

## Infinito and protection against tuber blight – modes of action

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### SUMMARY

Bayer's late blight product Infinito® has become established in recent years as a key product in European late blight control strategies. This summary focuses on mechanisms explaining the tuber blight performance under field conditions. The impact of foliar applications of Infinito on spore inactivation and sporulation reduction in the canopy and its subsequent effect on expression of infections in tubers was studied in specific and general field trials. The trial results confirm the existence of direct sporicide activity of Infinito present at the canopy surface and anti-sporulant activity via reduction of lesion growth and spore production, resulting in solid protection against tuber blight.

### KEYWORDS

*Phytophthora infestans*, late blight, tuber blight, fungicides, sporicide, lesion growth, sporulation, fluopicolide, propamocarb

### INTRODUCTION

Late blight in potatoes, caused by the oomycete pathogen *Phytophthora infestans*, is an economically important disease in Europe threatening yield and quality of potatoes in storage. When not controlled adequately, late blight epidemics have the potential to destroy canopies (leaf blight) resulting in considerable yield losses. Late blight epidemics in the canopy also have the potential to infect tubers during the growing season (tuber blight), resulting in soft rot of potatoes either pre- or post-harvest. Tuber blight may unexpectedly result in severe losses during storage when latent infections become apparent and soft rot sets in.

Potato crops need adequate protection against late blight and protective fungicide spray programs are important to secure yield and yield quality. Fungicide spray programs usually consist of a number of fungicide products applied according to a certain timing strategy considering the risk of leaf and tuber blight. Bayer's late blight product Infinito® has become established in recent years as a key product in European late blight control strategies.

Infinito combines the activity of the active substances fluopicolide and propamocarb-HCl. Infinito has been presented at previous Euroblight Workshops<sup>1,2,3</sup>. Various characteristics have been

presented, including translaminar activity, impact on spore viability and sporicidal activity. Infinito has been evaluated in Euroblight leaf and tuber blight trials in the period 2006-2011, its protection level has been rated<sup>4,5</sup> and these ratings are included in the Euroblight fungicide table published on the Euroblight website. Infinito has been categorised in the Euroblight table as a 'systemic + translaminar' product.

The mode of action of Infinito against late blight has been shown to be multifold, turning Infinito effective whether late blight attacks occur in leaves, stems, tips or tubers. This summary focuses on the modes of action of Infinito against tuber blight under field conditions. Which mechanisms explain the tuber blight protection by Infinito?

The impact of foliar applications of Infinito on spore inactivation and sporulation reduction in the canopy and its subsequent effect on expression of infections in tubers have been studied in specific field trials conducted in The Netherlands and their results will be presented. In addition, reference is made to field trials in which the impact of Infinito spray sequences on tuber blight has been studied.

## **MATERIALS AND METHODS**

### *Spore inactivation*

Inactivation of spores was studied according to a protocol applied to three trials conducted in The Netherlands in 2005 and 2006. Trials consisting of 4 replicates and 15-30 m<sup>2</sup> plots were conducted at sites with silt soil types considered supportive for tuber infections, in regions without nearby potato production to minimize the risk of natural late blight epidemics.

Potato canopies (cv. Bintje) were completely protected against late blight with cover sprays (mancozeb) until mid-late flowering (BBCH 67), when a program of three experimental treatments started. Treatments were made at weekly intervals. A broadcast inoculation with sporangiospores was conducted 2 days after the third experimental treatment in the evening hours. The inoculation was followed by sprinkler irrigation simulating a 10 mm rain event the same evening and another 10 mm rain event the next day. The canopy was desiccated with diquat dibromide 1-2 days after the inoculation event. Potatoes were harvested 2-3 weeks after complete desiccation. Tubers from net plots (20 plants) were assessed for the presence of late blight infections and tuber blight was expressed as % incidence.

