers and the unwounded got approx. 35 to 70% blighted tubers. In 2009 the experiment was disturbed by pink rot (*Phytophthora erythroseptica*) infections.

Tuber blight field trials

There was no significant effect of irrigation at critical days on tuber blight. At Rygge the disease developed fast both in 2008 and 2009 and the haulm was killed by late blight by the middle of August. At Solør almost all the haulm was attack by late blight by the end of August in 2008 and 2009. In 2008 some tuber blight developed at all harvest dates, but more blight developed on the first dates than on the last dates. Hence, most of the tuber infection must have happened at harvest and not in the growing season. In 2009 we dipped half of each sample in chlorine immediately after harvest to be able to distinguish between infection in the growing season and at harvest. Tubers dipped in chlorine developed hardly any tuber blight. Tubers not dipped in Chlorine developed much more tuber blight on the first two harvests than on the last two harvest dates. On the two last harvest dates both years the haulm was totally killed by late blight.

CONCLUSIONS

The experiments confirm that it is possible to reduce the fungicide input in late blight control by exploiting the host resistance to reduce the fungicide dose. By using the rules in Table 1 it was possible to reduce the fungicide input in these four inoculated field trials by about 40 to 60% in the cultivar with high resistance without increasing the level of infection in the haulm compared to the treatment with full dose.

Most of the *P. infestans* inoculum remain in the top soil and less than 0,1% of the inoculum is washed down to 4 cm depth in soil without cracks.

No clear evidence was found in 2008 that tubers became less susceptible to tuber blight infection at increasing maturity .

In the four tuber blight field trials the tuber blight was mainly caused by infection at harvest.

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Experimental control strategies reducing the fungicide input at a practical scale

GEERT KESSEL, JOANNEKE SPRUIJT, BERT EVENHUIS,
PETRA VAN BEKKUM AND HUUB SCHEPERS

Plant Research International, Wageningen University and Research Centre.
PO Box 69, 6700 AB Wageningen, The Netherlands

SUMMARY

Phytophthora infestans is the most devastating disease in potato cultivation. Chemical control is necessary to ensure a healthy crop. At the same time Dutch governmental policy asks for a reduction of the environmental impact of potato late blight control by 75% in 2012 as compared to 1996-1998. The aim of the experiments was to compare Decision Support Systems with different approaches to blight risk management for their ability to reduce the fungicide input without compromising control efficacy.

Considerable savings, up to 81% when compared to weekly, full dose rate, spray schedules, can be achieved by using information on cultivar resistance, length of the critical period and disease pressure to decide whether or not to spray. The subroutine calculating the potential for viable transport of spores is only effective on resistant varieties as the threshold was exceeded with every critical period on less resistant cultivars.

Implications of the experimental control strategies for agricultural practise are discussed.

KEYWORDS:

Solanum tuberosum; potato; late blight; *Phytophthora infestans*

INTRODUCTION

Phytophthora infestans is the most devastating disease in potato cultivation. Chemical control is necessary to ensure a healthy crop. At the same time Dutch governmental policy asks for a reduction of the environmental impact of potato late blight control by 75% in 2012 as compared to 1996-1998. Fungicide choice (Schepers *et al.*, 2009) and reduced dose rates (Evenhuis *et al.*, 2009) contribute directly to reduce the environmental impact. Additionally, the goal can be achieved by using resistant cultivars and by increasing the efficacy of the fungicide applications by:
• matching operational requirement and fungicide characteristics
• using reduced dose rates on more late blight resistant cultivars (Spits *et al.*, 2009)
• matching of the spray timing with potential infection events considering
   - potential infection events in the near future
   - the atmospheric ability for viable transport of sporangia (Skelsey *et al,* 2009a and 2009b)

The aim of the experiments was to compare Decision Support Systems with different approaches to blight risk management for their ability to reduce the fungicide input without compromising control efficacy.

MATERIALS AND METHODS

Treatments
Field experiments were carried out at 5 locations (Table 1). Spray application were carried out according to Decision Support Systems ProPhy provided by Agrovision, Plant Plus provided by Dacom and WUR-blight developed by Wageningen UR.

Table 1. *Cultivars and decision support systems used in the 2009 experiments at 5 locations in The Netherlands.*

		A	B	C	D
		Plant Plus or ProPhy	WUR - Blight 1		WUR-Blight 2
Location	Purpose	Susceptible cultivar	Susceptible cultivar	Intermediately resistant cultivar	Resistant cultivar
Lelystad	Ware	Bintje	Bintje	Agria	Sarpo Mira
Westmaas	Ware	Lady Olympia	Lady Olympia	Agria	Bionica
Valthermond	Starch	Starga	Starga	Seresta	Festien
Vredepeel	Ware	Premiere	Premiere	Hansa	Innovator
Slootdorp	Seed	Spunta	Spunta	Agria	Toluca

Potato cultivars were planted in the spring of 2009. The crop was treated according to good agricultural practise. Late blight control varied between treatments.

Decision support systems
Treatment A was sprayed according to ProPhy (Westmaas and Slootdorp) or PlantPlus (Lelystad, Valthermond and Vredepeel). According to PlantPlus a spray application was only carried out when the threshold of 200 points was reached. Advice to consider a spray application in the range between 50 and 200 points was ignored. Treatments B and C were sprayed according to WUR-blight 1 which takes the following factors into consideration:
- Spray only when critical weather is predicted and the previous spray application does not protect the potato crop sufficiently.
- Default dose rates depend on the level of resistance of the cultivar (Spits *et al.,* 2009) and are reduced to a minimum of 25% of the recommended dose rate on resistant cultivars.
- Dose rates can be reduced depending on the fungicide degradation during the predicted critical period.

Treatment D was also sprayed according to the above criteria, but on top of that disease pressure was taken in to account. A sub routine calculates the capacity of the atmosphere to transport spores viably. according to Skelsey *et al.,* (2009a, b). If this capacity is low, despite the fact that a critical period is predicted, the crop will not be sprayed.
Decision Support Systems were consulted daily and spray advice was given accordingly.

Spray application
Spray applications were carried out using a SOSEF-sprayer with Teejet XR110.04 nozzles approximately 50 cm above the foliage, or a comparable spraying device. Spray applications were carried out with a volume of 250 l/ha. Haulm killing was carried out at the end of the season, timing depending on the purpose of the potato crop.

Observations

During the growing season the foliar infection was assessed at weekly intervals. To evaluate the epidemic, the Area Under the Disease Progress Curve (AUDPC) was determined. At the end of the season the crop was harvested. Yield and tuber blight incidence were determined. The number of spray applications and the full dose rate equivalents of the spray applications were assessed. If a spray application is carried out with a 50% dose rate it is defined as 0.5 dose rate equivalents. Total dose rate equivalents are determined by adding all individual dose rate equivalents sprayed during the season.

Statistical analyses

Five experiments were carried out. Each experiment was laid out as a randomized complete block design.

Analysis of Variance (ANOVA) was performed on yield, late blight severity and tuber blight incidence based on weight, measured per experimental plot, using Genstat release 12.1 (Payne *et al.,* 2002).

RESULTS

In the south of the Netherlands two infection periods occurred in May (Westmaas) and June (Westmaas & Vredepeel), early in the season. Followed by 4 additional infection events in Vredepeel and 5 at Westmaas.

In general, late blight infection risks first occurred at the beginning of July in the north of The Netherlands (Lelystad, Slootdorp, Valthermond). On average, the number of spray applications varied little with the treatments, although regional differences occurred. In the starch potato area the number of sprays was highest, due to the long growing season. The total dose rate equivalents applied however varied considerably between treatments (Table 2). On average the dose rate equivalent decreased with 19% by just spraying to cover the critical period (B compared to A). Taking also the cultivar effect into account a reduction of 42% was achieved (C compared to A). With the most resistant cultivar also the effect of disease pressure came into the equation and a reduction of 65% was achieved on average. Even higher reduction rates would have been achieved when the spray schedules were compared to weekly spray schemes with full dose rates, varying from a minimum of 46% to a maximum of 81% reduction.

Table 2. *Full dose rate equivalents of the sprays applied according tot the different late blight control strategies. The number of spray applications is denoted between parenthesis*

Location	Weekly interval	A Plant Plus or ProPhy Susceptible cultivar	B WUR - Blight 1 Susceptible cultivar	C WUR - Blight 1 Medium resistant cultivar	D WUR-Blight 2 Resistant cultivar
Lelystad	13	4.0 (4)	5.25 (7)	3.75 (7)	2.25 (6)
Westmaas	14	11 (11)	5.75 (7)	3.75 (7)	1.75 (4)
Valthermond	16	9.0 (9)	11.75 (15)	8.0 (15)	3.75 (12)
Vredepeel	15	4.8 (5)	5.0 (7)	4.0 (9)	2.75 (8)
Slootdorp	11	9.0 (9)	3.0 (4)	2.5 (4)	2.5 (4)
Average	13.8	7.6 (7.6)	6.2 (8.0)	4.4 (8.4)	2.6 (6.8)

No foliar or tuber blight occurred in the field during the experiments at Vredepeel and Slootdorp. Foliar blight occurred at Lelystad and Valthermond (Table 3), whereas tuber blight was found at Lelystad and Westmaas.

Table 3. *Foliar blight (AUDPC) and tuber blight as a result of the control strategies assessed, at three locations in 2009, in The Netherlands. No late blight occurred at Slootdorp and Vredepeel.*

Treatment	AUDPC						Tuber blight					
	Lelystad		Westmaas		Valthermond		Lelystad		Westmaas		Valthermond	
A	469	.b	0	a	180	.b	2.0	.b	0.06	a	0	a
B	6	a.	0	a	40	a.	0.4	a.	0.02	a	0	a
C	12	a.	0	a	14	a.	0.1	a.	0.02	a	0	a
D	0	a.	0	a	66	a.	0.6	a.	0.00	a	0	a

DISCUSSION

Considerable savings, up to 81% when compared to weekly, full dose rate, spray schedules, can be achieved by using information on cultivar resistance, length of the critical period and disease pressure to decide whether or not to spray. The subroutine calculating the potential for viable transport of spores is only effective on resistant varieties as the threshold was exceeded with every critical period on less resistant cultivars (Skelsey *et al.,* 2009a).

Late blight control was satisfactory apart from a few treatments. Plant Plus was used strictly with the threshold of 200 points. In agricultural practise PlantPlus advices to consider spraying at 50 points or above. In our experiments the 200 threshold was applied strictly, which may have caused the significant late blight development in treatment A at Lelystad and at Valthermond. Late blight control in cultivar Festien (treatment D Valthermond) lacked somewhat also. From previous experiments a dose rate reduction of 75% should still control late blight satisfactory in this cultivar (Evenhuis *et al.*, 2009). However the current late blight population might contain higher levels of virulent genotypes leading to erosion of the resistance level of cultivar Festien. Therefore it is necessary to monitor the *P. infestans* population for changes in aggressiveness an virulence.

At Westmaas no foliar blight was found, nevertheless some tuber blight occurred. Possibly some blight in the crop remained undetected. Alternatively sporangia might have been deposited on the crop after haulm killing and infested the tubers subsequently.

WUR-blight had on average no effect on the number of spray applications compared to the other Decision Support Systems. However the timing of the spray applications was much different. The WUR blight system protects the crop principally only during critical periods with a minimal fungicide input. This calls for flexibility of the farmer, since the DSS has to be consulted daily, also shortly after a spray application. In a period with high disease pressure sometimes two spray applications within a week are advised using WUR-blight, whereas in other periods long spray intervals of 30 days or more occurred. Farmers have a preference to a more or less weekly spray schedule. Strategies have to be developed in which a weekly schedule can be combined with lowering dose rates based on cultivar resistance and infection periods.

CONCLUSIONS

On average the dose rate equivalent decreased with 19% by just spraying to cover the critical period. Taking also the cultivar effect into account a reduction of 42% was achieved.

With the most resistant cultivar also the effect of disease pressure came into the equation and a reduction of 65% was achieved on average.

Compared to weekly spray schemes with full dose rates, a reduction varying from a minimum of 46% to a maximum of 81% can be achieved using WUR-blight.

In 2009 a limited number of critical periods occurred. At Westmaas, Slootdorp and Vredepeel the potato crop was not infected despite reduced and minimal fungicide input.

A low level late blight infection was found in Lelystad using WUR-blight.
A 25% dose rate proved to be too low to protect cultivar Festien sufficiently.
The modules can be further improved for future use.

ACKNOWLEDGEMENTS

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POSTERS

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Early Dormancy Break
in Blighted Progeny Tubers

HOLLY RIVETT, RUAIRIDH BAIN AND MARK MCQUILKEN

SAC
West Mains Road
Edinburgh, EH9 3JG, Scotland

Early Dormancy Break in Blighted Progeny Tubers

SAC

Holly Rivett, Ruairidh Bain and Mark McQuilken
SAC
West Mains Road
Edinburgh, EH9 3JG, Scotland
Email: Ruairidh.Bain@sac.co.uk

Introduction

In several of the previous 10 years in the potato blight trial field at SAC, Auchincruive Estate, Ayrshire, Scotland, many actively growing King Edward plants were observed many weeks after the trials had been completely desiccated. These plants were not the result of re-growth due to ineffective desiccation because when some were pulled out of the ground in earlier years the new stems clearly originated from progeny tubers, not the stems of the desiccated plants. The reason for the early break in tuber dormancy and the growth of these new plants after the haulm of the original crop was dead was investigated in more detail in 2009.

Stems growing from blighted progeny tubers

Sprouted progeny tuber showing typical symptoms of blight
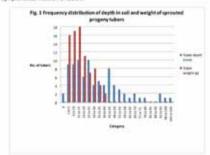
Methods

In early to mid-November 2009 83 actively growing King Edward plants were dug up from areas that contained infector plants, not treated with fungicide. The severity (%) of blight on the tuber surface area, the weight of individual tubers and the depth of tubers in the soil were recorded.

Also, the plots of some treatments in a fungicide trial, that had been desiccated with 4 l/ha of a diquat product but not yet harvested, were assessed for the number of actively growing stems in each of the two centre rows. The cultivar used in the trial was King Edward and plots had been treated with fungicides known to have different efficacies in controlling tuber blight. The fungicides were Laminator Flo (mancozeb) @ 3.3 l/ha, Electis (zoxamide + mancozeb) @ 1.8 kg/ha, Shinkon (amisulbrom) @ 0.5 l/ha, Shirlan (fluazinam) @ 0.4 l/ha and Ranman A + B (cyazofamid + adjuvant) @ 0.20 + 0.15 l/ha. The untreated control was also assessed.

Results and Discussion

All 83 of the progeny tubers that had sprouted were blighted. This confirms the finding by Montarry et al. (2007) that tuber infection by P. infestans can induce early sprouting. In this study the extent of the tuber surface area that was blighted varied considerably but was generally high, i.e. greater than 20% (data not shown). The fact that two of the 83 emerged stems originated from progeny tubers with very little tuber blight suggests that it may be the location of the tuber blight relative to the eyes that is crucial and therefore early sprouting will be a function of both blight lesion location and size. Further experiments are also required to determine if premature sprouting of tubers can be induced by latent, i.e. pre-symptomatic, infection of tubers.

Fig. 1 frequency distribution of depth in soil and weight of sprouted progeny tubers

Results and Discussion (continued)

As expected more of the blighted progeny tubers had been closer to the soil surface than deeper in the ridge (Fig. 1). However, premature sprouting was observed for 12 tubers that had been 12 cm or deeper. Tubers of a large range of sizes were blighted (Fig. 1). The average tuber size was small because the King Edward plants were not protected from foliar blight and therefore died relatively early.

Table 1 shows the current Euroblight ratings for direct control of tuber blight by the five fungicides and the number of emerged stems per row. The higher the Euroblight fungicide rating the lower the mean number of emerged stems. This inverse relationship was highly significant (R^2=0.93, P=0.008). Treatment with the four tuber blight fungicides Electis, Shinkon, Shirlan and Ranman TP resulted in significantly fewer emerged stems than the mancozeb treatment (F_{DF} = 0.010, LSD 1.29) but differences between the four fungicides with activity against zoospores were not significant.

There are several implications of blight-induced premature sprouting of progeny tubers in the field. For most crops with some tuber blight, harvest will take place before shoots from infected tubers can emerge therefore there isn't the risk of haulm produced by infected progeny tubers late in the season becoming blighted and being a source of inoculum to infect healthy tubers during harvesting.

Table 1 The number of stems emerging in late autumn from progeny tubers in desiccated field plots that had been treated with different late blight fungicides

Fungicide	Euroblight rating for tuber blight control	Mean no. of stems emerged per row
Untreated	–	2.1
Laminator Flo	0	2.4
Electis	++	1.0
Shinkon	++(+)	1.0
Shirlan	++(+)	0.8
Ranman TP	+++	0.0

The impact of blight-induced early sprouting is likely to be greater on the quality of the infected tubers, e.g. loss of moisture and loss of processing quality. Clearly the impact on the quality of the crop will be directly related to the number of infected tubers. In the fungicide experiment described above only stems that had emerged were counted. It is likely that other infected progeny tubers had sprouted but the sprouts had not yet reached the soil surface. The decline in quality associated with sprouting will be of little consequence for those tubers in which the blight causes an extensive rot because those tubers will be graded out. Establishing if premature sprouting is induced by latent infection, or very small lesions that fail to develop further, will allow the true relationship between the incidence of tuber infection and the effect of such infection on tuber quality parameters to be known.

Irrespective of the implications of this phenomenon for the risk of additional spread of blight in the field or tuber quality one aspect of the phenomenon is fascinating. There is now clear evidence from two independent studies that the pathogen P. infestans when it infects progeny tubers in the autumn can accelerate dormancy break and the production of stems. This contrasts sharply with the finding that the same pathogen inoculated into seed tubers prior to planting in spring can prevent or delay emergence, reduce the number of stems per plant and reduce plant height (Kelly, unpublished). Further experiments are necessary to determine if the different results obtained are due to the method of infection, time of infection and/or other factors.

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Acknowledgements

Crossability of wild potato species and advanced breeding lines resistant to late blight

B. U. CARLSON-NILSSON[1], N. M. ZOTEYEVA[2]

[1] Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Box 101, SE-230 53 Alnarp, SWEDEN, ulrika.carlson@ltj.slu.se
[2] N.I. Vavilov Institute of Plant Industry (VIR), RUSSIA, nzoteyeva@gmail.com

SUMMARY

In order to reduce the damage from *Phytophthora infestans* the use of new resistant cultivars is one of the viable approaches for late blight management. In crosses made at SLU, Alnarp (Sweden), wild and cultivated species, *Solanum tuberosum* L. cultivars and breeding lines possessing leaf or tuber resistance were used. The main aim of our study was to examine the crossability of several potato species and *S. tuberosum* breeding lines/cultivars resistant to *P. infestans*. This paper reports the results of crosses of two hexaploid (6x, 4EBN), one tetraploid (4x, 2n=48) and one diploid (2x, 2n=24) potato species with *S. tuberosum* cultivars and breeding lines (4x, 2n=48). Hybrid seeds were obtained in crosses where accessions of the Mexican species *S. demissum* Lindl. and *S. guerreroense* Corr. were pollinated with the Swedish cultivar Superb and *S. tuberosum* L. subsp. *andigenum* (Juz. and Bukasov) Hawkes. Hybrid seeds were also obtained from a direct cross between a selection from the cultivar Aurora (female) and the Bolivian diploid species *S. ruiz-ceballosii* Card. In several crosses within *S. tuberosum* genotypes the cultivars Ora, Kiva and Superb were found as effective pollinators. The crossability of the cultivars Kiva and Ora as well as of *S. tuberosum* breeding lines depended much on the choice of the other parental accession.

KEYWORDS

Phytophthora infestans, potato late blight, resistance, hybrid seedlings, wild species, breeding lines

INTRODUCTION

In the European Union almost 6 Mha of potatoes are grown, representing a value of close to €6,000,000,000. Late blight caused by *Phytophthora infestans* causes estimated annual losses (costs of control and damage) of more than €1,000,000,000 (Haverkort *et al.* 2008). Breeding achievements using large-scale approaches have not been able to significantly decrease yield losses caused by late blight. The most effective and environmentally friendly way to defeat *P. infestans* is by incorporation of resistance genes from new sources like wild potato species and advanced breeding lines.

Solanum demissum has been actively used in breeding programs targeting the development of cultivars resistant to *P. infestans*. Evaluation of diverse potato germplasm has been conducted already during nearly one century. Resistance genes of many species were successfully transferred to cultivated *S.*

tuberosum. In laboratory tests for resistance to *P. infestans* in accessions of wild potato species done in the late 1990s, extreme resistance was also found in plants of the Mexican species *S. guerreroense* (Zoteyeva, 1999). This species is phylogenetically close to *S. demissum.* In the same evaluation, an accession of *S. ruiz-ceballosii* (VIR-7370) was identified as highly resistant in both leaves and tubers (Zoteyeva, 1999, Zoteyeva *et al.*, 2004).

In the 1970s potato breeding with emphasis on vertical resistance was replaced with breeding for horizontal resistance (Wastie, 1991). Several authors have examined the relationship between race specific resistance and field resistance in potatoes and have found evidence for a beneficial effect of R-genes. There is also evidence that defeated R-genes may contribute to late blight resistance and a combination of R-genes and high levels of field resistance is therefore a desirable goal (Steward *et al.*, 2003). Another application of long-term resistance may be to make the optimal selection of R genes due to results of monitoring the *P. infestans* population for each potato growing area.

The main aim for the present study is to examine the crossability of wild species and breeding lines resistant to *P. infestans* and to obtain hybrids combining different types of resistance via interspecific crosses.

Advanced breeding lines from the collection at the plant breeding program at SLU, Sweden, are promising parental material combining resistance to *P. infestans* and reasonably good consumer qualities. These lines were evaluated in the field under severe infection pressure as well as in laboratory tests.

Solanum demissum Lindl. and the phylogenetically close species *S. guerreroense* Corr. were used as genetic sources of extreme resistance to *P. infestans.* Accessions that showed a hypersensitivity reaction (HR) were identified in leaflet inoculation tests performed earlier. These accessions were pollinated with pollen of cultivar (cv.) Superb and *S. tuberosum* subsp. *andigena* Hawkes respectively. One accession from the species *S. ruiz-ceballosii* Card. (VIR-7370) was identified as highly resistant in both leaves and tubers (Zoteyeva, 1999). Plants from this accession were hybridized with selections of the cv. Aurora.

MATERIALS AND METHODS

Pathogen material
In the evaluation of resistance of accessions from different wild species, two Polish *P. infestans* isolates from the collection of pathogens of IHAR-Mlochow Research Center (Poland) were used. The concentration of the inoculum comprised 75 sporangia/mm^3. The resistance of Swedish cultivars and *S. tuberosum* breeding lines were tested using an aggressive Swedish isolate. The concentration of the inoculum comprised 25 000 zoospores/ml. Tests were performed on detached leaflets and decapitated tubers.

Plant material
Hybridizations were performed on cut branches in the summer of 2009. The branches were kept in jars with water in a greenhouse. Plant material used in the crosses is represented in Table 1.

Table 1. *Parental accessions used in the crosses. Levels of resistance to Phytophthora infestans in leaves and tubers and consumer qualities.*

Parental accessions	Resistance to *Phytophthora infestans**)		Consumer qualities
	leaf	tuber	
Kiva	S*	R	Good
Ora	M	R	Good
Rosamunda**	not tested	R	Good
Superb	S	M	Very high
08-9-Aurora	R	M	High
05-A3 adg	M	R	Satisfying
93-1015	ER	S	Satisfying
04-2081	M	R	Satisfying
04-2662	R	R	Satisfying
04-3262	M	R	Satisfying
S. demissum L.	R	M	Wild type
S. guerreroense Corr.	R	M	Wild type
S. ruiz-ceballosii Card.	R	R	Wild type

*Levels of resistance, ER - extreme, R - high, M - medium, S – susceptible
**Immune to wart, resistant to Globodera rost, race 5

RESULTS AND DISCUSSION

In crosses performed the Swedish cultivar Superb was found as an effective pollinator and yielded a high number of seeds in all crosses performed. A high number of seeds were also obtained in one single cross between the cultivar Rosamunda (female) and the breeding line 04-3262 (Table 2). In reciprocal crosses between cv. Ora and the breeding line 04-3262 higher number of seeds was found when Ora was used as female parent. In combination where Ora was used as pollinator the number of seeds was two times lower. Two breeding lines, 04-2081 and 04-2662 (both female parents), were crossed with the cultivars Kiva and Ora. With both breeding lines seed production was lower in combinations with Ora compared to Kiva. In opposite, in combinations of these two cultivars with the breeding line 93-1015, used also as female parent, number of seeds was higher in crosses with Ora compared to with Kiva. Obtained results showed that seed formation in crosses using the same cultivars or breeding lines depends much on the other parental accession.

Table 2. *Results from crosses performed with accessions of wild species and S. tuberosum genotypes.*

Successful crosses	Nr of seeds per fruit	Unsuccessful crosses
04-2081 × Kiva	110,3	04-2081 × 04-3262
04-2081 × Ora	80	04-3262 × 04-2662
04-2662 × Kiva	65,7	04-2662 × 04-3262
04-2662 × Ora	47,5	04-2662 × Superb
04-2662 × Superb	102	93-1015 × 04-3262
04-3262 × Ora	68,8	Kiva × 04-3262
Ora × 04-3262	120	Ora × 04-3262
Ora × Superb	144,7	Kiva × 08-A3 adg
Rosamunda × 04-3262	170	08-A3 adg × 04-3262
93-1015 × Kiva	94	08-A3 adg × 04-2662

Successful crosses	Nr of seeds per fruit	Unsuccessful crosses
93-1015 × Ora	120	08-A3 adg × Ora
08-A3 adg* × Kiva	102	grr VIR-18407 × 05-A3 adg
08-A3 adg × Superb	206	dms VIR-3355 × Superb
08- 9-Aurora × rcb* VIR-7370	29	
dms* VIR-3355 × Superb	45,5	
grr* VIR-18407 × Superb	40	
dms VIR-3355 × 05-A3-adg	33,7	
grr VIR-18407 × 05-A3- adg	54	

Abbreviations of Solanum species: adg – S. tuberosum subsp. andigenum, rcb – S. ruiz-ceballosii, dms – S. demissum, grr – S. guerreroense.

Crosses done on different genotypes of *S. guerreroense* pollinated with different sets of pollen from *S. tuberosum subsp. andigenum* resulted in three unsuccessful (nine pollinated flowers) and three successful (seven pollinated flowers) crosses. One successful and one unsuccessful cross were noted in combinations where *S. guerreroense* and *S. demissum* were pollinated with *S. tuberosum* (cv. Superb). Obtained results confirmed the data obtained by Hermsen and E. Sawicka (1979) who found differences in ability to cross among different genotypes within species.

Plants of the diploid species *S. ruiz-ceballosii* were used as male parents in crosses with a selection of cv. Aurora. Regardless the difference in chromosome number of parental accessions this combination resulted in seed production (Table 2).

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Phenotypic characteristics of North-West Russian populations of *Phytophthora infestans* (2003-2008)

N.M. ZOTEYEVA[1], M.V. PATRIKEEVA[2]

[1] All-Russian Institute of Plant Industry (VIR), St. Petersburg, Russia   (nzoteyeva@gmail.com)
[2] All-Russian Institute of Plant Protection, St. Petersburg, Russia

SUMMARY

Frequencies of *Phytophthora infestans* genes for virulence had been examined for the samples collected in North-West Region of Russia in 2003-2008. The total of 459 isolates have been collected in seven districts of St. Petersburg where the populations of *P. infestans* are very complex and possess high phenotypic diversity. Virulence to resistance genes R1 –R11 was tested using set of Black's differentials. It was detected that the mean numbers for the virulence genes per isolate increased from 7.7 in 2003 to 8.1 in 2008. The most commonly detected genes for virulence were R1 and R3. The lowest frequency was observed in gene for virulence 9 which was detected only in epidemic seasons of 2003 and 2008. The frequencies of genes for virulence R2, R5 and R6 were lower than that of other genes, which were defined in isolates collected. The analyses of isolate subgroups sampled from cultivar Newsky in 2003-2006 showed that the proportion for the number of isolates where genes for virulence R2 and R6 have not been detected was very similar. The significant difference (p<0.0001) was found in genes for virulence frequency in isolates sampled during two years (2004 and 2005) in the same districts from leaves of potato cultivars Snegir and Nevsky.

INTRODUCTION

Phytophthora infestans is the causal agent of late blight, which is the most devastating disease in potato worldwide. In the European Union almost 6 Mha of potatoes are grown representing a value of close to €6,000,000,000. The direct and indirect costs of Late blight (costs of control and damage) estimated at more than €1,000,000,000 per one year (Haverkort *et al.* 2008). More pathogenic isolates appeared in Europe when the old clonal lineage of *P. infestans* was replaced by new more diverse population in 1990s (Goodwin and Drenth, 1977). The occurrence of A2 mating type led to sexual reproduction of *P. infestans*. Many studies conducted in potato production worldwide discovered a large spectrum of isolates with complex races (Drenth *et al.* 1994, Flier *et al.* 2003, Forbes *et al.* 1997). Both mating types had been detected in *P. infestans* populations in St. Petersburg Region in the late 1990s (Vedenyapina *et al.,* 2002). Analyses of phenotypic structure of *P. infestans* populations in North-Western Russia in two epidemic seasons (1998 and 2003) reflected that the average numbers of virulence genes per isolate increased significantly (Zoteyeva, Patrikeeva, 2008). The main goal of the performed evaluation was to characterize *P. infestans* populations from St. Petersburg Region and to detect changes in genes for virulence (R1-R11) frequencies. This is important considering that frequent occurrence of genes for virulence in *P. infestans* populations increases the risk of destruction of potato plants.

MATERIALS AND METHODS

Isolates were collected from fields of small-scale farms, experimental fields of All-Russian Institute of Plant Industry, All-Russian Institute of Plant Protection, North-West Institute of Agricultural Research and from several private seed production fields (Table 1). Fungicide treatments have not been frequently applied in majority of these fields, especially in small-scale farms. Two to seven sets were inspected in different years (Table 1).

The main goal of the research was to characterize the *P. infestans* isolates by virulence phenotypes. Four hundred fifty nine isolates were tested in total. *P. infestans* isolates were sampled from the leaflets with single lesions from the beginning of *P. infestans* manifestation until the end of vegetative period. The fragments of the infected leaflets were placed into the tuber slices of potato cultivars without R-genes. The isolates were maintained on tuber slices of susceptible cultivars (Priekulskij Rannij and Lotona) placed in glass Petri dishes (6 days of incubation, at 17-18 C°). Mating types were identified in 23 isolates collected in 2003, 2004 and 2007.

Virulence to 11 Black's R-gene differential set R1 – R11 (Black *et al.,* 1953; Malcomson & Black, 1966) each possessing a single *R*-gene from *Solanum demissum* was defined in *P. infestans* isolates in detached leaflet assays. Differential set was made available by IHAR-Mlochow Research Center (Poland). Two replications of three leaflets of each differential genotype were inoculated using isolates tested with 20 µl drop of sporangial suspension (50 sporangia/ mm³). The mating type tests were done by co-growth of isolates to be tested and A1 or A2 tester strains in Petri dishes with rye agar.

