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Preface

EuroBlight Workshop Limassol, Cyprus 12-15 May 2013

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 14th Workshop was hosted by the Cyprus University of Technology in Limassol, Cyprus. The Workshop was attended by 94 persons from 16 European countries, Russia, Chile, Argentina, China and Israel. Representatives from all countries presented the late blight epidemic in 2012 and recent research results regarding integrated control, decision support systems, resistance of varieties, late blight in organic potatoes and population biology of the late blight pathogen. Since early blight is an increasing problem in Europe reports on this disease are also included.

The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, PPO-Special Report no. 16. The current and previous Proceedings are also available on the EuroBlight website www.EuroBlight.net.

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Papers

The development and control of Late Blight (*Phytophthora infestans*) in Europe in 2012

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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. This paper reports the development and control of late blight in Europe, 2012.

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 between institutions and across countries.

At the workshop in Limassol attention was drawn to major issues of relevance to policy making in Europe i.e. the rapid changes in *P. infestans* populations causing late blight in Europe, America and Asia, including the emergence of strains with altered pathogenicity or reduced fungicide sensitivity. Constant monitoring of populations and characterization of invasive genotypes in order to understand and predict changes is a prerequisite for the deployment of IPM strategies, as required by Directive 2009/128/EC on the sustainable use of plant protection products. It directly influences the development and deployment of resistant cultivars, the performance of disease warning systems and the efficacy of plant protection products. A coordinated and continuous monitoring effort was suggested and included in the EuroBlight statement produced after the meeting (see www.euroblight.net). Subsequently, an initiative was launched aiming to collect 1000-1500 late blight samples from the main potato growing regions in Europe. The goal is to capture as much genotypic variation as possible by sampling as many fields as possible. Samples are analysed using standardised 12 multiplex EuroBlight SSR genotyping. The results will provide insight in the international, national and regional structures of the *P. infestans* EU

population through e.g. the proportion (and dynamics) of the different currently known clonal lineages (e.g. Green33, Blue13, Pink6 etc.) and unique genotypes in the population. This paper reports the development and control of late blight in Europe, 2012 and thereby describe the foundation for the further insight in the structure and behaviour of the European *P. infestans* (meta) population.

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 only slightly edited.

THE DEVELOPMENT AND CONTROL OF *PHYTOPHTHORA INFESTANS* IN EUROPE IN 2012

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 2012 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. The weather based risk at selected stations in Europe is shown in Fig. 4. The level of tuber blight attack is given in Fig. 5 and problems with tuber blight is shown in Fig. 6.

Belgium 2012

About two thirds of the ware crop acreage was planted relatively early, between March 23 and April 8. Due to excessive rains during rest of April, the remaining third of the crop was planted relatively late, in the second half of May and the first week of June. These late planted potatoes were exposed to a high risk on late blight attacks. After all, early attacks were present on dump piles and very early crops from the second week of May, threatening the emerging and highly sensitive young plants. Moreover, the continuous changeable weather remained favourable for the disease. This was also the case during the very wet months of June (wettest since 1981) and July. Despite the short average spraying intervals – 6 days in June and only 5 days in July – late blight attacks became widespread in the fields. Dry and sunny weather from the beginning of August led to a considerable drop in disease pressure. Conditions became eventually too dry for harvesting and caused very high dry matter content. When the rain finally came, it brought a record wet month of October and difficult harvesting conditions. Nevertheless, the level of diseased tubers turned out to be very low.

Czech Republic 2012

Potato late blight had a very diverse development in the individual localities of the Czech Republic, based on weather progress and/or precipitation amounts; however, generally the disease occurrence could be considered as a moderate one. The year 2012 was characterized by delayed onset of epidemic disease spreading, moderate infection pressure and low tuber infection. First outbreaks were already found in the second decade of June; however, they were of a local character and late blight only spread in the surroundings of primary sources. Torrential rains with subsequent sunshine and rapid crop drying were not sufficient for intensive disease development and high temperatures in the end of July stopped further development. Precipitation in July, especially in the second half of the month, resulted in surface spreading of late blight and epidemic onset. It also continued in August; however, in the second decade of the month epidemic was broken by short drought and extreme high temperatures. Precipitation and temperature decline toward the end of the month and in September contributed to recovery of further disease spreading. Tuber infection occurred, but mostly only locally and to lower extent. Seed crops due to early vegetation ending were not significantly affected by late blight and tubers were not infected at all, when the infection was not caused through re-growths. In ware potatoes problems only arose, where last treatments were neglected, vegetation was not ended in time or foliage was in touch with active late blight at harvest. Generally, disease management in practice was relatively good. Fungicide control had lower yield effect than usually due to delayed epidemic onset and differences in efficacy of individual fungicides were relatively small.

Cyprus 2012

Potato cultivation in Cyprus is concentrated in two crops. The main one is the spring crop, planted during November/January and harvested during April/May and early June, and the winter crop that is planted in July/August and harvested in November/December. In October, November and partly in December of 2011 short showers made conditions favourable for blight infections of the winter crop that was close to harvest, thus that crop suffered moderately tuber blight. Since the conclusion of the winter crop is overlapping with the initiation of the spring crop, *P. infestans* inoculum has a continuum, mainly in escapes, since the production area is relatively concentrated. In 2012, the majority of the crops at the coastal line of the Kokkinochoria region were set in mid-November and the first blight hits were recorded in mid-February. Nevertheless, the disease progression was slow, due to several dry spells the following

months. A short rainy period close to harvest caused some tuber blight problems. No DSS is available in Cyprus and the common practice for potato late blight management is the empirical application of fungicides, which may lead to up to twelve sprayings during the spring crop. Months from May until early October were very dry.

Denmark 2012

The spring 2012 was characterised by high amounts of precipitation, the planting of the potatoes was delayed until end of April and first days of May. The soil temperature remained low for an extended period and the potatoes stayed for a long time in the soil before germination. There were no indication of infection from oospores and the incidence and severity of blight remained low during most of the season due to low temperatures, despite the very humid conditions with relatively high risk indications. In the northern part of Jutland, there were some severely damaged fields in both ware and starch potatoes which were initiated from a potato cull pile and non-protected late, planted fields. The main control strategy was based on mancozeb, mandipropamid, cyazofamid and metalaxyl. Metalaxyl had limited effect in the Northern part of Denmark due to the widespread occurrence of the late blight isolates blue 13.

England & Wales 2012

Planting in 2012 was delayed in many regions due to poor weather. Cooler temperatures in April and May delayed crop emergence. Many regions received 50% more rainfall than the long term average in May, June and July. Waterlogging and flooding were common during the entire season and caused severe crop loss in some cases. Two hundred and fifty-four outbreaks of late blight were reported in 2012 as part of the Potato Council funded outbreak maps, with the earliest reported on 2 May in the South West (Cornwall). Five outbreaks were reported in May, 21 in June, 183 in July, 34 in August, 6 in September and 1 in October. Severe cases of late blight meant some crops were desiccated early. Agronomists were routinely finding lesions in commercial crops. Achieving complete control of late blight was difficult. Late planting and slow emergence meant some crops were infected as they emerged or soon after. According to the UK pesticide usage survey report 235 using 2010 figures, the most frequently applied active ingredients to ware crops were fluazinam, mancozeb/cymoxanil, cyazofamid, mandopropamid and fluopicolide/propamocarb-hydrochloride. For seed crops, the most frequently applied active ingredients were fluazinam, cyazofamid, cymoxanil, mandipropamid and mancozeb/cymoxanil.

Estonia 2012

Due to late spring there was only short time difference between planting, development and late blight infection of early and main-crop potatoes. After the very low incidence of late blight in the previous year, the late blight established very late in 2012. The dry weather in first half of growing season did not favour the development of late blight. The weather conditions were more favourable for development of early blight than for late blight. Also leaf blotch of potato, caused by *Botrytis cinerea* caused essential damages in potato foliage. The weather changed in mid-July, when intensive rains occurred in northern and western parts of Estonia and favoured infection. First late blight attacks were recorded on July 14. The weather in central and southern parts of Estonia remained dry until the end of first decade of August. Rains covering whole Estonia since mid-August created very favourable conditions for late blight. Infected potato foliage was destroyed within a week in these conditions. The new network consisting of 13 iMetos stations was established in collaboration of Jõgeva PBI and farmers' cooperative Talukartul for DSS in late blight control. Use of DSS saved 1-2 fungicide applications in average.

Finland 2012

First late blight observations in conventional crop were reported simultaneously at several sites in the middle of July. This is approximately two weeks later than in average during 2000s in Finland. From the end of July to the harvest there were very heavy rains and severe floods at certain regions. At those regions farmers were not able to spray against late blight and many fields were fully destroyed by blight, flood and bacterial rots. However in regions with more moderate rainfall late blight was in good control and majority of potato crop was not severely attacked. Tuber blight was no specific problem but at very rainy regions pink rot (*Phytophthora erythroseptica*) and bacterial rots caused considerable losses.

Germany 2012

Crops were planted in good conditions. The crop emergence was normal 15 to 25 May. The first outbreak of late blight in potatoes was end of April (very early) in plastic covered fields. Attacks in different regions and ware potatoes were found in June. The weather conditions for the development of late blight was high in the North and moderate in the South. The number of fungicide treatments was normal in 2012. All kind of products were used. Attacks of *Alternaria* seems to be an increasing problem in the east and southern part of Germany.

Republic of Ireland 2012

Following a warm and dry spring most crops were planted in good conditions. As the summer months were one of the wettest on record those planted later suffered as they were developing in epidemics. The extremely wet conditions led to high disease pressure being experienced throughout the country, with the highest experienced in the south-west where 600 mm of rain fell in between May and August. Under these conditions control was difficult and was reliant on routine fungicide applications, often involving mixtures of fungicides (including actives with good curativity) and with short intervals between applications. Although rainfall was well above average in June, due to the slightly colder weather disease epidemics did not occur until mid-July. Although there was high disease pressure and the presence of disease in crops no major problems with tuber blight were recorded. No information is available as to the genetic structure of the population in 2012.

Lithuania 2012

Overall weather conditions in 2012 were conducive to the spread and development of late blight disease in potato crop. Unusual warm and wet July had significant influence on very high potato crop yield. Most farmers without irrigation managed to obtain about 40.0 t/ha potato yield. By the average 4 - 6 fungicide application was needed for late blight control. Crop rotation and fungicide application are most popular tools for reducing outbreaks of the disease. *Alternaria* is still a very rare disease in Lithuania. Decision support systems are not used by farmers at all.

The Netherlands 2012

After a dry period in March potato were planted rather early at the end of March until the beginning of April. Crop emergence was not that early (half of May) because of moderate temperatures afterwards. The weather conditions after emergence were not very favourable for late blight. First outbreaks were reported between 20 and 25th of June in the south-east region of the country. The disease pressure during the months July and August was high because of the rainy weather conditions during these summer months. Thanks to a frequent use of fungicides there was hardly any infestation at harvest of the ware potatoes.

Northern Ireland 2012

After a slow start (April was unusually cold and both April and May had below average rainfall), the weather favoured blight from the third week in June and the first outbreak was confirmed on 22 June. Blight was subsequently identified in crops throughout Northern Ireland, but mostly at low levels. Despite conditions apparently very favourable to infection, blight was not as severe as might have been expected, possibly because crop growth was very poor due to the cool weather and lack of sunlight. Growers were mostly very diligent in applying fungicides using a wide range of products and blight was generally well-controlled in both foliage and tubers.

Norway 2012

A dry spring resulted in little late blight in the early potatoes; the first attacks came in the middle of June. The weather was late blight favourable from mid-June and onwards, this resulted in infections 2-3 weeks earlier than normal in the main crop. Infected seed tubers were probably the main cause of primary infections. On average the potato fields received eight fungicide applications. About 50 % of the fields had leaf blight in early August, but at low levels. The precipitation was very high during the whole season. However it was not reported much more tuber blight than normal. Typically one treatment with Ridomil or Tyfon is used early and then Ranman or Revus. 80 % of the treatments were carried out by these two products. In Norway the decision support system for potato late blight is available for free at www.vips-landbruk.no and consists of four parts - A map of the blight attacks found, the Negative prognosis to predict the first fungicide application and Førsund's rules and a new late blight model to predict days with high risk of blight infections. The system is used both by the advisory service and by farmers.

Poland 2012

The date of crop emergence was relatively early, 10-20 May, despite April and May were dry. Indications of oospores were found on a trial site (in the North) and in a few other fields (central Poland). First attacks of late blight were recorded on 10 June in central region. In many regions optimal conditions for the first appearance of *P. infestans* were observed in the first half of June (central and southern part of Poland) and in the second half of June (northern regions). Weather conditions in the next months didn't favour development of the disease and very often the total stopping its development was recorded. The second outbreak of late blight was observed in the second half of July. Weather conditions did not favour infection pressure and rate of the disease development wasn't very high. The active ingredients used on the largest areas were mancozeb, metalaxyl-M+mancozeb, fluopicolide+propamocarb-hydrochloride and fenamidone+propamocarb-hydrochloride. The farmers applied 1-9 sprays, the most often 1-4. The yield in 2012 was relatively high and the level of tuber blight was very low. *Alternaria* was observed early in the season on 28th May in west-southern region of the country and caused some problems during all growing season.

Russian Federation 2012

A severe late blight development was observed on potato fields of the Kaliningrad, Pskov, Leningrad, Novgorod, Tver, and Smolensk regions (yield losses exceeded 20%). A moderate disease development was observed in Arkhangelsk, Vologda, Murmansk, Kirov, Moscow, and Bryansk regions. On the other territory of the European part of Russia, the disease development was rather weak. The main source of the primary infection was infected seed tubers. The most popular fungicides were Tanos, Shirlan, Infinito, Ridomil Gold MZ, and Acrobat MZ. The total number of treatments varied from 2 to 10. Owners of allotment gardens did not use any

fungicides. In Russia the DSS VNIIFBlight is available for free at www.kartofel.org. This system is used by some advisory services and the owners of small potato farms.

Scotland 2012

One hundred and twenty-five confirmed outbreaks in Scotland were reported on the Potato Council-funded blight outbreak maps up until the 26th of November. The progression of crop outbreaks (119 in number) was 0% in May, 1.7% in June, 70.6% in July, 26.1% more in August, 0.8% in September, 0% in October and 0.8% in November, up to the 26th. There were five confirmed outbreaks on outgrade piles of potatoes (30 May, 30 June (x 2), 11 and 12 July) and one outbreak on volunteers (23 August). 2012 was an exceptional season. Several factors combined to make blight control particularly difficult. Planting was late, with some growers still planting in July. Prolonged heavy rain in late June into early July made fungicide application difficult for many weeks with late starts to the fungicide programme and extended intervals. For many crops the prolonged wet weather coincided with rapid canopy development. The season was wet with 129, 120, 98, 103 and 135 mm of rain recorded in June, July, August, September and October at Auchincruive. During the growing season conditions were generally overcast and cooler than the average. The cost of the blight fungicide programme was considerably greater than normal. Application was more difficult, e.g. for some crops rows of potatoes were sacrificed to allow tractors/sprayers fitted with wider tyres to apply fungicides. Fungicides with curative activity were particularly effective in the cooler temperatures. Harvest was very difficult and protracted. Some crops were not harvested or parts of fields were left not harvested. A few crops were harvested in the spring of 2013.

Sweden 2012

The spring was warm and dry in most of Sweden 2012 resulting in good conditions for planting. The first blight reports in 2012 came 23rd of May from a covered early potato field on the South west coast. A few reports of attacks on dump piles and in organic potato fields came in late June. In July, attacks of late blight were reported from all over the country, but the situation was in most cases less severe than in 2011. The rainfall during 2012 was very unevenly distributed both geographically and over time. Some areas in the South had good or even dry conditions while other areas further north had difficulties getting out in the fields to spray resulting in difficulties to control late blight. In addition, the harvest conditions was very bad in these areas.

Switzerland 2012

Weather conditions during the potato growing season 2012 were very favourable for late blight. Though the onset of the season was rather dry during March and April, it started to rain at the beginning of May and several MISPs (main infection and sporulation period) were registered in all parts of Switzerland. Two first late blight attacks were observed early on May 9 and 11 in two potato fields covered with fleece in the south-western part of Switzerland. This part is isolated by high mountains and the Lake of Geneva, therefore these attacks were of no significance for all other potato growing regions. On May 18 and May 22 three further late blight attacks were observed. During the first two weeks of June, it was very rainy and our decision support system PhytoPRE registered up to seven consecutive MISPs for almost all MeteoSwiss weather stations. So, weather conditions were very favourable for the development of late blight and the number of observed late blight attacks almost exploded until the end of June. During the first two weeks of July and August, weather conditions were again very favourable for the development of late blight and late blight spread over the potato growing regions. Only the two last weeks of August were very warm and dry. Even though it was difficult to conduct fungicide applications accurately

timed, plant protection officers informed us, that farmers could control late blight well. As 2011, late blight attacks which were registered in our DSS PhytoPRE were mainly from untreated monitoring plots, potatoes planted in gardens or from fields with insufficient fungicide protection. Number of announced attacks was comparable to the year 2009 (2008: 224, 2009: 95, 2012: 102).

EARLY ATTACKS OF LATE BLIGHT

In North-West Europe, early attacks of late blight is often found on dump piles or in potatoes covered with plastic. In 2012 the first outbreak of late blight was recorded on the south of Germany end of April (very early) in plastic covered fields. Widespread attacks in Germany were found Mid-June. This is 1½ month later in the season, indicating that the very early attacks were not initiating widespread attacks in commercial fields. Early attacks in late April or early May was recorded in UK South West (Cornwall), Belgium, France, Germany and Switzerland. In Sweden the first blight reports in 2012 came 23rd of May from a covered early potato field on the South west coast. In most other regions of Europe early attacks were recorded in June (Fig. 1 and Fig 3). Widespread attacks in conventional fields in Europe were found in June in the central part of Europe and in July in the Northern part of Europe (Fig. 2 and Fig. 3). Oospores were mentioned as possible source of inoculums in the reports from Estonia and Poland.

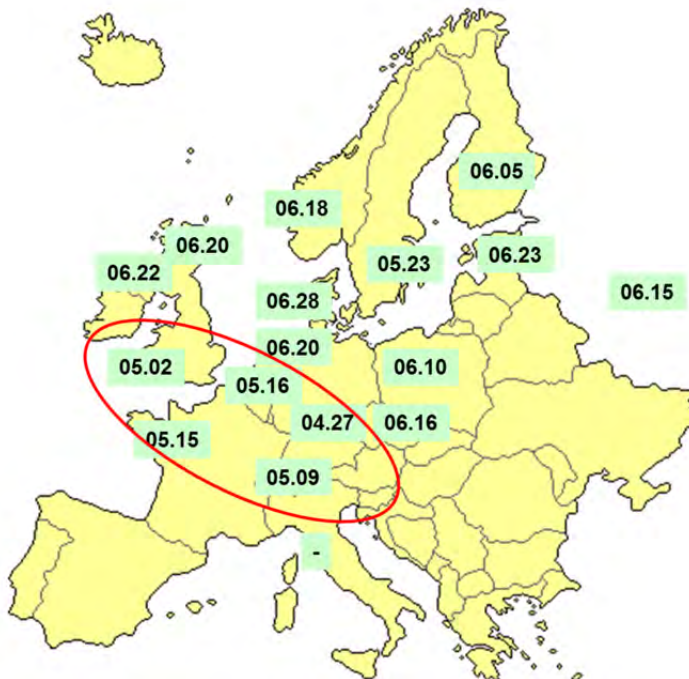


Figure 1. Date of first observation of late blight in covered or very early planted potatoes, 2012

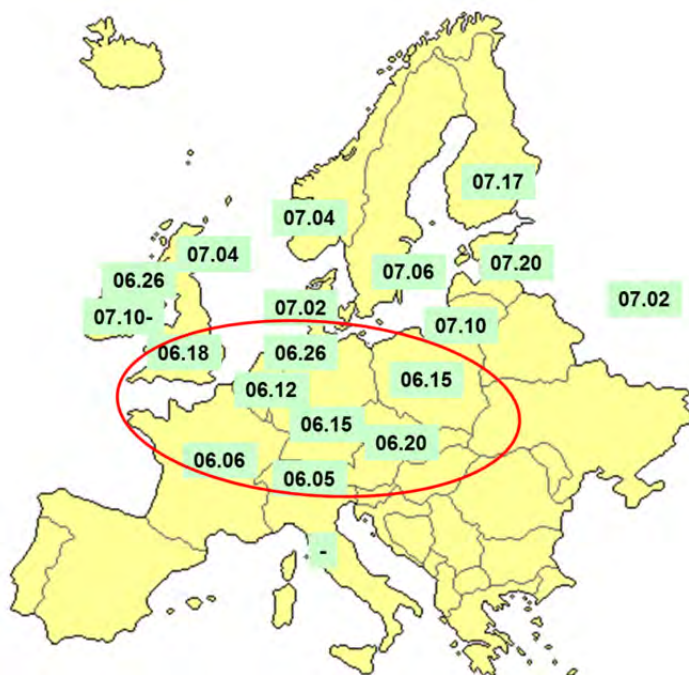


Figure 2. Date when first infections were reported in more than 5 conventional, normally planted potato fields in 2012

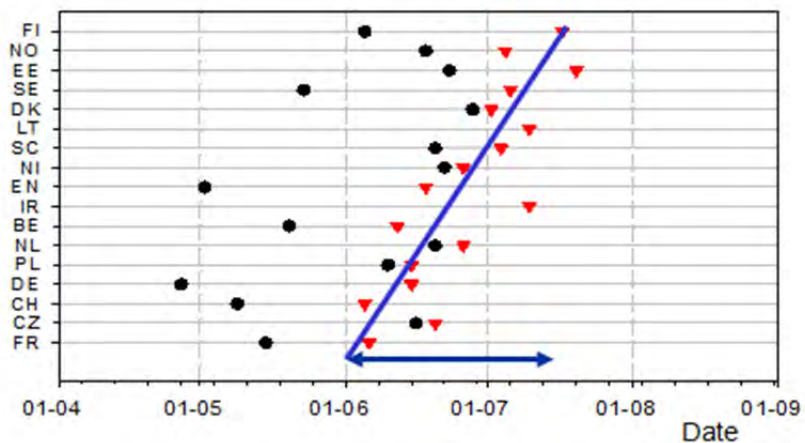


Figure 3. Date of first observation of late blight in covered or very early planted potatoes (black dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (red triangles), 2012. Distance from early attacks to late attacks in conventional fields is approximately 1½ months (FR & CH compared to Estonia and Finland)

WEATHER BASED RISK OF LATE BLIGHT DEVELOPMENT IN 2012

The weather based risk of late blight is estimated or calculated in Fig. 4. The late blight risk was not calculated by EuroBlight for 2012, as carried out during previous years. Instead the information is based on inputs from each of the country editors. In Ireland extremely wet conditions led to high disease pressure being experienced throughout the country, with the highest experienced in the south-west where 600 mm of rain fell in between May and August. In all other countries in Europe, the risk of late blight was low-medium in May. Unfavourable conditions for blight were also experienced in June in East Europe and in the Nordic / Baltic region (Fig. 4). Blight favourable spells in September in the North-East Europe resulted in medium to high levels of tuber blight as indicated from Finland, Estonia and Sweden. In the remaining Europe the risk of late blight in August and September were mostly medium resulting in low levels of tuber blight (Fig. 5)

Country	May	June	July	August	September
Finland	Low	Low	High	High	High
Norway	Low	Medium	High	High	High
Estonia	Medium	Medium	High	High	High
Sweden	Low	Medium	High	High	High
Denmark	Low	Medium	High	High	High
Lithuania	Low	Low	Medium	High	High
Scotland	Low	High	High	High	Low
N. Ireland	Low	High	High	High	Low
England	Medium	High	High	High	Medium
Ireland	High	High	High	High	High
Belgium	Medium	High	High	Medium	Low
Netherlands	Low	Medium	Low	Medium	Medium
Poland	Low	Medium	Low	Medium	Medium
Germany	Low	Medium	Low	Medium	Medium
Switzerland	Medium	High	High	Medium	Medium
Czech republic	Low	Low	High	Medium	Medium
France	Medium	Medium	High	Medium	Medium

Figure 4. The weather based risk of late blight in Europe from May-September, 2012

TUBER BLIGHT IN 2012

The level of tuber blight was reported as low in all countries in Europe, except for Sweden, Finland and Estonia, probably due to a combination of effective leaf blight control and favourable weather conditions during harvest (Fig. 4 & 5).



Figure 5. The level of tuber blight attacks (low, medium or high) in 2012 compared to normal

ALTERNARIA 2012

The level of attack of Alternaria is shown for 2012 in Fig 6. Alternaria seems to be a minor problem in North/West Europe. Some countries stress that attacks of Alternaria is an increasing problem, but severe attacks were only found in countries in Central Europe in 2012.



Figure 6. Problems with *Alternaria*, 2012 in three classes compared to normal

USE OF DSSs

Several decision support systems for late blight forecasting and control are used in Europe (Table 1). In **Germany** there are two decision support systems, PhytophthoraModel Weihenstephan (www.krautfaeule.de) and ISIP (www.isip.de). The majority of the potato growers are directly informed by fax or e-mail. In many regions the state advisory services 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 PhytoPRE web pages with information on the weather based infection risk and maps with late blight attacks are visited intensively by many growers (approx. 200'000 clicks/growing season). In addition the PhytoPRE data sheet with LB-attacks is weekly published in farmer's newspapers. A lot of farmers have learned due to PhytoPRE to consider the critical facts/periods of late blight. For the coming season, a PhytoPRE Web App Service for mobile phones will be available. In **Estonia**, Jõgeva Plant Breeding Institute provided information on first outbreaks of late blight and recommendations for timing of fungicide applications on a web-platform. The network consisting of 13 iMetos stations was used in collaboration of Jõgeva PBI and farmers' cooperative Talukartul for DSS in late blight control. The advice of fungicide timing is based on negative prognosis and Fry model. The DSS recommended proper start of fungicide applications. Following applications were recommended on label intervals or for 1-2 days shorter interval than label intervals. In

Norway the decision support system for potato late blight is available for free at www.vips-landbruk.no and consists of four parts - A map of the blight attacks found, the Negative prognosis to predict the first fungicide application and Førsund's rules and a new late blight model to predict days with high risk of blight infections. The system is used both by the advisory service and by farmers. In **England** and **Wales**, Blightwatch, supported by Potato Council and industry sponsors, was available free to registered users in 2012 to give e-mail/SMS alerts to inform users of high-risk weather conditions and also provide information on late blight outbreaks in their selected postcode areas. Other decision support systems are available but less widely used, including Plant Plus. Information on weather-related blight risk was also available through BlightCAST and provided free to registered users by Syngenta Crop Protection. In **Belgium** approximately 2000 potato growers receive advice on late blight control from one of the two warning services, depending on the region (Flanders and Walloon Region). A network of more than 80 automatic weather stations in Belgium collects the necessary meteorological data. The disease models in use have their origin in 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 and lesion growth) have been added, leading to a much more quantitative disease model. Additionally, the model has been integrated with GIS software and supplemented with a late blight attacks monitoring service. Advices are updated several times per day and communicated via internet, e-mail, fax or post. A separate advice for organic growers is available, pointing out critical days for preventative applications with copper fungicides. A web application is also available for field specific advice, where cultivar and the effect of fungicide sprayings are taken into account. In **the Netherlands** two commercial companies supplying DSS's, Dacom and Agrovision. Many growers get information on late blight by fax, online, telephone or via a PC Program. The use of DDS's hasn't changed a lot during the last years. New is the introduction of a Phytophthora App by Apps for Farming in 2012. The first release made it possible to get an region specific advice. In **Northern Ireland** growers and advisers 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 sign up to receive Blight Warnings by SMS text message. Warnings of Infection Periods are also given on local radio and in the local farming press. In addition, growers can 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 **Poland** some farmers cooperate with Research Institutes using NegFry, some cooperate with Bayer Company using proPlant expert.com, a few cooperate with Syngenta using the DACOM system. In **Russia** A small number of Russian farmers used the Plant Plyus (Dacom) and VNIIFBlight DSSs. In the **Czech Republic**, national late blight forecasting is done by the State Phytosanitary Administration; the data are free-accessible on internet. It is based on negative prognosis of late blight. The Potato Research Institute Havlíčkův Brod, Ltd. provides contract-based Negative prognosis, NegFry and NoBlight of late blight and the advisory services do similar services for selected agricultural enterprises. In **Cyprus**, currently no late blight DSS is used by farmers. Several systems are tested by Cyprus University of Technology.

Table 1. *DSSs currently used in European countries in 2012*

Country	DSS in use in 2012
Belgium	Improved Guntz-Divoux, GIS + surveillance
Czech Republic	Negative prognosis, NegFry and NoBlight
Denmark	Blight Management on web + APP
England & Wales	Blight-Watch, Plant Plus and BlightCAST
Estonia	Jögeva PBI web system
France	Mileos (MiIPV + MildiLIS)
Germany	PhytophthoraModel Weihenstephan, ISIP
Netherlands	ProPhy, Plant Plus and Phytophthora App
N. Ireland	DARD Blight-Net, and Plant Plus
Norway	VIPS (Försund, negative prognosis, New model)
Poland	Negative Prognosis, NegFry and ProPlant
Russia	Plant Plus, VNIIF-3 and SimCast+VNIIF-3
Sweden	Plant Plus , DK Blight Management and NO VIPS
Switzerland	PhytoPRE+2000, PhytoPRE Web App Service

Tuber blight in relation to *Phytophthora infestans* genotype

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SUMMARY

National incidences of tuber blight were generally low during the years that genotype 13_A2 dominated the GB population. This study examined if lower than expected tuber blight was related to the aggressiveness of this genotype. Isolates of 13_A2 were generally more aggressive on tubers than isolates of older genotypes (but there was considerable variation within genotypes). This result implies that greater problems with tuber blight should have been experienced in the years that 13_A2 dominated. There is some evidence to support the idea that 13_A2 causes a higher incidence of tuber blight initially during the growing season but due to faster decay of tubers infected with 13_A2 the incidence of tuber blight at harvest could be lower than for less aggressive genotypes. The experiments demonstrated that not only did the more aggressive 13_A2 colonise tuber tissue more quickly than isolates of older genotypes but the severity of secondary bacterial soft rotting was significantly greater for 13_A2. The more rapid soft rotting associated with 13_A2 requires more detailed experimental work.

KEYWORDS

Late blight, *Phytophthora infestans*, tuber blight, 13_A2

INTRODUCTION

The national incidence of tuber blight was low in recent years (2007 to 2011). This coincided with the domination of the GB *P. infestans* population by isolates of the 13_A2 genotype. It isn't clear whether this was simply a coincidence or whether there was a causal relationship between genotype of *P. infestans* and the incidence of tuber blight, with isolates of genotype 13_A2 resulting in low incidences. This study examined the influence of *P. infestans* isolate on tuber blight using two different direct inoculation methods. The first attempted to simulate the natural infection process in the field. The second was a more artificial point inoculation method. In addition the impact of isolate on the period of survival of blighted tubers was investigated.

MATERIALS AND METHODS

Tuber infection in situ in relation to P. infestans genotype (2011)

Six chitted seed tubers (20-30 mm diam.) (cv. Rocket) were shallow planted at a depth of 5 cm on 13 April 2011 in 49-litre tubs containing John Innes No 1 compost. Four replicate tubs were planted. Each tub was labelled with replicate and isolate. The tubs were placed in a polytunnel for 15 weeks to encourage rapid tuber initiation and development and to keep the foliage dry.

Tap water (130 litres) was stored at room temperature for at least 48 hours. This was used for watering the tubs after they were moved to the controlled temperature room. Another c. 130 litres were stored at 10°C for watering the inoculum into the compost on the day of inoculation. The haulm from each tub was cut c.10 cm above the compost surface to allow the tubs to be stacked in the controlled temperature room. The compost was saturated with the tap water, which had been stored at room temperature, to ensure that tuber lenticels were open prior to inoculation and the tubs incubated on 25 July 2011 for 24 hours at 4°C. Tubers were watered from below and overhead so that the compost was wet throughout the complete depth profile.

P. infestans isolates were grown on King Edward potato leaves for 10 days at 16°C, 16-hour day length. Sporangial suspensions were prepared by washing leaves with a 9:1 mixture of sterile distilled water and potato tuber extract (McKee, 1964) that had been pre-incubated at 16°C for 24 hours. This temperature was to prevent zoospores being produced prior to the time of tuber inoculation. Once the concentration of sporangia for each test isolate was determined, the isolate with the lowest concentration was eliminated and replaced with a *P. infestans*-free control (water only) to check for natural infection. The concentration of sporangia for individual isolates was adjusted to 8.25×10^4 sporangia in 100 ml per tub. To assess direct and indirect germination, aliquots of the sporangial suspensions were incubated at 10°C for 24 hours, then fixed and assessed.

All leaf material was removed from the surface of the compost and solid trays were kept in place below the tubs during the inoculation process to prevent cross contamination between tubs. The inoculum suspensions (100 ml) were dribbled evenly onto the surface of the compost using Sarstedt plastic disposable cups with a small hole drilled in the bottom of each cup. A clean cup was used for each isolate. Immediately after inoculum was dribbled onto the surface, sporangia were watered in using 5.4 litres of tap water per tub. The water had been stored at 10 °C for 24 hours. Tubers were stacked two high and arranged as a randomised complete block. Two days after inoculation, trays were drained of any water to alleviate anaerobic conditions, particularly at the base of the tubs. The temperature of the room was increased to 10°C and the tubs were incubated for 21 days. The progeny tubers were harvested, washed, dried and destructively assessed to determine the incidence of blight. Four replicates were assessed, however only the results from two were analysed because of severe bacterial soft rot in tubs of the other two reps. The bacterial soft rot was sufficiently severe to prevent accurate assessment of tuber blight. A similar experiment was carried out in 2012.

Determine the influence of P. infestans genotype on the survival of inoculated tubers (2011)

Tubers (cv. Saxon) were washed, dried and surfaced sterilised. Tubers were placed in rows on trays containing damp tissue. Each tuber was wounded once using a cork borer of 15 mm diameter (sterilised between rows with IMS and flaming), which was pushed into the tuber to a depth of 5 mm. A random sample of intact tubers was tested for contamination by

Pectobacterium atrosepticum and/or *Pectobacterium carotovorum* subsp. *carotovorum*. A total of eight isolates of *P. infestans* and five replicates were used. Four replicates were for tuber burial in the field whilst the fifth replicate was used to determine blight severity in relation to isolate (destructive assessment). In total 100 tubers per isolate were inoculated (20 tubers per replicate).

P. infestans isolates were grown on King Edward potato leaves for 7 days at 16°C, 16-hour day length. Sporangia were washed from the leaflets using sterile distilled water, the concentration was adjusted and 20 µl of sporangial suspension (containing 20 sporangia) were point inoculated into each wound site on 15 September 2011. Individual suspensions were thoroughly mixed between each row of tubers using a Gallenkamp® Spinmix (The Technology Centre, Loughborough, UK). Control tubers were inoculated with sterile distilled water only. Trays containing the inoculated tubers were labelled, placed in large black bags and the bags sealed before being stored at 4°C and high relative humidity for 15 days (Table 1). The black bags were removed after 7 days. Trays were arranged as a randomised complete block. Following the incubation period, trays were placed in an ambient store for 7 days prior to planting.

Due to heavy and persistent rainfall towards the end of September 2011, burial was delayed to allow ground conditions at the site to improve. To limit disease progress prior to planting, tuber samples were stored at 4°C for a further 4 days. Tubers from replicate five were transferred to the ambient store, 24 hours prior to destructive assessment, to encourage disease development. Replicate five was destructively assessed on 12 October 2011. Tubers were cut transversely through the wound/inoculation point and each tuber assessed for blight severity (%).

Table 1. Details of dates and timings (2011 experiment)

Task	Date
Inoculation	15 September
Incubation (4°C)	15 - 30 September
Bags removed	22 September
Incubation (12°C)	30 September – 07 October
Incubation (4°C)	07 – 11 October
Incubation (12°C) (Rep. five)	11 – 12 October
Burial (South Holm)	11 October

Before burial each tuber was individually weighed and numbered. Tubers were transferred to the field in their individual trays and laid out next to the relevant plot. Each plot was 3.4 m (4 rows) x 7.50 m with 2 m spacing between plot ends and 2.6 m between plot sides. Tubers were hand planted in the middle two rows to a depth of 14.5 cm with 20 cm spacing between tuber centres, using a randomised complete block design (four replicate blocks). Trowels were thoroughly washed and gloves changed between plots to prevent cross contamination. Fifteen extra tubers were inoculated with isolate 2008_6082F (13_A2) to allow monitoring of tuber rotting throughout the period of burial. Two weeks after burial five tubers were individually harvested and destructively assessed to monitor disease progression. All tubers were harvested by hand 6 weeks after burial (21 and 22 November 2011) and individual tubers were washed thoroughly to remove all soft rotted tissue and each tuber re-weighed. Tubers were also assessed for the

presence or absence of blight symptoms. A similar experiment was carried out in April to June, 2012.

RESULTS AND DISCUSSION

Tuber infection in situ in relation to P. infestans genotype (cv. Rocket 2011)

In the 2011 experiment, on average 13_A2 isolates caused a significantly higher incidence of tuber blight than representative isolates of the old population (Table 2). However, there was considerable variation between isolates of 13_A2 (Fig. 1). Only one 6_A1 isolate was included in the experiment due to the poor growth of 6_A1 isolates prior to inoculation. The 6_A1 isolate tested gave the lowest incidence of infection. This isolate was grouped with the 13_A2 ones to compare new genotypes with old. The new genotypes resulted in a higher incidence of tuber infection but the difference was just statistically significant (Table 2). Similar results were obtained in the 2012 experiment (data not shown).

Table 2. Mean in situ infection for isolates of old and new genotypes, cv. Rocket, 2011

	13_A2	6_A1	Old
	2008_6082F	2008_6090A	2008_7006D (2_A1)
	07/39		2009_7122A (7_A1)
	2009_7654A		2008_6422F (8_A1)
		New (13_A2 and 6_A1)	
Mean infection (%)	29.2	23.8	14.7
F pr. 13_A2 vs. Old	0.008		
F pr. New vs. Old	0.046		

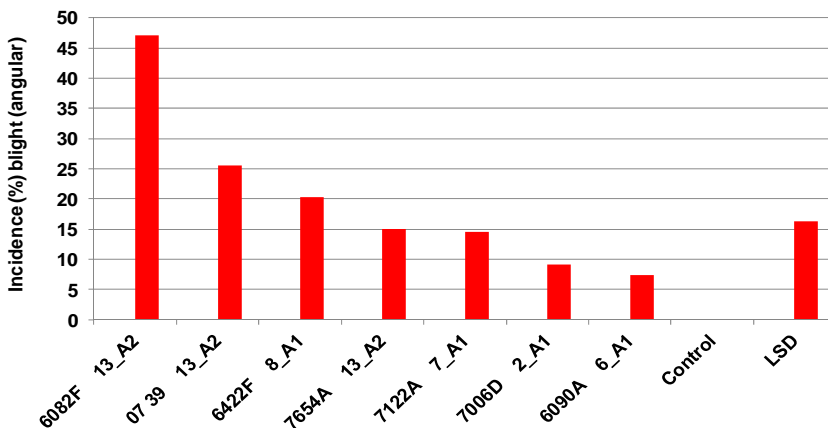


Figure 1. Incidence of in situ infection of cv. Rocket tubers by different isolates of *P. infestans*, 2011

Compared with the old isolates the 13_A2 isolates tested resulted in a significantly higher *in situ* infection (14.7% and 29.2% respectively) and yet the 13_A2 isolates had significantly lower indirect germination percentages (28.6% and 23.4% respectively) and direct germination percentages (15.5% and 10.4% respectively).

Determine the influence of P. infestans genotype on the survival of inoculated tubers

In both experiments, 2011 and 2012, tubers infected with 13_A2 isolates developed significantly more severe tuber blight than those inoculated with older genotypes (Tables 3 and 4). The presence of genotype 13_A2 also accelerated bacterial soft rotting (Tables 3 and 4). This result was anticipated because it is known that blight infection of tubers predisposes them to secondary rot by bacteria (Sicilia *et al.*, 2002). However, the absence of a strong relationship between tuber blight severity and soft rot severity was not expected. The weak relationship was partly due to limited soft rot development for some isolates in relation to the tuber blight severity they caused, e.g. isolate 2008_7006D in the 2011 experiment (Fig. 2). Less severe bacterial soft rot than expected was generally limited to isolates of the older genotypes, i.e. 2008_7006D in the 2011 experiment and 2008_7006D, 2010_8042B and 2008_6422F in the follow-up experiment (Fig. 3).

However, although results were consistent in both experiments for isolate 2008_7006D they were not for 2008_6422F. The lack of a consistent result was for soft rot, not blight. This suggests that a factor other than *P. infestans* genotype influenced tuber decay by bacteria. It's assumed that the bacterial soft rot was caused by the *Pectobacterium carotovorum* subsp. *carotovorum* detected in low numbers on non-wounded, non-inoculated tubers. The mean number of bacteria per tuber was 7.0 in the 2011 experiment and 295 in 2012. It's possible that tuber contamination by *Pectobacterium* varied within the tuber stocks used. However, this explanation appears unlikely because the three replicates of each stock tested for contamination gave a similar result. Also, a large number of tubers were inoculated with each *P. infestans* isolate. The *P. infestans* inoculum for tuber inoculation was prepared from inoculated leaf material. This was a deliberate decision to avoid the issue of axenic culture influencing the aggressiveness of isolates on host tissue. However, one drawback of this method is that the concentration of pectolytic bacteria in the *P. infestans* suspensions may have been affected by the condition of the leaves used to prepare the inoculum. A higher concentration of bacteria may have been washed from leaf lesions with more advanced blight development. Further experiments, in which bacterial contamination of sporangial suspensions will be controlled, will re-test the influence of *P. infestans* isolate on the rate of tuber soft rotting.

In the absence of oospores, survival of *P. infestans* between growing seasons is in infected tubers. The above result suggests that the survival of blighted buried progeny tubers is less likely if infected by 13_A2 compared with older, less aggressive genotypes. However, in the one study that specifically examined overwinter survival of inoculated tubers in relation to isolate aggressiveness, survival was unaffected by isolate even although aggressiveness differences were substantial (Montary *et al.*, 2007).

The more rapid, and more severe, bacterial rotting of tubers infected with 13_A2 compared with older genotypes has a potential implication for seed tuber-borne blackleg. A key early step in blackleg development is rotting of the seed tuber by *Pectobacterium atrosepticum*. If this happens more quickly after planting then either there is a higher incidence of non-emergence (blanking) or, if the plant emerges, blackleg symptoms are likely to develop earlier in the

growing season. Seed crops entered in classification schemes are more likely to be affected because of the low tolerances for blackleg. Tuber blight incidences are normally low therefore any impact on blackleg development in ware crops is likely to be small.

Table 3. Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of *P. infestans* and buried in soil, 2011 experiment

Genotype	13_A2	6_A1	Old
Isolates	2006_3928A	2008_6090A	2008_7006D
	2008_6082F	2009_7126A	2009_7122A
	2009_7654A		2008_6422F
Mean blight severity (%)	26.9	15.7	20.3
Mean soft rot severity (%)	35.5	31.6	28.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	<0.001	0.004	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.002	0.039	

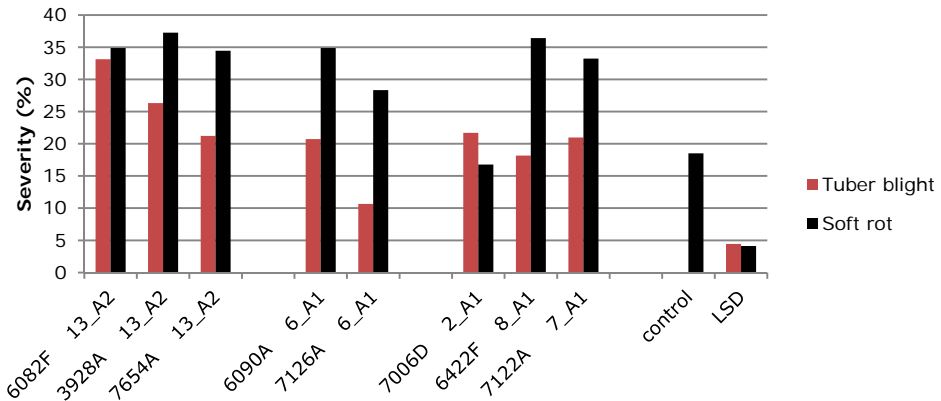


Figure 2. Severity of blight and bacterial soft rot for tubers inoculated with different isolates of *P. infestans* and buried in the field, 2011 experiment

Table 4. Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of *P. infestans* and buried in soil, 2012 experiment

Genotype	13_A2	6_A1	Old
Isolates	2008_6082F	2008_6090A	2008_7006D
	07/39	2011_8406A	2010_8042B
	2008_6102A	2011_8986A	2008_6422F
Mean blight severity (%)	31.4	27.5	24.0
Mean soft rot severity (%)	42.8	46.5	11.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	0.004	0.007	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.009	<0.001	

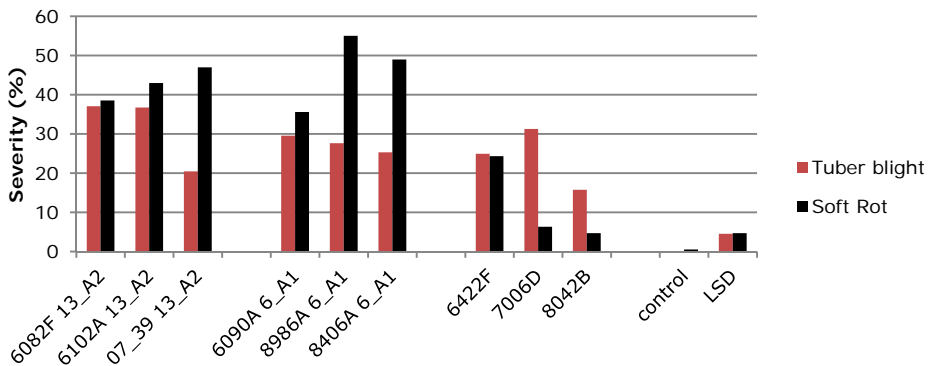


Figure 3. Severity of blight and bacterial soft rot for tubers inoculated with different isolates of *P. infestans* and buried in the field, 2011 experiment

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Where Do We Go After Smith?

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SUMMARY

In Britain growers rely on ‘Smith Periods’ to predict the occurrence of potato late blight; one of the most destructive plant diseases world-wide. A Smith Period describes a set of environmental conditions during which disease is able to develop. These conditions were, however, defined from field observations in the 1950s and current evidence suggests that the criteria should be updated to provide a better predictor of blight. The Smith Period is also limited in that it uses historical (recent) weather data and is location-specific, i.e., it does not account for disease pressure in surrounding regions. We aim to develop more accurate and comprehensive methods of assessing blight risk. Predictions will be based on the properties of the contemporary pathogen population that consolidates the current epidemiological understanding of the disease, weather forecast data, and sophisticated models that predict risk of spatial spread at the landscape-scale.

KEYWORDS

Phytophthora infestans, late blight, epidemic, dispersal, landscape, risk, simulation, decision support system

BACKGROUND

The Smith Period has been the mainstay of potato late blight forecasting in the UK since 1975. The Smith rule is currently used in a slightly modified version compared to the original rule (Chapman, 2012): a full Smith Period has occurred if, on each of 2 consecutive days: the minimum air temperature was at least 10°, and 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 totaled only 10 hours on one or both days. Smith Period data are provided free of charge as a collaborative service (<http://www.blightwatch.co.uk/>) supported by the Potato Council and industry sponsors that covers the whole of the UK down to individual postcode level (a broad subdivision of the UK into approximately 125 geographic regions). It’s a robust prediction model that has served the industry well for decades, but there is mounting evidence that the temperature and humidity thresholds need to be updated to match the newer, more aggressive strains of the pathogen that we now have in Britain (Chapman, 2012, Cooke *et al.*, 2012). The potato industry in the UK also benefits from another Potato Council funded

source of blight risk data: disease outbreaks are reported by scouts as part of the 'Fight Against Blight' campaign (<http://www.potato.org.uk/fight-against-blight>). This provides valuable confirmation of blight activity, but it is clear that not every outbreak is reported. Critically, neither source of blight risk data accounts for disease build up nor pathogen dispersal at wider spatial scales than the individual infected crops, and neither consider prognosis for future infection and spread of disease. Here, we set out to develop a more comprehensive disease forecasting system that provides the UK potato industry with information on the historical, current, and future risk of disease.

PROPOSED METHODOLOGY

Environmental parameters

The precise relationship between humidity, temperature, and infection will be examined using current cultivars and a representative selection of isolates for the contemporary *P. infestans* population. The experiments will be conducted in growth chambers set at a range of temperatures with different controlled periods of high humidity. This will provide data to refine the Smith Period criteria, resulting in a dynamic set of decision rules that changes with the genetic make-up of the UK pathogen population. The new Smith criteria will be used to parameterize a sophisticated, spatiotemporal simulation model of the potato late blight pathosystem for UK weather conditions (Skelsey *et al.*, 2005, Skelsey *et al.*, 2009b, Skelsey *et al.*, 2009c, Skelsey *et al.*, 2010); they will define when conditions are conducive for infection, and when they are not.

Model validation

Historical weather and late blight outbreak data (from the Fight Against Blight campaign) will be used to provide a 'real-world' validation of new Smith Period criteria and allow the project team to beta-test model prototypes and fully understand the requirements of industry users. These data will also be combined with historical records on the spatial coverage of potato production areas in the UK to provide a unique landscape-scale validation of the potato late blight pathosystem simulator. The process of model parameterization and validation is illustrated below, using Scotland as an example (Figure 1a).

A SPATIALLY EXPLICIT DECISION SUPPORT SYSTEM

The late blight pathosystem simulator (with the new Smith Period criteria as a component) can produce a wide variety of output pertaining to historical, current, and future risk of disease occurrence and epidemic spread. The following predictors of risk were highlighted as being particularly useful during knowledge exchange events within the potato industry.

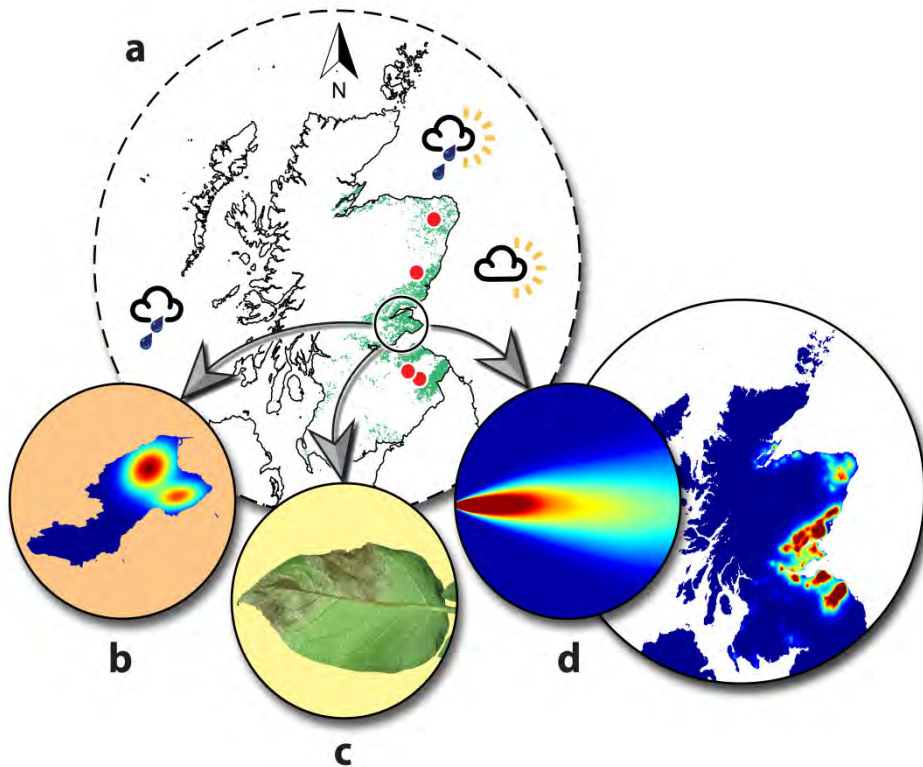


Figure 1. A spatially explicit decision support system for potato late blight. **a)** Historical weather, outbreak (red circles), and crop distribution data (green areas show potato crops in Scotland in 2012) will be used to refine and test new Smith Period criteria, which will be implemented within an existing late blight pathosystem simulator. Simulator output: **b)** historical risk of late blight activity in an example geographic region (red areas are high risk); **c)** risk of disease occurrence for any given area according to new Smith Period criteria, using either current weather observations or weather forecast data; and **d)** future risk of viable spore transport over distance for any given area assuming a hypothetical inoculum source, or the cumulative inoculum risk from all known reported outbreaks of disease

Historical risk of blight activity: primary inoculum risk

The simulator can quantify and map the connectivity of potato fields for the spread of disease for any specific geographic region and time period (Figure 1b). If the distribution of crops and weather data from the previous growing season is used, a map highlighting the high risk areas for primary inoculum in the upcoming season is obtained (assuming that last year's hot-spots for late blight are next year's hot-spots for primary inoculum). This could be useful for adjusting volunteer plant control strategies or fungicide plans. If this analysis is repeated using many years of weather and crop rotation data, we may find a long-term geographic trend in blight activity, which could serve as a map of expected blight risk for the whole upcoming season, and

beyond. This could be useful for making longer-term strategic decisions about varietal or product choices.

Current / future risk of disease occurrence

During the season the simulator can quantify and map the current risk of disease occurrence according to the new Smith Period criteria (Figure 1c). We can use weather data from the previous 48 hours (current practice), or from various upcoming meteorological 'lead times' (e.g., 1-day, 2-day forecast) in order to provide the industry with advanced warning of conducive conditions for blight, affording greater time to act.

Risk of inoculum dispersal among fields

The late blight pathosystem simulator has an aerobiological component that is used to determine the spread of disease between fields (Skelsey *et al.*, 2008, Skelsey *et al.*, 2009a). This has proven to be a useful tool for modifying spray recommendations (Kessel *et al.*, 2009, Kessel *et al.*, 2012, Skelsey *et al.*, 2009a); i.e., even if a Smith Period is predicted, growers may not be at risk if viable spores cannot travel very far. The distance that spores can travel is dependent on wind speed and atmospheric turbulence, and detached sporangia are sensitive to the dose of UV radiation received during transportation. The simulator can therefore predict the average distance that viable spores will travel using weather forecast data for any specific area, and predict actual spore deposition patterns from known outbreaks of disease (Figure 1d).

CONCLUSIONS

This modeling framework will constitute the first spatially explicit decision support system to provide a daily forecast for the risk of disease spread for any crop pathosystem. These maps and model output, or the model itself (as an interactive tool for subscribers), would be ideal for publication on the blightwatch.co.uk website. This will provide a further striking visual aid to decision making, and facilitate knowledge transfer in a manner meaningful to the industry. We will continue to work closely with the industry and the Potato Council to ensure we develop methods that are relevant and applicable to day-to-day management decisions and are appropriate for implementation on a GB scale.

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Potato late blight forecasting and initial inoculum sources in Norway

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SUMMARY

Initial sources of inoculum of *Phytophthora infestans* were investigated in ten potato fields with early outbreaks of potato late blight. Infected plant samples and isolates from these fields were examined with respect to mating type prevalence, fungicide resistance and genotypes based on microsatellites. A high proportion (91 %) of the isolates recovered were of mating type A1. However, both mating types were found in 3 of 9 fields with more than one isolate recovered, and sometimes both mating types were found on the same plant. Most of the isolates recovered from fields treated with metalaxyl-M prior to sampling had reduced sensitivity or were resistant to metalaxyl-M, and most of the isolates recovered from fields without metalaxyl treatment were sensitive. The isolates recovered from fields treated with propamocarb prior to sampling had a higher frequency of reduced sensitivity to propamocarb than isolates from fields without propamocarb treatment. We found that most plants contained more than one *P. infestans* SSR-genotype. Clustering analysis of the infected samples revealed that most samples clustered together according to fields. By combining information from *P. infestans* isolates and DNA extracts from the leaf lesions we found examples of both mating type A1 and A2 having the same multilocus genotype. This result indicates that both of these genotypes have a common ancestor, hence the inoculum originates from oospores. Although this is a minor study of only 10 fields with a limited amount of isolates and plant samples, the results indicate oospores in the soil is an inoculum source. Hence the forecasting model to predict outbreaks of potato late blight should be modified to include this.