### *Sporulation reduction*

The effect on spore production was studied according to a protocol applied to one trial conducted in The Netherlands in 2009. The trial consisting of 4 replicates and 30 m<sup>2</sup> plots was conducted at a site with silt soil. The potato canopy (cv. Bintje) was completely protected against late blight with cover sprays (mancozeb) until mid-late flowering (BBCH 67), when a program of four experimental treatments was implemented. Treatments 2, 3 and 4 were made at 4-5 day intervals. Treatment 1 was made 12 days before treatment 2. Treatments 2, 3 and 4 were postponed as the leaf blight epidemic did not spread from infector rows to experimental plots. A broadcast inoculation with sporangiospores was conducted 6 days before the second experimental treatment to induce a late blight epidemic in the canopy.

Throughout the experimental period (except treatment dates), the canopy was sprinkler irrigated daily from 7:00 AM to 9:00 PM during 4 minutes per hour to maintain a humid microclimate supportive for disease progress. Sprinklers were used to simulate 10 mm rain events 1 day after treatment 3 and 4 days after treatment 4.

Leaf blight incidence was assessed at regular intervals to monitor the progress of the epidemic in each plot. For assessment of sporulation parameters, leaflets with a single lesion were collected randomly in each plot (25 leaflets per plot) 1-3 days after experimental treatment 2, 3 and 4. For each leaflet, lesion size was measured (in mm<sup>2</sup>). Leaflets were dipped in water to collect spores and spores were counted. Sporulation intensity (the number of spores per cm<sup>2</sup> lesion) was calculated from spore counts and lesion size. Spore production per lesion was calculated from sporulation intensity and lesion size. The canopy was desiccated with diquat dibromide 18 days after the last experimental treatment. Potatoes were harvested 2 weeks after complete desiccation. Tubers from net plots (15 m<sup>2</sup>) were assessed for the presence of late blight infections and tuber blight was expressed as % incidence.

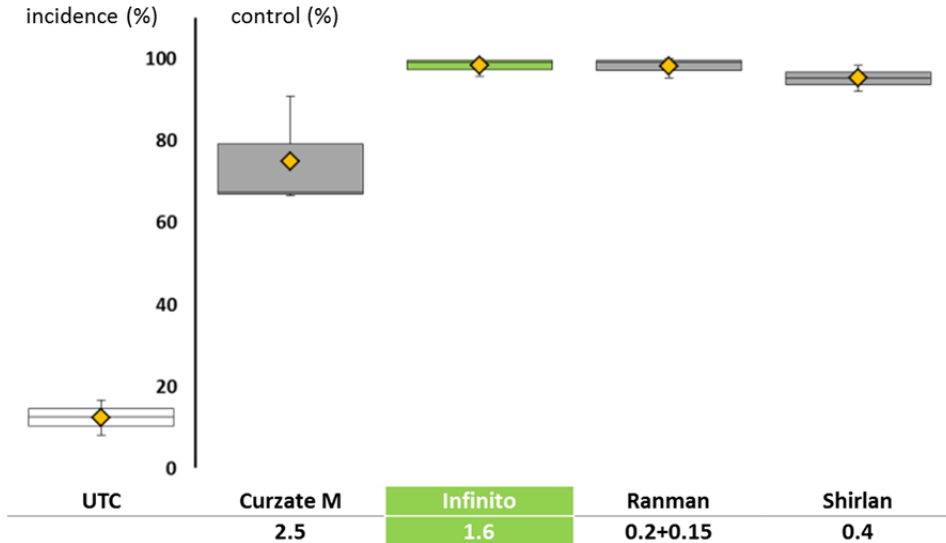
#### *Tuber blight control*

The performance of Infinito against tuber blight was studied according to a protocol applied to 21 trials conducted in the Netherlands in 2006-2011. Trials consisting of 4 replicates and 38 m<sup>2</sup> plots were conducted at various sites with silt soil types. A program consisting of 10-14 experimental treatments applied in sequence at approximately 7-day intervals was implemented from emergence complete until desiccation. In case a natural epidemic had not yet become apparent, late blight was introduced in the first week of July via inoculation with sporangiospores of individual plants in infector rows positioned in between replicates. Leaf blight incidence was assessed at regular intervals to monitor the progress of the epidemic in each plot. The canopy was desiccated with diquat dibromide 1-4 days after the last experimental treatment. Potatoes were harvested 2 weeks after complete desiccation. Tubers from net plots (15 m<sup>2</sup>) were assessed for the presence of late blight infections and tuber blight was expressed as % incidence.