Table 1. *P. infestans isolates sampled in 2003-2008*

Year	Number of sampled isolates	Sources	Localities
2003	88	14 potato cultivars (p. cvs), 3 interspecific hybrids, 3 potato species	7 (Vyborg, Pushkin, Tosno, Gatczina, Luga, Vsevolozhsk)
2004	69	7 p. cvs, 3 potato species	3 (Pushkin, Tosno, Gatczina)
2005	145	4 p. cvs, 1 potato species, 1 tomato cv.	3 (Pushkin, Tosno, Gatczina)
2006	52	3 p. cvs	4 (Pushkin, Gatczina, Luga, Vsevolozhsk)
2007	54	2 p. cvs, 1 interspesific hybrid, 1 potato species	3 (Pushkin, Gatczina, Luga)
2008	51	10 p. cvs	3 (Pushkin, Gatczina, Luga)

RESULTS AND DISCUSSION

As it was expected, the first *P. infestans* manifestation in St. Petersburg Region usually occurred in the last days of June - first days of July. The seasons 2003 and 2008 were epidemic. In 2006 and 2007 *P. infestans* manifested later, these seasons were characterized by much lowest infection spread in the first part of July in comparison with other 2003-2008 seasons. Isolates tested for mating types in 2003, 2004 and 2007 contained the both types (A1 and A2).

The numbers of pathotypes identified during the period of evaluation were: 17 in 2003, 16 in 2004, 17 in 2005, 6 in 2006, 3 in 2007 and 4 in 2008. In both 2003 and 2004 years the most frequent pathotype was 1.2.3.4.5.6.7.8.10.11. – 24% and 40%, respectively. The largest shares of other isolates from 2003 were represented by races: 1.2.3.4.6.7.8.10.11. (11%) and 1.3.4.10.11. (10%). Thirty five percent of pathotypes sampled in 2004 were of race 1.3.4.5.7.8.10.11. In the next three seasons the share with pathotype 1.2.3.4.5.6.7.8.10.11. increased to 58% in 2005, to 78% in 2006 and to 82% in 2007. In 2005 the following pathotypes were also detected: 1.3.4.5.7.8.10.11. (14%),

1.3.4.5.6.7.8.10.11. (7%), 1.2.3.4.5.7.8.10.11. (6%), 1.2.3.4.10.11. (4%) and 12 different pathotypes represented by single isolates. As it is mentioned above in 2006 and 2007 the largest shares of isolates were represented by race 1.2.3.4.5.6.7.8.10.11. In epidemic 2008 the share of this pathotype decreased to 13%, the predominant race was 1.2.3.4.6.7.8.10.11. (71%).

The overall evaluation reflected that potato late blight populations in St. Petersburg Region are very complex. The average number of virulence factors per isolate in 2003-2008 is increasing in time, particularly the number of virulence factors in late 1990s was 6.3 compare to 7.7 in 2003 and to 8.1 in 2008. In late 1990s genes for virulence 5 and 8 have been rarely detected in the collected isolates and the gene for virulence 9 was not detected at all (Vedenjapina *et al.* 2002). The frequency of gene for virulence 8 was highest in 2003 (63%) and in 2007 (100%). The higher number of genes for virulence per isolate found in 2003-2008 in comparison with data obtained in late 1990s indicates changes in phenotypic structures of late blight populations in this region. Due to the detection of A2 mating type the sexual reproduction in *P. infestans* populations can be assumed.

In all isolates collected during 2003-2008 genes for virulence R1 and R3 were common for 100% of the isolates. Genes for virulence 2, 5, 6 and 8 were less frequent. The lowest frequency was noted for gene for virulence 9. In 2003 and 2008 the frequencies of genes for virulence 5 and 8 was significantly lower then in the other seasons. The numbers of detected genes for virulence 2 and 6 during full period of evaluation were relatively close. The data obtained for isolates collected from cultivar Nevsky in 2003 - 2006 showed a positive correlation between numbers of isolate with/ without these genes for virulence. It is possible to assume that cultivar Nevsky possess the ability for phenotypic selection in *P. infestans* isolates due to significant differences (p<0.001) in genes for virulence frequency in isolates sampled during two years (2004 and 2005) from leaves of two cultivars: Snegir (47 isolates) and Nevsky (68 isolates) (Fig.1).
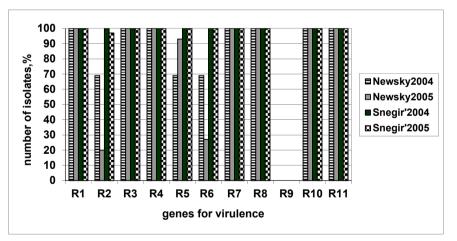
Fig. 1. *Genes for virulence (R1 – R11) expression in Phytophthora infestans isolates sampled from leaves of potato cultivars Newsky and Snegir in 2004, 2005*

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Latent infection rate of potato seed tubers with *Phytophthora infestans* (Mont.) de Bary

KEIL S., BENKER M. AND ZELLNER M.[*]

Bavarian State Research Centre for Agriculture, Lange Point 10, 85354 Freising, Germany
[*]Corresponding author: Email: Michael.Zellner@LfL.bayern.de

SUMMARY

From 2007 to 2009 17 batches of certified potato seed tubers were tested for latent infections with late blight (*Phytophthora infestans*). Using PCR diagnosis it was possible to show that latent infestation is a common problem. Infection rates from 2% up to 38% were found while only 3 out of the 17 batches were free of *P. infestans*. The given data show no significant difference of infestation rates between seed tubers produced organically or conventionally.

KEYWORDS

Late blight, PCR-test, *Solanum tuberosum*, stem blight, tuber blight

INTRODUCTION

The oomycete *P. infestans* causes late blight in potatoes which still is one of the most important diseases in potato production worldwide (Shtienberg *et al.* 1990). In most cases the source of an infestation is the fungus overwintering in tubers on cull piles or in volunteers (Kadish and Cohen 1992) where the pathogen can grow and sporulate in the next growing season and can then spread to other plants by wind. Another nowadays more important possibility is the overwintering in tubers in storage (Zellner 2004). Today's optimal modern storage conditions prevent the pathogen from spreading within the tubers and so no visible symptoms appear. Once planted, at high soil humidity after rainfall sporangia can be produced on the infected tubers (Adler 2000, Bäßler *et al.* 2002 and 2004). These are spread via soil water and infect neighbouring tubers and plants leading to primary stem blight (figure 1). As no visible symptoms appear on the seed tubers the late blight infestation is not assessed during the official certification process. So seed tubers with a latent late blight infestation carry *P. infestans* right into new planting sites leading to early infestation.

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Fig. 1. *Stem blight*

METHODS

From 2007 to 2009 a total of 17 batches of certified seed tubers were tested for latent infection with *P. infestans*. From each charge 47 tubers (respectively 94 in 2007) were randomly chosen and tested. Five batches were from organic and 12 from conventional production.

Samples were prepared and extraction of DNA was performed with the DNeasy Plant Mini Kit.

The extracted DNA of the pathogen was amplified with a primer setting recommended by Judelson & Tooley (2000) with the primers 5´-GAAAGGCATAGAAGGTAGA-3´ (forward primer 08-3) and 5´-TAACCGACCAAGTAGTAAA-3´ (reverse primer 08-4). Concentration of the reaction-mixture was as following: 10ng DNA/µl, 10% PCR-Buffer y, 0.4µM of each primer, 2mM MgCl2, 200µM deoxynucleotide triphosphates and 0.67 units TaqDNA polymerase. Amplification was performed in a MJ Research PTC-200 thermal cycler. The products were resolved by electrophoresis in 0.9% agarosis gels in Tris-Borate-EDTA buffer and stained with 0.005% ethidium bromide. Images were captured digitally (figure 2).
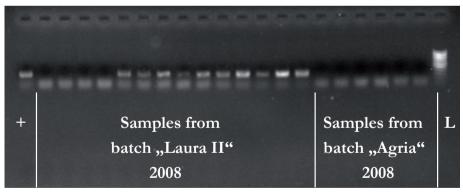
Fig. 2. *Image of resolved amplification products on 0.9% agarosis gel stained with ethidium bromide. (L: Low range mass ruler DNA Ladder; +: positive standard e.g. DNA extracted from pure P. infestans mycelium)*

RESULTS

In 2007 2 out of 5 tested batches showed latent infections on more than 10% of the seed tubers and one charge was without infection (figure 3). The mean infection rate was 11.2%, on average every ninth tuber was infected.

The average rate of latent late blight was 12.7% in 2008. No charge was free of infections and 4 out

of 6 showed infestation rates above 10% (figure 4).

In 2009 2 out of 6 tested batches were free of latent infections and the same number showed infestations rates above 10% (figure 5). The mean infection rate was 9.2%.

The overall average of infestation throughout all 17 tested seed tuber batches was 11% (figure 6). The highest rate of latent infestation was 38%. 17.6% (3 out of 17) were tested negative on *P. infestans*.

The results from comparing organically and conventionally produced seed tubers showed no significant differences (t-test, p<0.05) in the average percentage of latent infected seed tubers.
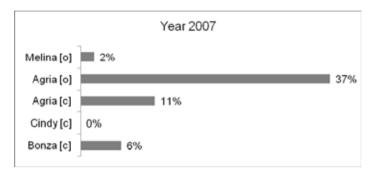
Fig. 3. *Latently infected seed tubers in %, 2007 (n=94); [o] organically [c] conventionally produced seed tubers*
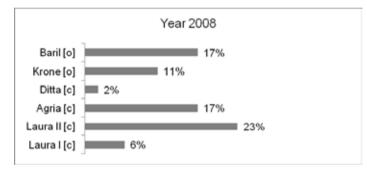
Fig. 4. *Latently infected seed tubers in %, 2008 (n=47); [o] organically [c] conventionally produced seed tubers*
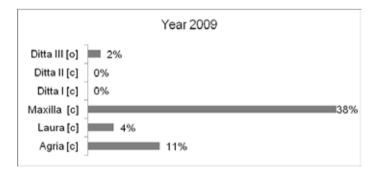
Fig. 5. *Latently infected seed tubers in %, 2009 (n=47); [o] organically [c] conventionally produced seed tubers*
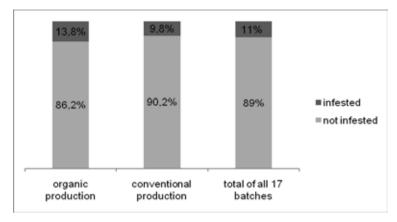
Fig. 6. *Mean infestation rate of seed tubers with P. infestans*

DISCUSSION

The given data illustrates that there is a considerable risk of bringing *Phytophthora infestans* into the field with seed tubers even when using certified and symptomless tubers. Latent infections with *Phytophthora infestans* seem to be a general problem since the data shows no significant difference between tubers from organic and conventional production. As a result seed tubers may look healthy, but can still be infested, thus lead to an early primary outbreak of this disease (stem blight). Because of the high risk of infected seed tubers a timely usage of protective fungicides is recommended. This type of infection can only be treated with curative fungicides and the aim of the first treatment is to contain the growth of the fungus from the tuber through the stem of the potato plant. Systemic products spread into the plant tissue and seize the fungus when it grows upwards. Therefore in conventional potato farming infections can be reduced by systemic fungicides. Thus latent infected seed tubers are a more serious problem in organic farming since no curative fungicides are available. For organic as well as for conventional seed potato production it is important to reduce the latent infection rate.

Further research should deal with the question of how to clearly reduce tuber infections with *P. infestans*. Especially for seed tubers overwintering in storage the aim is to have an infection rate as low as possible as they initiate new infections in the field the following year.

ACKNOWLEDGMENTS

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Posters

Role of oospores in the overwintering and year-on-year development of the late blight pathogen on tomato en potato

M.A. KUZNETSOVA, T.I. ULANOVA, A.N. ROGOZHIN,
T.I. SMETANINA AND A.V. FILIPPOV

All-Russian Research Institute of Phytopathology, Bolshie Vyazemy,
Moscow Region, 143050 Russia; e-mail: kuznetsova@vniif.ru

SUMMARY

The role of oospores as a source for the regular late blight renewal has been studied in a long-term experiment using tomato strains of *P. infestans* and two tomato cultivars. The obtained results showed that oospores, generated after the mating of strains of different mating types (A1 and A2), maintained their infection ability after their overwintering in the soil. The presence of the infectious agent in the soil causes the spreading of the late blight to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem. Therefore, at least in the course of the next vegetation season, *P. infestans* oospores can be an important source of infection, causing the appearance of first late blight lesions on plants.

KEY WORDS

potato, tomato, late blight, overwintering of oospores

INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) dBy, is the most harmful disease of potato and tomato plants. During epiphytoties, potato production often drops in 1.5-2 times, and tomato crops can be fully lost. In Russia the main place of the pathogen overwintering and, therefore, the main source of the year-to-year infection renewal, is infected seed potato in the storage facilities. After the planting of infected tubers, *P. infestans* zoosporangia are formed on their surface, which then generate zoospores, infecting the underground parts of a plant and, as a result of a zoospore migration through soil capillars, bottom leaves, which contact with the ground (Boguslavskaya and Filippov, 1976). It was considered that before 1980 the primary manifestation of the disease on tomato plants was caused by pathogen spores, generating on potato fields.

Oospores represent another structure able to the overwintering. *P. infestans* is a heterothallic species with two mating types, A1 and A2. The contact of strains with two different mating types results in an oospore generation (Miller, 2001).

The formation of *P. infestans* oospores on potato was firstly revealed by Gallegy and Galindo in 1957. This study was carried out in Toluca valley (Central Mexico), which is considered to be a place of origin of this pathogen (Fry, Spielman, 1991). Until this time, the *P. infestans* population outside Central Mexico was represented by a single clonal line (A1). This line had only vegetative reproduction. The first non-Mexican A2 strains were revealed on potato field of Switzerland (Hohl,

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Iselin, 1984), and then in the Great Britain on potato tubers, imported from Egypt (Shaw *et al.*, 1985). A sexual reproduction occurs when two different types of mycelium, A1 and A2, contact with each other; in this case they form male and female reproductive organs, oogonium and antheridium. Each mating type can form both types of reproductive organs, though certain genotypes play rather male or female role (Judelson 1997). The meiosis takes place within reproductive organs, and, after their fusion, the fertilization can occur, which results in the oospore formation.

The sexual reproduction not necessarily results in an outcrossing; some of the offspring can represent a selfing product (Knapova *et al.*, 2002), other can be identical to the one of the parents (Carter 1999). Besides, many genotypes can be self-fertile, i.e. they are able to generate oospores at the absence of the opposite mating type (Smart *et al.*, 2000).

The first information about the revealing of the A2 mating type of *P. infestans* on potato in Russia was published in 1993 (Vorobyeva and Gridnev, 1993). Oospores, differing in their origin, were revealed in the Moscow region in 1998-1999 (Smirnov, 1998; Smirnov *et al.*, 1999). Meanwhile, in 1967 P.A. Kvartskhava and A.I. Maglakelidze revealed both mating types and the generation of *P. infestans* oospores on kangaroo apple (*Solanum laciniatum*) in Western Georgia (Maglakelidze, 1971).

Many scientists consider that the A2-containing "new population" was imported from Central Mexico to Europe and other continents in 1970 with the infected seed potato. However, the simultaneous revealing of A2 strains in Europe, Asia, and America allows us to consider that such global change in the structure of *P. infestans* population could be caused by any other reasons. The appearance of the A2 type coincided with significant changes of the population structure of *P. infestans* and the increase in its aggressiveness. The pathogen became less dependent on the temperature and humidity, and the first disease symptoms are often manifested too early for many places (Flier, 2001). It is considered that this is caused by a sexual recombination and the influence of an additional infection source, i.e. overwintered oospores (Andersson *et al.*, 1998; Hannukkala *et al.*, 2006).

The role of oospores as the primary source of infection was investigated under natural conditions and in the course of special laboratory and field experiments. According to the data, obtained by Turkensteen and Flier, in Netherlands oospores remain viable for 4 and 3 years in the sandy and clay soil, respectively (Turkensteen, Flier, 2000). There was also a report that in Finland they remain viable at least within one winter (Lehtinen *et al.*, 2002). It was also showed that on the fields, where potato was cultivated for several seasons, the late blight appeared by 9 days earlier on average, than on the fields with other advanced crops (Bødker *et al.*, 2006). We observed the cases, when the late blight initially appeared on the planted tomato seedlings and then spread on the adjacent potato plots. In such case we can suppose that overwintered oospores were the primary source of infection. Some authors consider that the presence of both mating types in a primary nidus of infection, the variety of genotypes, isolated from such nidus, and the dominating infection of bottom leaves, contacting with a soil, are typical signs of the fact that this nidus originated from overwintered oospores (Lehtinen, Hannukkala, 2004; Widmark *et al.*, 2007). According to Evenhuis and others (Evenhuis *et al.*, 2004), infected seed tubers represent the later late blight source than overwintered oospores. They also consider that all cases of the early disease manifestation (within first week after the appearance of shoots) in Netherlands were caused by oospores.

At the same time, other authors consider that oospores play a minor role as the source of a primary infection (Cook *et al.*, 2007). In many regions oospores are not generated at all or generated in a small number due to the domination of a monoclonal *P. infestans* population, represented by only one mating type. For example, such situation is typical for the most part of Siberia, where only A1 type was found (Elansly *et al.*, 2001) and several countries of Western Europe, where the earlier genotypes were almost fully replaced with the 13A2 genotype (Lees *et al.*, 2009).

The aim of this study was to obtain direct evidences of the significance of oospores as a source for the regular late blight renewal in Russia.

MATERIALS AND METHODS

The study was carried out using tomato strains of *P. infestans* and two tomato cultivars. We did not include potato into this study, since the possible hidden infection of tubers could influence on the results of our experiments.

Six pairs of monozoosporic *P. infestans* isolates of A1 and A2 types were examined for their ability to the oospore formation. The isolates were cultured on Petri dishes using rye medium (according to Caten and Jinks, 1968) and also were used for the inoculation of detached tomato leaves (cv. Otradniy). To determine the presence of oospores, infected leaves were boiled in 96% alcohol for 2-3 min to remove chlorophyll, then bleached in 10% solution of a chlor-containing substance ("Belizna") and microscoped in 50 fields of vision (1 mm^2 each). The oospore frequency below 51, from 51 to 250, and above 250 oospores per field of vision was evaluated as rare, moderate, and frequent, respectively (Amatkhanova *et al.*, 2004). Among six examined pairs, only two demonstrated an abundant oospore formation on Petri dishes and detached leaves. In our further experiments we used one of these two pairs, 14a (A1) and 36b (A2).
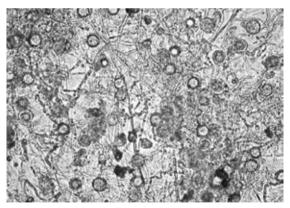
In 2007-2009 we completed the following series of experiments:
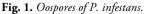
I. Study of the overwintering of **P. infestans** *oospores under field conditions.*
90 tomato plants (cv. Bomax) were grown in a greenhouse and in autumn of 2007 were infected with three variants of inoculum: 1) 14a (A1); 2) 36b (A2); 3) 14a (A1) + 36b (A2). In the third variant, the A1:A2 ratio was 1:1. The inoculum of the examined isolates was cultivated on rye medium. The used dose was 6000 zoosporangia per 1 m^2. Each variant included 10 plants.
After the first disease manifestation, detached leaves were analyzed for the oospore presence.
The oospore formation was observed only for plants, inoculated with the A1+A2 mix (Fig. 1); in other two variants, where plants were inoculated with only A1 or A2 type isolates, we did not reveal any oospores.

Fig. 1. *Oospores of P. infestans.*

In all three variants the infected plants were taken out of a greenhouse and buried into improved sod-podzol heavy soil at a depth of 20 cm. Plants of each variant were buried on a separate isolated place. Next spring we took soil samples, containing overrotten debris of infected plants, from each plot. These samples were used to prepare a suspension for the inoculation of potato tuber slices (cv. Sante) and detached leaves of tomato plants (cv. Otradniy), grown in a greenhouse. The suspension was prepared in the following way: 1 g of the soil was diluted in 10 ml H$_2$O. The suspension was dropped on the surface of leaves (18 leaves per variant) and fresh tuber slices (20 slices per variant).

Inoculated leaves and tuber slices were placed into wet chambers (trays covered with polyethylene film). After 5-day incubation we started everyday observation of the pathogen development (Fig. 2 A, B).
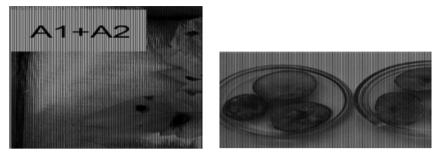
Fig. 2. *Late blight manifestation on tomato leaves (A) and potato tuber slices (B), inoculated with soil samples, containing overwintered P. infestans oospores (A1+A2 variant).*

In 2008-2009 we expanded our program of investigations. In spring 2009 we grew the seedlings of tomato plants (cv. Otradniy) in a greenhouse and then planted it on an experimental field (Fig. 3) in several different variants:

During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A1 type isolate, into each planting hole;

During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A2 type isolate, into each planting hole;

During a planting, we added 1 kg of soil, containing overwintered plant debris, infected with the A1+A2 mix of isolates, into each planting hole;

During a planting, we did not add any soil samples into planting holes (control).

Each plot was isolated from others by spring wheat. We provided the thrice repeatability of each variant. After the planting, we observed the late blight development each 5-7 days.

Fig. 3. *Plots with tomato plants, surrounded by spring wheat.*

II. Study of the way of transmission of the infectious agent from soil to the overground parts of a plant
The experiment was carried out in a greenhouse using tomato plants (cv. Bomax). After the development of 4-5 leaves, plants were taken out of the soil, and their roots were immersed into the suspension of *P. infestans* zoosporangia (A1 and A2 types, 6000 zoosporangia/ml) for 5 min.

Then the contaminated seedlings were planted into 5-liter soil-containing flowerpots. Plants were grown under daylight. The soil humidity was maintained at the level of 60% of the total soil water capacity. Ten days after planting we started our everyday examinations of each plant to determine the moment of a disease manifestation and the character of possible lesions. Plants, which roots were immersed into water, were used as a control. Each tested variant included 30 tomato plants.

RESULTS AND DISCUSSION

I. Overwintering of P. infestans oospores under field conditions

During both years of our study the inoculation of tomato leaves and tuber slices with the examined soil samples was successful only in the case when these samples contained overrotten debris of plants, infected with the A1+A2 mix of isolates (Table 1). In 2008 and 2009 we registered the late blight lesions with the fruiting on 27% and 17% of detached leaves and 20% and 25% of tuber slices, respectively.

Table 1 *Infectiousness of Phytophthora infestans after the overwintering in the soil depending on the type of inoculum (ARRIP, 2008-2009)*

Inoculum type	Presence of oospores	Late blight manifestation on leaves/tuber slices, %
A1 type isolate	No	0/0
A2 type isolate	No	0/0
1+2 mix (1:1)	Yes	22,0/22,5

In two other variants (soil samples with debris of plants, inoculated with A1 or A2 strains) we did not reveal any disease manifestations on both leaves and tuber slices. The obtained results show that the oospores, generated after the mating of 14 (1) and 36b (2) strains, maintained their infection ability after the overwintering.

This fact was also confirmed by the observations on the late blight development on tomato plants, grown in the field. The first disease manifestation on tomato plants, grown on the plot with plant debris, infected with A1+A2 variant, was registered two weeks after the planting (June 24). Late blight lesions were observed on 17% of plants; they were located mainly on the bottom leaves and the bottom part of the stem. In the case of two other variants and the control, we observed first lesions only 25 days after the planting (July 17; Table 2). However, the fact that the infection was simultaneously manifested in control plants, makes it possible to suppose that in this case its development was caused by any outer sources of infection. In this period the late blight symptoms were registered in the nearest potato and tomato fields. Therefore, our results can be considered as a direct evidence of the fact that oospores, generated after the mating of A1 and A2 type strains, can overwinter in the soil and cause the renewal of the late blight in the next season.

Table 2. *Late blight development after the pathogen overwintering in the soil depending on the inoculum type (ARRIP, 2009)*

Inoculum type	Presence of oospores	Late blight manifestation on tomato plants in the field
Control	No	17.07.09
A1 type	No	17.07.09
A2 type	No	17.07.09
1+2 mix (1:1)	Yes	24.06.09

II. Way of transmission of the infectious agent from soil to the overground parts of a plant

Two weeks after the root inoculation, we observed the first visible late blight symptoms on the bottom part of several stems. After two more weeks, we observed isolated late blight lesions on other

plants; these lesions were located at the different height of the stem (up to 15 cm). Such character of lesions allowed us to suggest the development of a pathogen mycelium took place within the stem and was asymptomatic for a long period. The lesions appeared mainly near internodes. A longitudinal cut of such stems demonstrated the necrotization of their tissues. The placement of stem pieces into a wet chamber, caused the generation of zoosporangia on necrotizing tissues and tissues adjacent to vessels. In our experiment we observed such type of lesion in 23% of samples (Fig. 4).

Fig. 4. *Late blight manifestation on tomato plants, grown on the oospore-containing soil.*

Thus, basing on these experiments, we can conclude that, in the case of a presence of the infectious agent in the soil, the disease can spread to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem.

The obtained data show that *P. infestans* oospores can overwinter in the soil and, at least in the course of the next season, be a source of infection, causing the appearance of first late blight lesions on plants.

The modern *P. infestans* populations from many Russian regions include now both mating types, and A1:A2 ratio is often close to 1:1. In these cases this source of infection can play an important role, especially in private gardens, where usually there is no crop rotation. We can also suppose that, in the case of any other A1:A2 ratio in a population, the role of oospores as the source of primary infection becomes less important.

Unlike zoosporangia, oospores are generated within plant tissues. According to our observations, they acquire the germination ability only after the decay of surrounding plant tissues. It is known that the rate of decay and mineralization of plant debris and, therefore, the oospore's acquisition of the ability to infect plants, significantly depends on the temperature, moisture, and microbiological activity of the soil. This process seems to be slower for the northern latitudes. In the case of a prolonged soil moistening, oospores either directly infect underground parts of plants, or generate zoosporangia with the subsequent release of zoospores. After the germination of an oospore into a zoosporangium, plants are infected with zoospores, which move to the surface of the soil using soil capillaries and infect stems and leaves, which contact with the soil. In our opinion, it is not possible to distinguish late blight infection focuses, caused by oospores, from those caused by contaminated seed material. We also can not agree with the hypothesis that the typical feature of the oospore infection is the earlier disease manifestation, comparing to the infection, caused by a seed contamination. We consider that the period of the disease manifestation depends mainly from both the number of infected seed tubers and the level of contamination of the soil with oospores.

Basing on the results of our studies, we can conclude that in many regions of Russia oospores,

generating by the mating of A1 and A2 types of *P. infestans* strains, can overwinter in the soil and cause the late blight development in the next vegetation season.

CONCLUSION

Thus, basing on these experiments, we can conclude that, in the case of a presence of the infectious agent in the soil, the disease can spread to the above ground parts of plants, manifesting itself on the bottom and other parts of the stem.

The obtained data show that *P. infestans* oospores can overwinter in the soil and, at least in the course of the next season, be a source of infection, causing the appearance of first late blight lesions on plants.

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Potato resistance to late blight as related to the *R1* and *R3* genes introgressed from *Solanum demissum*

EMIL E. KHAVKIN[1], EKATERINA A. SOKOLOVA[1], MARIA P. BEKETOVA[1, 3], ARTEM A. PANKIN[1], MARIA A. KUZNETSOVA[2], IRINA N. KOZLOVSKAYA[2], SVETLANA YU. SPIGLAZOVA[2], NATALIA V. STATSYUK[2], ISOLDA M. YASHINA[3]

[1]Institute of Agricultural Biotechnology, Moscow, 127550 Russia
[2]Institute of Phytopathology, Bolshiye Vyazemy, 143050 Russia
[3]Institute of Potato Industry, Korenevo, 140052 Russia

SUMMARY

New races of *Phytophthora infestans* are known to rapidly defeat potato late blight (LB) resistance introgressed with the germplasm of *Solanum demissum*, the early source of race-specific resistance genes (*R*-genes). Nonetheless, the presence of the major *demissum* gene *R1* in potato cultivars was associated with higher field indices of LB resistance (Stewart *et al.*, 2003; Gebhardt *et al.*, 2004; Beketova *et al.*, 2006) suggesting that these *demissum R*-genes in some way add to plant defense response. We developed and verified SCAR markers recognizing the race-specific genes *R1* and *R3* of *S. demissum* and *S. stoloniferum* and the germplasms of these species. By screening wild *Solanum* species and potato accessions reportedly free from wild *Solanum* germplasm (Chilotanum varieties and old cultivars), we established that these markers reliably discerned between germplasms of cultivated *Solanum tuberosum* ssp. *tuberosum* and wild sources of LB resistance. Screening 161 potato cultivars demonstrated that the presence of SCAR markers of *R1* and *R3* genes was significantly related to higher indices of LB resistance. Similar results were obtained when the presence of *demissum R*-genes was recognized using specific races of *Ph. infestans*. Such association presumes that both *R1* and *R3* genes contribute to overall LB resistance of potato cultivars.

KEYWORDS

Phytophthora infestans, *Solanum* species, race-specific genes, overall resistance

INTRODUCTION

For several decades, breeding for potato late blight (LB) resistance has heavily relied on germplasm introgression from *Solanum demissum*, the wild Mexican species comprising the race-specific resistance genes *R1-R11*. New pathogen races are known to rapidly overcome such resistance; nonetheless, many potato cultivars comprising the *R*-genes from *S. demissum* maintain higher field resistance than the genotypes lacking such genes (for review of the earlier evidence see Gebhardt *et al.*, 2004; Stewart *et al.*, 2003; Trognitz and Trognitz, 2007).

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Stewart *et al*. (2003) reported that potato cultivars containing the *R1* gene identified with the specific races of *Ph. infestans* manifested considerably higher field LB resistance than the cultivars free of *R*-genes. Cloning *R*-genes (Hein *et al*., 2009) makes it feasible to detect these genes in potato and its wild relatives using the molecular markers developed from the particular gene sequences. Thus, Gebhardt *et al*. (2004) used the *R1* sequence to develop the marker R1-1400. This marker was found in *S. demissum* and *S. stoloniferum*, and by screening 415 potato cultivars, these authors established significant association between the presence of R1-1400 and the indices of LB resistance collected from cultivar passports (passport resistance). Similar relationship between the presence of R1-1400 marker and the passport resistance indices was reported from our laboratory for cultivars bred mostly in the former Soviet Union (Beketova *et al*., 2006). Trognitz and Trognitz (2007) demonstrated that the R1-1400 fragments cloned from differentials and cultivars were completely identical to the prototype gene *R1*.

In this study we employed both molecular and phytopathological methodologies for recognizing the *R1* and *R3* genes and both field and laboratory assays for LB resistance. The significant association between the presence of these genes and high LB resistance presumes that *R1* and *R3* someway contribute to LB resistance of potato cultivars.