KEYWORDS

Phytophthora infestans, mating type, fungicide resistance, Simple-sequence repeat (SSR), tuber blight, inoculum source.

INTRODUCTION

Potato late blight, caused by *Phytophthora infestans*, is the most important potato disease in Norway. To control the disease multiple fungicide applications are necessary. In average 8

fungicide applications per field were used to control potato late blight disease in Norway in 2012 (based on the amount of fungicides sold in 2012 and the total potato production area). The relatively high number of applications in 2012 was probably caused by the humid weather with an early onset of the potato late blight epidemics in both 2011 and 2012. Advisors and farmers can use the decision support tools, freely available in VIPs (www.vips-landbruk.no), to time their fungicide applications. In VIPs the Negative prognosis model (Ullrich & Schrödter, 1966) is used to predict the onset of the potato late blight epidemics. Normally the accumulated risk values from this model exceed the threshold (150) before the first appearance of late blight in potato fields, but the last years the model has failed. In order to improve the forecast for risk of early onset of potato late blight, it is important to have knowledge of the initial inoculum sources. Therefore we have sampled and genetically characterized early outbreaks of potato late blight in a systematic way to study the relative importance of infected seed tubers versus oospores in the soil. Another aspect of importance to disease control is the presence of fungicide resistance in the late blight population; hence isolates were tested for resistance to both metalaxyl and propamocarb.

MATERIALS AND METHODS

Samples were collected from early outbreaks of potato late blight in 10 potato fields distributed in three districts in Norway, in July 2012. In each field five potential inoculum source plants were sampled. The potential inoculum source was defined as the plant in center of the disease foci, with more late blight than the surrounding plants and one or more stem lesions. From each plant one stem containing minimum three leaflets with single lesions and one stem lesion were sampled. These lesions were excised and put into separate bags. The mother tuber from each plant was collected in a separate bag with a piece of the stem of interest left on, while the other stems were pulled away, to indicate which area of the mother tuber to sample from. However some of the mother tubers were completely decomposed and not possible to sample. In one field (S) mainly single lesion plants were detected, and consequently more plants were sampled.

In the lab, the area of the mother tubers close to the stem of interest were cut into thin slices to look for symptoms and for DNA extraction. From each leaf lesion one half was used for isolation of *P. infestans* and the other half was used for DNA extraction. The stem lesions were only used for DNA extraction. DNA was extracted according to Cullen *et al.* (2001) and purified using a spin column filled with polyvinylpyrrolidone. Six polymorphic SSR regions were amplified using PCR with primers developed previously: Pi02, Pi04, Pi26, Pi33 (Lees *et al.*, 2006); 4B and G11 (Knapova & Gisi 2002). The fluorescently labeled PCR products were analyzed by using an automated ABI 3730 DNA analyzer as described by Brurberg *et al.* (2011).

P. infestans was isolated from the leaf lesions by using potato tuber slices of cultivar Bintje as selective "growth media" before transferred to pea agar as described by Lehtinen *et al.* (2008). The mating type of the isolates were determined by the presence or absence of oospores after pairing them with standard isolates on pea and rye B mixed agar as described by Hermansen *et al.*, 2000. The sensitivity to metalaxyl-M and propamocarb were determined by the isolates ability to grow and sporulate on leaf discs floating on water with different fungicide concentrations as described by Lehtinen *et al.* (2008). The effects of the fungicides were calculated as the % inhibition of growth and sporulation in comparison to growth on leaves floating in water without fungicides.

RESULTS AND DISCUSSION

All the 10 potato fields sampled had outbreaks of late blight earlier than forecasted with the Negativeprognose model at the respective locations. Most of the fields had low level of late blight at the time of sampling (Table. 1). From 148 leaf samples with lesions, 73 *P. infestans* isolates were recovered. The recovery rate was relatively low, which is probably because the samples were collected from fungicide treated fields. Surprisingly most (91%) of the recovered isolates were of mating type A1 (Fig. 1). In previous studies, where we have sampled fewer isolates per field and later in the epidemic, we have found a more even distribution of mating types (Hermansen *et al.*, 2000; Lenhtinen *et al.*, 2008). In 3 of 9 fields, with more than one isolate recovered, both mating types were found. In the two fields with both mating types recovered and more than one isolates recovered per plant (fields L and V), we found both mating type on the same plant.

Table 1. Origin and background of potato / *P. infestans* samples. Ridomil contains metalaxyl-M and mancozeb, Tyfon contains propamocarb and fenamidone, Revus contains mandipropamid and Ranman contains cyazofamid

District	Field	Cultivar	% late blight at sampling in the haulm	Fungicide treatments before sampling	Number of plants sampled	Sample size		
						Leafs	Stems	Tubers
Vestfold	S	Folva	0.01	Revus, Ridomil, Tyfon	12	14	2	1
Vestfold	M	Berber	0.5		6	16	5	6
Vestfold	L	Folva	0.1	Ridomil, Ranman, Tyfon	5	15	5	5
Buskerud	P	Asterix	10	Ridomil, Revus, Tyfon	5	15	5	5
Buskerud	G	Kerrs Pink	0.1		5	15	5	5
Buskerud	O	Pimpernel	0.5		5	15	5	5
Hedmark	K	Beate	0.2		5	15	5	2
Hedmark	N	Folva	0.1		5	15	5	1
Hedmark	H	Asterix	0.2	Ridomil	5	15	5	5
Hedmark	V	Asterix	0.2		5	15	5	5

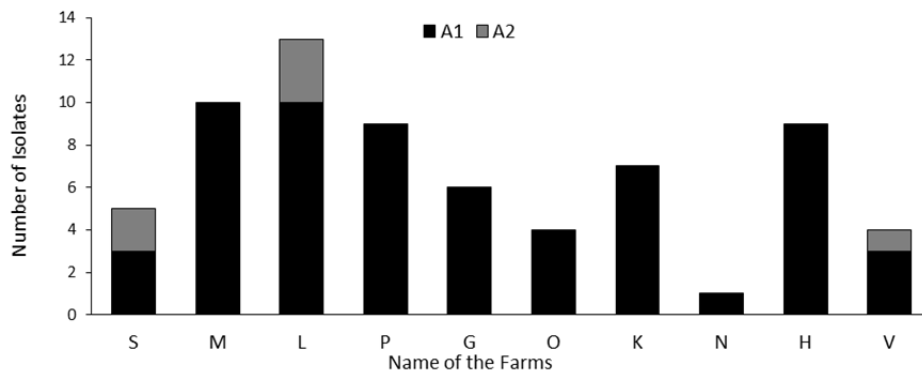


Figure 1. Number of isolates recovered from leaves from different fields and their mating type

Many of the isolates recovered from fields treated with metalaxyl-M prior to sampling, were resistant (19 of 39) or had reduced sensitivity (16 of 39) to metalaxyl-M. Only one of the isolates recovered from fields not treated with metalaxyl containing fungicide prior to sampling was resistant, and two had reduced sensitivity (Fig. 2). This indicates that application of metalaxyl-M exerts a strong selection pressure. It is only allowed to apply metalaxyl once per season in Norway. The results show that it is advisable to continue to limit the number of metalaxyl applications to one treatment per season to prevent metalaxyl resistance problems.

Two of the 70 isolates were resistant to propamocarb; they grew and developed spores on the highest concentration of propamocarb (Fig. 3). They were recovered from a field treated with propamocarb containing fungicide prior to sampling. Some isolates (14 of 70), had reduced sensitivity to propamocarb, they were able to grow and sporulate on some of the leaf discs at 100 ppm propamocarb. Most of these isolates (10 of 14) came from fields treated with propamocarb prior to sampling. Only 19 of 70 isolates were fully inhibited of the lowest propamocarb concentration, 10 ppm. They came from both types of fields. The difference between fungicide resistance in isolates sampled from fields with or without treatments with fungicide containing propamocarb prior to sampling was not so clear cut as for metalaxyl, but it was a tendency of higher tolerance to propamocarb among the isolates recovered from treated fields.

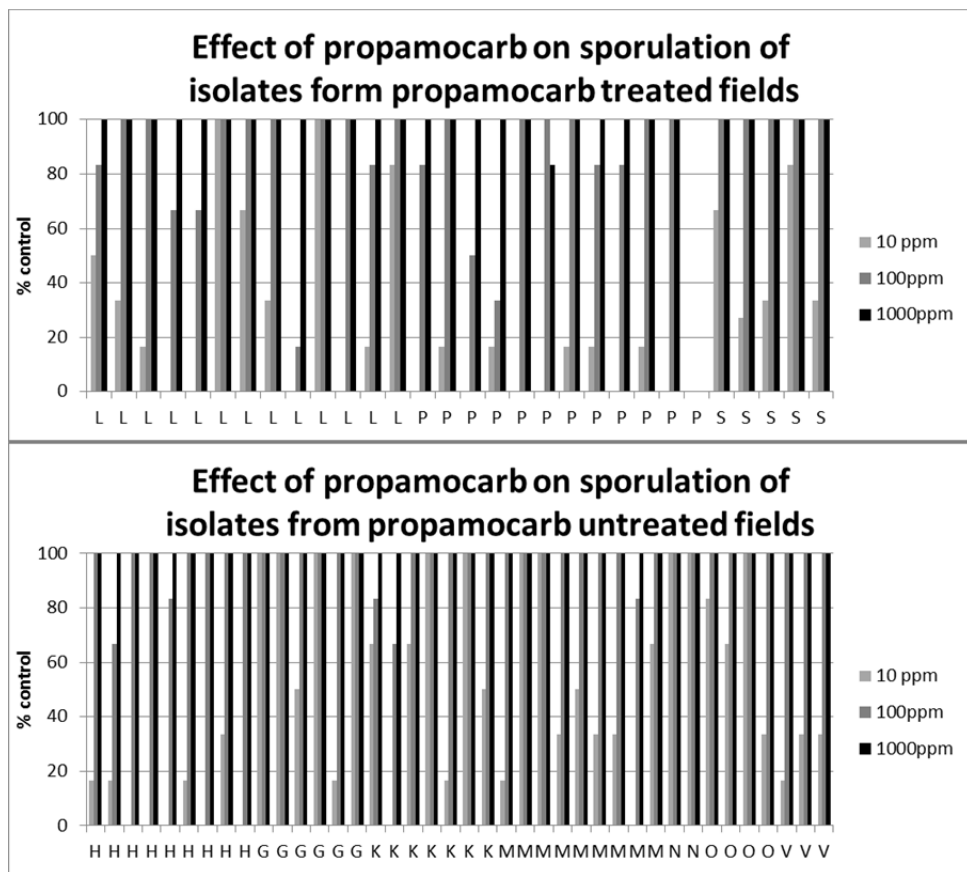


Figure 3. The ability of propamocarb-HCl to control potato late blight, calculated as the inhibiting effect on sporulation of *P. infestans* isolates growing on potato leaf disks floating on water with different concentrations of metalaxyl in comparison to sporulation on potato leaf disks floating on water without fungicide. The top graph presents the effect of propamocarb-HCl on isolates recovered from fields treated with fungicides containing propamocarb before sampling. The lower graph presents the effect of propamocarb -HCl on isolates recovered from fields not treated with fungicides containing propamocarb before sampling. The letters on the x-axis represent the field the isolates come from

DNA was isolated from all plant samples and genotyped using SSR primers. 187 of the 197 leaf and stem samples were successfully fingerprinted with all six SSR primers. None of the tubers had typical late blight symptoms. DNA was nevertheless extracted, but failed to give PCR products with most of the SSR primers. In total 32 alleles were detected at six SSR loci (Table 4). An allele not previously detected in Norway (146 of PIG11), was found in 26 samples originating from different fields. Several of the samples produced more than two alleles per loci, a phenomenon that also has been reported previously (Lees et al 2006; Brurberg et al 2011). We found that most plants contained more than one genotype, and the few tuber samples that gave SSR products had mainly different alleles than the corresponding stem and leaf samples. Clustering analysis (UPGMA) of leaf and stem samples revealed that most samples clustered

together according to fields (Fig. 4). By combining information from *P. infestans* isolates and DNA extracts from the leaf lesions we found examples of both mating type A1 and A2 having the same SSR multilocus genotype. This result indicates that both of these genotypes have a common ancestor, hence the inoculums originate from oospores.

Table 2. Allele frequencies for SSR markers in 187 *P. infestans* infected samples from leaves and stems

SSR locus	Allele	Allele frequency	SSR locus	Allele	Allele frequency
PiG11	142	0.50	Pi04	160	0.04
	146	0.14		166	0.82
	148	0.04		168	0.10
	152	0.20		170	0.90
	154	0.34	Pi26	173	0.14
	156	0.40		177	0.42
	158	0.11		179	0.36
	160	0.25		181	0.72
	162	0.30		183	0.14
	166	0.01		185	0.11
PiO2	152	0.28		187	0.13
	158	0.61	Pi4b	205	0.44
	160	0.58		213	0.63
	162	0.76		217	0.60
	164	0.09	Pi33	203	0.99
		206		0.31	

CONCLUSIONS

We found indications of significant genetic variation of *P. infestans* within fields, even though we only sampled 5 plants per field. There seems to be a strong shift in the *P. infestans* population in the field after treatments with fungicide (in particular metalaxyl-M), increasing the frequency of resistant clones. We found *P. infestans* in some of the seed tubers, but we found no clear indications of tuber inoculum leading to outbreaks of late blight. However, we found indications of both mating types having the same SSR multilocus genotype in one field and most fields seemed to have their own family of *P. infestans*. Although this a minor study of only 10 fields with a limited number of isolates and plant samples, the results indicate that oospores in the soil could be an important inoculum source. Hence the forecasting model to predict outbreaks of potato late blight should be modified to include this.

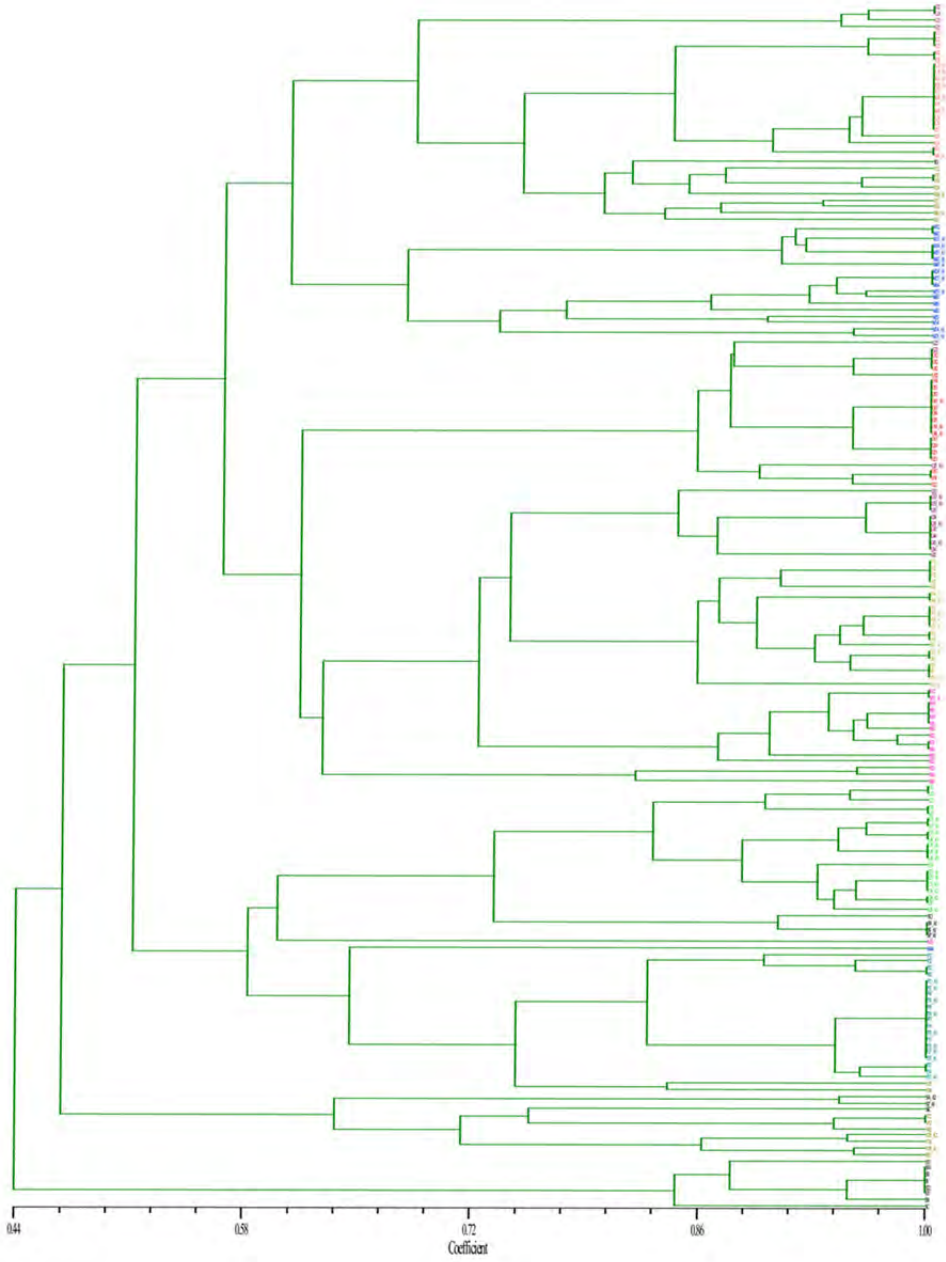


Figure 4. Dendrogram of 187 *P. infestans* infected samples from 10 fields generated from matrices of similarity based on SSR cluster analysis

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Strategies to reduce primary *Phytophthora* infections in conventional and organic potato production

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SUMMARY

Early primary stem infections developing from infected seed tubers are the most important starting points for early and massive late blight epidemics. As revealed by PCR analyses, about 10% of the seed tubers are latently infested with *P. infestans*, and thus carry the inoculum of the pathogen. In conventional farming, primary infections can be effectively prevented by early foliar applications of systemic fungicides, ideally 1-2 weeks ahead of the first visible symptoms. Seed treatments with contact fungicides might be an additional way to reduce incidence of primary infections. In organic farming, postponing the onset of infection originating from infected seed tubers is even more important, but systemic fungicides are not available. Seed treatments and foliar treatments with copper and/ or alternative products can be part of a management strategy aiming at a retardation of disease onset and a reduction of leaf infections to minimize the deposition of sporangial inoculum on the soil surface and the potato crop.

KEYWORDS

disease management, inoculum, late blight, latent infection, *Phytophthora infestans*, seed tubers, stem infection

INTRODUCTION

Late blight caused by *Phytophthora infestans* is one of the most devastating diseases of potato. In general, there are two major pathways of how the pathogen will infect a given potato field to start a late blight epidemic: i) infection will start from outside the field as sporangial inoculum originating from diseased volunteer plants, from infected refuse tubers, or from an infected neighboring stand, and blown into the field by wind; ii) infection will start from within the field, with infected seed tubers being the main source of inoculum (Johnson, 2010; Zellner *et al.*, 2011; Wharton *et al.*, 2012). Starting from an infected seed tuber, the pathogen can take several different ways to infect a potato plant and start an epidemic. When soils are sufficiently wet, sporulation on tubers might occur, leading to an infection of developing stems, or neighboring plants and tubers via the soil. Furthermore, the pathogen can grow on or inside the stem up to a certain height, and cause symptoms on aboveground parts of the stem. All these

pathways will eventually lead to primary *Phytophthora* stem infections from which – via sporulation and secondary leaf infections – disease spots will develop that soon will affect the whole field. Such primary infections usually will occur relatively early in the season, earlier than secondary infections via airborne sporangia, as the primary inoculum originates directly from the tuber, and the pathogen can establish on the tuber and in the soil for a certain time before and during sprouting. In particular, this will happen when weather conditions are suitable for the pathogen and for the development of disease (i.e. moist soils and temperatures above 10° C).

Previous studies based on PCR analyses have shown that an average of 10% of the European seed tubers are (latently) infected with *P. infestans* (Zellner *et al.*, 2011) and as such, potentially carry the inoculum into the fields. Even if only 1% of these produce plants with diseased sprouts (Powelson *et al.*, 2002), this would eventually result in approx. 40 disease spots per hectare (assuming a density of 40,000 plants/ha).

This study has investigated different ways to reduce the extent of these primary stem infections in both conventional and organic potato production by fungicide applications on the foliage or on the tuber. In both conventional and organic production modes, a timely and effective control of primary *Phytophthora* infections is crucial for any further disease management efforts and for the prevention of substantial yield and quality losses.

MATERIALS AND METHODS

Field tests

All field tests were carried out using a “double setting” technique of potato seed tubers (Keil *et al.*, 2010), with one healthy tuber and one artificially infected tuber planted on the same position in the field. The artificially infected tubers (cv. Désirée) were produced by injecting approx. 50-100 sporangia in about 50 µl H₂O into each tuber with a syringe several days before planting. These tubers served as a source of inoculum for the neighboring healthy tubers (cv. Agria), thus facilitating the development of early stem infections and at the same time ensuring the sprouting of at least one plant per planting position. In case of the tuber treatment tests, the healthy tuber received the fungicide seed dressing, while the infected tuber remained untreated. In case of the foliage treatment tests none of the two tubers was treated. All tests were set up in a completely randomized block design with four replications per treatment, plot size ca. 25 m², at two sites in southern Germany in 2011, or 2012.

Conventional farming

Foliar applications: The treatments applied to potato foliage in this test are listed in Table 1. Dosage and timing of application were according to standard recommendations and common practice. Extent of late blight and stem blight were recorded in July. Data presented here are those of the cultivar planted as a healthy tuber (cv. Agria).

Table 1. Treatments applied in field test for the prevention of stem infection by foliar treatment, conventional farming

treatment / product	active ingredient	dosage	fungicide mode of action
untreated control	-	-	-
Infito	propamocarb + fluopicolide	1.5 l/ ha	systemic (propamocarb)
Ridomil Gold MZ	metalaxyl M + mancozeb	2.0 kg/ ha	systemic (metalaxyl M)
Proxanil Pack	propamocarb + cymoxanil	2.0 l/ ha	systemic (propamocarb)
Fantic M	benalaxyl M + mancozeb	2.5 kg/ ha	systemic (benalaxyl M)
Revus	mandipropamid	0.6 l/ ha	locally systemic/ translaminar

Tuber treatments: The treatments applied to potato seed tubers several days before planting in this test and their dosages are listed in Table 2. Seed dressings were applied using ULV spray nozzles on an automatic potato grader. Extent of late blight and stem blight were recorded in July. Foliage treatments during the season were made with contact fungicides only to avoid any influence by systemic products on primary stem symptoms. Data presented here are those of the cultivar planted as a healthy, treated tuber (cv. Agria).

Table 2. Treatments applied in field test for the prevention of stem infection by tuber treatment, conventional farming

treatment/ product	active ingredient	dosage	fungicide mode of action
untreated control	-	-	-
Cuprozin fl.	Cu hydroxide	16 ml per 100 ml H ₂ O per 100 kg	contact
Zetanil M	mancozeb + cymoxanil	120 g per 200 ml H ₂ O per 100 kg	locally systemic/ translaminar
Fantic M	benalaxyl M + mancozeb	100 g per 200 ml H ₂ O per 100 kg	systemic (benalaxyl M)
Monceren	pencycuron + prothioconazole	60 ml per 100 ml H ₂ O per 100 kg	[Rhizoctonia treatment]

Organic farming

Foliar applications: Field (and laboratory) tests with copper and various alternative substances for foliar applications are described in another paper by the same authors in this proceedings (Nechwatal & Zellner, 2013).

Tuber treatments: The treatments were applied to potato seed tubers either in autumn shortly after harvest (before storage at 5°C), or in spring several days before planting, using ULV spray nozzles on an automatic potato grader. The preparations and their dosages are listed in Table 3. Extent of late blight and stem blight were recorded during June and July. Data presented here are those of the cultivar planted as a healthy, treated tuber (cv. Agria).

Table 3. Treatments applied in field test for the prevention of stem infection by tuber treatment, organic farming

treatment/ product	dosage	application time
control	-	-
copper (Cuprozin fl.)	16 ml per 100 ml H ₂ O per 100 kg	autumn 2011
Na phosphonate (test product)	40 ml + 160 ml H ₂ O per 100 kg	autumn 2011
Bacillus subtilis (Serenade)	320 ml per 100 kg	autumn 2011
chitosan (ChitoPlant)	2 g in 200 ml H ₂ O per 100 kg	spring 2012
copper (Cuprozin fl.)	16 ml per 100 ml H ₂ O per 100 kg	spring 2012
Na phosphonate (test product)	40 ml + 160 ml H ₂ O per 100 kg	spring 2012
Bacillus subtilis (Serenade)	320 ml per 100 kg	spring 2012

LABORATORY TESTS

In addition to the detached leaf tests described by Nechwatal & Zellner (2013) in this proceedings, laboratory tests were performed to test alternative substances for their ability to reduce tuber blight (brown rot) in artificially infested potato tubers. Tubers were infested by a short full immersion in a sporangial suspension (5 sporangia/ μ l) and incubation at room temperature for 24 – 48 h to initialize the infection. This treatment was meant to mimic natural tuber infestation at the end of the season with sporangia being washed onto the tubers from infected foliage. After that, dried tubers were treated by spray application with various substances, as indicated in Table 6, at a rate of approx. 0.7 ml/ tuber. After 6-8 weeks at 15°C in the dark, number of tubers with brown rot symptoms and extent of the rot for each tuber was recorded. Up to 6 tests were performed with each product, with 10 or 30 tubers per treatment.

RESULTS

Field tests

Conventional farming

Foliar applications: The results of the foliar application test are shown in Table 4. The combination of the use of artificially inoculated tubers and favorable weather conditions in 2011 generally caused high rates of primary stem blight. All three systemic fungicides caused a significant reduction in stem blight incidence as compared to the control. The reduction achieved by the use of a non-systemic fungicides was not significant.

Table 4. Field test for the prevention of stem infection by foliar treatment in conventional farming. Incidence of stem infection. Different letters indicate significant differences according to a squared ranks test for variances

treatment/ product	incidence of stem infection (%)
control	63a
Infito	21b
Ridomil Gold MZ	20b
Ranman Proxanil Pack	15b
Fantic M	24b
Revus	33ab

Tuber treatments: The results of the tuber treatment trial are shown in Table 5. Again, incidence of stem infection was comparably high in all variants, due to the use of artificially infected tubers and suitable weather conditions in 2011. In this test, stem infection could be significantly reduced by the two contact or locally acting fungicides. The systemic fungicide could not reduce the incidence of stem infection, just as the seed dressing active against *Rhizoctonia*.

Table 5. Field test for the prevention of stem infection by tuber treatment in conventional farming. Incidence of stem infection. Different letters indicate significant differences according to a squared ranks test for variances

treatment/ product	incidence of stem infection (%)
control	41a
Cuprozin fl.	14b
Zetanil M	13b
Fantic M	31ab
Monceren	38a

Organic farming

Foliar applications: see another paper by the authors in this proceedings (Nechwatal & Zellner, 2013).

Tuber treatments: A field tests was performed in 2012 to test the influence of several alternative substances for use in organic farming for their ability to reduce stem blight. Application was done in autumn or in spring. However, although artificially infected seed tubers were used, incidence of stem blight was low in these trials. This was most likely due to relatively dry weather conditions during spring 2012. Therefore, no data on the effect of the seed dressing on stem infections are available. However, when the (secondary) leaf infection data as of August 2012 were considered, an effect of all treatments on the degree of leaf infections could be observed in one stand, and an effect of the spring copper treatment in the other (data not shown).

LABORATORY TESTS

Several alternative, copper-free products and substances for potential use in organic farming were applied to artificially infected tubers to evaluate their effect on the establishment of tuber infections and their ability to prevent/ reduce the amount of brown rot. Some of the products/ preparations were able to clearly reduce the number of successfully infected tubers and the amount of brown rot developing on the tubes after 6-8 weeks (Table 6). These effects were non-significant in most cases, due to the high variability of the data. Clove oil and a commercial mustard preparation proved to be the most effective, and are planned to be included in field tests in 2013.

Table 6. Incidence and severity of brown rot in artificially infested tubers after application of alternative seed treatments. Tests consisted of 10 or 30 tubers per treatment. * denotes significant differences from the control at $p \leq 0.05$ (Dunnett's Multiple Comparison Test)

treatment/ product	dosage	no. of tests	mean percentage of brown rot per tuber (relative to control)	mean no. of successfully infected tubers (relative to control)
control	-	6	100%	100%
garlic product (AMN BioVit)	1%	5	128%	105%
copper (Cuprozin fl.)	16%	6	112%	105%
Pythium oligandrum (Polyversum)	7%	6	111%	92%
Aureobasidium pullulans (Boniprotect)	1%	3	102%	105%
44 °C water (heat treatment)	-	3	102%	74%
Bacillus subtilis (Serenade)	1%	5	101%	90%
Brassica juncea powder (non-commercial)	10%	1	95%	103%
chitosan (ChitoPlant)	0.1%	5	83%	82%
knotweed product (Regalia)	0.25%	3	81%	83%
Na phosphonate (test product)	1%	6	75%	75%
clove oil (non-commercial)	1%	6	68%	65%
mustard powder product (Tillecur)	20%	4	41%	49%*

DISCUSSION

Early primary stem infections originating from infected seed tubers are the most important starting point for early and massive late blight epidemics in both conventional and organic potato production (Powelson *et al.*, 2002; Johnson, 2010; Zellner *et al.*, 2011; Wharton *et al.*, 2012). In contrast to any secondary infections originating from airborne sporangial inoculum these occur earlier in the season, making them particularly significant for the further development of the disease on a field scale (Zellner *et al.*, 2011).

In conventional farming foliage treatments are meant to directly affect the pathogen before or during its development in/ on the newly growing stems, and thus to directly reduce stem infections. Our results show that primary stem infections can be effectively prevented by an early foliar application of a systemic fungicide. Systemic fungicides are taken up into the tissue being distributed in the plant, and thus can reduce the growth of the pathogen from within the plant. In contrast, non-systemic or only locally systemic fungicides are not able to achieve this

effect, as they cannot reach the pathogen growing in the stem or infecting the stem from below the soil line. The first application of a systemic fungicide to prevent the initiation of an epidemic ideally takes place approximately 1-2 weeks ahead of the first visible symptoms to reach optimum efficiency. Such optimum timing for the beginning of the first application can be determined approximately, when considering the regional infection pressure, weather data, and disease incidence in neighboring high risk areas (like river plains, depressed areas or other moist sites).

Seed treatments might be an additional way to reduce incidence of primary infections in conventional potato farming. They are meant both to reduce the risk of infection of the developing sprouts and to affect sporulation on the infected tuber in the soil. This will also provide protection of neighboring tubers within the potato hill. Our test has shown that in this case, contact or locally systemic fungicides are effective against primary stem blight, most likely by acting against *Phytophthora* propagules located on the tuber surface and in the surrounding soil. Systemic fungicides, in contrast, are not active on the tuber surface, as they are translocated quickly upwards the plant within the growing stems, and are therefore not optimized for an effect on the tuber surface or the surrounding soil.

In organic potato production, early primary infections are a key factor for development and economic significance of late blight disease, as they determine the onset of an epidemic and thus, have a strong influence on potato yield at harvest. Therefore, the time when an infection and an epidemic starts is even more important for organic farmers. As systemic fungicides are not available, copper currently is the only fungicide able to control late blight in organic farming (in Germany and other countries). Foliar treatments in this production type are rather considered a method to postpone the onset of a *Phytophthora* epidemic and to minimize the extent of leaf infections than to prevent the occurrence of primary stem infections itself. This will also minimize the deposition of sporangial inoculum on the soil surface and on the potato crop, and as such will reduce the extent of (latent) infection in the harvested tubers – an important prerequisite for the production of disease free seed tuber material.

As in conventional farming, seed treatments with copper or alternative products also can be part of a management strategy for late blight in organic production (Benker *et al.*, 2006; Wharton *et al.*, 2012). The effect of a seed treatment on secondary leaf infection that was found in our field test could not be unambiguously proven to be an effect of the tuber treatments on the incidence of primary stem blight, due to weather conditions unsuitable for the development of this type of symptom. However, it might still be considered as a retardation effect, the mechanism for which remains unclear. The different treatments might have caused a reduction of sporulation on the tubers, causing a decrease of sporangia present in the soil, and a later onset of massive leaf infections. Lab tests have shown that several alternative substances might have the potential to provide protection against primary stem blight when applied as a seed dressing. Further field tests to be carried out will show whether they are also effective under field conditions.

In both conventional and organic potato farming, the use of both leaf fungicides and seed treatments (seed dressing) might be able to achieve a further reduction and/ or retardation of a late blight epidemic and thus, might help to better control the disease and its impact on potato yield. In organic production, this strategy might further reduce the copper input and help to produce disease free seed tubers.

ACKNOWLEDGEMENT

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Dacom Phytophthora advice going mobile

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SUMMARY

In 1996, an advisory module for accurate *P. infestans* control was developed by Dacom. Over the years, this model has been intensively tested by researchers and used by farmers around the world. The advice is calculated and presented on the PC of the farmer. To be able to advise growers without a PC, a warning system by phone based on the Dacom model was introduced in the Netherlands and operated for 11 years until 2012. But the world of communication is changing rapidly. The development of smartphones and the use of social media has an impact on the use of smartphones by farmers. Instant information at any place right on the smartphone or tablet is now demanded. In order not to compromise on quality, Dacom has completely re-build her *P. infestans* advice to present it on iOS and Android platforms.

KEYWORDS

P. infestans, models, Dacom, advice, mobile platforms

INTRODUCTION

In the early 90's, Dacom developed her model for the management of *P. infestans*. In that same period, there was great concern by the potato industry and the Dutch government about the extensive usage of fungicides. The potato sector joined forces to reduce the dependency of fungicides. As a part of the chosen strategy, Decision Support Systems, the so-called DSS's, were promoted. Also, a general advice system that alerted potato growers by telephone was initiated. This system was powered by the Dacom model and used for 11 years. In the meantime, applying a fungicide only when needed, was becoming commonly accepted in the Netherlands.

Dacom BV is a Dutch company founded in 1987 and provides farmers with advice systems to optimize crop yield based on ICT and sensor technology. For processors, Dacom makes field information transparent for traceability. Also, yield can be forecasted and afterwards benchmarking of the carbon footprint and the water footprint can be done.

MOTIVATION

With the fast changes in society with the social media and the quick acceptance of smartphones, Dacom decided to make her DSS modules accessible for a broader market by going mobile with her fungus advice. At the same time, farmers are more in need of instant information regarding the situation on the farm and want to focus their attention on possible problem spots.

DATA COLLECTION

In order to operate the model, data from different sources is needed. The weather data is measured by automatic weather stations that are located near the potato field. In order to predict an infection event, the weather forecast is also included in the data set. The weather parameters needed for the calculation are: air temperature (1.50 m), air humidity (1.50 m), rain fall, wind speed, and solar radiation. An option is to include the wind direction. All this data is collected automatically at the Dacom databank and processed into hourly data. Each potato field has a unique weather station assigned (Figure 1).



Figure 1

The farmer will create a potato field in the system. He will record the potato variety, the planting date and the date of emerge. During the growing season he will record at a regular interval crop conditions as crop status, crop growing speed and crop density. Furthermore, the farmer will record the fungicides including the time and the amount he used on the potato crop.

The farmer or agronomical advisor can report possible infection in fields nearby the farmer field in the Dacom system.

THE MODEL

As the model has been described in earlier editions, this description will be just a summary.

The model consists of two main modules: crop coverage by fungicide calculation and development of the *P. infestans* and displayed in the top part of the graph (Figure 2 - top part).

Based on the available information, the (un)protected status of the crop is calculated based on the wear off and the half time value of the fungicide used (including dosage). The other factor is the production of new leaves. Again, depending on the product used, new, unprotected leaves

will be formed. Based on the combination of these two factors, the unprotected status of the crop is calculated through time from the last spraying.

The calculation of the development of the fungus starts with the calculation of the number of spores on a virtual lesion. If spores are calculated, these spores will be ejected and distributed under certain conditions. Depending on the information from neighboring fields, the number of spores attacking the field are calculated. Next, the moment of penetration into a leaf of the spore is calculated based on the presence of free water, the temperature and the variety (Figure 2 - bottom part).

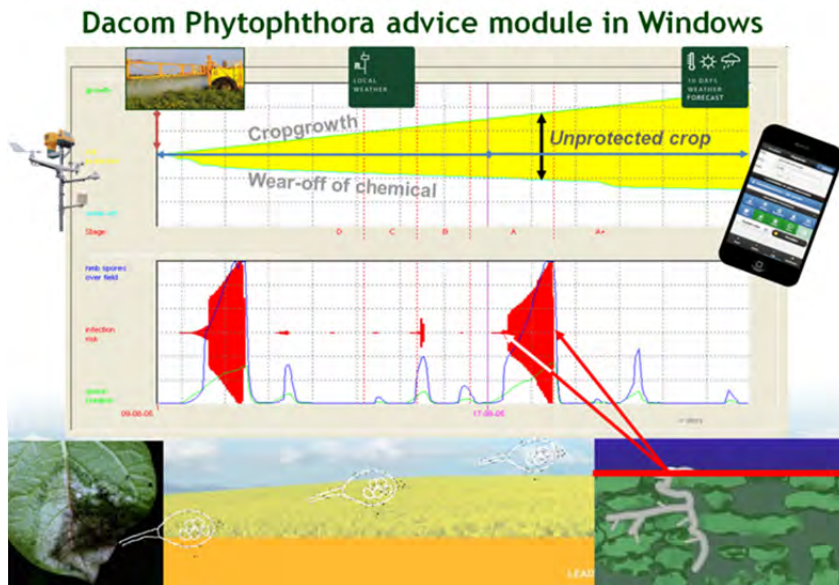


Figure 2

The combination of the unprotected leaf area and the severity of the infection event (duration and number of spores) an advice to spray is calculated. Either for the coming days with a contact fungicide. Or, if an event is missed, with a systemic fungicide.

FIELD DATA

Recording new information can be done either in the traditional Dacom system on the farmer PC or through the Crop-R geo crop recording platform on a web browser or on an app on the smartphone or tablet.

DACOM YIELD MANAGER

The *P. infestans* app is part of a broader suite of applications for the cell phone under the "Dacom Yield Manager" heading. The app can be downloaded for the iOS operating system or

the Android system from the respective web stores. For the first time usage, the app has to be initiated by a username and password.

In the background at the Dacom databank a farmer can indicate that he wants to view the blight situation on his smartphone. All the potato fields of this farmer belonging to the current crop year will be processed. Each hour, the latest available information will be used to calculate the current status of the field regarding the danger for an infection of *P. infestans*. The fields will be ordered where the field most in need of a fungus application will be on top of the list. On request, the information is sent to the farmer's smartphone (Figure 3).

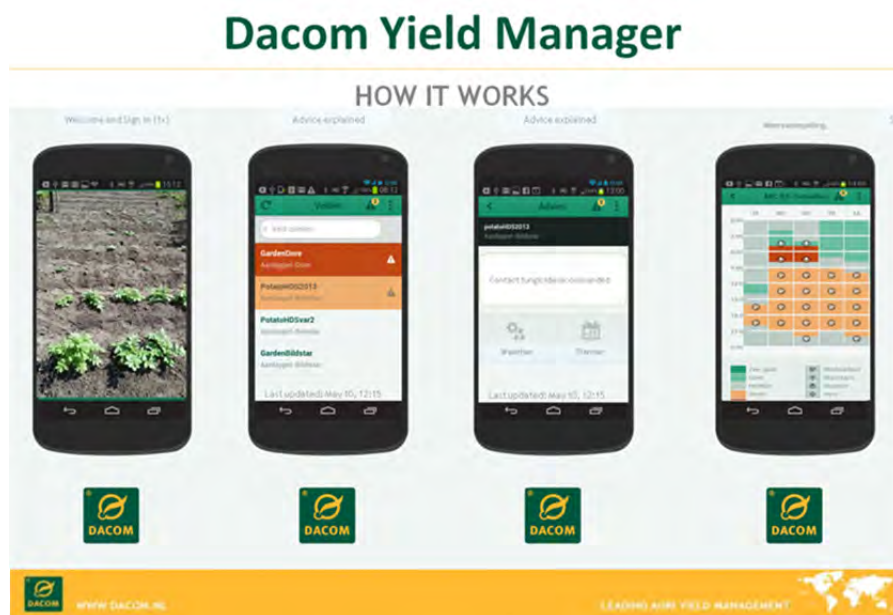


Figure 3

When the farmer wants information about the status of his field, he simply presses the “Dacom Yield Manager” button on his smartphone. Within about 6 to 8 seconds the information of his fields will appear on the screen. The fields are color codes: “red” means “an application is recommended”, “orange” means “an application should be considered” and “white” will generate the advice that “no application is needed”. The color “blue” means that “there is not sufficient information of this potato field to generate an advice”. By touching a field, a further screen with spraying information will appear. On this screen, it is also possible to have an overview of the spraying condition in the coming 10 days and also the weather forecast over this period.

DACOM BLIGHT APP

For 11 seasons, Dacom has warned farmers through a telephone messaging system about the occurrence of *P. infestans* near a farm location in order of the Dutch Potato Organization. This project ended because of the end of the Masterplan it was part of. Dacom decided to make a

general *P. infestans* app. This app can be downloaded for free and works on the available weather data near the location where the user is at that moment. It will calculate the infection risk of *P. infestans* for yesterday, today and tomorrow (Figure 4).

Dacom Blight APP's

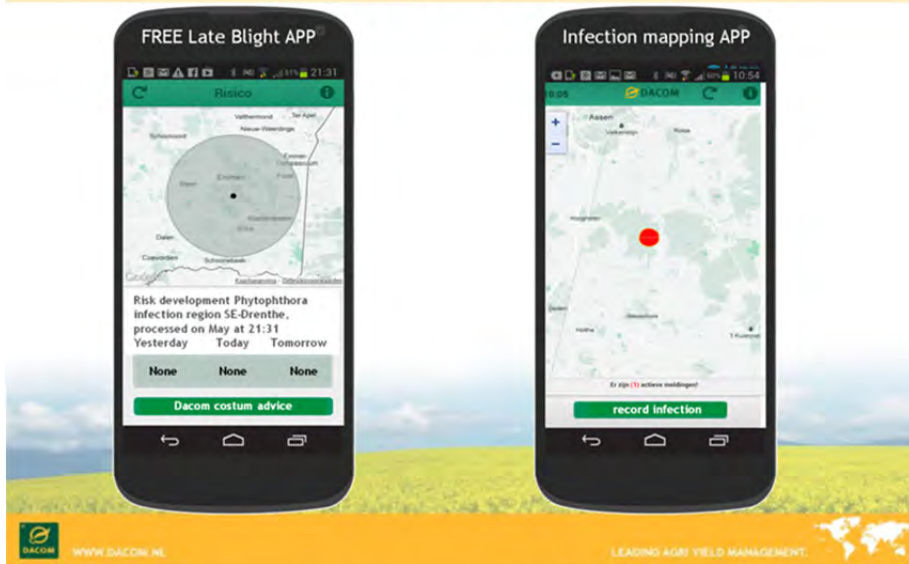


Figure 4

CONCLUSION

Instant availability of information is becoming rapidly a common feature. For agricultural science, it is a necessity to make the output of DSS models available on these new social platforms. In that way, research knowledge will be implemented by a broad group of farmers in an effective way. Dacom made a first step by transferring her *P. infestans* model to mobile platforms.

Impact of fungicide input on leaf blight (*Phytophthora infestans*) development on different potato cultivars

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SUMMARY

A shift towards more aggressive and virulent late blight (*Phytophthora infestans*) genotypes in GB, including 13_A2 and 6_A1, has resulted in the resistance ratings of previously resistant cultivars being downgraded in 2011. The use of 13_A2, the dominant A2 strain in GB, in untreated cultivar screening trials has increased the disease pressure and discrimination between varieties is now less clear. Integrated control of late blight using cultivar resistance and reduced fungicide inputs requires robust information on the resistance ratings of varieties and downgrading of resistance ratings may be considered a set back to its implementation. The results of thirteen experiments conducted from 2009 to 2011, however, provide evidence that the contribution of cultivar resistance differs substantially for different levels of fungicide input. The contribution of moderately resistant varieties (resistance ratings of 5 to 7) is considerably greater when plants are fungicide treated than left unprotected, with fungicide application of 0.5 dose (as a proportion of the full recommended label rate) sufficient to demonstrate cultivar differences in small plot screening trials. Previous experiments have demonstrated that the rank order of varieties is similar regardless of whether fungicide is applied or plants are left untreated. The inclusion of fungicide treatment in resistance screening trials could be used to slow the epidemic. Fungicide treatment would therefore allow the contribution of cultivar resistance to season long late blight control to be determined and also offer clearer discrimination between varieties.

KEYWORDS

Late blight, *Phytophthora infestans*, foliar blight, cultivar resistance, fungicides, integrated control

INTRODUCTION

EU legislation requires member states to promote lower pesticide inputs and encourage incorporation of non-chemical control measures into crop disease management practices. This includes the control of late blight (*Phytophthora infestans*) on potato. Cultivar resistance, in

combination with reduced fungicide input, has been shown to successfully reduce foliar blight severity in previous experiments (Fry 1978; Neilsen 2004; Kirk *et al.*, 2001 & 2005; Kessel *et al.*, 2006; Naerstad *et al.*, 2007; Bain *et al.*, 2011). A shift in the late blight population in GB towards more aggressive and virulent *P. infestans* genotypes, including 13_A2 and 6_A1, resulted in the foliar resistance ratings of several cultivars being downgraded from resistant (e.g. Cara with a rating of 7 in 2010) to moderately resistant (Cara with rating of 5 in 2012) (Lees *et al.*, 2012). Sufficiently large differences in foliar resistance between varieties are a key part of integrated control; however 99% of the potato hectareage in GB is of cultivars with a resistance rating of 5 or below. Cultivar resistance ratings are based on disease progress on test varieties (as determined by the area under the disease progress curve) relative to disease progress on two standard (anchor) varieties (one susceptible and one resistant). Recent trials in GB have shown that, in the presence of the more aggressive genotypes, differences between varieties tended to be smaller in untreated compared with fungicide-treated plots and gave a preliminary indication that the inclusion of fungicide would allow better discrimination between varieties (Bain *et al.*, 2008, Bain *et al.*, 2011). The inclusion of fungicide treatment in variety screening trials could therefore offer more robust information on varieties for use in an integrated control strategy.

This work was carried out as part of a government and industry funded Sustainable Arable LINK project which aims to deliver robust information on the use of integrated late blight control to the GB industry. The first objective was to test whether the use of fungicides improves discrimination between cultivars when disease pressure is high. The second objective was to test whether the downgrading of cultivar resistance ratings due to the presence of more virulent and aggressive genotypes will affect the use of cultivar resistance as part of an integrated control strategy during both rapid haulm growth and stable canopy.

MATERIALS AND METHODS

Discrimination between varieties: cultivar resistance ratings in relation to fungicide dose

In 2012, an experiment with five varieties with resistance ratings ranging from 3 (least resistant) to 8 (most resistant) was conducted at the SRUC site at Auchincruive Estate, Ayrshire, Scotland (Table 1). The trial was laid out in a randomised split plot design with six replicates. Plots consisted of four plants of each cultivar (two in each row, 30cm apart) in the centre two rows, with an outer row of King Edward on each side of the plot. These rows of King Edward acted as spreader rows but were treated with the same fungicide input as the four test plants in each plot. Three treatments were included: two fungicide programmes of Shirlan (0.4 or 0.2 L/ha) alternating at 7 day intervals with Quell Flo (1.65 or 3.3 L/ha)) applied season long plus an untreated control (Table 2). Fungicides were applied as main plot treatments with cultivars included as sub-plots.

In 2010, a similar experiment consisting of 19 varieties with resistance ratings from 2 (least resistant) to 8 (most resistant) was conducted at the ADAS site near Cilcennin, near Aberystwyth, Ceredigion, Wales. Results from five varieties with similar cultivar resistance ratings to the SRUC trial in 2012 were selected and presented in this paper (Table 1). The trial was laid out in a randomised split plot design with three replicates, with plots arranged as described previously for the SRUC site. In this experiment and in contrast to the SRUC site in 2012, the single spreader rows of King Edward were left untreated. Three treatments: two

fungicide programmes of Shirlan (0.4 or 0.2 L/ha) applied season long plus an untreated control were included as main plot treatments with the cultivars included as sub-plots (Table 2).

The cultivar x fungicide experiment was inoculated on 12 July 2010 at Cilcennin using a *P. infestans* isolate of 13_A2 representative of the GB population. The trial at Auchincruive was not inoculated directly but became infected from a neighbouring trial that had been inoculated with 13_A2.

Table 1. The six cultivars included in the cultivar x fungicide dose trials and their foliar resistance ratings at SRUC Auchincruive in 2012 and ADAS Cilcennin in 2010

Cultivar resistance rating	Varietal resistance rating (from the British Potato Variety Database, 2012)
3 (least resistant)	King Edward
4	Maris Piper
5	Cara
6	Axonaa
7	Ambob
8 (most resistant)	Sarpo Mira

^aIncluded in ADAS Cilcennin experiment in 2010 only; ^bIncluded in SRUC Auchincruive experiment in 2012 only.

Table 2. Fungicides, rates and intervals in the cultivar x fungicide dose trials at SRUC Auchincruive in 2012 and ADAS Cilcennin in 2010

Treatment	Product	Active ingredient(s)	g/kg or L product	Concentration (g a.i./ha)	Rate/ha	Interval (days)
1	Untreated	-	-	-	-	-
2	Shirlan SCa	fluazinam	500g/L	200	0.4 (L)	7 days
3	Shirlan SCb	fluazinam	500g/L	100	0.2 (L)	7 days

^aalternated with 1.65 L/ha Quell Flo (455 g/L mancozeb; 750g a.i./ha) for SRUC Auchincruive experiment in 2012.

^balternated with 3.30 L/ha Quell Flo (455 g/L mancozeb; 1500g a.i./ha) for SRUC Auchincruive experiment in 2012.

Integrated control trials: cultivar resistance ratings in relation to fungicide dose across two sites and three years

IN 2009, 2010 and 2011 a total of twelve experiments were conducted to determine the effectiveness of integrated control treatments incorporating reduced fungicide inputs and cultivar resistance to control foliar late blight during rapid canopy growth and stable canopy. Six experiments, 3 investigating integrated control strategies during rapid haulm growth and 3 during stable canopy were conducted at the SRUC site near Auchincruive, with a similar 6 trials

conducted in parallel at the ADAS site near Cilcennin. Experiments were laid out in a randomised split plot design with 4 replicates. Each sub-plot consisted of either King Edward (resistance rating 3) or Cara (resistance rating 5) and was 4 rows wide by c. 3m long, with seed spacing determined by tuber size. All foliar assessments were done on the centre 2 rows of each sub-plot. In the rapid haulm growth trials, treatment fungicide applications were started as soon as plants started to meet within the rows or earlier if late blight risk was high. In the stable canopy trials, all plots including the untreated were oversprayed with Merlin 2.5 L/ha (propamocarb-HCL + chlorothalonil) during rapid canopy growth at 7 or 10-day intervals depending on early season risk or as soon as plants started to meet within the rows. One fungicide was tested in the rapid haulm growth trial (Revus; 250g/L mandipropamid: full label rate 0.6 L/ha). Three fungicides were tested in the stable canopy trial (Infinito: 62.5g/L fluopicolide + 625g/L propamocarb-hydrochloride, full label rate 1.6 L/ha; Revus and Shirlan). Fungicides were applied at 7-day or 10-day intervals in both rapid haulm and stable canopy trials at 0, 25, 50, 75 and 100% of the recommended label rate. Dithane NT (mancozeb 75% w/w) at 1.7 kg/ha or an alternative mancozeb product at an equivalent rate (1275g a.i./ha) was applied to the stable canopy trial for the remainder of the season once test treatment applications were completed. Dithane NT at 2.0 Kg/ha was applied to the rapid haulm trials once treatment applications were completed. Data were averaged across application interval and fungicide product for the stable canopy trial results presented in this paper.

Experimental sites were inoculated on 12, 12 and 3 July (Cilcennin) and 7, 12 and 8 July (Auchincruive) in 2009, 2010 and 2011 respectively. At Cilcennin, fungicides were applied in 250 litres of water per hectare using a hand held Oxford Precision Sprayer operating at 200 kPa through 110° flat fan nozzles. At Auchincruive, fungicides were applied in 200 litres of water per ha using a tractor-mounted, modified AZO compressed air sprayer, operating at 3.5 bars (350 kPa) to give a medium/fine spray quality using Lurmark F03-110 nozzles.

The percentage leaf area destroyed by foliar blight was assessed at regular intervals during the epidemic using a modified version of the keys Large (1952) and Anon (1976). Data are presented as the percentage of leaf area affected by foliar late blight or used to calculate the Area Under the Disease Progress Curve (AUDPC) as appropriate. AUDPCs were subjected to ANOVA to test whether there was an interaction between fungicide treatment and cultivar resistance rating, with the least significant difference (LSD) for specific comparisons included.

RESULTS AND DISCUSSION

Discrimination between varieties in untreated and fungicide treated situations

In untreated plots at the SRUC experiment site in 2012, there was little or no separation in the progress of foliar late blight on varieties with a resistance rating of 3 (King Edward) or 4 (Maris Piper) (Figure 1A to C). Cara, with a resistance rating of 5, appeared to be more resistant than Ambo which has a resistance rating of 6 and little foliar blight developed on Sarpo Mira (Figure 1A). Following application of Shirlan at 0.2 L/ha (half the recommended label rate), separation between the varieties became more distinct, with progress of foliar blight clearly slower on Maris Piper than King Edward (Figure 1B). Foliar blight development was still slower on Cara than Ambo where Shirlan at 0.4 L/ha (the full recommended label rate) was applied. Application of fungicide moved the progress of foliar late blight on moderately resistant varieties away from the susceptible anchor variety, King Edward, and closer to the more resistant anchor variety, Sarpo

Mira. Where Shirlan at 0.4 L/ha (the full recommended label rate) was applied, moderately resistant varieties were giving control closer to Sarpo Mira than King Edward (Figure 1C).

At the ADAS experiment site in 2010, differences between varieties in untreated plots were less distinct (Figure 1D to F). Progress of foliar late blight on varieties in untreated plots with resistance ratings of 3, 4 and 5 (King Edward, Maris Piper and Cara) was similar, with disease development on Axona and Sarpo Mira also similar (Figure 1D). With application of Shirlan at 0.2 L/ha and 0.4 L/ha, progress of foliar late blight on moderately resistant varieties (Cara and Axona) was closer to the resistant anchor variety Sarpo Mira than the susceptible anchor variety King Edward (Figure 1E and 1F).

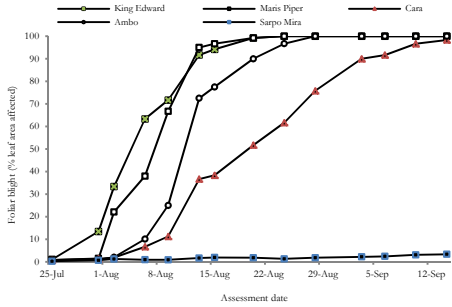
Comparison of the AUDPCs in the ADAS Cilcennin trial in 2010 showed no statistically significant differences between varieties with resistance ratings of 3 to 5, King Edward, Maris Piper and Cara respectively, where varieties were left untreated (Table 3). Where Shirlan at 0.4 L/ha was applied, however, control of foliar late blight on Cara was not statistically different from that on Axona and Sarpo Mira.

Table 3. Effect of fungicide input (Shirlan at 7 day intervals) on AUDPC values for five varieties with resistance ratings from 3 to 8 grown at the ADAS Cilcennin site in 2010

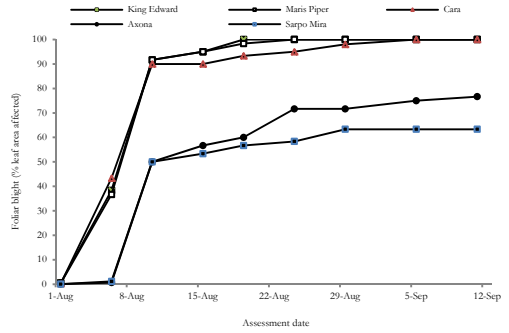
Fungicide rate applied	Variety (resistance rating)				
	King Edward (3)	Maris Piper (4)	Cara (5)	Axona (6)	Sarpo Mira (8)
Untreated	3561	3545a	3484 a	2284b	2024
0.2 L/ha	1971	1308	956	502 b	340
0.4 L/ha	1152	536	387 b	136 b	159
F pr. treatment x variety	<0.001				
LSD (P=0.05) (for same level of fungicide)	335.9				

^aAUDPC not significantly different from King Edward for the same level of treatment; ^bAUDPC not significantly different from Sarpo Mira for the same level of treatment.