## **RESULTS**

#### *Spore inactivation*

All trials conducted according to this protocol showed tuber blight incidence in the untreated control plots (UTC). Mean tuber blight incidence in untreated plots ranged from 8 to 17% in individual trials, resulting in a mean incidence level of 12% for all trials (figure 1). All treatments showed distinctively lower levels of tuber blight incidence compared to the untreated control. Mean control levels ranged from 75% for Curzate M to 95% for Shirlan and 98% for Ranman (+ surfactant) and Infinito. The reduction of tuber blight incidence by these treatments was consistent in all three trials.

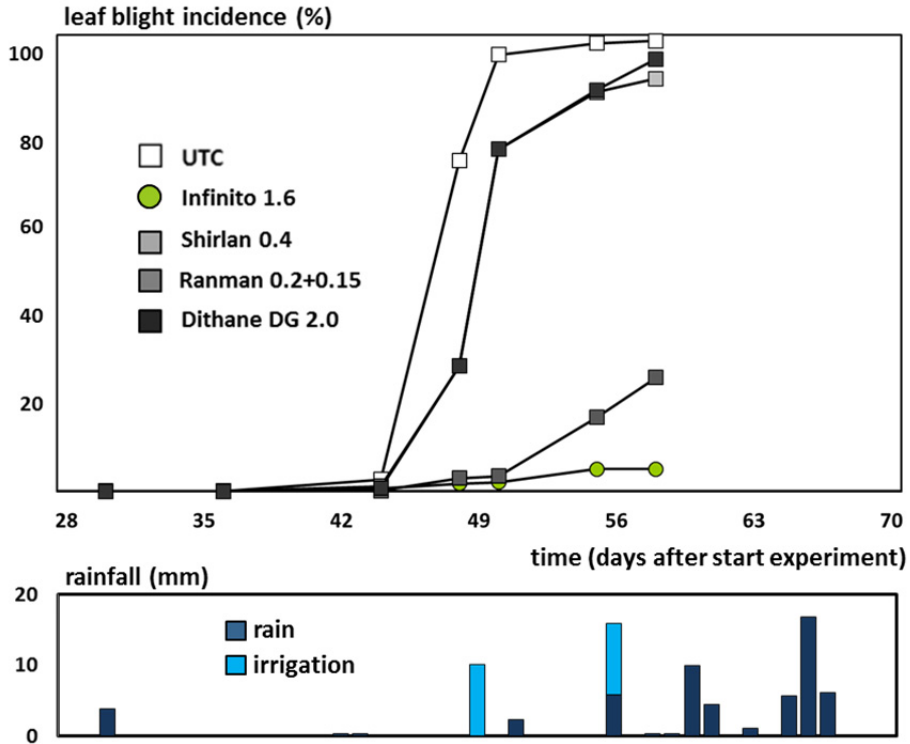


**Figure 1.** Tuber blight incidence (%) for untreated crops and tuber blight control (%Abbott) for treatments with means of 3 trials indicated by diamonds (◊)

#### Sporulation reduction

The progress of leaf blight incidence over time is shown in figure 2. Experimental treatments 1, 2, 3 and 4 were made on day 31, day 43, day 48 and day 52, respectively. Mean late blight incidence over plots was 0.048% (ranging from 0.003 to 0.1% incidence) on day 36, 1 day before the inoculation event (day 37), with no apparent relation with the experimental product applied at treatment 1. The inoculation proved successful and resulted in 2.6% incidence in untreated plots (range 2-4%) and 0.69% in treated plots (range 0.1-1.5%) on day 44, 6 days after the broadcast inoculation event and 1 day after treatment 2.