MATERIALS AND METHODS
Plant Material
Potato tubers for this study arrived from the collections of the Institute of Potato Husbandry (Korenevo, Moscow region, Russia), the Institute of Plant Industry (St. Petersburg, Russia), the Institute of Phytopathology (Bol'shiye Vyazemy, Moscow region, Russia), and the Research and Practical Centre for Potato, Fruit and Vegetable Growing, (Samokhvalovichi, Minsk region, Belarus). As a whole, we screened 161 potato accessions. The pedigree information, particularly on the presence of the germplasm of *S. demissum* and *S. stoloniferum*, was obtained from the already published evidence in the catalogs of the institutions mentioned above and the electronic catalogs of the European Cultivated Potato Database (www.europotato.org/varietyindex.php) and the Dutch-German Potato Collection (www.plantbreeding.wur.nl/potatopedigree). Seeds of *S. demissum* and *S. stoloniferum* accessions were obtained from the collections of the Institute of Plant Industry (St. Petersburg, Russia) and the United States Potato Genebank, NRSP-6 (Sturgeon Bay, WI).

Development of DNA markers
Standard protocols were employed for genomic DNA isolation from plant leaves, PCR analysis, and cloning and identifying genome fragments. Specific primers for sequence characterized amplified regions (SCAR markers) were designed following multiple alignment of the *S. demissum* and *S. stoloniferum* sequences (Table 1) with their structural homologs from the NCBI Genbank using the programs BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Vector NTI Suite 8 package (Invitrogen). The marker R1-1205 modified from R1-1400 (Gebhardt *et al*., 2004) provided more reliable scoring. Other markers were developed in this laboratory.

Table 1. *SCAR markers of the R1 and R3 genes of S. demissum and S. stoloniferum and the germplasms of these species.*

Markers and their size, bp	Prototype clone	Chromosome	Position in the prototype clone
R1-1205	AF447489	5	5126-6331
R3-1380	AY849382	11	1677-3056
Ssto-448	EU041625	5	100-548
Sdms-523	AY875783	5	59138-59530

Phytopathological assays

Identification of *R*-genes in potato plants was accomplished by the artificial inoculation of detached leaves with a set of *Ph. infestans* races (1; 3; 4; 10; 11; 1.2; 1.3; 1.4; 2.4; 3.4; 1.2.4; 1.3.4.; 1.2.3.4). The leaflets of the tested plants were placed into a wet chamber, representing a frame (30 × 40 × 4 cm) with the bottom made of metal gauze. The lower surface of each leaflet was inoculated, using a micropipette, with two drops of a pathogen spore suspension. The concentration of suspension corresponded to 10 spores in a microscopic field at 100x magnification. The frames were placed onto wet filter paper and covered with glass plates. After 24-h incubation, the drops were shaken off, and the leaves were turned upside down. The sporulation registered after 4-6 days of incubation testified a compatible reaction.

Field assays of potato LB resistance were carried out under natural infection conditions. Leaves were examined every 7-10 days, starting from the first blotches on a susceptible cultivar, until the complete leaf decay. The percentage of lesions on the leaf was recorded using a scale of the Britain Mycological Society (James, 1971), transformed into the area under the disease progress curve and ranked by the 1-9 score scale. For laboratory assays of LB resistance, we used the express method based on the combined laboratory and field tests following the artificial inoculation with *Ph. infestans* spores (Filippov *et al.,* 2004). The field indices of LB resistance for potato foliage and the laboratory indices for detached leaves were registered in 2008 and 2009. By the U-test of Mann-Whitney (1947), field indices for two years were highly consistent. The field and laboratory indices for a particular cultivar differed, on the average, by two scores; nonetheless they were closely correlated, and we further employed an integrated index calculated as the average resistance over two field and two laboratory scores for the particular potato cultivar.

Statistical methods

To link the presence of the *R*-genes to LB resistance indices in potato cultivars, we used the nonparametric U-test of Mann-Whitney (1947) implemented in SPSS Statistics 17.0 software (http://www.spss.com).

RESULTS AND DISCUSSION

Verification of SCAR markers

To verify marker specificity towards *R1* and *R3*, we screened *S. demissum* and *S. stoloniferum* plants, potato cultivars comprising the germplasm of *S. demissum* (demissoid cultivars), including the *R*-gene differentials, and potato cultivars reportedly free of wild *Solanum* germplasm. In addition, we cloned and sequenced genome fragments corresponding to markers R1-1400/1205 and R3-1380 from several potato cultivars and *S. stoloniferum*.

All four markers under study reliably discerned cultivated *S. tuberosum* ssp. *tuberosum* from two wild *Solanum* species and potato cultivars comprising the germplasm of these species (Table 2). However, only three markers passed through several crosses into the modern potato cultivars. The marker Sdms-523 recognizing *demissum*-specific polymorphisms of selectively neutral internal transcribed spacer (ITS) was lost as soon as after two meiotic generations.

The marker R1-1400/R1-1205 was present in the differentials R1, R5, R6 and R9, thus corroborating data by Trognitz and Trognitz (2007), whereas the marker R3-1380 was found in Black's differentials R3, R7, R8 and R9 (Black *et al.*, 1953). To explain such discrepancies we would suggest three possible reasons. First, Huang (2005) reported that functional *R5-R11* genes are structurally similar

to *R3* gene located in the same gene cluster with *R5-R11* on *demissum* chromosome 11; therefore the marker R3-1380 probably did not discern between these loci. Second, the differentials R5-R9 apart from the corresponding *R5–R9* genes may contain non-functional homologues of *R1* and *R3* genes corrupted by the nucleotide changes, which were nevertheless tagged by the *R1*- and *R3*-specific markers. Finally, the differentials initially selected for the presence of single *demissum R* gene could be in fact not monogenic and need further genetic improvement. The sequences of genome fragments of *S. stoloniferum*, differentials and potato cultivars corresponding to the markers R1-1400/R1-1205 and R3-1380 were 98-100% identical to the prototype *S. demissum R1* and *R3a* genes (Ballvora *et al.*, 2002; Huang, 2005).

Table 2. *Frequencies of the markers of R-genes and germplasms of Solanum species in potato and its wild relatives*

Genotypes (number of accessions)	SCAR markers			
	R1- 1400/1205	R3-1380	Ssto-448	Sdms-523
S. demissum (35)	0.40	0.11	0.80	1.0
S. stoloniferum (51)	0.18	0.16	0.80	0.0
S. tuberosum ssp. *tuberosum*: Chilotanum forms (6) and old cultivars free from *dms/sto* germplasm (11)	0.0	0	0	0.0
Demissoid potato cultivars (144)	0.40	0.38	0.84	0.0

Comparison of two methods for discerning R-genes

When *R*-genes were assessed in potato cultivars using simple *Ph. infestans* races, the data obtained by phytopathological *R*-genotyping in most cases matched the evidence for the presence of *R*-genes recognized with the molecular markers (Table 3). The agreement was higher for *R1* than for *R3* probably because the marker R3-1380 does not discriminate between *R3* and the structurally related *R5-R11* genes (see above).

Table 3. *Agreement of the data obtained by molecular and phytopathological methods of R-genotyping*

	Recognized genes		
	R1	*R3*	*R1* and *R3* together
The number of matches between two methods	53	44	33
Per cent	75	62	47

Association of LB resistance with the presence of R-genes

We investigated association between the presence of *R*-genes discerned by molecular and phytopathological genotyping and the indices of LB resistance assessed in the field and laboratory trials.

Potato cultivars with well-established pedigrees were divided into several subpopulations by their marker patterns (Table 4). The initial three subpopulations comprised the cultivars with the marker R1-1205, the marker R3-1380, and two markers present together. The fourth subpopulation is a batch of three previous sets. The control 1 subpopulation lacking the markers R1-1205 and R3-1380 is a heterogeneous group combining the genotypes with the germplasm of *S. demissum* and *S. stoloniferum* (control 2) and the genotypes free of such germplasm (control 3). The percentage of highly resistant potato accessions (scores 7-9) in genotypes comprising the marker R1-1205 or both R1-1205 and R3-1380 was notably higher than in the genotypes devoid of these markers (Table 4). The statistical analysis using the Mann-Whitney U-test demonstrated highly significant association between the presence of both markers R1-1205 and R3-1380 and high LB resistance. However, the

association of high LB with the presence of the markers R1-1205 and R3-1380 separately was not evident. LB resistance in the control subpopulation 2 devoid of the markers R1-1205 and R3-1380 exceeded that in the control 3 free of the *S. demissum* and *S. stoloniferum* germplasm; these data suggest that *S. demissum* *R*-genes other than *R1* and *R3* are present in some genotypes comprising the control 2. Indeed, *R*-genotyping with *Ph. infestans* simple races discerned the genes *R2* and *R4* in some of these cultivars.

LB resistance indices in the subpopulation conferring the *R1-R4* genes, as discerned by the phytopathological method, and in the subpopulation devoid of these genes differed by two scores of resistance revealing highly significant effect of the *R*-genes (Table 5).

In most potato accessions under study, *R1* and *R3* were transferred from *S. demissum*; however, many of these cultivars reportedly comprise the germplasm of *S. stoloniferum*. The presence of several *R*-genes in *S. stoloniferum* was first attested by phytopathological methods (McKee, 1962; Toxopeus, 1964; Grünwald and Flier, 2005). Gebhardt *et al*. (2004) reported the marker R1-1400 in *S. stoloniferum*, and recently several functionally active *R3a* sequences were revealed in this genome (Champouret, 2010). It is therefore of considerable interest to discover whether introgression of *stoloniferum R1* and *R3* genes into potato cultivars has ever occurred and to evaluate the relative inputs of these genes introgressed from each species.

Table 4. *Association of potato LB resistance with the presence of markers of the R1 and R3 genes (The number of potato accessions in each subpopulation is given in parentheses)*

Subpopulations of potato cultivars	Percentage of highly resistant cultivars (scores 7-9)	Mean scores by the 9-score scale	Standard deviation
comprising only R1-1205 (22)	27	5.3[c]	1.30
comprising only R3-1380 (23)	13	5.4[c]	1.24
comprising both R1-1205 and R3-1380 (20)	45	5.9[ac]	1.58
all accessions comprising the *R*-gene markers (65)	28	5.5[bc]	1.38
control 1: accessions devoid of R1-1205 and R3-1380 markers (44)	16	4.76[c]	1.61
control 2: cultivars comprising the germplasm of *S. demissum* and *S. stoloniferum* but devoid of R1-1205 and R3-1380 markers (36)	19	5.1[c]	1.56
control 3: *tuberosum* accessions free of the germplasm of *S. demissum* and *S. stoloniferum* (8)	0	3.2[ae]	0.46

[a]*Significantly different from the control 1 at 1% confidence level*
[b]*Significantly different from the control 1 at 5% confidence level*
[c]*Significantly different from the control 3 at 1% confidence level*
[d]*Significantly different from the control 3 at 5% confidence level*
[e]*Significantly different from the control 2 at 5% confidence level*

Table 5. *Association of potato LB resistance with the presence of R-genes discerned with Ph. infestans races (The number of potato accessions in each subpopulation is given in parentheses)*

Subpopulations of potato cultivars	Mean resistance scores by 9-score scale	Standard deviation
comprising the *R1-R4* genes (54)	5.8*	1.37
free of the *R1-R4* genes (18)	3.86	1.42

Difference significant at 0.1% confidence level by the Mann-Whitney U-test

Thus, using two independent methodologies for recognizing *demissum R*-genes, we established highly significant association between the presence of the *R1* and *R3* genes and high LB resistance. Further experiments are required to prove that such statistical association is derived from the activity of functional *R*-genes.

CONCLUSIONS

The highly significant correlation between the presence of the *demissum R* genes and high LB resistance described in this paper corroborates the earlier evidence from several authors (Stewart *et al.*, 2003; Gebhardt *et al.*, 2004; Beketova *et al.*, 2006). The attested effect of both *R1* and *R3* genes from *S. demissum* and probably *S. stoloniferum* presumes that these genes contribute to overall LB resistance of potato cultivars.

Tan *et al.* (2008) discussed several models explaining the participation of *R*-genes in overall potato LB resistance, including (1) non-durable race-specific resistance rapidly defeated by new races of *Ph. infestans*, (2) durable broad-spectrum resistance conferred by several *R*-genes from *S. bulbocastanum* and other wild *Solanum* species, (3) QTLs involved in resistance and (4) residual resistance with poorly understood mechanisms. Recent studies of race specificity of *S. bulbocastanum* genes (Champouret e*t al.*, 2010; Halterman *et al.*, 2010) suggest that they are also prone to defeat, and the difference between the models (1) and (2) is inconsiderable and transient. Meanwhile, rapid advance in physical mapping reveals new *R*-genes behind earlier established QTLs for LB resistance (Hein *et al.*, 2009). The current progress in elucidating the specific interactions between R-receptor kinases and pathogen effectors (Jones and Dangl, 2006; Vleeshowers *et al.*, 2008) will undoubtedly help better understand the role of defeated *R*-genes in overall LB resistance.

ACKNOWLEDGMENTS

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Late blight-resistant tuber-bearing *Solanum* species in field and laboratory trials

ELENA V. ROGOZINA[1] , MARIA A. KUZNETSOVA[2] , MARIA V. PATRIKEYEVA[3] ,
SVETLANA Y. SPIGLAZOVA [2], TATJANA I. SMETANINA[2], NATALIA N. SEMENOVA[4],
KENNETH L. DEAHL[5]

[1] N.I.Vavilov Institute of Plant Industry, St-Petersburg, 190000, Russia
[2] All-Russian Research Institute of Phytophatology, Bol'shiye Vyazemy, Moscow region, 143050, Russia
[3] All-Russian Research Institute of Plant Protection, Pushkin, St-Petersburg, Russia
[4] State St-Petersburg University, St-Petersburg, Russia
[5] USDA-ARS Genetic Improvement of Fruits and Vegetables Laboratory,
Beltsville, Maryland, USA
E-mail: rogozinaelena@gmail.com

SUMMARY

The effects of a current *Phytophthora infestans* population in Russia on genotype of Mexican tuber-bearing *Solanum* species were assessed in field and laboratory trials. Two-year experiments documented disease progress in 78 accessions of 19 species under high infection pressure. Detached leaf tests conducted independently in laboratories of two scientific institutions identified the response of 19 species to isolates collected in different regions of Russia. As a whole, we characterized *Solanum* genotypes with a broad range of resistance to late blight.

KEYWORDS

Phytophthora infestans, late blight, Solanum species.

INTRODUCTION

Wild *potato* collection at VIR (N.I.Vavilov Institute of Plant Industry) is a source of useful breeding characteristics, in particular the resistance to pests and diseases. To describe the breeding potential of wild species the results of evaluation of potato germplasm preserved at VIR has been provided by many authors (Bukasov & Kameraz, 1972, Budin *et al*., 1984, Zoteeva *et al*., 2004). Since most evaluation data on wild potato comes from screening populations, such information provides only a general guide for selecting prospective germplasms (Hoekstra *et al*., 1997). The reaction of potato germplasm to late blight (LB) must be additionally verified due to the changes in *Phytophthora infestans* populations in Russia (Elansky *et al*., 2001). Mexican wild potatoes have been recognized as an outstanding source of late blight resistance. Here we report the evidence on 78 accessions of Mexican tuber-bearing *Solanum* species assessed for LB resistance to the current *P. infestans* population in the field and to a complex race of *P. infestans* in the laboratory. The well-characterized

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Solanum genotypes manifesting the broad range of resistances can be further employed in breeding programs and concurrent molecular-genetic studies.

MATERIALS AND METHODS

Plants

The plant genotypes used in this study belong to 78 accessions representing 19 species of eight series as listed in Table 1. To compare the behavior of Mexican species to a cultivated potato, the plants of *S. chilotanum* were included into the experiment. Each accession grown from greenhouse seedlings was presented by one genotype selected because of abundant tubers and lack of virus symptoms. The tubers of each individual genotype were harvested in the greenhouse. The clones were propagated in the greenhouse annually and used for field and laboratory tests.

Table 1. *Accessions of Solanum tuber-bearing species tested for LB resistance.*

Series	Species	Accession numbers in the VIR collection
Demissa	*S. brachycarpum*	k-2830
	S. demissum	k-15173, k-18521
	S. hougasii	k-8818
Longipedicellata	*S. fendleri*	k-5747, k-5751, k-23335, k-23841, k-24218, k-24221
	S. hjertingii	k-15194, k-19276, k-21409, k-24223, k-24387
	S. papita	k-16889, k-24417
	S. polytrichon	k-7426, k-16905, k-18142, k-19164, k-23561, k-23556, k-23563, k-24298, k-24462, k-24463
	S. stoloniferum	k-3336, k-19196, k-20106, k-21547, k-21618, k-23652, k-24420
Borealia	*S. wightianum*	k-24250
Polyadenia	*S. polyadenium*	k-23553
Pinnatisecta	*S. brachystotrichium*	k-22919, k-23200, k-24197
	S. jamesii	k-15203, k-22619, k-23397, k-23398, k-23399, k-24395, k-24397
	S. pinnatisectum	k-21955, k-23569, k-24239, k-24243
	S. stenophyllidium	k-20105, k-24255
	S. tarnii	k-23936
Cardiophylla	*S. cardiophyllum*	k-3319, k-4464, k-10456, k-16827, k-16828, k-17370, k-18086, k-24203, k-24206, k-24207, k-24209, k-24375
	S. ehrenbergii	k-18085, k-18225, k-19059, k-19061, k-19257, k-21300, k-23277, k-23279, k-24279, k-24373
Bulbocastana	*S. bulbocastanum*	k-19981, k- 21266
Tuberosa	Control - *S. chilotanum*	k-1671
Total	19	78

Field test

Field assessments were carried out during the 2008 and 2009 plant growth periods at the Pushkin Experimental station of VIR situated 20 km south of St-Petersburg in the Leningrad region, the North-Western part of Russia. Each year 2-5 plants per genotype were grown in the field. The experimental plots were positioned between adjacent border rows of potato cultivars. Foliage LB assessments were taken on each plant weekly for 5 weeks in 2008 and for 4 weeks in 2009 using the 1 to 9-score scale, where 1 corresponds to 100% necrotic tissue and 9, to no visible lesions. These scores were converted into mean percent defoliation for the corresponding range (i.e., 1=0%,

5=50%, and 9=100%) and used to calculate the area under the disease progress curve (AUDPC) for each individual accession. To compare AUDPC across two-year experiments the relative AUDPC (RAUDPC) was calculated using Microsoft Excel (Anonymous, 2007).

Laboratory test
Detached leaf tests (see Eucablight protocol – Detached leaf test for foliage blight resistance: www. euroblight. net/) were conducted independently in two laboratories: at the Institute of Phytopathology (IP) and the Institute of Plant Protection (VIZR). Leaflets were collected from 30-55-day-old greenhouse plants. Single pathogen isolates collected in the Leningrad and Moscow regions (both containing the race 1,2,3,4,5,6,7,8,9,10,11) were used in this study. Three to five leaflets per each tested accession were inoculated with zoospore suspension at the concentration of 30-40 × 1000 per ml according to standard protocol. The leaflets of cvs.: Alpha, Bintje, Eersteling, Escort, Robijn, Gloria, Sapro Mira and Sante (Eucablight standard set) were used as the control in IP and cvs. Latona and Elizaveta, in VIZR. Disease assessments were carried out for six days following inoculation. Both the leaf area affected by pathogen and the extent of sporulation were scored.

Statistical analysis
Data were analyzed by ANOVA to compare late blight damage in the accessions of wild potato species across two-year field tests. The correlation between area under disease progress curve (AUDPC) and the resistance of the wild potato infected in the laboratory was estimated with the Spearman's coefficient. *STATISTICA* 6 1 package (StatSoft Inc. /StatSoft Russia) was used for processing the data.

RESULTS AND DISCUSSION

Field trials
Epidemic performance of *P. infestans* was recorded for both years of field trials. Isolates of *P. infestans* from those fields were very complex and virulent to all 11 *R*-gene differentials tested, except R9. In both years, the diseases emerged at the same period, 55-60 days after planting, but the time-course of disease progress varied substantially. The AUDPC scores and their variations for individuals of 19 *Solanum* species in the two years are given in Table 2.

Table 2. *AUDPC indices in 19 Solanum species in two-year field evaluations.*

Species name	Number of individual genotypes	2008 year				2009 year			
		mean	±SD	min	max	mean	±SD	min	max
S. chilotanum	1	0.575				0.370			
S. brachycarpum	1	0.350				0.000			
S. demissum	2	0.612	0.159	0.500	0.725	0.240	0.311	0.020	0.460
S. hougasii	1	0.500				0.270			
S. fendleri	6	0.729	0.090	0.550	0.800	0.715	0.172	0.440	0.900
S. hjertingii	5	0.570	0.186	0.350	0.775	0.454	0.312	0.150	0.890
S. papita	2	0.375	0.000	0.375	0.375	0.310	0.155	0.200	0.420
S. polytrichon	10	0.725	0.171	0.350	0.950	0.483	0.223	0.250	0.880
S. stoloniferum	7	0.571	0.266	0.175	0.925	0.344	0.242	0.000	0.650
S. wightianum	1	0.625				0.080			
S. polyadenium	1	0.600				0.040			
S. brachystotrichium	3	0.775	0.225	0.550	1.000	0.670	0.370	0.260	0.980
S. jamesii	7	0.782	0.087	0.625	0.925	0.281	0.207	0.000	0.630
S. pinnatisectum	4	0.406	0.096	0.350	0.550	0.185	0.194	0.000	0.450
S. stenophyllidium	2	0.837	0.053	0.800	0.875	0.685	0.021	0.670	0.700
S. tarnii	1	0.400				0.090			
S. cardiophyllum	12	0.679	0.150	0.475	1.000	0.293	0.215	0.080	0.710
S. ehrenbergii	10	0.719	0.096	0.525	0.800	0.565	0.237	0.200	0.910
S. bulbocastanum	2	0.137	0.053	0.100	0.175	0.100	0.141	0.000	0.200
Total	78	0.642	0.197	0.100	1.000	0.398	0.271	0.000	0.980

Variability for infestation by *P. infestans* was found both among and within *Solanum* species and in both years of trial. The rate of increase of infected leaf area across the tested material was greater in 2008 than in 2009. The average AUDPC value for the Mexican species was 0. 64 (from 0. 10 to 1. 00) in 2008 and 0. 39 (from 0 to 0. 98) in 2009 as compared to 0.58 and 0.37 respectively, in the control of *S. chilotanum*. In both years we observed a highly significant species effect. The most resistant were *S. bulbocastanum* plants, its final defoliations were not more than 20%. Some individuals of ssp. *S. brachycarpum* (k-2830), *S. demissum* (k-15173), *S. hougasii* (k-8818), *S. tarnii* (k-23936) and *S. cardiophyllum* (k-24207) were significantly resistant to the local population of *P. infestans*. Their final defoliations did not exceed 50% in both years of trial. Plants of *S. pinnatisectum* and *S. papita* displayed a different level of LB resistance. Their AUDPC values were either below or occasionally equal to those of control *S. chilotanum* plants. Plants of *S. brachystotrichium, S. stenophyllidium* and *S. ehrenbergii* were extremely susceptible and their final defoliation was almost 100%. Most individuals of those species were damaged even more than *S. chilotanum* (Table 2). All other Mexican tuber-bearing *Solanum* species showed variable degrees of damage depending on the year. Two-year observations indicated a wide-range response of *S. stoloniferum* plants LB infection. For instance, the accession k- 21618 was resistant in 2008 (AUDPC 0.175) and showed medium susceptibility in 2009 (AUDPC 0.48). On the other hand, the accession k-3336 was resistant in 2009 (AUDPC 0.00) and showed medium susceptibility in 2008 (AUDPC 0.48). Thus, the response of *S. stoloniferum* individuals under natural infection was not consistent over years. Similarly, the performance of individuals of *S. fendleri, S. polytrichon, S. hjertingii, S. jamesii* was depended on the year. Factorial ANOVA indicated that plant species and the year of testing significantly affected the final defoliation of tested accessions, whereas the interaction of these two factors was insignificant.

Laboratory trial

Classification of accessions of 19 *Solanum* species according to the results of detached leaf tests (DLT) is presented in Table 3. Of 78 accessions tested in the field trial, 64 were evaluated for resistance to the *P. infestans* isolate collected in the Leningrad region in the test performed at VIZR and 24 were evaluated at IP using the isolate collected in the Moscow region.

Table 3. *Number of individuals from 19 Solanum species in resistance categories (detached leaf tests)*

Species	Number of individual genotypes *	Resistance category **			
		R	MR	MS	S
S. chilotanum	1\				1\
S. brachycarpum	1\	1\			
S. demissum	2\1	2 \1			
S. hougasii	1\	1\			
S. fendleri	6 \3	1\	\1		5 \ 2
S. hjertingii	2\	1\			1\
S. papita	2\1		\1		2\
S. polytrichon	10\5	4\			6\5
S. stoloniferum	6\2	2\			4\2
S. wightianum	1\	1\			
S. polyadenium	1\1	1\1			
S. brachystotrichium	2\1				2\1
S. jamesii	5\1			2\	3\1
S. pinnatisectum	4\3	2\1	\1	1\1	1\
S. stenophyllidium	2\1				2\1
S. tarnii	1\1		1\1		
S.cardiophyllum	7\2	5\2			2\
S.ehrenbergii	8\1			\1	8\
S.bulbocastanum	2\1	2\1			
Total	64\24	23\6	1\4	3\2	37\12

* *VIZR\IP data*

** *R-resistant, MR-medium resistant, MS-medium susceptible, S- susceptible*

Detached leaf tests conducted at two scientific institutions produced a similar pattern. In general, the proportion of resistant and susceptible individuals greatly exceeded that of the intermediate type of reaction. The proportion of susceptible individuals in both tests was approximately equal (58 and 50 %, respectively). Similar to field trials, variation for infestation by *P. infestans* was found both among and within *Solanum* species in laboratory tests. All individuals of *S. demissum* and *S. bulbocastanum* species as well as in single accessions of *S. brachycarpum, S. hougasii, S. polyadenium* ssp. were highly resistant to infestation. All tested genotypes of *S. brachystotrichium* and *S. stenophyllidium* species as well as all controls plants were susceptible. A broader distribution of response to *P. infestans* inoculums was observed in *S. fendleri* and *S. pinnatisectum* (Table 3). The individuals of *S. demissum* (k-18521), *S. polyadenium* (k-23553), *S. pinnatisectum* (k-24239), *S. cardiophyllum* (k-16828, 24375) and *S. bulbocastanum* (k-19981) were recognized as resistant to both *P. infestans* isolates.

In general there was a good agreement between the results of the field and laboratory trials for tested genotypes. Correlation coefficients between resistance ratings obtained for genotypes tested in the two-year field experiment and infected with two *P. infestans* isolates are significant (Table 4).

Table 4. *Spearman's coefficients of correlation between field and laboratory test results: significant at P=0.05*

	AUDPC 2009	DLT of VIZR	DLT of IP
AUDPC 2008	0.500	0.391	0.491
AUDPC 2009		0.445	0.527
DLT of VIZR			0.781

The highest correlation (*r*= 0.78) was obtained when two laboratory tests were compared. The Spearman's rank order correlation is lower when comparing two field tests. A medium correlation was obtained between field and laboratory tests for each pair of experimental data (Table 4). These data suggest different levels of resistance in inoculated detached leaves and intact plants. As a whole, the results obtained by both test methods show that only the individual plants of such *Solanum* species as *S. brachycarpum, S. demissum, S. pinnatisectum, S. cardiophyllum* and *S. bulbocastanum* possess high levels of LB resistance. *Solanum* genotypes which belong to *S. brachycarpum* (k-2830)*, S. demissum* (k-15173), *S. pinnatisectum* (k-24239)*, S. cardiophyllum* (k- 18086, 24206, 24207, 24375) and *S. bulbocastanum* (k-19981, 21266) are resistant to late blight both in inoculated detached leaves and intact plants in the field.

Indeed, in our experiments, some genotypes resistant in the field were infected in the laboratory test: *S. pinnatisectum* (k-21955), *S. polytrichon* (k-24463) and *S. stoloniferum* (k-21547). In contrast, four individuals recognized as resistant to both *P. infestans* isolates, *S. demissum* (k-18521), *S. fendleri* (k-5751), *S. cardiophyllum* (k-16828) and *S. polyadenium* (k-23553), manifested greater resistance to the artificial than natural infection. There is more than one explanation for these phenomena. *Solanum* accessions involved in this study belong to genetically distant groups and vary in many characteristics including plant architecture and physiological status. As plants senesce, they become more susceptible to LB attack than younger plants. A strong pubescence or a widely spaced arrangement of leaves may cause some difference in foliage attack or *P. infestans* penetration in the tissue. On the other hand, in the laboratory trials, the uniform conditions of the experiment minimize the effects of plant age and morphology. However, in the latter case, the short period of plant-pathogen interaction is far from natural. Field tests, in which the observations were carried out over a longer period, reflect the reaction of intact plants and probably provide an assessment of several components of race-nonspecific resistance. However, the laboratory method can determine the race-specific reaction and allow detecting wild potato plants with non-compatible reaction to a complex race of *P. infestans*, crucial for the search for new genes for LB resistance.

Currently, there is rapid progress in mapping and isolating genes from wild potato species that confer resistance to *P. infestans* in. Molecular insight into the complex processes involved in potato-pathogen interactions is believed to be a necessary precondition for breeding for durable LB resistance (Hein *et al.,* 2009). Based on data from the field and the laboratory tests, the genotypes of *Solanum* species with related genome constitutions and contrasting responses to *P. infestans* invasion are identified within series Cardiophylla and Pinnatisecta. These *Solanum* genotypes could be a subject of further study.

CONCLUSIONS

The response of *Solanum* species to LB differed markedly depending on the test to which they were exposed. *Solanum* genotypes which possessed of resistance to late blight both in field and laboratory trials belong to *S. brachycarpum* (k-2830)*, S. demissum* (k-15173), *S. pinnatisectum* (k-24239)*, S. cardiophyllum* (k- 18086, 24206, 24207, 24375) and *S. bulbocastanum* (k-19981, 21266)..