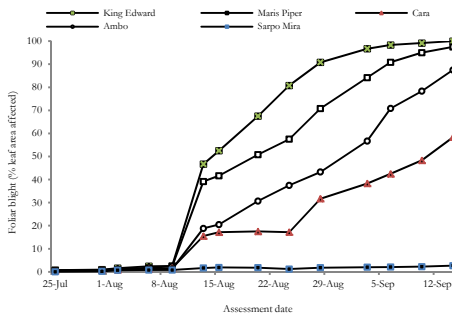
A: Untreated SRUC Auchincruive, 2012



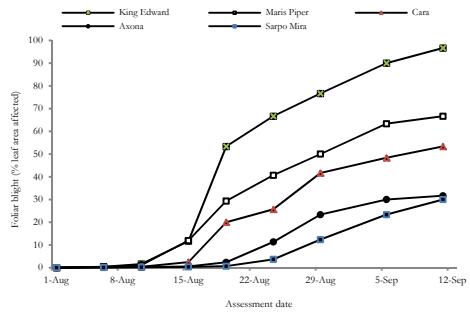
D: Untreated ADAS Cilcennin, 2010



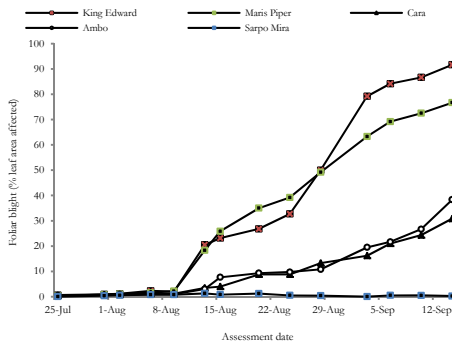
B: Shirlan 0.2 L/ha SRUC Auchincruive, 2012



E: Shirlan 0.2 L/ha ADAS Cilcennin, 2010



C: Shirlan 0.4 L/ha SRUC Auchincruive, 2012



F: Shirlan 0.4 L/ha ADAS Cilcennin, 2010

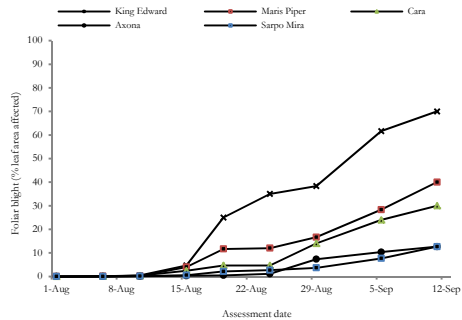


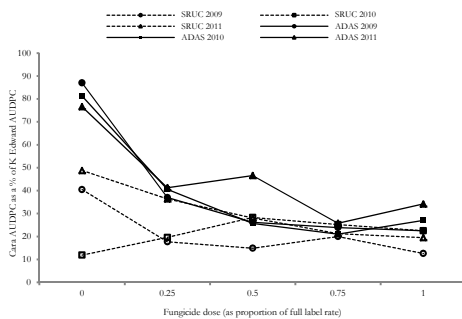
Figure 1. Progress of foliar late blight (as the percentage leaf area affected) in untreated and fungicide treated plots on varieties with resistance ratings ranging from 3 to 8 in two trials; one at SRUC Auchincruive in 2012 and one at ADAS Cilcennin in 2010. Shirlan at the rate specified was applied season long in the ADAS Cilcennin trial in 2010 but alternated with half of full rate Quell Flo as appropriate in the SRUC Auchincruive trial in 2012

Comparison of integrated control strategies for control of foliar late blight

During rapid canopy growth discrimination between King Edward and the more resistant Cara was greater where fungicides had been applied compared with completely untreated plots (Figure 2A and 2B). In all but one trial the difference between the two cultivars progressively

increased with increasing fungicide dose. The exception was the rapid canopy trial in 2010 at the SRUC Auchincruive site, where low disease pressure meant discrimination between varieties was little affected by fungicide dose (Figure 2A). Application of fungicide doses above 0.5 (as the proportion of the full recommended label rate) offered diminishing discrimination between the two varieties. For treatments applied during stable canopy, disease progress was also closer on varieties left untreated than when fungicides were applied (Figure 2B). With these trials also differences between varieties increased as fungicide dose increased but there was a greater response to fungicide dose during stable canopy. This could either be due to the growth stage at the time of treatment application or the different fungicides used. One fungicide (Revus) was used in the rapid canopy trial but three fungicides (Revus, Infinito and Dithane NT) were applied in the stable canopy trial.

A: Rapid Canopy



B: Stable canopy

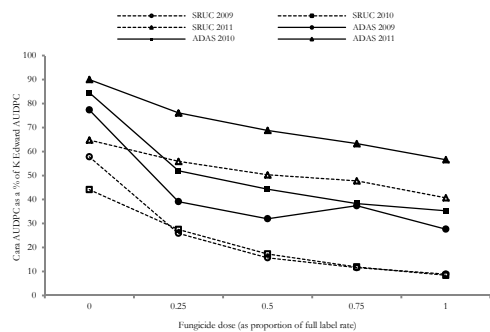


Figure 2. Change in AUDPC between the more resistant variety (Cara) and the more susceptible variety (King Edward) expressed as a percentage of AUDPC with increasing fungicide dose across twelve field experiments at SRUC and ADAS. Data presented are average of six trials conducted at two sites (SRUC Auchincruive and ADAS Cilecennin) over three years (2009 to 2011) during rapid canopy growth (A) and stable canopy (B). Where the percentage is higher there was a greater degree of similarity in disease progress on the two varieties

The results of thirteen out of fourteen experiments presented here provide clear evidence that relative AUDPCs for varieties differ substantially for different levels of fungicide input. The contribution of moderately resistant varieties (resistance ratings of 5 to 7) to foliar late blight control is considerably greater where plants are fungicide treated than left unprotected. In these experiments, applying 0.5 dose (as a proportion of the full label rate) was sufficient to pull apart varietal differences in small plot variety screening trials. Fungicide use slows the epidemic by indirectly or directly limiting sporulation and allows the assessment of cultivar resistance over a wider range of growth stages and leaf ages. There is evidence to suggest that leaf position/age has an impact on cultivar resistance to late blight, with no significant differences in the growth of late blight on basal leaves on varieties with resistance ratings from 2 to 8. Discrimination between the growth rates of late blight on susceptible and resistant varieties occurred to a much greater extent on the apical leaves (Visker *et al.*, 2003). The impact this would have on implementation of integrated control strategies is unknown and warrants further investigation, however, the inclusion of fungicide treatment in resistance screening trials would be beneficial to

determine how varieties would perform as part of a season long integrated control strategy and also their contribution to the control of late blight in standard commercial practice.

It has been demonstrated previously in experiments comparing the performance of varieties in untreated and fungicide protected conditions that the rank order of varieties is similar in untreated and treated situations (Bain *et al.*, 2011). Although the downgrading of resistance ratings due to the dominance of more virulent and aggressive genotypes could be regarded as problematic to the use of integrated control in GB, it has been demonstrated that substantial differences between varieties do exist that can be exploited in combination with reduced fungicide inputs for the successful control of late blight.

ACKNOWLEDGEMENTS

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First results of an EU-wide genotype monitoring of *Phytophthora infestans* using FTA cards

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SUMMARY

The aim of this study was to characterize the genotypic structure of *P. infestans* isolates collected over the 2012 season in several European countries to investigate whether populations underwent major changes over the last few years and to establish a baseline for further population change studies. The success rate (genotype specified) of the methodology using FTA cards for the sampling was generally quite high. From the data it can be concluded that, in general, in each of the fields sampled one genotype is dominating the population. The frequency of some well-known genotypes was different across the countries where samples were taken, however in almost every country the genotype 13_A2 was detected. The genotype 6_A1 was most frequent in the United Kingdom, 1_A1 was mainly found in Belgium and France. The methodology used in this study was proven as a good tool to investigate the population changes of *Phytophthora infestans* in Europe.

KEYWORDS

Phytophthora infestans, genotype, monitoring

INTRODUCTION

The Oomycete *Phytophthora infestans* is the causal organism of potato late blight, the most important disease in potato, the second most important arable crop in Europe. Migration of genotypes between Mexico, North America and Europe has occurred several times throughout history, likely linked to the movement of infected tubers used as seeds. Until the 1980s, the A2 mating type was not present in Europe but now both A1 and A2 mating-types co-occur in many European regions (Spielman *et al.*, 1991) allowing for sexual recombination and the formation of oospores. Apart from tubers, oospores are another possibility for the pathogen to survive the sometimes harsh North-European winters. Apart from Mexico, in Europe oospores have only been reported as a source of primary inoculum in Scandinavia and the North-East part of the Netherlands. However, increased genetic diversity has been observed in other parts of Europe which could be a consequence of sexual reproduction. Although both mating types have been

present since the late 1990s, *P. infestans* populations in France and Switzerland, as characterized with SSR's (simple sequence repeats) have been described as more or less clonal (Knapova and Gisi 2002). Also, significant population changes have been described recently as the likely result of import of foreign *P. infestans* genotypes or more frequent sexual recombination. Over the past decade this has resulted in a higher level of aggressiveness of *P. infestans* isolates (Kiezebrink and Shaw 2006) making disease outbreaks more severe and disease control more difficult. Recently the appearance of new SSR genotypes in European populations was described with novel SSR markers (Lees *et al.*, 2006). Using this methodology in 2009 Lees *et al.* detected a new frequent genotype with a characteristic 154 bp allele in locus D13 which was named as 13_A2 or 'blue13'. Another example of a new SSR genotype is the 33_A2 or 'green 33' isolate recently found in locations in The Netherlands (Schepers 2012). The aim of this study was to characterize the genotypic structure of *P. infestans* isolates collected over the 2012 season in several European countries to investigate whether populations underwent major changes over the last few years and to establish a baseline for further population change studies. Due to the high level of polymorphism, random distribution throughout the genome and co-dominance, microsatellites (SSR) are ideal molecular markers to determine the genetic structure of populations.

MATERIAL AND METHODS

Sampling and Genotyping P. infestans

300 FTA classic Cards (GE Healthcare\Whatman) with 4 sampling zones each were distributed within the network of Syngenta field workers together with sampling instructions. Four lesions were sampled from each infected field. Data on the origin of the sample (location, host, and cultivar) were also recorded and stored. Following the field season, cards were collected and processed. SSR genotypes were determined using the standardized 12-plex Euroblight set of *P. infestans* SSR's (Li *et al.* 2010) and GeneMapper software. Known clonal lineages (e.g. Blue 13, Green 33 etc.) were identified. All other genotypes were assigned to the "miscellaneous group". This group therefore contained many different genotypes in contrast to the other groups containing one clonal lineage.

RESULTS AND DISCUSSION

Distribution of samples and intralocation variability

From all countries in Europe 402 samples were received. 322 samples could be successfully processed giving an overall success rate of the genotype determination of 80%. However, there were big differences across countries, in CH, DE and PL the success rate was between 40 and 75 %, in the other countries (BE, CZ, DK, FR, NL, UK) the success rates were from 92 to 100 %. The 322 successful samples originated from 83 locations. More than one sample was available for 72 locations.

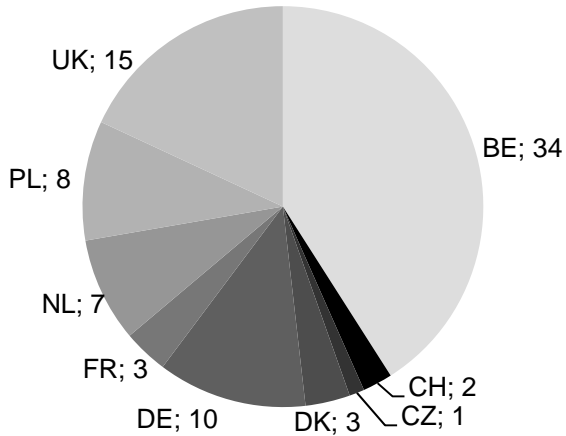


Figure 1. Sample location country distribution

The 72 locations with multiple successful samples were evaluated for the variability of the genotypes. For each location 2 to 16 (four FTA cards) samples were available, however the majority (89 %) was limited to one card (2 to 4 samples) and only in 8 cases more than one card was received from the same location. To allow for a simple discrimination, the results of the analysis were clustered into 3 classes representing the percentage identical genotypes within the samples from one field: 83 – 100 % / 60 – 75 % / 43 – 50 %. As can be seen in Figure 2 in about 50 % of the cases the genotype of all samples taken from the same location were nearly identical and in 80 % of the cases one genotype was dominant in more than half of the samples taken from one location.

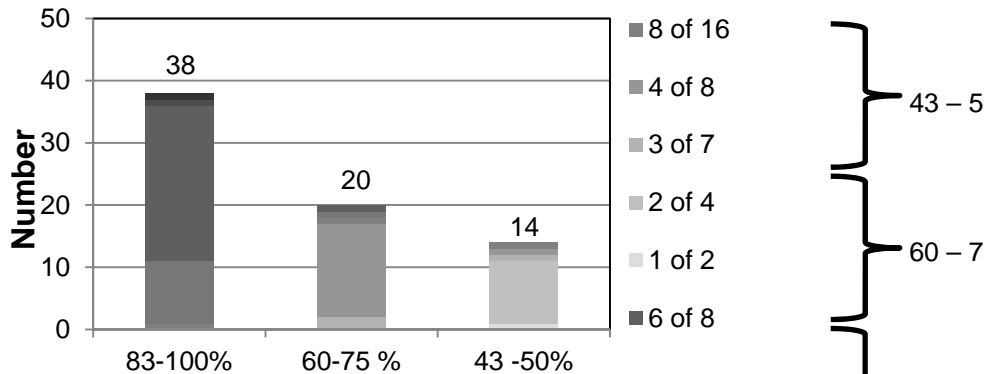


Figure 2. Percentage identical genotypes within the samples from one location based on samples from the available 72 multi-sample locations

DISTRIBUTION OF GENOTYPES

Taking all samples across Europe together, the most dominant genotype identified was 13_A2 ("Blue13", Table 1). Also a lot of genotypes not belonging to the four defined genotypes were found ("Others"). The genotype 6_A1 with about 10 % of the total samples was with the exception of 2 locations in Belgium exclusively found in the UK; here it was the dominant genotype. 1_A1 with about 9 % of the total samples was mainly found in Belgium and France (one location with 8 samples). Also interesting to notice was the distribution in Poland with the vast majority of the samples being classified as "Others".

Table 1. Genotypes of samples from different countries

Country	Number of samples with genotype					Total number per country	Relative per country (%)
	13_A2	6_A1	1_A1	33_A2	"Others"		
Belgium	72	2	19	7	16	116	36.0
United Kingdom	25	29	1	-	12	67	20.8
Germany	21	-	-	-	21	42	13.1
Poland	3	-	-	1	25	29	9.0
Netherlands	8	-	2	1	16	27	8.4
France	9	-	8	-	3	20	6.2
Denmark	-	-	-	-	11	11	3.4
Switzerland	6	-	-	-	-	6	1.9
Czech Republic	1	-	-	-	3	4	1.2
Total (#)	145	31	30	9	107	322	
Relative (%)	45.0	9.6	9.3	2.8	33.2		100

However, as shown before we have about 50 % of locations where multiple genotypes were detected. Therefore, the distribution of the genotypes in the different locations as shown in Table 2 is also quite interesting. The geographical distribution of the most frequent genotypes 13_A2, 6_A1 and 1_A1 are shown in Figures 3 and 4, however, some locations are too close together to be distinguished.

Table 2. Genotypes of samples from different locations

Country	Number of locations	Number of locations with genotype				
		13_A2	6_A1	1_A1	33_A2	"Others"
Belgium	34	21	2	9	3	12
United Kingdom	15	9	9	1	-	6
Germany	10	8	-	-	-	8
Poland	8	3	-	-	1	7
Netherlands	7	4	-	3	2	6
France	3	2	-	1	-	2
Denmark	3	-	-	-	-	3
Switzerland	2	2	-	-	-	-
Czech Republic	1	1	-	-	-	1
Total	83	50	11	14	6	45

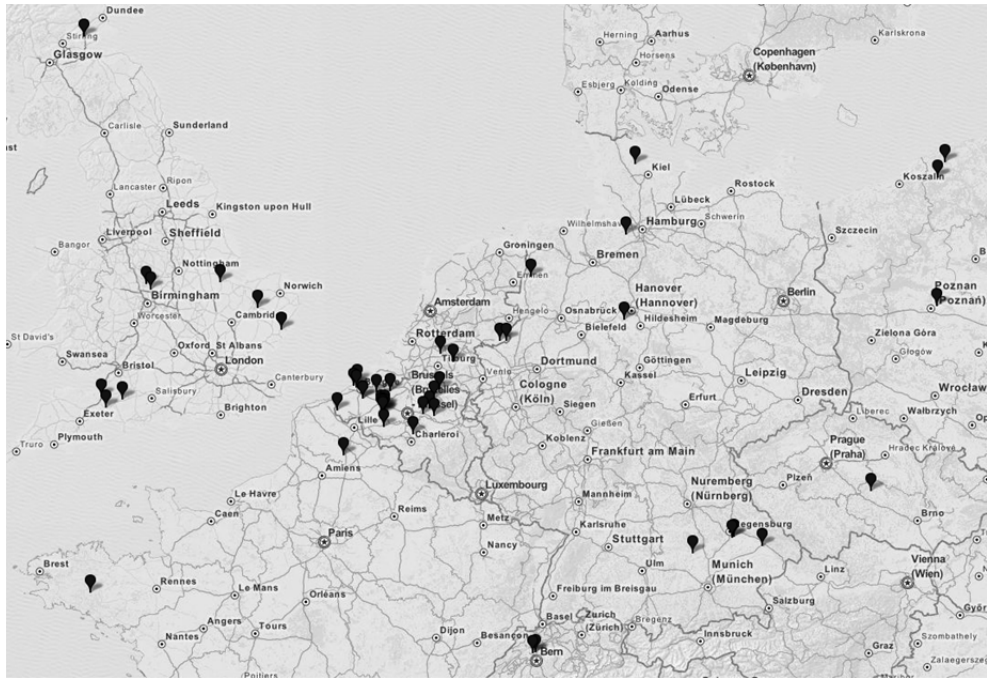
**Figure 3.** Locations where 13_A2 ("Blue13") was present



Figure 4. Locations where 6_A1 ("Pink 6", light) and 1_A1 ("GS008", dark) were present

CONCLUSIONS

The success rate (genotype specified) of the methodology was generally quite high with the exception of FTA cards coming from Germany and Poland which had a much higher failure rate (34 and 60 %) than the cards from the other countries (average 5 %). The reason for this is still under evaluation.

80 % of the samples from the same location had a dominant (> 50 %) genotype; in 50 % of the locations only one genotype has been found. Assuming that there is no bias due to "close by" sampling (samplers have been asked to sample from distant parts on one field) it can be concluded that in general in each field sampled one genotype is dominating the population.

The frequency of some genotypes was different across the countries where samples were taken, however in almost every country the genotype 13_A2 was detected. The genotype 6_A1 was frequent in the United Kingdom and rarely also in Belgium where 1_A1 was dominating the population. This genotype was also important in France but only minor in The Netherlands and The United Kingdom. A few isolates of the "new" genotype 33_A2 were detected in Belgium and also in The Netherlands and Poland. However, other studies have shown that it is also present in the United Kingdom (D. Cooke, pers. communication). In the majority of samples from Poland, The Netherlands and Germany different genotypes than the ones mentioned so far were found ("Others").

The methodology used in this study has proven as a good tool to investigate the population changes of *Phytophthora infestans* in Europe.

ACKNOWLEDGEMENTS

The authors want to thank all the people involved in the sampling of the 402 samples in the field.

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The early blight situation in Sweden – species abundance and strobilurin sensitivity

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SUMMARY

Early blight is a very common potato disease in the south-eastern part of Sweden, but has also been reported from other areas of the country. The disease has increased in severity during the last decades, but the true causal agent of the lesions is often not known. Identification of the causal agent of lesions with symptoms resembling early blight started in 2009, focusing on detection of *Alternaria solani* and *A. alternata*. Potato leaflets showing symptoms of early blight were collected three times during August and September from 2009 to 2012 in south-eastern Sweden. In addition, in 2010 and 2012, samplings were performed in the middle part of Sweden. Using diagnostic PCR methods (Edin, 2012; Zur 2002) the two *Alternaria* species were commonly identified in samples from south-eastern Sweden, most often found in co-occurrence. The two species were only found in a few samples from other areas, separate or in co-occurrence.

Despite repeated applications of strobilurin-based fungicides, the incidence of early blight was high in many of the sampled field. Loss of sensitivity toward strobilurins has been associated with various substitutions in the gene encoding cytochrome *b* in several plant pathogen species. In order to identify any substitution in *A. solani*, the PCR-products of all the confirmed samples were sequenced using the same primer combination as above. All *A. solani* samples were wild type, except for the samples from one farm collected in 2012 where the gene encoding cytochrome *b* diverged considerably from the wild type. The PCR product was shorter than the ordinary one. These strains may be of another genotype more resembling the American strains of *A. solani* (B. Adolf, pers. comm). These diverging samples as well as other samples with a shorter PCR-product will be analysed using the primers developed by Pasche *et al* (2005).

For *A. alternata*, a section of the cytochrome *b* gene of was amplified and cut using a restriction enzyme to identify the occurrence of G143A substitution (B. Vega, pers. comm.). The substitution was found in the majority of the samples from 2011 and 2012. This implies that the efficacy of strobilurin-based fungicides may have been reduced in the *A. alternata* population. However, the consequences for the potato crop are unknown since the pathogenicity of *A. alternata* may be low on potato, but further analyses are needed to confirm this.

During the collections in August and mid-September 2011 and 2012 scattered lesions with symptoms similar to early blight were found on *Solanum nigrum* in three different fields. In 2011, *A. alternata* was found in 14 of these lesions, eight of which had the G143A substitution. Two of the lesions collected in 2012 were caused by *A. solani*.

KEYWORDS

Alternaria solani, early blight, fungicide sensitivity

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Pathogenicity of *Alternaria*-species on potatoes and tomatoes

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SUMMARY

Various experiments were performed to analyze the virulence of *Alternaria solani* and *Alternaria alternata* on tomatoes and potatoes. Isolates were made from potatoes from different regions worldwide and a number of isolates were used as pure cultures for inoculum on tomatoes in the greenhouse and potatoes in the greenhouse and in the field. Conditions and host cultivars were varied in order to achieve infections. However, in all trials *A. solani* isolates were highly virulent while *A. alternata* isolates showed low or no symptoms after inoculation. Therefore doubts are justified if *A. alternata* is a causal agent of Early Blight or if it is rather a secondary invader which lives saprophytic on lesions and is therefore often isolated from leaf spots.

KEYWORDS

Alternaria solani, *Alternaria alternata*, Early Blight, Pathogenicity

INTRODUCTION

Early blight is in several potato growing regions a serious disease. The disease can be controlled by well-timed fungicide applications. In Western Europe two different *Alternaria* species are mainly discussed as causal pathogens, *Alternaria solani* and *Alternaria alternata* (Philippi 2011, Boehme 2013). Studies from Russia postulate also that the species *A. tomatophila*, *A. tenuissima*, *A. infectoria* and *A. arborescens* are involved in the Early Blight disease (Orina *et al.*, 2012). In Brasil *A. grandis* has been published as causal agent of Early Blight (Rodrigues *et al.*, 2010). *A. alternata*, *A. tenuissima*, *A. arborescens* and *A. infectoria* produce small spores, *A. solani*, *A. tomatophila* and *A. grandis* produce larger spores. The importance of the *Alternaria* species in Early Blight is seen differentially. Especially the impact of the small spored species, mainly *A. alternata* is controversially discussed. While some researchers see both species as causal agents or discuss a pathogen complex of *A. solani* and *A. alternata* (Leiminger and Hausladen 2012, 2013), others are convinced that only *A. solani* is pathogenic (Turkensteen *et al.*, 2010). In the last case *A. alternata* would be a saprophyte, which colonizes leaf lesions wherever this lesion came from (e.g. ozone damage, variation specific, caused by *A. solani* etc.) and is therefore a secondary invader. Between the different opinions consensus is found that *A.*

solani is pathogenic. Different experiments were performed with the objective to contribute to the elucidation of the importance of *A. alternata* in the Early Blight disease.

MATERIALS AND METHODS

Isolates

Isolates from 2010, 2011 and 2012 were made from leaf samples from potatoes. Leaf samples were sent dried to BASF, Limburgerhof and leaves with typical Early blight symptoms were sterilized, lesions were cut out and placed on 2 % malt agar (at 16°C or 22°C). Outgrowing mycelium was transferred on new petri dishes. Pure cultures were made by additional transfers. Isolated species were determined by their spore size and shape. Different isolates from different regions were used in the following experiments.

Greenhouse trials

Pathogenicity trials with single isolates on tomatoes in the greenhouse

Three weeks old tomato plants were inoculated with spore suspensions from the different isolates in order to determine the pathogenicity of each isolate. 10 ml spore suspension of each *A. alternata* and *A. solani* isolate was made with deionized water and 2 % malt solution, respectively. This experiment was conducted twice. The concentration of the spore suspensions were around $\sim 10^6$ spores per milliliter for *A. alternata* and $\sim 10^5$ spores per milliliter for *A. solani*. Spores were suspended in deionized water, 0.2 % malt extract or 2 % malt extract. Inoculated plants were incubated in a moist chamber with 20 °C and 95 % relative humidity. During the first 24 hours a lid covered the plants in order to prevent the spore suspension from washing off the leaves. The plants were kept for one week and rated regularly.

Additional trials were performed under same conditions but with mixtures of different isolates of *A. alternata* and *A. solani*. The idea behind was if a complex of both species leads to stronger infections.

In another experiment, tomato plants were wounded slightly with a tooth brush or stronger with a needle with the intention to enhance the infection by *A. alternata*. Infection was carried out with 2 % malt and the same conditions as described above.

Additional experiments were performed with older tomato plants in order to vary the age of the plants at inoculation time point since there are hints in literature that older leaves are more susceptible than younger ones (Rotem 1994).

Virulence trial with isolates in mixture on potatoes in the greenhouse

Host plants were three weeks old potatoes of the variety Aveka and Kuras which were inoculated with 50 ml of each single suspension as described above. An air sprayer with a nozzle size of 0.8 mm and 0.5 bar was used to coat the leaves with the suspensions. Two plants of each cultivar were sprayed with 2 % malt solution to serve as control plants. In between two suspensions the sprayer was washed with water in order to prevent contamination. Inoculated plants were incubated in a moist chamber with 20 °C and 95 % relative humidity. During the first 24 hours a lid covered the plants in order to prevent the spore suspensions from washing off the leaves. The plants were kept for two weeks and were visually rated regularly.

Field trial

Two potato varieties, Aveka and Kuras, were planted into a field. The field consists of two separate fields each with cultivar Aveka and Kuras, respectively. Every subfield contains 100 completely randomized plots, implying there are four replications per treatment. One plot of the size of 3 m x 1.5 m comprises two rows of potatoes. The field was fertilized once with 350 kg*ha⁻¹ ENTEC® perfect, which is equivalent for 25.5 kg N*ha⁻¹. To avoid infections with *Phytophthora infestans* (Late blight) one application with Ranman® (0.2 kg*ha⁻¹) and one application with Revus® (0.6 kg*ha⁻¹) was carried out. There were additional treatments against weeds and *Leptinotarsa decemlineata* (Colorado potato beetle). Inoculation date was five weeks after emergence of potato sprouts. The preparation of inoculum started a few weeks prior to the field inoculation.

For each *A. solani* strain one two weeks old fungal petri dish was pureed (Ultra Turrax) with 100 ml deionized water. Then 15 flasks with 50 ml of V8 medium were inoculated with 500 µl of the fungal-agar-puree. The flasks were placed in an agitating chamber (150 rpm, 22 °C). After one week the resulting mycelium was pureed for one minute and 1 ml mycelium puree was pipetted on 2 % malt agar plates and incubated for another two weeks at 22 °C and 12 h photoperiod. With this method it was possible to inoculate more than 400 petri dishes for each strain in a relatively short period of time.

As *A. alternata* produces enough spores only 60 plates per strain were needed for the inoculum preparation. Thus fungal material was transferred in the usual five-point manner on 2 % malt agar containing petri dishes two weeks previous to the inoculation date.

The spore suspensions were prepared with 2 % malt solution and adjusted to a spore density of $1.78 \cdot 10^4$ spores per milliliter. Afterwards the spore suspensions were mixed according to the treatments and 1.4 liters of each treatment were taken into the field. In turns every suspension was poured into a backpack sprayer and sprayed with 2 bar onto the plots. After each spore suspension the backpack sprayer was cleaned with water in order to prevent contamination. To achieve better conditions for infection the field was irrigated before and after inoculation. In frequent time intervals the plots were rated visually. The trial plan is shown in Table 1.

Table 1. Trial plan for the field trial 2012 in potatoes (var. Aveka and Kuras). As 121, As 185, Aa 204 and Aa 257 represent isolates of *A. solani* and *A. alternata*, respectively

Treatment number	Isolates	Ratio <i>A. solani</i> (As) : <i>A. alternata</i> (Aa)
1	Not inoculated control	-
2	As 121	100
3	Aa 204	100
4	As 121 : Aa 204	10 : 90
5	As 121 : Aa 204	50 : 50
6	As 121 : Aa 204	90 : 10
7	As 185	100
8	Aa 257	100
9	As 185 : Aa 257	10 : 90
10	As 185 : Aa 257	50 : 50
11	As 185 : Aa 257	90 : 10
12	As 121 : Aa 257	50 : 50
13	As 185 : Aa 204	50 : 50

RESULTS AND DISCUSSION

Isolates from diseased leaf samples

Both species, *A. alternata* and *A. solani* were isolated from diseased leaves. Interestingly, the temperature during the incubation of the leaves in the agar plates played an important role in the success of isolation of *A. solani* or *A. alternata*. Lower temperatures favored isolation of *A. solani* and higher ones the isolation of *A. alternata* (Figure 1). This is a very important finding because it shows that the isolation success depends at least on the temperature. However the fact that both species could be isolated from typical lesions does not necessarily show that both species are virulent. To show this, the Koch's postulates needed to be fulfilled. Therefore we made inoculation trials under various conditions which are described in the next chapters.

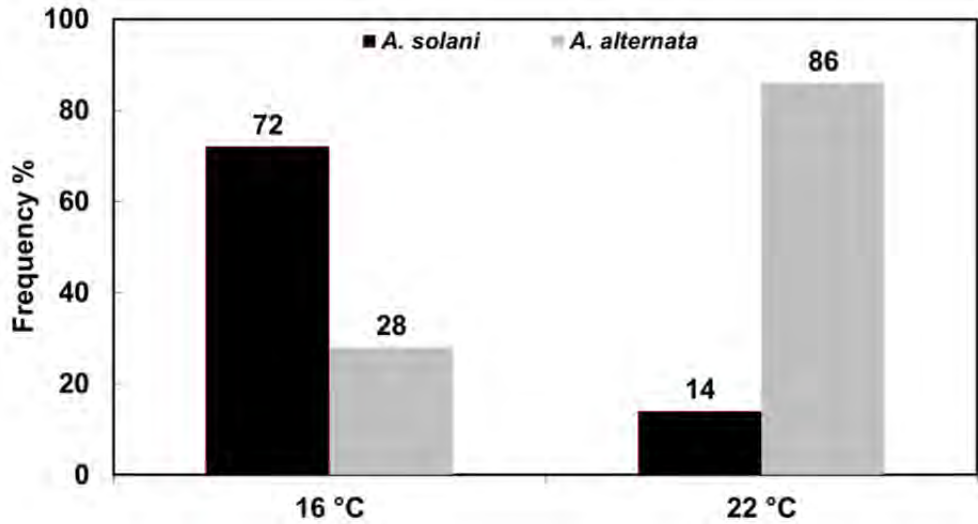


Figure 1. Influence of temperature during isolation process on the success of isolation of *A. solani* or *A. alternata*. Data represent 161 isolates which were made from 10 samples

Greenhouse trials with tomatoes

In all greenhouse trials no typical infections were achieved with various isolates of *A. alternata* but easily with all isolates of *A. solani* (Figure 2, 3). Mixtures did not show higher infection rates, which indicates that a complex of two species did not enhance virulence. When spores were suspended in 2 % malt extract, the infection was much higher. The very weak symptoms caused by *A. alternata* in the 2 % malt extract experiments were untypical black spots on the leaf surface which could be physically removed.

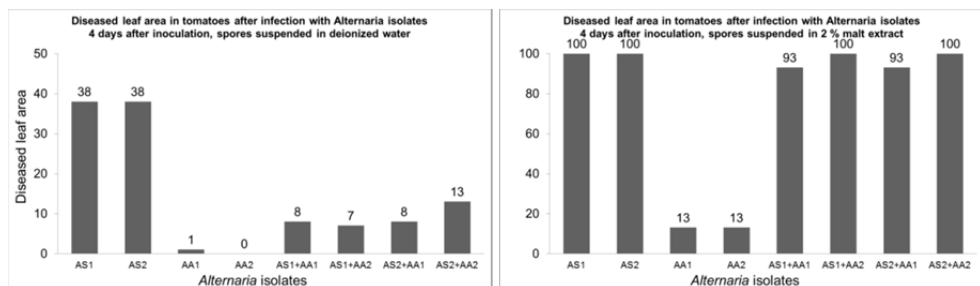


Figure 2. Infection of tomatoes with strains of *A. solani* (AS1, AS2), *A. alternata* (AA1, AA2) or mixtures of the isolates (AS1+AA1, AS1+AA2, AS2+AA1, AS2+AA2). Spores were suspended in deionized water (left) or 2 % malt extract (right)

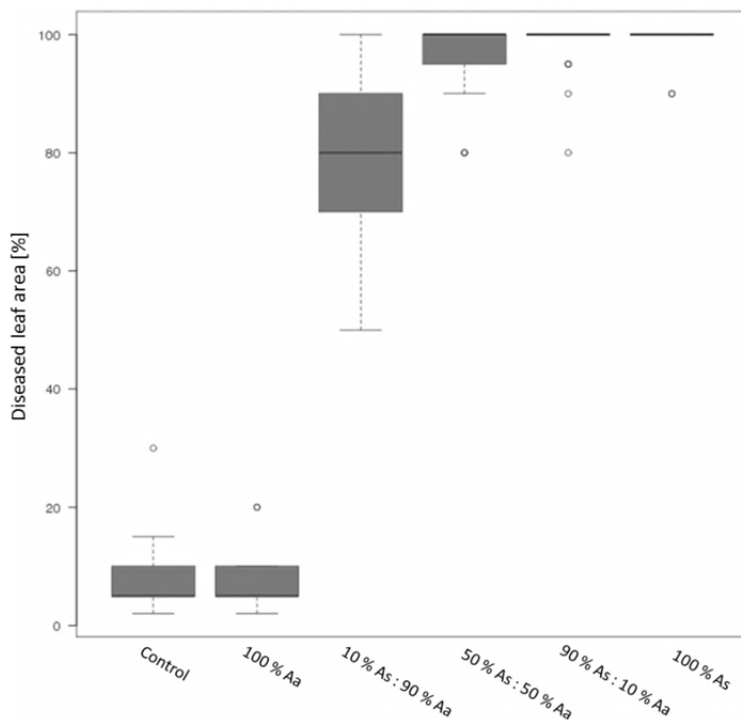


Figure 3. Box and Whisker plot of a greenhouse trial with tomatoes. Plants were infected with isolates of *A. solani* (As), *A. alternata* (Aa) or mixtures of the isolates in different ratios (90:10, 50:50, 10:90). Spores were suspended in 2 % malt extract

A trial on two months old tomato plants was done according to the previous experiments in the greenhouse. Ten leaves of two months old tomato plants were inoculated in order to divide the whole plant into sections with differently old leaves. The idea was to see whether each section shows different susceptibility against Early Blight. There were already Early Blight symptoms visible after three days post inoculation. Plants that were inoculated with *A. solani* showed lesions and the leaves started to hang (Figure 4). On the contrary the treatment with 100 % spore suspension of *A. alternata* looked as good as the control plants with no inoculation.

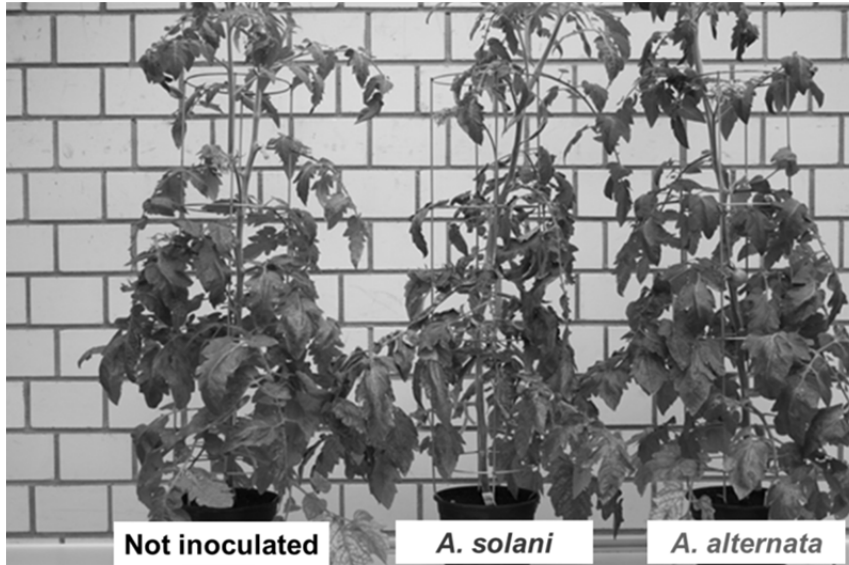


Figure 4. Two months old tomato plants inoculated with spore suspensions made with 2 % malt solution. From left to right: Control, plant inoculated with a spore suspension of 100 % *A. solani* (As) and one with 100 % *A. alternata* (Aa), 3 days after inoculation

After seven days post inoculation plants inoculated with high percentages of *A. solani* in the spore suspension lost already most of their leaves and showed high rates of necrosis as to compare in Figure 5. On the other hand high percentages of *A. alternata* in the mixture led only to weak symptoms and plants looked almost like the untreated control. The plants were divided into lower, middle and upper leaf level. As there was no visible differentiation in disease severity between the middle and the lower section during the rating process, they were combined to lower leaf level. So there were in total 2 sections, upper and lower leaf level, to rate and evaluate.

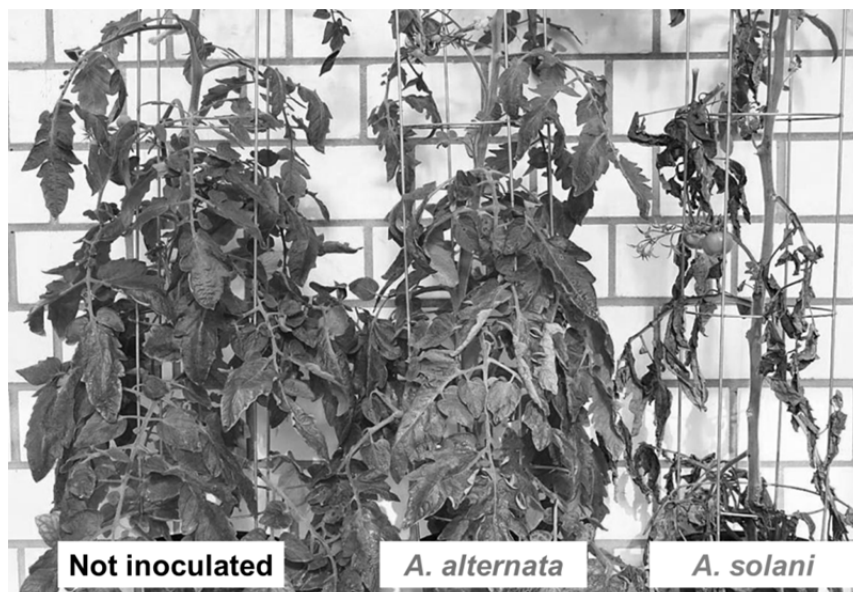


Figure 5. Two months old tomato plants inoculated with spore suspensions made with 2 % malt solution. From left to right: Control, plant inoculated with a spore suspension of 100 % *A. alternata* and one with 100 % *A. solani*, 7 days after inoculation

At the end of the period of observation the rating results were used to calculate the area under disease progress curve (AUDPC) to determine the intensity of disease. Figure 6 shows the results concerning the differences between the treatments. The tendency is the same as in previous experiments. The higher the content of *A. solani* in the mixture the more severe is the infection. Infections caused by a 100 % spore suspension of *A. alternata* cause only weak infections. Thus AUDPC is small and significant smaller than in the other treatments but not significantly different from the untreated control.

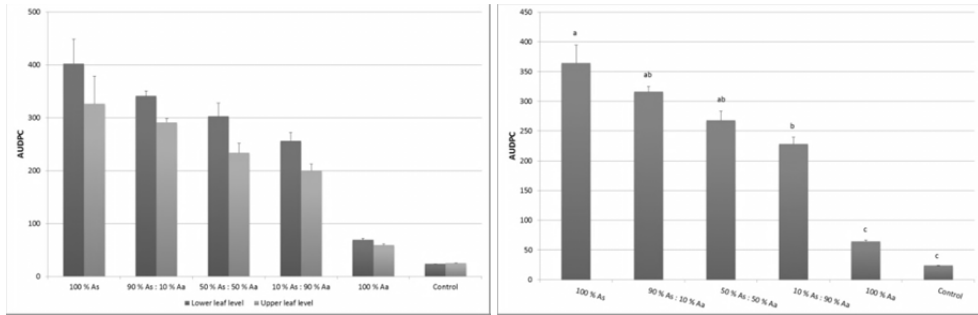


Figure 6. Left: AUDPC for infection progress in the mixture experiment on two months old tomato plants in the greenhouse for upper and lower leaf level. Rating took place 3 - 7 dpi. Simple means with standard error. Right: Statistical analysis of AUDPC of the mixture experiment on two month old tomato plants in the greenhouse. Means with standard error. Same letter means no significant differences ($\alpha=0.05$, LSD). 7 dpi

Even wounding (slight with tooth brush on upper and lower leaf side or stronger with needles) did not lead to infections with *A. alternata* (Figure 7). This experiment was not done with *A. solani*, because under this incubation conditions a 100% disease severity would occur even without wounding.

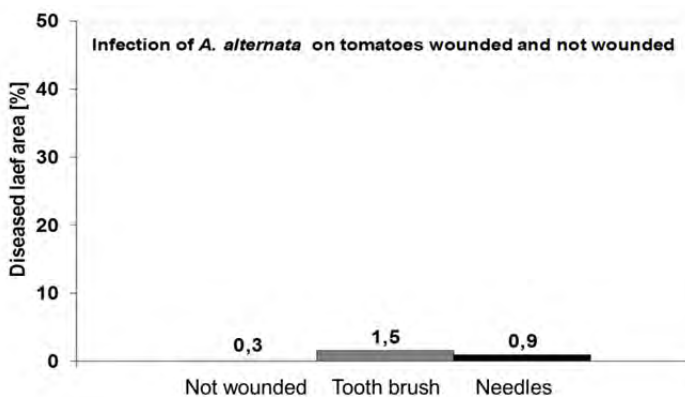


Figure 7. Mean values of 2 trials with 3 replicates of 8 isolates each on 14 days old tomato plants. Inoculation was done directly after wounding with 2×10^6 Spores/ml. Evaluation 9 days after inoculation

Greenhouse trials with potatoes

Figure 8 shows a typical result of inoculation experiments with potatoes in the greenhouse. Infected leaves are sometimes dropped soon after symptoms appeared. *A. solani* infected potato plants but *A. alternata* did not. No differences in infection between the two potato cultivars Aveka and Kuras were observed.

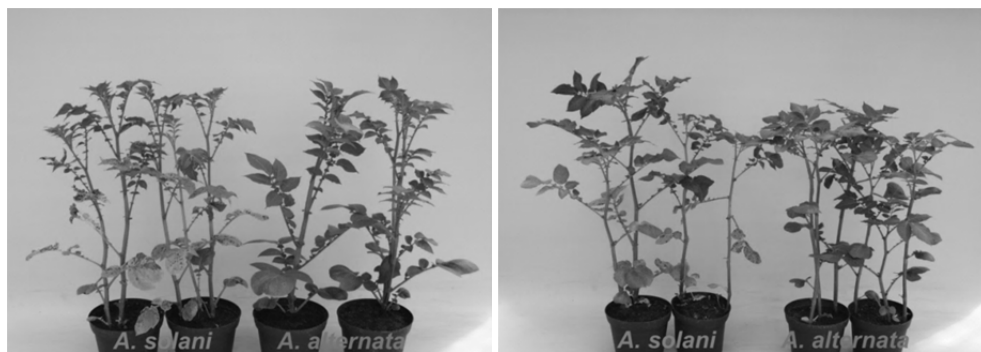


Figure 8. Infection of potato plants in the greenhouse with *A. solani* and *A. alternata*. Lower leaves were inoculated. Plants infected with *A. solani* showed yellowing of leaves, the typical brown target spots and dropping of leaves. No symptoms occurred on plants infected with *A. alternata*. Left: cultivar Kuras. Right: cultivar Aveka

Field trials with potatoes

Field trials were performed with the varieties Aveka and Kuras. Two isolates of *A. solani* and 2 isolates of *A. alternata* were used for inoculation alone and in mixtures with different ratios. Only isolates of *A. solani* or mixtures of *A. solani* and *A. alternata* infected the plants. Four days after infection first lesions were visible in the *A. solani* infected plots (Figure 9). In *A. alternata* plots, no or very low symptoms occurred. From most of the spots which occurred in *A. alternata* plots, *A. solani* was reisolated (20 isolates were made, 18 were *A. solani*, 2 were *A. alternata*) which indicates a natural “background” infection. This was also seen in the not inoculated control plots. Figure 10 shows the infection of the different isolates and mixtures during the time period of the trial. The values are mean values over both trials in Aveka and Kuras. Data show that the more *A. solani* is in the inoculation suspension, the higher the disease rating is.



Figure 9. Infection of potato plants in the field with *A. solani*. First symptoms occurred four days after inoculation (left) and disease developed further within the next three weeks (right) to very high disease levels and many dropped leaves. Here symptoms on variety Aveka are shown

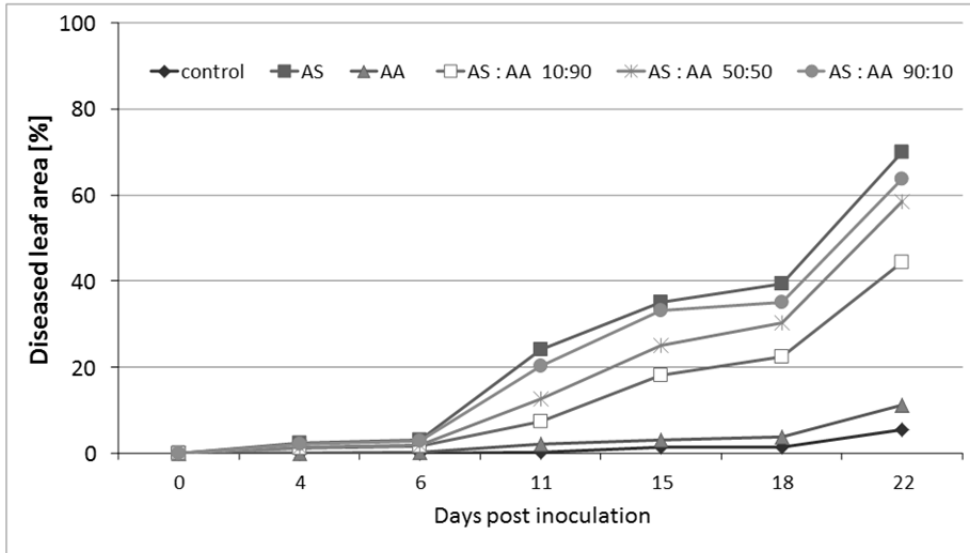


Figure 10. Infection of potato plants in the field over the time period of evaluation. Data show that the more *A. solani* was in the spore suspension, the higher the disease level was. AS means *A. solani* and represents two different isolates, AA means *A. alternata* and represents two different isolates. The values given are mean values of two isolates and mixtures in two different varieties from 4 replicates, which is then the mean value of 16 plot estimations

CONCLUSIONS

Different trials were made to evaluate the virulence of *A. solani* and *A. alternata* in potatoes and tomatoes. All trials showed that the species *A. solani* is highly virulent, while *A. alternata* is not or very low virulent under the different conditions we used. Since mixtures of *A. solani* and *A. alternata* did not lead to higher infection levels than *A. solani* alone, the theory of a disease complex is not justified from our view. Therefore our conclusion is that the main pathogen of Early Blight is *A. solani*. If other large spored *Alternaria* species, such as *A. grandis* and *A. tomatophila* play a role in Early Blight in Europe is currently under investigation. The fact that *A. alternata* can often be isolated from lesions on potato plants is from our view the result that *A. alternata* grows as a saprophyte there and is a secondary invader on such lesions.

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Comparing pathogenicity of *Alternaria solani* and *Alternaria alternata* in potato

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SUMMARY

Large-scale surveys of leaf lesions on potato in the period of 2009-2012 showed that many symptoms were not caused by *Alternaria solani*, but were of physiological origin. As a large percentage of these lesions contained the fungus *Alternaria alternata*, the question arose whether or not *A. alternata* is capable of causing necrosis. In this study we show and confirm that *A. alternata* appears to be unable to infect potato leaflets and cause lesions, suggesting there is no need to specifically treat against this saprophytic fungus.

KEYWORDS

Koch, lesions, *Alternaria*, ozone

INTRODUCTION

Early blight receives more attention in recent years as problematic pathogen on potatoes. Due to climate changes and more specific treatments for late blight, an increase in the amount of early blight was observed. However, as the symptoms strongly resemble symptoms caused mostly by deficiencies and ozone damage, people in the field often have difficulty distinguishing early blight symptoms from others. As a result in practice there have been numerous cases of people applying fungicides to a non-biologic cause of symptoms, with little to no result as consequence. In a few instances, they went as far as saying they were dealing with fungicide-resistant early blight.

On top of this problem with diagnostics is the discussion whether or not *A. alternata* is capable of causing lesions like *A. solani* does, therefore warranting a fungicide treatment. As *A. alternata* is present in high concentrations in the air, it is often found inside lesions. Lacking other obvious biological causes, such as *A. solani*, it is often assumed that *A. alternata* is the cause of lesions. In this study we test and compare the pathogenicity of *A. solani* and *A. alternata* on various cultivars of potato. The study is performed both in a detached leaf assay and in a field trial. Additionally, the survey database allows for statistical analysis of co-occurrence, suggesting either competitive or non-competitive interactions between both species.

MATERIALS AND METHODS

Isolates of A. solani and A. alternata

For both species, three different isolates were used. Two *A. solani* isolates were obtained from practice fields in 2011. The third isolate was obtained from the Fungal Biodiversity Centre (CBS, CBS107.61). Likewise, two *A. alternata* isolates were obtained from practice fields, while the third isolate, A906NL11.2 was included as a commonly used isolate in studies.

Field trial

Plants of seven cultivars (Innovator, Markies, Miranda, Seresta, Festien, Aveka, Valiant) were planted in sandy soil (Wijster, the Netherlands). Three treatments were included: inoculation with *A. solani*, inoculation with *A. alternata* and an untreated control. Each treatment contained three replicates of at least 8 plants per cultivar per replicate. Inoculation was performed using a cocktail of the three aforementioned isolates at the flowering stage of potato growth. 10.000 spores/ml were applied during calm wind conditions. Lesions were counted and analysed in the laboratory for presence for either *Alternaria* species.

Detached leaf assay

Leaflets were obtained from the same plants used in the field trial (Wijster, The Netherlands, sandy soil). These plants had not received any treatments with fungicides with efficacy against early blight. Leaflets were placed on water agar (15g/l), abaxial side up.

In order to give both fungi maximum chances to infect, small wounds were made on the abaxial side of half the leaflets using a sterile scalpel. The other leaflets remained unwounded. Per leaflet 10 drops of 10 μ l each, containing spore suspensions (10000 spores/ml) were placed. Suspensions contained spores of one of three *A. solani* isolates, one of three *A. alternata* isolates or no spores (untreated control). Petri dishes were sealed and placed into a climate chamber for 7 days for disease development (20°C, 16h light). Lesion development and size were assessed at this point.

RESULTS

Detached leaf assay

Lesions only appeared on leaflets inoculated with *Alternaria solani* (Figure 1). *Alternaria alternata* was unable to create lesions under any condition. Wounding of the leaves increased the size of lesions caused by *A. solani* (Figure 2), most likely due to the easier access the fungus has to the target cells, starting the disease earlier. Isolate 3 of *A. solani* contained a spore solution with less than 10.000 spores/ml, but high amounts of mycelium. It is shown that when wounded, this does not affect the ability of the fungus to cause lesions, but in unwounded leaves, lesions are smaller, pointing to the importance of spores in spread of the disease.



Figure 1. Detached Leaf Assay. Lesions caused by *A. solani* appear on all cultivars, whereas *A. alternata* is unable to create lesions on leaves. Shown here is cultivar “Seresta”, although results apply to all cultivars. Wounding did not allow *A. alternata* to create any lesions

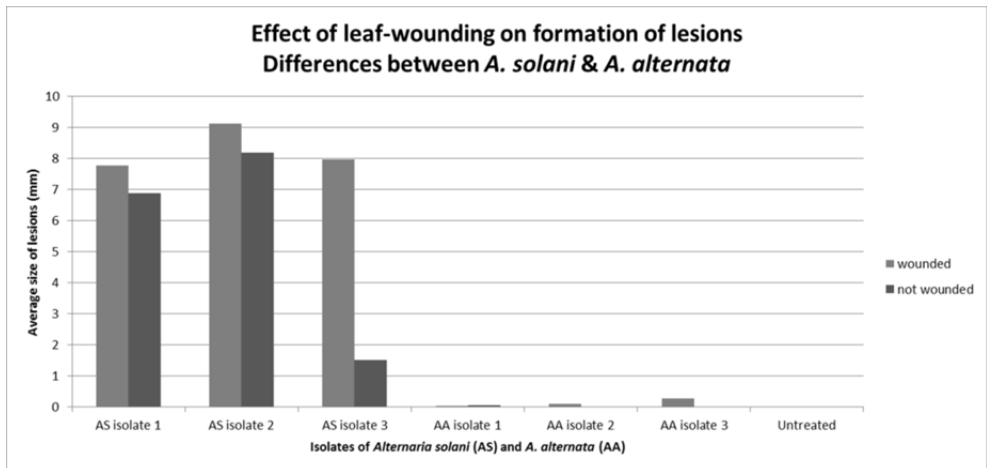


Figure 2. Effect of wounding of leaves on the lesion size. Seven cultivars are included in the trial. *A. solani* (AS isolate 1-3) causes lesions on both wounded and unwounded leaves, showing its role as a pathogen. *A. alternata* (AA isolate 1-3) shows no significant lesions compared to the untreated control

While *A. alternata* does not create lesions on its own, the discussion arose whether or not it can make lesions created by other causes, like *A. solani*, bigger. This would still warrant treatment of *A. alternata*. On top of lesions caused by *A. solani*, we applied spores of *A. alternata*. Measuring the lesions after one week showed that *A. alternata* did not enhance lesion growth (Figure 3).