The broadcast inoculation set off a leaf blight epidemic which resulted in complete defoliation of the canopy in a period of 25 days, with an exponential phase in between day 44 and 49. The spray programs based on sequences of 4 treatments with either Dithane DG, Shirlan, Ranman (+ surfactant) or Infinito resulted in various levels of leaf blight protection. Leaf blight incidence ranged from 90-95% in plots treated with Dithane or Shirlan, to 20% in plots treated with Ranman and 5% in plots treated with Infinito.



**Figure 2.** Progress of late blight incidence in the canopy of untreated and treated crops (up) and occurrence of natural/ artificial rain events in time (down). Time expressed in days after start of the experiment (01.07 = 0)

Rain events were scarce in the main phase of the experimental period (day 28-60); natural rain events added up to 22 mm rainfall in total until day 60 and there was no relevant natural rain event. Sprinkler irrigations on days 49 and 56 were the only relevant rain events in the period until complete canopy destruction in the untreated plots. There were 5 rain events in the period from complete canopy destruction in the untreated plots until desiccation (day 60-70), adding up to 34 mm rainfall.

Late blight lesions present in plots treated with Infinito differed in appearance from lesions treated with contact fungicides. There was an apparent tendency that lesions in Infinito plots were sporulating to a lesser extent compared to lesions in plots treated with Dithane, Ranman or Shirlan (figure 3). Furthermore, it was apparent that lesions in plots treated with Infinito were extending at a slower pace compared to lesions in plots treated with contact fungicides (figure 4).