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Characteristics of the *Phytophthora infestans* population in Russia

N.V. STATSYUK[1], M.A. KUZNETSOVA[1], I.N. KOZLOVSKAYA[1], B.E. KOZLOVSKY[1], S.N. ELANSKY[2], E.V. MOROZOVA[1], E.V. VALEVA[1], AND A.V. FILIPPOV[1]

[1]All-Russian Research Institute of Phytopathology, VNIIF,
Bolshie Vyazemy, Moscow region, 143050 Russia; e-mail: natafg@rambler.ru
[2] Department of Algology and Mycology, Biological Faculty,
Moscow State University, Vorob'evy Gory, Moscow, 119992 Russia

SUMMARY

A collection of 434 *Phytophthora infestans* isolates, obtained during 2007-2009 from potato and tomato fields of different parts of European Russia, has been assessed for several phenotypic and genotypic markers, including mtDNA haplotype, Pep1 and Pep2 loci, mating type, metalaxyl sensitivity, and the virulence. The comparison of phenotypic and genotypic characteristics of *P. infestans* population of the Moscow region in 2008-2009 with the similar data, obtained in 1997-1998, has shown significant changes occurred within a 10-year period: (1) the frequency ratio of Ia and IIa MtDNA haplotypes has shifted towards the predominance of the Ia type, (2) the percent of strains belonged to the A2 mating type has increased, (3) the virulence gene 9 has appeared in the current population, which now contains all 11 virulence genes, and (4) the percent of metalaxyl-susceptible strains has significantly decreased from 78 to 58%. The analysis of the studied populations has shown that the Leningrad, Astrakhan, and Nizhni Novgorod populations are rather uniform, whereas the Moscow, Mariy El and Kostroma populations are characterized by a high diversity. The Astrakhan "tomato" population has a unique pattern of virulence gene frequencies and seems to be monoclonal.

KEYWORDS

late blight, potato, tomato, population characteristics

INTRODUCTION

Phytophthora infestans, the causal agent of the late blight disease of potato and tomato, is the most damaging microbial pathogen of these crops world-wide, including Russia and Eastern Europe. In the XIX century this pathogen caused significant potato yield losses in the Northern Europe that resulted in the Irish Potato Famine. In the last decades, new agrotechnical and plant pest control methods, such as the use of certified seeds, breeding programs, crop rotation, and highly-effective fungicides, significantly decreased potato yield losses caused by the late blight. At the same time, due to a high pathogen variability and a possible introduction of new strains by a potato shipment from

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central Mexico to Europe, the European population of *P. infestans* has undergone significant changes [1-2]. The "old" population was represented by the A1 mating type and Ib mitochondrial (Mt) DNA haplotype, whereas the "new" population consisted of the isolates of both A1 and A2 mating types and Ia and IIa MtDNA haplotypes [3-7]. Among other changes in *P. infestans* population traits, one should also mention an increase in the metalaxyl resistance that decreased the effect of the use of popular metalaxyl-based fungicides on the late blight control [8].

The interaction between strains of different mating types can cause the sexual reproduction of *P. infestans,* increasing the genetic diversity of the progeny; this can possibly result in an increase in the virulence and fungicide resistance of newly developed strains.

In our previous studies we analyzed the phenotypic and genotypic characteristics of *P. infestans* populations from the Moscow region, Siberia, and Far East [9]. In this study we present the data, obtained for the Moscow region (2008-2009) and 5 new regions, including North Western Russia and several regions of the Eastern part of European Russia.

MATERIALS AND METHODS

P. infestans isolates were collected from commercial potato and tomato fields, located in the following regions of the European Russia (Fig. 1): Leningrad (21 isolate), Moscow (100 isolates, including 20 from tomato plants), Nizhni Novgorod (13 isolates), Astrakhan (31 isolates from tomato plants), Kostroma (105 isolates), Smolensk (49 isolates), and the Mariy El Republic (115 isolates, including 93 from tomato plants). The total number of the studied isolates was 434.

Allozyme analysis. Genotypes at two peptidase loci (Pep1 and Pep2) were analyzed using a cellulose acetate gel electrophoresis according to [10] with some modifications [11]. The genetic diversity for the Pep1 locus in Russian *P. infestans* populations is significantly lower than that of the Pep2 locus, represented by two alleles (100 and 112) and stained simultaneously with the Pep1 locus (Fig. 2), so we analyzed both these markers.
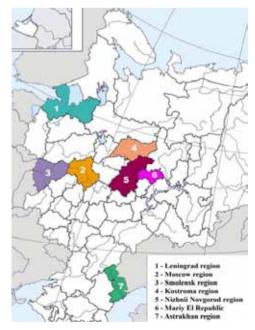
Fig. 1. *Locations of sampling sites.*

The mtDNA haplotype identification was carried out according to [12] with some modifications. The *mating type* was tested by the growing isolates on rye agar with the known reference strains of the A1 and A2 mating types. Agar blocks with the studied and reference strains were placed by pairs into Petri dishes, containing rye-vegetable agar, at a distance of 4-5 cm from each other. The Petri dishes were incubated in the dark at 18°C or 14 days, and then we microscoped the place of a hypha contact between the strains to determine the presence or absence of oospores. If the studied isolate generated oospores only with the A2 isolate, it was referred to the A1 type. If the isolate generated oospores only with A1 isolate, then it was referred to the A2 type. If the isolate generated oospores with both reference strains, then it was referred to A1A2 type.

Metalaxyl sensitivity. The sensitivity of isolates to metalaxyl-containing fungicides was determined by the inoculation of fungicide-treated tuber discs [13] or fungicide-containing

medium [14] with the tested isolates at different fungicide concentrations. According to the obtained results, isolates were considered as sensitive (S), moderately resistant (MR), or resistant (R).
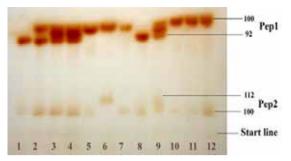
Fig. 2. *Cellulose acetate plate showing typical allozyme banding patterns of two peptidase loci for P. infestans isolates.* **Pep1 locus***: 92/92 (lanes 1 and 8); 100/100 (lanes 5-7, 10-12); 92/100 (lanes 3, 4, 9).* **Pep2 locus***: 100/100 (lanes 1-5, 7, 8, 10-12); 112/112 (lane 6); 100/112 (lane 9).*

Virulence. To study the virulence of isolates, we used a set of differentiator cultivars, obtained from the International Potato Center (CIP, Peru) and containing 22 genotypes, including all known resistance genes in different combinations. We also used the test set, containing R_0-R_{11} genotypes and obtained from the Institute of Plant Cultivation and Acclimatization (IHAR, Poland). The analysis was carried out under laboratory conditions using detached potato leaves. The leaves were placed into a wet chamber, representing a frame (30x40 cm) with the bottom made of a metal gause. The chamber was covered with a glass sheet. To inoculate the leaves, we used a 4-5-day culture of *P. infestans*, grown on the tuber slices of a susceptible potato cultivar, or 10-12-day culture, grown on nutrient medium. A pathogen suspension was prepared in a concentration, corresponding to 10-15 conidia in a microscopic field at 100x magnification. Tubes with the suspension (5 ml) were placed into a refrigerator (6-7ºC) to initiate the release of zoospores. The lower surface of each leaf was inoculated by 2 drops of suspension using a micropipette; after 24 h the drops were shaken off, and the leaves were turned upside down. The leaves were incubated at 18-20ºC. The level of the disease development on the leaves was determined after 4-6 days of incubation. The presence of the fruiting testified a compatible reaction and was designated by a "+"; the absence of any fruiting was designated as "-".

RESULTS AND DISCUSSION

Allozyme analysis.
The results of the allozyme analysis for Pep1 and Pep2 loci are shown in Fig. 3.
In all populations the predominant genotype of the Pep1 locus was 100/100; all three "tomato" populations were represented by only this genotype. The presence of all three possible variants (92/92, 92/100, and 100/100) was revealed only in the Moscow "potato" population, like in the case of our previous study [9].
In the case of Pep2 locus, the genetic diversity was higher. All three possible variants were revealed in 4 populations at different proportions. Again, the most frequent genotype was 100/100, excepting the Leningrad population, represented by the only 112/112 genotype, Smolensk population, represented mainly by 100/112 genotype, and Kostroma population, where the 112/112 genotype predominated. It is interesting that the 100/100 genotype was predominant for all three tomato populations (100% for Astrakhan and Moscow regions and 47.8% for the Mariy El Republic).
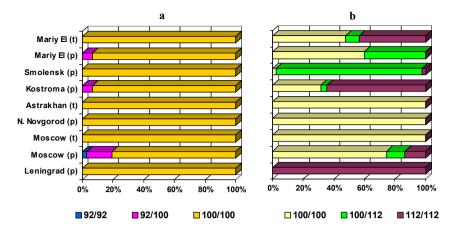
Fig. 3. *Frequency of Pep1 (a) and Pep2 (b) genotypes of P. infestans samples from different regions of European Russia; (p) and (t) mean "potato" and "tomato" populations, respectively.*

Analysis of mitochondrial DNA haplotype

The results of the mtDNA analysis are shown in Fig. 4.

We revealed only two mtDNA haplotypes (Ia and IIa). The Ia genotype was predominant for all populations, excepting the Mariy El "tomato" population; two populations (Leningrad and Astrakhan "tomato") were presented by only this genotype.

In the case of the Moscow region, some changes in the ratio of these MtDNA haplotypes were registered comparing to the previous data, obtained in 1997-1998 [9]. According to the earlier study, the Ia : IIa ratio was about 36 : 64, whereas the recent data show a tendency to the increase in the Ia percentage (55 : 45).
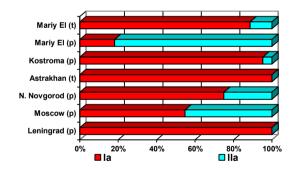
Fig. 4. *Mitochondrial DNA haplotypes of P. infestans samples from different regions of European Russia; (p) and (t) mean "potato" and "tomato" populations, respectively.*

Mating type

Mating type frequencies, determined for all studied populations, are shown in Table 1.

The A1 mating type predominated in most of the examined populations; in the case of Leningrad (potato) and Astrakhan (tomato) populations, it was the only variant revealed. The A2 mating type prevailed in the Nizhnii Novgorod, Smolensk, and Mariy El (potato) populations (100, 94.4, and 64.3 percents, respectively). Populations from the Moscow and Kostroma regions included a small percent of A1A2 strains, able to generate oospores with both reference strains.

Comparing the data obtained for the Moscow region in 1997-1998 [9] and 2008-2009, one can

note that the A1:A2 ratio in both potato and tomato population of *P. infestans* changed from 72:28 (potato) and 88:12 (tomato) to 61:35.4 and 65:35, respectively, i.e. the percent of the A2 strains increased.

Table 1. *Occurrence of A1 and A2 mating types in Russian P. infestans populations*

Region	Mating type, %		
	A1	A2	A1A2
Leningrad (potato)	100	0	0
Moscow (potato)	61	35.4	3.6
Moscow (tomato)	65	35	0
Nizhni Novgorod	0	100	0
Astrakhan (tomato)	100	0	0
Kostroma (potato)	80.1	19.4	0.5
Smolensk (potato)	5.6	94.4	0
Mariy El (potato)	35.7	64.3	0
Mariy El (tomato)	77.8	22.2	0

Virulence

The results of our virulence study are shown in Fig. 5.

According to our data, populations from the Kostroma and Leningrad regions were similar to each other: the frequencies of single genes were about the same, and both populations did not include the gene 9. Both populations have similar values of the factor of virulence (FV). The most frequent races in both populations included 8-10 virulence genes.

Both tomato and potato populations of *P. infestans* from the Mariy El Republic were represented by complex races and had the similar structure. The most frequent races were 1.2.3.4.6.7.8.10.11 (28.2%) and 1.2.3.4.5.6.7.8.10.11 (50%). The total percent of these two races in these two population was 78.2%.

The Moscow population included all virulence genes and was presented mainly by complex races, containing 7-11 genes (including gene 9, which was absent in the most of the studied populations). The most frequent races were 1.3.4.7.8.10.11; 1.2.3.4.6.7.8.11; 1.2.3.4.6.7.8.10.11; 1.2.3.4.5.6.7.8.10.11; and 1.2.3.4.5.6.7.8.9.10.11. Comparing to the earlier data, the gene 9 appeared in the population.
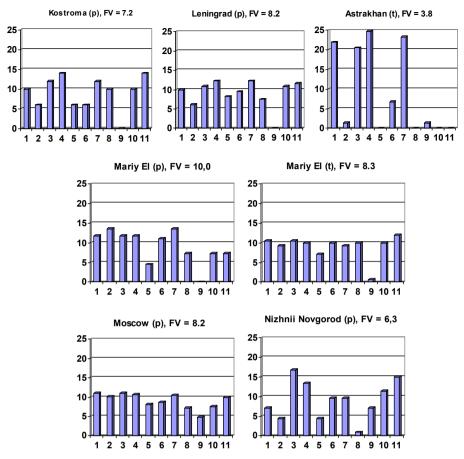
Fig. 5. *Frequencies of separate virulence genes in the studied P. infestans populations. FV, factor of virulence; (p) and (t) mean "potato" and "tomato" populations, respectively.*

The population from the Nizhnii Novgorod region consisted mainly of complex races, containing 6-11 virulence genes (57.9%). Like the Moscow population, this population also contained the gene 9; it is also interesting that the frequency of gene 8 was unusually low. The FV value (6.3) was lower than for the above-mentioned populations (7.2-10.0).

In the case of the Astrakhan population, we revealed a significant predominance of virulence genes 1, 3, 4, 7. Genes 5, 8, 10, and 11 were not revealed; the frequencies of genes 2 and 9 were very low. The FV value for this region was very low (3.8), and the most frequent race was the race 1.3.4.7 (47%). Thus, this population significantly differed from other ones.

In general, one should note that some of the tested populations contain the gene 9, which was not determined in the Russian P. infestans populations during our earlier studies.

Metalaxyl resistance

The results of the analysis of the studied *P. infestans* populations for their metalaxyl resistance are shown in Fig. 6.

Both Mariy El populations were represented by only susceptible isolates; the most of isolates from Kostroma and Moscow regions were also susceptible (98 and 58%, respectively). The percent of susceptible strains in the Moscow "potato" population significantly decreased comparing to the

earlier data [9] (from 78 to 58%). Astrakhan "tomato" and Leningrad populations were moderately resistant (100% and 76%, respectively). Finally, Nizhnii Novgorod population consisted mainly of the resistant isolates (73%).
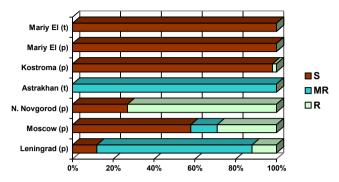
Fig. 6. *Frequency of P. infestans strains with different level of the metalaxyl resistance in different regions of European Russia. S, susceptible strains, MR, moderately resistant strains, R, resistant strains; (p) and (t) mean "potato" and "tomato" populations, respectively.*

CONCLUSIONS

The comparison of phenotypic and genotypic characteristics of *P. infestans* population of the Moscow region in 2008-2009 with the similar data, obtained in 1997-1998 showed that some significant changes occurred within a 10-year period. The frequency ratio of Ia and IIa MtDNA haplotypes shifted from 36:64 to 55:45, increasing the percent of Ia type. The A1:A2 ratio in both potato and tomato *P. infestans* populations of the region shifted from 72:28 (potato) and 88:12 (tomato) to 61:35.4 and 65:35, respectively, i.e. the percent of A2 strains increased. The virulence gene 9 appeared in the current population, which now contains all 11 virulence genes. The percent of susceptible strains in the "potato" population decreased from 78 to 58%.

The analysis of the studied populations showed that the Leningrad, Astrakhan, and Nizhni Novgorod populations are rather uniform. The Astrakhan "tomato" population has a unique pattern of virulence gene frequencies and seems to be monoclonal. The Moscow population has the highest diversity; a high diversity level was also observed for the Mariy El and Kostroma populations that can be explained by the fact these 3 regions represent potato-growing regions with a high volumes of imported seed potato.

The data obtained for the studied "potato" and "tomato" populations shows the first ones have a greater diversity in some parameters, such as the MtDNA haplotype and Pep1/Pep2 loci.

Most of the analyzed populations contained the virulence gene 9, which was not revealed in the populations, studied in our previous investigation.

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Rating of fungicides used for the potato late blight control

A.V. FILIPPOV

All-Russian Research Institute of Phytopathology, VNIIF, Bolshye Vyazemy,
Moscow region, 143050 Russia; e-mail: filippov@vniif.ru

SUMMARY

A method for the evaluation of the consumer qualities of fungicides, applied for the potato late blight control, has been proposed. The rating of fungicides, basing on their functional properties and cost, was calculated for three stages of a potato plant development.

KEY WORDS

potato, late blight, protection efficacy, rain resistance, cost

In 2010 the Russian market of fungicides, used to protect potato against the late blight, included 15 preparations.

The most frequent questions, asked by Russian potato growers, are related to the optimal choice of fungicides. In our study we tried to make a quantitative characteristic of the consumer qualities of the offered preparations.

In this paper we considered only functional and economical indices of the consumer qualities of compared fungicides, which are directly connected with the profit of a potato-growing company. We did not take into account any anthropological indices, such as the comfortability of application, safety, and ecological compatibility.

Functional properties of a fungicide are characterized mainly by its ability to protect leaves, new growing leaves (during a rapid growth of tops), tubers, and also by its rain resistance. The economical characteristic represents the cost of a fungicide dose, required for the protection of 1 hectare of a potato field.

To compare the efficiency of different fungicides, we used the assessment of these parameters made by the group of independent international experts of the Euroblight association (N.J. Bradshaw, 2007; see Table 1) and also the results of our own studies (M.A. Kuznetsova *et al.*, 2010). We transferred the efficiency indices, expressed in pluses, into numbers:

+ + +	3
+ + (+)	2,5
+ +	2
+ (+)	1,5
+	1
(+)	0,5

The data on the cost of preparations were obtained from the on-line price lists of manufacturers.

Posters

Table 1. *Characteristics of the efficiency of compared fungicides (Euroblight PPO-Special Report 12, 2007)*

Preparation	Efficiency			Resistance to rain
	Leaves	New growing leaves	Tuber	
Copper preparations: **Abiga Pik**	+	0	+	+
Dithiocarbamates: **Dithan M45, Mancoceb, Penncoceb, Polyram**	+ +	0	0	+ (+)
Chlorothalonyl: **Bravo**	+ +	0	0	+ + (+)
Fluazinam: **Shirlan**	+ + +	0	+ + (+)	+ + (+)
Dimethomorph + mancoceb: **Acrobat MZ**	+ + (+)	0	+ +	+ + (+)
Cymoxanyl + copper: **Ordan, Kurzat**	+ + (+)	0	0	+ +
Phamoxadon+ cymoxanyl: **Tanos**	+ +	0	n/r	+ + (+)
Phenamydon + mancoceb: **Sectin Fenomen**	+ + (+)	0	+ +	+ +
Methalaxyl + Mancoceb: **Ridomil Gold MZ, Metaxyl**	+ + +	+ +	n/r	+ + +
Propamocarb-HCl + fluopicolid: **Infinito**	+ + +	+ +	+ + +	+ + (+)

+ + + excellent; + + good; + satisfactory; 0, zero effect (or no any data about the effect); n/r, not recommended for the tuber protection

According to our concept of chemical protection of potato, the assortment of used fungicides depends on the potato development stage. Therefore, we calculated the rating of fungicides separately for three stages: **I**, from the shoot emergence to the time of row closing; **II**, from the time of a row closing to the flowering; and **III**, from the flowering to the natural destruction of haulm.

In the case of the stage I, the most important parameters are the leaf protection efficiency, rain resistance, and the cost of a fungicide. In the case of the stage II, these parameters are the efficiency of protection of new growing leaves, rain resistance, and the cost of a fungicide. In the case of the stage III, the important parameters are the tuber protection efficiency, rain resistance, and the cost of a fungicide.

The ratings of fungicides concerning their consumer qualities were assessed using a 5-score scale (Tables 2-4). To do this, we compared all preparations and chose the best and the worst value of each parameter, setting them as 5 and 1 score, respectively. All other compared preparations obtained intermediate scores, corresponding to their position between the leader and the outsider. For example, if for the stage I the leader and the outsider showed 3 and 1 pluses in the leaf protection efficiency, respectively, then the preparation, which had 2.5 pluses, obtained 4 scores. In the case of a cost evaluation, 5 scores were assigned to the cheapest preparation, and 1 score – to the most expensive one.

To evaluate the preparations according to their functional properties, we used the equation 1; to evaluate preparations by only their cost, we used the equation 2.

$$R_x = 4\frac{y_x - \min(y)}{\max(y) - \min(y)} + 1. \qquad (1)$$

$$R_x = 4\frac{\max(y) - y_x}{\max(y) - \min(y)} + 1. \qquad (2)$$

In both formulas FR_x is the quantitative value of the studied characteristics of the fungicide X (in scores), y_x is a quantitative value of the studied parameter of the fungicide X, expressed in the number of pluses (Euroblight assessment) for the formula (1) or the cost of treatment, rubles/hectare, for the formula (2).

For each stage of potato development, the total scores of the compared fungicides were determined as the average values of intermediate scores.

Table 2. *Consumer qualities of fungicides, applied at the stage I (from the shoot emergence to the time of a row closing)*

Fungicide	Leaf protection		Resistance to rain		Cost of fungicide		Total score
	1*	2**	1*	2**	Cost, rub./hectare	Intermediate score	
Shirlan	3,0	5	2,5	5	1168	2,4	4,13
Acrobat MZ	2,5	4	2,5	5	1338	1,6	3,53
Mancozeb	2,0	3	1,5	2,3	553	5	3,43
Ordan	2,5	4	2,0	3,6	1120	2,6	3,40
Kurzat R	2,5	4	2,0	3,6	1150	2,4	3,33
Penncozeb	2,0	3	1,5	2,3	632	4,7	3,33
Dithane M45	2,0	3	2,0	2,3	699	4,4	3,23
Polyram	2,0	3	1,5	2,3	680	4,4	3,23
Tanos	2,0	3	2,5	5	1366	1,5	3,16
Bravo	2,0	3	2,5	5	1491	1,0	3,00
Sectin Fenomen	2,5	4	2,0	3,6	1475	1,1	2,90
Abiga Pik	1,0	1	1,0	1	570	4,9	2,30

1* Efficiency, number of pluses;
2** Intermediate score.

Table 3. *Consumer qualities of fungicides, applied at the stage II (from the time of a row closing to the flowering)*

Fungicide	Protection of new growth		Resistance to rain		Cost of fungicide		Total score
	1*	2**	1*	2**	rub./hectare	Intermediate score	
Methaxyl	2,0	5	3,0	5	1445	2,1	4,02
Infinito	2,0	5	2,5	1	1297	5	3,66
Ridomil Gold MZ	2,0	5	3,0	5	1500	1	3,66

1* Efficiency, number of pluses;
2** Intermediate score.

Table 4. *Consumer qualities of fungicides, applied at the stage III (from the flowering phase to the time of natural haulm destruction)*

Fungicide	Tuber protection		Resistance to rain		Cost of fungicide		Total score
	1*	2**	1*	2**	rub./hectare	Intermediate score	
Infinito	3,0	5	2,5	5	1297	1,78	3,92
Shirlan	2,5	4	2,5	5	1168	2,35	3,78
Acrobat MZ	2,0	3	2,5	5	1338	1,60	3,20
Abiga Pik	1,0	1	1,0	1	570	5	2,33
Sectin Fenomen	2,0	3	2,0	2,7	1475	1	2,23

1* Efficiency, number of pluses;
2** Intermediate score.

The tables show that, according to the consumer qualities for fungicide preparations, the best fungicides are Shirlan, Acrobat MZ, and Mancoceb (for stage I); Metaxyl, Ridomil Gold MZ, and Infinito (for stage II); and Infinito, Shirlan, and Acrobat MZ (for stage III).

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Wild Tuber Bearing Solanum Species screening for Late Blight Resistance under natural conditions

PELLÉ ROLAND, KERMARREC MARIE-PAULE AND CHAUVIN JEAN-ERIC

UMR APBV, INRA Agrocampus Rennes,
Université Rennes 1, Keraïber 29260 Ploudaniel, France

Posters

Wild Tuber Bearing *Solanum* Species screening for Late Blight Resistance under natural conditions

Pellé Roland, Kermarrec Marie-Paule and Chauvin Jean-Eric
UMR APBV, INRA Agrocampus Rennes, Université Rennes 1, Keraïber 29260 Ploudaniel, France

Introduction

The INRA *Solanum* wild relative species collection maintained in Ploudaniel (West Brittany—France) is composed of 26 species represented by 863 clones. Those accessions introduced since the seventies has never been tested for the Late Blight resistance. In 2004 we started to screen for foliage resistance under semi-natural conditions. The idea is to detect new unexploited sources of resistance to *Phytophthora infestans* which could be then introduced in the cultivated *S. tuberosum* germplasm. At this moment, about half of our collection has been tested.

Material and Methods

Solanum polytrichon

Solanum berthaultii

- Plants grown in 5 liters containers (50% peat, 35 % sand and 15% pine bark)on a concrete area. Planting date was in the mid-May.
- 2 years of testing :
  - Year 1 : 2 replicates for each clone ;
  - Year 2 : a randomised block design with 4 blocks and one replicate per clone in each block. In the second year, were experimented only the clones that were detected resistant during the first year.
- Natural infection by local strains of *P. infestans* (Virulence characterised by Black's differentials R1 to R11 included in the expérimental design (Table 1)).
- Spreader plants of cv « Bintje » include to ensure a reliable source of inoculum during the epidemic.
- Six additional controls to ensure the reliability of the evaluation method (Arka, Alpha, Eerstelling, Robijn and Gloria).
- Plants watered by dripping water and sprinklers as often as necessary.
- Disease scored weekly as soon as the first late blight attack was observed according to B.M.S. scale (James, 1971) during 6 to 10 weeks according to year conditions.
- For each plot calculation of :
  - the Area Under Disease Progress Curve (AUDPC) ;
  - the delay between the first visible symptoms on the tested genotype and the susceptible control cultivar « Bintje » ;
  - the slope of the logarithmic transformation of the DPC.

Results

Table 1 : Observed virulences of the local strains of P. infestans in the expérimental design from 2004 to 2008.

Year	r	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
2004	NT	+	+	+	+	+	-	+	+	-	NT	+	+
2005	+	+	+	+	+	-	+	+	+?	-	+	+
2006	+	+	+	+	+	-	+	+	-	-	+	+
2007	+	+	+	+	+	-	+	+	+?	-	+	+
2008	+	+	+	+	+	+	+	+	+?	-	+	+

NT = not tested   - = uninfected
(red) = infected   +? = uncertain symptoms

Among the 362 screened clones belonging to 19 species, 71 clones (20%) representing 13 species proved to have high or intermediate level of *P. infestans* resistance (Table 2). Both R-genes and quantitative resistance have been described in these sources, according to DPC shape.

Table 2 : Number of tested clones for each species, number of resistance sources detected, with indication of probable type of resistance.

Plant Species	Number of clones tested for resistance	Number of resistant clones	Major R gene	Quantitative resistance
S. acaule	1	0		
S. andigna	115	3	Unlikely	Probably
S. berthaultii	11	7	Yes	Probably
S. brachistotrichum	4	2	Yes	Probably
S. bulbocastanum	7	4	Yes	Probably
S. cardiophyllum	3	0		
S. chacoense	88	5	No	Probably
S. fendleri	1	0		
S. gourlayi	8	0		
S. hougasii	5	5	Yes	probably
S. kurtzianum	1	0		
S. phureja	34	11	No	yes
S. polytrichon	11	10	Unlikely	yes
S. sparsipilum	12	1	No	yes
S. spegazzinii	2	0		
S. stenotomum	26	2	Probably	probably
S. stoloniferum	17	17	Yes	probably
S. tarijense	13	2	Unlikely	yes
S. trifidum	3	2	Yes	probably
Total	362	71		

Discussion—Perspectives

Some crosses between detected resistant clones and *S. tuberosum* at the diploid level produced a sufficient number of seeds to progress in introduction of resistance to late blight in cultivated potato ; it is the case for *S. berthaultii*, *S. bulbocastanum*, *S. chacoense*, *S. hougasii*, *S. polytrichon*, *S. stenotomum* and *S. tarijense*. Unfortunately, for *S. trifidum*, *S. brachistotrichum* and *S. stoloniferum* there was no success with the attempted crosses. However, one solution could be to use bridge species to be able to exploit these new resistant sources.

Efficacy of different fungicides for the control of early blight

B. HEREMANS, L DE LANGE, J. CARRETTE AND G. HAESAERT

University College Ghent, Faculty of Biosciences and landscape Architecture,
Voskenlaan 270, 9000 Ghent, Belgium

Posters

Efficacy of different fungicides for the control of early blight

Heremans B., De Lange L., Carrette J. & Haesaert G.

University College Ghent, Faculty of Biosciences and Landscape Architecture, Voskenslaan 270, 9000 Ghent, Belgium

Introduction

Early blight caused by two species of the genus *Alternaria* occurs commonly worldwide on potato crops and other Solanaceae. *A. solani* and *A. alternata* are destructive pathogens, particularly in regions with high temperature and alternating dry and high humidity periods. Early blight results in premature dying of foliage and yield losses.
Early blight was normally controlled by fungicide treatments against *Phytophthora infestans*, but in the last years the disease gained in importance. This change is due to several reasons: reduction of nitrogen supply to the crop, climatic change, the growing of more susceptible potato varieties and the use of new fungicides against late blight with less efficacy against early blight. In recent years some specific fungicides against early blight were developped.
In this study the efficacy of several fungicides was tested against *A. solani* and *A. alternata* in *in vitro* tests.

Materials and methods

Isolates of *Alternaria* species were collected in Flanders, Belgium at the end of the growing season of 2009. Two *A. alternata* and two of *A. solani* isolates were included in this study, as well as a German isolate for both *Alternaria* species.
Isolates of *A. solani* and *A. alternata* were maintained on potato dextrose agar (PDA). Plugs of one week old fungal mycelium were inoculated on PDA containing different fungicides in different doses. The tested fungicides are summarized in table 1. Fungicides were applied at 3 doses: dose recommended for field application and a 10 and 100 times lower dose. Criterium for assessment was the colony diameter whereby the different fungicide treatments were compared to the control (% growth after 10 days).

Results and Discussion

The tested fungicides showed differences of efficacy in controlling the two *Alternaria* species (Fig 1 and 2). *A. alternata* 101 and 103 were more sensitive to the fungicides tested than the German isolate and than the *A. solani* isolates.
Azoxystrobine (Amistar) and boscalid plus pyraclostrobine (Terminett) were developped for the control of *Alternaria* species in potatoes. Azoxystrobine (Amistar) and boscalid plus pyraclostrobine (Terminett) completely inhibited the mycelium growth of *A. alternata* 101 and 103. Nevertheless, an efficiency of 70 % and 50 % was observed for respectively the field dose of boscalid plus pyraclostrobine (Terminett) and azoxystrobine (Amistar) on the German isolate of *A. alternata*. For azoxystrobine (Amistar) a mean inhibition of 32 % was observed for the *A. solani* isolates. Boscalid plus pyraclostrobine (Terminett) completely controlled the growth of the tested *A. solani* isolates.
The other fungicides tested are fungicides used to control late blight in potatoes. These fungicides controlled very well *A. alternata*. Only the treatment whereby the field dose was reduced 100 times was less efficient on the German isolate: the efficacy fluctuated between 18 and 74 % with a mean efficiency of 61 %. The field dose of these fungicides completely inhibited the growth of all the *A. solani* isolates tested. The Belgian isolates were less sensitive to the lower doses of these fungicides then the German isolate. The efficiency of the 10 times lower dose fluctuated between 32 and 100 %. The efficiency of the 100 times lower dose fluctuated between 27 and 58 %.