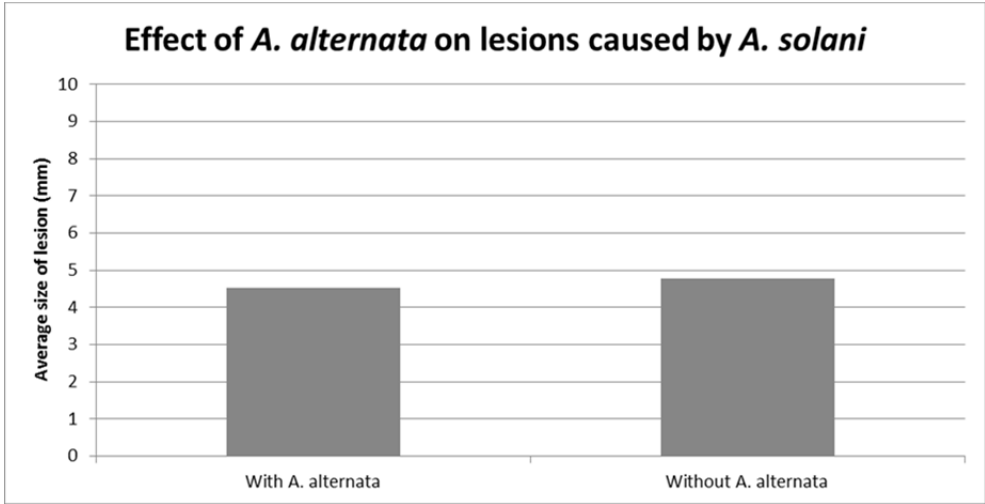


Figure 3. *A. alternata* on top of *A. solani*. Based on this small experiment, where *A. alternata* was placed on top of lesions caused by *A. solani*, there is no enhancing effect of *A. alternata* on lesion growth

Field trial

Similar to the detached leaf assay, presence of *A. alternata* did not lead to more lesions in the field. Problematic with field trials concerning *A. alternata*, is the naturally high concentration of the fungus in the air. Spores of the fungus are a known allergen for hay fever-like reactions. Due to this high concentration, *A. alternata* is often found in lesions caused by pathogens or physiological damage. Inoculation of plants in the field with *A. alternata* therefore did not lead to more or bigger lesions (Figure 4). All fields contained a certain amount of lesions before inoculation, most likely caused by ozone. The fields inoculated with *A. solani* contained more lesions, caused by this fungus.

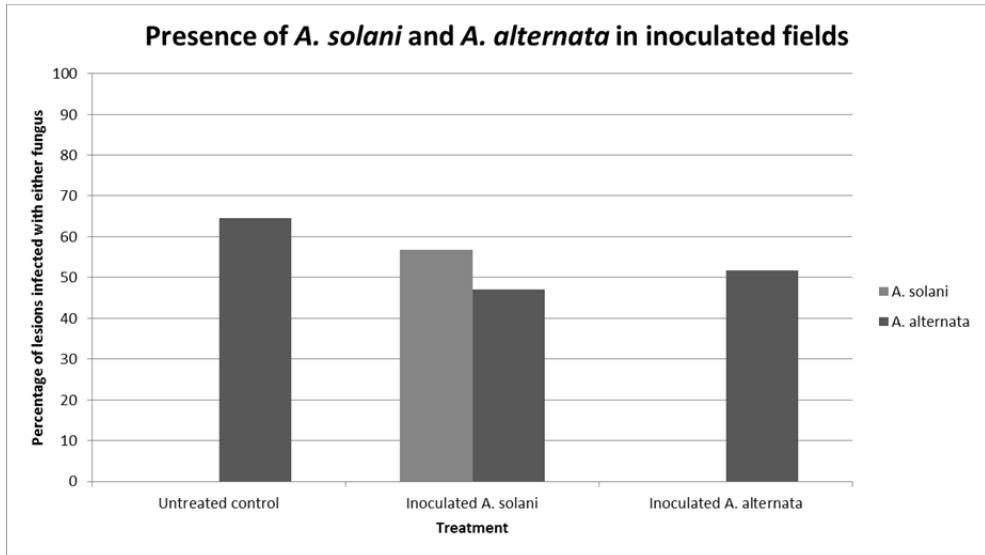


Figure 4. Inoculation with *Alternaria alternata* does not lead to development of lesions. As a saprophytic fungus on potato, it does find its way into lesions already present. *A. solani* was only found in the trial fields inoculated with this fungus, confirming its role as a pathogen and showing there was no cross-contamination to other fields

Statistical analysis of co-occurrence

Based on the hypothesis that two different species that are both pathogens on the same host plant are competitors of each other, we analysed our data on co-occurrence of both *Alternaria* species. If both *A. solani* and *A. alternata* are pathogens, presence of one fungus would depend on the presence or absence of the other. In the treatment where both fungi are present, we gathered 265 lesions and determined the presence of either fungus. Table 1 shows the statistical analysis of the results. *A. alternata* appears independent of *A. solani*. This is in line with our expectations. *A. alternata* lives on dead tissue, regardless of the cause. It appears in 51,7% of the lesions, regardless of whether or not these are infected with *A. solani*.

Table 1. Analysis of co-occurrence of *A. alternata* and *A. solani*. Based on the chi-square test, both fungi appear independent of each other

2012 trials		
Total # of lesions	265	
Total # of lesions infected with <i>A. alternata</i>	137	
% of total lesions infected with <i>A. alternata</i>	51,7	
Total # of lesions infected with <i>A. solani</i>	197	
	With <i>A. alternata</i>	Without <i>A. alternata</i>
Observed # of lesions of <i>A. solani</i>	101	96
Expected # of lesions of <i>A. solani</i> (51,7% of 197)	101,85	95,15
Chi-square test	p = 0,9041	

DISCUSSION

Based on our large-scale studies into lesions on foliar tissue of potato in recent years, we started to have our doubts about the pathogenic capabilities of *Alternaria alternata*. If the fungus is a true pathogen and present in such high quantities that it warrants hay fever alerts, there should hardly be any healthy plant left standing in the field. Combined with the fact that damage caused by ozone and deficiencies is an underestimated problem this led us to investigate the role of the fungus compared to its big brother *A. solani*.

In the study we presented here, we applied the postulates of Koch. Surprisingly, in literature we were unable to find a study where this was already done for *A. alternata*. In the field, *A. alternata* is already present in high concentrations, which is why it is often found in lesions and named as the cause of it, especially if the lesion lacks other fungi due to being caused by physiological damage. When plants were inoculated with high concentrations of *A. alternata*, this did not lead to the appearance of more lesions (figure 4). *A. solani*, as expected, did create more lesions.

In the lab, we provided both fungi with ideal circumstances: applied directly on the leaf; wounded to ease access and development under controlled conditions. *Alternaria alternata* once again failed to create lesions on any of the cultivars.

While our experiments were relatively simple, they are important. In Dutch agricultural practice, growers sometimes apply fungicides specifically for *A. alternata*. Our results indicate that this is not necessary. *Alternaria solani* is the true pathogen in the family and is the one that should be treated with fungicides. These results are confirmed elsewhere in these Proceedings by Stammler *et al.* Other causes of leaf spots, such as damage caused by ozone or nutrient deficiencies, are the bigger danger to potato yield.

State of the art and important research questions: Report from the new EuroBlight Alternaria group

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1. PROTOCOLS

The discussion started with the key question of the causal agent of early blight in potatoes (*A. solani*, *A. alternata*, other *Alternaria* species).

The first step is to harmonize the different protocols of the Early Blight research groups with the aim to get comparable results.

The EuroBlight *Alternaria* group decided to summarize the different protocols for isolation, spore production, artificial inoculation (greenhouse and field) and molecular detection of the participants.

2. PROTOCOL FOR FUNGICIDE TRIALS TO PROVIDE RATINGS FOR ALTERNARIA FUNGICIDES

Hans Hausladen presented the following outline of the protocol to initiate discussion.

Field trial

- Susceptible variety
- Weekly applications of Revus or Ranman Top to prevent late blight
- Two to five applications of *Alternaria* fungicides
- Test fungicides to commence before the start of the epidemic (approximately 7 to 8 weeks after emergence)
- *Alternaria* test fungicides to be applied at intervals of 14 days and at the highest label dose rate in Europe
- Two or more reference fungicides, i.e. mancozeb (1500 g a.i. per ha), Signum (0.25 kg/ha) and Ortiva (0.5 L/ha)

Fungicide rating trials according to the protocol will be conducted 2013 in Germany, Netherlands, Denmark, Belgium and Poland (supervisor: Bent Nielsen).

3. HOMEPAGE

All the protocols as well as relevant publications (*Alternaria* on potato and tomato) will be uploaded and updated on the EUROBLIGHT homepage.

4. NEW INITIATIVES

The members of the subgroup decided to initiate a cooperating project dealing with the widespread of the QoI fungicide resistance of *A. solani* in European potato growing areas. The project calls "Monitoring of sensitivity to fungicides (QoI) of *A. solani* isolates".

The project starts in 2013 with a limited number of *Alternaria solani* isolates and locations.

Report of the Control Strategies Subgroup meeting on 15 May 2013: Discussion and agreements reached

BAIN RA

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

CHAIRMAN: HUIB SCHEPERS

Initially members of the Alternaria Subgroup attended a joint meeting with the Control Strategies Subgroup to allow aspects of Alternaria fungicide ratings to be fully discussed.

1. ALTERNARIA BLIGHT

1.1 Efficacy rating for Revus Top

Prior to the workshop the rating assigned to Revus Top was undecided but between ++(+) and +++. Consequently it was agreed in Limassol that the full 0 to ++++ rating scale needed to be used. Azoxystrobin and Signum were re-rated as +++(+), allowing Revus Top to be assigned +++. The ++++ rating will be kept in reserve for future products with very high efficacy.

1.2 Protocol for fungicide trials to provide ratings for Alternaria fungicides

Hans Hausladen presented the following outline of the protocol to initiate discussion.

Field trial

- Susceptible variety
- Weekly applications of Revus or Ranman Top to prevent late blight
- Two or three applications of Alternaria fungicides (after the meeting this was revised to two to five applications)
- Test fungicides to commence before the start of the epidemic (approximately 7 to 8 weeks after emergence)
- Alternaria test fungicides to be applied at intervals of 14 days and at the highest label dose rate in Europe
- Two or more reference fungicides, i.e. mancozeb (1500 g a.i. per ha), Signum (0.25 kg/ha) and Ortiva (0.5 L/ha)

Proposal: The protocol should be tested in existing trials in 2013 (Agreed)

Proposal: Tuber yield should be assessed (Not agreed)

Proposal: The Alternaria Subgroup should decide when fungicide ratings trials are to start (Agreed)

Proposal: Out of season trials could be outwith Europe, e.g. in South America, to speed up testing of the protocol. The trials would be arranged through the fungicide companies. (Decision deferred pending further discussion at the Alternaria Subgroup meeting later in the day)

1.3 Miscellaneous

The rates of fungicide products have to be included in the Alternaria fungicide table.

Proposal: A disclaimer to cover possible fungicide insensitivity in Alternaria spp. was also required for the Alternaria table (Agreed). The wording should be 'Insensitivity genes have been found in the European population but there has been no loss of efficacy in the field' (Agreed).

2. CONTROL STRATEGIES SUBGROUP ALONE, LATE BLIGHT

There were thirty attendees.

2.1 Ratings

Proposal: From 2013 the 0 to +++ ratings decided by the fungicide experts will be allocated between workshops (Agreed).

All updates of the fungicide ratings table are to be notified by e-mail or by a message on the EuroBlight website.

Proposal: Revus Top is to be included in the table with identical ratings to those for Revus (straight mandipropamid). Revus and Revus Top are to be included on the same row of the table (Agreed).

2.2 Fungicide table

Proposal: The late blight and Alternaria fungicide tables should be combined (Not agreed). There are different rating scales for the two diseases.

Proposal: That the A and B tables should be combined because of the move to objective, trials-based ratings (Agreed).

Proposal: Include in the table the date that a product was first registered in Europe but there is no need to include the country of first registration (Agreed).

Proposal: Products no longer marketed are to be removed from the table (Agreed). The following two products are to be removed: propamocarb + mancozeb and propamocarb + chlorothalonil. The cyoxanil + metiram mixture is to remain, together with chlorothalonil.

Concern was expressed that there are too few products with decimal ratings for tuber blight control compared the number with earlier subjective ratings. Jens G. Hansen has to include a statement on the EuroBlight website that previous 0 to +++ ratings can be obtained from the workshop proceedings.

Proposal: There should be a disclaimer included with the fungicide table to cover possible fluazinam insensitivity. The disclaimer should read ' Isolates have been found in The Netherlands resulting in lower field efficacy of fluazinam' (Agreed).

Proposal: There should be links from the EuroBlight late blight and *Alternaria* fungicide ratings tables to the FRAC website (Agreed).

2.3 Trials

There will be three EuroBlight leaf blight ratings trials in 2013. However, there will be no EuroBlight tuber blight fungicide rating trials in 2013 due to an insufficient number of new products being put forward.

Fantic M (benalaxyl-M (4%) + mancozeb (65%)) has been rated for leaf blight control and is therefore to be included in the ratings table. Data to inform subjective ratings for some characteristics need to be obtained from Isagro Ricerca.

Proposal: Leaf blight ratings trials should have more reference products than just mancozeb (Decision deferred until after Huub Schepers and Bert Evenhuis considered this question).

2.4 New initiatives and developments

Huub Schepers outlined the EU-wide population monitoring that was starting in 2013.

He also described the potential role of EuroBlight in the forthcoming ERA-net call.

Proposal: EuroBlight should progress the development of LatinBlight in South America (Agreed). The initial step would be to organise a LatinBlight event as part of the next ALAP meeting in Columbia in 2014.

Fungicide companies are to contact Huub Schepers with details of a local contact in South America (Agreed by all companies present at the meeting that have interests in South America).

Proposal: An App should be developed for the fungicide tables (Not agreed).The tables were not considered to be sufficiently dynamic for an App and access via the EuroBlight website was considered perfectly adequate.

Proposal: That following the increased importance of fungicide curative activity commercially in 2012, especially in the UK, decimal ratings trials should be carried out for curative activity to provide improved information on this property (Not agreed).

3. RECORD OF FUNGICIDE TABLES

The most up to date versions of the late blight and *Alternaria* fungicide tables should be accessed via the EuroBlight website. The fungicides tables in this paper are a record of the tables as at 1 September 2013, prior to the agreements reached above being implemented.

GENERAL COMMENTS ABOUT THE RATINGS TABLES FOR LATE BLIGHT FUNGICIDES (LATE BLIGHT TABLES A AND B)

The scores for individual products 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. The dose rates in brackets are those used in the EuroBlight field trials to determine the leaf blight and tuber blight ratings. Only compounds included in EuroBlight trials are rated for foliar and tuber blight control. Ratings will be lower where fungicide insensitive strains are present.

The ratings given in Table A are for late blight fungicides currently registered in several EU countries and are for commercially available products containing one active ingredient, or two active ingredients as a co-formulated mixture. The ratings are NOT for the active ingredients themselves. Table A 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 those for leaf and tuber blight control, are based on field experiments and experience of the performance of products when used in commercial conditions. Ratings for leaf blight and tuber blight control were calculated from the results of EuroBlight field trials, and only compounds included in a minimum of six of these trials are rated. Ratings, other than leaf and tuber blight control ones, are intended as a guide only and will be amended in future if new information becomes available. Tables A and B are available on the EuroBlight website and the website versions are updated more frequently.

Late Blight Table B 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 either calculated from dedicated trials (leaf blight and tuber blight efficacy only) or are the consensus view of the Control Strategies Subgroup and are based on information from non-EuroBlight 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 (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.

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.

Late Blight Table A. The effectiveness of fungicide products/co-formulations for the control of *P. infestans* based on the highest dose rate registered in Europe (as at 1 September 2013)

Product [Dose rate (l or kg/ha)]	Effectiveness		Stem blight	Tuber blight ⁴	Mode of Action			Rainfastness	Mobility in the plant
	Leaf Blight ³	New growth			Protectant	Curative	Anti-sporulant		
Copper			+		+(+)	0	0	+	contact
dithiocarbamates (2.0) ¹	2.0		+	0.0	++	0	0	+(+)	contact
Chlorothalonil			(+)		++	0	0	++(+)	contact
cyazofamid (0.5)	3.8	++	+	3.8	+++	0	0	+++	contact
fluzinam (0.4)	2.9		+		+++	0	0	++(+)	contact
zoxamide+mancozeb (1.8)	2.8		+ 5		+++	0	0	++(+)	contact+contact
famoxadone+cymoxanil			+(+)		++	++	+	++(+)	contact+translaminar
mandipropamid (0.6)	4.0	++	+(+)		+++	+6	+(+)	+++	translaminar+contact
benthiavalicarb+mancozeb (2.0)	3.7		+(+) ⁵		+++	+(+)	+	++(+)	translaminar+contact
cymoxanil+mancozeb			+(+)		++	++	+	++	translaminar+contact
cymoxanil+metiram			+(+)		++	++	+	++	translaminar+contact
cymoxanil+copper			+(+)		++	++	+	++	translaminar+contact
dimethomorph+mancozeb (2.4)	3.0		+(+)		++(+)	+	++	++(+)	translaminar+contact
fenamidone+mancozeb (1.5)	2.6		+(+) ⁵		++(+)	0	+(+) ⁵	++	translaminar+contact
benalaxyl+mancozeb ²		++	++		++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+mancozeb ²		++	++		++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+fluzinam ²		++	++		++(+)	++(+)	++(+)	+++	systemic+contact
propamocarb-HCl+mancozeb		+(+)	++		++(+)	++	++	+++	systemic+contact
propamocarb-HCl+chlorothalonil (2.7)	3.4	+(+)	++		++(+)	++	++	+++	systemic+contact
propamocarb-HCl+fenamidone (2.0)	2.5	+(+)	++		++(+)	++	++	+++	systemic+translaminar
propamocarb-HCl+flupicolide (1.6)	3.8	++	++ 3.9		+++	++	++(+)	++(+)	systemic+translaminar

See caveats listed in the section entitled 'General comments about the ratings tables'

¹ Includes maneb, mancozeb, propineb and metiram. ² See text for comments on phenylamide resistance. ³ Based on EuroBlight field trials in 2006-2011.

⁴ Based on EuroBlight field trials 2009-2011 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 ; Blank = no rating

The scale for leaf blight is a 2 to 5 scale (2=least effective, 5= most effective).

The scale for tuber blight is 0 (no effect) to 5 (complete control).

Disclaimer : this is given in the text of this paper.

Late Blight Table B. Provisional ratings for the effectiveness of new fungicide products for the control of *P. infestans* in Europe (as at 1 September 2013)

Product [Dose rate (l or kg/ha)]	Effectiveness		Mode of Action					Rainfastness	Mobility in the plant
	Leaf Blight ¹	New growth	Stem blight	Tuber blight ²	Protectant	Curative	Anti-sporulant		
amisulbrom + mancozeb (0.5 + 2.0)	4.5	+	+	3.7	++(+)	0	?	+++	contact+contact
initium + mancozeb (2.5)	3.7	? ³	? ³		++(+)	0	0	+++	contact+contact
dimethomorph + fluazinam (1.0)	3.7			3.3					translaminar+contact
(propamocarb + cymoxanil) + cyazofamid ((2.0) + 0.5))				4.6					(systemic+translaminar)+contact
propamocarb + cymoxanil (2.0)					+(+)	++(+) ⁴	++(+)		systemic+translaminar

See caveats listed in the section entitled 'General comments about the ratings tables'

¹Calculated from EuroBlight trials ²Based on EuroBlight field trials 2009-2011. ³Observations from some field trials indicated that both new growth and stem blight efficacy were ++. ⁴In some trials the curative activity was +++.

Key to ratings : 0 = no effect ; + = reasonable effect ; ++ = good effect ; +++ = very good effect ; Blank = no rating

The scale for leaf blight is a 2 to 5 scale (2=least effective, 5=most effective).

The scale for tuber blight is 0 (no effect) to 5 (complete control).

Disclaimer : this is given in the text of this paper.

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

Product	Efficacy
azoxystrobin	+++
fluazinam	(+)
metiram/mancozeb ¹	++
propineb	++
chlorothalonil	+(+)
famoxadone+cymoxanil	++
fenamidone+mancozeb or propamocarb ²	++
zoxamide+mancozeb	++(+)
pyraclostrobin + boscalid	+++

Key to ratings : 0 = no effect ; + = some effect; ++ =reasonable effect ; +++ = good effect ; ++++ very good effect

¹This rating applies to products containing mancozeb when used at the highest dose rates (>1500 g/ha). This rating may not be appropriate where the rate of mancozeb used is lower, particularly where the second active substance is not effective against *Alternaria*. ²In some trials there were indications that the rating was ++(+). Ratings will be lower where fungicide insensitive strains are present.

Disclaimer: this is given in the text of this paper.

Efficacy of fluazinam for control of potato late blight (*Phytophthora infestans*) in Danish field trials

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SUMMARY

Shirlan (fluazinam) and mancozeb-based products were for many years the main products used for control of potato late blight (*Phytophthora infestans*) in Denmark. In 2006-2007 field trials were seen for the first time in which a significantly lower effect of Shirlan against late blight was observed. The low effect was mainly seen in field trials with artificial inoculation at Research Centre Flakkebjerg (AU). Improved effect of Shirlan was seen from 2008 when isolates for use as artificial inoculation were changed but there was still a large variation in efficacy (2012 with very high effect). The use of Shirlan has declined drastically since 2008, and Shirlan is now only recommended when the risk of infection is low and the active new growth of the potato plant is limited.

KEYWORDS

Potato late blight, *Phytophthora infestans*, disease control, fluazinam

INTRODUCTION

Shirlan (fluazinam) was introduced on the Danish potato market in 1998. Together with mancozeb, Shirlan was for many years the main product used for control of potato late blight (Cooke *et al.*, 2011). Spraying was recommended at weekly intervals with a label dose of 0.4 l/ha, and the effect against late blight was normally high, even under high disease pressure. In the same period the first DSS models were developed in which Shirlan was used with different dose levels depending on disease pressure (Nielsen, 2004). In 2006 field trials were carried out for the first time in which a significantly lower effect of Shirlan against late blight was observed. The same low effect was seen again in 2007, especially in field trials at Research Centre Flakkebjerg (Aarhus University).

FIELD TRIALS BEFORE 2006

Results from field trials 2005 are shown in fig. 1. Spraying was performed at weekly intervals, 0.4 l/ha, 12 sprayings per season and with start before the first attack in the plots. Spreader rows between the blocks were inoculated with a mixture of isolates collected in 2003 from

Denmark. There was a severe disease development in the trials but spraying with Shirlan always resulted in a high efficacy level. The same picture was seen in other trials in Denmark, and the results shown in fig. 1 are more or less representative of the situation in trials before 2006.

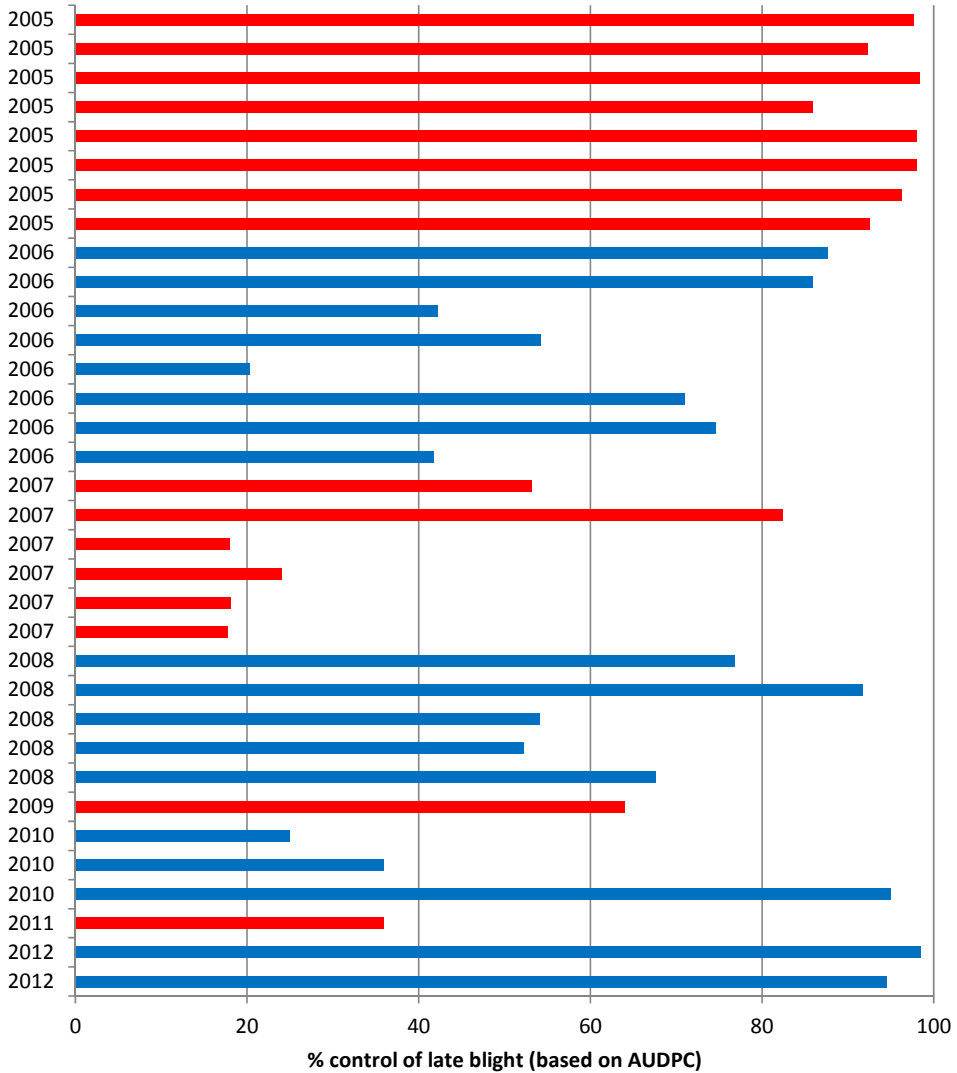


Figure 1. Control of potato late blight (*P. infestans*) in Danish field trials 2004-2012. Spraying with Shirlan 0.4 l/ha at weekly intervals in susceptible starch varieties and artificial inoculations with mixed isolates. % control is based on calculations of AUDPC in the trials

FIELD TRIALS 2006-2007

In 2006 a very low effect against late blight (20-54% control) was observed in four trials at Research Centre Flakkebjerg. Trial design, dose (0.4 l/ha), susceptible varieties, intervals, etc. were the same as in previous years, but the isolates used for artificial inoculation in early July were changed. A mixture of eight isolates collected from Flakkebjerg in the summer of 2005 was used as inoculum in the spreader rows. Variation in efficacy in spray trials with Shirlan had been seen before but never in so many trials at the same locality. Next year, 2007, the same low efficacy was seen again in five field trials at Research Centre Flakkebjerg (18-53% control, fig. 1). Same design, etc. as previously but in 2007 only four of the "2005" isolates were used as artificial inoculum in early July (these isolates were also used in 2006).

The low effect observed in 2006-2007 was mainly seen in field trials at Flakkebjerg Research Centre, but low-medium effect was also observed in other trials in Denmark in which disease pressure was high. Good disease control was observed at other locations in Denmark in which disease pressure was more moderate.

The significantly reduced effect observed at Flakkebjerg 2006-2007 was not reported from other countries. Previous trials at Flakkebjerg showed a variation in the effect against late blight but not as pronounced as seen in the trials 2006-2007.

LATER RESULTS

Due to the low effect observed in the field trials recommendations for use of Shirlan in Denmark were changed, and the use of Shirlan in the potato production declined. Also, the number of trials with Shirlan at Flakkebjerg declined. New isolates collected in potato fields in Denmark in 2007 (Flakkebjerg and other localities) were used as artificial inoculum. The effect after spraying with Shirlan (0.4 l/ha, same set-up as previously but with new isolates) varied in the trials 2007-2011 (25-95% control), but generally the effect was higher than in 2006-2007 (fig. 1). In 2012 only one field trial was performed at Research Centre Flakkebjerg, and here the effect was very high (95-99% control). The same isolates were used for artificial inoculation as in 2008-2011 supplemented with two isolates from 2011.

TEST OF ISOLATES

Some of the isolates from 2007 and later years were tested for sensitivity to fluazinam, and the results showed that the sensitivity was within the standard range (Syngenta, unpublished). Isolates from 2008-2012 were also tested for genotype, and no "green33" or "blue13" was found (Geert Kessel, Wageningen and David Cooke, Hutton Institute; unpublished results).

CHANGE IN USE AND RECOMMENDATIONS

The recommendations for use of Shirlan in Denmark were changed from 2008. Shirlan (0.3-0.4 l/ha) is only recommended at low disease pressure and not in high-risk periods and periods with active new growth. Recommended spray interval is 7 days and only at low risk of infection up to 10 days. The use of Shirlan in Denmark drastically declined from 2008, and new products came on the market (Ranman and Revus) replacing Shirlan. The DSS models were also replaced with new products (Nielsen *et al.*, 2010) ensuring a high disease control.

CONCLUSIONS ON FIELD TRIALS WITH FLUAZINAM

- Stable and high effect against late blight before 2006
- Low-medium effect in many trials 2006-2007
- Mainly in inoculated trials at Research Centre Flakkebjerg
- Variation in effect 2008-2011 (low-moderate-high)
- High effect 2012 (one trial)

- Inoculated trials
 - New isolates (inoculum) 2006-2007
 - New isolates (different) again from 2008
 - No signs of reduced sensitivity (Syngenta test)
 - No "green32" or "blue13" genotypes observed

- Continuous and high disease pressure at Research Centre Flakkebjerg
- Problems observed in susceptible varieties with rapid growth at start of late blight epidemic
- Changes in recommendations: no use in high-risk periods and periods with active new growth

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Infito[®]: Protection against different *Phytophthora infestans* isolates of the A2 & A1 mating type

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SUMMARY

Infito[®] is a liquid coformulation of fluopicolide and propamocarb-HCl, a well-established potato fungicide with strong translaminar and curative properties, and has been specifically developed for effective and long-lasting control of potato late blight. In this study, we investigated the efficacy of Infito[®] and three other fungicides against recent field isolates with different MLGs of *Phytophthora infestans* under greenhouse conditions. The results in this study indicate no shift in sensitivity to Infito[®] in either A1 or A2 multilocus genotypes from European potato fields. Infito[®] demonstrated its reliable high efficacy on both A2 genotypes tested, 33_A2 MLG (A2_Green 33) and 13_A2 MLG (A2_Blue 13).

KEYWORDS

Infito[®], *Phytophthora infestans*, potato late blight, increased aggressiveness, A1 & A2 mating type, multilocus genotypes

INTRODUCTION

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans* is considered to be the economically most important potato disease globally (Kroon *et al.*, 2011). *P. infestans* has migrated from Central or South America to Europe in the 1840's and spread rapidly throughout Europe in the next decades (Yoshida *et al.*, 2013). The population of *P. infestans* remained stable until beginning of the 1980's due to asexual reproduction of the solely appearing A1 mating type. With the arrival of the new A2 mating type in Europe in 1976, sexual reproduction and gene recombination between the two mating types allowed new populations to develop. These population changes have resulted in significant adjustments in the epidemiology of the pathogen especially in recent years, such as shorter life-cycles, reduced latent periods and earlier primary infections (also at lower temperatures) leading to increased aggressiveness and more severe damage to the potato crop (Lees *et al.*, 2008).

The pathogen's genetic diversity can be monitored using different genetic markers. The simple sequence repeats (SSRs) recently have been proved effective for defining multilocus genotypes (MLGs) (Lees *et al.*, 2006). The most recent examples of aggressive new genotypes in Europe

are 13_A2 MLG, that first emerged in 2004 and rapidly displaced other genotypes (Cooke *et al.*, 2012), and 33_A2 MLG, first emerging in 2009 in The Netherlands.

In conditions of higher and more severe disease pressure, farmers need effective and reliable fungicides to assure a good protection against potato late blight without increasing the overall number of applications. Fluopicolide is a novel fungicide of the new chemical class, acyl picolides, developed by Bayer CropScience. It exhibits a high level of efficacy against a wide range of oomycete diseases. Fluopicolide has a novel biochemical mode of action and does not show cross resistance with other chemical families, including phenylamides (metalaxyl-M, mefenoxam). It combines strong translaminar properties and contact activity, with long-lasting disease control at low dose rates.

Infito[®] is a liquid coformulation of fluopicolide and propamocarb-HCl, a well-established potato fungicide with systemic and curative properties. The suspension concentrate (SC) contains 62.5 + 625 g a.i./liter of the respective active ingredients and has been specifically developed for effective and long-lasting control of potato late blight. Combining the two different modes of action with complementary fungicidal properties, Infito[®] is also a tool for a solid anti-resistance management. Infito[®] is rated in the EuroBlight fungicide table for leaf and tuber blight protection and is established in the European market as a key product against late blight.

In the present study, we investigated the efficacy of Infito[®] and several other fungicides against different multilocus genotypes of *P. infestans* (A1 and A2 mating types, field isolates from different European countries from 2008 and 2012) on four-week old potato plants under greenhouse conditions.

MATERIALS AND METHODS

Fungicide preparation: Four commercially formulated fungicides commonly used on potatoes or tomatoes were evaluated in this study: Infito[®] (active ingredients: fluopicolide and propamocarb-HCl), Shirlan[®] (active ingredient: fluazinam), Revus[®] (active ingredient: mandipropamid) and Ranman Top[®] (active ingredient: cyazofamid). We evaluated commercial formulations of the fungicides rather than the active ingredient alone to more realistically represent compounds that are used by growers in the field. The concentrations tested were according to the label recommendations. Fungicide solutions were prepared in sterile deionized water.

Fungal culture: Wild type isolates of *P. infestans* were obtained from infected leaf samples collected from commercial fields from 2008 to 2012 in Belgium, France, Germany, Poland and The Netherlands. The multilocus genotypes (MLGs) of the isolates were characterized by Dr. David E.L. Cooke using multiplex simple sequence repeat genotyping (Li *et al.*, 2013). In this study, three different *P. infestans* isolates were analyzed: A reference isolate from Germany (2008), a 33_A2 MLG isolate from The Netherlands (2012) and a 13_A2 MLG isolate from Germany (2012).

Whole-plant assay: For fungicide application, four to five-week-old greenhouse grown potato plants (cv Arkula) were sprayed to runoff with an automated spraying cabinet. 24 hours after application the plants were inoculated by spraying them with a sporangial suspension of 10.000 sporangia per ml of the appropriate *P. infestans* isolate. Inoculated plants were kept in a climatic chamber with 95% relative humidity and 20°C with a 16 hour photoperiod for 7 days. There were three replications for each multilocus genotype. Data regarding the proportion of leaf and plant blighted were visually estimated to calculate percent disease index (%DI).

RESULTS AND DISCUSSION

In this study, the protective efficacy of four different commercially available fungicides was compared against different European field isolates of *Phytophthora infestans* from 2012 under greenhouse conditions. All four tested fungicides were able to control disease development of the *P. infestans* reference isolate from 2008 on four-week old potato plants under the chosen conditions (figure 3). Mean control levels ranged from 76% for Shirlan® to 93% for Infinito® and 98% for Revus®.



Figure 1. Efficacy of three selected fungicides used at the recommended dose rates against a *Phytophthora infestans* field isolate 13_A2 MLG under greenhouse conditions (A: Infinito® applied at 1,6 l/ha, B: Shirlan® applied at 0,4 l/ha, C: Revus® applied at 0,6 l/ha). Evaluation was done 7 days after inoculation

Against the two recent MLG isolates 13_A2 and 33_A2 from 2012 however, a clear difference in the efficacy of the four tested fungicides was observed (figure 3). MLG 13_A2 isolates are ranked amongst the most aggressive *P. infestans* isolates, showing among the shortest latent periods and the largest lesions on different potato cultivars (Cooke *et al.*, 2012). This highly aggressive genotype 13_A2 has been spreading in Western Europe from 2004 onwards. In 2006-2007 it was dominating in the Netherlands, France, United Kingdom, Switzerland, Germany and Belgium (Gisi *et al.*, 2011). Infinito® showed an excellent preventative efficacy against leaf infections of MLG 13_A2 (figure 1) while Shirlan®, Revus® and Ranman Top® failed to limit the leaf infection of this isolate (figure 2). The mean efficacy levels against leaf infections of MLG 13_A2 were 75% for Infinito®, 13% for Revus®, 3% for Ranman Top® and 0% for Shirlan® (figure 3).

33_A2 MLG is a recent genotype that is detected in the Netherlands from 2009 onwards. 33_A2 MLG is an aggressive isolate able to replace other aggressive A1 and A2 isolates of *P. infestans* and dominates the *P. infestans* population in fluazinam treated fields (Schepers & Kessel, 2011). In the greenhouse experiments presented in this study Infinito® and Ranman Top® were able to suppress disease development of this MLG on potato leaves. The infested foliage area was 3% in the plants treated with Ranman Top® and 5% in the ones treated with Infinito® while it reached 63% in the Shirlan® treated plants (figure 2). In the potato plants treated with Revus® the infested leaf area was 27%. The efficacy of the four tested fungicides against 33_A2 MLG ranged from 97% for Ranman Top® and 95% for Infinito®, to 73% for Revus® and 37% for Shirlan® (figure 3).

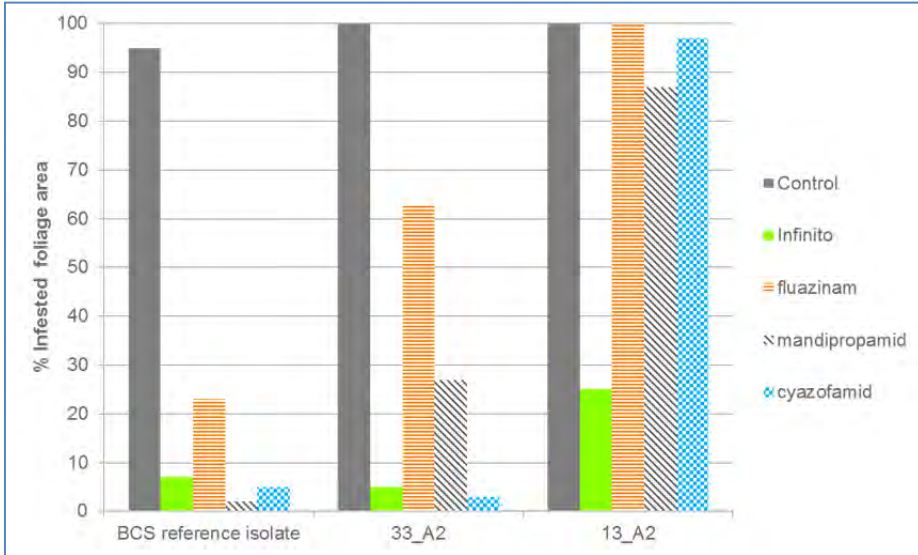


Figure 2. Mean intensity of infestation measured 7 days after inoculation of potato plants with 3 different isolates of *Phytophthora infestans*

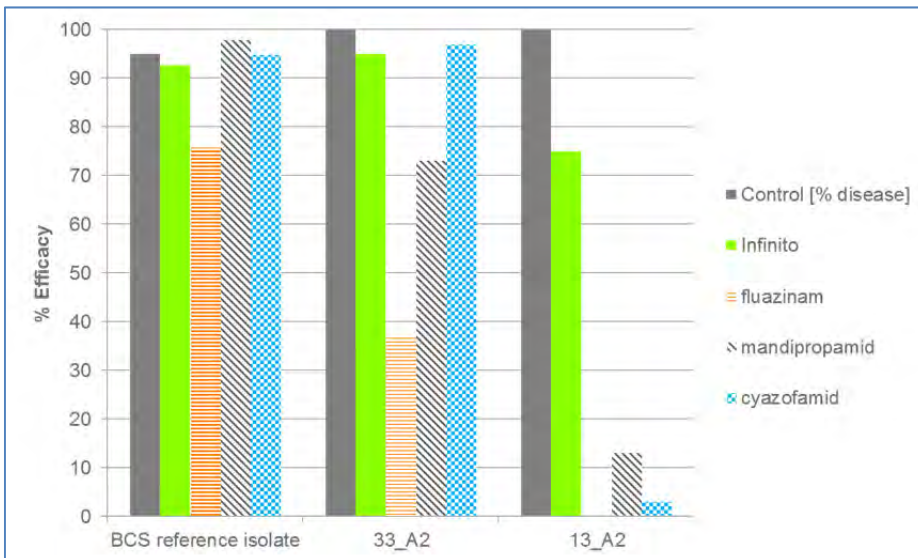


Figure 3. Mean efficacy of 4 different fungicides, measured 7 days after inoculation of potato plants with 3 different isolates of *Phytophthora infestans*

CONCLUSIONS

BCS conducts annual sensitivity monitoring programs to evaluate shifts in the European population of *Phytophthora infestans* against fungicides with different modes of action. As a result of this annual monitoring from 2008 to 2012 a change of importance of different isolates & genotypes of *Phytophthora infestans* in the main potato growing areas has been shown. The research work presented indicates no shift in sensitivity to Infinito® in either A1 or A2 multilocus genotypes from European potato fields. These genotypes can be found in increasing percentage in infected potato fields with some challenges in control with introduced standard products. Nevertheless Infinito® proved to have a continuously high efficacy on both A2 genotypes tested, 33_A2 (A2_Green 33) and 13_A2 (A2_Blue 13). In the European market Infinito®, is a leading fungicide with top performance against *Phytophthora infestans*. BCS recommends the use of Infinito® in spray programs in alternation with other fungicides.

ACKNOWLEDGEMENTS

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Infito and protection against tuber blight – modes of action

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SUMMARY

Bayer's late blight product Infito® 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 Infito 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 Infito 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 Infito® has become established in recent years as a key product in European late blight control strategies.

Infito combines the activity of the active substances fluopicolide and propamocarb-HCl. Infito has been presented at previous Euroblight Workshops^{1,2,3}. 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^{4,5} 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 m² 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 m² 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 mm²). Leaflets were dipped in water to collect spores and spores were counted. Sporulation intensity (the number of spores per cm² 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 m²) 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 m² 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 m²) 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.

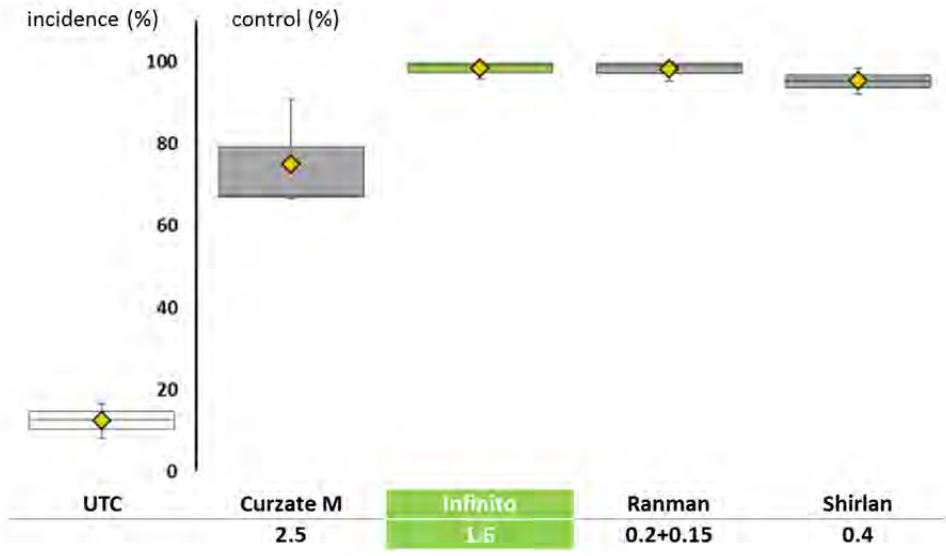


Figure 1. Tuber blight incidence (%) for untreated crops and tuber blight control (%Abbott) for treatments with means of 3 trials indicated by diamonds (◊)

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.

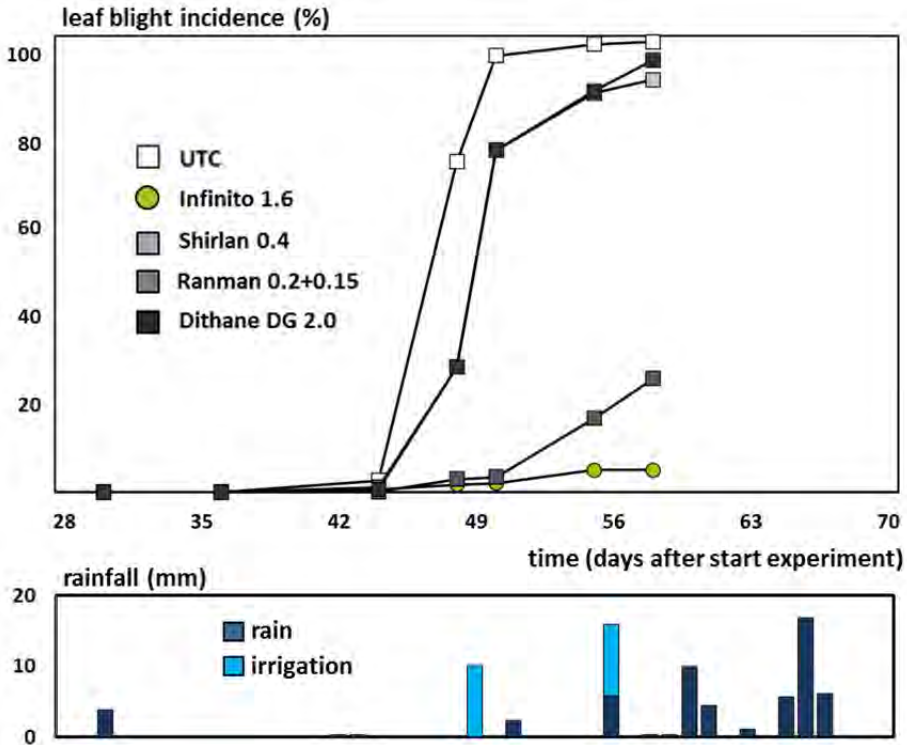


Figure 2. Progress of late blight incidence in the canopy of untreated and treated crops (up) and occurrence of natural/ artificial rain events in time (down). Time expressed in days after start of the experiment (01.07 = 0)

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.

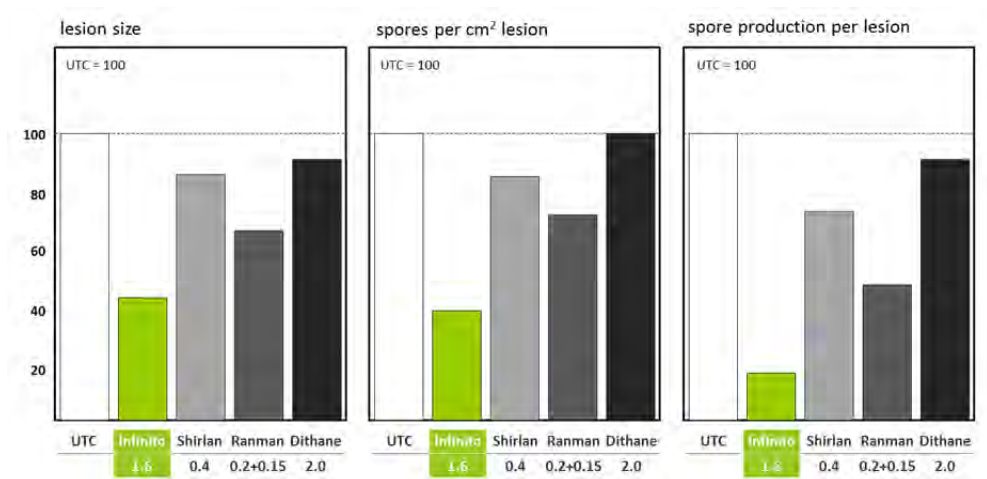


Figure 5. Sporulation parameters lesion size (in mm²), sporulation intensity (in spores per cm² lesion) and spore production per lesion. Parameter values relative to the values recorded for the untreated control (indexed at 100)

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.

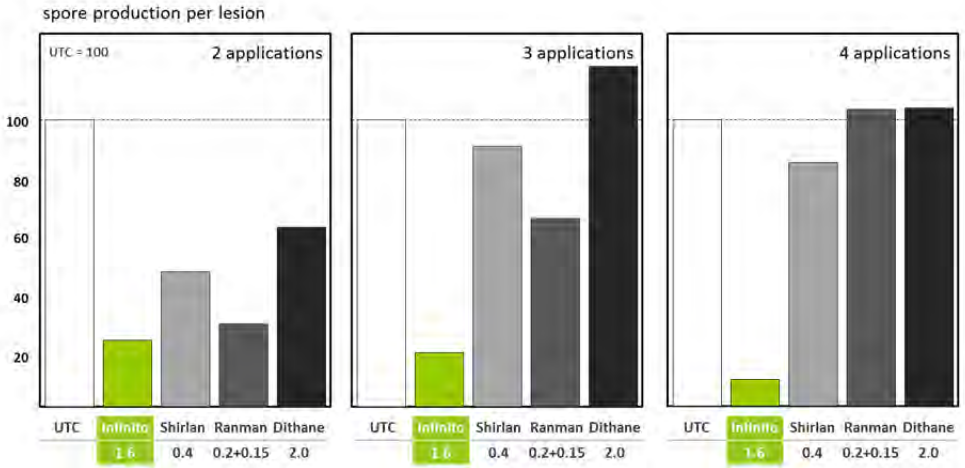


Figure 6. Evolution of spore production in relation to the number of experimental treatments. Spore production means relative to the spore production in the untreated control (indexed at 100)

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).

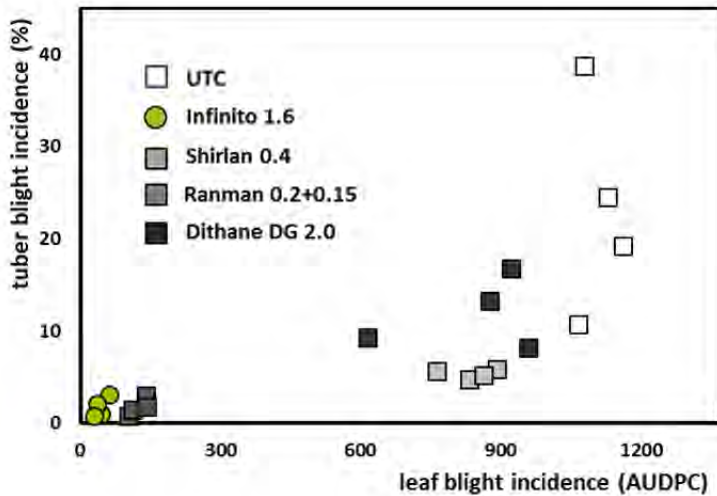


Figure 7. Relation between tuber blight incidence and overall leaf blight incidence in individual plots (left). Tuber blight incidence expressed in %, leaf blight incidence expressed in AUDPC

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%.

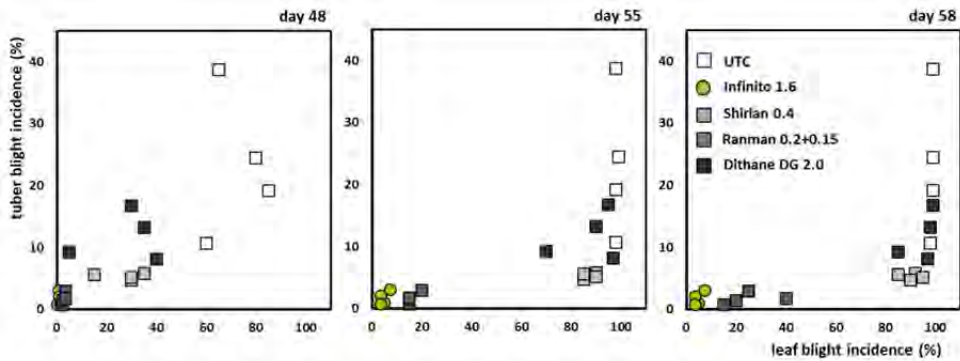


Figure 8. Relation between tuber blight incidence and leaf blight incidence at days with artificial rain events (day 48 and 55) and at the final leaf blight incidence recording (day 58). Tuber and leaf blight incidence expressed in %

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).

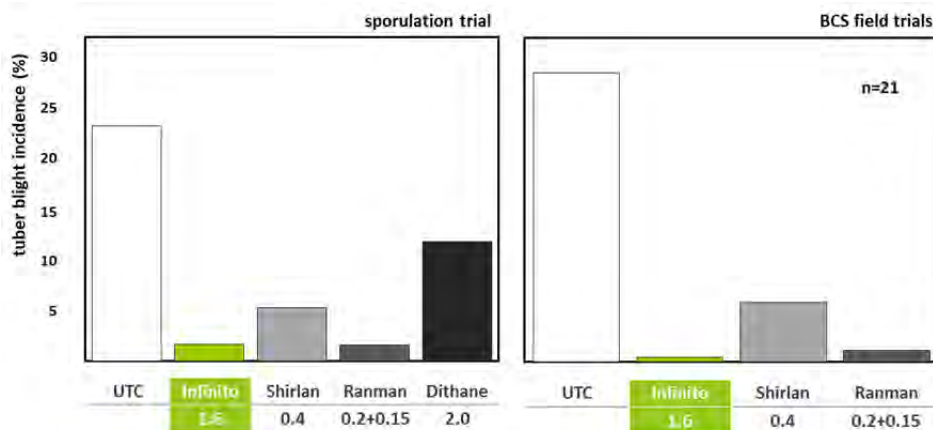


Figure 9. Mean tuber blight incidence in the sporulation trial (left) and in (21) BCS trials conducted in The Netherlands in the period 2006-2011

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 activity¹. 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 tests¹. 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 HCl¹. 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

This summary is based on the works of PPO-AGV, HLB BV and Proeftuin Zwaagdijk and on the works of the country team of BCS in The Netherlands.

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Late blight management in Israel

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Makhteshim Agan Industries is a leading manufacturer and distributor worldwide of crop-protection solutions and the largest producer of post-patent products in the industry. The Company supplies farmers with efficient solutions that assist them in combating disease and increasing yields. Makhteshim Agan is characterized by its know-how, advanced technological-chemical abilities, expertise in product registration, and observance of strict standards of environmental protection, stringent quality control and global marketing and distribution channels.

Consumption of potatoes in Israel has been increasing steadily. Along with the consumption, the production of potatoes has also been increasing and reached 18,000 ha in 2012. Potatoes are grown in the Negev region (>80% of total production), in the Sharon area, in the Chula valley and in the Arava desert. The potato production occurs in various soils, from sandy to medium-heavy soil and also with saline water.

Potatoes are grown in Israel in two seasons: the fall season planting starts in September and harvested in January, while the spring season starts in December and harvested until June. Tubers are imported to Israel from Europe for the fall season, and the production is exported back to Europe both for seeds and consumption (fresh market and industry).

The two seasons differ in their growth conditions: the spring growing season is short and fast, characterized with days that have more light hours. The fall season is slow and characterized with days that are going shorter. Therefore, the main disease during the fall season is Early Blight caused by *Alternaria solani*. In the spring, differences between the cold nights and warmer days, coupled by massive canopy, high levels of long lasting dew is formed creating favorable conditions for the *Phytophthora infestans*, the agent of Late Blight.

Eighty percent of the potatoes are grown in the dry region (Negev) and therefore are regularly irrigated twice a week. Late blight control in Israel is based on protectant fungicides (mancozeb and chlorothalonil) that are sprayed from 70% emergence following each irrigation. Potatoes are monitored on daily basis for late blight infections. In case of favorable disease conditions (rainfalls or heavy fog), translaminar fungicides "Super protectants" are being sprayed (dimethomorph, benthiovalicarb, mandipropamid, propamocarb-HCL). In a typical season 2-3 treatments are sprayed with those fungicides. In case of infection, curative (systemic) fungicides are being used (metalaxyl-M, cymoxanil). In addition, a sample of infected leaves is sent to prof. Yigal Cohen (Bar-Ilan University) for resistance monitoring. Results are reported to "Yacham" (potato growers cooperative) and delivered back to the growers (2-3 days). In case of metalaxyl resistance - super protectants are being used again.

Banjo Forte (fluazinam 200 + dimethomorph 200 SC) is a new innovative product for potatoes, containing two AIs with complementary modes of action: a local translaminar AI, dimethomorph, and a very effective contact AI, fluazinam. Due to this unique combination, efficacy of **Banjo Forte** on different *P. infestans* strains is high. **Banjo Forte** has a wide disease spectrum including excellent leaf and tuber blight (*P. infestans*) prevention as well as white mold (*Sclerotinia sclerotiorum*) control. **Banjo Forte** perfectly fits the mid application timing, the products used with a low dose and is therefore simple to use.