contact fungicides



Infinito

**Figure 3.** Appearance of late blight lesions in plots treated with contact fungicides (left) and Infinito (right)



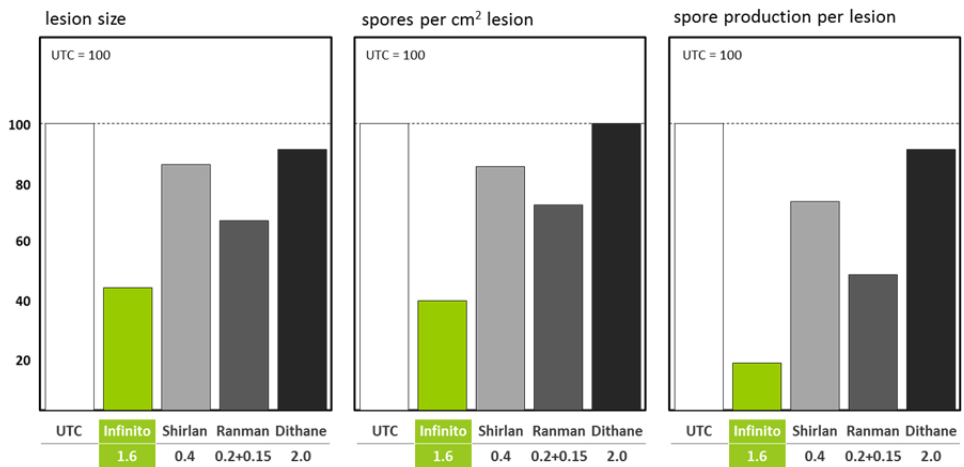
contact fungicides



Infinito

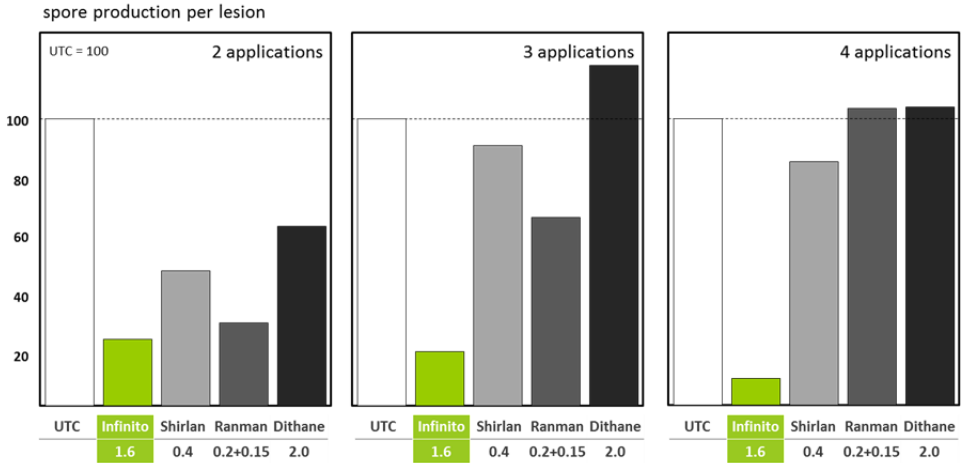
**Figure 4.** Growth of late blight lesions in plots treated with contact fungicides (left) and Infinito (right)

Sporulation parameters lesion size, sporulation intensity and spore production per lesion were assessed for each series of leaflets – collected after the second, third and fourth experimental treatment. Parameter means across the three samplings are shown in figure 5, with values for treatments shown relative to the value in the untreated control (indexed at 100). Mean lesion size was lowest in plots treated with Infinito. Treatments with contact fungicides also had an effect on lesion size when compared to the untreated control, but the effect was distinctively smaller. Sporulation intensity in plots treated with Infinito was also distinctively lower when compared to the untreated control and when compared to contact fungicides. As a consequence of both effects, the spore production by lesions in plots treated with Infinito tended to be significantly lower compared to the spore production by lesions in untreated plots and in plots treated with contact fungicides.



**Figure 5.** Sporulation parameters lesion size (in mm<sup>2</sup>), sporulation intensity (in spores per cm<sup>2</sup> lesion) and spore production per lesion. Parameter values relative to the values recorded for the untreated control (indexed at 100)

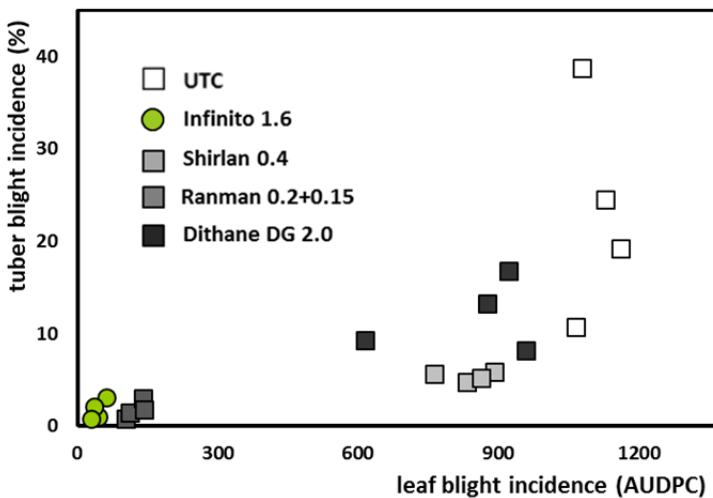
Figure 6 shows the evolution of spore production in time from the first to the third sampling, representing the impact of 2, 3 or 4 experimental treatments. The sampling after 2 treatments shows that all treatments had a considerable effect on spore production. The subsequent samplings show that the effect of Infinito on spore production per lesion persisted and improved after 3 and 4 applications, whereas the effect of contact fungicides disappeared.