Table 1: Dose of the tested fungicides

Product	Active ingredient		Dose product	Dose active ingredient
Amistar	azoxystrobine	250 g/l	0,25 l/ha	62,5 g
Terminett	boscalid	267 g/kg	0,2 kg/ha	53,4 g
	pyraclostrobine	67 g/kg		13,4 g
Dequiman	mancozeb	750 g/kg	2,1 kg/ha	1575 g
UnikatPro	mancozeb	667 g/kg	1,5 kg/ha	1000,5 g
	zoxamide	83 g/kg		124,5 g
Acrobat Extra	dimethomorf	75 g/kg	2 kg/ha	150 g
	mancozeb	667 g/kg		1334 g
Curzate M	cymoxanil	45 g/kg	2 kg/ha	90 g
	mancozeb	680 g/kg		1360 g
Sereno	fenamidone	100 g/l	1,25 kg/ha	125 g
	mancozeb	500 g/l		625 g
Tanos	famoxadone	250 g/kg	0,5 kg/ha	125 g
	cymoxanil	250 g/kg		125 g
Valbon	benthiavalicarb	17,5 g/kg	1,6 kg/ha	28 g
	mancozeb	700 g/kg		1120 g
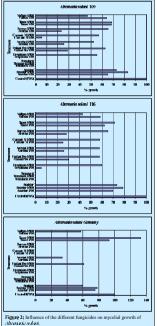
Conclusion

Fungicides may be less efficient on different isolates in an *Alternaria* population and *Alternaria* isolates may develop resistance to some azoxystrobine.

Aknowledgements

Figure 1: Influence of the different fungicides on mycelial growth of *Alternaria alternata*.

Figure 2: Influence of the different fungicides on mycelial growth of *Alternaria solani*.

Modelling the effects of spatially distributed cropping systems on the epidemics of potato late blight and on the durability of cultivar resistances

TOKY RAKOTONINDRAINA [1], JEAN-ÉRIC CHAUVIN [2], ROLAND PELLE [2],
CATHERINE CHATOT [3], FRÉDÉRIQUE AUROUSSEAU [3], JEAN-NOËL AUBERTOT [1]

[1] UMR AGIR INRA / INP Toulouse – ENSAT,
BP 52627, 31326 Castanet Tolosan Cedex, France;
[2] UMR APBV INRA / Agrocampus ouest / Université Rennes 1,
Domaine de Kéraïber, 29260 Ploudaniel, France;
[3] ACVNPT(Association des Créateurs de Variétés Nouvelles de Pomme de Terre)

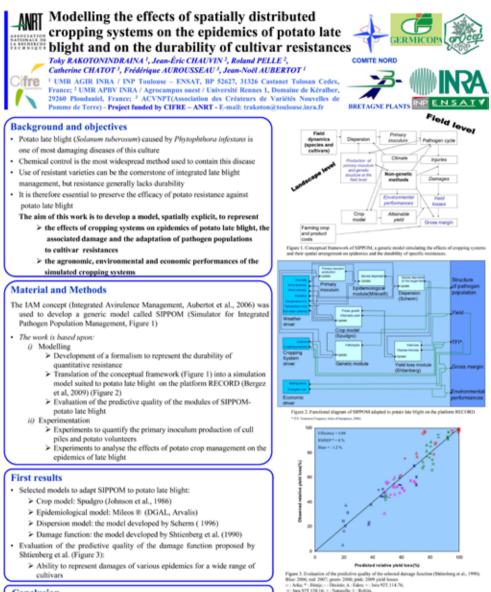# Modelling the effects of spatially distributed cropping systems on the epidemics of potato late blight and on the durability of cultivar resistances

Toky RAKOTONINDRAINA [1], Jean-Éric CHAUVIN [2], Roland PELLE [2], Catherine CHATOT [3], Frédérique AUROUSSEAU [3], Jean-Noël AUBERTOT [1]

[1] UMR AGIR INRA / INP Toulouse – ENSAT, BP 52627, 31326 Castanet Tolosan Cedex, France; [2] UMR APBV INRA / Agrocampus ouest / Université Rennes 1, Domaine de Kéralber, 29260 Ploudaniel, France; [3] ACVNPT(Association des Créateurs de Variétés Nouvelles de Pomme de Terre) - **Projet funded by CIFRE – ANRT** - E-mail: trakoton@toulouse.inra.fr

Background and objectives

- Potato late blight (*Solanum tuberosum*) caused by *Phytophthora infestans* is one of most damaging diseases of this culture
- Chemical control is the most widespread method used to contain this disease
- Use of resistant varieties can be the cornerstone of integrated late blight management, but resistance generally lacks durability
- It is therefore essential to preserve the efficacy of potato resistance against potato late blight

  The aim of this work is to develop a model, spatially explicit, to represent
  - ➤ the effects of cropping systems on epidemics of potato late blight, the associated damage and the adaptation of pathogen populations to cultivar resistances
  - ➤ the agronomic, environmental and economic performances of the simulated cropping systems

Material and Methods

The IAM concept (Integrated Avirulence Management, Aubertot et al., 2006) was used to develop a generic model called SIPPOM (Simulator for Integrated Pathogen Population Management, Figure 1)

- *The work is based upon:*
  - i) Modelling
    - ➤ Development of a formalism to represent the durability of quantitative resistance
    - ➤ Translation of the conceptual framework (Figure 1) into a simulation model suited to potato late blight on the platform RECORD (Bergez et al, 2009) (Figure 2)
    - ➤ Evaluation of the predictive quality of the modules of SIPPOM-potato late blight
  - ii) Experimentation
    - ➤ Experiments to quantify the primary inoculum production of cull piles and potato volunteers
    - ➤ Experiments to analyse the effects of potato crop management on the epidemics of late blight

First results

- Selected models to adapt SIPPOM to potato late blight:
  - ➤ Crop model: Spudgro (Johnson et al., 1986)
  - ➤ Epidemiological model: Mileos ® (DGAL, Arvalis)
  - ➤ Dispersion model: the model developed by Scherm (1996)
  - ➤ Damage function: the model developed by Shtienberg et al. (1990)
- Evaluation of the predictive quality of the damage function proposed by Shtienberg et al. (Figure 3):
  - ➤ Ability to represent damages of various epidemics for a wide range of cultivars

Conclusion

- SIPPOM late blight will help in designing strategies for integrated, collective and durable management of potato late blight
- The created tool will help in identifying appropriate ideotypes to limit the risk of potato late blight and to enhance the epidemiological modelling of *Phytophthora infestans* life cycle

EUROBLIGHT – A potato late blight network for Europe, Arras, France 3-6 May 2010

Figure 1. Conceptual framework of SIPPOM, a generic model simulating the effects of cropping systems and their spatial arrangement on epidemics and the durability of specific resistances.

Figure 2. Functional diagram of SIPPOM adapted to potato late blight on the platform RECORD.

Figure 3. Evaluation of the predictive quality of the selected damage function (Shtienberg et al., 1990).

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Phenotypic variation within a clonal lineage of *Phytophthora infestans* from Nicaragua

U. BLANDÓN-DÍAZ[1,2], A-K. WIDMARK[2], A. HANNUKKALA[3], N. HÖGBERG[2], J.E. YUEN[2]

[1]Universidad Nacional Agraria, Managua, Nicaragua. Apdo 453;
[2]Swedish University of Agricultural Sciences, Dept of Forest Mycology and Pathology, PO Box 7026, S-750 07 Uppsala, Sweden;
[3]MTT Agrifood Research Finland, Plant Production Research, FI-31600 Jokioinen, Finland.

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Phenotypic variation within a clonal lineage of *Phytophthora infestans* from Nicaragua

U. Blandón-Díaz[1,2], A-K. Widmark[2], A. Hannukkala[3], N. Högberg[2], J.E. Yuen[2]

[1]Universidad Nacional Agraria, Managua, Nicaragua. Apdo 453; [2]Swedish University of Agricultural Sciences, Dept of Forest Mycology and Pathology, PO Box 7026, S-750 07 Uppsala, Sweden; [3]MTT Agrifood Research Finland, Plant Production Research, FI-31600 Jokioinen, Finland.

Introduction

Late blight caused by the fungus-like oomycete *Phytophthora infestans* (Mont.) De Bary is one of the main constraints affecting both potato and tomato crops in the northern highlands of Nicaragua (Figure 1). The main objective of this study was to assess comparatively the genotypic and phenotypic variation of *Phytophthora infestans* isolates collected in potato and tomato growing areas.

Figure 1. Potato experimental plot planted with the susceptible cultivar CalWhite apparently healthy (A) and two weeks later under favourable conditions for potato late blight development (B)

Materials and Methods

Sampling of *Phytophthora infestans* (Figure 2).

Figure 2. Single lesion isolates were collected in three northern departments of Nicaragua from 2007 to 2010. In each department many sites were sampled.

Genotypic analysis

• Simple sequence repeats (SSR) markers: Pi4B, PiG11, Pi16, Pi70, PiD13, Pi63 and Pi04 (Knapova and Gisi, 2002; Lees et al., 2006).
• Mitochondrial DNA (mtDNA) haplotyping (Griffith and Shaw, 1998).

Phenotypic analysis

• Mating type determination (conventional pairing)
• Virulence testing and fungicide sensitivity were done as described by Lehtinen et al., 2008. Mean number of virulence factors per isolate (Ci) and pathotype (Cp) were calculated as described by Andrivon (1994).

Results

From 2007 to 2010, 248 isolates of *P. infestans* were collected. All of them were tested for mating type, 132 isolates were used for genotypic analysis and 98 were used for virulence and fungicide testing. SSR genotyping revealed no polymorphism among tested isolates of *P. infestans*. Mitochondrial DNA haplotyping detected the Ia haplotype (Table 1).

Table 1. Alleles detected by 7 SSR markers and mtDNA haplotype of *Phytophthora infestans* isolates collected in Nicaragua.

	SSR markers							mtDNA
Loci	4B	G11	Pi16	Pi70	D13	Pi63	Pi04	
Alleles	205	122	176	162	86	148	166	Ia
	213	156	178	162	107	157	170	

Ninety-eight percent of the isolates were resistant to metalaxyl, 1% intermediate and 1% sensitive, while 82% sporulated in propamocarb-HCl at 10 mg.l^{-1}, 28% sporulated in propamocarb-HCl at 100 mg.l^{-1} and no isolate sporulated at 1000 mg.l^{-1} (Figure 3).
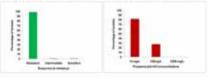
Figure 3. Response of *Phytophthora infestans* isolates from Nicaragua to metalaxyl and propamocarb-HCl.

The virulence testing showed a high variation among isolates. A total of 37 different races were identified. The percentage of isolates overcoming R-genes varied. Only one isolate overcame R9 and no one overcame R8 (Figure 4). The Ci and Cp were 6.2 and 5.4 respectively.

Figure 4. Percentages of *P. infestans* isolates overcoming R-genes

References

Conclusions

• Nicaraguan population of *P. infestans* is dominated by a clonal lineage (based on 7 SSR markers) that has the A2 mating type and the Ia haplotype
• The virulence spectrum within this clonal lineage is highly variable.

Marker-assisted selection of QTL *PiXspg* in potato diploid backcross populations

FLORENCE ESNAULT, ROSANNE QUELENNEC, JEAN-PAUL DANTEC, STEPHANE MAUGER & JEAN-ERIC CHAUVIN

INRA UMR APBV, Domaine de Keraïber, 29260 Ploudaniel, France

SUMMARY

Using a stem assay, a major QTL originating from the wild potato relative *S. spegazzinii* has been identified and mapped to chromosome X. Named *PiXspg*, it explains between 30 to 40% of the phenotypic variation for the stem resistance component. A fine mapping of *PiXspg* has been carried out, leading to the development of two SSR markers (SSR223 and SSR74) and three CAPS markers (P10c8, TG403F1 and P8h11) that are closely linked to the QTL. The usefulness of these markers in a marker-assisted selection program has been evaluated in four diploid backcross populations. A good correlation between the phenotypic data and the genotypic data has been observed for three backcross populations. However, as the presence of *PiXspg* is not always correlated with stem resistance, it is likely that other genomic regions and/or epistatic interactions are involved in the expression of this trait.

KEYWORDS

Potato, Late blight resistance, Quantitative Trait Loci, *S. spegazzinii*, Marker-assisted selection

INTRODUCTION

As most of the R-genes identified in *Solanum* wild species have been overcome by *Phytophthora infestans*, the UMR APBV team aims at identifying and studying quantitative trait loci (QTL) involved in late blight resistance.

A segregating population (96D32) obtained by crossing a susceptible dihaploid *S. tuberosum* clone (Rosa H1) with a resistant clone belonging to the wild potato relative *S. spegazzinii* has been studied for late blight resistance using a stem assay. A major QTL originating from the wild species has been identified. Using genetic map developed by Caromel (2004), this QTL has been mapped to chromosome X and is named *PiXspg*. It explains between 30 to 40% of the phenotypic variation for the stem resistance component. A fine mapping of *PiXspg* has been carried out, leading to the development of two SSR markers (SSR223 and SSR74) and three CAPS markers (P10c8, TG403F1 and P8h11) that are spread over a 15 cM region including the QTL (Quélennec *et al*., 2009). The objective of this study is to evaluate the usefulness of these markers in a marker-assisted selection program.

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MATERIALS AND METHODS

Among the 96D32 population, four clones (96D.32.6, 96D.32.26, 96D.32.66 and 96D.32.124) which carry *PiXspg* and show a good level of resistance in field assay were selected. They were crossed with a susceptible dihaploid *S. tuberosum* (Caspar H3), leading to the obtention of four backcross populations (06D.23, 06D.24, 06D.27 and 06D.29 respectively). These populations, which comprise 137, 149, 136 and 140 clones respectively, were evaluated for stem resistance using a stem assay as described by Danan (2009). The following variables were studied: L32 (Necrosis length 32 days after inoculation), REC (Receptivity) and IND (Inductibility).

The parents and 6 clones of each of these populations were genotyped with the SSR markers SSR223 and SSR74 (SGN database) and the CAPS markers P10c8/TaqI, TG403F1/CfoI and P8h11/HaeIII. When the markers were polymorphic, they were used on the entire population. For the CAPS markers, specific primer pairs were designed based on the DNA sequence available in the PoMaMo database using Primer3 program. PCR amplifications were performed in a PTC200 thermal cycler (Bio-Rad).

Concerning the SSR markers, each 10µl PCR reaction volume contained 1X buffer (Promega), 2 mM MgCl2, 150 µM dNTP, 0.1 µM M13-tailed forward primer, 0.2 µM reverse primer, 0.1 µM IRD 700-labeled M13 tail, 0.35 U Taq DNA polymerase (Promega) and 20 ng of genomic DNA as template. The cycling protocol consisted of an initial denaturation step at 94°C for 4 min, then 12 cycles of denaturation for 30 s at 94°C, annealing for 1 min from 65°C to 54°C (-1°C per cycle) and extension for 30 s at 72°C, then 25 cycles of denaturation for 30 s at 94°C, annealing for 1 min at 53°C and extension for 30 s at 72°C, followed by a final 10 min extension step at 72°C. Fluorescence labeled fragments were separated on a LI-COR DNA Analyser using 5.5% acrylamide gels.

Concerning the CAPS markers, each 17µl PCR reaction volume contained 1X buffer (Promega), 2 mM MgCl2, 150 µM dNTP, 0.3 µM of each of the primers, 0.03 U Taq DNA polymerase (Promega) and 20 ng of genomic DNA as template. The cycling protocol consisted of an initial denaturation step at 94°C for 3 min, then 30 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 55°C and extension for 1 min 30 s at 72°C, followed by a final 10 min extension step at 72°C. After digestion with restriction enzymes, ethidium bromide-stained PCR fragments were visualized on 1.5%-agarose gels.

Goodness-of-fit between observed and expected segregation ratios at marker loci was tested by a chi-square analysis. A one-way analysis of variance (GLM procedure, SAS software) was used to test the correlation between the phenotype and the genotype.

RESULTS AND DISCUSSION

As the CAPS marker P10c8/TaqI is linked to the susceptible allele of the *S. spegazzinii* parent, it can not be used in backcross populations. The CAPS marker TG403F1/CfoI was monomorphic in the four backcross populations. The CAPS marker P8h11/HaeIII was polymorphic in two populations: 06D.24 and 06D.27. The two SSR markers were polymorphic in all four populations. According to chi-square tests, the segregation of these molecular markers in the backcross populations did not deviate significantly from to the 1:1 expected ratio (Figure 1).
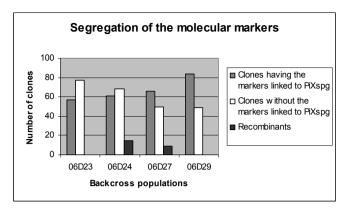
Figure 1: *Segregation of the molecular markers in the backcross populationfor 06D.23 and 06D.29, segregation of SSR74; for 06D.24 and 06D.27, segregation of SSR74 and P8h11*

The analysis of variance showed that in populations 06D.23 and 06D.24, the clones having the markers linked to *PiXspg* have significantly lower values of L32 and IND than the clones without these markers (Table 1). In population 06D.29, this is observed only for L32. In population 06D27, no significant correlation is observed.

In population 06D24, the presence of the markers is associated with lower REC values whereas no QTL for REC was detected in the 96D32 population.

Table 1: *Mean values of L32 (cm), IND (cm/day2) and REC (cm/day) according to the presence or not of the markers linked to PiXspg and results of the ANOVA. ***, **, * : significant at the 0.001, 0.01, 0.05 probability level respectively*

Backcross population	Variable	Clones having the markers linked to PiXspg	Clones without the markers linked to PiXsp$_g$	ANOVA	
				F	R^2
06D23	L32	5.21	7.58	**11.67****	0.06
	IND	0.008	0.017	**8.70****	0.05
	REC	0.07	0.06	0.05	0
06D24	L32	4.25	6.04	**16.36*****	0.12
	IND	0.007	0.012	**2.95***	0.02
	REC	0.05	0.1	**15.88****	0.12
06D27	L32	4.91	5.68	1.7	0.02
	IND	0.008	0.011	0.83	0.01
	REC	0.07	0.07	0.03	0
06D29	L32	6.32	7.47	**4.27***	0.02
	IND	0.013	0.017	2.13	0.01
	REC	0.07	0.07	0.45	0

CONCLUSIONS

In 3 out of the 4 studied backcross populations, the molecular markers linked to *PiXspg* appear to be useful to predict the late blight stem resistance of the clones. The usefulness of these markers will also be evaluated for marker-assisted selection at the tetraploid level.

However, as the presence of *PiXspg* is not always correlated with stem resistance, it is likely that other genomic regions and/or epistatic interactions are involved in the expression of this trait.

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Definition of thresholds for soil moisture to assess zoospore infections by *Phytophthora infestans*

JEANETTE JUNG, MARTIN EBLI & BENNO KLEINHENZ

Central Institution for Decision Support Systems in Crop Protection, Germany

SUMMARY

Potato tubers, which are infected with *Phytophthora infestans*, are able to release sporangia and zoospores in the ambient soil, when temperature and soil moisture are optimal for the fungus. In a field-experiment inoculated potato tubers were buried next to healthy tubers. In this way the possibilities for movements of zoospores through water-filled pores within the soil should be tested. Because of the diameter of zoospores at least all pore sizes smaller than 10 µm have to be filled completely with water. This fact led to a determination of a "limiting infection value" in relation to soil moisture. It was assumed that if soil moisture is below this limit zoospore movements are impossible. In the field-experiment could be seen, that the localisation of the limiting infection value varies among the different irrigated plots. For this reason further studies are required to specify the influence of soil type and maybe other so far unknown soil factors on the localisation of the limiting infection value.

KEYWORDS

Phytophthora infestans, soil moisture, zoospore infections, latent infected tubers, SIMBLIGHT1

INTRODUCTION

The prediction model SIMBLIGHT1 calculates the first appearance of *Phytophthora infestans* in potatoes in fields. These forecasts are based on meteorological data and crop characteristics (Figure 1). Studies done in the past have shown, that a correlation between high soil moisture after planting and early occurrence of *P. infestans* can be assumed. The aim of this current study is to specify the relation between soil characteristics and first appearance of *P. infestans*.

In addition the possibilities of soil water content simulations were tested. Therefore a bucket model was verified with the data collected in this study. Both, the relation between soil characteristics and appearance of *P. infestans* as well as the possibility of simulating the soil water content, should lead to an integration of a soil-module in SIMBLIGHT1.

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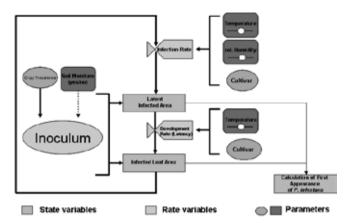
Figure 1: *Structure diagram of SIMBLIGHT1 (Kleinhenz et al. 2007)*

HYPOTHESIS

Potato tubers, which are infected with *P. infestans*, are able to release sporangia and zoospores in the ambient soil, when temperature and soil moisture are optimal for the fungus (Zan, 1962; Lacey, 1967; Sato, 1980; Adler, 2000; Porter, 2005).

With a diameter from up to 36 µm sporangia of *P. infestans* are hardly able to pass through soil pores. Zoospores of *P. infestans* instead have only a diameter of 10 µm (Porter *et al.*, 2005). They have the ability to move through water-filled pores within the soil. In this way zoospores are able to infect potato sprouts from the tuber they arise and neighbour tubers respectively (Figure 2).

Spore movement through soil depends on pore size distribution and size of water-filled pores. Saturation of soil starts within small pore sizes, which have a high water potential. For movement of zoospores all pore sizes smaller than 10 µm have to be filled completely with water.

In years, which offer high soil moisture on the plots over a period of at least 4 days the possibility for movements of zoospores exists. This process increases the risk of an early appearance of *P. infestans* in field. The effect of soil moisture on potato tuber infection due to *P. infestans* was assessed in a field-experiment.

MATERIAL AND METHODS

Inoculated potato tubers were buried next to healthy tubers in a sugar-beet field. It was necessary to do the field experiment surrounded by culture not susceptible to *P. infestans* and in a potato free growing area to avoid *P. infestans* infections from outside the trial plot. Afterwards the potato field was divided into four plots. Each plot consisted of 10 rows with a length form 7 meters planted with potatoes. Inoculated tubers were only buried in the middle of each plot. In this way twenty inoculated tubers were used as inoculum source to infect healthy tubers in every plot. To approve the theory of a higher transfer from zoospores out of an infected seed stock in relation to a longer existence of high soil moisture, each plot was treated with a different number of irrigation-days (8, 4, 2 days of irrigation and one plot with no irrigation – Figure 2). In the irrigated plots 50 litre water per square meter were given each day.

Soil moisture was measured gravimetric every two days. Therefore an undisturbed soil sample was taken with a soil sample ring. In each plot six soil samples were taken. In this way the average soil moisture of each plot could be analysed. In addition the pore size distribution of the plots was analysed weekly. In this way the possibility for movements of zoospores could be analysed.

VP_0	VP_2	VP_4	VP_8
no irrigation	2 days of irrigation (100 mm/m² within 2 days)	4 days of irrigation (200 mm/m² within 4 days)	8 days of irrigation (400 mm/m² within 8 days)
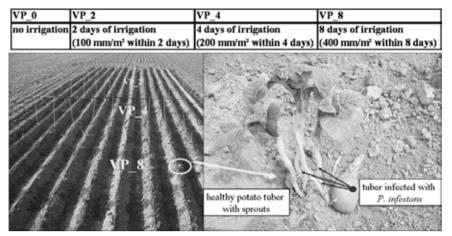
Figure 2: *Arrangement of field-experiment and concept of buried transfer of zoospores from infected potato tubers*

RESULTS AND DISCUSSION

Field experiment

The hypotheses that spore movement through soil depends on pore size distribution and size of water-filled pores led to the determination of a "limiting infection value" in relation to pore size distribution (Figure 3). Water saturation of soil starts within small pore sizes, which have a high water potential. For movement of zoospores all pore sizes smaller than 10 µm have to be filled completely with water. This threshold is identical with the created limiting infection value. For possible movements of zoospores the soil moisture has to be above this border.
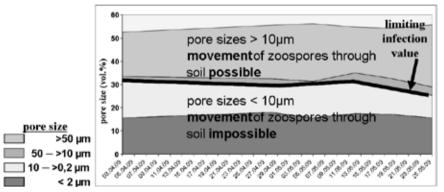
Figure 3: *Determination of the limiting infection value on movement of zoospores from P. infestans through soil*

In the field-experiment it could be seen, that the localisation of the limiting infection value depends on the amount of irrigation. As irrigation leads to consolidation, there must be a dislocation of the limiting infection value. Figure 4 shows the different limiting infection values of each plot. These facts lead to some different effects: The mean soil moisture in the plots differs about 5 % by vol. (Figure 4 – 1.), but in all cases the limiting infection value for the related plot is reached. The mean soil moisture in each plot is nearly identical, but the limiting infection value is only reached in one plot (Figure 4 – 2.).
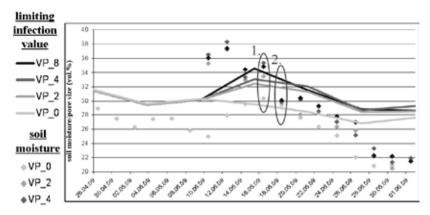
Figure 4: *Comparison of the development of the limiting infection value in relation to soil moisture*

Simulation of soil moisture

Soil moisture was measured directly every two days in a very time-intensive procedure within the field-experiment. A practical solution could be using so called bucket models. They simulate the soil water content with simple and generally available data from weather stations.

A first correlation between field-data and simulation shows promising results. Most of the deviations lie in a range of 5 % by vol. (Figure 5). This is identical with the variability of field-data within a plot.
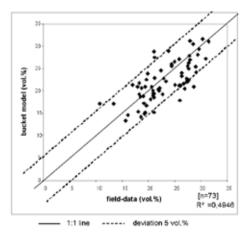
Figure 5: *Correlation of the results of the bucket model with field-data*

CONCLUSIONS

The influence of soil moisture on the limiting infection value could be shown in this field-experiment. Further studies are required to specify the influence of soil type and maybe other so far unknown soil factors on the localisation of the limiting infection value. The verification of the bucket model for the simulation of soil water content showed promising results (deviations were within the range of variations in field). These results build a good fundament for the integration of soil parameters in a soil-module in SIMBLIGHT1.

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Searching among wild *Solanum* species for homologues of *RB/Rpi-blb1* gene conferring durable late blight resistance

ARTEM A. PANKIN[1], EKATERINA A. SOKOLOVA[1], ELENA V. ROGOZINA[2],
MARIA A. KUZNETSOVA[3], KENNETH L. DEAHL[4],
RICHARD W. JONES[4] AND EMIL E. KHAVKIN[1]

[1] Institute of Agricultural Biotechnology, Moscow, Russia
[2] Institute of Plant Industry, St. Petersburg, Russia
[3] Institute of Phytopathology, Bol'shiye Vyazemy, Russia
[4] USDA-ARS, Agriculture Research Center, Beltsville, MD, USA

SUMMARY

Solanum bulbocastanum comprising a CC-NBS-LRR gene *RB/Rpi-blb1* confers broad-spectrum resistance to *Phytophthora infestans* and is currently employed in potato breeding for durable late blight (LB) resistance. Genomes of several *Solanum* species were reported to contain *RB* homologues with confirmed broad-spectrum defence function. With the discovery that novel *P. infestans* races break LB resistance mediated by the genes of broad-spectrum specificity, pyramiding several *RB*-like genes from various *Solanum* species in a single potato cultivar seems a promising approach to durable LB resistance. Here we report early evidence on *RB*-like sequences in the wide range of *Solanum* species section *Petota*. The panel of *Solanum* species was screened with three *RB*-related PCR markers. *RB*-like sequences were found in every tested *Solanum* accession suggesting universal distribution of *RB* structural homologues among *Solanum* genomes, while the marker RB-629 corresponding to the *RB* gene was found in 14 species. The phylogenetic analysis of RB-629 sequences suggested highly conserved pattern of polymorphisms that was neither species- nor series-specific. Apparently, duplication and evolution of *RB*-like loci preceded *Solanum* speciation. Marker presence and particular haplotypes were not immediately associated with high LB resistance.

KEYWORDS

Phytophthora infestans, *Solanum spp.*, late blight resistance, *R* genes, potato

INTRODUCTION

Late blight (LB) caused by *Phytophthora infestans* (Mont.) de Bary is still challenging potato fields around the globe. Disease resistance mediated by the *R* genes is one of the integral elements of the plant immune system. Products of *R* genes directly or indirectly recognise the cognate effector (Avr) which is introduced into the plant cell by the pathogen and induces the hypersensitive response (Dangl and Jones, 2001). Cultivated potato (*Solanum tuberosum* L.) lacks *R* genes active against *P. infestans,* primarily due to the practice of vegetative propagation that excludes natural selection for

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functional *R* loci under recurrent pathogen attacks. In contrast, wild *Solanum* species inhabiting the regions with the most diverse populations of *P. infestans* acquired numerous *R* loci that are functional against LB and are indispensable genetic resources for potato introgression breeding.

The set of eleven *R* genes was identified in the Mexican species *S. demissum* and introgressed into potato varieties. However, such resistance was reportedly defeated in the field by rapidly evolving *P. infestans* races (Fry, 2008; Hein *et al.*, 2009). Nonetheless, the presence of some *demissum R* genes in potato cultivars was explicitly associated with high LB resistance indices (for more details see Khavkin *et al.* in this issue). Several QTLs and genes for LB resistance have been mapped on the linkage groups of various wild *Solanum* species (Hein *et al.*, 2009). A cluster of four resistance gene analogues (RGAs) located on chromosome 8 of *S. bulbocastanum* was cloned, and *RGA2* (*RB*/*Rpi-blb1*) was shown to confer LB resistance in both transient and stable expression systems (Song *et al.*, 2003; van der Vossen *et al.*, 2003). Potato transformation with *RB* homologues isolated from *S. bulbocastanum* (*Rpi-bt1*), *S. stoloniferum* (*sensu* Spooner *et al.*, 2004; *Rpi-sto1, Rpi-pta1*), and *S. verrucosum* (*RB^{ver}*) confirmed specificity of these genes against a broad spectrum of *P. infestans* races (Liu and Halterman, 2006; Vleeshouwers *et al.*, 2008; Oosumi *et al.*, 2009). Recently, *P. infestans* races lacking Avr effectors compatible with RB ligand and thus virulent on potato plants transformed with *RB* have been identified (Champouret *et al.*, 2009; Förch *et al.*, 2010; Halterman *et al.*, 2010). From the breeding prospect, many wild *Solanum* species exhibiting high levels of LB resistance cannot be crossed with *S. tuberosum* by conventional breeding methodologies. Thus, cloning and functional characterisation of the genes underlying broad-spectrum LB resistance would promote immediate exploitation of wild *Solanum* germplasms in potato breeding. Pyramiding, by cisgenesis, in potato genome broad-spectrum *R* genes from various sources with different specificity to pathogen races and with additive effect is a promising approach to durable LB resistance of potato cultivars (Tan *et al.*, 2010).