Chemical Control Strategy of Potato Late Blight Based on the DSS 'China-blight'

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SUMMARY

Potato late blight is the most devastating disease of potato in China. Due to the shortage of resistance of cultivars in most cases, chemical control is still the main method in use today to manage the disease. In order to improve the control efficiency, a web based DSS (Decision support system) on potato late blight management in China --- "China-blight" (www.china-blight.net) was developed in 2008. In order to know when to start spray program could save more sprays, a field trial was carried out in Hebei China during 2012 growing season. The current results showed that when the spray program started according to first late blight symptom forecasting of "China-blight" can save 1 out of 6 sprays compared to the routine spray program.

KEYWORDS

Potato late blight, *Phytophthora infestans*, Chemical control strategy

INTRODUCTION

At present, China has become the top potato production country in the world. Potato, the fourth important food crop in China, is planted mainly in 22 provinces, municipalities and autonomous regions. Potato late blight caused by *Phytophthora infestans* has become the major limitation to potato production worldwide. In China, it causes 10~40% yield loss in common years or even worse in special years (Song and Xie, 1997). Due to the shortage of resistance of cultivars in most cases, chemical control is still the main method in use today to manage the disease. There are two existing strategies to start spray program for late blight management in China: (1) spray program starting at a fixed time or fixed plants height widely used in large scale commercial planting; and (2) spray program starting when late blight epidemic already developed in the field normally two weeks after first symptom appearance. In order to improve the control efficiency, a web based DSS (Decision support system) on potato late blight management in China --- "China-blight" (www.china-blight.net) was developed in 2008 (Hu *et al.*, 2012). A field trial was carried out in Hebei China during 2012 growing season with the aim to compare the control efficacy and fungicide input of spray programs based on "China-blight" as well as the two existed spray strategies.

MATERIALS AND METHODS

Location of the field trial: Weichang, Hebei, China;

Size of plots: 6 rows x 7 meters (35 cm between plants, 65 cm between rows);

Lay out of plots of the field trial: randomized complete block design with 4 replicates;

Potato Cultivar: Favorita (highly susceptible to *P. infestans*);

Fungicide: Infinito (687.5 g/L (propamocarb-HCl + fluopicolide), Bayer Crop Science (China) Co. Ltd.), label dose (a.i 700 g./ha) was used in the field trial;

Treatments: see Table 1 and Table 2.

First symptom of late blight observation: daily check for all plots after emergence till first symptom observed in one of the plots;

Late blight assessment: weekly visual assessment;

Yield assessment: two rows in the center of each plot were harvested for yield assessment, tubers more than 150 grams were weighed as commercial yield.

Table 1. Treatments in the field trial for the control of potato late blight in 2012

Treatment	First spray	Followed sprays	Fungicide	Spray interval
A	Plants height ca. 18 cm	Before the risk day	Infinito	See
B	According to the first symptom appearance forecasted by 'China-blight'	according to 'China-blight' (MISP model)	a.i. 700 g/ ha	Table 2
C	When the first symptom appear in the field			
D	Two weeks after the first symptom appear in the field			
E	—	—	—	—

Table 2. Spray intervals based on weather conditions

Number of risk days since last spray	Spray interval / days
0	10-14
1	7-10
≥2	5-7

Note: 1 'highly risk day = 2 risk days', the model used is MISP model developed by Cao et al., 1996.

RESULTS AND DISCUSSION

Time of first late blight symptom appearance in the field

The planting date of all the plots was 5 May 2012, and the date of plant emergence was 1 June 2012. Time of first late blight symptom in the trial field forecasted by "China-blight" was 14 to 21 July 2012, the first symptom was observed in the trial field on 17 July 2012.

Date of first spray and number of total sprays of different treatments

The first spray of treatment A was carried out on 2 July 2012, which was the earliest spray in all the treatments, followed by B with the first spray on 13 July, and then C and D with first spray on 19 July and 30 July respectively. The total sprays in the whole growing season of treatment A, B, C, D and E (unsprayed control) were 6, 5, 4, 2, and 0, respectively. (Table 3)

Table 3. Spray record of the field trial 2012

Treatment	Date of spray					
	2 Jul.	13 Jul.	20 Jul.	25 Jul.	30 Jul.	7 Aug.
A	√	√	√	√	√	√
B	—	√	√	√	√	√
C	—	—	√ 19 Jul.	√	√	√
D	—	—	—	—	√	√
E	—	—	—	—	—	—

“√” means spray, “—” means no spray.

Late blight development in different treatments

Late blight progress of different treatments (Figure 1) showed that treatment A, B and C had the same disease level during the whole growing season, the final disease severity were all below 2%. Even though the number of sprays of these treatments were different from each other, with the same control efficacy treatment B saved 1 spray compare to A, and treatment C saved 2 sprays and 1 sprays compare to A and B, respectively. However, treatment D and E had very severe disease epidemics, the final disease severity were more than 88%. Actually there were no significant difference between treatment D and E in terms of late blight control, although the two sprays of D reduced a little of disease severity compared to the unsprayed control (E).

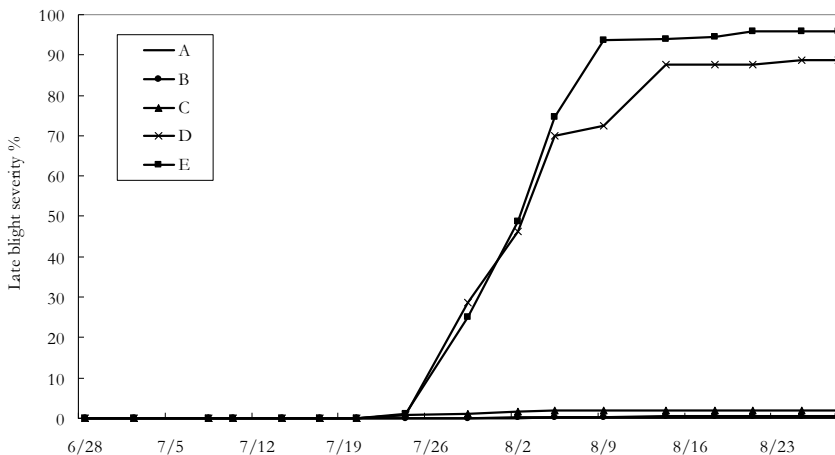


Figure 1. Disease progress of different treatment in field trial 2012

Yield of tubers in different treatments

Yield of tubers of different treatments showed in Figure 2, it indicated that treatment A, B and C had the same level of gross yield and marketable yield, and there were no statistical significant difference among them although there were differences among the number of sprays among these treatments during the whole season. Likewise, treatment D and E had the same level of gross yield and marketable yield without statistical significant difference between each other, although there were two sprays for D whereas no spray for E (unsprayed control) during the growing season. However, yield of treatment A, B and C was significantly higher than that of treatment D and E.

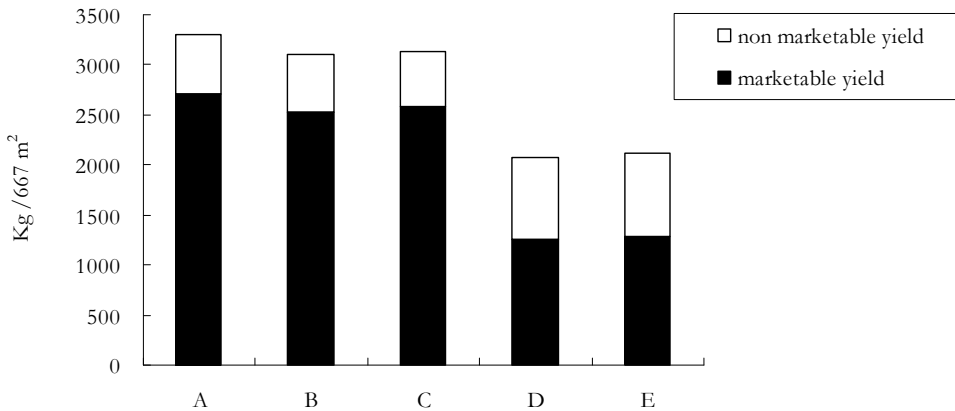


Figure 2. Yield of different treatment in field trial 2012

CONCLUSIONS

Based on the field trial of 2012 in Hebei China, some conclusions can be made as follows: (1) Compared to the routine spray program the DSS “China-blight” could save 1 spray (16.7% of total fungicide input) without reduction of control efficacy to potato late blight; (2) Good monitoring of first late blight symptom in the field can help the growers save 33% fungicide input (2 sprays) in the whole growing season compared to the routine spray program; and (3) Spray program started more than 2 weeks after the first symptom appearance in the field could not give meaningful control for potato late blight.

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The potato blight population in Northern Ireland in 2012: ongoing changes and fungicide performance

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SUMMARY

Nearly 100 isolates of *Phytophthora infestans* obtained from all potato-growing regions of Northern Ireland in 2012 were characterised for metalaxyl resistance, mating type and *Pep* allozyme genotype. A small sub-sample was also analysed for SSR genotype. The marked decline in the incidence of the A2 mating type between 2010 and 2011 (from over 70% to 10%) was partially reversed: 37% of isolates proved to be A2. All A2 isolates were metalaxyl-resistant and the vast majority were from the south and east of Northern Ireland (Cos. Down and Antrim). Overall, 62% of isolates were metalaxyl-resistant. All A2 isolates were *Pep* 96/96 and SSR analysis of three showed that they were Blue 13 (13_A2). All but two A1 isolates were *Pep* 100/100; these two, which were *Pep* 96/96, were shown to belong to the Pink 6 (6_A1) genotype by SSR (the first finding in Ireland since 2009), whereas the other A1 isolates analysed by SSR were all 8_A1. The Northern Ireland *P. infestans* population remains highly clonal. In small-scale field trials in Belfast in 2010, 2011 and 2012, a standard programme of two applications of mandipropamid + fluazinam followed by eight applications of fluazinam gave good control of foliar and tuber blight and there was no evidence for a decline in performance of fluazinam, which is the fungicide most widely used for potato blight control in Northern Ireland.

KEYWORDS

Phytophthora infestans, Northern Ireland, mating type, metalaxyl resistance, allozyme genotype

INTRODUCTION

Since the 'new' *Phytophthora infestans* population arrived in Northern Ireland in the early 1980s (Cooke *et al.*, 1995), the population has remained clonal. Up to 2007, it was dominated by a few A1 clonal lineages with the A2 mating type at low frequency (Carlisle *et al.*, 2001; Cooke *et al.*, 2006). From 2007 onwards, the population has undergone major upheavals following the appearance in Northern Ireland (Cooke *et al.*, 2009) of genotype 13_A2, also referred to as 'Blue 13' (Cooke *et al.*, 2012a). Genotypic and phenotypic characterisation of *P. infestans* isolates from the island of Ireland was initiated as part of an all-Ireland project on potato late blight and

this showed the presence of 'Blue 13' throughout Ireland in 2008 and by 2009 it was the dominant genotype (Kildea *et al.*, 2010). The 2010 season was not conducive to late blight and only 51 viable isolates were obtained, all from Northern Ireland; over 70% were A2 mating type and over 80% were metalaxyl-resistant, but most were from a single site (Cooke *et al.*, 2012b). In 2011, blight was widespread and nearly 200 isolates were obtained from across the island of Ireland, of which 100 were from Northern Ireland. In both Northern Ireland and the Republic of Ireland there was a dramatic reduction in the incidence of the A2 mating type; only 10% and 22% of isolates from Northern Ireland and the Republic of Ireland, respectively, proved to be A2. In Northern Ireland, the A2 isolates were from only five sites, all were metalaxyl-resistant and SSR analysis showed that all were the 13_A2 Blue 13 genotype, while the A1 isolates belonged to older genotypes, mainly 8_A1; the situation was similar in the Republic of Ireland. In contrast, in Great Britain, although the incidence of Blue 13 also declined abruptly to only 10% of isolates (Cooke, D.E.L., personal communication), the population was dominated by the newer genotype 6_A1 ('Pink 6'), which was found in Northern Ireland at low frequency only in 2009. The all-Ireland late blight project concluded in 2012 and did not include population characterisation for that year, so to obtain information on whether the decline of Blue 13 would continue a short-term investigation was initiated. Here we report results of this and of fungicide performance of standard programmes in 2010-2012.

MATERIALS & METHODS

Collection, isolation and storage of Phytophthora infestans isolates

Blighted potato leaf material was collected mainly from commercial seed crops by members of the Northern Ireland Department of Agriculture and Rural Development (DARD) Agri-food Inspection Branch. Once received, the blighted material was incubated and isolates established as previously described (Kildea *et al.*, 2010).

Mating type, metalaxyl sensitivity and SSR determination

Mating type was determined as described by Cooke *et al.* (2006). The sensitivity of isolates to the fungicide metalaxyl was determined using a floating leaf disk assay (Cooke *et al.*, 2006). For selected isolates, genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase) and *Pep-1* (peptidase), were determined using cellulose acetate electrophoresis (Carlisle *et al.*, 2001). A sub-sample of isolates was genotyped by SSR analysis at the James Hutton Institute (JHI) using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) in accordance with the protocol developed by EUCABLIGHT.

Field evaluation of fungicide performance against foliar blight 2010-2012

Tubers cv. Up-to-Date were planted in April or May of each year (Table 1) at AFBI Headquarters, Newforge, Belfast, Northern Ireland in fully randomised blocks with five replicate plots per treatment as described by Cooke & Little (2010). 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 recent Northern Ireland isolates of *P. infestans*. In 2010 and 2011, 50% of leaves were inoculated with A2 metalaxyl-resistant isolates, 25% with A1 resistant isolates and 50% with A1 sensitive isolates, while in 2012 (following the decline in the incidence of the A2 mating type), 25% were inoculated with A2 resistant isolates, 25% with A1 resistant

isolates and 50% with A1 sensitive isolates. 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, 2010-2012: dates of field operations*

Year	Planting date	Fungicide application dates		Inoculation	Desiccation	Harvest
		First	Last			
2010	28 April	18 June	19 August	7 July	25 August, 1 September	21 September
2011	4 May	29 June	31 August	6 July	15, 20 September	31 October
2012	21 May	26 June	30 August	2 July	6, 13 September	9 October

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 mandipropamid (150 g /ha as 'Revus', Syngenta) tank-mixed with half-rate fluazinam (100 g/ha as 'Shirlan', Syngenta) followed by eight sprays of full-rate fluazinam (200 g/ha). Other programmes included in the trial are not reported here (for results of 2012 trial mandipropamid + fluazinam/mancozeb programme, see poster Cooke & Nugent, in this volume).

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 and tubers harvested in September or October (Table 1). The yield from each plot was graded and recorded; the number and weight of blighted, soft-rotted tubers was recorded and they were then discarded. The number and weight of firm blighted tubers >35 mm was assessed (and diseased tubers discarded) in November-December in each year. The remaining healthy tubers were stored and re-assessed in late January-February, after which the final marketable yield was determined. In each trial, the Area Under the Disease Progress Curve (AUDPC) was calculated from the untransformed percentage foliage blight for each plot.

RESULTS

Population characterisation 2012

The weather in summer 2012 was very cool and wet between May and September. Until the third week in June, minimum temperatures were generally well below 10°C and so conditions were too cold for development of blight. The first outbreak was reported on 22 June as it became warmer. Blight was encouraged by the high proportion of rainy days as well as by very high rainfall on certain days (22 June, 44.4 mm; 27 June, 53.6 mm; 24 September, 52.0 mm) and outbreaks were reported in all potato-growing areas of the Province. A total of 34 crops were sampled (Co. Antrim, 11; Cos. Down & Armagh, 15; Co. Londonderry & Tyrone, 8) and 99 *P. infestans* isolates obtained (up to five isolates per site).

Overall, 62% of isolates were metalaxyl-resistant (Figure 1) with the highest proportion of resistant isolates found in Cos. Down & Armagh (74%) and Co. Antrim (69%) compared with only 27% in Cos. Londonderry & Tyrone (Figure 2). This compares with the 2011 figure of 33% metalaxyl-resistant isolates overall.

Eighty-four isolates from 31 crops were tested for mating type and 37% proved to be A2 (Figure 3), compared with only 10% in 2011. All of the A2 isolates were metalaxyl-resistant and the majority were obtained from the south-east (Figure 2), while 30% of A1 isolates were metalaxyl-resistant. Only one A2 isolate was from Cos. Londonderry & Tyrone (4.5%, n=22), whereas 36% from Co. Antrim (n=33) and 62% from Down & Armagh (n=29) were A2.

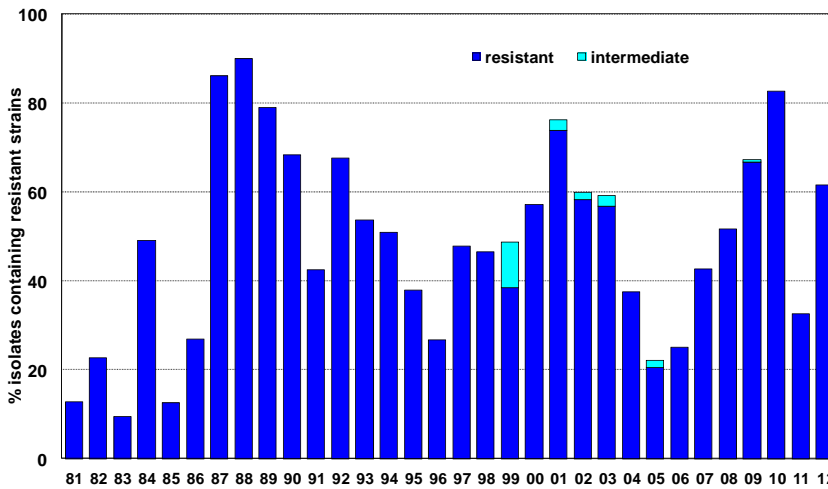


Figure 1. The percentage of Northern Ireland *Phytophthora infestans* isolates containing metalaxyl-resistant strains, 1981-2012

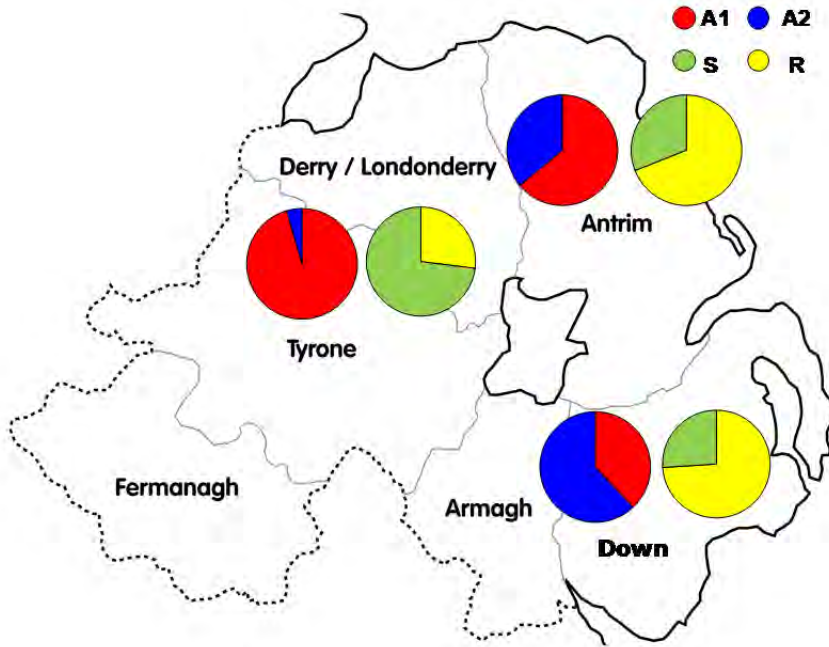


Figure 2. Mating type and metalaxyl sensitivity of Northern Ireland *Phytophthora infestans* isolates, 2012

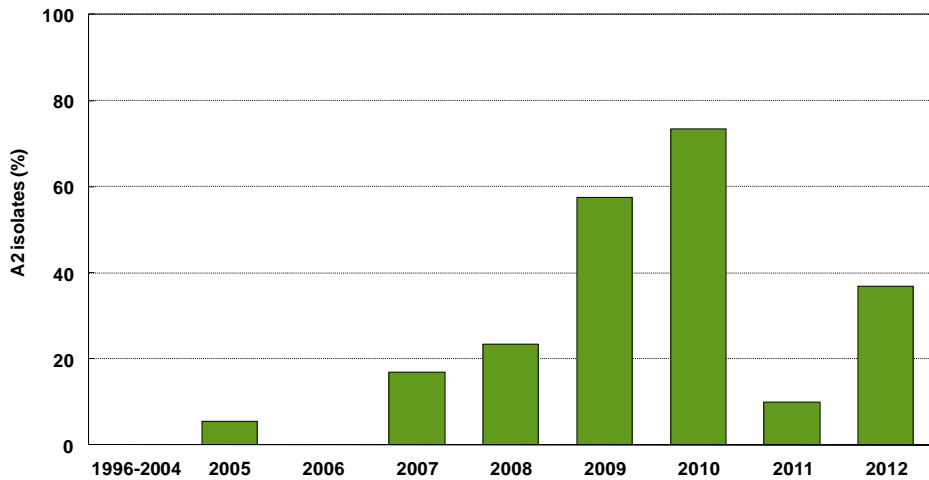


Figure 3. The percentage of Northern Ireland *Phytophthora infestans* isolates of A2 mating type, 1996-2012

All isolates characterised for *Gpi* proved to be 100/100. Of 82 isolates characterised for *Pep*, all of 30 A2 isolates were 96/96, while of 52 A1 isolates, 50 were *Pep* 100/100 and two were 96/96. Nine of the isolates were SSR genotyped at JHI and this showed that the three A2 isolates (from three different sites) were all Blue 13 (two 13_A2_1 and one 13_A2_37), while of six A1 isolates, four (from three sites) were 8_A1 (two 8_A1, two 8a_A1) and two (from a single site) were 6_A1. The two 6_A1 isolates were the two A1 isolates with *Pep* 96/96.

Field evaluation of fungicide performance against foliar blight 2010-2012

In each year, the standard programme (two applications of mandipropamid + half-rate fluazinam followed by eight fluazinam applications) achieved good foliar blight control (Table 2), while the adjacent, inoculated infector drills had 95-100% foliar infection by the final assessment. Foliage blight was greatest in 2012 probably as a result of some tuber blight in the seed and conditions very conducive to infection, but was still under 5% at the final assessment, whereas plots where the first two applications of another programme were made at very reduced rate were completely killed by blight (data not shown). Tuber blight was also well controlled, although in 2012 extensive soft rotting made accurate assessment impossible. Yields were extremely low in 2012 reflecting the very poor growing conditions, with low temperatures and little sunshine as well as a high incidence of blackleg (*c.* 30% of plants affected), but were similar in all programmes.

Table 2. Performance of standard mandipropamid + fluazinam/fluazinam programme in field trials for the control of potato blight, 2010-2012

Year	Final foliar blight assessment		AUDPC ^a	Tuber blight (% by number)	Total yield >35 mm (kg/plot)	Marketable yield (kg/plot)
	Date	Foliar blight (%)				
2010	24 August	1.24	7.7	3.63	65.0	58.6
2011	14 September	1.80	27.2	7.49	56.7	48.6
2012	4 September	4.00	87.4	3.73	30.1	26.7

^a Area Under the Disease Progress Curve for foliage blight development

DISCUSSION

Mating type determination combined with *Pep* allozyme genotyping provided a useful predictor of genotype in the Northern Ireland *P. infestans* population. All A2 isolates genotyped since 2008 have proved to be Blue 13 and all of these examined to date for *Pep* allozyme genotype were 96/96. It is therefore reasonable to assume that all A2 *Pep* 96/96 isolates identified in the current study were Blue 13. In contrast, the vast majority of A1 isolates in the current population in Northern Ireland were *Pep* 100/100, but the two which were *Pep* 96/96, proved to belong to the Pink 6 genotype, the first finding of this since 2009. In a study of Pink 6, allozyme genotyping of 18 isolates from Great Britain, Northern Ireland and the Republic of Ireland showed that all had *Pep* 96/96 (Kildea *et al.*, 2013). Therefore the low frequency of *Pep* 96/96 in A1 isolates of *P. infestans* from Northern Ireland in 2012 clearly indicated that the Pink 6 genotype was rare. As in previous years, it is likely that the vast majority of A1 isolates belonged to older genotypes such as 8_A1 as was indicated by the limited SSR genotyping.

However, it should not be assumed that A1 isolates of *P. infestans* from other populations with *Pep 96/96* belong to the Pink 6 genotype, since this allozyme pattern can be associated with quite different multi-locus genotypes e.g. in the Hungarian *P. infestans* population (Nagy *et al.*, 2006).

The current study clearly indicates that Northern Ireland *P. infestans* population is continuing to change: the Blue 13 genotype, which declined so markedly in 2011, underwent a resurgence in 2012 (to 37% of isolates), although it did not return to dominating the population as it did in 2009 and 2010. The increase in Blue 13 was associated with an increase in the proportion of metalaxyl-resistant isolates within the population (since Blue 13 is invariably metalaxyl-resistant) and, as the A1 isolates found belonged to genotypes that may be either metalaxyl-resistant or -sensitive (unlike Pink 6, which is always metalaxyl-sensitive), it is probably inadvisable to encourage the use of phenylamide-containing formulations for blight control in Northern Ireland. The limited characterisation of the 2012 Northern Ireland isolates indicated that the population remained highly clonal, but distinct from that in Great Britain where Pink 6 dominated (Cooke, D.E.L., personal communication).

Field trials between 2010 and 2012 indicated continuing good performance of programmes based on mandipropamid and fluazinam. Growers are encouraged to make use of a wide range of active ingredients with different modes of action and not to rely heavily on a single fungicide (*viz.* fluazinam), but for the purposes of trials, it is useful to have a simple standard programme for comparative purposes. The performance of the standard indicated consistent good control by fluazinam and no evidence of the poor performance associated with the occurrence of the Green 33 *P. infestans* genotype as occurred in The Netherlands in 2011 (Schepers *et al.*, 2013). Examination of genotyping results from the previous all-Ireland study failed to find any isolates with the Green 33 profile in the Irish population between 2008 and 2011 (S. Kildea, personal communication) and Green 33 is rare in Great Britain (Cooke, D.E.L., personal communication), so at present this and similar genotypes are not affecting fluazinam performance in the UK and Ireland; anecdotal evidence is of good performance in Northern Ireland. Any occurrence of *P. infestans* genotypes with reduced sensitivity to fluazinam could seriously impact on late blight control in Northern Ireland as fluazinam is the most popular fungicide for blight control, used by 63-75% of seed growers in the years 2010-12 (Cooke, L. R., unpublished) and is the most widely used fungicide (in terms of spray hectares) on seed, early and maincrop potatoes (Withers *et al.*, 2013). It is therefore important to continue to monitor changes in the *P. infestans* population in Northern Ireland so that control programmes can take into account the characteristics of current genotypes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge a British Society for Plant Pathology Undergraduate Vacation Bursary which supported Emma Walker. We thank DARD's Agri-food Inspection Branch for obtaining potato blight samples and data and David Cooke and colleagues at the James Hutton Institute for generously carrying out SSR analysis of selected isolates.

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Changes in epidemiology and population structure of *P. infestans* in Finland 1847-2011

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SUMMARY

The history of potato late blight occurrence was studied from 1845 to 2011. The first blight epidemics were most probably reported in Finnish newspapers in 1847. Thereafter the disease has been regularly present causing severe epidemics few times per decade. The onset of blight until late 1980s was relatively stable, usually in August. Before 1980s, there were only very few years when late blight was present in July. In 1990s the outbreak of the disease rapidly came 4 to five weeks earlier and the epidemics nowadays start at the last week of June or the first week of July. The early starting epidemics have considerably increased the number of fungicide applications needed to control the disease. In the average 5 to 6 applications in conventional growing are currently the normal practice.

KEY WORDS

Phytophthora infestans, epidemiology, fungicides

INTRODUCTION

Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive diseases of potato globally, including Finland (Fry 2008, Zadoks 2008). The disease is present in Finnish potato fields almost annually but severe crop failures have been relatively rare due to late onset of epidemics. The obvious change of aggressiveness of epidemics in late 1980s awoke interest to study the disease in more detail. Also new control strategies were needed to keep the late blight in control again.

The first indication of possible changes in the potato late blight population was the rapid selection of phenylamide resistant *P. infestans* strains at the beginning of the 1980s, soon after commercialisation of the group of phenylamide fungicides. The next indication of some serious change in potato late blight was the discovery of A2 mating types in potato fields in 1981 in Switzerland. A2 mating type had never before been reported in potato stands outside Mexico. Also the importance of oospores as a source was raised to discussions.

It is characteristic of *P. infestans* that prior to the 1980s the population outside Mexico was dominated by one clonal lineage, US-1. Thereafter populations have become more diverse, undergoing rapid seasonal changes. Moreover, in certain regions populations have become almost clonal, or consist of very few dominant clonal lineages. Particularly in northern Europe, current *P. infestans* populations consist of numerous unique or almost unique genotypes among which no obvious clonal lineages exist (Cooke *et al.*, 2011).

Potato late blight can be effectively controlled by several fungicides. However the public pressure to reduce use of agricultural chemicals has made development of integrated control strategies very important subject in potato late blight management. Also the risk of development of fungicide resistant strains of *P. infestans* has forced to target the fungicide applications more carefully than before.

The aim of this article is to summarise historical and recent migrations of *P. infestans* into Finland. The changes due to the migrations in disease epidemiology and phenotypic traits of the pathogen are emphasised. The article is based on Doctoral thesis in Plant Pathology defended in University of Helsinki May 4th 2012 (Hannukkala 2012). The opponent was Prof. William Fry from Cornell University, US.

MATERIALS AND METHODS

The history of late blight occurrence in Finland from 1845 to the 1980s is described based on newspaper articles (from 1845 to 1900) and scientific reports (from 1910 to 1982). The occurrence and severity of potato late blight from 1982 to 2011 is based on monitoring untreated plots of cv. "Bintje" in annual variety trials and untreated plots in fungicide efficacy trials at 6 to 9 Research Stations of MTT Agrifood Research Finland, and similar experiments at the Potato Research Institute. The effect of climatic factors on first late blight outbreaks was modelled for the period 1993–2002. From 1999 to 2002 aspects of suspected soil-borne epidemics were studied in detail. In total 4927 *P. infestans* isolates were collected from 1990 to 2010. Mating type (2703 isolates), R-gene virulence race (1100 isolates) and response to fungicides metalaxyl (3912 isolates) and propamocarb-HCl (2541 isolates) were determined. Haplotype based on mitochondrial DNA was determined for 154 isolates collected from 1992 to 2000. Development in fungicide use from 1953 to 2010 is described based on statistics for fungicide sales and the area devoted to potato growing in Finland.

RESULTS AND DISCUSSION

Leaf and tuber symptoms on potato most probably caused by the late blight pathogen were first described in eastern Finland in 1845. In 1847 and 1848 the disease was widespread, occurring in various parts of the country. During 1850s there were several extremely dry summers when the disease seemed to be absent (Figure 1).

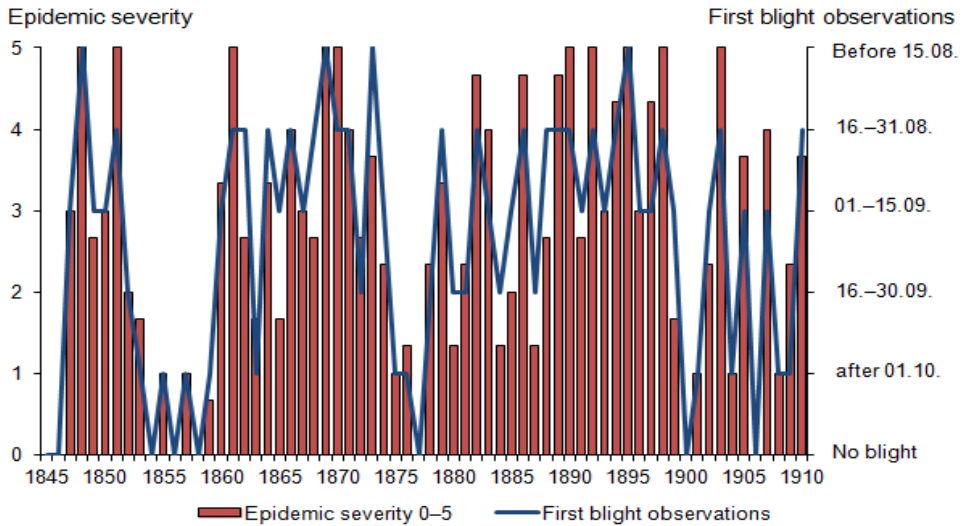


Figure 1. Dates of first blight observations and estimated severity of epidemics in Finland in 1845–1910

From 1849 to the 1980s one to five severe late blight epidemics were reported per decade. During this period late blight usually appeared in the fields during the latter part of August or early September. From 1930s to late 1980s the onset of epidemics was relatively stable, usually at the latter half of August (Figure 2).

At the end of the 1990s there was a rapid shift towards early outbreaks of late blight and since then the first late blight outbreaks have been reported at the end of June or during the first week of July (Figure 3). The shift towards early epidemics coincided with the increase of A2 mating type of *P. infestans* in the Finnish late blight populations. This indicates that oospores as primary source of inoculum are involved. In Finnish table and starch potato production growers mostly have very short crop rotations or potato is grown in almost continuous monoculture. This enables accumulation of oospores into soil especially when many growers at the end of season often become somewhat careless with continuing fungicide applications. It is very typical that occasional late blight lesions are present in crop at the end of season.

The chemical blight control was very rare in Finland until second half of 1970s. Until 1990s the total potato acreage was theoretically sprayed less than once based on fungicide sales and area under potato cultivation. The control was fully based on dithiocarbamate products. Metalaxyl product as pre packed mixture with mancozeb was introduced to markets at the latter half of 1980s. At that time metalaxyl product was recommended to be applied only after numerous blight lesions had appeared to crop. Moreover no straight mancozeb product was applied after metalaxyl application. It was no wonder in light of current knowledge that in the beginning of 1990s the *P. infestans* population was fully resistant to metalaxyl in Finland.

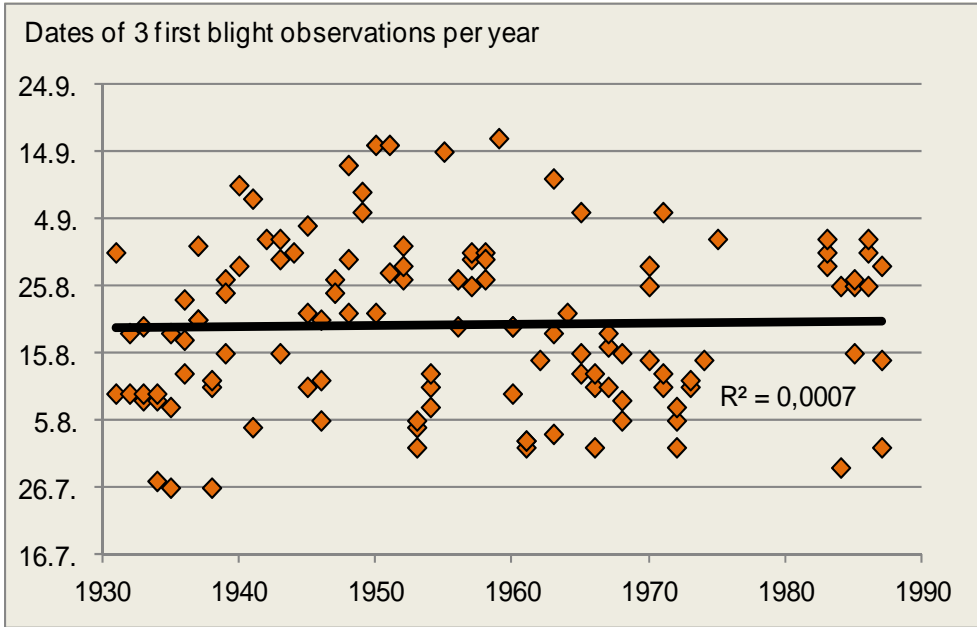


Figure 2. Dates of first blight observations and in Finland in 1930–1983

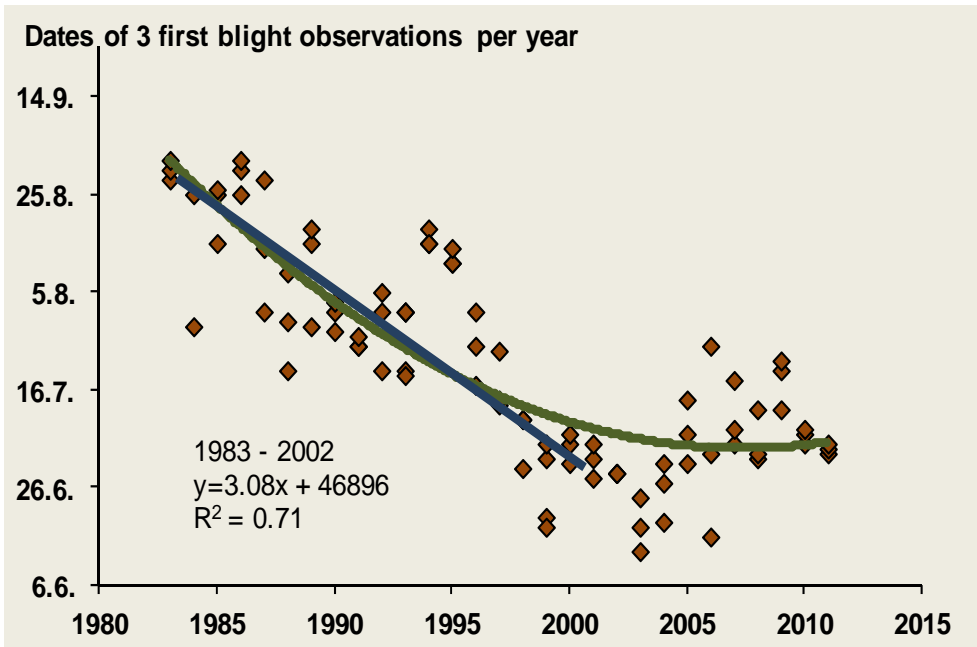


Figure 3. Dates of first blight observations and in Finland in 1983–2011

Strict anti-resistance strategies were introduced and followed in 1990s. Also novel fungicides with different modes of actions were registered. Until the end of 1990s the metalaxyl resistant strains had almost disappeared from the Finnish *P. infestans* populations.

Towards the end of 1990s it became obvious that the prevailing practice to start fungicide applications against late blight at the beginning of August was not valid any more. The rapid increase in the number of annual fungicide applications during 1990s (Figure 4) was simply due to the fact that most growers started their spray program in the beginning of July instead of August. Currently the theoretical number of annual blight fungicide applications is between 4 and 5 based on fungicide sales and acreage under potato cultivation.

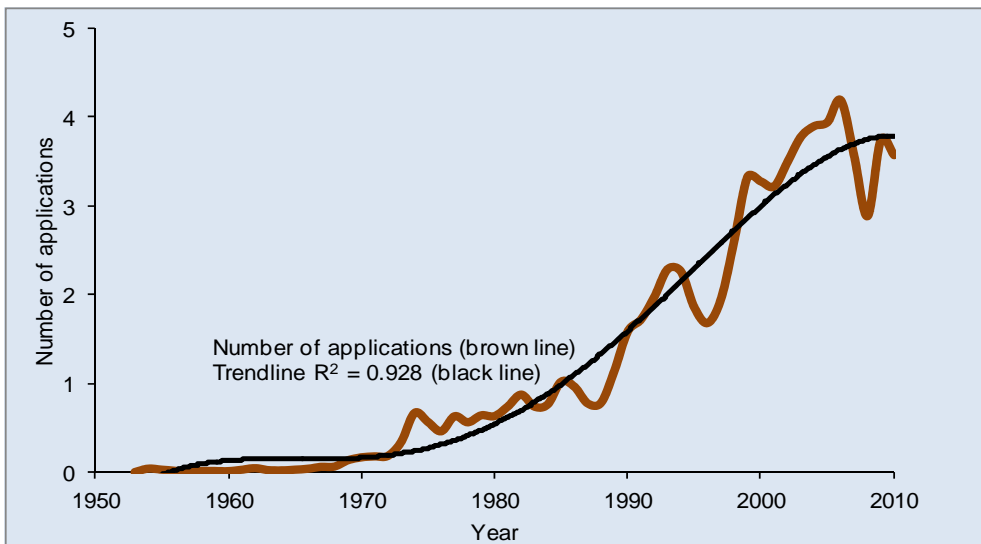


Figure 4. Average number of sprays against potato late blight in Finland from 1953 to 2010

In 1990s and especially during 2000s the number of different active ingredients registered for potato late blight control in pre-packed mixtures and straight products has increased considerably. It seems again that onset of potato late blight epidemics has stabilized to first week of July and growers have learned to live with the current sexually reproducing *P. infestans* population.

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Monitoring the Danish population of potato late blight pathogen, *Phytophthora infestans* in 2011-2012 and occurrence of 13_A2

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SUMMARY

Samples of the potato late blight pathogen (*Phytophthora infestans*) were collected from different potato fields in Denmark in 2011 and 2012 and genotyped using simple sequence repeat (SSR) markers. The results showed that the Danish *P. infestans* population is highly diverse with only a few minor clones that were spread locally. The diversity in the data indicate oospore-borne inoculum is important in Danish potato crops. The only dominant genotypes, 13_A2 and dk_6, were present in both seasons with 13_A2 comprising 12% in 2011 and 25% in 2012.

KEYWORDS

Potato late blight, *Phytophthora infestans*, monitoring, Danish population, genotype 13_A2, dk_6

INTRODUCTION

Knowledge of the genetic diversity and structure of pathogen populations advances applied and fundamental science. At a practical level, it helps the industry understand sources of primary inoculum and, if combined with data on the traits of the local pathogen population (e.g. fungicide resistance) it can improve disease management decisions. Such knowledge also provides insights into the rate and mechanisms of evolutionary change within the pathogen population.

Previous surveys have indicated a diverse population across the Nordic region (Brurberg *et al.*, 2011, Sjöholm *et al.*, 2013) that suggests sexual recombination and a significant role of oospores as a source of primary inoculum (Widmark *et al.*, 2011, Yuen & Andersson, 2012). In contrast, other regions of Europe are characterised by relatively few dominant clonal lineages with a lower proportion of genetically unique recombinant isolates (e.g. Li *et al.*, 2012, Cooke *et al.*, 2012). One aggressive lineage in particular, termed 13_A2 (Cooke *et al.*, 2012), spread rapidly from 2004 onwards to become locally dominant. It is now widespread in some parts of Europe (Chmielarz *et al.*, 2013, Cooke, unpublished data) and found in China (Li *et al.*, 2013a) and India (Chowdappa *et al.*, 2012). It has not, to date, been reported in the Nordic regions. In

this study we investigated the structure of the Danish *P. infestans* population in more detail than previous studies by sampling late blight outbreaks potato crops over the 2011 and 2012 seasons. A specific emphasis in this study was to examine the causes of any incursion of the 13_A2 lineage into the Danish potato production regions.

MATERIALS AND METHODS

Late blight infected leaf samples were collected from potato fields in Denmark in early September 2011 and from July to September 2012. The sampling targeted potato fields from across different potato growing areas with one single-lesion leaflet chosen per field. Lesions were pressed onto FTA cards (Whatman) for later genotyping. Identification of genotypes using a 12-plex SSR protocol was performed according to a published protocol (Li *et al.*, 2013b). The resultant fingerprints were compared to other defined lineages in published and unpublished data held at the James Hutton Institute.

RESULTS

In 2011, a total of 74 samples were collected from 52 fields and 9 samples (12%) were of the genotype 13_A2 ("Blue 13") lineage. Most of the 13_A2 samples (6) came from North Jutland (starch potato producing area). In 2012, a total of 112 samples from 102 fields were tested and 28 samples (25%) were of the genotype 13_A2. In this season, 22 of the 28 13_A2 samples came from North Jutland and 6 samples of 13_A2 from mid-Jutland. No samples matching the "green33" lineage were found in either year. Other samples were of locally distributed clonal types or unique genotypes found only once (Fig. 1).

DISCUSSION

The results show that the Danish *P. infestans* population is highly diverse (only two clones comprised more than 3% of the population). Sub-clonal SSR variation is evident and other minor clones are mostly locally spread. The only dominant genotypes, 13_A2 and dk_6, were present in both seasons. The diversity in the data indicates that oospore-borne inoculum is important in Denmark and that some inoculum from these initial sources is propagated within crops and spread locally as it was sampled again later in the same season.

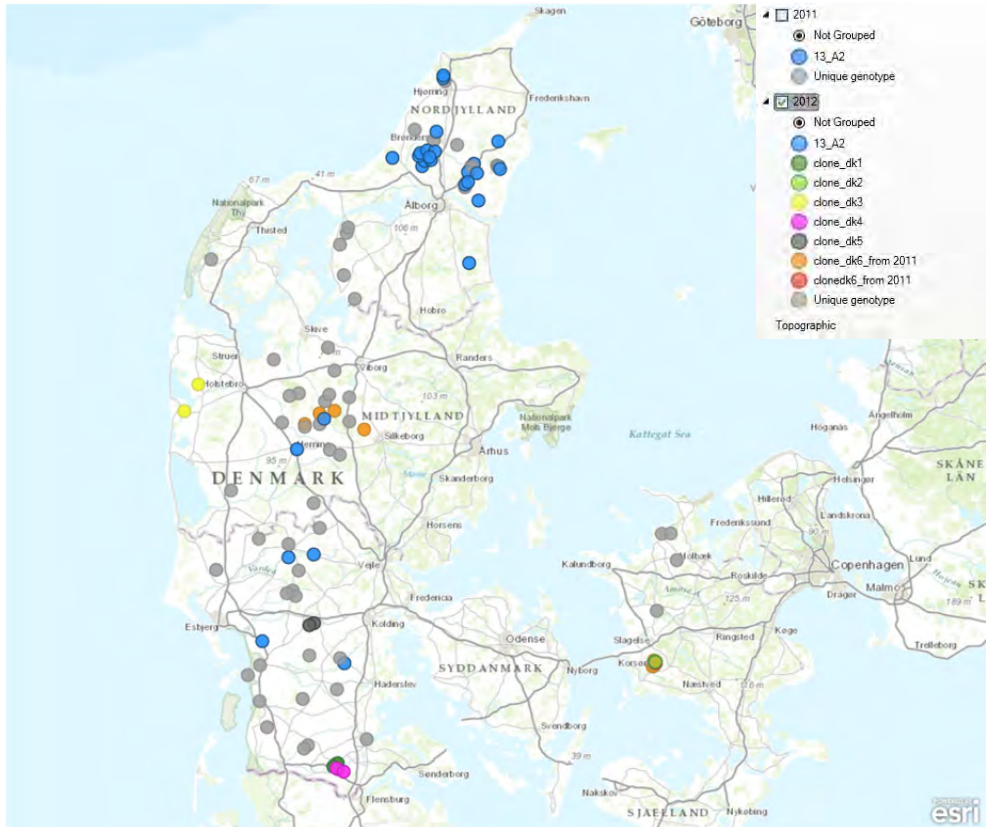


Figure 1. Distribution of *P. infestans* genotypes in Denmark 2012. Isolates were collected July – September 2012 from different potato fields and conserved on FTA cards for subsequent SSR genotyping

Most of the 13_A2 genotype samples came from North Jutland where there is a high concentration of varieties grown for starch production. In 2012, the first record of late blight infection was 3th July and over the following 2-3 weeks many fields were sprayed with metalaxyl (Ridomil Gold) curatively to control the outbreaks. The collection of isolates in the area was started from the 13th-19th of July and many isolates were of the 13_A2 genotype.

Resistance to metalaxyl has been demonstrated in the 13_A2 genotype (Gisi *et al.*, 2011, Cooke *et al.*, 2012) and the prevalence of 13_A2 in North Jutland may thus be a consequence of a selection pressure generated by curative metalaxyl usage. Prior metalaxyl testing in Denmark has shown that 5% of the *P. infestans* isolates sampled were resistant in 2003 and 25% in 2008 (based on the leaf disk method, Hannukkala, unpublished). In another study, 8% of isolates collected in 2006 to 2007 were resistant (Gisi *et al.*, 2011).

Further sampling in 2013 and a detailed analysis of SSR fingerprints, combined with spatial analysis and crop management information from three consecutive potato growing seasons will shed more light on the drivers of *P. infestans* population change in Denmark.

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Evaluating the potential of *Phytophthora infestans* to genetically adapt to the Rpi-blb1 (RB) source of blight resistance

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SUMMARY

The *P. infestans* resistance gene *Rpi-blb1* is derived from the wild potato species *Solanum bulbocastanum* and the corresponding gene product recognizes the presence of the *ipiO* virulence effector of *P. infestans*, triggering a resistance response when this effector is introduced during invasion by *P. infestans*. The goal of this study was to compare the sequence variation in the *ipiO* gene following passage through detached leaves of Maris Peer (MP) expressing *Rpi-blb1* with that resulting from passage through untransformed Maris Peer. After 10 repeat passages through MP + *Rpi-blb1*, 4 regions of the *ipiO* sequence were identified as containing up to 12 hotspots where sequence variants occurred at frequencies of between 1.79 % and 59.91%. Significantly, comparable levels of variation were also recorded following passage through untransformed MP - *Rpi-blb1* leaf tissue, suggesting that the *ipiO* sequence is in a steady state of flux as a result of being exposed to host tissue, irrespective of whether the host is equipped with the corresponding R gene or not.

KEYWORDS

Phytophthora infestans, Rpi-blb1, 454 sequencing, ipiO.

INTRODUCTION

P. infestans, like all plant pathogens, has to negotiate intricate defence systems in order to infect its host. During the biotrophic phase of *P. infestans* infection, the pathogen forms a structure called the haustorium with which it invaginates the host cell membrane, delivering pathogenicity factors ('effectors') into the host cytoplasm and acquiring nutrients from the host cell (Dodds and Rathjen, 2010). Plants may respond to this attack by attempting to restrict colonization, often through effector-triggered immunity which involves a form of programmed cell death

popularly known as the 'hypersensitive response'. To facilitate successful colonization, the *P. infestans* genome encodes effectors which operate inside the host cell (Haas *et al.*, 2009). The best known *P. infestans* effectors are those containing the RXLR (arginine-any amino acid-leucine-arginine) motif, which represents a conserved sequence determinant of host translocation (Birch *et al.*, 2006). Such effectors include the *ipiO* gene family which is highly diverse among *P. infestans* populations worldwide with class I IPI-O occurring in the majority of pathogen genotypes (Champouret *et al.*, 2009).

The wild potato *S. bulbocastanum* displays strong partial resistance to *P. infestans* mediated by *R* genes including the *RB* gene, also known as *Rpi-blb1* (Song *et al.*, 2003). The resistance response conferred by the *Rpi-blb1* gene includes the induction of a classical hypersensitive response and up-regulated transcription of pathogenesis-associated defence genes (Chen and Halterman, 2011). However, *Rpi-blb1* only confers strong partial resistance and can permit the growth and sporulation of *P. infestans*, albeit at significantly lower rates compared to the non-*Rpi-blb1* expressing control (Song *et al.*, 2003). The *Rpi-blb1* gene product recognizes the presence of the *ipiO* virulence effector of *P. infestans* and triggers a resistance response when this effector is introduced during invasion by *P. infestans* (Vleeshouwers *et al.* 2008). The fact that the *ipiO* locus is prone to mutation (Haas *et al.*, 2009) suggests that in a scenario where *P. infestans* parasitizes and sporulates on a partially resistant host, each subsequent generation may exhibit increased virulence against the formerly resistant host.

To investigate whether continued parasitism on a host harbouring the *Rpi-blb1* gene would lead to pathogen adaptation and breakdown of resistance, this study experimented with a representative isolate of the recently occurring 6_A1 *P. infestans* genotype ('Pink 6'), which was passaged ten times through detached leaflets of transgenic potato plants carrying one copy of the *Rpi-blb1* gene. At the end of the repetitive passaging, levels of polymorphism within the *ipiO* sequence of each cycled isolate were assessed and compared with the initial isolate.

MATERIALS AND METHODS

Plant and pathogen preparation

The potato cultivar Maris Peer (MP) was used for this study along with an *Rpi-blb1*-expressing potato line of the same cultivar containing a single copy of *Rpi-blb1* (Wendt *et al.*, 2012). Plants were propagated from tubers under glasshouse conditions of 20°C and a minimum of 16 h day length. For the passaging experiments, leaflets (fourth / fifth leaf) below the uppermost fully expanded leaves were collected from glasshouse maintained 6 to 8 week old plants. Three independent biological replicates per treatment were included in a single experiment. *P. infestans* isolate DL43B_A1 [SSR genotype 6_A1 (Pink 6), A1 mating type] was maintained on rye A media slants and subcultured every 4 months.

Passaging of P. infestans isolate DL34B_A1 through detached leaflets of Rpi-blb1-expressing transgenic potato

Prior to commencing, the isolate was firstly passed through leaflets of the susceptible cultivar, Kerr's Pink to restore any virulence lost in culture. Viable inoculum was produced 7 days post-inoculation by washing leaflets with sporulating lesions in 5 ml of sterile distilled water in 50 ml Falcon tubes to dislodge sporangia into suspension. The resulting suspensions were standardized with a haemocytometer to a concentration of 2×10^4 sporangia/ml and incubated at 4°C for

2 hours to release zoospores. To conduct the first passage, inoculations were performed using inoculum prepared as described above. A single leaflet was deemed an experimental unit. The leaflets were placed lower surface uppermost in 9 cm diameter inverted Petri dishes with a layer of dampened paper towel at the base before being inoculated in the centre with 20 μ l of the appropriate sporangial/zoospore suspension. Inoculated leaflets were incubated in a growth chamber at 18°C for 7 days with a 16 h photoperiod. For mean area under lesion progress curve (AULPC) assessment visible lesion area was calculated for each inoculated leaflet as $1/4\pi ab$ for area of an ellipse with a = breadth of lesion and b = lesion length (Colon *et al.*, 1995). Lesion diameter along the main leaf vein and diameter perpendicular to the main leaf vein were deemed length and breadth respectively for consistency. Seven days post inoculation leaflets of each isolate/cultivar interaction were washed separately in sterile 50 ml Falcon tubes containing 2.5 ml of sterile distilled water to obtain inoculum for the next passage. As the same leaves were used for lesion area measurements and for inoculum production for the next passage, caution was observed to avoid the cross contamination among samples at all stages of the experiment. The same procedure for inoculum preparation and leaflet inoculation was followed for ten consecutive cycles. Cycle 0 was *P. infestans* inoculum that was not exposed to MP leaf tissue. Pure cultures were obtained upon completion of the tenth cycle by transferring sporangia from sporulating lesions individually onto pea agar without antibiotic. Three plates were prepared for each isolate during transfer of sporangia onto pea agar to reduce the risk of loss of isolates through contamination.

DNA extraction, ipiO amplification and 454 sequencing

Approximately 50 mg of *P. infestans* mycelia were harvested from 10 day old pea agar cultures of isolates using sterile scalpels and transferred into 2 ml Eppendorf tubes. The mycelia were freeze dried for 24 h and pulverized using a mixer mill with sterile glass beads. Extractions were performed using the procedure of Raeder and Broda (1985).

To determine the level of polymorphism within the ipiO gene, the target sequence was firstly PCR amplified under conditions of 0.2 mM dNTPs, 1X thermal buffer, 100 nM forward primer, 100 nM reverse primer, 1 unit Taq polymerase, 50 ng/ μ l genomic DNA and 13.8 μ l water in a total volume of 20 μ l. The reactions were completed in a Biometra 3500T Thermo Cycler with an initial cycle of denaturation of 5 min at 95°C followed by 40 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 58°C and extension for 40 sec at 72°C and a final extension for 5 min at 72°C. The ipiO primers used were: 5' ATG GTT TCA TCC AAT CTC and 5' CTA TAC GAT GTC ATA GCA TGA CAC. PCR products were loaded onto 1% (w/v) agarose gel stained with ethidium bromide (1 μ g/ml) and submerged in 0.5 M TBE buffer. A size standard (100 bp ladder NEB, UK) was loaded alongside the samples and 70 V of current applied for 30 min. Bands were visualized and imaged using a Kodak Imager (Image Station 440 CF, Kodak Digital Science TM, USA). Once identified, the requisite bands were excised with a scalpel and transferred into sterile 1.5 ml tubes. DNA was eluted using the Qiaex II Kit (Quiagen, Germany) following the manufacturer's instructions. The resulting supernatant containing the purified amplicons from each sample was transferred into fresh 1.5 ml Eppendorf tubes and quantified using a fluorescent Qubit DNA Assay (Invitrogen, USA). Generated amplicons were sequenced on a Roche GS Junior 454 sequencing platform according to manufacturer's recommendations for sample preparation and run completion. Data analysis was performed using the Roche proprietary AVA™ software, to analyse variant frequency. Variants were selected as positive when they dominated in >2% of the reads collected per *P. infestans* sample.