**Figure 6.** Evolution of spore production in relation to the number of experimental treatments. Spore production means relative to the spore production in the untreated control (indexed at 100)

*Tuber blight control*

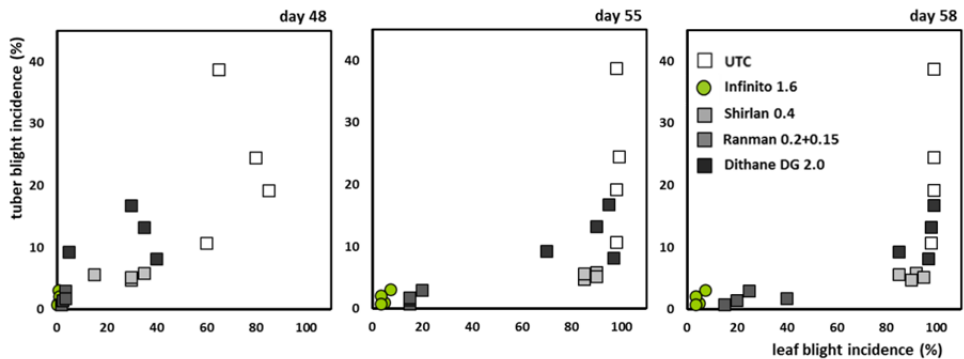
The relation between tuber blight incidence and leaf blight epidemics for individual plots is shown in figure 7. Infinito combined lowest leaf and tuber blight values. The figure also shows the consistency of products with acknowledged tuber blight properties in comparison to Dithane and the untreated control. The leaf blight epidemic resulted in 10-40% tuber blight in untreated plots (figure 7); mean tuber blight incidence in untreated plots was 23% (figure 9, left).



**Figure 7.** Relation between tuber blight incidence and overall leaf blight incidence in individual plots (left). Tuber blight incidence expressed in %, leaf blight incidence expressed in AUDPC

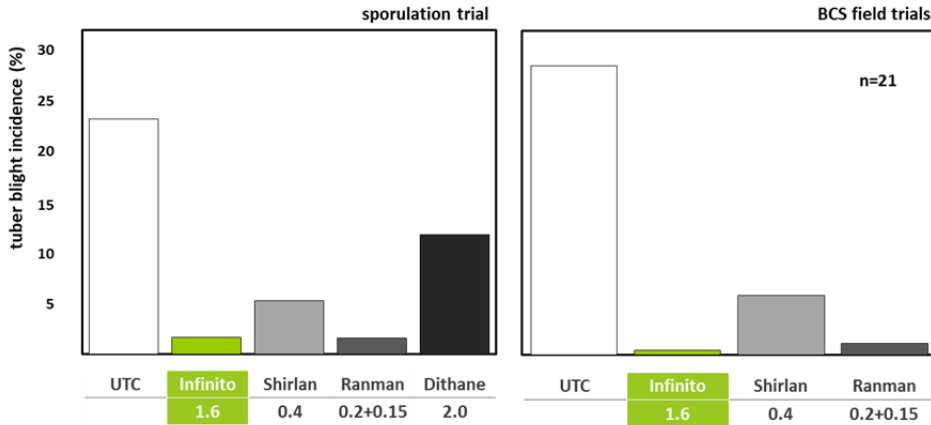


The relation between tuber blight incidence recorded after harvest and leaf blight incidence (%) at the days of irrigation and at the final assessment date is shown in figure 8. The separation of untreated plots from treated plots was established on day 48 already. Treatment separation had become apparent as well, but to a lesser extent compared to subsequent recordings (day 55 and day 58). On day 48, leaf blight incidence in treated plots ranged from 1.5 to 40% and the correlation between leaf and tuber blight incidence appeared to follow a linear trend rather than an exponential trend apparent in the plots for day 55 and day 58. Shirlan plots showed lower tuber blight incidence levels within the leaf incidence range of 10-40% compared to Dithane. Infinito and Ranman plots showed similar tuber blight incidences (0.5-2%) in a similar leaf blight incidence range (0.5-2%) which was however lower compared to the leaf blight incidence range for Shirlan and Dithane. On day 55, leaf blight incidence in plots treated with Dithane or Shirlan ranged from 70 to 90% whereas leaf blight incidence in plots treated with Ranman or Infinito ranged from 2-20%.