In the present study, we employed an effective and efficient allele mining approach to demonstrate the universal distribution and diversity of *RB*-like candidate *R* genes within wild *Solanum* germplasm. We revealed conserved patterns of polymorphisms specific for paralogous *RB*-like loci rather than for *Solanum* species and tentatively suggest that *RB* homologues duplicated and diverged preceding *Solanum* speciation.

MATERIAL AND METHODS

Plant material and DNA isolation

Seeds of wild *Solanum* species were obtained from The Centre for Genetic Resources (CGN), the Netherlands, NRSP-6 Potato Genebank (PI), USA, and The Vavilov Institute of Plant Industry (VIR), Russia. Genomic DNA was isolated from individual plants of 134 accessions representing 18 wild *Solanum* species, section *Petota* (Table 1), by modified CTAB isolation (Doyle and Doyle, 1987) and AxyPrep™ Multisource Genomic DNA Miniprep Kit.

SCAR markers design, amplification and cloning

RB-like homologues were amplified from genomic DNA using universal sequence characterised amplified region (SCAR) markers RB-1223 tagging several *RB*-like loci and marker RB-629 specific for the *RB* gene. PCR primers were optimised using the Oligonucleotide Properties Calculator (http://www.basic.northwestern.edu/biotools). Allele-specific PCR primers 1 and 1' recognising functional allele of *S. bulbocastanum RB* (Colton *et al.*, 2006; RB-226) were modified to increase reaction specificity. The amplification reactions contained 1 µl of 10x PCR buffer, 100-150 ng of

genomic DNA, 1 μl 2.5 mM dNTP, 10 pmol each of two primers, 1 U of either *Pfu* DNA polymerase (Fermentas) for cloning) or *Taq* DNA polymerase (Syntol) for screening and sterile water to a volume of 10 μl and were run in an MJ PTC-200 thermocycler (Biorad). PCR products were separated by electrophoresis in 1.5% w/v agarose and stained with ethidium bromide. Amplified fragments were cloned using InsTAclone™ and CloneJET™ PCR Cloning Kits (Fermentas) and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI 3730 DNA Analyzer (Applied Biosystems).

Phylogenetic analysis of RB-629 sequences
DNA sequences were analysed using BLAST 2.2.23 (Altschul *et al.*, 1990), Lasergene 6.0 (DNAStar), and ExPASy Translate tool (http://www.expasy.org). Cluster analysis was performed using Maximum likelihood, Neighbor-joining and Maximum parsimony algorithms implemented in Phylip 3.69 (Felsenstein, 1989).

LB resistance assays
LB resistance scores of individual *Solanum* plants were independently determined in the laboratory and field trials at the Institute of Phytopathology and at the Institute of Plant Industry (for the protocols see Rogozina *et al.*, this issue).

RESULTS AND DISCUSSION

Comparative analysis of *RB/Rpi-blb1* homologues exposed conserved structure of exonic regions (over 90% identity) and dramatically polymorphic introns supposedly diverged already after duplication of the *RB*-like loci.
Based on discovered polymorphisms, the functionally active *RB*-like loci can be provisionally arranged into three distinct groups: *RB*-group (*RB*, *Rpi-blb1*, *Rpi-sto1*, *Rpi-pta1*), *RB^{ver}*-group and *Rpi-bt1*-group. Apparently, these groups represent orthologous loci, which emerged from the different *RB*-like paralogues duplicated in ancient *Solanum* genotypes and independently acquired defence function against LB under the selective pressure of the pathogen invasion events following *Solanum* speciation.

In order to investigate the distribution of *RB*-like genes in the wild *Solanum* germplasm, three SCAR markers were designed: RB-1223 tagging all three groups of *RB*-like loci, RB-629 specific for *RB*-group and allele-specific RB-226 (Fig. 1). Marker RB-1223 was used to screen 19 accessions representing 11 species (*S. bulbocastanum*, *S. cardiophyllum* ssp. *ehrenbergii*, *S. demissum*, *S. hjertingii*, *S. hougasii*, *S. iopetalum*, *S. pinnatisectum*, *S. polyadenium*, *S. polytrichon*, *S. stenophyllidium*, *S. stoloniferum* and *S. verrucosum*). This marker was universally present in every tested accession, suggesting ubiquitous distribution of the *RB* homologues in *Solanum* genomes. The RB-1223 marker was present in several copies (1-3 copies per accession) and greatly varied in size (~800 to 1300 bp). Sequencing experiments showed that polymorphic bands of this marker in various *Solanum* accessions corresponded to paralogous *RB*-like loci. The observed variation in size was mainly due to the polymorphisms in the intron (Pankin *et al.*, unpublished data).
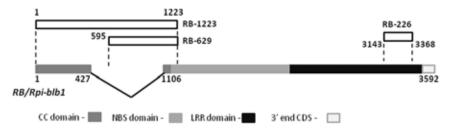
Figure 1. *Schematic structure of the RB/Rpi-blb1 gene and locations of SCAR markers. Numbers are nucleotide positions in base pairs (bp) in relation to the full-length RB/Rpi-blb1 gene sequence (AY426259, 3528 bp). Lines angled downwards indicate the position of 680-bp long intron.*

The panel of the 134 accessions of 18 *Solanum* species was screened with the locus-specific RB-629 and allele-specific RB-226 markers. RB-629 was present in 54% of accessions representing 14 species, whereas allele-specific RB-226 recognising characteristic 18-bp long indel was found only in 7% of accessions from five species (Table 1). Our data suggest much wider distribution of *RB*-group loci in *Solanum* germplasm than reported earlier (14 species vs. two species reported by Wang *et al.*, 2008 and four species reported by Lokossou *et al.*, 2010). In addition to *S. bulbocastanum*, *S. cardiophyllum* subsp. *cardiophyllum*, and *S. stoloniferum* reported by Lokossou *et al.* (2009), three more species *S. caridiophyllum* subsp. *ehrenbergii*, *S. jamesii* and *S. pinnatisectum* were found to contain marker RB-226. It is remarkable that we found RB-226 attributed to the functionally active *S. bulbocastanum RB* allele (Colton *et al.*, 2006) both in resistant and susceptible *Solanum* accessions including *S. bulbocastanum*. It follows that RB-226 cannot be universally used to discern the active *RB* allele even in *S. bulbocastanum* accessions.

Table 1. *Results of the screening of Solanum germplasm with RB-group specific SCAR markers RB-629 and RB-229.*

Specific amplification		*Solanum* accessions
RB-629	RB-226	
+	+	**blb** PI 243510, 275198, VIR 21266-432, 23174-510; **ehr** *VIR 24373-425 (ehr)*; **jam** *VIR 15203-349 (jam1)*; **pnt** VIR 19328-362; **sto** PI 365401, VIR 24263
+	-	**blb** PI 243506, 243508, 243509, 243512, VIR 21274-509-1, 21274-509-2, 23181-511; **bst** VIR 23201, 23201-344, *24197-345 (bst)*; **crd** VIR 4464, *16828-390 (cph)*, 23030-410; **dms** PI 161366, 175408, 230487, *VIR 15173 (dms)*, 23315-277; **ehr** VIR 21301, 23276, 24572-520-2; **fen** *VIR 23335-286 (fen)*, 23841, 24221; **hjt** PI 283103, *VIR 15194-291 (hjt2)*, *21409 (hjt1)*, 24223; **hou** *VIR 8818 (hou)*, 8818-278; **jam** PI 612455, *VIR 22619-351 (jam2)*, 24397, 24397-359; **pld** *VIR 23546-532 (pld2)*, *23553-334 (pld1)*; **pnt** PI 186553, 275234, VIR 19327, 21955, *21955-363 (pnt1)*, 24239, *24239-367 (pnt2)*, *24243-368 (pnt3)*, 24415, 24415-370; **pta** VIR 21547, 24417; **plt** VIR 16905, *23556-315 (plt1)*, 23561-316, 24298-318, 24410, 24462-321, *24463-322 (plt2)*; **sph** PI 320265, *VIR 24255-380 (sph)*; **sto** CGN 17606, PI 161178, 205510, 230490, 255533, 255534, 310964, 338621, VIR 3360, 3336-324, 18925, 19196-325, 21618-327, 23652, 23652-329, 24420-330
-	-	**bcp** VIR 2830-273; **blb** PI 255516, 275191, 275200, VIR 19981-431, 21274-509-3, 23181-511-1, 23181-511-2; **bst** VIR 20105-378, 24197; **crd** VIR 24375-426; **dms** PI 160221, 161167, 161176, 175404, 186552, 218047, 275211, VIR 15174, 18521-275; **ehr** PI 255520, VIR 18086, 18224, 18225, 23277, 23279-414, 24206-419, 24207-420, 24572-520-3; **fen** VIR 24218-288; **hjt** VIR 19276-293, 24387; **iop** PI 230459; **jam** VIR 15203, 23397-352, 23398-353, 23399-354; **pld** PI 275237, 275238; **plt** VIR 8815, 24463; **pnt** PI 275233, VIR 4213; **pta** VIR 8816, 16889; **trn** VIR 23936-381; **sto** CGN 17607, PI 558477; **ver** PI 365404, VIR 23015-555, 23760-556

RB-629 was cloned from 16 accessions representing 12 *Solanum* species (Table 1). The phylogenetic analysis of RB-629 sequences produced four distinct clusters: cluster 1 of *S. bulbocastanum*-like haplotypes; cluster 2 comprising pseudogenes, except one *pinnatisectum* RB-629 fragment (pnt2); cluster 3 specific for *S. polytrichon;* and cluster 4 combining other *RB*-group sequences with open reading frame (Fig. 1). Remarkably, the Maximum likelihood, Neighbor-joining and Minimum parsimony algorithms when applied to the same dataset produced congruent trees with high bootstrap values, thus suggesting high reliability of the revealed clustering.

Abbreviations: *Solanum* species (Hawkes *et al.*, 1994) - *S. brachycarpum* Correll (bcp), *S. brachistotrichum* (Bitter) Rydb. (bst), *S. bulbocastanum* Dunal (blb)*, S. cardiophyllum* John Lindley (cph), *S. cardiophyllum* ssp. *ehrenbergii* Bitter (ehr), *S. demissum* Lindl. (dms), *S. fendleri* (fen), *S. hjertingii* Hawkes (hjt), *S. hougasii* Correll (hou), *S. iopetalum* (Bitter) Hawkes (iop), *S. jamesii* Bitter (jam), *S. papita* Rydb. (pta), *S. pinnatisectum* Dunal (pnt), *S. polyadenium* Greenm. (pld), *S. polytrichon* Rydb. (plt), *S. stenophyllidium* Bitt. (sph), *S. stoloniferum* Schltdl. (sto), *S. tarnii* Hawkes & Hjert. (trn), *S. verrucosum* Schtdl. (ver). Genbanks: CGN – The Centre for Genetic Resources, the Netherlands, PI - NRSP-6 Potato Genebank, USA and VIR – the Vavilov Institute of Plant Industry, Russia. Accession numbers with sequenced RB-629 marker and corresponding sequence names in parentheses as shown in Fig. 1 are italicised. Resistant accessions (R and MR) are highlighted in grey. Accessions without LB resistance scores are underlined.

The described pattern of polymorphisms was neither species- nor series-specific. Therefore, we suggest that the observed diversity of *RB*-group loci emerged before *Solanum* speciation. Apparently, each cluster combines allelic variants of *RB* orthologues whereas inter-cluster polymorphisms were indicative of different *RB* loci. Despite the defence function against LB unequivocally demonstrated in complementation experiments with *RB* genes (Song *et al.*, 2003; van der Vossen *et al.*, 2003; Vleeshouwers *et al.*, 2008), the presence and polymorphisms of *RB* sequences in various *Solanum* species was not immediately associated with higher LB resistance. Apparently, *RB*-like genes duplicated in *Solanum* genomes are of ancient origins (van der Vossen *et al.*, 2003). Defence function of *RB* orthologues could emerge after speciation independently in various *Solanum* species, under the selective pressure of the pathogen, as they spread over the Americas. Another model explaining abundance of non-functional *RB* loci in *Solanum* species is the loss of function of either *RB* genes due to the frame-shifting nucleotide mutations or any downstream elements involved in the signalling cascade of the defence response.

Redundant copies of *RB*-like paralogues apparently serve as a backup pool essential to the adaptive evolution of *R* gene-related pathogen recognition when *Solanum* species respond to novel races of pathogen (for review see Hubert *et al.*, 2001).
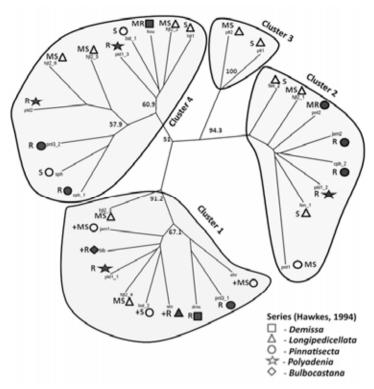
Figure 2. *Phylogenetic analysis (Maximum likelihood) of the RB fragments (RB-629).*
'+' – presence of allele-specific RB-226. LB resistance ranks are as follows: S – susceptible, MS – moderately susceptible, MR - moderately resistant, R – resistant. Filled are the pictograms of the resistant accessions. Bootstrapping was performed with 1000 replicates, and values higher than 50% are shown at the nodes. Cluster 1, haplotypes that joined with functional RB/Rpi-blb gene; cluster 2, pseudogenes, except for pnt2 haplotype; cluster 3, S. polytrichon-specific haplotypes; cluster 4, other haplotypes. For the list of sequences refer to Table 1.

CONCLUSIONS

Environmentally-friendly strategies for managing durable LB resistance are partially based on introgressing the *R* genes from wild *Solanum* species into commercial potato cultivars. Gene-specific markers proved to be effective and efficient tools when searching for orthologous *R* genes of broad-spectrum LB resistance, such as *RB/Rpi-blb1*, in wild *Solanum* species. Allele-mining approach helps to screen extensive collections of wild *Solanum* germplasm and to identify prospective candidates for comprehensive cloning experiments and potato introgression breeding for durable LB resistance. Using gene-specific SCAR markers, we found that *RB*-like structural homologues were universally distributed across wild *Solanum* species section *Petota*. An 18-bp long indel characteristic of functional *S. bulbocastanum RB* allele tagged by marker RB-226 was found in several *Solanum* species, and its presence was not always associated with high LB resistance. Sequencing and comparative analysis of *RB*-like gene fragments revealed ancient origins of the duplicated *RB*-like loci and characteristic patterns of nucleotide substitutions and indels most of which apparently arose before *Solanum* speciation.

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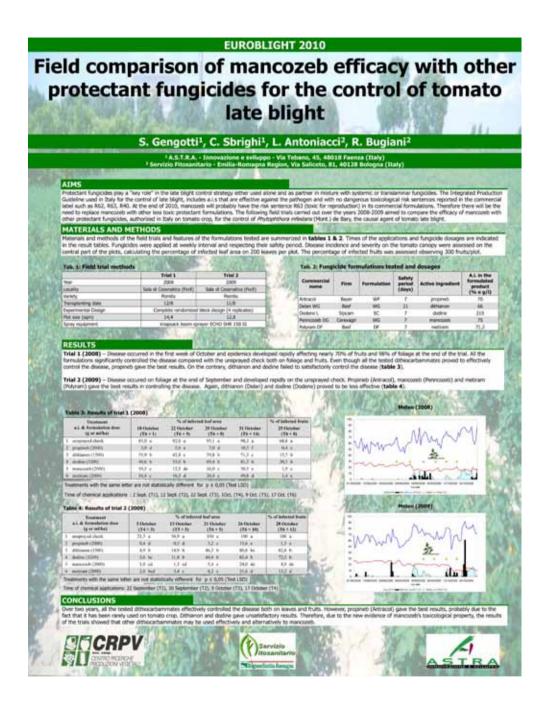
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Field comparison of mancozeb efficacy with other protectant fungicides for the control of tomato late blight

S. GENGOTTI[1], C. SBRIGHI[1], L. ANTONIACCI[2], R. BUGIANI[2]

[1] A.S.T.R.A. - Innovazione e sviluppo - Via Tebano, 45, 48018 Faenza (Italy)
[2] Servizio Fitosanitario - Emilia-Romagna Region, Via Saliceto, 81, 40128 Bologna (Italy)

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S. Gengotti[1], C. Sbrighi[1], L. Antoniacci[2], R. Bugiani[2]

[1] A.S.T.R.A. - Innovazione e sviluppo - Via Tebano, 45, 48018 Faenza (Italy)
[2] Servizio Fitosanitario - Emilia-Romagna Region, Via Saliceto, 81, 40128 Bologna (Italy)

AIMS

Protectant fungicides play a "key role" in the late blight control strategy either used alone and as partner in mixture with systemic or translaminar fungicides. The Integrated Production Guideline used in Italy for the control of late blight, includes a.i.s that are effective against the pathogen and with no dangerous toxicological risk sentences reported in the commercial label such as R62, R63, R40. At the end of 2010, mancozeb will probably have the risk sentence R63 (toxic for reproduction) in its commercial formulations. Therefore there will be the need to replace mancozeb with other less toxic protectant formulations. The following field trials carried out over the years 2008-2009 aimed to compare the efficacy of mancozeb with other protectant fungicides, authorized in Italy on tomato crop, for the control of *Phytophthora infestans* (Mont.) de Bary, the causal agent of tomato late blight.

MATERIALS AND METHODS

Materials and methods of the field trials and features of the formulations tested are summarized in **tables 1 & 2**. Times of the applications and fungicide dosages are indicated in the result tables. Fungicides were applied at weekly interval and respecting their safety period. Disease incidence and severity on the tomato canopy were assessed on the central part of the plots, calculating the percentage of infected leaf area on 200 leaves per plot. The percentage of infected fruits was assessed observing 300 fruits/plot.

Tab. 1: Field trial methods

	Trial 1	Trial 2
Year	2008	2009
Locality	Sala di Cesenatico (ForlÌ)	Sala di Cesenatico (ForlÌ)
Variety	Perella	Perella
Transplanting date	12/8	11/9
Experimental Design	Complete randomized block design (4 replicates)	
Plot size (sqm)	14,4	12,8
Spray equipment	Knapsack boom sprayer ECHO SHR 150 SI	

Tab. 2: Fungicide formulations tested and dosages

Commercial name	Firm	Formulation	Safety period (days)	Active ingredient	A.i. in the formulated product (% x g/l)
Antracol	Bayer	WP	7	propineb	70
Delan WG	Basf	WG	21	dithianon	46
Dodene L	Sipcam	SC	7	dodine	215
Penncozeb DG	Cerexagri	WG	7	mancozeb	75
Polyram DF	Basf	DF	7	metiram	71,2

RESULTS

Trial 1 (2008) – Disease occurred in the first week of October and epidemics developed rapidly affecting nearly 70% of fruits and 98% of foliage at the end of the trial. All the formulations significantly controlled the disease compared with the unsprayed check both on foliage and fruits. Even though all the tested dithiocarbammates proved to effectively control the disease, propineb gave the best results. On the contrary, dithianon and dodine failed to satisfactorily control the disease (**table 3**).

Trial 2 (2009) – Disease occured on foliage at the end of September and developed rapidly on the unsprayed check. Propineb (Antracol), mancozeb (Penncozeb) and metiram (Polyram) gave the best results in controlling the disease. Again, dithianon (Delan) and dodine (Dodene) proved to be less effective (**table 4**).

Table 3: Results of trial 1 (2008)

Treatment a.i. & formulation dose (g or ml/ha)	% of infected leaf area			% of infected fruits
	19 October (T6 + 1)	22 October (T6 + 5)	29 October (T6 + 8)	31 October (T6 + 14)	29 October (T6 + 8)
1 unsprayed check	89,8 a	92,0 a	97,1 a	98,2 a	68,8 a
2 propineb (2000)	3,9 d	5,9 e	7,0 d	14,5 f	8,4 c
3 dithianon (1500)	16,9 b	42,8 c	54,8 b	71,2 c	15,7 b
4 dodine (3200)	40,6 b	55,0 b	69,4 b	82,3 b	28,3 b
5 mancozeb (2000)	9,9 c	12,3 de	16,9 c	38,9 e	1,9 c
6 metiram (2000)	30,4 c	46,3 d	30,4 c	49,8 d	1,4 c

Treatments with the same letter are not statistically different for p ≤ 0,05 (Test LSD)

Time of chemical applications : 2 Sept. (T1), 12 Sept. (T2), 22 Sept. (T3), 1Oct. (T4), 9 Oct. (T5), 17 Oct. (T6)

Table 4: Results of trial 2 (2009)

Treatment a.i. & formulation dose (g or ml/ha)	% of infected leaf area			% of infected fruits
	5 October (T4 + 3)	15 October (T5 + 5)	21 October (T6 + 5)	26 October (T6 + 10)	26 October (T6 + 12)
1 unsprayed check	21,3 a	36,9 a	59 a	100 a	100 a
2 propineb (2000)	6,4 d	8,2 d	3,2 c	15,6 c	1,3 e
3 dithianon (1500)	6,9 b	14,9 b	46,3 b	80,8 bc	62,8 b
4 dodine (3200)	3,6 bc	11,8 b	44,4 b	85,8 b	72,3 b
5 mancozeb (2000)	1,6 cd	1,3 cd	5,4 c	24,0 de	8,9 de
6 metiram (2000)	2,6 bcd	3,4 c	6,2 c	35,6 d	15,2 d

Treatments with the same letter are not statistically different for p ≤ 0,05 (Test LSD)

Time of chemical applications: 22 September (T1), 30 September (T2), 9 October (T3), 17 October (T4)

Meteo (2008)

Meteo (2009)

CONCLUSIONS

Over two years, all the tested dithiocarbammates effectively controlled the disease both on leaves and fruits. However, propineb (Antracol) gave the best results, probably due to the fact that it has been rarely used on tomato crop. Dithianon and dodine gave unsatisfactory results. Therefore, due to the new evidence of mancozeb's toxicological property, the results of the trials showed that other dithiocarbammates may be used effectively and alternatively to mancozeb.

Efficacy evaluation of different fungicides for the control of potato late blight in Italy

S. GENGOTTI[1], C. SBRIGHI[1], L. ANTONIACCI[2], R. BUGIANI[2]

[1] A.S.T.R.A. - Innovazione e sviluppo - Via Tebano, 45, 48018 Faenza (Italy)
[2] Servizio Fitosanitario - Emilia-Romagna Region, Via Saliceto, 81, 40128 Bologna (Italy)

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[1] A.S.T.R.A. - Innovazione e sviluppo - Via Tebano, 45, 48018 Faenza (Italy)
[2] Servizio Fitosanitario - Emilia-Romagna Region, Via Saliceto, 81, 40128 Bologna (Italy)

AIMS

Two new fungicides (dimethomorph+pyraclostrobin and fluopicolide+propamocarb) were recently authorized in Italy for the control of late blight on solanaceous crops. Their toxicological properties and environmental impact make them suitable to be introduced in the list of fungicides allowed in the Integrated Production Guidelines for the disease control in Italy. Therefore, two field trials were setup over the years 2007-2008 with the aim to evaluate and compare the efficacy of new formulations introduced in the italian market for the control of *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight.

MATERIALS AND METHODS

Materials and methods of the field trials and features of the tested formulations are summarized in **tables 1 & 2**. Times of the applications and fungicide dosage are indicated in the result tables. Fungicides were applied respecting the safety period. Disease incidence and severity on the potato canopy were assessed on the central part of the plots, calculating the percentage of infected leaves and the percentage of infected leaf area by observing 200 leaves per plot. In 2008, potato was sown very late in the season (August) to benefit for the wet climate in autum and higher disease pressure. To avoid early blight infections, 4 sprays with mancozeb at 6-7 days interval were applied on all the experimental plots.

Table 1 – field trial methods

	Trial 1	Trial 2
Year	2007	2008
Locality	Imola (Bologna)	Sala di Cesenatico (Forlì)
Variety	Primura	Bologna
Sowing date	27/3	26/8
Experimental design	Complete randomized block design (4 replicates)	
Plot dimension (sqm)	24,0	21,0
Spray equipment	knapsack boom sprayer ECHO SHR 150 SI	

Table 2 – tested fungicides and dosage

Commercial name	Firm	Formulation	Safety period (days)	Active ingredient	A.i. in the formulated product (% or g/l)
Cabrio Duo	Basf	SC	3	dimethomorph + pyraclostrobin	72 + 40
Electis	Dow	WG	7	zoxamide + mancozeb	8,3 + 66,7
Forum R	Basf	WP	20	dimethomorph + copper oxychloride	6 + 40
Melody compact	Bayer	WP	20	iprovalicarb + copper oxychloride	4,2 + 20,3
Ridomil Gold R	Syngenta	WP	20	metalaxyl-m + copper oxychloride	2,5 + 40
Volare	Bayer	SC	7	fluopicolide+ propamocarb	62,5 + 625

RESULTS

Trial 1 (2007) – Disease occurred at the end of May and developped rapidly affecting more than 90% of the check plot. All the tested formulations effectively protected the crop. However, formulation containing metalaxyl-m + copper oxychloride (Ridomil Gold R), again, confirmed to be the best to contain the disease (**table 3**). No tuber blight was recorded.

Trial 2 (2008) – Disease symptoms occurred on 10 October. All the formulations showed a good efficacy. The efficacy on foliage of formulations recently introduced on the market fluopicolide+propamocarb (Volare) and dimethomorph+pyraclostrobin (Cabrio Duo), was similar to that provided by metalaxyl-m+copper oxychloride, still considered the best chemical reference. On the contrary, with high disease pressure (more than 87% of infected leaf area in the unsprayed check) the mixture zoxamide+mancozeb (Electis) and iprovalicarb+copper oxychloride (Melody compact) proved to be less effective (**table 4**). No tuber blight was recorded at harvest.

Table 3 - results of field trial n. 1 (2007)

Treatments a.i. and formulation dose (g or ml/ha)	% of infected leaves		
	18 June (T4 + 7)	26 June (T5 + 7)	3 July (T5 + 14)
1 Unsprayed check	61,3 a	80,0 a	91,0 a
2 zoxamide + mancozeb (1750)	3,38 b	2,61 c	2,36 bc
3 dimethomorph + copper oxychloride (3250)	4,06 b	3,94 bc	1,98 c
4 iprovalicarb + copper oxychloride (3500)	4,59 b	6,36 b	3,78 b
5 metalaxyl-m + copper oxychloride (4000)	0,23 c	0,82 d	1,31 c

Treatments with the same letter are not statistically different for p ≤ 0,05 (Test LSD)

Time of chemical applications: 7-May (T1), 18-May (T2), 30-May (T3), 11-June (T4), 19-June (T5)

Table 4 - results of field trial n. 2 (2008)

Treatments a.i. and dosage (g or ml/ha)	% of infected leaf area		
	15 October (T2 + 5)	22 October (T3 + 5)	31 October (T3 + 14)
1 Unsprayed check	35,4 a	65,6 a	87,5 a
2 zoxamide + mancozeb (1750)	3,3 bcd	22,7 bc	38,0 b
3 Dimethomorph + copper oxychloride (3250)	1,9 cd	16,8 cd	28,8 bc
4 metalaxyl-m + copper oxychloride (4000)	0,2 d	11,5 cd	25,3 c
5 iprovalicarb + copper oxychloride(3500)	10,7 b	30,1 b	40,0 b
6 dimethomorph + pyraclostrobin (2500)	1,3 cd	9,6 d	23,8 c
7 fluopicolide + propamocarb (1600)	1,4 cd	13,3 cd	22,0 c

Treatmetns with the same letter are not statistically different for p ≤ 0,05 (Test LSD)

Time of chemical applications 1-October (T1), 10-October (T2), 17-October (T3)

Meteo (2007)

Meteo (2008)

CONCLUSIONS

Under medium and severe disease pressure in 2007 and 2008 respectively, metalaxyl-m+copper oxychloride (Ridomil Gold R) confirmed to be the best chemical reference in controlling late blight on foliage. New formulations, fluopicolide+propamocarb (Volare) and dimethomorph+pyraclostrobin (Cabrio Duo) showed, allthough in a single trial, an efficacy similar to that provided by the best chemical references, metalaxyl-m+copper oxychloride and dimetomorph+copper respectively. Iprovalicarb+copper oxychloride and zoxamide+mancozeb proved to be less effective.

Study of invasive French populations (2006-2008) of

Phytophthora infestans, the Oomycete causing potato late blight

ROSELYNE CORBIÈRE,, HÉLÈNE MAGALON,
FRÉDÉRIC BOULARD AND DIDIER ANDRIVON

INRA, UMR 1099 BiO3P - INRA / Agrocampus Ouest / Université de Rennes 1,
BP 35327, 35653 LE RHEU Cedex - FRANCE

Posters

Study of invasive French populations (2006-2008) of *Phytophthora infestans*, the Oomycete causing potato late blight.

Roselyne Corbière, Hélène Magalon, Frédéric Boulard and Didier Andrivon

INRA, UMR 1099 BiO3P - INRA / Agrocampus Ouest / Université de Rennes 1, BP 35327, 35653 LE RHEU Cedex - FRANCE.

Late blight is an important potato disease, caused by *Phytophthora infestans* which is responsable of aerial and polycyclic epidemics.
This Oomycete is heterothallic with two mating-types named A1 and A2. Before 2003, French populations contained almost only A1 isolates. Then, drastic changes in *P. infestans* populations have been observed : the frequency of A2 isolates dramatically increased with a very fast and steady progression of these A2 isolates.

This invasion was first observed in Northern France (6% A2 in 2003 to 76% in 2007), then in all potato production areas (5% A2 in 2005 to 75% in 2008, in Brittany and 100% A2 in Center of France in 2007).
To understand the population dynamics of this pathogen, we explored the genetic and phenotypic variations of isolates sampled from the three main potato production areas during 2006 to 2008.

Microsatellite markers reveal that A1 and A2 isolates are two genetic distinct populations.

The genotypes of 480 isolates collected over 3 years (2006 to 2008) from Brittany, North and Center of France are explored using 12 microsatellite loci (Knapova and Gisi, 2002; Lees *et al.*, 2006).

This analysis reveals :
- a structuration according to mating types.
- a clonal, but large genotypic diversity with many unique multi-locus genotypes.
- a larger diversity among A1 isolates than A2 isolates.
- a clonal lineage making more than 50% of the A2 isolates (named 13_A2 genotype), in North and in Brittany.
- no structuration according to regions and years.
- no relationship between genotypes and virulence patterns.

Distribution of multilocus genotypes per year and per mating-type, according to the three regions.

Each color represents a repeated MLG.

unique multi-locus genotype (MLG)

Factorial correspondence analysis

All A2 isolates are resistant to metalaxyl fungicide.
Fungicide resistance is assessed with a floating leaf disk method, with 10 and 100 µg metalaxyl /mL.

A1 – 50 isolates (2007 - 2008)   **A2** – 70 isolates (2007 - 2008)

76 % sensitive   100 % resistant

Aggressiveness does not explain the fast expansion of A2 isolates in France.

Aggressiveness of 111 A1 and 127 A2 isolates (collected from 2004 to 2007) is tested under controlled conditions, by comparing lesion size and sporulation of each isolate inoculated on detached leaflets of a susceptible cultivar (Bintje).