RESULTS AND DISCUSSION

Disease progression

For the conventional Maris Peer DL34B_A1 host pathogen interaction, disease progressed steadily from cycle 1 (AULPC = 647.19 +/-24.20) up to cycle 10 (AULPC = 1105.67 +/-27.52). In the case of the MP+*Rpi-blb1* line (equipped with a single copy of *Rpi-blb1* transgene), an initial AULPC score of 54.27 +/-4.14 was recorded after the first infection cycle. Notably, the disease lesions caused by DL34B_A1 enlarged progressively in subsequent cycles of inoculations (data not shown), with an AULPC of 1003.65 +/-44.82 recorded at cycle 10. One possible explanation to account for the occurrence of disease on RB-bearing hosts is delayed or ineffective triggering of the hypersensitive response (HR) (Vleeshouwers *et al.*, 2000) and/or the positioning of the integrated *Rpi-blb1* transgene within the Maris Peer genome. As the *Rpi-blb1* confers strong partial resistance in the form of a disease-rate-reducing phenotype which is different from immunity (Song *et al.*, 2003), delayed or ineffective HR is inevitable and allows escape and proliferation of pathogen hyphae which are necessary for establishment of the biotrophic phase of parasitism (Vleeshouwers *et al.*, 2000).

IpiO sequencing

Diversity across the *ipiO* sequence was assessed for nine DL34B_A1 *P. infestans* samples; that is from the three replicates of cycle 0, the three replicate isolates passaged through the MP + *Rpi-blb1* line and the three replicate isolates passaged through the conventional Maris Peer control for 10 cycles. The number of reads ranged from 5,356 to 8,662, with an average read number per template of 7,070 +/-415. As a result, a consensus of the *ipiO* sequence before exposure to potato leaf tissue was prepared from 19,172 reads (cycle 0) (Table 1), which acted as the reference sequence with which to compare against the *ipiO* reads obtained after the 10 repeated passages through the MP + *Rpi-blb1* material.

Table 1. Number of 454 reads per individual *P. infestans* DL34B_A1 isolate before (cycle 0) and after (cycle 10) passaging through MP / MP + *Rpi-blb1* potato leaf tissue

Host	Cycle	Replicate	No. 454 reads	Mean (+/- SD)
MP	10	1	8337	--
MP	10	2	8662	---
MP	10	3	5945	7648 +/- 856
MP + <i>Rpi-blb1</i>	10	1	7711	---
MP + <i>Rpi-blb1</i>	10	2	7910	---
MP + <i>Rpi-blb1</i>	10	3	5895	7172 +/- 641
---	0	1	7789	---
---	0	2	5356	---
---	0	3	6027	6390 +/- 725

Following 10 successive cycles on MP + *Rpi-blb1* material the *P. infestans* sequence underwent substantial genetic change with a comparative analysis of each replicate's batch of reads against the respective *ipiO* consensus sequence (from cycle 0) indicating the prevalence of 12 'hotspots'

across approximately 4 regions of the *ipiO* sequence (90bp, 163 – 167bp; 278 – 304bp and 340 – 368bp) (Table 2).

Table 2. Percentage sequence variation in the *ipiO* gene in three replicate *P. infestans* DL34B_A1 isolates (R1, R2 and R3) after 10 cycled passages through MP / MP + *Rpi-blb1* potato leaf tissue

Variant No.	bp position	MP			MP + <i>Rpi-blb1</i>		
		R1	R2	R3	R1	R2	R3
1	90	59.91	57.01	57.19	53.09	56.8	57.03
2	163	2.33	3.02	0.98	2.97	2.57	2.73
3	167	2.83	3.47	1.80	3.67	3.2	3.2
4	278	13.45	12.73	13.49	14.62	31.61	13.87
5	289	13.46	12.78	13.49	14.61	31.68	13.87
6	290	13.47	12.77	13.49	14.61	31.68	13.87
7	304	13.34	12.66	13.41	14.54	31.54	13.81
8	340	13.46	12.72	13.47	14.55	31.62	13.73
9	343	4.16	4.06	2.07	1.79	1.93	4.13
10	352	20.56	18.71	17.80	15.81	21.86	18.79
11	367	6.00	6.48	6.64	8.94	13.65	7.22
12	368	20.69	20.19	21.68	22.82	47.44	21.14

Variant frequency varied across the population, ranging from 1.93% to 59.91%, with variant no. 1 occurring in an average of 58.03% of *ipiO* sequences following exposure to leaf tissue expressing *Rpi-blb1* transcripts. While this contrasts with a recent study which concluded that the presence of the *RB* sequence did not promote adaptive parasitism in *P. infestans* (Halterman and Middleton, 2012), this report relied on direct sequencing of cloned *ipiO* amplicons which is not comparable to the coverage obtained through the 454 platform. Of significance for this study, however, was the comparable level of variation recorded across the *ipiO* sequence following passage through the control, non-transgenic plant material, which is not equipped with the *Rpi-blb1* transgene. While the level of variant occurrence was variable, the same variants were recorded irrespective of whether the infecting *P. infestans* isolate was passaged through host leaves with and without the *Rpi-blb1* resistance. This result was unexpected owing to the specific gene-for-gene interaction that exists between *ipiO* and the *Rpi-blb1* gene from *S. bulbocastanum*. In fact it was hypothesised that the absence of the R gene in the corresponding host would not result in any genetic change in the corresponding effector coding sequence in *P. infestans*. Based on the results presented here, this does not appear to be the case as the *ipiO* effector sequence in *P. infestans* is registering a constant state of flux during the potato – *P. infestans* host-pathogen interaction.

CONCLUSIONS

Employing a next generation sequencing approach, this preliminary study indicates the substantial potential for sequence change in an effector coding sequence after successive passages through clonal host tissue, irrespective of whether the potato leaf tissue was expressing the corresponding R gene. Further experimentation is required with additional genotypes of *P. infestans* to ensure the observed phenomenon is not genotype-specific and also

to investigate the impact of this repeat passaging on the sequence integrity of other effector coding sequences in the *P. infestans* genome.

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Alternaria diseases of potatoes: epidemiology and management under Israeli conditions

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SUMMARY

Potatoes are grown in Israel in two main seasons. For the autumn crop, potatoes are planted in late August to early September and harvested during December and January. For the spring crop, potatoes are planted in late January to February and harvested during May to July. Two *Alternaria* species infect potato plants in Israel: *A. solani*, the causal agent of early blight and *A. alternata*, the causal agent of necrotic lesions. Nevertheless, the most destructive pathogen is *Phytophthora infestans*, the causal agent of late blight. Analysis of disease progress curves revealed that early blight is more important in the autumn than in the spring season. Results of field experiments suggested that *A. solani* intensifies towards the end of the season, in mature plants and that the yield was reduced and application of fungicides was profitable in the autumn but not in the spring seasons. Based on these experiments we developed an integrated strategy for management of both early and late blights. The strategy was evaluated in field experiments, under natural infections, and found accurate. In observations carried out in commercial fields it was found that necrotic lesions appear suddenly in large areas, often after heavy rain events and that the phenomenon was more common in crops growing in sandy soils. *Alternaria alternata* was isolated from necrotic lesions and the Koch postulates were completed and proved the pathogenicity of that fungus. Based on these observations it was hypothesized that heavy rains wash the nitrogen fertilizer from the root zone and that necrotic lesions appear in plants suffering from stress imposed by sudden reduction in nitrogen content in the foliage. These hypotheses were tested and it was found that necrotic lesions develop primarily in nitrogen-deficient plants and that applying supplemental N fertilization reduces necrotic lesion severity. Accordingly, it was decided not to recommend fungicide spraying for suppression of necrotic lesions.

KEYWORDS

Alternaria alternata, *A. solani*, disease management, *Solanum tuberosum*

INTRODUCTION

Potatoes are grown in Israel (30° E, 31° N) in several regions. The main area of production (ca. 12,300 ha, 80% of the national cultivated area) is in the northern Negev. The climate there is

semi-arid with mild winters and hot, rainless summers. There are two main seasons for potato production, autumn and spring. For the autumn season crop, potatoes are planted in late August – early September and harvested during December and January; for the spring crop they are planted in late January – February and harvested during late May, June and July. The growing seasons differ markedly in respect to the environment: for the autumn season crop, growth starts when temperatures are high and the days are relatively long, and continues under decreasing temperatures, day length and radiation; for the spring season crop, growth starts when temperatures are relatively low and days are relatively short, and continues under increasing temperature, day length and radiation (Levi *et al.*, 1986).

Early blight (caused by *Alternaria solani*), necrotic lesions (caused by *A. alternata*) and late blight (caused by *Phytophthora infestans*), are the main foliar diseases of potatoes in Israel. Severe epidemics of early blight may restrict potato yields by up to 20-30% and late blight may result in complete destruction of the yield in severe epidemics (Rotem, 1994; Shtienberg *et al.*, 1996). Quantitative estimations on the effects of necrotic lesions on yield are currently not available. In order to suppress the diseases, fungicides are often applied to the foliage. During a typical growing season, potato fields are sprayed with fungicide eight to twelve times. In the current paper we review the experience gained over the years in coping with these potato diseases under the semi-arid conditions prevailing in Israel.

EARLY BLIGHT, CAUSED BY *ALTERNARIA SOLANI*

Observations and epidemiological considerations

The response of potatoes to *A. solani* changes as the host ages. Immature potato plants are relatively resistant to early blight. However, after the initiation of tuberization, susceptibility increases gradually and mature plants are very susceptible to *A. solani* (Pscheidt & Stevenson, 1988; Rotem, 1994). Cultivars differing in genotype resistance usually follow a similar pattern of changes in age-related resistance. Thus, early blight is principally a disease of senescing plants and early sprays had little, if any, effect in overall suppression of *A. solani* (Christ & Mazuga, 1989; Pscheidt & Stevenson, 1988; Shtienberg *et al.*, 1996). Analysis of epidemics of early blight in the autumn and spring seasons in Israel revealed that early blight is more important in the autumn than in the spring season. The variable effects of the disease in the two growing seasons was attributed to the different seasonal patterns of environmental conditions such as temperatures and radiation (Shtienberg *et al.*, 1996). This implies that management of early blight in the autumn is essential and may lead to significant yield increases, but the need for its suppression in the spring season is questionable.

Late blight may develop in both growing seasons but epidemics are more likely to occur and are more severe in the spring, due to the prevalence of more conducive weather. Often, the disease does not develop at all in a certain season or in certain fields but due to its destructive potential growers apply fungicides as a precocious measure. Since adequate control efficacy depends on timely initiation of fungicide sprays (*i.e.*, before the disease had infected the crop) spraying is initiated soon after emergence and continues, in 7-10 days intervals, until vine kill.

Conceptual model for early and late blights management

Results of previous studies enabled us to develop concepts for integration of genotype resistance, age-related resistance and fungicide in the management of *A. solani* and *P. infestans*. An attempt was made to develop an effective, reliable and cost-effective solution for changing environment and under uncertain conditions. The basic principles are as follows: late blight is the

most important disease and the crop has to be protected before the pathogen had invaded the field; thus, sprays are applied in a prophylactic manner as an insurance measure. The frequency of sprays is dependent on the response of the cultivar to *P. infestans* and on the season: sprays are applied less frequent to moderately resistant cultivars and in the autumn. To quantify the general risk a specific field is facing, three "risk" categories were defined. Risk category "A" implied that late blight was not observed at all in the region; risk category "B" implies that late blight was observed in the region but not in the field and risk category "C" implies that symptoms were observed in the specific field. A regional system has to be developed to notify growers on the current situation of the disease in the region. The type of fungicide to be applied and the frequency of sprays are determined according to the current risk category. In general, protectant fungicides at half rate are to be applied under risk category A and protectant fungicides at full rate are to be applied at risk category B. Under risk category C, protectant fungicides are to be applied if conditions are unfavorable to *P. infestans* and systemic fungicides are to be applied when environmental conditions are highly favorable to *P. infestans* (*i.e.*, cool, rainy weather).

Early blight is to be considered specifically only in the autumn season employing the concepts presented in Figure 1. Application of fungicides is not needed in plants at the vegetative stage because they are relatively resistant. Accordingly, spraying should be initiated only when host response to *Alternaria* shifts towards increasing susceptibility, *i.e.*, at the initiation of the reproductive stage (Shtienberg *et al.*, 1989). The frequency of subsequent sprays should be determined according to the genotype resistance of the cultivar to *A. solani* and the efficacy of the fungicide, in relation to changes in age-related resistance. Accordingly, protectant fungicides should be applied initially at relatively long intervals, which will shorten as the crop ages. Towards the end of the season, more effective control, by means of a systemic fungicide, is recommended. The maturity class of the cultivar and its genotype resistance to *A. solani* should be considered as well by adjusting the frequency of spraying according to the changes in host response to the pathogen (Figure 1). Towards the end of the autumn season, if late blight alert B or C is issued, a systemic fungicide used against early blight is to be mixed with a full rate of a fungicide against late blight (protectant or systemic). In the spring season, the environment promotes host growth and the production of new leaves precludes a significant reduction in yield and management of early blight should not be considered specifically. The protectant sprays applied against late blight in the spring season are sufficient for adequate suppression of early blight.

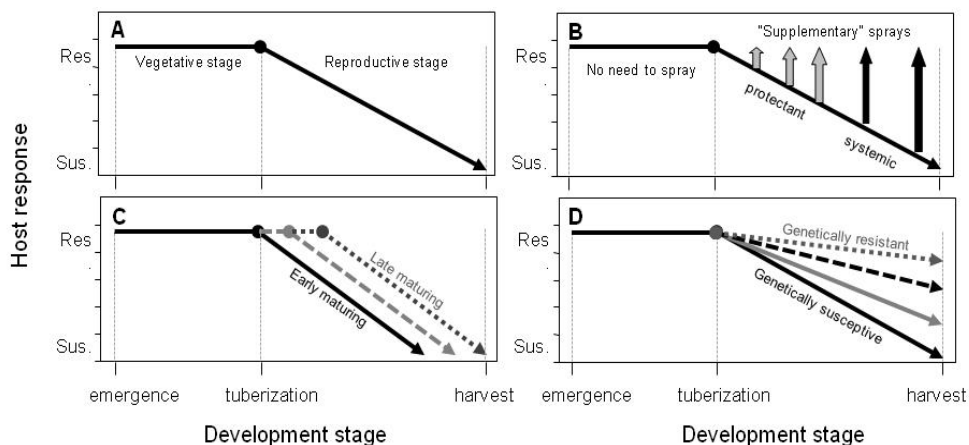


Figure 1. Graphical presentation of components of the conceptual model for integration of control measures for suppression of *Alternaria solani*, the causal agent of early blight in potatoes. **A.** Changes of host response over time (i.e., age related resistance); **B.** The need for applying supplementary fungicidal sprays to compensate changes in host resistance over time; **C.** Effects of maturity class of the cultivar; **D.** Effects of genotype resistance. Host response: Res. = resistant; Sus: susceptible

Experimental results

The conceptual mode described above was evaluated in seven experiments conducted in the northern Negev region of Israel; 4 experiments were carried out in the autumn season and 3 in the spring season. All experiments were carried out in commercial fields so that the results would reflect the complexity and uncertainty prevailing in normal situations. In this report results recorded in one autumn-season experiment and in one spring-season experiment are presented but those recorded in the other experiments were similar. Susceptible cultivars were used in the experiments, Mondial in the autumn experiment and Alpha in the spring experiment. Plots consisted of four 7-m long rows and were separated from each other by fallow areas approximately 1 m wide. Fungicides (in 260-300 L water/ha) were applied by means of a motorized back-sprayer at a pressure of 275 kPa with cone-jet X6 nozzles. In each trial one protectant and one systemic fungicide (for each pathogen) were used. The protectant fungicide was mancozeb and the systemic fungicides were tebuconazole (Folicur) and cymoxanil+mancozeb (Mancur; CM). The following five treatments were included in all trials: (i) untreated control; (ii) management of both early and late blights by protectant fungicides (application of mancozeb on a 7-day schedule); (iii) optimal management of early blight (application of tebuconazole on a 10-14 day schedule); (iv) optimal management of late blight (application of CM on a 10-14-day schedule); and (v) application of protectant and systemic fungicides according to the concepts of the integrated management strategy. In treatments iii spraying was initiated after observing the first early blight symptoms in the plots; in the other treatments, prophylactic sprays were applied. Two individuals assessed disease severity visually independently and the average scores were computed. Assessments were made every 7-14 days from the appearance of disease symptoms until the end of the season. Since it was not always possible in the field to distinguish between symptoms induced by *A. solani* or *P. infestans*, their combined intensity was assessed. Thus, disease severity records reflect the intensity of both pathogens and towards the end of the season also the natural senescence of the crop. Severity

records were used to calculate the Area Under the Disease Progress Curve (AUDPC) for each of the treatments. Results were subjected to statistical analysis and where F values showed significant differences, Fisher's protected LSD Test was applied at $P = 0.05$.

In the autumn experiment early blight was observed 40 days after emergence; late blight was observed in the region (risk category B) about three weeks after emergence and in the field (risk category C) about 7 weeks later. By the end of the season, plots treated only with tebuconazole and plots treated only with CM were significantly more diseased than plots that were treated against both pathogens (the integration treatment). Presumably, the higher disease severity resulted from inadequate suppression of *P. infestans* and *A. solani*, respectively, in this plots. AUDPC values in the fungicide treated plots differed significantly from the value recorded in untreated plots (Figure 2).

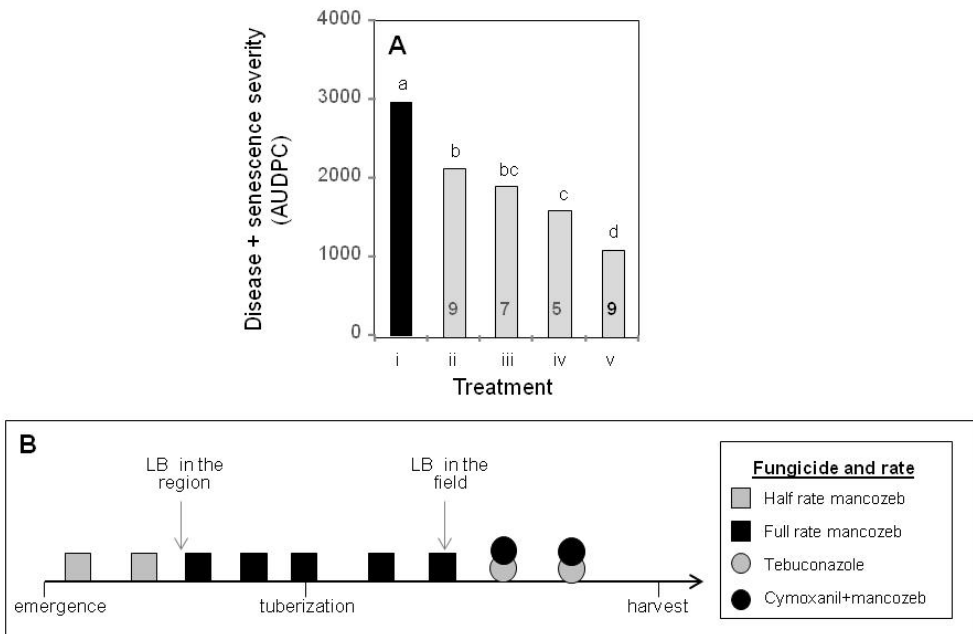


Figure 2. Evaluation of the integrated management strategy in a field experiment carried out in the autumn season. The following five treatments were included in the trials: (i) untreated control; (ii) mancozeb; (iii) tebuconazole; (iv) cymoxanil+mancozeb (CM); and (v) the integrated management strategy. **A.** Effects of the treatments on the AUDPC. The number of sprays applied in each treatment is indicated within the bars; **B.** Timing of spraying in the integrated management treatment. Values of bars accompanied by different letters differ significantly as determined by the LSD test at $P < 0.05$

In the spring experiment late blight was more destructive than early blight (although both diseases prevailed in the experiment). Both pathogens were adequately suppressed in the CM treatment and also in the integrated management treatment. This control was achieved in spite of the fact that only 2 CM sprays were applied in the integration treatment, as compare to 5

sprays in the CM treatment. Disease control (presumably late blight) in the tebuconazole treatment was inadequate as reflected by the higher AUDPC values (Figure 3).

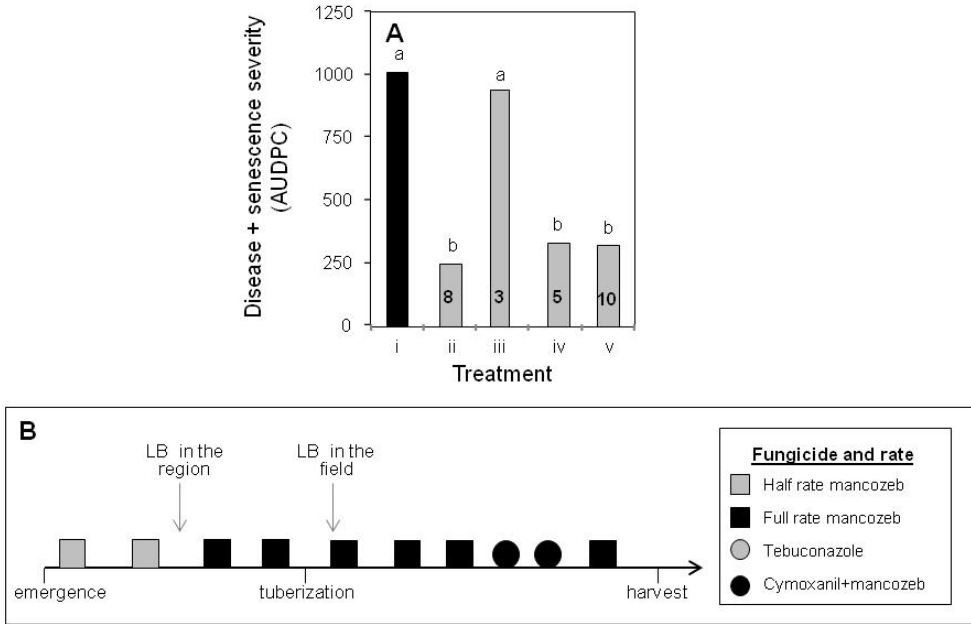


Figure 3. Evaluation of the integrated management strategy in a field experiment carried out in the spring season. The following five treatments were included in the trials: (i) untreated control; (ii) mancozeb; (iii) tebuconazole; (iv) cymoxanil+mancozeb (CM); and (v) the integrated management strategy. **A.** Effects of the treatments on the AUDPC. The number of sprays applied in each treatment is indicated within the bars; **B.** Timing of spraying in the integrated management treatment. Values of bars accompanied by different letters differ significantly as determined by the LSD test at $P < 0.05$

NECROTIC LESIONS, CAUSED BY *ALTERNARIA ALTERNATA*

Observations and epidemiological considerations

Observations in commercial fields in the northern Negev reveal that, occasionally, brown spots develop on the lower side of potato leaves. The spots are scattered on the leaves in large numbers (Figure 4). In accordance to the symptoms the phenomenon was called "necrotic lesions". The fungus *A. alternata* was consistently isolated from these spots and its pathogenicity was confirmed by completing the Koch's postulates (Droby *et al.*, 1984). In their study, Droby *et al.* (1984) found that the fungus infects the leaf by direct penetration and *via* stomata. Young plants, at the 10-12 leaf stage, were less susceptible than adult plants; a differential susceptibility of the leaves was observed in which the middle leaves of the plant showed the highest disease incidence at any given growth stage. They also reported that susceptibility varied according to the cultivar and the quantity of sprinkler irrigation. Observations in commercial fields, under natural conditions revealed that necrotic lesions appear suddenly in

large areas, often after heavy rain events (> 50 mm rain). Furthermore, the phenomenon is more common in crops growing in sandy soils. Application of fungicides against *Alternaria* slightly reduced the intensity of necrotic lesions but control efficacy was relatively low (D. Shtienberg; *unpublished results*). Based on these observations it was hypothesized that necrotic lesions appear in plants suffering from stress imposed by sudden reduction in nitrogen content in the foliage and that heavy rains, or large quantities of over-head irrigation, wash the nitrogen fertilizer from the root zone.

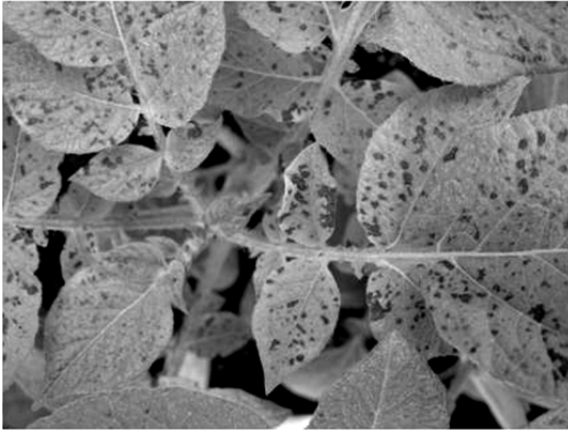


Figure 4. Potato leaves severely infected by necrotic lesions, caused by *Alternaria alternata*

Experimental results

The hypothesis that deficit in nitrogen fertilizer predispose the development of necrotic lesions was examined in a set of field experiments in 2003 to 2005 (Shtienberg *et al.*, 2006). In these experiments the quantitative interaction between various N rates and rain quantities were used. Nitrogen was applied *via* the irrigation system (fertigation) as done in commercial production at rates varying from 0 to 250 kg/ha; "rain" was mimicked by over-head irrigation system at a rate equivalent to 60 mm occurring once (33 or 45 days after planting) or twice (33 and 45 days after planting). To minimize the possibility that "real rain" will fall and mask our treatments, the experiment was carried out in the spring season at a location where rains are infrequent. It was found that necrotic lesions severity was governed by nitrogen rate and by the timing and number of mimicked-rain events. Necrotic lesions severity was the highest in plants that were not fertilized with nitrogen at all (0 fertilization treatment) and were exposed to mimicked-rain twice; the lowest necrotic lesions severity was observed in plants that were fertilized at with high nitrogen rate (245 kg nitrogen/ha) which were not exposed to mimicked-rain at all (Figure 5). Nitrogen content was determined in leaf petioles sampled from the experimental plots. It was found that necrotic lesion severity was significantly related to nitrogen contents in the petioles (Figure 6). The experiment was repeated once with similar results.

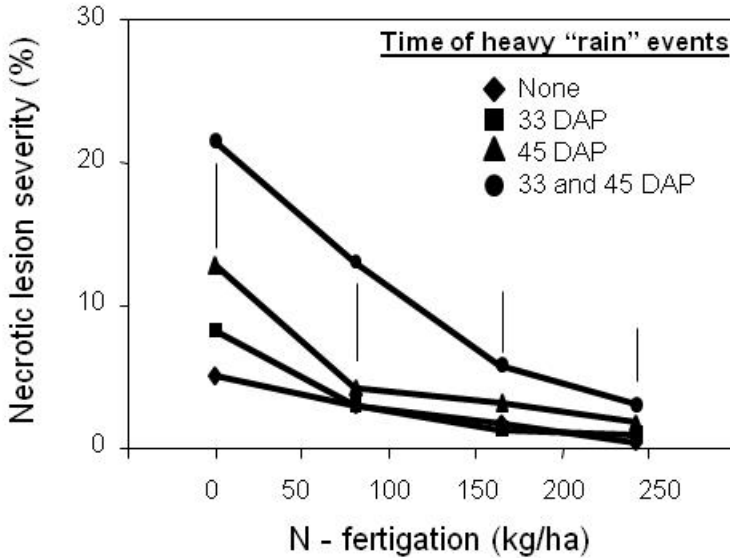


Figure 5. The interacting effects of nitrogen fertilization and the number of heavy "rain" events (mimicked by over-head irrigations) on the severity of necrotic lesions, caused by *Alternaria alternata*, in potato foliage. Vertical lines indicate the LSD at $P = 0.05$. DAP = days after planting

In another set of experiments attempts were made to reduce necrotic lesion severity by application of nitrogen soon after the occurrence of (mimicked) heavy rain event or by application of foliar sprays. Heavy rain event was mimicked over-head irrigation (applied once, at quantity equivalent to 80 mm of rain). After the termination of the mimicked rain, N-fertilizer was applied at a rate of 180 kg N/ha. For the fungicide treatment, tebuconazole was applied 4 times in bi-weekly intervals, starting 3 weeks before the time of mimicked-rain. Thus, two sprays were applied before and two sprays were applied after the occurrence of the mimicked-rain event. Disease severity (*i.e.*, the proportion of the foliage exhibiting disease symptoms) was assessed one month after the simulation of rain. It was found that spraying of fungicides did not affect necrotic lesions severity but application of surplus nitrogen, soon after the occurrence of the mimicked heavy rain event, significantly reduced necrotic lesions severity as compared with the plants that did not receive the surplus fertilization (Figure 7).

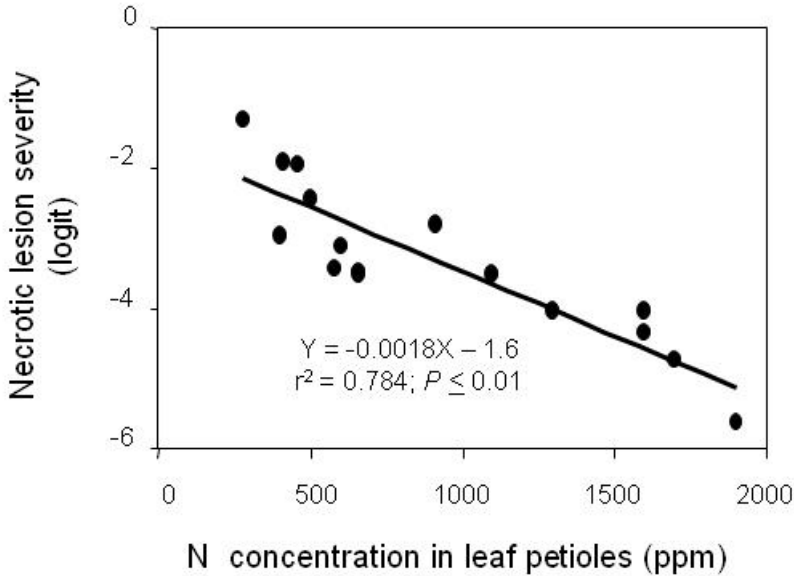


Figure 6. The relationship between N concentration in leaf petioles and severity of necrotic lesions. Necrotic lesion severity is expressed in logit units ($\text{logit} = \ln(X/(100-X))$ where $X = \text{disease severity}$)

Based on our previous observations and on the experiments described herein, it was concluded that necrotic lesions develop primarily in nitrogen- stressed plants. Application of fungicides did not reduce necrotic lesions severity significantly. On the other hand, applying supplemental N fertilization soon after the occurrence of the heavy rain event reduced necrotic lesions severity significantly. As a consequence, to our opinion application of fungicides for the suppression of necrotic lesions is not required; application of supplemental nitrogen soon after the occurrence of heavy rain event may be considered.

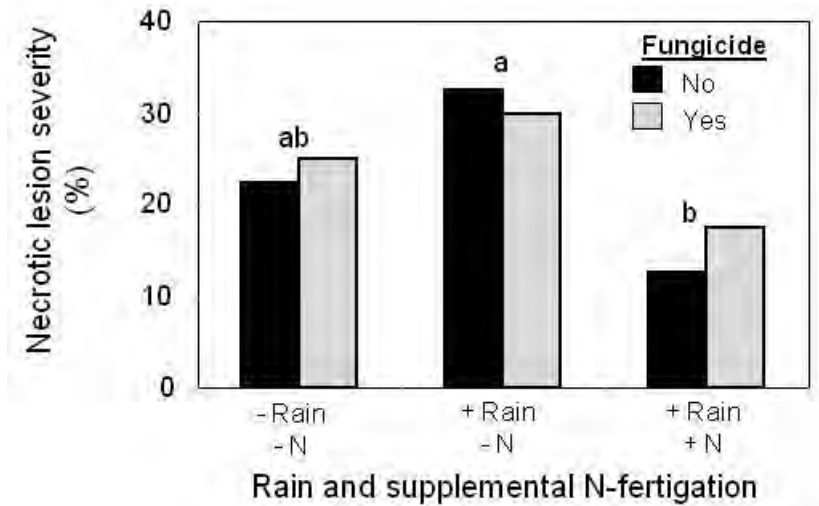


Figure 7. The interactive effects of heavy “rain”, supplemental N fertilization and fungicides, on necrotic lesions severity. Rain was mimicked by over head irrigation at quantity equivalent to 80 mm; Nitrogen was implemented after the mimicked rain event in quantity of 180 kg N/ha. Four tebuconazole sprays were applied, two before and two after the mimicked rain event. Effects of spraying were insignificant; Values of bars accompanied by different letters differ significantly as determined by the LSD test at $P < 0.05$

DISCUSSION

Development of more than one pathogen in a certain field is a well-known situation. Potato growers in the northern Negev region of Israel had to combat simultaneously with *A. solani*, *A. alternata* and *P. infestans*. The most important measure employed by growers during the growing season for the suppression of foliar diseases is application of fungicides. Several fungicides of various groups are currently available for the control of each pathogen. However, their efficacy may differ markedly and certain fungicides cannot be used for the suppression of all pathogens. For example, triazole fungicides are highly effective against *Alternaria* but not effective at all against *P. infestans*. On the contrary, if CM is applied against *P. infestans*, only the protectant portion of the product (*i.e.*, mancozeb) is effective against *Alternaria*. The protectant fungicides are effective against all pathogens but their efficacy, in general, is inferior to that of the specific systemic fungicides. Other important factors that should be taken into account are the cost of the fungicides and the ability of the pathogens to develop resistance against the commonly used fungicides. The cost of systemic fungicides applied to a unit area is 2-10 times higher than the cost of the comparable protectant fungicides. The costs, and the need to reduce the number of applications to lower the probability for development of fungal populations resistant to the effective systemic fungicides, motivate growers to apply them as less frequent as possible.

The assumption underlying the concepts of the integration management strategy presented in this study is that the effects of different control measures are complementary and additive. Accordingly, application of one measure may compensate for a decrease in another measure.

Integration of three measures was considered, *viz.*, genotype resistance, age-related resistance and fungicide type and efficacy. Genotype resistance and age-related resistance were considered as measures in which their contribution is more or less predetermined. Fungicides were used as a flexible measure by which it was possible to respond to the situations develops in the field during the growing season.

The relative importance of the pathogens may differ between years, growing seasons and individual fields. Based on our experiments and observations we concluded that in the northern Negev, late blight is the predominant disease and should be managed properly in all crops; early blight should be considered only in the autumn season and necrotic lesions develop sporadically and since it has no significant effects on yield, specific control measures should not be applied for its management. The integrated management strategy presented here provided adequate suppression of the foliar diseases threatening potato production in commercial fields under diverse conditions. It should be noted that the number of sprays applied according to the integrated strategy was not necessarily lower than that applied by the grower's routine program. However, disease suppression was always adequate and calculations of the cost/benefit ratio revealed that the net income, after deduction of spraying expenses was markedly higher in the integrated management treatment than in the other treatments (results not shown). Potato growers in the Negev region implement components of this strategy with considerable success.

ACKNOWLEDGMENTS

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An integrated concept for early blight control in potatoes

ANDREA VOLZ, TONGLE HU, HANS HAUSLADEN

SUMMARY

In an attempt to develop an integrated early blight management strategy in potatoes, we tested different soil treatments in order to reduce the primary inoculum in the soil. Besides incorporation of calcium cyanamide or biofumigant plant tissue into the furrows, we varied the crop rotation and compared scorings between potato plots with previous crops potatoes and barley, respectively. While we are still working on proving the causal relationship between our treatments and reduced early blight severity, we have already observed significant effects of all of our treatments.

KEYWORDS

Alternaria spp., calcium cyanamide, biofumigation, crop rotation, soil inoculum

INTRODUCTION

Early blight in potatoes is mainly caused by the pathogen *Alternaria solani*. In potato fields we observe a pathogen complex consisting of *A. solani* and *A. alternata*. Yield losses can add up to 25% and in Germany late maturing varieties are affected more heavily than early maturing varieties. So far early blight has been controlled by fungicides, most effectively by the strobilurin azoxystrobin. But only two years after registration of azoxystrobin in the USA, *A. solani* isolates with reduced sensitivity to azoxystrobin have been found (Pasche *et al.*, 2004). The altered sensitivity is caused by the F129L mutation of the cytochrome b gene. Until now there have been no proven reports of a loss of sensitivity among European isolates in the field, but the mutation is also spreading among isolates in Europe (Leiminger *et al.*, 2013). So we have to pose the question: do we have alternatives or complements for early blight control?

In the last decades early blight control has been achieved by inhibiting or reducing the secondary spread of *A. solani* spores between plants. Yet, the primary source of inoculum is fungal material which is overseasoning on plant debris within the soil. Therefore we thought of ways to affect this primary inoculum and we conducted several field trials with different soil treatments. The factors we varied were the previous crop, the nitrogen fertilization, the soil application of azoxystrobin, and biofumigation. Biofumigation means the suppression of soil-borne pathogens, pests, and weeds by isothiocyanates (ITCs), which derive from hydrolysis of glucosinolates by myrosinase in disrupted plant cells (Angus *et al.*, 1994, Kirkegaard *et al.*, 1993). Shredded plants of the brassicaceous plant family can be incorporated into infested soil

to release their ITCs and in this way to inhibit pathogens. We expected that the early blight pressure in potatoes with previous crop potato should be higher than after the previous crop barley. Furthermore we believed that biofumigation and even soil application of azoxystrobin after planting would reduce early blight severity. As fourth element we varied the type of nitrogen fertilizer and expected calcium cyanamide to have a fungicidal side effect on *Alternaria* spores and mycelium.

MATERIALS & METHODS

Field trials

The field trials were conducted in two locations near to Munich, Bavaria. As early blight susceptible potato variety we chose the late maturing "Maxilla". Plant density was 40,000 plants per hectare. Trials were designed as a randomized complete block consisting of four replications. Field plots were comprised of six rows (0.66 m between rows) and were 7.5 m long (29.7 m²). For suppression of late blight infections we sprayed Ranman (active ingredient: cyazofamid) weekly in all plots of our trials. In our biofumigation trial in 2012 we incorporated leaf material of different crops into the furrows of the potato plots (table 1) for simulating different crop rotations.

Table 1. Crop species, variety, and amount of green manure amendments of the biofumigation field trial and the reported characteristic isothiocyanate (ITC)

Treatment	Green manure amendment	Released ITC	Amount per plot (kg fresh weight)
1	Dried infested leaves of <i>S. tuberosum</i> "Kuras"	_____	0.5
2	none (control)	_____	—
3	<i>Solanum tuberosum</i> "Maxilla"	_____	7.5
4	<i>Phacelia tanacetifolia</i> "Lila"	_____	7.5
5	<i>Sinapis alba</i> "Albatros"	4-hydroxybenzyl-ITC	7.5
6	<i>Raphanus sativus</i> "Defender"	4-(Methylsulfinyl)-3-butenyl-ITC	7.5

In a second trial (2010-2012) we compared calcium cyanamide (CaCN₂) fertilization to a calcium ammonium nitrate (CaNH₄NO₃) fertilizer and to Ortiva (active ingredient: azoxystrobin) soil application. In the years 2011 and 2012, this trial was planted in two fields with comparable soil parameters except for the crop rotation. So we had eight replications for each treatment, four with potatoes and four with barley as previous crop. Details can be seen in table 2.

Table 2. Nitrogen fertilization, fungicide application, and previous crops of our multiannual second trial

Treatment	Fertilizer	Fungicide	Previous crop	Years
1	0 kg N	—	barley	2010-2012
2	CaNH ₄ NO ₃ (160 kg N)	—	barley	2010-2012
3	CaCN ₂ (160 kg N)	—	barley	2010-2012
4	0 kg N	Ortiva (3 l/ha)	barley	2010-2012
5	CaNH ₄ NO ₃ (160 kg N)	Ortiva (3 l/ha)	barley	2010-2012
6	0 kg N	—	potato	2011-2012
7	CaNH ₄ NO ₃ (160 kg N)	—	potato	2011-2012
8	CaCN ₂ (160 kg N)	—	potato	2011-2012
9	0 kg N	Ortiva (3 l/ha)	potato	2011-2012
10	CaNH ₄ NO ₃ (160 kg N)	Ortiva (3 l/ha)	potato	2011-2012

For revealing the effects of the treatments of interest on early blight development we compared the area under the disease progress curve (AUDPC) of potato plants according to the formula of Das *et al.* (1992). Scores were taken as percent value of necrotic leaf area every ten days. Of each plot we scored ten plants and thereof three leaf levels (top, middle, and bottom).

Lab experiment

In a lab trial we observed the effect of biofumigant ITCs at direct contact with spores of *A. solani*. A dilution series of different ITCs was added to a standard culture medium and poured into petri dishes. Final concentrations of ITCs ranged between 1 μ M and 10 mM. After solidification, a spore solution was streaked on the medium resulting in approximately 800 spores per petri dish and dishes were sealed with parafilm afterwards for inhibiting evaporation of volatiles. The spore solution was derived from 14 day old single spore isolates growing on SNA medium (Nirenberg, 1981). Each ITC was tested repeatedly with four different single spore isolates. The ITCs of interest were allyl-, ethyl-, methyl-, benzyl-, and phenylethyl-ITC (Sigma Aldrich Corp., MO-St. Louis). Spore germination was evaluated after an incubation period of four days by counting 100 spores per petri dish and differentiating between germinated and non-germinated spores.

Scorings and AUDPC values were tested for significant differences by SPSS version 21.0.0.0 (IBM Corp., NY-Armonk).

RESULTS

The biofumigation trial in 2012 revealed a differentiated early blight development. Amendments of white mustard and leaf radish resulted in significantly reduced leaf symptoms compared to the amendments with potato leaves, whereas Phacelia ranged in between.

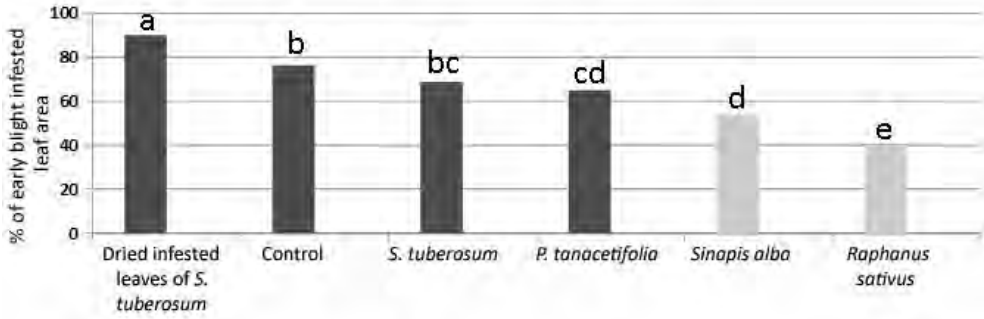


Figure 1. Effect of green manure amendments on early blight symptoms of top leaves in the late season of 2012. Different letters above columns mean significant differences (Tukey, $\alpha=0,05$)

Lab experiments showed a clear inhibition of spore germination by ITCs. Yet, the effective concentration varied between ITCs and allyl-ITC has been the most potent one with 100 μM being enough to inhibit germination.

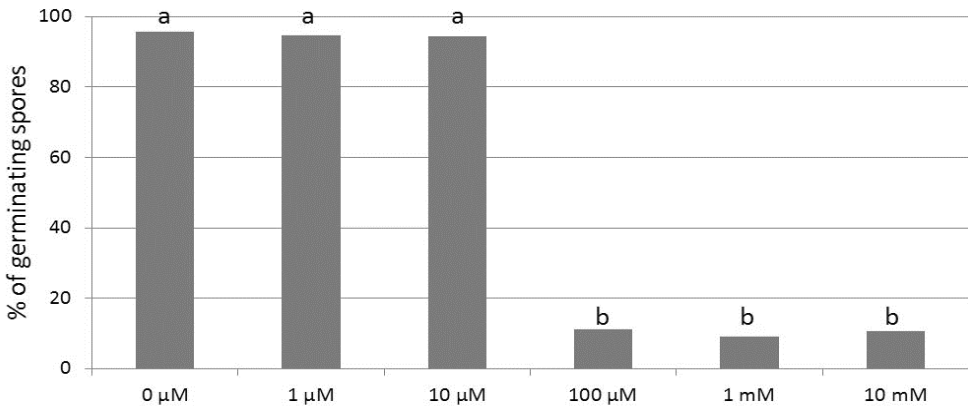


Figure 2. Spore germination of *A. solani* on media with increasing allyl-ITC concentrations. Different letters above columns mean significant differences (Tukey, $\alpha=0,05$)

In our second trial be observed that early blight epidemics are delayed by approximately 15 to 20 days after wheat as previous crop compared to plots with potatoes as previous crop. The mean AUDPC of the plots with previous crop potato was significantly higher than the mean AUDPC of plots with previous crop barley ($\alpha=0.05$).

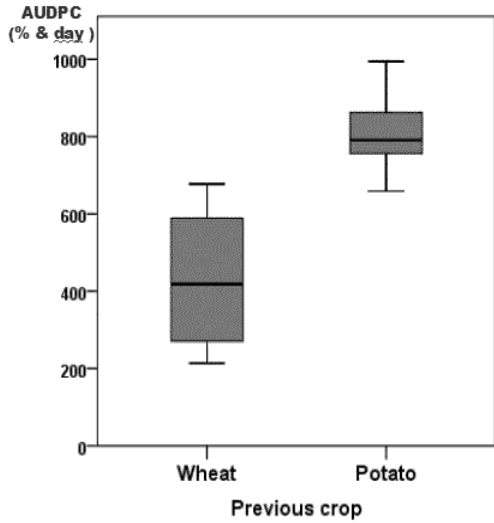


Figure 3. Effect of previous crops wheat and potatoes on the early blight AUDPC of potatoes in 2011

With regard to nitrogen fertilization, calcium cyanamide-fertilized plants had significantly less early blight symptoms than plants treated with calcium ammonium nitrate ($\alpha=0.05$). The AUDPC of calcium cyanamide-treated plants was comparable to plants sprayed with Ortiva.

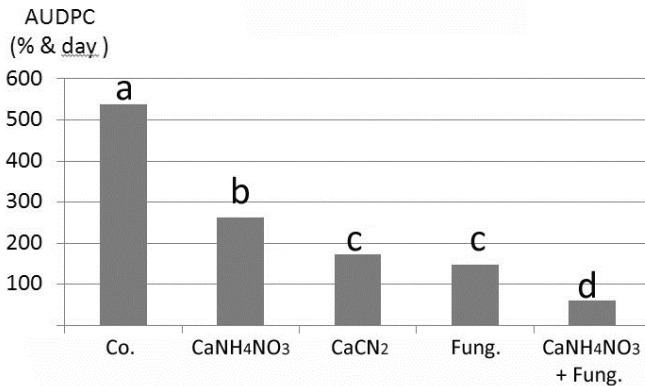


Figure 4. Comparison of calcium cyanamide, calcium ammonium nitrate, and the fungicide Ortiva with regard to their effect on the early blight AUDPC in 2010. Different letters above columns mean significant differences (Tukey, $\alpha=0.05$)

DISCUSSION

Our experiments give us reason to believe that there is more than an explicit fungicide treatment to control early blight in potatoes. Integrated disease management strategies include preventive aspects like growing disease resistant varieties and choosing an appropriate location for the respective crop. The aim to maintain healthy crops can be achieved by meeting their nutritional demands. Other factors are disease monitoring as well as biological and mechanical control methods. For some of these aspects we have already found promising approaches in early blight control. Now after having observed significant effects, we are trying to elucidate the causal relationship behind these effects.

The fungicidal side effect of degradation products of calcium cyanamide which has been reported by Finck and Börner (1985) can until now only assumed to be the reason for the delayed early blight development in cyanamide-treated plots. In lab assays, we have no evidence for any lethal effects of the degradation products of calcium cyanamide on spores or mycelium of *Alternaria* spp. so far. But assays are still under progress. As an alternative explanation to the fungicidal potential we consider the slower degradation of calcium cyanamide and thus the more adequate availability of nitrogen during early blight epidemics to be a reason for obviously healthier plants. Nevertheless, effects are significant and constant over years.

A very promising method for early blight control seems to be biofumigation. There are many factors which affect the efficiency of the procedure like fertilization and growth stage of biofumigant plants, the time span between maceration of biofumigant plant tissue and incorporation into soil, and soil temperature and humidity during and after incorporation, and some more. Therefore further research is necessary to support farmers for a better practicability of this method. But if conditions are optimal, a significant reduction of early blight symptoms is possible.

First scorings of the field season 2013 seem to confirm the tendency of last year: after wheat as previous crop early blight epidemics are delayed by approximately 15 to 20 days compared to plots with potatoes as previous crop. The most probable reason for accelerated and enhanced early blight development in the plots with previous crop potatoes is a higher concentration of soil inoculum. We have already developed a DNA extraction method for soil samples and aim to provide evidence for the presence and for the amount of soil inoculum by PCR and qPCR, respectively. This will help us to compare the extent of leaf infestation with the concentration of *A. solani* and *A. alternata* inoculum in soil and to reveal a suspected correlation.

Encouraged by our findings about the effects of crop rotation, biofumigation, and calcium cyanamide on early blight development on potatoes, we hope to find even more modules for a comprehensive strategy to control this disease.

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Differentiation of *Alternaria* species and quantification of disease development using real-time PCR

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SUMMARY

An *Alternaria*-specific real-time PCR assay was developed to clearly discriminate *A. solani* and *A. alternata*. The use of real-time PCR allowed a quantitative estimation of fungal biomass in plant tissues. With this work we report real-time PCR applications to accurately assess the extent of *Alternaria* spp. colonization during disease development. This assay provides a useful tool to quantify pathogen levels during initial latent stage of infection and disease development.

KEYWORDS

Alternaria solani, *Alternaria alternata*, disease quantification, molecular diagnosis,

INTRODUCTION

Early blight (EB), caused by fungi of the genus *Alternaria*, belongs to one of the most devastating diseases of potato. Pathogens, which are discussed to be involved in EB disease are *Alternaria solani* as well as *A. alternata*. Several studies had shown that *A. solani* and *A. alternata* could be isolated simultaneously out of EB typical symptoms (Bäßler *et al.*, 2004; Leiminger, 2009; Latorse *et al.*, 2010). Up to now, techniques of *Alternaria* identification mainly rely on agar plate methods, where *Alternaria* species are differentiated macroscopically by the morphology of their spores. Similarly, disease scorings based on the assessment of visual symptoms do not guarantee a distinct differentiation of pathogens. Although methods exist to detect *Alternaria* species in potato, current techniques do not allow a quantification of the fungi *in planta*. The most commonly used molecular technique for the identification of *Alternaria* species in general is conventional PCR with species-specific primers based on the ribosomal DNA internal transcribed spacer (ITS). The application of real-time PCR represents a highly sensitive and specific technique for the detection and quantification of nucleic acids (Taylor *et al.*, 2001). With this work we firstly describe real-time PCR applications to accurately assess the extent of *Alternaria* colonization of potato leaves during early disease development and to differentiate both species. The use of this assay opens the opportunity to track the specific progression of *Alternaria* species within the host which may contribute to a better understanding of EB

epidemiology. This may then be used in epidemiological investigations as well as in disease management strategies.

MATERIAL AND METHODS

Field studies

Leaf samples were taken from naturally infected field trials. Sampling schemes were designed as a randomized complete block and were replicated four times. Experimental plots were situated within a commercial potato field which remained untreated against EB, thus providing natural inocula for EB disease development. In 2003 and 2004 field trials were carried at Weihenstephan, in 2005 at the location Straubing. All plots were fertilized and cultivated according to general agricultural practice.

Visual evaluation of EB infection

Disease progression was observed weekly from potato emerge until death of the potato plants. Within each of the replications, 10 plants per plot were monitored for disease progression of EB or other diseases (e.g. late blight) and percentage of EB infected leaf area was assessed visually. The disease severity per plant was calculated as a mean value. For leaves which were either completely dead or where the lower leaves had senesced and dropped from the plant, the disease evaluation was omitted.

Collection of plant probes

From the onset of first visible symptoms on older leaves, leaf samples were taken within an approx. 14 day interval out of EB untreated control plots. Each plot was divided into 4 subplots and leaves were collected out of lower, middle and apex leaf sections. Ten leaves were taken for each subplot and were mixed for further analysis. Each plot was repeated 4-times and was randomly distributed. Tissue was harvested and immediately frozen in liquid nitrogen at the site. For levels, which did not contain any leaves due to premature defoliation caused by EB, sampling was discontinued.

Genomic DNA extraction and PCR amplification

Samples were carefully ground in liquid nitrogen and crushed with a pestle to a fine powder. DNA was extracted out of 100 mg homogenized plant material. The final DNA was finally dissolved in 100 µl Tris EDTA (TE buffer, pH 8). 1µl part of this DNA solution was used for quantitative PCR (real-time PCR) which was performed in 25 µl reactions with Absolute QPCR SYBR Green® ROX Mix. Real-time PCR analysis has been carried out to elucidate which of both species was dominating. Therefore species-specific primers were developed. *Alternaria* specific primers derived from the base-sequences of the internal transcribed spacers (ITS) of ribosomal RNA (rRNA) genes. Quantitative PCR of *A. solani* produced a 152 bp fragment and a 95 bp fragment of *A. alternata*, but no amplicon was obtained from uninfected potato leaves. In order to determine the species specificity of the primers, purified genomic DNA of 50 different fungal species, including major tomato and potato pathogens were included in the PCR assays.

RESULTS AND DISCUSSION

Visual quantification of EB disease development

The infection process of *Alternaria* spp. was monitored by visual disease scorings as well as by real-time PCR analysis. Disease ratings revealed that first disease symptoms occurred at a very young age of potato plants. Here, EB was the primary foliar disease among all observed diseases. Over all years, disease epidemics progressed slowly till the end of July and more rapidly thereafter (Figure 1). As the season progressed, EB symptoms rapidly increased and spread onto higher leaf levels. Secondary spread of EB was observed in all years starting from the end of July or the beginning of August. Heavy EB epidemics occurred throughout all five years of investigation. EB resulted in premature defoliation of potato plants in mid September.

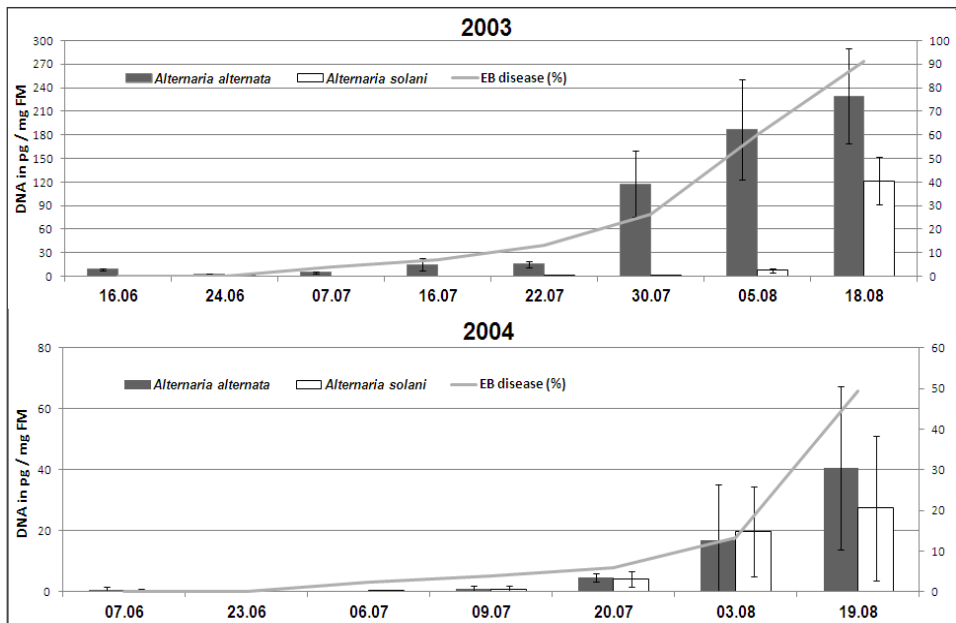


Figure 1. Specific disease progression of *A. solani* and *A. alternata* measured by real-time PCR compared to disease progression of early blight in 2003 and 2004, location Weihestephan

Quantifying EB disease in leaves

Within the developed PCR system both *Alternaria* species could be clearly identified and differentiated. No positive amplification signals were obtained on the non-target fungal species. Primers revealed that the targets were well conserved within the repetitive DNA sequence of either *A. solani* and *A. alternata*. The development of fungal species within the leaves was monitored. The real-time PCR analysis detected the presence of *Alternaria* DNA in almost all samples at quite early stages of plant development. Higher amounts in DNA levels of *A. alternata* were observed in 2003, showing *A. alternata* as the predominant species from the beginning of sampling (Figure 1). In 2004, DNA of both species were found to be present at almost equal levels throughout the season (Figure 1). In 2005, *A. solani* was found to be the

predominant species with high quantities of fungal DNA. Although small amounts of DNA has been detected at the beginning of the season for both species, significantly higher amounts of *A. solani* DNA could be detected with progressing season. In contrast, in 2005 low *A. alternata* DNA values were observed which remained at low levels till the end of the season (Figure 2).