**Figure 8.** Relation between tuber blight incidence and leaf blight incidence at days with artificial rain events (day 48 and 55) and at the final leaf blight incidence recording (day 58). Tuber and leaf blight incidence expressed in %

Mean tuber blight incidence in the untreated plots was 23%. Mean tuber blight incidence in treated plots ranged from 12% for Dithane to 5% for Shirlan and 2% for Ranman and Infinito (figure 9, left).



**Figure 9.** Mean tuber blight incidence in the sporulation trial (left) and in (21) BCS trials conducted in The Netherlands in the period 2006-2011

The performance of Infinito against tuber blight in the specific sporulation trial was confirmed by the results of 21 field trials conducted in The Netherlands in the period 2006-2011, in which products were sprayed in sequence (figure 9, right). The average incidence level of tuber blight was 29% for untreated canopies, 6% for Shirlan, 1% for Ranman and 0.5% for Infinito.

## DISCUSSION

### *Spore inactivation*

Infinito has been reported to reduce the viability of sporangiospores produced in a canopy via translaminar activity<sup>1</sup>. Sporangiospores collected from the downward surface of leaves treated only at the upward surface proved significantly less viable and less infective compared to sporangiospores collected from untreated leaves or from leaves treated with contact fungicides. The translaminar activity of Infinito will contribute to protection against tuber blight when late blight is present as a source of sporangiospores within the canopy, but will not contribute when sporangiospores arrive from a nearby or distant source outside the canopy. This study was set up to study the contribution of the direct sporicide potential of Infinito present on the canopy surface to protection against tuber blight.

The trial set-up in the sporangiospores inactivation trials was designed to mimic the situation in which sporangiospores arriving from a source outside the treated canopy reach the tubers in the soil via rainwater dripping from leaves or running along petioles and stems towards the stem basis into the upper soil layer. In all trials, sporangiospores were brought onto the canopy by a single broadcast inoculation event. There was no sporangiospore source within the canopy in any of the trials as late blight epidemics had not become established before the inoculation event. The probability of inoculum pressure from other sources in the vicinity of the trial sites was minimized by selecting sites distant from potato fields. The inoculation event was likely to result in leaf infections but canopies were desiccated before lesions appeared. The consistency in tuber blight incidence patterns across trials supports the assumption that all trials mimicked the pursued situation.

It then appears reasonable to interpret the reduction of tuber blight in treated objects by direct sporicidal activity upon exposure of spores to the fungicides present on the surface of leaves and stems. The performances of acknowledged sporicide tuber blight protectant products Ranman and Shirlan vs. Curzate M fit to this interpretation. Under the field conditions of these tests, a sequence of 3 applications of Infinito demonstrated a high and consistent level of direct sporicide activity comparable to the performance of a similar sequence of 3 applications with Ranman.

#### *Sporulation reduction*

Infinito has been reported to reduce the formation of sporangiospores produced in a canopy via translaminar activity in in-vitro tests<sup>1</sup>. Lesions produced significantly less sporangiospores at the downward surface of leaves treated at the upward surface with Infinito 2 days after inoculation. The anti-sporulant activity of Infinito has been rated 2.5 at a 0-3 scale in the Euroblight rating table. The anti-sporulant activity of Infinito could be observed in regular field trials but had not been recorded and studied specifically in relation to tuber blight incidence.

The trial set-up was designed to mimic the situation in which sporangiospores arrive from a source outside the treated canopy and initiate a leaf blight epidemic in the canopy. In this set-up, tuber blight might have been initiated by the sporangiospores brought into the canopy at the broadcast inoculation event and by the sporangiospores produced in the canopy during the leaf blight epidemic. Different from the set-up in the spore inactivation trials, the broadcast inoculation was not followed by irrigation as it was not intended to wash down inoculum to the soil. Sporangiospores may nevertheless have reached the soil at the inoculation event, and direct sporicide activity of the products applied may have had an impact on the tuber blight protection levels recorded.