Sporulation capacity on Bintje leaflets of A1 and A2 isolates (2007) from 3 French regions

A1 isolates are slightly more aggressive than A2, under our experimental conditions.

The highest sporulation is in A1 isolates from Northern France, where Bintje has been the dominant host since several decades.

A2 isolates have more complex virulence profiles (7 to 11 virulence factors), but they are less diverse than those of A1.

Virulence patterns are determined using an international set of potato clones, each possessing one or more pathotype-specific resistance genes (R1to R11)

A1 - 13 virulence profiles   **A2 - 7 virulence profiles**

2 isolates with 11 virulences | 54% with 11 virulences | 23% with 7 to 9 virulences

40 % with 7 to 9 virulences | 56 % with 5 to 6 virulences | 23% with 10 virulences

A2 isolates are genetically and phenotypically different from A1 isolates. The majority of A2 isolates shows new virulence patterns : they overcome most or all resistance genes from *Solanum demissum*, including those which have not been introduced into potato cultivars so far (e.g. R9). They are also insensitive to metalaxyl, although this fungicide has been scarcely used during the past years.

On the opposite, A1 population shows more genotypic and phenotypic diversity than the A2 population and seems more aggressive. Other life history traits, possibly related to environmental or climatic changes, should now be considered to explain the emergence of A2 isolates. The role and importance of sexual reproduction also has to be assessed to understand these population changes.

12th EuroBlight Workshop
A potato late blight network for Europe
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Occurrence of late blight in Algeria during 2009 and evaluation of potato cultivars for resistance to *Phytophthora infestans*

AMINA GALFOUT [1], ABDELAZIZ KEDAD [1], ROSELYNE CORBIERE [2]
AND ZOUAOUI BOUZNAD [1]

[1]Laboratoire de Phytopathologie et Biologie Moléculaire, Ecole Nationale Supérieure Agronomique (ENSA), El-Harrach, ALGIERS, Algeria
[2]INRA, UMR Inra - Agrocampus Ouest - Université Rennes I-1099 BIO3P, BP 35327, 35653 LE RHEU, France
E-mail: z.bouznad@ensa.dz

SUMMARY

Prospections carried out in different production areas of potato in Algeria during spring 2009 showed severe epidemics of late blight in Western coastal (Mostaganem) and Central Western (Ain Defla and Mitidja) regions. In contrast, in Mascara location, the prospected fields revealed a low late blight frequency. Potato cultivars were tested for foliar resistance to late blight under controlled conditions and in a field trial. Our results showed a high susceptibility of most of the 13 tested cultivars and especially of cv. Spunta, dominant in Algeria, but an interesting resistance of cv. Sarpo Mira. Moreover, some cultivars exhibited variable level of resistance, according to the spore production of the two isolates used in the bio-assay.

KEYWORDS

Solanum tuberosum L., *Phytophthora infestans*, potato late blight, cultivar ranking, resistance components, *in vitro* evaluation, field trial.

INTRODUCTION

In the last decade, potato production areas in Algeria have known a large development. Late blight caused by *Phytophthora infestans* remains one of the most severe diseases of this crop. Since 2007, the disease caused drastic losses in the yields, particularly in regions where climatic conditions are highly favourable. In this situation, it was necessary to assess the frequency and intensity of this disease in various Algerian production areas. On the other hand, few informations are available about late blight resistance of potato cultivars grown under Algerian conditions. Two experiments were carried out in 2009 to evaluate foliar resistance level of several cultivars grown in Algeria : 1) an assay under controlled conditions, on detached leaflets with artificial inoculations and 2) a field trial under natural conditions of infection.

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MATERIALS AND METHODS

Prospections for late blight occurrence

The prospections were carried out during April and May 2009 in two main regions : the central West (Mitidja and Ain Defla) and the West (Mostaganem and Mascara) of Algeria (Fig. 1). These locations have a high potential of potato crop, where they yield nearly 60% of the national production. In the prospected fields, seed tubers were planted during February until mid-March.

Disease occurrence was estimated by late blight frequency in each field (percent of diseased plants per field) and by disease severity (estimated on a scale from 1 to 9, where 9 value was completely necrosed plant).

Resistance test on detached leaflets

Nine potato cultivars were selected : Amorosa, Arinda, Armada, Arnova, Atlas, Liseta, Sarpo Mira, Spunta and Timate. Cultivar Bintje was used as reference because it is highly susceptible to late blight in European countries, but it is not grown in Algeria. The resistance test was carried out by artificial leaflet inoculations and incubation in humid chamber. Experiment was performed on four leaflets per cultivar and isolate. Each cultivar leaflet was separately inoculated with two A2 *P. infestans* isolates (Z1 and Z5), previously characterized by Beninal *et al.* (2009) and which are the two prevalent pathotypes in Algeria (Corbière *et al.*, 2010). Isolates Z1 and Z5 were respectively avirulent to *R9* and virulent to *R9*, and they overcome all the other 10 *R*-genes of Black's differentials. Leaflets were inoculated with a 20 µL drop of a suspension at 5×10^4 sporangia/mL. The necrose diameter and the sporulation intensity were respectively estimated after 4 and 6 days of incubation at 18°C. To quantify spore production, each leaflet was washed in 10 mL of water and sporangia production per lesion was determined with a haemocytometer, with three replicates per leaflet.

Field trial

Trial was performed in an experimental field in El Harrach (near Algiers). A total of 11 cultivars was evaluated: Amorosa, Arinda, Arnova, Atlas, Bintje, Désirée, Fabula, Kondor, Sarpo Mira, Spunta and Timate. Potato seeds were planted on 10th March according to a randomized complete block design with four replications. Trial was exposed to natural infections from local inoculum of *P. infestans*. Notations were weekly recorded from 25th April to 29th May, on 40 plants per cultivar. The number of diseased plants was evaluated in each micro field and late blight was scored on each plant, by a scale from 1 to 9 values, where 9 value was totally destroyed plant. An attack index (IAM) was calculated in order to evaluate the disease intensity on each cultivar, according to the formula :

$$IAM = \frac{\Sigma \text{ Index of attack for each plant (1 to 9)}}{\text{Number of plants noted}}$$

RESULTS

Occurrence of late blight in Center and West of Algeria during 2009

A total of 40 fields were prospected during April and May 2009 (Fig. 1).
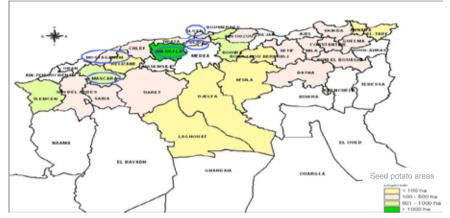
Figure 1. *Main Algerian areas prospected for occurrence of late blight (in circles)*

The mean late blight frequency and the mean disease severity per location, evaluated on May, is given in Table 1. The disease was the most important in the Central Algeria (Mitidja), with a frequency of 100% and an average severity of 7,5. In contrast, the weakest frequency was noticed in Mascara location where the disease severity did not exceed a level of 5 and with a weak frequency (not more than 10%). In the Western Algeria (Ain-Defla and Mostaganem), the mean late blight frequencies were respectively 50 and 66%, but it grew up to 100% in 6 fields of the 17 prospected ones. In Ain Defla region, late blight frequency fast increased from 2,5% to 50% in three weeks, from 13th April to 5th May.

Table 1. *Frequency and severity of late blight in Central and Western Algeria on May 2009*

Location	Mean area of fields (ha)	Prospected cultivars	Late blight frequency	Disease severity
Center : Mitidja - Blida	10 ha	Spunta, Fabula	100 %	7,5
West Center : Ain Defla	15 ha	Spunta	50 %	5,5
West : - Mostaganem,	1 ha	Spunta, Kondor, Désirée,	66 %	7
- Mascara	3 ha	Atlas	10 %	3

Assessment of potato cultivars for resistance on detached leaflets

Development of lesion diameters

After four incubation days, eight cultivars exhibited necrosis with the two isolates Z1 and Z5. Lesion diameter varied from 1 mm on cv. Armada with isolate Z1, to 27,5 mm on cv. Bintje with isolate Z5 (Fig. 2). In contrast, two cultivars, Arinda and Sarpo Mira, presented no symptom with one isolate. Cv. Arinda had no necrosis with Z5 and very small lesion with Z1, and cv. Sarpo Mira, no necrosis with Z1 and small lesion with Z5. Then, according to lesion diameter, seven cultivars were scored as susceptible, especially cvs Bintje and Spunta. Cultivar Armada was intermediate, because necrosis were observed with the two isolates, but these lesions were small. On the other hand, cvs Arinda and Sarpo Mira showed a high level of resistance to the two isolates. Moreover, these cultivars did not present any infection respectively with Z5 isolate (which overcome *R9* gene) and Z1 isolate (which did not overcome *R9*). Thus, these two cultivars expressed different and interesting resistance levels to *P.infestans*.

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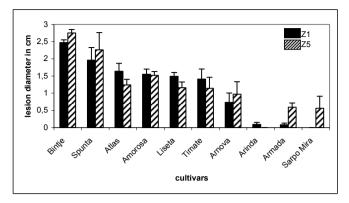
Figure 2. *Lesions diameter of two different P. infestans isolates (Z1 and Z5) on ten potato cultivars and measured on detached leaflets, after 4 days of incubation.*

The variance analysis of the values showed significant differences between the cultivars. The Newman and Keuls test (at 5% of significance) for lesion diameters, discriminated three rather homogeneous groups. The first one was composed of cvs Bintje and Spunta; the second one of cvs Amorosa, Atlas, Liseta and Timate, and the third one of cvs Arnova, Armada, Arinda and Sarpo Mira.

Sporulation intensity of the lesions

Sporulation, after six incubation days, was observed on all cultivars, with the two isolates, except on cvs Arinda and Sarpo Mira (Fig. 3). Spore production was variable according to the cultivar and to the isolate. The highest sporulation was noticed for cv. Bintje with isolate Z5 (2×10^5 sporangia/mL), while the weakest value was recorded for cv. Armada (10^4 sp/mL) with isolate Z1. Interestingly, no spore was produced on cv. Sarpo Mira with the two isolates, and on cv. Arinda, only few spores were produced with isolate Z5 and none with isolate Z1. On cvs Armada, Arnova and Atlas, sporulation was higher with isolate Z5 than with isolate Z1. Moreover, on cv. Atlas inoculated with Z1, sporulation was low, although lesion size was large.
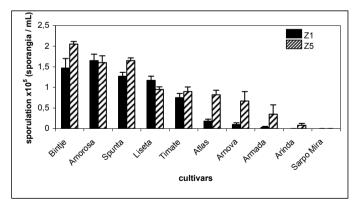
Figure 3. *Sporangia production of two different P. infestans isolates (Z1 and Z5) on ten potato cultivars and measured on detached leaflets, after 6 days of incubation.*

Variance analysis for sporulation data revealed significant differences (P<5%) between the cultivars, but rankings were different according to the isolates. With isolate Z1, three rather homogeneous

groups were discriminated; the first one was composed of cvs Bintje and Amorosa, the second one of cvs Spunta, Liseta and Timate and the third one of cvs Atlas, Arnova, Armada; Arinda and Sarpo Mira. With isolate Z5, cultivars were ranged into four rather homogeneous groups : the first one of cv. Bintje, the second one of cvs Spunta and Amorosa, the third one of cvs Liseta, Timate, Atlas; Arnova and Armada, and the fourth one of cvs Arinda and Sarpo Mira.

According to the two resistance components, cultivars could be classified into four groups :
- Sarpo Mira and Arinda were the most resistant ones.
- Armada, Arnova and Atlas moderately susceptible cvs.
- Amorosa, Liseta and Timate, susceptible cvs.
- Spunta and Bintje, the most susceptible cultivars.

Assessment of potato cultivars for foliage resistance, in a field trial
In the field trial, the average of attack index on the 11 cultivars ranged from 3 (resistant) to 7,5 (highly susceptible). Variance analysis of this attack index showed a highly significance between the cultivars and allowed to discriminate four homogeneous groups (Table 2). The most resistant cultivars were Sarpo Mira and Arinda, with the weakest attack index. Four cultivars, Atlas, Amorosa, Arnova and Kondor, had a relatively weak attack index and were moderately susceptible. Two others cultivars, Timate and Fabula, were susceptible. Finally, the most susceptible cultivars were Spunta, Bintje and Desiree with the highest attack index.

Table 2. *Attack index on 11 potato cultivars in a naturally infected field trial*
(late blight scored by a 1 to 9 scale values where 9 value = completely necrosed plant)

Cultivars	Attack index
Sarpo Mira, Arinda	3
Atlas, Arnova, Amorosa, Kondor	5,3
Timate, Fabula	6,2
Spunta, Desiree, Bintje	7,5

DISCUSSION AND CONCLUSION
Our prospections showed that late blight epidemics were dramatic in all the prospected regions during spring 2009, except in Mascara. Disease was severe in Mitidga, Aïn Delfa locations and in the coastal Western part of Algeria (Mostaganem). In these regions, late blight epidemics were favoured by conducive weather conditions.
Most of the 13 cultivars tested for foliage late blight resistance were moderately susceptible to susceptible. The assay, carried out on detached leaflets with two isolates with different virulence patterns, confirmed the behavior of most of the cultivars assessed in the field trial, and especially the resistance of cv. Sarpo Mira. This cultivar, with pathotype-non specific resistance, seems to be promising, but it has been newly introduced in Algeria and is not frequently grown in this country. Our results are consistent with usually cultivar resistance ratings, *e.g.* for cvs Atlas and Arnova, moderately susceptible to late blight on foliage. Cultivar Spunta, dominant in Algeria, was highly susceptible to late blight and cv. Kondor, moderately susceptible, as it was also noticed with Moroccan *P. infestans* populations by Hammi (2003). However, these results need more investigations, *e.g.* resistance of cv. Arinda must to be confirmed. Indeed, in the European cultivated potato database, this cultivar has a low to medium resistance to late blight on foliage. Furthermore, according to Andrivon *et al.* (2007), Moroccan *P. infestans* isolates were locally adapted to cv. Désirée and were more aggressive on cv. Désirée than on cv. Bintje. In our field trial, we did not notice clear local

adaptation of Algerian isolates to cv. Désirée in comparison with cv. Bintje, and in the bio-assay, cv. Bintje was highly susceptible to the two Algerian *P. infestans* isolates.

Under controlled conditions, the cultivars, especially the most resistant ones, exhibited variable level of resistance, according to the spore production of the two different isolates. The expression of cultivar resistance and its spatial and temporal stability depend on environments, but also on characteristics of the local *P. infestans* isolates in each country. In Algeria, *P. infestans* isolates showed variability in their pathogenic traits (Corbière *et al*., 2010). The accurate host resistance and knowledge of cultivar responses to disease has then to be assessed with current and diverse Algerian *P. infestans* isolates. Therefore, further work needs to be performed to evaluate cultivars for durable resistance in Algeria, under various environmental conditions, and also to explore diversity and pathogenic traits of Algerian *P. infestans* populations. However, this study provides informations on potato cultivar resistance usefull to develop effective, integrated disease management programmes in Algeria.

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In vitro evaluation of difenoconazole and chlorothalonil on conidial germination and mycelial growth of *Alternaria alternata* and *A. solani* causal agent of early blight in Algeria

FERIEL ISSIAKHEM AND ZOUAOUI BOUZNAD

Laboratoire de Phytopathologie et Biologie Moléculaire, Ecole Nationale Supérieure Agronomique (ENSA), El-Harrach, Algiers, Algeria, E-mail: z.bouznad@ensa.dz

SUMMARY

Diseases are still the main cause of reduction of yield in potato crops in Algeria. After late blight which is the most destructive disease, early blight is also an important foliar disease, reported to be caused by *A. alternata* and *A. solani,* responsable of yield losses in our Algerian climatic conditions. This research was initiated to examine in laboratory conditions the efficacy of two fungicides used in Algeria. The results showed that Difenoconazole had a better effectiveness than Chlorothalonil in inhibition of mycelial growth and conidial germination of *A. solani and A. alternata. A.solani* showed also a higher sensitivity than *A. alternata* to the two tested fungicides.

KEY WORDS

Early blight, potato, difenoconazole, chlorothalonil, fungicides effectiveness.

INTRODUCTION

Potato (*Solanum tuberosum*) is traditionally one of the most cultivated crops in Algeria. Among biotic stresses, early blight is an important foliar disease reported to be caused by *Alternaria alternata* and *A. solani* responsable of yield losses under our climatic conditions. Control of these two pathogens can be accomplished through various means: use of resistant potato cultivars, appropriate farming techniques such as careful tillage, crop rotation, etc., as well as fungicide application that may directly affect the growth of fungi.

The present research was conducted to evaluate the efficacy of the two fungicides chlorothalonil and difenoconazole used in Algeria towards *Alternaria solani* and *A. alternata*.

In-vitro experiments were conducted on mycelial growth and conidial germination of the early blight causal agents, using two fungicides available on the Algerian market.

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MATERIALS AND METHODS

Fungicides

The tests were performed *in vitro* to evaluate the effectiveness of two fungicides: difenoconazole (250 g i.a./l) and chlorothalonil (720 g i.a. /l), on conidial germination and mycelial growth of *A. solani* and *A. alternata*, and to compare them with the concentrations used in field. Concentrations of difenoconazole and chlorothalonil were then calculated from dose used in field treatments as shown in Table 1.

Fungal material, and estimation of mycelial growth and conidial germination

Isolates of *A. solani* and *A. alternata* (Fig.1a and 1b) were obtained from leaves of potatoes showing characteristic symptoms of early blight (Fig. 2a and 2b) collected in Algeria.
The tests were carried out in Petri dishes for mycelial growth and on slides for conidial germination

Fig. 1a. *Symptoms of A. alternata*   **Fig. 1b**. *Symptoms of A. solani*
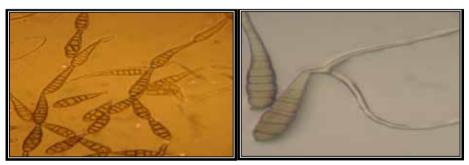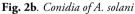
Fig. 2a . *Conidia of A. alternata*   **Fig. 2b**. *Conidia of A. solani*

For mycelial growth, the tests were conducted on malt agar medium and diameter of the colonies was measured after 7 days of incubation at 18-22°C.
Tests were performed with twelve cultures (3 x 4 repetitions), per fungicide concentration and per isolate.

The inhibition of mycelial growth (CI50) was evaluated by:

$$Im \% = \frac{V0-V}{V0} .100$$

V0 mycelium growth on medium without fungicide and V growth on medium with fungicide.

For conidial germination, a drop of conidial suspension of each fungicide concentration was deposited on glass slide recovered by agar. Conidial germination was observed under microscope,

after 24 hours of incubation at 18-22 °C. One hundred conidia were observed per isolate and fungicide concentration.

The inhibition of conidial germination was evaluated by: $Ic\% = \dfrac{Q - Q_0}{100 - Q_0} \cdot 100$

Q0 was the number of germinated conidia on medium without fungicide and Q, the number of germinated conidia on medium with fungicide

Table 1. *Doses used for mycelial growth and conidial germination test*

active substance	Chemical group	Species of fungi	Doses used for mycelial growth test		Doses used for conidial germination test	
			ppm	µl i.a. /l	ppm	µl i.a. /l
difenoconazole	triazoles	*A. solani*	250	1000	0	0
			125	500	1.95	7,81
		A. alternata	62,5	250	0.48	1,95
			31,25	125	0.122	0,48
			15,62	62,5	0.030	0,12
			7,81	31,25		
			3,90	15,62		
			1,95	7,81		
			0,97	3,90		
			0,48	1,95		
			0,24	0,97		
			0,122	0,48		
			0,061	0,24		
			0,030	0,12		
			0	0		
chlorothalonil	chloronitriles	*A. solani*	2880	4000	0	0
			1440	2000	2880	4000
		A. alternata	720	1000	720	1000
			360	500	180	250
			180	250	45	62,5
			90	125		
			45	62.5		
			22,5	31,25		
			0	0		

RESULTS

Effectiveness of fungicides on development of mycelial growth

The results obtained (Fig. 4 a,b,c and d) showed that difénoconazole had a strong inhibition effect on *A. solani* (89%) with a concentration of 0,97 ppm (or 7.81 µl i.a. /l) , whereas it was only 56% with the same concentration (Fig.) for *A. alternata*. With this level of inhibition, the mycelial growth speed was only respectively 0,33 mm/j and 1,5 mm/j for *A. solani* and *A. alternata*, whereas the control mycelial growth speed was 3.25 mm/j for *A. solani* and 3.45 mm/j for *A. alternata*.

On the other hand, chlorothalonil (Fig. 3 a,b,c and d) showed a lower effectiveness with always a difference between the two pathogens. Thus, *A. solani* was inhibited at 89% with a concentration of

1440 ppm (or 2000µl i.a. /l), and *A. alternata* was inhibited at 57% for the same concentration. These inhibitions had an effect on the mycelial growth speed of both pathogens *A. solani* and *A. alternata*, which were respectively 0.33 mm/j and 1.46 mm/j. This difference showed that this product is more effective on *A. solani* than on *A. alternata*.

The curves of regression (y=ax +b) for each fungicide, obtained by transformation of the percentages of inhibition into probits (Finney, 1952), allowed to determine the CMI and the CI50 for the two products. It was noted that the CI50 of difenoconazole was weaker than that of chlorothalonil, with 0.446µl i.a. /l and 44.59 µl i.a. /l respectively for *A. solani* and 2.720 µl i.a. /l and 970.9 µl i.a. /l respectively for *A. alternata*.

Effectiveness of fungicides on conidial germination

Our results (Table. 2) clearly showed that the percentages of inhibition of conidial germination were strongly related with doses used. We noted that difenoconazole strongly reduced the conidial germination of *A. solani* and reached 92% at 1.95 ppm (7.81µl i.a. /l), whereas the conidial germination of *A. alternata* was only reduced to 65% for the same concentration

Table 2. *Effectiveness of fungicides on conidial germination: Percentage of inhibition and probits.*

Active substance	Species	Doses (µl i.a/l)	Log (10xC)	% Inhibition	Probits
Difenoconazole	*A.solani*	7,81	1,89	92	6,41
		1,95	1,29	71	5,55
		0,48	0,68	60	5,25
		0,12	0,08	38,4	4,69
	A.alternata	7,81	1,89	65	5,39
		1,95	1,29	41	4,77
		0,48	0,68	34	4,59
		0,12	0,08	20	4,16
Chlorothalonil	*A.solani*	4000	3,60	78	5,77
		1000	3 ,00	69	5,50
		250	2,40	31	4,50
		62,5	1,80	23	5,26
	A.alternata	4000	3,60	53	5,08
		1000	3 ,00	34	4,59
		250	2,40	26	4,36
		62,5	1,80	16	4,01

From results obtained with chlorothalonil, we noticed that the reduction of conidial germination was less important for *A. solani* than for *A. alternata*. It was respectively 78% at 720 ppm (1000 µl i.a./l) for *A. solani*, whereas it was 53% at the same concentration for *A. alternata* (table 2).

Inhibition was determined for the two products by the CI 50 which is the minimal concentration which inhibits 50% of conidial germination. Results are represented in Table 3.

Table 3. *CI 50 of difenoconazole and chlorothalonil for conidial germination of A. solani and A. alternata*

pathogens	Active substance	
	difenoconazole CI50 en µl i.a./l	chlorothalonil CI50 en µl i.a./l
Alternaria solani	0.30	490,68
Alternaria alternata	2.60	3600,41

DISCUSSION AND CONCLUSION

The results obtained showed that the two fungicides tested, difenoconazole and chlorothalonil, had an effect *in vitro* on mycelial growth and spore germination of both *Alternaria* species. Furthermore, *A. solani* was more sensitive than *A. alternata* in regard to the two products, whose IC50 allowed to rank the two fungicides as follows : difenoconazole>chlorothalonil. Then, our results showed that difenoconazole had a better effectiveness than chlorothalonil in inhibition of mycelial growth and conidial germination of *A. solani* and *A. alternata*.

In previous works, Tofoli *et al* (2003) also showed efficacy of chlorothalonil against *A. alternata* and Badoc (2005) obtained efficacy of azoxystrobin on germination and mycelial growth of *A. alternata*, the causal agent of fruit storage rots. In fields, more recent report (MacDonald *et al.*, 2007) showed efficacy against *A. solani* of other active ingredients belonging to the same family of strobilurin (azoxystrobin, pyraclostrobin). *In vitro* results do not always reflect what happens in the field. This study should be complemented by field trials to prove or disprove the effectiveness of these products on *Alternaria* inoculum on the plant, and to compare them to new fungicides.

ACKNOWLEDGEMENTS

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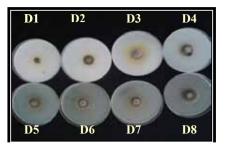
Fig. 3a. *Effect of Chlorothalonil against A. solani.*

Fig. 3b. *Effect of Chlorothalonil against A. alternata*

Fig. 3c. *Effect of chlorothalonil on diametral growth of mycelium*
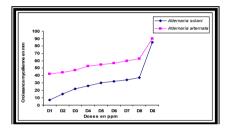
Fig. 3d. *Inhibition percentage of Chlorothalonil on diametral growth of mycelium*

Fig. 4a. *Effect of difenoconazole against A. solani.*
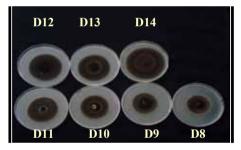
Fig.4b. *Effect of Difenoconazole against A. alternata*

Fig. 4c. *Effect of difenoconazole on diametral growth of mycelia*
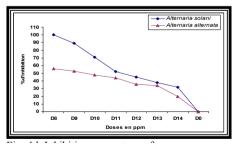
Fig. 4d. *Inhibition percentage of difenoconazole on diametral growth of mycelia*

Late blight resistance of potato mapping populations in front of naturally evolving pathogen populations

MARHADOUR[1], S., PELLE[2], R., ABIVEN[3], J.M., AUROUSSEAU[4], F., DUBREUIL[5], H., LE HINGRAT[6], Y. AND CHAUVIN[2], J.E.

[1] FN3PT INRA UMR APBV Keraiber 29260 Ploudaniel France
[2] INRA UMR APBV Keraiber 29260 Ploudaniel France
[3] Bretagne Plants, Station de Création Variétale, Kerloï, 29260 Ploudaniel France
[4] Station de recherche du Comité Nord, 76110 Bretteville du Grand Caux, France
[5] CROCEP, Station de Lavergne, 87370 Lauriére, France
[6] SFN3PT, Roudouhir, 29460 Hanvec, France

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Late blight resistance of potato mapping populations in front of naturally evolving pathogen populations

Marhadour[1], S., Pellé[2], R., Abiven[3], J.M., Aurousseau[4], F., Dubreuil[5], H., Le Hingrat[6], Y. and Chauvin[3] J.E.

Context

Non specific resistance to Phytophthora infestans has become an objective for our breeders. To be able to deal with such a complex polygenic trait in breeding programs, one can use molecular markers linked to quantitative resistance loci (QRL).

Here we present phenotypical results obtained on two segregating families. Experiments have been done during years of important evolution in the pathogen populations (2005 to 2009). However, despite we could observe the erosion of R gene(s) efficiency, partial resistance factors present in the source of resistance were still efficient.

Which type of material did we study ?

Genealogy of the material is detailed on the figure.

INRA89Tx13.3 is the source of resistance coming from CIP A population.
Berber is a susceptible variety.
GA3 is a susceptible genotype of another mapping family.
B2G6 progeny exhibited specific resistance associated with partial resistance.
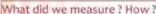
Standards were included in the experiment. Results obtained on Robijn (partial resistance) and Désirée are presented.

Blacks differentials were also included each year in the experiments.

What did we measure ? How ?

B2 family was experimented each year between 2005 and 2007. B2G6 was experimented in 2009.
The experimentations took place in Ploudaniel, France (oceanic conditions) under natural conditions of contamination.
Bintje was used as a spreader.
Disease progress curves were constructed using a mean of 10 notations each year. Each genotype was visually scored using a scale of foliage destruction (Dowley et al, 1999).
AUDPC, deltaa and deltat (Andrivon et al, 2006) parameters were computed.
Each genotypes was observed in 3 replicates (except in 2009, 2 replicates for B2G6 family)
Statistical tests were made using SAS software.

Perspectives
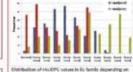
- Continue the evaluation of the new generation of material and the behavior of resistance factors in front of the new pathogen populations,
- Evaluate the proportion of QRL detected in the first generation which is also efficient in the second one,
- Compare QRL detected across the progenies.

Erosion of the R gene(s) efficiency

In the first generation of material (B2 family), segregation of the resistance factors is observed and is subjected to a strong year effect. Important Year effect is also observed on parental clones and standards
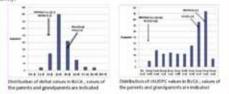
Distribution of AUDPC values in B2 family depending on the year

A rather rapid erosion of the R gene(s) efficiency has been observed through the decreasing value of the period without symptom on the material as compared with susceptible control (deltat)

Factors of partial resistance are not masked by R gene(s) in 2009

Additional intra progeny crosses have been made leading to a new generation that has started to be observed in 2009, concomitantly to the observed loss of efficiency of R gene(s). It was then easier to observe the efficiency of partial resistance factors that were previously masked by R gene(s) and only visible in the R gene free proportion of the family.

Distribution of deltat values in B2G6, values of the parents and grandparents are indicated

Distributions of AUDPC values in B2G6, values of the parents and grandparents are indicated

Interests and limits to experiment in natural conditions of contamination

- Our site of experiment can be considered as a "hot spot" for experimentation on late blight resistance. Late blight occurs every year without the need to use artificial inoculum. The pathogen pressure is high due to optimal climatic conditions.
- Isolates sampled each year and characterized since a long time by phytopathologists (Andrivon's team), are characterized by a complex pattern of virulences (Corbière, pers. comm.) and were able to overcome most of the 5 dominant R genes except R1 in 2006 and R9 which was never infected during the period of the experiments (differentials results).
- Between 2005 and 2009, changes have occurred in the pathogen population in France (Andrivon, communication during this congress) including the increase of A2 mating type and the pattern of virulences. Our site was concerned despite it was slightly different from the others in France probably due to the presence of the INRA breeding material where a lot of resistance genes are experimented (Corbière, pers. comm.).