In 2005 leaf samples were taken simultaneously out of three different plant sections. Analysis of the middle and top third of the canopy revealed that Alternaria-DNA could be detected prior to the development of EB symptoms. Here, real-time PCR allowed the detection of fungal growth already during the latent phase of disease development. The largest increase of the amounts of DNA took place at the middle leaf section in 2005. The largest increase of the amounts of fungal DNA took place during the symptomatic stage of the infection with severe EB symptoms (Figure 2). Real-time PCR allowed to quantify Alternaria species in field infected potato leaves. Detection of fungal DNA failed on the very last sampling dates. Here, real-time PCR did not yield any results although leaves were heavily infected by EB. This is probably due to the lack of live mycelia which had been completely converted to spores, which had obviously not been disrupted by powdering in liquid nitrogen.

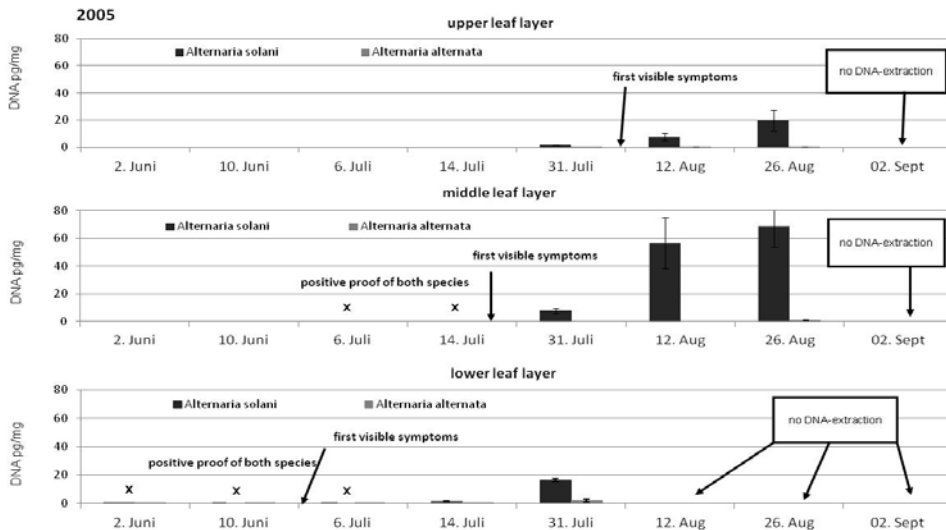


Figure 2. Disease progression of *A. solani* and *A. alternata* measured by real-time PCR in 2005, location Straubing. To follow up species development in more detail, potato plants were divided into three leaf layers (bottom, middle and upper leaf section). Detection of fungal DNA failed on the very last sampling dates

Analysis yielded significantly different amounts of Alternaria-DNA of both pathogens and among all investigated years. Although both pathogens were present, their intensity of infestation differed strongly in different years. Only in 2003 *A. alternata* was solely found at higher DNA levels. In the following year 2004 a more equal distribution of both species was revealed by real-time PCR. In fact, *A. solani* was found at higher DNA amounts throughout the season at the location Straubing over the years 2005 to 2007 (data not shown). Even though both species

were found in almost all years, stable and higher DNA levels were expressed by real-time PCR analysis for *A. solani* rather than for *A. alternata*. Higher DNA values of *A. solani* can be explained by its higher pathogenicity and faster disease development (Rotem, 1994, Viskonti and Chelkowski, 1992).

The sensitive real-time PCR detection technique enabled to measure the degree of fungal development at different plant ages. It could be shown that older leaves from lower leaf sections were infected earlier than other leaves from higher leaf sections. With increasing leaf age successively higher located leaves were affected by EB, reflecting higher DNA levels. These confirm older results of Harrison and Venette (1970), who found that higher leaf levels were increasingly affected as the season progressed. These results demonstrate that the developed real-time PCR protocol is a sensitive and reproducible method for *in planta* quantification of *Alternaria* spp. during potato leaf colonization. The gradual upward progression of EB disease can be seen in the real-time PCR results.

Although disease pressure was very low and symptom appearance was very inconspicuous, real-time PCR has proven to be very effective for the quantification of *Alternaria* DNA levels at very early or even latent stages of infections. Early samplings from leaves of the middle and upper leaf sections confirmed *Alternaria* DNA, although no visual symptoms were found. PCR analysis enabled the screening of pathogen colonization already during the initial latent stage of infection, which would help to improve diagnosis.

CONCLUSIONS

Real-time PCR has proven as diagnostic assay to investigate the impact on species development and represents a more objective manner in the observation of fungal development. This technique can now be used in routine inspections to screen material for the presence of EB relevant pathogens and would thus help in the understanding of the dynamics of *Alternaria* spp. in potato field grown material. The use of this technique will be a useful tool in effective EB disease management as well as in plant pathogen interaction studies.

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The F129L mutation of the cytochrome b gene in German *A. solani* isolates and its impact on their sensitivity towards QoI fungicides

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SUMMARY

Early blight, caused by *Alternaria solani*, is one of the most widespread fungal diseases of potato (*Solanum tuberosum*). The most efficient fungicides for early blight control are quinone outside inhibitors (QoIs) like azoxystrobin, when applied as preventive treatments.

However, loss of sensitivity to QoIs has previously been reported for *A. solani* in the United States.

In Germany, we collected 203 *A. solani* isolates from 81 locations between the years 2005 and 2011 and screened them for the presence of the F129L mutation in the cytochrome *b* (*cytb*) gene. 74 isolates carried the F129L mutation. Sequence analysis revealed that isolates contained two different structures of the *cytb* gene – one having an intron after codon G 131 (genotype II), the other one lacking it (genotype I). 63% of our isolates were genotype I. The F129L mutation occurred in genotype II isolates only and there in the majority of isolates (97%).

Additionally we determined sensitivity to azoxystrobin and pyraclostrobin in conidial germination assays. Reduced sensitivity to azoxystrobin was observed for all isolates carrying the F129L mutation. The loss of sensitivity to pyraclostrobin was less pronounced. According to our results of the conidial germination assay, we pooled the isolates in sensitive and less sensitive isolate groups and inoculated potato eye cuttings with the different spore solutions. After treatment with azoxystrobin, plants inoculated with the less sensitive isolate pool developed significantly stronger early blight symptoms than plants sprayed with the sensitive isolates. Our results suggest an increasing amount of F129L isolates in the German population of *A. solani* between the years 2009 and 2011. The loss of fungicide efficacy may be caused by the application of QoIs and related to this the selection for the F129L mutation.

The data are published (1).

KEYWORDS

Alternaria solani, cytochrome *b*, fungicide resistance, QoI

PUBLICATION

- (1) J.H. Leiminger, B. Adolf, H. Hausladen, 2013. Occurrence of the F129L mutation in *Alternaria solani* populations in Germany in response to QoI application, and its effect on sensitivity. Plant Pathology Doi: 10.1111/ppa.12120.

Posters

Alternaria control, What method to decide the sprays?

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Alternaria control, What method to decide the sprays?

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Context: I am not any more in research or experimentations, but when I am with growers or technicians, as consultant, I have a lot of questions about the early blight : Is it necessary to control this one? In what fields? When it is useful to spray? Is there a method to decide?

Objective: To build a simple method to decide the sprays, particularly the first one

SOME DATA

Some epidemiological models and DSS are proposed: **Physiological Days** (P Days), **Growing Degree Day** (D Days) based on the growth of the potato with temperatures maxi and mini, **Tomcast** based on the epidemic(Severy values: mean day temperature and hours of leaf wetness), **early blight DSS of Dacom**. The research stations in **Belgium** are working on new models.

But, it seems that the results are different according to the area. Probably the physiology of the plant is more important for the early blight than for the late blight epidemic.

The disease threshold values as tool for effective control tested by J. Leiminger and H. Hausladen shows that the first treatment is around 50% of the disease incidence on the lower leaf level of the potato. It is probably difficult for a grower to observe this one.

The age and the stresses of the crop are predominant: The first symptoms of early blight seem to appear when the foliage is stabilised, around the beginning of the flowering time. C. Ducatillon in Belgium, and other technicians in France as Denis Jung, has shown that a low dose of nitrogen is favorable for Alternaria, the carences of magnesium, manganese, sulphur are also favorable. Other conditions are also favorable: bad soil structure, water deficiency.

There are big differences in the cultivar susceptibility: markies, amyla, gourmandine, daisy, maestro, sarpo mira, desibelle, marabel are very susceptible. Brice Dupuis (Libramont 2006) has given four classes of susceptibility.

The primary inoculum should act upon the first contaminations: Proximity of contaminated field the earlier year.

The decrease of using dithiocarbamate increases the development of early blight.

WHY NOT A SCALE OF RISK

Is it necessary to protect this field against early blight? (specific fungicides)

→ Proposition of a scale to evaluate risk of early blight attack:

Note of susceptibility of the cultivar	0: « not susceptible »	1 :light	2:medium	3: very susceptible
Soil type, water stock if no irrigation	0: correct	1:medium	2: light	
Soil structure or planting conditions	0: correct	1:bad	2: very bad	
Dose of N	0: correct or excess	1: low dose	2: deficiency	
Deficiencies (mg, mang, s)	0: no	1: some symptoms	2: high symptoms	
Phytotoxicity	0: no	1: some symptoms	2: high symptoms	
Dryness stress with early flowering if no irrigation	0: no	1: limited effect	2: high effect	
Near field contaminated the earlier year			2 : juxtaposed fields	
Number of dithiocarbamate (1500g/ha) before flower	0: 3 sprays	1: one spray	2: no dithiocarbamate	
Alternating periods of warm days (T>26°C) and rains at flowering time			2	

The rule could be: note: 0 to 6: no risk, 7 to 10: moderate risk >10: high risk

Date of the first specific spray:

• **Now:**

- a common advice (phytosanitary companies, advisers...) is the beginning of flowering time
- or symptoms at the lower level of the plant, or higher on the plant in the part of field with « bad soil »
- or first symptoms in the region

• **Very soon:** epidemiological modeles, and DSS adapted at each region (temperature, rain.....)

CONCLUSIONS AND CAUTION

This approach is a reflection, not a result of experimentation. Nevertheless it could be evaluated.

Effect of some pesticides on the *in vitro* oospore formation and mycelial growth of *Phytophthora infestans* (Mont.) de Bary

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SUMMARY

The effect of some pesticides, including fungicides (fludioxonil and difenoconazole), insecticides (thiamethoxam and imidacloprid), and herbicide (metribuzin), on the oospore formation and radial colony growth of *Phytophthora infestans* on oatmeal agar has been studied. Fludioxonil demonstrates a statistically significant inhibition of the radial colony growth; other pesticides had no any effect on this parameter. A statistically significant effect on the oospore formation has been shown for all pesticides tested, excepting thiamethoxam. The maximum inhibiting effect has been shown for fludioxonil and difenoconazole; however, low concentrations of difenoconazole (1 µg/ml) stimulate the oospore formation.

KEYWORDS

Phytophthora infestans, oospore formation, potato late blight, oomycete, fungicide resistance

INTRODUCTION

Late blight of potato, caused by the oomycete *Phytophthora infestans* (Mont) de Bary, is a common disease for almost all potato-growing regions of Europe. In the case of severe infection, yield losses can reach 30% or even more (Ivanyuk *et al.*, 2005).

P. infestans is able to form thick-walled sexual structures called oospores. Though single isolates are also able to form single oospores in infected tissues, the most intensive oospore formation occurs in the case of a contact between strains of different mating types. The formation of oospores in different tissues of potato and tomato plants was observed under field conditions in many countries, such as Russia (Smirnov and Elansky, 1999), Norway (Hermansen *et al.*, 2002), Sweden (Strömberg *et al.*, 2001), Netherlands (Kessel *et al.*, 2002), etc. Oospores, together with infected seed material and overwintered tubers, represent one of the main sources of primary inoculum. Oospores can survive in soil for several years and are able to infect plants during the whole of this period. The most intensive plant infection occurs within two seasons

after the harvesting of potato or even later. In Norway oospores survived in the soil for 31 months (Bødker *et al.*, 2006). In the Moscow region of Russia and in Finland, oospores overwintered and still kept the ability to infect plants (Bagirova *et al.*, 1998, Ulanova *et al.*, 2010, Lehtinen, 2002). Oospores are also able to survive in tomato fruits for a long time (Rubin *et al.*, 2001). Hybrid oospores, resulted from different crosses, improve the genetic diversity of a pathogen population and, therefore, accelerate the process of the pathogen adaptation to new potato cultivars and fungicides.

The basic method to control the late blight disease is a chemical protection of crops via the treatment of fields with fungicides. It is known that sub lethal concentrations of fungicides, used to control late blight, cause the *in vivo* and *in vitro* reduction of the intensity of the oospore formation and the viability of oospores (Kessel *et al.*, 2002). In our study we investigated the effect of pesticides, which are not registered for the use against late blight, on the oospore formation and mycelial growth of *P. infestans*.

MATERIALS AND METHODS

In this study the effect of some fungicides (Maxim and Skor), insecticides (Aktara and Tanrek), and herbicides (Zenkor) on the radial mycelium growth and oospore formation was examined (Table 1).

Table 1. List of pesticides/active ingredients used in the study

Pesticide	Active ingredient	Pesticide type
Skor	difenoconazole	Fungicide
Maxim	fludioxonil	Fungicide
Aktara	thiamethoxam	Insecticide
Tanrek	imidacloprid	Insecticide
Zenkor	metribuzin	Herbicide

In our studies we used *P. infestans* isolates, collected from infected potato leaves in the Moscow (4 isolates, 2012), Ryazan (5 isolates, 2012), and Leningrad (1 isolate, 2008) regions, and one isolate collected from a tomato fruit in 2007 in the Mariy El Republic (Table 2).

Table 2. List of *P. infestans* isolates used in the study

Isolate name	Mating type	Host plant, region of origin, and information about the use of pesticides on this field before the sample collection
08LK 3	A1	Potato leaves, Leningrad region, first late blight foci, no any pesticide treatments.
07YTP40/1	A1	Tomato fruits, Mariy El Republic, Yoshkar-Ola, private plot, no any pesticide treatments.
12MGRK 19	A1	Potato leaves (cv. Sante), Moscow region, Odintsovo district, experimental field
12MGRK 25	A2	of the All-Russian Research Institute of Phytopathology, no any pesticide treatments.
12MGVK 15	A2	
12MGVK 55	A2	
12RKLS8	A1	Potato leaves (cv. Skarb), Ryazan region, commercial potato fields; the last fungicide treatment was applied 30 days before the sampling.
12RKLS15	A1	
12RKLS20	A1	
12RKLS47	A1	
12RKLYa15	A2	Potato leaves (cv. Yanka), Ryazan region, commercial potato fields; the last fungicide treatment was applied 30 days before the sampling.

The mating tests at different concentrations of fludioxonil and metribuzin were carried out using two pairs of isolates for the crossing; in the case of difenoconazole (18°C), thiamethoxam, and imidacloprid, such assessment was carried out using four pairs of isolates; finally, in the case of the same experiment with difenoconazole (25°C), six pairs of isolates were used (Table 3).

The assessment of the effect of the pesticide concentration on the radial colony growth was carried out using five *P. infestans* isolates. An agar block with the mycelium of each isolate was placed in the center of a Petri plate with oatmeal agar. All tests were made in three replications. The concentrations of active ingredients were the following: 0.1, 1, and 10 µg/ml for thiamethoxam and 1, 10, and 100 µg/ml for other substances. A radial colony growth was measured after 12-15 days of incubation, when the colony diameter in the control (pesticide-free) variant reached about 80% of the Petri plate diameter. For each variant, the results of measurements, obtained for all replications of the same concentration, were averaged.

Table 3. Isolate pairs used for crossings

Active ingredient used in the crossing experiments	Crossed pairs of isolates	
fludioxonil, metribuzin	12MGRK25 × 1MGRK19	12MGRK25 × 07YTP40/1
difenoconazole (18°C), imidacloprid, thiamethoxam	12MGRK25 × 12RKLS8 12MGRK25 × 12RKLS47	08LK3 × 12RKLYA15 08LK3 × 12MGVK15
difenoconazole (25°C)	12 RKLYA15 × 08LK3 12MGVK55 × 08LK3 12MGVK15 × 08LK3	12RKLS20 × 12MGRK25 12 RKLS15 × 12MGRK25 12RKLS47 × 1MGRK25

To study the effect of pesticides on the oospore formation, isolates of different mating types (see Table 3) were placed by pairs into Petri plates with oatmeal agar medium at a distance of 5 cm from each other. The medium was preliminary supplemented with the examined pesticides at the following concentrations: 0.1, 1, 10, and 100 µg/ml (difenoconazole); 0.1, 1, and 10 µg/ml (thiamethoxam); 1, 10, and 100 µg/ml (imidacloprid, fludioxonil, and metribuzin). Pesticide-free medium was used as the control variant. Each pair of isolates was planted in three repetitions (3 Petri plates) per each concentration variant, including the control. After the inoculation, Petri dishes were sealed with Parafilm. After 20-22 days of incubation at 18°C, the whole volume of medium from each Petri plate was re-suspended in 30 ml of distilled water. Three 30-µl samples were taken from each variant, placed onto an object-plate, covered with a cover glass, and then microscopied to detect oospores. For each Petri plate, 60 microscopic fields were examined; then the number of oospores per a microscopic field was recalculated to obtain their concentration per 1 mm³ of medium. The results of measurements were averaged for each pesticide concentration.

RESULTS

Effect of the pesticide concentration in agar medium on the radial colony growth rate

The obtained results showed that difenoconazole, thiamethoxam, and imidacloprid did not have any statistically significant effect on the radial colony growth of *P. infestans* (Table 4). Metribuzin caused a minor delay in the colony growth during the first 5-7 days; however, the colony diameter became close to the control value to the 10th day of incubation. Fludioxonil provided a statistically significant inhibition of the colony growth at the concentrations exceeding 10 µg/ml.

Table 4. *Effect of pesticide concentration in agar medium on the radial colony growth rate of P. infestans*

Active ingredient (AI)	Radial colony growth (mm) at different AI concentrations				
	Control (0 µg/ml)	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml
difenoconazole	82±7* (100%)	–**	76±9 (93%)	84±4 (102%)	81±6 (99%)
fludioxonil	82±6 (100%)	–	74±12 (90%)	56±10 (68%)	46±3 (56%)
thiamethoxam	82±6 (100%)	81±7 (99%)	82±6 (100%)	81±6 (99%)	–
imidacloprid	79±6 (100%)	–	76±9 (96%)	77±8 (97%)	76±5 (96%)
metribuzin	88±12 (100%)	–	85±12 (97%)	86±9 (98%)	80±5 (91%)

* A confidence interval for the significance level 0.05 is indicated after the ± symbol.

** The variant was not tested.

Effect of the pesticide concentration in agar medium on the oospore formation

A statistically significant inhibition of the oospore formation was observed for all pesticides tested (Table 5). A weak effect was observed only in the case of thiamethoxam. A strong inhibition effect was observed in the case of imidacloprid, fludioxonil, and difenoconazole; in the

last case, the effect was higher at 25°C, i.e., at the temperature uncomfortable for *P. infestans*. A significant inhibition of the oospore formation was observed in the presence of imidacloprid, though it did not inhibit the radial colony growth even at a high concentration (100 µg/ml).

Table 5. Effect of the pesticide concentration in agar medium on the oospore formation in *P. infestans*

Active ingredient (AI)	Number of oospores per a mm ³ at different AI concentrations				
	Control 0 µg/ml	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml
thiamethoxam	79.6 ± 3.6* (100%)	79.8 ± 3.8 (100%)	79.1 ± 3.9 (100%)	71.4 ± 3.7 (90%)	–
imidacloprid	79.6 ± 3.6* (100%)	–**	70.0 ± 3.4 (88%)	66.0 ± 3.1 (83%)	35.8 ± 2.8 (45%)
fludioxonil	112.7 ± 6.9 (100%)	–	98.4 ± 8.6 (87%)	73.6 ± 5.4 (65%)	42.3 ± 3.7 (36%)
metribuzin	135.0 ± 9.5 (100%)	–	103.0 ± 9.8 (76%)	118.2 ± 9.3 (88%)	74.8 ± 8.1 (55%)
difenoconazole (18°C)	79.6 ± 3.6 (100%)	72.5 ± 3.6 (91%)	82.2 ± 3.7 (103%)	54.9 ± 2.8 (69%)	35.8 ± 2.3 (45%)
difenoconazole (25°C)	29.7 ± 2.3 (100%)	14.1 ± 1.4 (47%)	22.6 ± 1.8 (76%)	12.0 ± 1.3 (40%)	10.8 ± 1.3 (36%)

* A confidence interval for the significance level 0.05 is indicated after the ± symbol.

** The variant was not tested.

DISCUSSION

The study of the effect of pesticides, which were not registered for the use against the late blight, on the radial colony growth of *P. infestans* showed the expected absence of any statistically significant growth inhibition. However, it was found that fludioxonil inhibits the mycelial growth of *P. infestans* at concentrations exceeding 10 µg/ml. An allowed fludioxonil concentration in a working solution is 10 (or even more) times higher than the levels tested (see Table 6) and is able to significantly influence on the *P. infestans* development in infected seed tubers, killing zoospores and zoosporangia on their surface. Probably this fact can explain a delay in the late blight development, observed in the case of the planting of seed tubers treated with the Maxim (AI - fludioxonil) preparation (A.V. Filippov, personal communication; Anisimov *et al.*, 2009).

As we have already mentioned earlier, all tested pesticides inhibited the oospore formation process. The tested pesticide concentrations were lower or, in the case of imidacloprid and thiamethoxam, about equal to concentrations allowed for the use in a working solution (Table 6). In our experiments, the level of inhibition of the oospore formation increased as the dosage of a preparation increased; therefore, one could suppose an increased effect in the case of a contact of the pathogen with a more concentrated working solution. A weak effect observed for thiamethoxam is possibly explained by a low value of the maximum experimental concentration (10 µg/ml) comparing to those of other preparations (100 µg/ml).

The inhibition of the oospore formation was earlier shown for fungicides used for late blight control. Kessel *et al.* (2002) studied more than 10 commercial fungicides recommended for late blight control. All these preparations taken in sub lethal concentrations inhibited the oospore formation to a greater or lesser extent; this effect was observed on both nutrient medium and potato leaves. Hanson and Shattock (1998) demonstrated an inhibiting effect of metalaxyl on the oospore formation by pairs of isolates, in which one or both parental isolates were sensitive to this fungicide; a decreased oospore formation was also observed for the crossing of two resistant isolates. Taking into account our results and results obtained by other researchers, we can conclude that almost all pesticides applied on potato are able to inhibit the oospore formation to a greater or lesser extent. A weak effect was revealed in the case of such fungicides as mancozeb and chlorothalonil (Kessel *et al.*, 2002) and the insecticide thiamethoxam (our studies).

Table 6. Concentrations of active ingredients used in the study and the recommended concentrations of the same ingredients in their working solutions

Active ingredient (AI)	AI concentration used in the study, µg/ml	Recommended AI concentration in the working solution*, µg/ml
difenoconazole	0.1, 1, 10, 100	188-625
fludioxonil	1, 10, 100	1000
thiamethoxam	0.1, 1, 10	37-75
imidacloprid	1, 10, 100	50-100
metribuzin	1, 10, 100	1630-4900

* According to the State Catalogue of pesticides and agrochemicals permitted for the use on the territory of Russian Federation.

In our experiments, difenoconazole, taken at the concentration equal to 1 µg/ml, was able to stimulate the oospore formation under optimal growth conditions (18°C) that was observed for all pairs of the isolates tested. The stimulating effect of low fungicide concentrations on the oospore formation was also described by Groves and Ristaino (2000), who studied the process of the oospore formation in a monoculture. Authors supposed that fungicide preparations are able to imitate the action of hormones and to influence on the mating type system. Probably the same reason explains the stimulating effect of difenoconazole on the oospore formation revealed in our study.

Difenoconazole represents a systemic pesticide able to penetrate into a plant. The difenoconazole concentration in plant tissues is lower than on the surface of a plant; it can be below the inhibiting level and close to the level optimal for the stimulation of the oospore formation. Therefore, in the case of a severe late blight infection, the treatment of potato plants with difenoconazole, especially at the minimum allowed dosage, is able to stimulate the oospore formation rather than to inhibit this process.

The obtained results show that pesticides applied on potato plants are able to inhibit the oospore formation even in the case when they do not possess any direct inhibiting effect on the pathogen growth. This improves a general phytosanitary situation on the fields and prevents the appearance of highly aggressive and fungicide resistant *P. infestans* strains caused by the hybridization process.

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Mileos® - the French Potato Late Blight DSS: continuous improvement over the past decade!

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Mileos® is a **web-based, on-farm** DSS available to potato growers to control potato late blight (LB), in France. It results from a collaboration between ARVALIS and the Ministry of Agriculture (SRAL Nord-Pas de Calais). The 2 pre-existing DSS's (MILPV and Mili-LIS) have been fused, in 2009, into an optimized tool, entirely reviewed and up-dated in order to better meet national demand and help farmers to comply with EU regulations. With Mileos® (see www.mileos.fr), the fungicide application on potato crops is optimized, triggered according a real-time LB risk assessment taking into account environmental data (climatic and disease pressure), agronomical data such as cultivar's LB resistance and crop health practices for the potato field as chemical input and irrigation.

Mileos® : model description & action thresholds

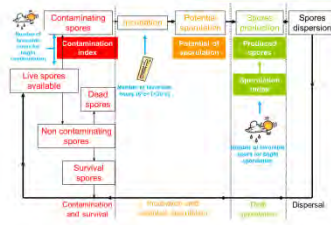


Figure 1: The Mileos® core unit is composed of 4 chronological modules based on the epidemiological cycle of *Phytophthora infestans* (inspired by Muckensturm, 1989)

Mileos® -**new version**- is an amalgamated model initially based on the epidemiological model Guntz-Divoux then implemented for hourly quantitative assessment of contamination index through the model Milsol by calculating the number of live spores available on the crop foliage. More recently, the fused model was revised and set up as **4 different compartments** (Figure 1) strictly simulating the different steps of a LB epidemiological cycle, from contamination to dispersal as well as the overlapping successive cycles. Required input variables are hourly temperature, relative humidity and rainfall. Additional environmental data (crop growth rate, cultivar, disease pressure, irrigation) are daily updated & contribute to the set up of action thresholds: treat or not, and what to treat with (Figure 2).



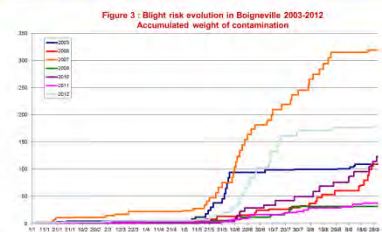
Figure 2: Screen-shot of Mileos® output with the different parameters: sporulation (potential and index), number of available spores

The most significant improvements ...

- ✓ for the core models
- Comparative analysis of computed VS observed data for a continuous adjustment that takes into account :
 - Genetic evolution (virulence & chem-resistance) of LB populations (Corbière, pers comm)
 - Better quantification of primary inoculum sources (in progress ; Toky *et al*, 2012)
 - Effect of low temperatures (6-8°C) on the germination of the spores taking into account
 - Incubation length better calibrated according to temperature,
 - More accurate value for "produced spores" for successive LB cycles
 - Integration of cultivar resistance to LB. (updated European Catalog)
 - Integration of weather forecast : + 3 days

- ✓ for the circulation of Information
- Thorough Redesign of the IT for an optimised transfer of the Information :
 - 4 servers: 2 for computing, 1 for the database and 1 as web-server
 - Met stations: most potato producing areas have a dense network of met stations, possibility of sharing and exchanging data
 - Easy down loading of output data as Excel spread sheet (eg. summary of all treatments, met data)
 - LB Risk Alert transmitted on new IT equipments: mobile, smart-phone etc

Mileos® : a tool to follow up LB epidemics



For a better understanding of environmental impact on LB epidemics, Mileos® is a very useful tool for comparing data (x=cumulated f(Log nb of spores) over years and per site (Figure 3). The same data analysis can be performed to characterize LB epidemics in different sites in a given year.

Mileos® : a tool for the National Crop Surveillance - BSV

For this purpose, a **simplified version** of Mileos® is used as a weekly LB risk analysis giving LB alerts at the level of a region. No treatment recommendation is given. The criteria of the number of potential LB generations according local met data allows the comparison of disease progression between regions.



Some Figures for 2012

- In 2012, the total amount of connections ...
 - for extension and technical teams, was ... **21**
 - for individual potato growers, ... **410**
 - **1380** different plots (field x cultivar x met station) were supervised, equivalent to ... **20.000 ha**, and representing all types of potato crops: earlies, fresh/ware, processing, starch and seeds; in most of the **potato growing Regions**: Nord-Pas de Calais, Picardy, Brittany, Beauce, Alsace and Champagne.
- The tool has also been recently experimented in Tunisia and Canada.

Mileos® is a sustainable tool for a sustainable control of potato LB. It has demonstrated its robustness and, as a DSS, is constantly tested, updated and adjusted according to the evolution of the biological environment as LB population genetic evolution (sexual reproduction, virulence or resistance to chemicals) or erosion of cultivar behavior to LB as well as according cultural practices or climate change. For these reasons, this device is in full agreement with the EU-Directive 2009/128/EC and fits in the National-French Ecophyto Plan Axe 2.

Evaluation of foliar resistance to *Phytophthora infestans* in potato varieties in Estonia

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EVALUATION OF FOLIAR RESISTANCE TO *PHYTOPHTHORA INFESTANS* IN POTATO VARIETIES GROWN IN ESTONIA

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Variety	Estimated scale values		
	2010	2011	Mean and SD
Arielle	2,1	2,6	2,3 ± 0,3
Ambition	4,6	4,2	4,4 ± 0,2
Evolution	3,6	3,7	3,6 ± 0,1
Fontane	5,1	3,2	4,2 ± 0,9
JP02-0053	4,8	3,9	4,3 ± 0,5
Madeleine	2,8	3,3	3,0 ± 0,3
Manitou	5,1	3,6	4,3 ± 0,7
Monaco	2,3	4,5	3,4 ± 1,1
OS01-1001	4,1	3,6	3,9 ± 0,2
Red Baron	5,1	4	4,5 ± 0,5
Rosagold	4,1	3,9	4,0 ± 0,1
Toluca	4,6	7,2	5,9 ± 1,3

Table 1. Estimated scale values and mean RAUDPC of potato cultivars evaluated for foliar resistance to *Phytophthora infestans* in the field in Estonia, 2010-2011

MATERIALS AND METHODS:

- Field trials were carried out at Einola Farm, Reola Tartumaa county, Estonia.
- In 2010 and 2011, ten potato varieties and two breeding lines of the Agricola breeding company were cultivated.
- In 2010, whole seed tubers were planted on 8 May, and in 2011, on 20 May.
- Potatoes were harvested on 10 September 2010 and on 9 September 2011.
- Trials were laid out according to a randomized block design with four replications.
- No fungicides, herbicides or insecticides were used in either year.
- Foliar disease was evaluated as a percentage of total foliage twice each week, every three or four days.
- Disease assessments were made after inoculation from 25 July to 30 August in 2010 (11 observations), and from 1 August to 8 September in 2011 (12 observations).
- Late blight infection was assessed according to the 0-100 % scale.
- Estimated scale values for varieties tested in both years were compared with scale values for the same cultivars from the European Cultivated Potato Database and breeding company Agricola website, where the scale for resistance against foliar blight is defined as: 1 (very low); 2 (very low to low); 3 (low); 4 (low to medium); 5 (medium); 6 (medium to high); 7 (high); 8 (high to very high); 9 (very high).



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson

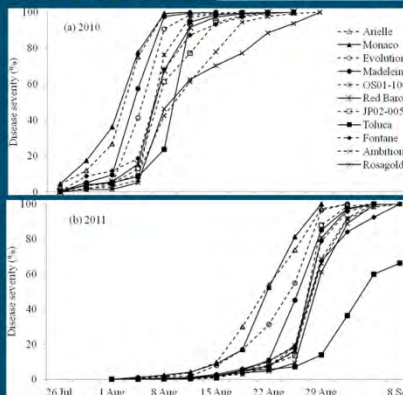


Figure 1. Development of foliar blight in tested potato varieties in 2010 (a) and 2011 (b).

INTRODUCTION:

- Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is one of the most important diseases affecting potato production worldwide.
- In northern areas, under favourable cool (15-20°C) and moist (leaves wet for at least three hours) conditions, the pathogen can cause considerable yield loss.
- Data from Finland and Estonia show that the first findings of blight now occur one month earlier than 20 years ago and blight infections are more severe.
- Potato growers are interested in growing new Western European potato varieties with good tuber quality, yield and earliness. Information about resistance to late blight in variety descriptions is not always appropriate for local conditions.
- The breeding company Agricola has been working on combining different blight resistance levels for a durable potato future and 'Toluca' is the best variety they have at present.

MAIN AIM OF THE RESEARCH:

- Evaluate foliar resistance of the Dutch breeding company Agricola potato varieties to find those most resistant to late blight and thereby diminish the number of fungicide sprays required while giving high yields in North-Eastern European conditions.
- Of special interest was the variety Toluca which is immune to foliar late blight and especially bred for the organic farming system.
- Based on trial information, more resistant and suitable potato varieties could be recommended directly to potato growers.



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson

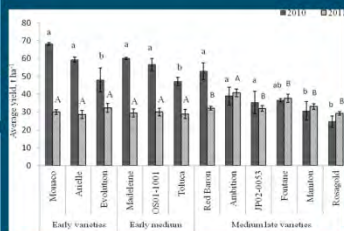


Figure 2. Average tuber yield (± SE) of potato varieties in 2010 and 2011. Different letters upon boxes indicate statistically significant differences (ANOVA, Tukey HSD test $\alpha < 0.05$)

RESULTS:

- In 2010, the first late blight symptoms were recorded on 26 July, when most of the potato varieties in the trial were infected, except the early medium breeding line OS01-1001 and the medium late variety Rosagold (Figure 1a).
- Late blight destroyed most of the foliage by 27 August and the growing period was short.
- In 2010, based on crop yields, we recommend the early varieties Monaco and Arielle, the early medium variety Madeleine and breeding line OS01-100 and the medium late variety Red Baron.

RESULTS:

- In 2011, the first late blight symptoms were found on the early variety Monaco on 1 August (Figure 1b). Most varieties were infected by 5 August, except medium late variety Manitou and the breeding line JP02-053.
- However, infection rate remained very low until mid-August with more intensive development after heavy rainfall on 12 and 13 August.
- With cooler and wetter weather conditions after 22 August, blight developed quite rapidly and most of foliage on early medium varieties, except Toluca, was destroyed by 2 September (Figure 1b).
- Toluca differed significantly from all other varieties with slower blight development, although infected at the same time as other early and early medium varieties its disease progressive curve area remained smaller (Table I).

DISCUSSION & CONCLUSION:

- Based on two years observation data we can conclude that most of the Agricola potato varieties we tested in our trials were susceptible or very susceptible to late blight.
- The only exception was the variety Toluca. In both years, this variety had the slowest late blight development at the beginning of the disease outbreak.
- According to this practical output, we can suggest from our trial results that potato growers shorten the spraying interval on the early variety Arielle and the early medium variety Madeleine.
- Other varieties were susceptible to late blight and should be controlled for late blight as recommended by pesticide producers (Table I).
- The early medium variety Toluca gave contradictory results in the two years being susceptible in 2010 (4.6 points) and quite resistant in 2011 (7.2 points).
- Crop yield in 2011 was on average 1/3 lower than in 2010 mainly because of late planting of tubers considering the conditions of that particular growing season (Figure 2).
- Further experiments are needed to find potato varieties more resistant to late blight in order to minimise the need for fungicide spraying.

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Late blight control in the specific conditions of Bârsa Land, Romania

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Introduction:

Integrated late blight control supposed to use cultural, biological and chemical measures. The aim is to use techniques at the right time without completely destroy of the disease, ensuring control methods to protect the environment and useful organisms. Therefore, between 2010-2011, two potato fields were setup with the purpose to evaluate and compare the efficacy of different fungicides used in Romania for potato late blight control.

Agronomic measures:

- Phyto - sanitary care represents a complex with prophylactic character to avoid the infection and to prevent the spread of the disease on an epidemic level.
- Eliminated tubers from sorting process represent a dangerous source of infection because the fungus produces spores and infected plants appears just when potato plants are raising up.
- Cultural methods are looking to provide normal and vigorous development of potato crops.
- Attentive selection of tubers, sprouting, planting in optimal time, correct fertilization, maintenance in time provided a normal development of plants and make shorter the favorable periods for disease attack.
- Unbalance fertilization, especially with nitrogen determine huge foliage, proper for late blight attack.
- Maintenance and protection against other diseases and Colorado potato beetle are important because stressed plants are sensitive to late blight.
- Weeds are in permanent concurrence with potato for all environmental factors.
- Their excessive foliage development reduces air currents making humid periods longer and increases the chance for late blight appearances.
- Vine killing in the case of diseased potato crops is a measure to reduce the frequency of tuber attack.
- To stop the late blight cycle on potato crops are recommended chemical products for haulm destruction.

Field trial methods

Year	2010	2011
Variety	Sante	Sante
Sowing date	29 April	13 May
Experimental design	Complete randomized block design (4 replicates)	
Plot size (sqm)	25	25
Spray equipment	Knapsack sprayer	
Harvest date	14 September	22 September

Field trials results

2010

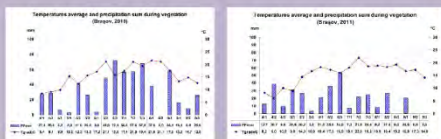
Plot	Foliar late blight (%)								Yield (t/ha)	Tuber blight (%)
	Start June 17	Treat June 28	Start July 8	Treat July 18	Treat July 28	Start Aug 8	Treat Aug 18	Start Aug 18		
Untreated	10.0 a	10.0 a	28.0 a	33.0 a	50.0 a	64.0 a	87.0 a	22.7 a	5.1 a	
No. 1	2.0 b	2.0 b	3.0 b	3.0 b	5.0 b	8.0 b	9.0 b	36.0 b	1.0 b	
No. 2	2.0 b	2.0 b	3.0 b	3.0 b	5.0 b	8.0 b	9.0 b	37.0 b	1.0 b	

2011

Plot	Foliar late blight (%)								Yield (t/ha)	Tuber blight (%)
	Start June 24	Treat July 4	Start July 15	Treat July 25	Treat Aug 5	Start Aug 15	Treat Aug 25	Start Aug 25		
Untreated	1.0 a	2.0 a	10.0 a	20.0 a	50.0 a	60.0 a	80.0 a	21.0 a	2.0 a	
No. 1	0.0 b	0.0 b	2.0 b	3.0 b	5.0 b	8.0 b	10.0 b	35.0 b	0.0 b	
No. 2	0.0 b	0.0 b	2.0 b	3.0 b	5.0 b	8.0 b	10.0 b	36.0 b	0.0 b	

Climatic conditions

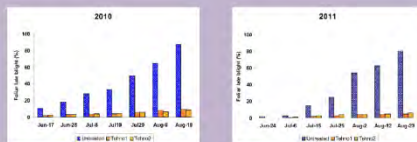
2010 - Epidemic phase of the disease was devastating and quickly installed (late blight apparition July 1st).
 On July and August heavy rains and high temperatures contributed to foliage dying.
 2011 - On June, rains accompanied by high temperatures led to an early late blight appearance. (June, 21th).
 From July to the end of potato growing season rainfall were increasingly reduced, (53.5mm, represented only 30.2% from the multiannual average).



- There are no significant differences between the two treated plots in any assessments.
- On both years, at the end of the growing season, treatments with all fungicides gave significantly (P<0.05) lower level of foliage blight compared with control (untreated) plot.
- The untreated plot (control) presented a high late blight attack.
- On the treated plots the disease evolving slowly due to applied fungicides.
- From the middle of July late blight was in sporulation phase, a difference between treated and untreated plots was noticed, with a higher frequency of attack in untreated plot.
- According to the experimental results the level of late blight on foliage was significantly higher in untreated plot.
- Thus, after two years of experiments, we observed that climatic conditions have a powerful influence on Phytophthora infestans attack on potato crops.
- To control this devastating disease it is necessary an efficient treatments program.

Fungicide spraying program

Plot	Spray 1	Spray 2	Spray 3	Spray 4	Spray 5	Spray 6	Spray 7
1	Untreated (control)						
2	Product: Odoxon 320 WG Dose: 1.0 kg Date: 15.06	Product: Elanthe 75 WG Dose: 2.5 kg Date: 25.06	Product: Curzate M 45 Dose: 2.5 kg Date: 05.07	Product: Armet 45 Dose: 2.5 kg Date: 15.07	Product: Diltane M 45 Dose: 2.5 kg Date: 25.07	Product: Odoxon 320 WG Dose: 1.0 kg Date: 05.08	Product: Baneq 500 SC Dose: 0.4 l Date: 15.08
3	Product: Armet M Dose: 2.5 kg Date: 15.06	Product: Fozoran 30 WDS Dose: 2.0 kg Date: 25.06	Product: Infante 80 S Dose: 1.4 l Date: 05.07	Product: Odoxon 320 WG Dose: 1.0 kg Date: 15.07	Product: Comment 450 Sc Dose: 2.0 l Date: 25.07	Product: Baneq 500 SC Dose: 0.3 l Date: 05.08	Product: Baneq 300 SC Dose: 0.4 l Date: 15.08



CONCLUSIONS:

- Integrated control of late blight is a managerial technique combination to maintain the disease on a low level and in the same time to protect the environment. Integrated control directories are:
- Cultural hygiene to limited the primary infection focus.
 - Using resistant varieties to limit late blight attack on foliage and tubers.
 - Fungicides apply using forecasting methods (Agroexpert system) and further treatments until harvest at recommended intervals.
 - Reduce application intervals (4 maximum 7 days) when the infection pressure is high

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Pyramiding *R* genes: genomic and genetic profiles of late-blight resistant interspecific potato hybrids

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SUMMARY

Clones of potato hybrids comprising genetic material from up to seven wild *Solanum* species were studied with phytopathological methods and DNA markers for *Solanum* genomes A, B and D and for late blight resistance genes *R1*, *R2/Rpi-blb3*, *R3a*, *R3b* and *RB/Rpi-blb1*. Late blight resistance of these clones was obviously associated with the presence of DNA markers for the *R* genes and significantly related to the number of these markers discerned in particular interspecific hybrids.

KEYWORDS

Phytophthora infestans, wild *Solanum* species, potato hybrids, late blight resistance, stacking genes, DNA markers.

INTRODUCTION

Stacking (pyramiding) several resistance genes of diverse race specificity by hybridization or genetic transformation is presently seen as an upcoming venue to broad-spectrum and durable late blight (LB) resistance (Tan *et al.*, 2010; Kim *et al.*, 2012; Zhu *et al.*, 2012). Here we describe two sets of interspecific potato hybrids, which comprise germplasms from several *Solanum* species per genotype, manifest high LB resistance and therefore are promising sources for breeding new potato cultivars with wide-range LB resistance (Rogozina *et al.*, 2013). SCAR markers for *Solanum* genomes A, B and D and for five LB resistance genes were used to screen these hybrids. We found that the profiles of genome markers were in good agreement with the reported pedigrees and breeding histories of the hybrids. In parallel, we demonstrated that the patterns of *R* genes in the hybrids were in most cases explained by the evidence on the *R* genes in wild diploid and polyploid *Solanum* species that were reportedly involved in breeding these

hybrids. The data from collated molecular and phytopathological studies infer that stacking *R* genes in interspecific hybrids significantly fortifies their LB resistance.

MATERIALS AND METHODS

Two sets of interspecies hybrids (Table 1) comprising germplasms from two to ten *Solanum* species were developed in the Institute of Potato Husbandry, Korenevo, Moscow (IPH) and the Institute of Plant Protection, Pushkin. St. Petersburg (IPP). Most hybrids displayed high foliage LB resistance in the field trials under natural infection conditions and high-to-moderate resistance in the laboratory assays with detached leaves infected with highly virulent complex race isolates of *Phytophthora infestans*. As far as it was possible, our study also included wild *Solanum* species reportedly involved in breeding these hybrids (Table 2).

Standard protocols were employed to extract DNA from young leaves and PCR-amplify genome fragments. Hybrids were screened with SCAR markers for *Solanum* genomes A, B and D developed from low-copy *LEAFY* (Drobyazina and Khavkin, 2012) and COSII genes (Wu *et al.*, 2006). In the latter case, SCAR markers were designed using the sequences from several *Solanum* species cloned by the authors with the previously described primers (Wu *et al.*, 2006; Rodriguez *et al.*, 2009). The profiles of CC-NBS-LRR genes for LB resistance (*R* genes) were assessed with SCAR markers specific for the genes *R1*, *R2/Rpi-blb3*, *R3a*, *R3b*, and *RB/Rpi-blb1* (Sokolova *et al.*, in press).

The data were processed using the Statistica 6 package (StatSoft, <http://www.statsoft.com/>).

RESULTS AND DISCUSSION

All interspecific potato hybrids under study comprise markers for genomes A and D derived from *S. tuberosum* and *S. demissum*, and some hybrids contain the markers for genome B apparently transferred with the germplasm of *S. polytrichon* = *S. stoloniferum*. The hybrids comprising genome B markers contain the marker for *Rpi-blb1*, the gene found only in *S. bulbocastanum* and *S. stoloniferum* (Fadina *et al.*, in press). There were two exceptions: hybrids IPP10 and IPP12 reportedly comprising the AB germplasm from *S. stoloniferum* and *S. vallis-mexisi*; here we find the *Rpi-blb1* marker in the absence of the genome B marker (Table 1). The evidence on genomes and *R* genes in the hybrids agrees fairly well with the profiles previously established in wild *Solanum* species (Table 2), thus supporting the pedigrees of the hybrids.

Table 1. Genome and R-gene patterns in interspecies hybrids bred in the Institute of Potato Husbandry (IPH) and the Institute of Plant Protection (IPP)

Hybrids	Hybrid pedigrees (<i>Solanum</i> species) *	<i>Solanum</i> genomes recognized with SCAR markers	R genes for LB resistance established with SCAR markers	LB resistance***
IPH1	<i>chc, cmm, dms, mga, tbr</i>	A1-A3, D	<i>R3a, R3b</i>	MS/4
IPH2	<i>adg, chc, dms, sem, tbr</i>	A1-A3, D	<i>R3b</i>	S/3
IPH3	<i>chc, cmm, dms, mga, tbr</i>	A1-A3, D	<i>R1, R3a, R3b</i>	MR/7
IPH4	<i>dms, tbr</i>	A, D	<i>R1, R2/Rpi-blb3, R3a, R3b</i>	MS/4
IPH5	<i>adg, chc, dms, sto, tbr</i>	A1, A3, D	<i>R1, R2/Rpi-blb3, R3a, R3b</i>	MR/6
IPH6	<i>adg, chc, dms, tbr</i>	A1, A3, D	<i>R2/Rpi-blb3, R3a, R3b</i>	MS/5
IPH7	<i>adg, chc, cmm, dms, edn, mga, ryb=phu, tbr</i>	A, A1, A3, D	<i>R2/Rpi-blb3, R3a, R3b</i>	MR/6
IPH8	<i>dms, tbr</i>	A, A1, A3, D	<i>R2/Rpi-blb3, R3b</i>	MS/5
IPH9	<i>dms, tbr</i>	A, A1, A3, D	<i>R1, R2/Rpi-blb3, R3a, R3b</i>	R/8
IPP10	<i>adg, dms, mcd, plt=sto, tbr, vlm</i>	A1-A3, D	<i>R2/Rpi-blb3, R3a, R3b, RB/Rpi-blb1</i>	MS/4
IPP11	<i>adg, dms, mcd, plt=sto, tbr, vlm</i>	A1-A3, B, D	<i>R2/Rpi-blb3, R3a, RB/Rpi-blb1</i>	S/3
IPP12	<i>adg, ber, dms, mcd, plt = sto, pnt, tbr, vlm</i>	A, A1, A3, D	<i>R2/Rpi-blb3, R3a, RB/Rpi-blb1</i>	MR/6
IPP13	<i>adg, dms, pnt, tbr</i>	A, A1, A3, D	<i>R2/Rpi-blb3, R3a</i>	MS/5
IPP14	<i>adg, ber, dms, mcd, plt=sto, pnt, tbr, vlm</i>	A1-A3, B, D	<i>R2/Rpi-blb3, R3a</i>	MS/4
IPP15	<i>adg, ber, dms, mcd, phu, plt=sto, pnt, tbr, vlm, vrn</i>	A1-A3, B, D	<i>R2/Rpi-blb3, R3a, RB/Rpi-blb1</i>	MR/6.5
IPP16	<i>adg, ber, dms, mcd, plt=sto, tbr</i>	A1-A3, D	<i>R1, R2/Rpi-blb3, R3a, RB/Rpi- blb1</i>	MR/7
IPP18	<i>dms, mcd, pnt, tbr</i>	A1, A2, D	<i>R1, R2/Rpi-blb3, R3a</i>	MS/4
The EUCABLIGHT standard cultivars				
Alpha	<i>tbr</i>	A	none	S/3
Bintje	<i>tbr</i>	A	none	S/3
Eersteling	<i>tbr</i>	A	none	S/3
Robijn	<i>tbr</i>	A	none	MS/4
Escort	<i>dms, tbr</i>	A, D	<i>R2/Rpi-blb3, R3a, R3b</i>	MR/7
Sarpo Mira	<i>dms?, tbr</i>	A, D	<i>R3a, R3b**</i>	R/8

*Abbreviations of *Solanum* species. *adg* - *S. andigenum*, *ber* - *S. berthaultii*, *chc* - *S. chacoense*, *cmm* - *S. commersonii*, *dms* - *S. demissum*, *edn* - *S. edinense*, *mcd* - *S. microdontum*, *mga* - *S. megistacrolobum*, *phu* - *S. phureja*, *plt* - *S. polytrichon* (= *S. stoloniferum*), *pnt* - *S. pinnatisectum*, *ryb* - *S. rybinii* = *S. phureja*, *sem* - *S. semidemissum*, *sto* - *S. stoloniferum*; *tbr* - *S. tuberosum*, *vlm* - *S. vallis-mexisi*, *vrn* - *S. vernei*.

** According to Rietman et al. (2012), Sarpo Mira comprises at least four R genes.

***Grades/points of LB resistance in detached leaf assays: R, resistant (points 8-9), MR, moderately resistant (points 6-7), MS, moderately susceptible (points 4-5), S, susceptible (points \leq 3).

We also compared the patterns of *R*-gene markers in the interspecific hybrids with their LB resistance evaluated in the detached leaf assays. The EUCABLIGHT standard cultivars free of wild *Solanum* germplasm (Alpha, Bintje, Eersteling, and Robijn) served as a negative control, and cultivars Escort and Sarpo Mira, as a positive control group with high LB resistance. The *R* genes obviously fortified LB resistance of potato hybrids, and the correlation between the number of *R*-gene markers and LB resistance was highly significant, with the Spearman's coefficient of 0.63 (Table 3; Fig. 1).

Table 2. Some wild *Solanum* species reported in pedigrees of interspecies potato hybrids

Series	Species	Genomes established		<i>R</i> genes established by molecular studies**
		by classical genome analysis	by molecular technologies*	
Tuberosa	<i>S. berthaultii</i>		A1A1, A3A3	<i>R1</i> , <i>R3b</i>
	<i>S. microdontum</i>	AA	A1A1, A3A3	<i>R1</i> , <i>R3a</i> , <i>Rpi-mcd1</i>
Longipedicellata	<i>S. stoloniferum</i> , <i>S. vallis-mexisi</i>	AABB	AABB, A1A1BB	<i>R1</i> , <i>R2/Rpi-blb3</i> , <i>R3a</i> , <i>R3b</i> , <i>Rpi-blb1</i>
Demissa	<i>S. demissum</i>	AADDD'D', A1A4[B,C,D]	AAPPPP, AABB, A1A1D	<i>R1</i> , <i>R2</i> , <i>R3a</i> , <i>R3b</i>
Bulbocastana	<i>S. bulbocastanum</i>	AbAb	BB	<i>R2/Rpi-blb3</i> , <i>R3a</i> , <i>R3b</i> , <i>Rpi-blb1</i> , <i>Rpi-blb2</i> , <i>RB-bt1</i>
Pinnatisecta	<i>S. pinnatisectum</i>	ApiApi	BpiBpi, BB	<i>R2</i> , <i>R3a</i> , <i>R3b</i>

*Compiled from Rodriguez et al. (2009); Drobyazina and Khavkin (2012).

** For more details see Sokolova et al. (in press).

Table 3. LB resistance of potato hybrids as affected by pyramiding the *R* genes

<i>R</i> -gene markers per plant	Groups of genotypes	Average resistance, points*
0	Standard <i>R</i> -gene free cultivars Alpha, Bintje, Eeertstellung, Robijn	3.3
1	IPH2	3
2	IPH1, IPH8, IPP13, IPP14	4.5
3	IPH3, IPH6, IPH7, IPP11, IPP12, IPP15, IPP18	5.3
4	IPH4, IPH5, IPH9, IPP10, IPP16	5.8

*Detached-leaf assays.

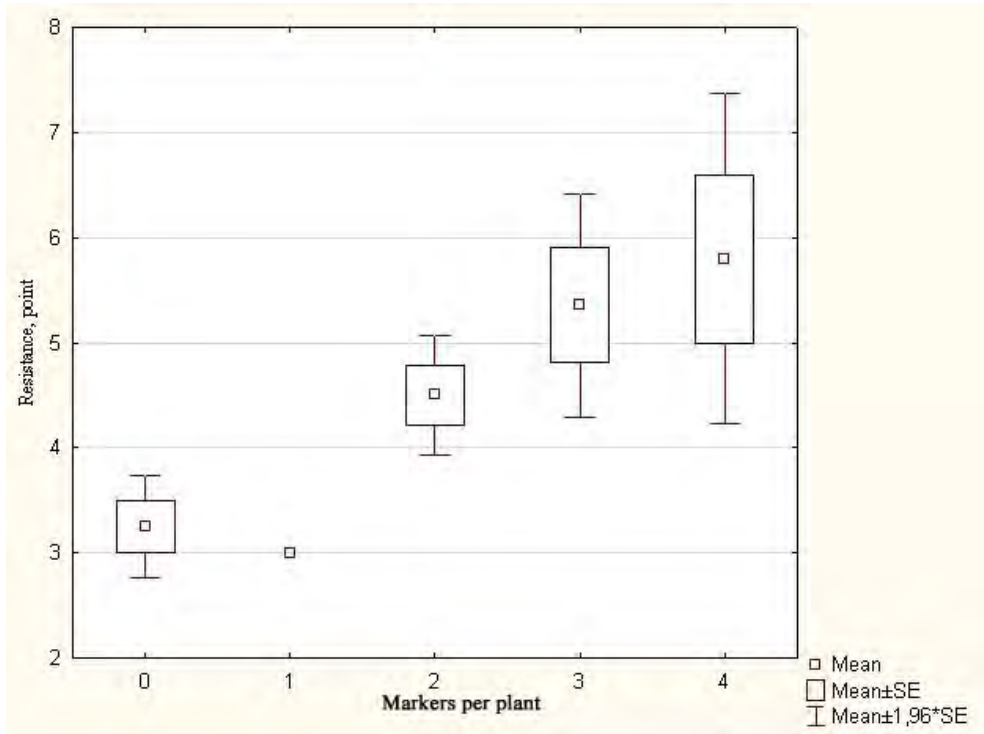


Figure 1. The effect of stacking *R* genes in interspecific potato hybrids on their LB resistance in detached-leaf assays

CONCLUSION

While the race-specific *R* genes are commonly held to be overcome (defeated) by new virulent races of *P. infestans*, our data support the hypothesis that these genes provide a discernible input to LB resistance (Gebhardt, 2013). Stacking *R* genes in interspecific hybrids obviously promotes their LB resistance.