The broadcast inoculation event was not intended in the original set-up. Infector rows were considered as the source of inoculum for treated plots but late blight did not develop sufficiently in the infector rows during a spell of dry weather. The first experimental treatment had already been made when weather conditions turned unfavorable for late blight. The second experimental treatment was postponed to allow the epidemic in the infector rows to gain momentum. As the intended inoculum source appeared ineffective for the purpose of the trial, it was decided to perform a broadcast inoculation event.

Late blight was present in treated plots at very low incidence levels by the time of the broadcast inoculation event, without an apparent relation between incidence level and the experimental product applied at treatment 1. The broadcast inoculation event resulted in a distinct increase of late blight incidence recorded 7 days after inoculation. The difference in late blight incidence between untreated and treated plots points at the residual activity of the products 6 days after treatment. Differences between experimental products were smaller and did not reveal an apparent advantage for Infinito at the start of the exponential phase of the leaf blight epidemic.

Infinito nevertheless proved to protect the canopy better than the other products tested when the epidemic progressed in the exponential phase. The distinct differentiation in performance against leaf blight is not helpful for the evaluation of tuber blight performance as there is a logical relation between the presence of leaf blight in the canopy and tuber blight risk. The question whether the tuber blight performance of Infinito is explained by its sporicide and anti-sporulant activity cannot be answered as the treatment sequence with Infinito resulted in the lowest incidence levels in the canopy quickly after the onset of the exponential phase of the epidemic. If one considers the leaf blight epidemic it is fair to state that the products perceived as tuber blight protectants all delivered distinct prevention of tuber blight.

The occurrence of tuber blight is considered to depend not only on the presence of inoculum but also on the occurrence of rain events necessary to direct sporangiospores towards the soil. The

main events responsible for the transport of sporangiospores via water were the sprinkler irrigation events by which 10 mm rain was simulated as natural rains were scarce and short-lasting. Sprinklers were also switched on every day for 4 minutes per hour (14 times per day) to maintain moisture in the canopy. The amounts of water supplied by these short irrigations are believed to be too small to be contributive to sporangiospore transport. They do support expression of tuber blight however as soil moisture is maintained at a level optimal for spore survival in the soil. Tuber blight incidence in this trial proved supportive to separate products according to performance.

Leaf blight incidence in the canopy at specific rain events might show a better correlation with tuber blight incidence than overall leaf blight incidence expressed by AUDPC. It would be recommendable to collect tuber samples after each rain event and relate the tuber blight incidence in these samples to the leaf blight incidence on that specific day. The final level of tuber blight incidence will consist of the inoculum flushes delivered by all rain event and the capacity of sporangiospores to survive in the soil and to reach the tubers.

The sporulation parameters all underline the anti-sporulant activity of Infinito which has been shown to depend on the activity of both fluopicolide and propamocarb HCl<sup>1</sup>. The evolution of sporulation reduction in response to the sequence of treatments suggest that the anti-sporulant activity of Infinito accumulates when the product is applied in a spray sequence. In this trial, treatments 2, 3 and 4 were applied at rather short 5-day intervals. The accumulation of the anti-sporulant activity of Infinito has been observed frequently in regular field trials in which Infinito was applied at 7-day intervals and is considered to contribute to the overall strong performance of Infinito against late blight in potatoes.

#### *Tuber blight control*

The tuber blight findings in the specific trials matched perfectly to the tuber blight performance of Infinito recorded in 21 regular field trials conducted in a period of 6 years, thus underlining the potential of Infinito to protect tubers very effectively against infection by late blight inoculum whichever the source.

## **CONCLUSION**

The data presented in this summary confirm the existence of direct sporicide activity of Infinito present at the canopy surface as well as anti-sporulant activity via reduction of lesion growth and spore production. Both can be considered as modes of action explaining Infinito's solid protection against tuber blight.

## **ACKNOWLEDGEMENTS**

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