Limits of experimenting in such conditions are:
- Composition of the inoculum is not controlled
- Population of isolates is always evolving

However, experimenting an overall selection in such conditions is interesting because
- Resistance factors are submitted to a high and diverse pressure of pathogen
- We can see what happen in "real life" to the resistance factors we are dealing with
- Partial resistance phenotype is now supposed to be accessible even if R gene(s) are segregating in the material

[1] FN3PT INRA GRR AFB7 Kerplouz 56500 Ploudaniel France
[2] INRA UMR APBV Keraiber 29260 Ploudaniel France
[3] Biogemma Plants, Station de Lorlères Kerblaïs, Kerlou, 29260 Ploudaniel, France
[4] Station de recherche du Comité Nord, 29670 Kerebroc du nord Laoc, France
[5] INRA CF, Station de Lavergne, 63200 Lavillet, France
[6] FN3PT, Ploudaniel, 29260 Kerplouz, France

Modern fungicides in control of early and late blight in Polish experiments

J.S. KAPSA

Plant Breeding And Acclimatization Institute - National Research Institute Radzików
Department of Potato Protection and Seed Science in Bonin, PL 76-009 Bonin 3

KEYWORDS

potato, late and early blight, protection, fungicide efficacy

INTRODUCTION

Late blight caused by *Phytophthora infestans* is a very serious economic threat in the vast majority of potato production systems. Two recent studies put the total loss (direct and through fungicides) at between $3 and $5 billion per year (Judelson and Blanco, 2005; Haldar *et al.,* 2006). After *P. infestans*, *Alternaria* is the second most important foliar fungal pathogen of potato crops world-wide. Early blight occurs in many parts of the world but is a problem mainly under the weather warm and dry with short periods of high moisture (Venette, Harrison 1973). According to Johnson *et al.* (1986) and Fry (1994), maximum documented yield reductions in USA are usually 20-30%. In Polish climatic conditions there were recorded high regional losses caused by the early blight, however, most related to cultivars with recognized susceptibility to this disease. The use of fungicides is necessary in controlling both early and late blights, but it is important to use fungicides that effectively protect potato plants against the diseases.

MATERIALS

Twelve field experiments to compare the fungicide effectiveness against early blight (EB) and eight against late blight (LB) were set up in the years 2005, 2007-2009. Studies were conducted in the Department of Protection and Seed Sciences of PBAI- NRI in Bonin with the emphasis on:

Comparison of time of the incidence and severity level of the early and late blight of potato in two/ three different locations: trials with LB in Bonin and Mierzym (north-west region of Poland) and trials with EB in Bonin, Mierzym (north-west region of Poland) and Stare Olesno (south region of Poland).

Evaluation to rate efficacy of selected fungicides (including newly registered in potato) in limiting the development of the early blight and late blight.

Fungicides tested in the different studies are listed in table 1. All fungicides were tested in all experiments.

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Table 1. *Fungicides used in the field trials*

Fungicide	Active ingredient	Dose rate Kg or L / ha
Amistar 250 SC[1]	azoxystrobine	0,5
Infinito 687,5 SC	fluopicolide + propamocarb-HCl	1,2-1,6 l
Tattoo C 750 SC	propamocarb-HCl + mancozeb	2,0 l
Ridomil Gold MZ 68 WG	mefenoxam + mancozeb	2,0 kg
Acrobat MZ 69 WG	dimethomorph + mancozeb	2,0 kg
Tanos 50 WG	cymoxanil + famoxate	0,5-0,7 kg
Revus 250 SC	mandipropamid	0,6 l
Altima 500 SC	fluazinam	0,4 l
Ranman 400 SC TwinPack*	cyazofamid + (adjuvant)	0,2 + 0,15 l

1) not registered in Poland for potato crops
* not in trials to control EB

Field trials for early blight control were carried out in 3 localities (Bonin, Mierzym and Stare Olesno) on cv. Bard (susceptible to the disease). Three sprayings were performed throughout the growing season against EB, beginning with the occurrence of the very first symptoms of the disease on the experimental plots. The next sprays were continued with the same program on all plots against late blight.

Evaluation of fungicide efficacy to late blight control was performed in 2 localities (Bonin and Mierzym) on cv. Irga (very susceptible to the disease). Control of the LB began based on DSS NegFry system. Six to seven sprayings were applied throughout the growing season, with intervals 7-10 days and 10-14 days.

All trials were carried out in four replicates; each size plot = 25 m2. The field trials were carried out in accordance with GEP.

The criteria for pathogen infection pressure assessment were evaluated on untreated plots (control) and assumed to be foliar blight severity at the end of growing season and relative area under the disease progress curve (rAUDPC).

The criteria for fungicide effectiveness assessment on protected plots was assumed to be the percentage of disease severity at the end of growing season, efficacy of tested fungicides compared to untreated control, relative area under the diseases progress curve (rAUDPC), the diseases development rate defining the increase of destruction of above ground plant parts in unit time and also tuber yield and its healthiness.

The results were analyzed in a 2-factorial ANOVA, the factors being years of experiments and the fungicides applied.

RESULTS AND DISCUSSION

Results of pathogen infection pressure assessment are presented in tab 2 and 3. The observations carried out at Bonin, Mierzym & Stare Olesno revealed that both time of occurrence and severity of early and late blight differed and were dependent upon meteorological conditions and upon the year. Generally, early blight appeared earlier in the South of Poland (exception season 2008). It was connected probably not only with weather conditions but also with viruses' infections. In this area (the South of Poland) climatic conditions also favor greater infection pressure of the viruses. Virus infections enlarged additionally early blight pressure under Stare Olesno conditions. 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).

In the field experiments early blight occurred the earliest at Stare Olesno in 2009 (at 10th June). The highest disease pressure, described as rAUDPC was observed in season 2005 and 2007 in Bonin, in 2007 in Mierzym and in 2005 in Stare Olesno (tab.2).

Table 2. *Time of occurrence of the early blight in the years 2005, 2007-2008*

Years	Bonin			Mierzym			St.Olesno		
	Data of disease appearance	Infected leaf area - %	rAUDPC	Data of disease appearance	Infected leaf area - %	rAUDPC	Data of disease appearance	Infected leaf area - %	rAUDPC
2005	27.06.	50,0	0,68	29.06.	33,1	0,33	24.06.	57,9	0,41
2007	19.06.	95,3	0,63	18.06.	98,2	0,61	16.06.	99,5	0,19
2008	20.06.	91,7	0,28	26.06.	95,3	0,16	24.06.	98,0	0,16
2009	19.06.	95,3	0,14	04.07.	71,4	0,28	10.06.	98,6	0,30

Time of the natural late blight epidemic start depended upon the year and differed between years up to 35 days in Bonin and up to 34 days in Mierzym. The disease occurred the earliest in Bonin in 2009 (at 20th June), followed 5 days later in Mierzym. The late blight epidemic started relatively late in both locations in 2005. The highest pressure of the disease was noted in Bonin in the years 2005 and 2007 with the highest foliar blight severity at the end of the season and highest level of rAUDPC. The differences of these factors were not so clear in Mierzym (tab.3).

Table 3. *Time of occurrence of the late blight in the years 2005, 2007-2008*

Years	Bonin			Mierzym		
	Data of disease appearance	Infected leaf area - %	rAUDPC	Data of disease appearance	Infected leaf area - %	rAUDPC
2005	25.07.	97,1	0,68	29.07.	98,9	0,33
2007	29.06.	99,5	0,63	25.06.	73,8	0,61
2008	17.07.	93,9	0,47	03.07.	99,8	0,01
2009	20.06.	81,7	0,14	25.06.	98,6	0,28

Generally speaking meteorological elements and their course during a growing season are the basic elements affecting occurrence of the diseases in the field and variability of infection pressure of *P. infestans* and *Alternaria* spp.

The carried out trials showed also, that all fungicides limited the early blight development compared to the untreated control but at different level (Fig.1). The best results were obtained for Infinito 687,5 SC (dose rate 1,2 l/ha) and the "old" fungicides Altima 500 SC, Antracol 70 WG, Bravo 500 SC. In Polish experiences (12 trials in 3 locations), Amistar 250 SC and Revus 250 SC were the least effective in control of EB.
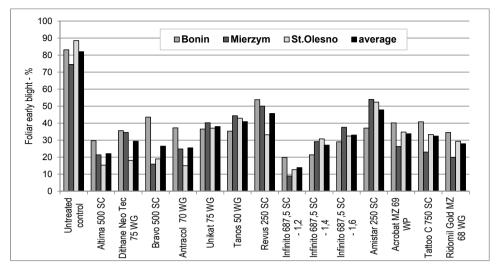
Fig.1. *Efficiency of selected fungicides in potato protection against early blight (12 field trials)*

The effectiveness of fungicides to control potato late blight epidemic for each experiments is given in table 4. The conducted trials showed that all fungicides, applied each 7-10 days, limited the LB development compared to the untreated control. Under climatic conditions in Mierzym, all tested fungicides revealed similar efficiency in inhibition of the late blight development, without any significant statistic differences. The lowest effectiveness in control of late blight was observed for Dithane Neo Tec 75 WG. In Mierzym, relative area under the disease progress curve (rAUDPC) for all fungicides was not too high and differences were not significant.

In Bonin the best effect of late blight control was observed after applications of fungicides Revus 250 SC and Infinito 687,5 SC; less effective in inhibition of LB development were Dithane Neo Tec 75 WG and Acrobat MZ 69 WP. The ranking of the fungicides' efficacy was very similar to the ranking based on rAUDPC index.

Similar good control of LB was observed when fungicides were applied each 10-14 days. Effectiveness of all tested fungicides was assessed above 80% (except for Acrobat MZ 69 WP in Bonin).

Over all years potato late and early blight were monitored as a destructive diseases, which caused significant yield losses due to premature defoliation.

The first priority for farmers and advisors is fungicide efficacy. Sometimes dose rates can be reduced in relation to weather conditions or cultivar resistance. In Poland it is not allowed to use lower dose rates than registered in the country.

Table 4. *Effectiveness of fungicides to control late blight during the whole season*

Treatment	Dose rate Kg, L /ha	Year	BONIN Foliar blight - %	Fungicide efficacy %	Rate of LB development	rAUDPC	Yield t/ha	Tuber blight - %	MIERZYM Foliar blight - %	Fungicide efficacy %	Rate of LB development	rAUDPC	Yield t/ha	Tuber blight - %
Unprotected control	-	2005	97,1		0.373	0,68	31,4	1,3	98,9		0.428	0,33	27,3	4,7
		2007	99,5		0.377	0,63	20,7	0,1	73,8		0.357	0,61	18,8	0,7
		2008	93,9		0.273	0,47	29,4	0,8	99,8		0.364	0,01	37,0	1,9
		2009	81,7		0.445	0,14	27,5	0,3	98,6		0.325	0,28	26,2	0,3
		mean	**93,1**		**0.367**	**0,48**	**27,3**	**0,6**	**92,8**		**0.369**	**0,31**	**27,3**	**1,9**
Altima 500 SC	0,4	2005	22,8	76,6	0.200	0,20	45,4	2,2	5,9	94,0	0.128	0,01	37,7	1,5
		2007	7,1	92,9	0.222	0,18	31,0	0,0	19,6	73,5	0.193	0,26	27,8	0,0
		2008	3,8	96,0	0.161	0,01	37,9	0,0	11,5	88,5	0.120	0,00	52,0	0,4
		2009	8,1	90,1	0.182	0,02	42,3	0,5	14,9	84,9	0.132	0,02	36,3	0,0
		mean	**10,5**	**88,9**	**0.191**	**0,10**	**39,2**	**0,7**	**13,0**	**85,2**	**0.143**	**0,07**	**38,5**	**0,5**
Dithane Neo Tec 75 WG	2,0	2005	57,9	40,4	0.248	0,39	42,4	1,5	32,1	67,5	0.219	0,05	35,5	3,5
		2007	29,7	70,2	0.280	0,31	25,9	0,0	36,5	50,4	0.232	0,38	23,0	0,0
		2008	2,9	96,9	0.225	0,01	37,2	0,7	37,6	62,3	0.177	0,00	46,0	0,7
		2009	28,7	64,9	0.225	0,04	37,5	0,5	33,1	66,4	0.177	0,06	37,7	0,0
		mean	**29,8**	**68,1**	**0.245**	**0,19**	**35,8**	**0,7**	**34,8**	**61,7**	**0.201**	**0,12**	**35,6**	**1,1**
Acrobat MZ 69 WP	2,0	2005	18,3	81,2	0.200	0,18	39,1	0,0	21,8	78,0	0.189	0,03	34,9	0,0
		2007	25,2	74,7	0.256	0,28	28,3	0,4	11,7	84,1	0.168	0,19	30,3	0,0
		2008	4,7	95,0	0.170	0,02	39,0	0,1	14,9	85,1	0.132	0,00	46,0	1,3
		2009	50,0	38,8	0.310	0,07	38,3	0,4	4,1	95,8	0.097	0,01	36,1	1,2
		mean	**24,6**	**72,4**	**0.234**	**0,14**	**36,2**	**0,2**	**13,1**	**85,8**	**0.147**	**0,06**	**37,1**	**0,6**
Ranman 400 SC	0,2+ 0,15	2005	4,7	95,2	0.128	0,05	46,6	0,5	11,7	88,2	0.159	0,02	44,1	2,1
		2007	13,9	86,0	0.364	0,34	27,4	0,0	11,7	84,1	0.206	0,27	28,9	0,3
		2008	1,1	98,8	0.187	0,00	40,6	0,0	14,9	85,1	0.132	0,00	47,6	0,0
		2009	18,3	77,6	0.237	0,03	38,1	0,0	21,8	77,9	0.155	0,08	39,0	0,0
		mean	**9,5**	**89,4**	**0.229**	**0,10**	**38,2**	**0,1**	**15,0**	**83,8**	**0.163**	**0,09**	**39,9**	**0,6**
Revus 250 SC	0,6	2005	2,9	97,0	0.128	0,04	45,1	0,2	25,2	74,5	0.189	0,04	41,3	4,9
		2007	9,5	90,5	0.233	0,21	34,5	0,0	17,4	82,5	0.151	0,02	41,7	1,6
		2008	3,9	96,0	0.216	0,01	39,3	0,0	18,3	81,7	0.144	0,00	45,0	0,0
		2009	4,1	95,0	0.168	0,01	41,4	4,2	8,7	91,2	0.120	0,01	38,8	0,0
		mean	**5,1**	**94,6**	**0.186**	**0,07**	**40,1**	**1,1**	**17,4**	**82,5**	**0.151**	**0,02**	**41,7**	**1,6**
Infinito 687,5 SC	1,2	2005	0,5	99,5	0.067	0,01	49,0	2,6	1,7	98,3	0.189	0,03	41,3	4,9
		2007	9,5	90,5	0.280	0,23	32,3	0,4	14,7	85,2	0.158	0,02	45,7	1,7
		2008	11,5	87,8	0.206	0,03	49,3	0,3	34,2	65,8	0.166	0,00	47,7	0,2
		2009	5,1	93,9	0.153	0,01	41,2	0,4	8,3	91,6	0.120	0,02	47,3	0,0
		mean	**6,7**	**92,9**	**0.177**	**0,07**	**43,0**	**0,9**	**14,7**	**85,2**	**0.158**	**0,02**	**45,5**	**1,7**
Infinito 687,5 SC	1,4	2005	0,7	99,3	0.055	0,01	47,6	0,7	1,1	98,9	0.128	0,01	35,8	0,8
		2007	8,3	91,7	0.211	0,17	35,8	0,0	8,0	91,7	0.123	0,01	40,9	0,3
		2008	1,1	98,8	0.152	0,00	40,4	0,0	18,3	81,7	0.144	0,00	40,0	0,0
		2009	4,1	95,0	0.138	0,01	45,3	0,5	4,7	95,2	0.097	0,01	46,9	0,0
		mean	**3,6**	**96,2**	**0.139**	**0,05**	**42,3**	**0,3**	**8,0**	**91,9**	**0.123**	**0,01**	**40,9**	**0,3**
Infinito 687,5 SC	1,6	2005	0,3	99,7	0.055	0,00	43,6	2,6	1,7	98,3	0.113	0,01	35,4	3,3
		2007	4,7	95,3	0.185	0,12	40,1	0,0	2,6	96,5	0.117	0,21	35,6	0,0
		2008	3,8	96,0	0.187	0,01	40,8	0,0	18,3	81,7	0.144	0,00	48,7	0,7
		2009	3,5	95,7	0.138	0,01	45,4	0,3	4,7	95,2	0.155	0,01	42,8	0,5
		mean	**3,1**	**96,7**	**0.141**	**0,04**	**42,5**	**0,7**	**6,8**	**92,9**	**0.132**	**0,06**	**40,6**	**1,1**
LSD (p=0,1) for treatments (mean of 4 year)			14,4	15,8	0.067	0.13	6,5	1,4	14,4	15,8	0.067	0.13	6,5	1,4
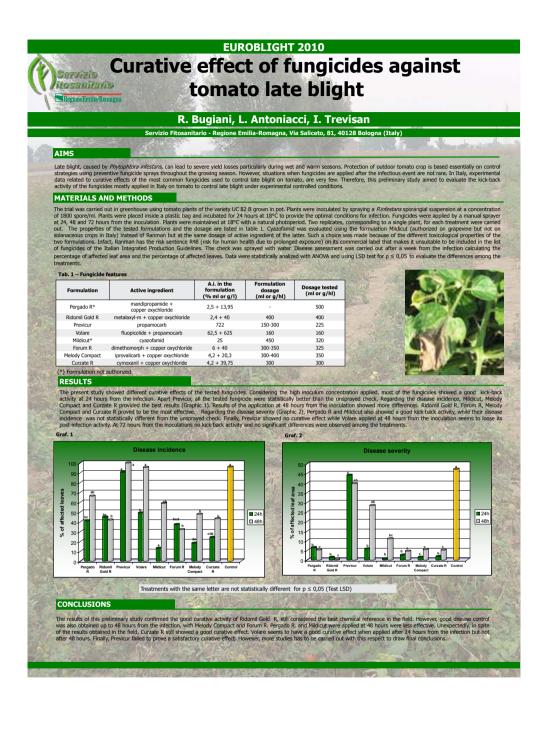
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Curative effect of fungicides against tomato late blight

R. BUGIANI, L. ANTONIACCI, I. TREVISAN

Servizio Fitosanitario -Regione Emilia-Romagna, Via Saliceto, 81, 40128 Bologna (Italy)

Posters

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R. Bugiani, L. Antoniacci, I. Trevisan

Servizio Fitosanitario - Regione Emilia-Romagna, Via Saliceto, 81, 40128 Bologna (Italy)

AIMS

Late blight, caused by *Phytophtora infestans*, can lead to severe yield losses particularly during wet and warm seasons. Protection of outdoor tomato crop is based essentially on control strategies using preventive fungicide sprays throughout the growing season. However, situations when fungicides are applied after the infectious event are not rare. In Italy, experimental data related to curative effects of the most common fungicides used to control late blight on tomato, are very few. Therefore, this preliminary study aimed to evaluate the kick-back activity of the fungicides mostly applied in Italy on tomato to control late blight under experimental controlled conditions.

MATERIALS AND METHODS

The trial was carried out in greenhouse using tomato plants of the variety UC 82 B grown in pot. Plants were inoculated by spraying a *P.infestans* sporangial suspension at a concentration of 1800 spore/ml. Plants were placed inside a plastic bag and incubated for 24 hours at 18°C to provide the optimal conditions for infection. Fungicides were applied by a manual sprayer at 24, 48 and 72 hours from the inoculation. Plants were maintained at 18°C with a natural photoperiod. Two replicates, corresponding to a single plant, for each treatment were carried out. The properties of the tested formulations and the dosage are listed in table 1. Cyazofamid was evaluated using the formulation Mildicut (authorized on grapevine but not on solanaceous crops in Italy) instead of Ranman but at the same dosage of active ingredient of the latter. Such a choice was made because of the different toxicological properties of the two formulations. Infact, Ranman has the risk sentence R48 (risk for human health due to prolonged exposure) on its commercial label that makes it unsuitable to be included in the list of fungicides of the Italian Integrated Production Guidelines. The check was sprayed with water. Disease assessment was carried out after a week from the infection calculating the percentage of affected leaf area and the percentage of affected leaves. Data were statistically analized with ANOVA and using LSD test for p ≤ 0,05 to evaluate the differences among the treatments.

Tab. 1 – Fungicide features

Formulation	Active ingredient	A.i. in the formulation (% ml or g/l)	Formulation dosage (ml or g/hl)	Dosage tested (ml or g/hl)
Pergado R*	mandipropamide + copper oxychloride	2,5 + 13,95	-	500
Ridomil Gold R	metalaxyl-m + copper oxychloride	2,4 + 40	400	400
Previcur	propamocarb	722	150-300	225
Volare	fluopicolide + propamocarb	62,5 + 625	160	160
Mildicut*	cyazofamid	25	450	320
Forum R	dimethomorph + copper oxychloride	6 + 40	300-350	325
Melody Compact	iprovalicarb + copper oxychloride	4,2 + 20,3	300-400	350
Curzate R	cymoxanil + copper oxychloride	4,2 + 39,75	300	300

(*) Formulation not authorized

RESULTS

The present study showed different curative effects of the tested fungicides. Considering the high inoculum concentration applied, most of the fungicides showed a good kick-back activity at 24 hours from the infection. Apart Previcur, all the tested fungicide were statistically better than the unsprayed check. Regarding the disease incidence, Mildicut, Melody Compact and Curzate R provided the best results (Graphic 1). Results of the application at 48 hours from the inoculation showed more differences. Ridomil Gold R, Forum R, Melody Compact and Curzate R proved to be the most effective. Regarding the disease severity (Graphic 2), Pergado R and Mildicut also showed a good kick-back activity, while their disease incidence was not statistically different from the unsprayed check. Finally, Previcur showed no curative effect while Volare applied at 48 hours from the inoculation seems to loose its post-infection activity. At 72 hours from the inoculations no kick-back activity and no significant differences were observed among the treatments.

Graf. 1

Graf. 2

Treatments with the same letter are not statistically different for p ≤ 0,05 (Test LSD)

CONCLUSIONS

The results of this preliminary study confirmed the good curative activity of Ridomil Gold R, still considered the best chemical reference in the field. However, good disease control was also obtained up to 48 hours from the infection, with Melody Compact and Forum R. Pergado R. and Mildicut were applied at 48 hours were less effective. Unexpectedly, in spite of the results obtained in the field, Curzate R still showed a good curative effect. Volare seems to have a good curative effect when applied after 24 hours from the infection but not after 48 hours. Finally, Previcur failed to prove a satisfactory curative effect. However, more studies has to be carried out with this respect to draw final conclusions.

The effect of late blight population changes on

on host resistance ratings

ALISON LEES [1], STUART CARNEGIE [2], DAVID SHAW [3] AND RUAIRIDH BAIN [4]

[1] Plant Pathology, SCRI, Invergowrie, Dundee, DD2 5DA, UK
[2] SASA, The Scottish Government, Roddinglaw Rd, Edinburgh, EH12 9FJ
[3] Sárvári Research Trust, Henfaes Research Centre, Llanfairfechan, LL33 0LB
[4] SAC, Ayr Campus, Auchincruive Estate, Ayr, KA6 5HW

Posters

The effect of late blight population changes on on host resistance ratings

Alison Lees[1], Stuart Carnegie[2], David Shaw[3] and Ruairidh Bain[4]

[1]Plant Pathology, SCRI, Invergowrie, Dundee, DD2 5DA, UK
[2]SASA, The Scottish Government, Roddinglaw Rd, Edinburgh, EH12 9FJ
[3]Sárvári Research Trust, Henfaes Research Centre, Llanfairfechan, LL33 0LB
[4]SAC, Ayr Campus, Auchincruive Estate, Ayr, KA6 5HW

E-mail: Alison.Lees@scri.ac.uk

Surveys of the composition of UK populations of *P. infestans* have revealed considerable changes in recent years, with a genotype designated as 13_A2 becoming dominant.

Anecdotal reports have also suggested that the historic late blight ratings for many existing potato varieties currently considered as having some resistance are not valid when these varieties have been challenged by isolates of genotype 13_A2.

Trials data from several sources has been combined in order to establish the reaction of these varieties to genotype 13_A2.

Materials and Methods

Data was made available from the following trials:
• SCRI, 2008 & 2009
• Sarvari Research trust at Llanbedrgoch 2008 & 2009, Henfaes, 2009
• SAC, 2008
• SASA, 2008 & 2009

Varieties and clones tested included commonly grown varieties, those undergoing Independent Variety Trialling and breeding and genetic resource material.

In general, a method similar to the agreed European protocol (www.eucablight.org) was used to assess host resistance and common reference varieties were included in all trials.

All trials were inoculated using an isolate of the 13_A2 genotype of *P. infestans*. In the SCRI and SASA trials artificially inoculated glasshouse-grown plants of cv. King Edward were placed at 1-2m intervals along infector drills of King Edward.

The percentage area of foliage of each test plant affected by late blight was assessed on at least four occasions during the epidemic. Scores were averaged and a 1-9 score calculated. An over-years and -trials analysis was conducted by Biomathematics and Statistics Scotland (BioSS) for varieties that were tested in at least two trials.
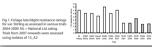
Results

There is evidence that some published foliar resistance ratings are inaccurate and these ratings fall into 2 categories:

Trials that have been conducted across Europe using a range of isolates including 13_A2 indicate that the original tests for some varieties may have over-estimated their degree of resistance. This discrepancy in ratings is therefore not related to the change in the *P. infestans* population.

In addition, some varieties that were originally determined to be resistant were found to be considerably more susceptible when challenged by 13_A2. An example is given in Figures.1. & 2., where the reaction of var. Stirling moves from an original published rating of 7 to a current rating of 4 when tested with 13_A2. Similar changes can be seen with, for example, Lady Balfour, Galactica and Romano (Fig 3)

Fig 1. Foliage late blight resistance ratings for var. Stirling as assessed in various trials 2004-2009. NL = National List rating. Trials from 2007 onwards were assessed using isolates of 13_A2

Fig 2. Late Blight symptoms on variety Stirling inoculated with isolates of 13_A2 and other genotypes

Fig 3a

Fig 3b

Fig 3c

Fig 3d

Variety Resistance Ratings

Results of trials conducted at the various sites using genotype 13_A2 are summarised in Fig3.

Each graph shows the rating from these tests compared with the previously published resistance rating for that group of varieties i.e. Fig 3a. shows the rating of varieties previously described as scoring 8 on the 1-9 scale etc.
In Fig 3d. varieties previously rated as either 5 or 4 are shown on the same graph.

Conclusions and future work

The published resistance ratings of some, but not all, cultivars appear to differ, markedly in some cases, from their reaction to the 13_A2 genotype which dominates the GB *P. infestans* population.

Sarpo Mira and Axona are amongst the most resistant varieties tested. Sarpo Mira was tested in all the trials whereas Axona was tested only in Wales. The varieties Toluca and Bionica were also very resistant with only hypersensitive lesions developing on the foliage.

Work to assess the current variety resistance ratings using isolates of the most common genotypes of *P. infestans* (including 13_A2 and 6_A1) will continue and changes in resistance ratings will be communicated to the industry.

Further information on protocols for late blight host resistance testing and results of trials conducted across Europe over several years can also be found via the Eucablight website: www.eucablight.org

The changing *Phytophthora infestans* population: implications for Late Blight epidemics and control

ALLISON CHAPMAN[1], ALISON LEES[1], DAVID COOKE[1] & LOUISE COOKE[2]

[1]Plant Pathology, SCRI, Invergowrie, Dundee DD2 5DA, UK
[2]Applied Plant Science & Biometrics Division, Agri Food & Biosciences Institute,
Newforge Lane, Belfast, BT9 5PX, UK

Posters

The changing *Phytophthora infestans* population: implications for Late Blight epidemics and control

Allison Chapman, Alison Lees & David Cooke
Plant Pathology, SCRI, Invergowrie, Dundee DD2 5DA, UK

Louise Cooke
Applied Plant Science & Biometrics Division, Agri-Food & Biosciences Institute, Newforge Lane, Belfast, BT9 5PX, UK

Introduction

In recent years there has been a change in the composition of the GB *P. infestans* population, with an increase in the A2 mating type being observed (Fig.1). This increase is largely due to the presence of the 13_A2 genotype, which was first identified in 2005. The proportion of 13_A2 isolates in the population increased from 12% in 2005 to 72%, 78% and 67% in the years 2007-2009 respectively.

Fig 1. Percentage of blight outbreaks in which A2 isolates of *P. infestans* were detected in GB between 1995 and 2009.

The dominance of the 13_A2 genotype of *P.infestans* compared with other genotypes may be due to a combination of increased aggressiveness, virulence and fungicide resistance, all of which are likely to make late blight more difficult to control.

For example, the ability of genotype 13_A2 to overcome host resistance in cultivars resistant to other genotypes of *P.infestans* is illustrated in Fig 2.

If some genotypes are found to have unusual phenotypic characteristics, such as the ability to infect at lower temperatures than previously thought, then this could have implications for disease forecasting and control strategies.

Fig 2. Plants of cultivar Stirling infected with isolates of genotype 13_A2 compared with isolates of genotypes 2_A1 6_A1, 8_A1 and 10_A2

Project Aims

Here we introduce a new study that will investigate the reasons why genotype 13_A2 is dominant in the GB *P. infestans* population by:

- Characterising the aggressiveness of isolates of 13_A2 compared with other genotypes
- Determining whether the temperature range at which *in vitro* growth, leaf infection and lesion development can take place is affected by genotype and whether there is an interaction with humidity
- Studying competition between isolates of various genotypes in laboratory and field experiments.

Ongoing and Future Experiments

Isolates - Fifty eight isolates of *P.infestans* collected between 2006-2008 from a diverse set of cultivars and geographical locations have been selected and characterised for genotype using SSR markers.

Aggressiveness - aggressiveness of these 58 isolates will be tested on five cultivars. Preliminary results using 7 isolates belonging to 6 genotypes tested on cultivar Craig's Royal are shown in Fig 3. This initial experiment shows that there are significant differences between isolates for aggressiveness. Conclusions regarding genotype differences cannot be made until all isolates have been tested.

Infection Efficiency - all isolates will be tested for infection efficiency and lesion development on detached leaves at 6°C, 8°C, 10°C, 12°C, 14°C, 16°C and 18°C using a temperature gradient plate incubator with a light/dark cycle of 16/8 hours respectively.

Competition Between Genotypes - Isolates belonging to various genotypes will be tested in competition with each other and the 13_A2 genotype *in vitro* and *in vivo* to elucidate the mechanisms of competition between genotypes.

Growth Rate - *In vitro* growth rates on Rye A agar plates incubated at 5°C, 10, 15°C, 20°C, 25°C and 30°C under a 16/8 hour light/dark cycle will be assessed. Early results showing differences in growth rates at 5°C are shown in Fig 4. Four of the 54 isolates tested did not show any growth at 5°C after 44 days incubation and growth of the remaining isolates was in a range 10–60mm. There does not appear to be a clear relationship between genotype and *in vitro* growth at 5°C. Results from additional temperatures and infection studies will allow a full analysis of genotype x temperature relationships to be made.
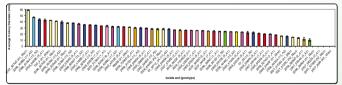
Fig 3. Mean lesion diameter (mm) of 7 isolates of *P. infestans* tested on detached leaflets of cultivar Craig's Royal after 7 days incubation at 15°C and 16 hour light/dark. Values are the mean of 30 leaves. Bars are coloured according to genotype and standard error bars are shown.

Fig 4. Average colony diameter (mm) of 54 isolates of *P. infestans* grown on Rye A agar at 5°C with a 16 and 8 hour light/dark cycle respectively for 44' days incubation. Bars are coloured according to isolate genotype and standard error bars are shown.