ACKNOWLEDGEMENTS

The authors thank T.V. Belyantseva, O.V. Makhan'ko and P.V. Voloshina who helped with experiments. The study was supported by the ISTC - ARS-USDA (project 3714p), the Ministry of Education and Science, Russian Federation (contract No.16. M04.12.0007), and the Russian Foundation for Basic Research (project 13-04-00163a).

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Sensitivity of Irish *Phytophthora infestans* to the CAA fungicide mandipropamid

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SENSITIVITY OF IRISH *PHYTOPHTHORA INFESTANS* TO THE CAA FUNGICIDE MANDIPROPAMIDE

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Introduction

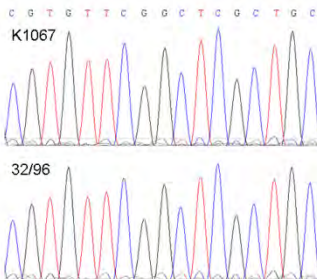
- Late blight caused by *Phytophthora infestans* most economically destructive disease of potato
- Fungicides relied upon for control
- Mandipropamide key fungicide in blight programmes
- No resistance detected in natural populations
- Mechanism of resistance known (Blau et al. 2010)

Materials & Methods

- 70 single lesion isolates selected based on genotype
- Sensitivity determined using agar plate assay
- Representative isolates tested *in planta*
- Sequence analysis of *PiCes3A* in selection of historical isolates

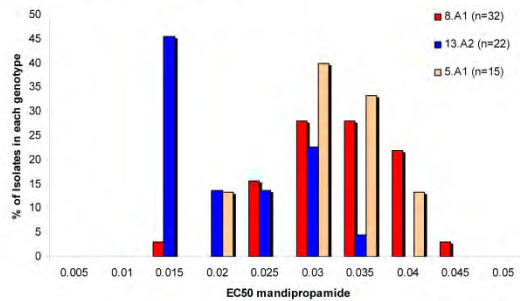


Devastating consequences of late blight on potatoes



Results & Discussion

- All isolates tested were sensitive to mandipropamide
- Differences between genotypes observed, with 13_A2 most sensitive
- KASP assay currently under development for early detection mutations associated with resistance



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A New Approach to Measure Potato Susceptibility to *Phytophthora infestans*, a Causal Organism of the Late Blight

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SUMMARY

The proposed method makes it possible to evaluate the level of the foliage and tuber susceptibility of potato cultivars to *Phytophthora infestans* under field and laboratory conditions using a mathematical simulation approach.

KEY WORDS

Phytophthora infestans, late blight, potato resistance

INTRODUCTION

Potato cultivar resistance to *Phytophthora infestans*, a causal agent of the late blight, still plays a key role in the control of this disease. The use of resistant plants requires no actions from potato growers during the season; it does not harm the environment and is usually compatible with other disease management techniques; finally, sometimes such approach is sufficient to reduce the disease development to a tolerant level (Fry, 1982). That is why the testing of potato cultivar for the late blight resistance is an important part of the selection process in the breeding of new potato cultivars.

There are two known resistance types: vertical (absolute) and horizontal (partial). The first-type resistance is race-specific, since it is related to dominant genes (R genes), which present in wild *Solanum* species (mainly *S. demissum* and *S. stoloniferum*), used by breeders in crossings. R genes provide a hypersensitive reaction of infected tissues that resulted in the localization of the pathogen penetration point by necrotized tissues. The pathogen perishes, leaving only a small necrotic lesion on a leaf.

Numerous attempts to obtain a long-term resistance using the mentioned R genes were unsuccessful because of the development of virulent races, always existing in any *P. infestans* population. As a result, breeders started to use another type of resistance, named partial (horizontal) or field resistance (Turkensteen, 1993; Colon et. al., 1995). In contrast to the race-

specific resistance, this type of resistance just controls the development of the disease and does not suppress it completely. It is usually considered that this type of resistance is polygenic, since it is efficient against all *P. infestans* races and, therefore, has a more stable and prolonged effect, than the race-specific resistance. However, the possibility of genetic recombinations, appeared in "new" *P. infestans* populations due to a sexual process, provided the appearance of more aggressive pathogen strains that caused a gradual decrease of this type of resistance. As a result, the partial resistance of some potato cultivars to various late blight populations can significantly vary. For example, cv. Santé is considered to be moderately resistant to late blight in France, moderately susceptible in Netherlands, and susceptible in the Moscow region of Russia. Due to this fact, there should be a permanent control on the late blight infection level of the cultivated potato varieties.

The common method of estimation of the late blight resistance of potato in the field is based on the scoring of the foliage destruction usually performed at any certain stage of the plant development. The late blight resistance is also scored under laboratory conditions using artificially inoculated detached potato leaves by the measurement of the size of necroses or the level of sporulation. Results of such estimation are expressed in accordance with the 9-score scale (Colon *et al.*, 1995). We consider that the traditional late blight assessment methods can be improved; to do this, one should use the quantitative value of the LB-caused yield loss, calculated from the dynamics of dying-off of infected tops during the whole vegetation period, as the key evaluation factor.

This paper describes procedures required to realize the above-described idea.

FIELD TESTS FOR FOLIAR BLIGHT RESISTANCE

The field assessment of the partial resistance of tested potato cultivars to late blight is carried out on the natural or artificial background by measuring the level of a leaf infection each 10-12 days using a special scale (Table 1).

Table 1. Scale for the assessment of the late blight infection of potato leaves (James, 1971)

Level of infection, %	Description
0	- No any signs of infection.
0,1	- First single spore-bearing spots.
1,0	- Weak level of infection (5-10 lesions per a plant).
5,0	- About 50 lesions per a plant; 1 of 10 leaf lobes is infected.
25,0	- Almost all leaves are infected, but plants still keep a normal form. The field looks green.
50	- Each plant is infected; about 50% of the leaf area is dead. The field looks green with brown spots.
75	- The infection is spread over 75% of the leaf area. The field looks brown-and-green.
95	- Plants have only single leaves, but the stems are green.
100	- All leaves died, and stems are died or dry.

Basing on this assessment data one can determine the area under the disease progress curve (AUDPC), in the course of the vegetation season, the corresponding yield losses caused by the early destruction of leaves (%), and the late blight resistance level (in scores).

The program of such calculation (Fig. 1) is placed at the website of the All-Russian Research Institute of Phytopathology (Rogozhin and Filippov, 2011; http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru).

This program is based on the known van der Plank hypothesis (1968), which assumes a direct ratio between the AUDPC on the potato foliage and yield losses. According to our long-term field studies (Gurevich, Filippov, and Tverskoy, 1977), this dependency can be expressed by the following equation:

$$\omega = \frac{AUDPC}{q} \cdot 100,$$

where ω is a yield loss (%) caused by an early leaf decay, q is the number of days between the bud formation phase and the decay of non-infected leaves. The average q value for the early, intermediate, and mid-late potato cultivars is 46, 52, and 84 days, respectively. If the foliage is killed by the frost or desiccant, or the harvesting is carried out before the natural dying-off of the foliage, then q is considered to be the number of days passed between the bud formation phase and the moment of the foliage death (Rogozhin and Filippov, 2012).

The calculated yield losses are then converted to the scores characterizing the level of the late blight resistance in accordance with the 9-score scale, where 9 scores represent the highest resistance level.

Calculation of potato late blight resistance indices for the field trials

Define the period of tuberization:

First early
 Early
 Mid-early
 Mid
 Mid-late
 Late
 in the case of a premature desiccation

Tuberization, days:

Insert the number of foliar blight assessments:

Assessment data

Number of assessment	Insert assessment dates (dd/mm)	Insert the level of infection, %
1	05/07	0.1
2	25/07	5
3	10/08	25
4	25/08	78

Calculate

AUDPC:

Calculated yield losses, %:

LB resistance, scores:

Figure 1. Working window of the program for the calculation of potato yield losses, caused by the late blight and the resulting early destruction of leaves

DETACHED LEAF TESTS FOR FOLIAGE BLIGHT RESISTANCE

The quantitative manifestation of the partial LB resistance within the same potato cultivar depends on the infection load, the level of aggressiveness of *P. infestans* strains, and weather conditions. Therefore, an objective assessment can be performed by the arrangement of field trials in regions, which are usually favorable for the late blight development (such as the Sakhalin island and Central Mexico), or under standard laboratory conditions with the use of special tests and the mathematical simulator of the epidemic development (Filippov et.al., 2004).

The laboratory assessment method, developed in our institute, is based on the joint use of the artificial inoculation of detached potato leaves and the mathematical model, simulating the late blight development under standard favorable meteorological conditions and at the given primary infection level. This model, based on the measurement of the inoculation efficiency, size of necroses, and sporulation productivity, reproduces the dynamics of the foliage destruction during a vegetation season and calculates the correspondence of this dynamics to the yield losses caused by the late blight of potato (Gurevich, Filippov, and Tverskoy, 1979). The method makes it possible to assess the cultivar resistance to the most aggressive *P. infestans* strains, including exotic ones under isolated laboratory conditions.

The tests are carried out on detached leaves, collected from the studied potato cultivars and inoculated with the studied *P. infestans* isolates, and, in parallel, on detached leaves of the standard potato cultivar, inoculated by the standard *P. infestans* strain.

Plants of the tested cultivars and the standard cultivar (30 plants of each cultivar) are grown under field conditions. During the phase of development of 7-9 leaves, a mid-level leaf is detached from each plant for the testing. Then leaves are transferred into laboratory premises and inoculated with the selected pathogen strains. Each tested "cultivar-isolate" pair is compared with the standard pair. The comparison of data, obtained in the course of experiments for each "cultivar-isolate" pair (number and diameter of necrotic lesions and the sporulation productivity) makes it possible to conclude about any differences in the aggressiveness of isolates from different regions and, therefore, about the level of resistance of tested cultivars.

In the proposed method we use the cv. Santé as a standard cultivar and the N161 *P. infestans* strain as a standard. The field yield loss of the above-mentioned cultivar, infected with the chosen strain, makes 30% under the weather conditions favorable for the disease development. Using the tests, one can measure the basic parameters of the infection cycle on each tested cultivar as compared to the standard cultivar.

1. Inoculation efficiency measurement

The test is carried out using 10 leaves for each cultivar. The leaves are inoculated by spraying with a suspension of zoosporengia (30000 spores/m²); the volume of the suspension is 5 ml per a cuvette. After the inoculation, leaves are incubated in a wet chamber for 3 days at 18°C; then the area of leaves was determined using a photoplanimeter, and the number of necrotic lesions per 1 cm² was calculated.

2. Measurement of necrotic lesions

Potato leaves are inoculated with a suspension of zoosporengia (1-2 drops per a leaf) using a microdispenser. The concentration of zoospores is the same as for the previous operation (stage 1). Inoculated leaves are incubated in a wet chamber for 18 h at 20°C. Then the drops of suspension are removed by a filter paper and the leaves are placed into a wet chamber again for 3 days. On the 4th day the diameter of necrotic lesions is measured (Fig. 2).

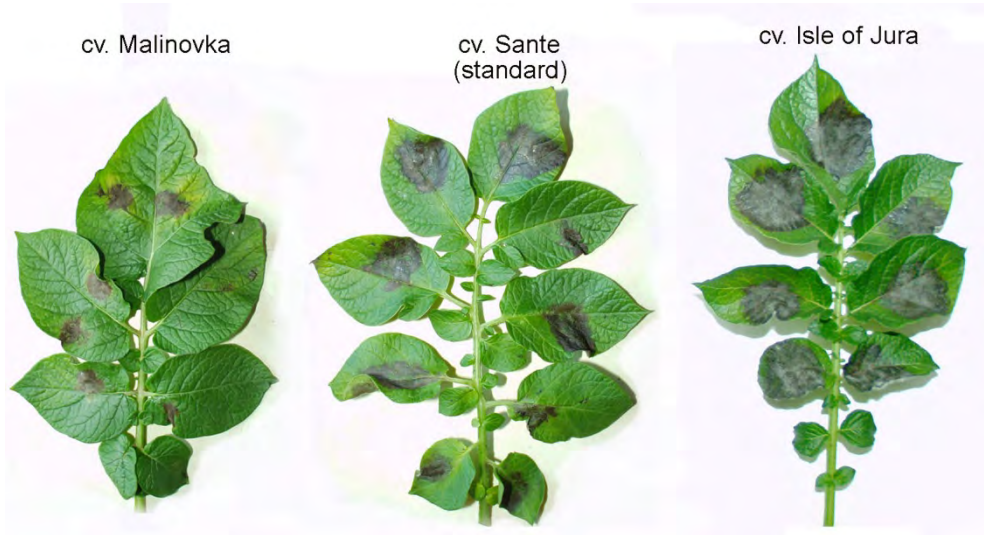


Figure 2. Example of infected potato leaves used to assess the size of necrotic lesions and the sporulation productivity

3. Measurement of the sporulation productivity

For this measurement one can use the leaves from the previous test.

The intensity of the spore formation is assessed using two methods. The more exact way is to calculate the number of conidia per one lesion using a Goryaev's count number. To do this, one should put 10 leaf lobes with necrotic lesions into a glass beaker and add 15 ml of distilled water (1.5 ml per a lesion). After shaking, the leaves should be removed, and the remaining water volume should be measured. Then the number of conidia per lesion should be calculated using the Goryaev's chamber.

All after-measurement calculations are performed separately for potato cultivars of three maturing groups. The program developed on the basis of the above-mentioned measurements, calculates the AUDPC value, yield losses, and the level of the late blight resistance of the tested cultivar under fixed conditions favorable for the disease development (Fig. 3). The program can be found on the above-mentioned website of our institute.

Losses and cultivar-pathogen interaction

Calculation of the potato late blight resistance indices basing on the results of laboratory trials

Authors: A.V. Filippov, A.N. Rogozhin, and B.I. Gurevich

Cultivar maturity group: Early-ripening, Mid-ripening, Late-ripening

Parameters of infection

	Tested cultivar-isolate pair	Standard "cultivar-isolate" pair	
Infection efficiency (lesions per cm ²)	12	5	AUDPC: 1592
Necrotic lesion size, mm	18	15	Calculated losses (%): 30,62
Sporulation efficiency (thousand spores/lesion or ball)	3	4	LB resistance, scores: 3,6

Calculation

Figure 3. Working window of the program calculating the level of the leaf blight resistance of potato cultivars under conditions, favorable for the disease development, on the basis of the measurement of the main parameters of the infection cycle on detached leaves

TUBER SLICE TESTS FOR TUBER BLIGHT RESISTANCE

To assess the late blight resistance of potato tubers under laboratory conditions, we propose to use a Lapwood method (Lapwood, 1965, 1967) with some modifications.

Potato tubers are sliced into pieces (7×5×40 mm) in the twenty-fold repeatability. One end of each piece is submerged for 3-5 seconds into a zoospore suspension poured into Petri dishes (2-3-mm layer). After a 6-day incubation, the length of the infected zone is measured by a ruler (mm), and the mycelial covering intensity is determined using a 4-score scale (Fig. 4).

Tuber slices of the cv. Santé, inoculated with the N161 strain, are used as a standard. According to the expert assessments, the level of the tuber resistance of the cv. Santé to the N161 strain is equal to 5.5 scores of the 9-score scale, where 9 scores correspond to the maximal resistance level. The cv. Santé and the strain 161 can be replaced by any other "standard" cultivar-isolate pair with the known result of their interaction, expressed in scores. From the practical point of view, it is desirable that the tuber resistance level of the selected "standard" cultivar towards the selected *P. infestans* isolate would be within the range of 4-7 scores.

Based on the measurements of the size of necrotic lesions and the level of the mycelial covering of tuber slices, the cultivar resistance index is calculated using the equation (1):

$$x = \frac{\sum(a \times b)}{n}, \quad (1)$$

where x is the resistance index, a is the average size of the lesion of the tested cultivar as compared with the standard one, b is the average mycelial covering intensity as compared with the standard (equal to 1), and n is the number of slices.

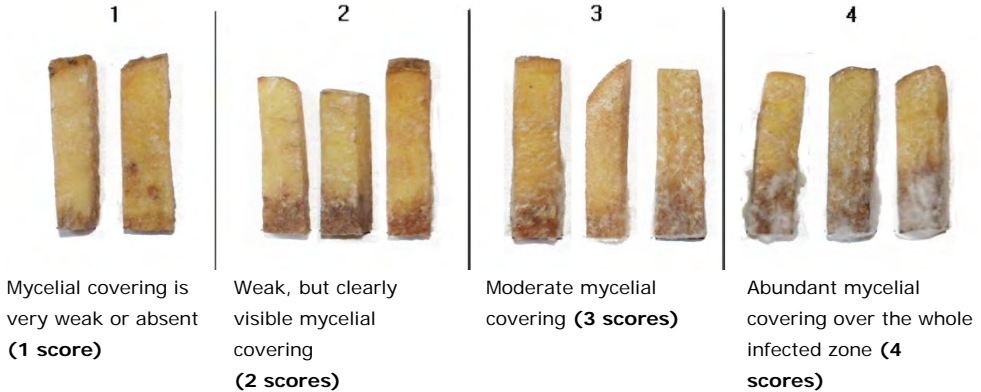


Figure 4. Scale for the visual assessment of the mycelial covering intensity

The calculated indices are then converted into scores using a special chart (Fig. 5). It is also possible to use a special program located at the ARRIP site (Fig. 6).

The methods presented in this paper are offered for the use as a procedure for the state registration of new potato cultivars in Russian Federation.

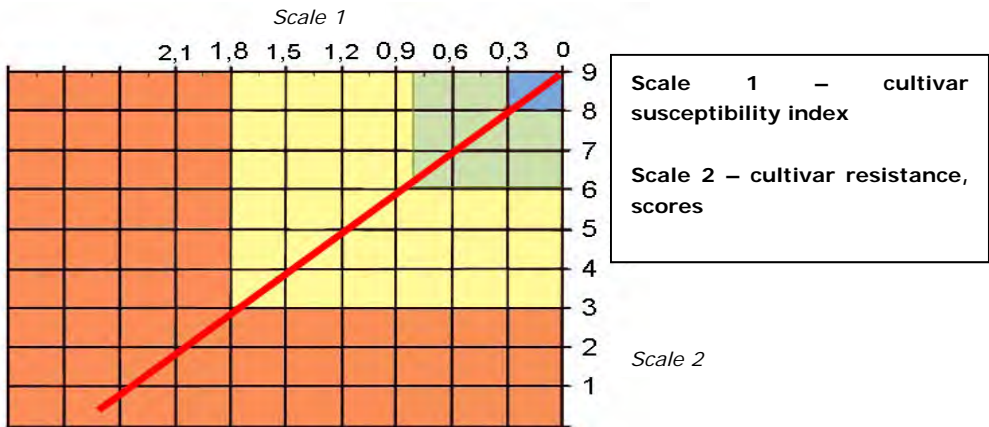


Figure 5. Chart for the assessment of the late blight resistance of potato tubers

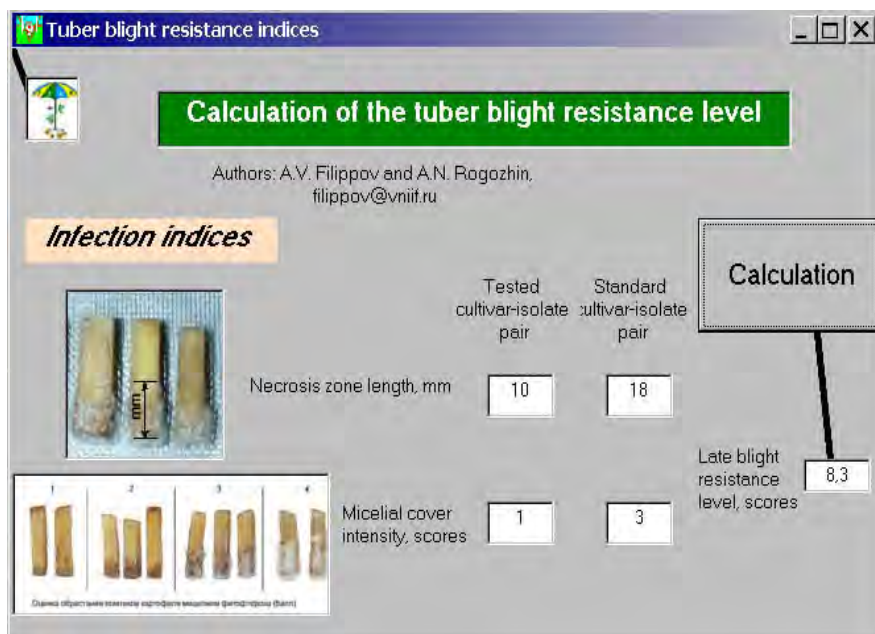


Figure 6. Working window of the program calculating the level of the tuber blight resistance of potato cultivars

ACKNOWLEDGMENTS

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BANJO FORTE

A new product in the late blight market

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BANJO FORTE

A NEW PRODUCT IN THE LATE BLIGHT MARKET

Introduction

Banjo Forte is the new potato late blight fungicide from Makhteshim-Agan. This product has been launched in 2013 and will be registered all over Europe. Because of the unique combination of two active ingredients, this is a very strong product against potato late blight.

Banjo Forte provides:

- A perfect solution against leaf- and tuber blight
- A strong side effect on white mold
- Very good rain fastness

Banjo Forte content

Banjo Forte is a SC formulation which contains two active ingredients, fluazinam and dimethomorph. The mode of action of fluazinam is preventive and anti sporulating. The mode of action of dimethomorph is effective against all different strains of phytophthora infestans. This active ingredient redistribute in the new leaves of the potato plant.

Multi year multi site EuroBlight trials

Makhteshim Agan participated in EuroBlight trials in different European countries. The average results are represented in Figure 1. *Based on EuroBlight trials in NL, UK, DK. 6 to 19 trials.

Banjo Forte controls white mold

An upcoming disease in Europe is white mold, caused by Sclerotinia sclerotiorum. In 2012 in the Netherlands, a trial has been conducted by PPO (Praktijkonderzoek Plant en Omgeving) for the Productschap Akkerbouw. Goal of this trial was to show the efficacy of different late blight fungicides towards white mold (figure 2).

Banjo Forte - Resistance testing

In 2011 and 2012 Makhteshim Agan did some resistance testing for Banjo Forte in Europe. In six countries P. infestans samples have been taken and tested by EPILOGIC in Germany. Infected leaves from different sites were placed in humid Petri dishes and incubated to encourage sporulation. The sporangia of the single colonies were transferred for maintenance and propagation onto fresh material. Afterwards the wet leaves were placed in a dark cold site to encourage the release of zoospores.

Results

Samples from France, Netherlands, United Kingdom, Germany, Sweden and Poland were sent to EPILOGIC to be analyzed. The highest MEC50 values were calculated from a French and German sample (9,65mg/l a.i.). The MEC50 of the sensitive standard was 4,4 mg/l a.i. There were no significant differences of MEC50 found values compared to the sensitive standard. All samples showed full sensitivity against dimethomorph. Also on new found P. infestans strains, the efficacy of Banjo Forte is good. In several pot trials in the Netherlands, Banjo Forte showed good control of P. infestans strains like Green33 and Blue13 (picture 2).

Summary

- Banjo Forte is one of the first late blight fungicides of Makhteshim Agan.
- Unique combination of two active ingredients, contributes to:
 - Better efficacy
 - Resistance management
- Very good side effects on other potato diseases
 - White mold



Fig. 1. Multi site efficacy towards late blight, 2006-2011

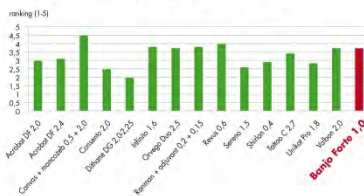


Fig. 2. Banjo Forte efficacy against white mold

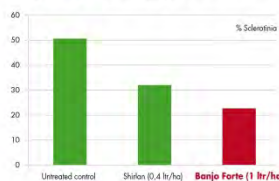


Fig. 2. Plants inoculated with Green33. Untreated control vs. Banjo Forte



Distribution of mating types and resistance to metalaxyl of *Phytophthora infestans* in southern Estonia

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DISTRIBUTION OF MATING TYPES AND RESISTANCE TO METALAXYL OF *PHYTOPHTHORA INFESTANS* IN SOUTHERN ESTONIA

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INTRODUCTION:

- *Phytophthora infestans* is one of the most serious and economically important pathogens in potato fields worldwide, including Estonia.
- Under favourable conditions it can destroy the whole potato haulm and cause a considerable yield loss.
- In Estonia, the average yield loss due to late blight can reach 20-25% and in untreated fields even more.
- Without control of potato late blight it is not possible to achieve high-quality crop yield.
- *P. infestans* isolated from potato leaves were collected from a region of Southern Estonia during 2010 and 2011.
- In total, 128 isolates were assessed for mating type and 71 isolates were analyzed for resistance to metalaxyl.

MAIN AIM OF THE RESEARCH:

- Survey the population structure of *P. infestans* in Estonia and characterise isolates by mating type and their resistance to metalaxyl

MATERIALS AND METHODS:

- In total, 128 isolates of *Phytophthora infestans* were collected from Estonia during 2010-2011.
- The isolates were sampled randomly from southern Estonia and the procedure was repeated two years.
- Blighted leaves (one per plant) were collected in the period from the emergence of disease until the end of the growing season in both years.
- Leaflets with single lesions, were collected from individual plants. Isolations were carried out as described in Runno-Paurson *et al.* (2009).
- For mating type determination was used a method as described by Lehtinen *et al.* (2007).
- The resistance to metalaxyl of 71 isolates was tested using a modification of the floating-leaflet method described by Hermansen *et al.* (2000).
- Leaflets of susceptible cultivar Berber were obtained from five week-old greenhouse-grown plants.
- The metalaxyl concentrations were 0.0, 10.0 or 100.0 mg l⁻¹ prepared from Analytical Master Standard, CGA 329351A
- Statistical analyses were performed with the program Statistica 11 (StatSoft, Inc., Tulsa, Oklahoma)

Table 1. Metalaxyl resistance among isolates of *Phytophthora infestans* in Estonia 2010 and 2011

Year	Percentage of isolates			Isolates tested (n)
	S (%)	I (%)	R (%)	
2010	76,2	14,3	9,5	21
2011	94	6	0	50
Total	88,7	8,5	2,8	71

S metalaxyl-sensitive, I, intermediate metalaxyl-sensitive, R, metalaxyl-resistant

CONCLUSION:

- The proportion of metalaxyl-resistant isolates in the Estonian population in 2010-2011 were quite low.
- Results showed that the use of metalaxyl-containing fungicides is still effective in Estonia.
- In Estonia the ratio of *P. infestans* A1:A2 (which is close to 1:1) mating types is suitable for sexual reproduction.
- During 2010-2011 13 fields had both mating types in the same potato fields.



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson

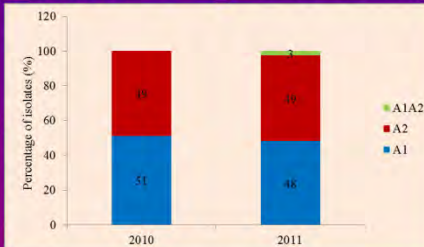


Figure 1. Percentages of mating types among isolates of *Phytophthora infestans* from southern Estonia (2010-2011)

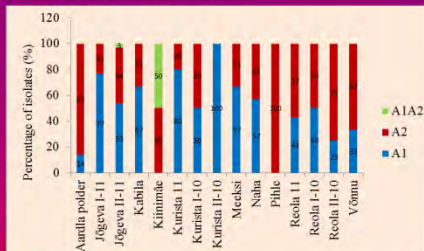


Figure 2. Percentages of mating types among isolates of *Phytophthora infestans* from southern Estonia potato fields

RESULTS:

- Among the 71 isolates, 9.5% were resistant, 8.5% intermediate and 88.7% sensitive to metalaxyl. In 2010 the percentage of resistant isolates was 9.5% and in 2011 it was 0%.
- In 2010 were 51% of the isolates A1 and 49% were A2 mating type. In 2011 were 48% of the isolates A1, 49% were A2 and 3% were self-fertile A1A2 mating type.
- There were thirteen fields that contained both A1 and A2 mating types.
- A1 mating type individuals were detected in one of the fifteenth fields, and A2 mating type individuals were also identified in one field.



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson



Photo: Eve Runno-Paurson

ACKNOWLEDGEMENTS: The study was supported by the Estonian Foundation grants no 9432 and 9450, Target Financing SF170057s09, project RESIST 3.2.0701.11-0003, European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, which is carried out by Foundation Archimedes

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Efficacy of various sources of resistance in protection of potato foliage and tubers against *Phytophthora infestans*

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Efficacy of various sources of resistance in protection of potato foliage and tubers against *Phytophthora infestans*

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Phytophthora infestans (Mont.) de Bary, the causal agent of potato late blight, can infect both foliar and tuber tissue. Although economic value of potato resides in its tubers, comparatively less effort is made in breeding for potato late blight resistance in tuber than in foliage. It is considered, that cultivars with a high level of foliage blight resistance show a good level of tuber blight resistance, however foliage resistance does not guarantee tuber resistance. Despite the abundance of major resistance genes introduced into potato cultivars, little is known about effectiveness of these genes in potato tubers. In many cases R genes which provide effective protection of potato foliage fail to function in the tubers.

To compare effectiveness of various resistance sources in foliage and tubers protection cv. Sarpo Mira and two potato clones; Balck's differential R9 and 04-IX-4 were chosen. Resistance of cv. Sarpo Mira and clone R9 is based on presence of multiple R genes (respectively: R3a, R3b, R4, *Rpi-Smira1*, *Rpi-Smira2* (Rietman et al. 2012) and R1, R2, R3a, R3b, R4, R8, R9 (Kim et al. 2012)). The resistance of clone 04-IX-4 is provided by single broad spectrum resistance gene (*Rpi-phu1*). This potato cultivar and clones proved to be highly resistant to *P. infestans* in field conditions (Fig. 1)

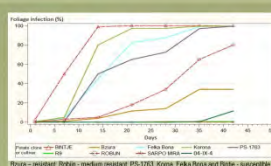


Fig. 1 Disease Progress Curve for cv. Sarpo Mira, clones R9, 04-IX-4 and standard potato cultivars/clones. Curves were plotted based on results of field trials from 2009.



Pic. 1 Sarpo Mira in field trial.

Clones R9, 04-IX-4 and cv. Sarpo Mira, as a donors of resistance, was crossed with susceptible potato cultivars. Among individuals from each progenies, five potato clones (outstanding in foliage resistance and in agronomic traits) were chosen for further evaluations. In 2011 and 2012 foliage and tuber resistance of donors and their selected progeny clones was assessed in detached leaflets and tuber slices tests (5 leaflets/slices in 2 replication, two times in each year). In both tests *P. infestans* isolate MP324 (race 1,2,3,4,5,6,7,10,11) was used for inoculation. The foliage resistance was also assessed in field trials in 2012.

	Leaflets test	Slices test	rAUDPC		Leaflets test	Slices test	rAUDPC		Leaflets test	Slices test	rAUDPC
Sarpo Mira	8,7	6,0	0,013	04-IX-4	8,9	8,8	0,003	R9	9,0	6,4	0,000
Purple × Sarpo Mira			Salad Blue × 04-IX-4			R9 × Felka Bona					
11-VIII-35	8,6	3,8	0,160	11-VIII-52	9,0	8,8	0,006	Bio-10	9,0	4,1	0,055
11-VIII-39	8,5	4,5	0,131	11-VIII-56	8,8	8,6	0,044	Bio-29	9,0	1,6	0,012
11-VIII-45	8,5	6,0	0,016	11-VIII-58	8,9	8,6	0,043	Bio-31	9,0	2,4	0,044
11-VIII-47	8,8	3,5	0,088	11-VIII-59	9,0	9,0	0,013	Bio-33	9,0	3,5	0,013
11-VIII-49	8,6	4,2	0,127	11-VIII-62	8,8	8,8	0,024	Bio-34	9,0	5,8	0,004

Pic. 2

Tuber slices test cv Sarpo Mira



Pic. 3

Tuber slices test clone 04-IX-4



Pic. 4

Tuber slices test clone R9



All tested clones show high level of foliage resistance in field and laboratory tests (Tab.1, 2, 3). Response of these clones for tuber inoculation was much more varied; clone R9, Sarpo Mira and their progeny clones show low and medium tuber resistance (scores range: 1,6 – 6,4). Clone 04-IX-4 and its progeny show extremely high tuber resistance; after inoculation with *P. infestans* no symptoms of infection was observed.

Resistance of cv. Sarpo Mira and clone R9 is based on presence of multiple R genes, but non of this genes proved to be effective in complete protection of tuber tissue. Although isolate MP324 was avirulent to *Rpi-Smira1* (and/or *Rpi-Smira2*) and R9 (and/or R8) in detached leaflets test, these genes did not provide complete resistance of tuber tissue. Resistance of clone 04-IX-4 is provided by single resistance gene *Rpi-phu1*, which is effective in both foliar and tuber tissue.

Currently, 'stacking' of broad spectrum R genes is one of the preferable solution in breeding against *P. infestans*. The use of foliage and tuber effective R genes can enhanced durability of foliage resistance and allows to avoid problems with tuber infections.

***Alternaria* spp. associated to potato crops and its epidemiology in southern Chile**

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SUMMARY

Early blight is one of the most important diseases on potato in southern Chile. In this study we could identify five *Alternaria* groups associated to potato crops (*A. alternata*, *A. tenuissima*, *A. arborescens*, *A. infectoria* and *A. solani*) using morphological and molecular characteristics. At the same time, the *Alternaria* spore release curve was determined using a spore trap and accumulation of physiological days. This information could be used to predict the appearance of the first symptoms of this disease, which would help the development of an early blight forecast system.

KEYWORDS

Alternaria, early blight, forecast system, spore release.

INTRODUCTION

Early blight, caused by *Alternaria* spp, is the second most important disease in potato in Chile, can infect potato plants developing variable damages depending of the cultivar susceptibility to the disease, agronomic management and favorable environmental conditions. Losses can be attained up to 30% in susceptible cultivars.

This disease affects both foliar and tuber. Initial leaf blight symptoms are observed in the lower part of the plants, on the older leaves. The main symptoms are brown circular spots with concentric rings, surrounded by a bright yellow ring and bounded to the leaflet veins.

However, it has been observed that the symptoms in field are variable, since the incidence and severity of the disease changes according to the season. This situation may be given by the presence of different *Alternaria* species, the environmental conditions and the amount of pathogen inoculum.

Alternaria species can have their own biological requirements and characteristics such as aggressiveness, fungicide resistance, optimum growth temperature and overwintering, among others. Then, many studies have been done in order to predict the appearance of early blight disease based on favorable environmental conditions and the spore release curve of the pathogen into the air.

Therefore, the aim of this study was to identify *Alternaria* spp. associated to potato plants in southern Chile and to determine the *Alternaria* spore release curve and their role in the disease development using potato physiological days (P-days).

MATERIALS AND METHODS

Identification of Alternaria spp. associated with potato plants through morphological characteristics and molecular identification.

Potato leaves with early blight symptoms were collected in potato crop from the Araucanía to Los Lagos regions in southern Chile. The pathogen was isolated from lesions and single-spore isolates were cultured on potato carrot agar (PCA) with fluorescent light cycles (8h light, 16 h dark) at 25° C for 5 days. Then, each isolate was characterized by colony morphology, sporulation patterns and conidial size. Finally, these results were compared with taxonomic keys following the method of Simmons (2007) and Piontelli (2011).

To perform the molecular analysis, three isolates were cultured of each morphological group on potato dextrose broth (PDB) for 4 days at 25°C with fluorescent light cycles (8h light, 16 h dark). Then, mycelium of each isolates were collected by filtration and were extracted by CTAB method. rDNA from ITS region was amplified with primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGTTATTGATATGC-3') (Chou et al, 2002). The PCR reaction was performed with an initial denaturation step at 94° C for 5 min, followed by 30 cycles of 94°C for 40s, 58°C for 40s and 72° C for 1 min, and a final extension step at 72° C for 5 min. Amplified fragments were cut from the agarose gel and purified with gel extraction kit. The ITS regions of 15 isolates were sequenced directly in both directions using the primers ITS5 and ITS 4, by Macrogen Korea.

DNA sequences obtained were aligned using the BioEdit Sequence Alignment Editor and were compared with database available in GenBank. Finally, phylogenetic analyses were performed with TreeFinder and the dendrogram was obtained by maximum-likelihood method.

Determination of the Alternaria spore release curve.

During the seasons 2010 to 2013 the *Alternaria* spore release curve was determined using a spore trap (Sporewatch spore & Pollen sampler, Burkard Scientific, England). This instrument was localized in the field near potato crop. It consist of a rotor carrying an adhesive tape. After 7 days this tape was removed and deposited on a slide for the conidia count under the microscope. P-days were calculated starting at potato plant emergence, using the maximum and minimum day temperatures. This model assumes 7° C, 21° C and 30° C as minimum, optimum and maximum, respectively according to crop development (Gent *et al.*, 2003).

RESULTS AND DISCUSSION

Identification Alternaria spp. associated to potato crops.

According to morphological characteristics that included colony morphology, sporulation patterns and conidial size, four small spore group (*A. alternata*, *A. tenuissima*, *A. arborescens* and *A. infectoria*) and one large group species (*A. solani*) were detected (Figure 1 and Table 1).

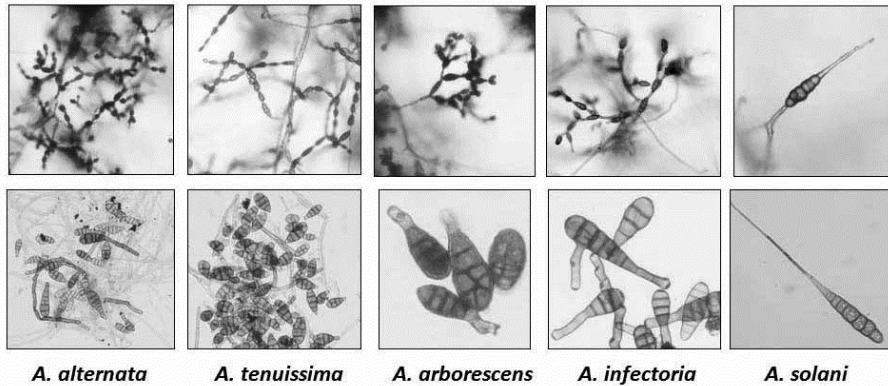


Figure 1. Morphology of *Alternaria* species associated to potato crops in southern Chile

Table 1. Comparison of morphological characteristics obtained with reference literature

Species	Conidial size			
	Reference range		Isolate range	
	(length x width)	trans.- long	(length x width)	trans.- long
	µm	N° septa	µm	N° septa
<i>A. alternata</i>	20-63 x 9-18	(3-8)-(0-1)	41,78 x 11,92	4-1
<i>A. tenuissima</i>	32-45 x 11-13	(4-7)-(0-4)	27,66 x 12,15	5-1
<i>A. arborescens</i>	12-30 x 7-11	(1-4)-(1-2)	29,28 X 11,44	3-2
<i>A. infectoria</i>	35-40 x 7-9	3-5	35,45 x 8,92	3-3
<i>A. solani</i>	150-300 x 15-19	(9-11)-(0-4)	200,90 x 19,05	8-2

Additionally to morphological criteria, three isolates of each morphological group were selected. Then, rDNA region containing the internal transcribed spacer 1, 5.8S rDNA and internal transcribed spacer 2 was amplified with primer pairs ITS5-ITS4 by PCR and then it was sequenced.

After sequencing, we obtained the consensus sequences for each isolate which were compared with database available in Genbank. It was possible to align *A. solani* and *A. infectoria*, corroborating its morphological identification.

As a result of this part of our study, the *Alternaria* spp. were divided into three groups: the first group included all small-spore isolates, excepting *A. infectoria*. The second group included *A. infectoria*. The last group correspond to *A. solani*. No relation was found between morphological and sequencing data in small-spored isolated.

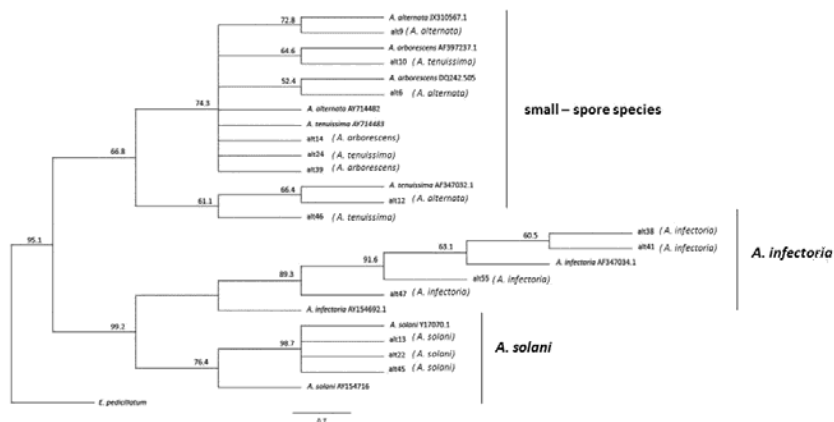


Figure 2. Dendrogram of *Alternaria* spp. obtained by maximum-likelihood method. Sequences from GenBank were incorporated for analysis, () shown identification according to the morphological analysis

Determination of *Alternaria* spore release curve.

The *Alternaria* spore release curve and its correlation with P-days could be used to predict the pathogen infection period. As a result, we observed a similar behavior during the three potato crop seasons evaluated, where the maximum conidia release were between 200 and 450 P-days. It was possible to differentiate 2 maximum peaks, one between 200 and 300 P-days and another between 350 y 450 P-days (Figure 3). Additionally, the first symptoms of the disease occurred in period of bloom, approximately one week after the first peak of the conidia release curve.

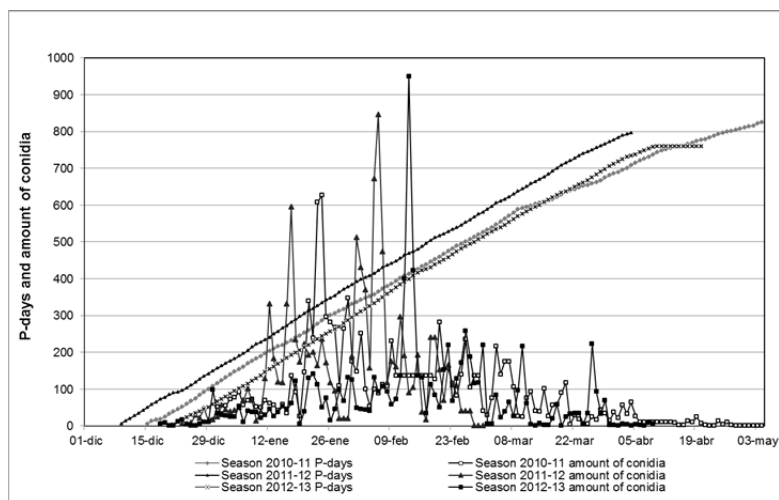


Figure 3. Potato physiological days value and *Alternaria* spore release curve, during 2010 to 2013 crop seasons. INIA Remehue, Región de Los Lagos, Chile

CONCLUSIONS

Five *Alternaria* groups were identified associated to potato crops in southern Chile: *A. alternata*, *A. arborescens*, *A. tenuissima*, *A. infectoria* and *A. solani* using morphological and molecular characteristics. This information is relevant for future studies of pathogenicity, virulence, fungicide resistance and field trials, because of the possibility of a differential behavior of each species.

The use of P-days and the *Alternaria* spore release curve can be used to predict the appearance of the first symptoms and support the integrated management of this disease, becoming an alternative for the development of a decision support system for the potato crop in southern Chile.

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Development of Late Blight (*Phytophthora infestans*) Resistant Potato Breeding Material for Organic Farming

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Development of Late Blight (*Phytophthora infestans*) Resistant Potato Breeding Material for Organic Farming

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Project

The German Federal Office for Agriculture and Food has funded a project to develop potato breeding material for organic farming in Germany. It aims at combining low susceptibility against late blight with other resistance and quality traits in potatoes. The network consists of the Bayerische Landesanstalt für Landwirtschaft (LFL), the Julius Kühn-Institut (JKI), the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), several potato breeders, organic farming organisations, as well as three organic potato farmers.

Field Trials

Field trials have been carried out since 2012 at three different locations in Germany. More than 140 potato varieties are grown and examined under organic farming conditions and natural *Phytophthora* infection.

By including potatoes from modern and historical gene pools, a large genetic diversity can be assessed for late blight tolerance, agronomic properties, starch content, and taste. Varieties are contributed by potato breeders, the IPK gene bank, the JKJ, and the LFL.



Breeding

For traditional breeding 10 000 potato seedlings per year are screened for phenotypic traits. 2000 seedling tubers are then chosen and revised under organic farming conditions. In a participative approach the organic farmers are actively involved in the selection process.

Simultaneously, DNA markers are analysed to give evidence for virus (PVY), nematode (*G. rostochensis*, *G. pallida*), and *Phytophthora* resistance. Molecular analyses are used to complement and accelerate traditional breeding methods.

Research

Field trials, trait assessment and DNA marker analyses will illustrate the phenotypic and genotypic diversity within the currently available breeding material. We will focus on using, evaluating, and developing DNA markers which are connected with *Phytophthora* resistance. Information gained in the process will be used for concerted potato breeding.

On the trial fields *Phytophthora* population will be monitored. Identification of the strain composition will be used to interpret differences in late blight tolerance between locations.



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Structural homologues of CC-NBS-LRR genes for potato late blight resistance in wild *Solanum* species

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SUMMARY

Breeding potato for durable resistance to late blight (LB) greatly benefits from expanding the resource of resistance genes provided by wild *Solanum* species. SCAR markers for race-specific CC-NBS-LRR resistance genes (*R* genes) have been employed to screen *Solanum* accessions representing six series of section *Petota*. Structural homologues of particular genes were found in many taxonomically distant species and widely differed in their distribution patterns. Such evidence suggests that the *R*-gene structures evolved before the divergence of genomes A and B and preceded *Solanum* speciation.

KEYWORDS

Phytophthora infestans, *Solanum* spp., CC-NBS-LRR genes, late blight, race-specific resistance genes.

INTRODUCTION

Late blight (LB) caused by *Phytophthora infestans* (Mont.) de Bary is among the most devastating potato diseases. New races of *P. infestans* rapidly defeat potato resistance based on germplasm transferred from *Solanum demissum*, and breeders search for new sources of durable LB resistance in genetic collections of wild *Solanum* species. *R* genes are highly conserved, and the candidate-gene approach, by using PCR amplification, opens the way to mine orphan *Solanum* species for new homologues of already known resistance genes (Hein *et al.*, 2009). We used five SCAR markers recognizing the race-specific resistance genes *R1*, *R2/Rpi-blb3*, *R3a*, *R3b* of *S. demissum* and *RB/Rpi-blb* of *S. bulbocastanum* to screen a collection of wild *Solanum* accessions representing six series from section *Petota* and clones developed from these accessions. The pattern thus produced will facilitate further search for new *R* genes for LB resistance and promote the comparative studies of rapidly evolving CC-NBS-LRR genes.

MATERIALS AND METHODS

Plant Material

Solanum accessions for this study arrived as microtubers from the collections of the Institute of Plant Industry (St. Petersburg, Russia) and the Institute of Phytopathology (Bol'shiye Vyazemy, Moscow region, Russia) and as seeds from the collections of the Institute of Plant Industry, Centre for Genetic Resources, the Netherlands, and the United States Potato Genebank, NRSP-6, Sturgeon Bay, WI. As a whole, we screened over 200 *Solanum* accessions.

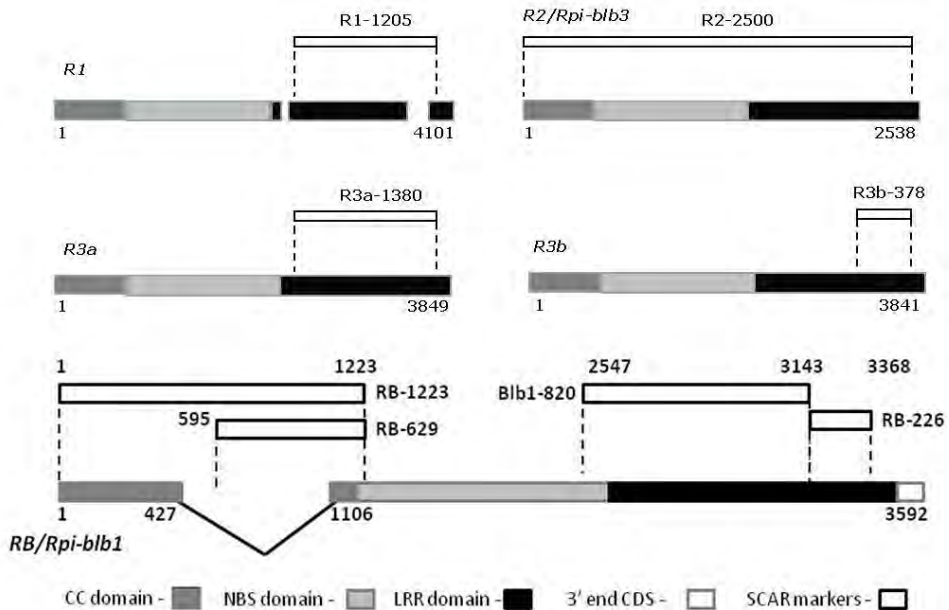


Figure 1. SCAR markers for R genes for LB resistance. Primer positions (base pairs) are shown against the structures of CC-NBS-LRR kinases for prototype genes R1, R2/Rpi-blb3, R3a, R3b and RB/Rpi-blb1. For more details see Table 1

Table 1. SCAR markers of *R* genes

Genes	Markers and their sizes, bp	5'-3' primer sequences	Chromosome	Prototype clone	Position in the prototype clone	Annealing temp., °C	References
<i>R1</i>	R1-1205	5'-CACTCGTGACATATCCTCACTA-3' 5'-GTAGTACCTATCTTATTTCTGCAAGAAT-3'	5	AF447489	5126-6331	61	Sokolova <i>et al.</i> , 2011
<i>R3a</i>	R3a-1380	5'-TCCGACATGTATTGATCTCCCTG-3' 5'-AGCCACTTCAGCTTCTTACAGTAGG-3'	11	AY849382	1677-3056	64	ibid.
<i>R3b</i>	R3b-378	5'-GTCGATGAATGCTATGTTTCTCGAGA-3' 5'-ACCAGTTTCTTGCAATTCAGATTG-3'	11	JF900492	94818-95195	64	Rietman <i>et al.</i> , 2012
<i>R2</i>	R2-2500	5'-ATGCTGATGCCTTTCTATCATTGTC-3' 5'-TCACAACATATAATTCCGCTTC-3'	4	FJ536325	1-2538	62	Kim <i>et al.</i> , 2012
<i>RB/Rpi-blb1</i>	RB-629	5'-GAATCAAATTATCCACCCAACTTTTAAAT-3' 5'-CAAGTATTGGGAGGACTGAAAGGT-3'	8	AY336128	595-1223	65	Pankin <i>et al.</i> , 2011
<i>RB/Rpi-blb1</i>	RB-226	5'-CACGAGTGCCCTTTTCTGAC-3' 5'-TTCAATTGTGTGCGCACTAG-3'	8	ibid.	3143-3368	50	Colton <i>et al.</i> , 2006
<i>RB/Rpi-blb1</i>	Bib1-820	5'-AACCTGTATGGCAGTGGCATG-3' 5'-GTCAGAAAAGGGCACTCGTG-3'	8	ibid.	2547-3143	62	Wang <i>et al.</i> , 2008

Development of DNA markers



Standard protocols were employed for genomic DNA isolation from young plant leaves, PCR analysis, and cloning and identifying genome fragments. Some specific primers for SCAR markers were already reported elsewhere, and some were designed following multiple alignment of the prototype gene sequences, their structural homologues and anonymous genome fragments lifted from the NCBI Genbank using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Vector NTI Suite 8 package (Invitrogen). The markers, primers and their positions as related to the prototype genes are shown in Fig. 1 and Table 1.

RESULTS AND DISCUSSION

SCAR markers for CC-NBS-LRR resistance genes have been employed to screen 14 wild *Solanum* species (Table 2). Structural homologues of particular *R* genes widely differ in their distribution patterns in the section *Petota*. The genes *R1*, *R2*, *R3a* and *R3b* initially identified in *S. demissum* were found in many taxonomically distant species. Structural homologues of the *RB/Rpi-blb1* gene, which was initially identified in genome B of *S. bulbocastanum* and *S. stoloniferum* (for review see Vleeshouwers *et al.*, 2011), were found in many genome A species; however, only *RBver* in *S. verrucosum* was shown to participate in LB resistance (Liu and Halterman, 2006). Such evidence suggests that many *R*-gene structures evolved before the divergence of genomes A and B and subsequent *Solanum* speciation. The presence of markers RB-226 and RB-820 consistently corresponds to the functional gene *Rpi-blb1*; whereas marker RB-629 may tag RB structural homologues poorly related to LB resistance (see also Fadina *et al.*, in press).

Table 2. Distribution of markers *R* genes in wild *Solanum* species

Series and species	The total number of screened accessions	The numbers of accessions comprising the particular markers						
		R1-1205	R2-2500	R3-1380	R3b-378	RB-629	RB-226	Blb1-820
<i>Bulbocastana</i> (<i>S. bulbocastanum</i>)	28	0						
<i>Demissa</i> <i>S. demissum</i>	38		n.d.					0
<i>S. hougasii</i>	7							0
<i>Longipedicelata</i> <i>S. hjertingii</i>	6	0		0			0	n.d.
<i>S. polytrichon</i>	12		0				0	
<i>S. stoloniferum</i>	51		0					
<i>Pinnatisecta/Cardiophylla</i> <i>S. cardiophyllum</i>	7	0	n.d.				0	0
<i>S. ehrenbergii</i>	17	0					0	0
<i>S. jamesii</i>	13	0	0	0			0	0
<i>S. pinnatisectum</i>	10	0		0			0	0
<i>Polyadenia</i> <i>S. polyadenium</i>	8	0	n.d.		0		0	n.d.
<i>Tuberosa</i> <i>S. verrucosum</i>	12	0	0	0		0	0	0
<i>S. berthaultii</i>	7		n.d.	0	0		0	0
<i>S. microdontum</i>	6		0		0		0	

 - frequent occurrence of the marker;  - rare occurrence of the marker; n.d. - no data.

When using this evidence for selecting the genotypes of paramount interest for further introgression programs, the crucial issue is the functional identity of newly found CC-NBS-LRR homologues. In some cases, the evidence for new alleles of the *R* genes in orphan *Solanum* species is supported by our data obtained by cloning and sequencing: thus cloned markers R1-1205 and R3a-1380 in wild *Solanum* species were 98-99% similar to the corresponding regions in the prototype genes. Therefore, we presumed that they represented the active genes (Sokolova *et al.*, 2011).

Other laboratories provide even more convincing data from co-expression studies in *Nicotiana benthamiana* and potato transformation (Table 3). To illustrate, the presence of the marker R3a-1380 in *S. stoloniferum*, *S. cardiophyllum*, *S. ehrenbergii*, and *S. microdontum* confirms the evidence by Champouret (2010), who found orthologues of the *R3a* gene in this species. The presence of the marker R2-2500 in *S. bulbocastanum* and *S. hjertingii* (Table 2) is in line with the report that the functional R2/Rpi-blb3 gene is present in these species (Champouret, 2010; Lokossou *et al.*, 2010). The presence of the markers RB-629 and Blb1-820 in the species *S. bulbocastanum*, *S. stoloniferum* and *S. polytrichon* is supported by the evidence from several studies using *Nicotiana* and *Solanum* transformation.

Table 3. Late blight resistance genes in wild *Solanum* species

Genes- prototype	Species where SCAR markers were found	Mapped and cloned genes and their homologues	Genes were cloned from the following species	Co-expression or transient complementation in <i>N. benthamiana</i>	Potato transformation
<i>R1</i>	<i>dms, hou, plt, sto, ber, mcd</i>	<i>R1</i> (Ballvora <i>et al.</i> , 2002; Kuang <i>et al.</i> , 2005)	<i>dms</i> (Ballvora <i>et al.</i> , 2002; Kuang <i>et al.</i> , 2005)	-	<i>R1 dms</i> (Ballvora <i>et al.</i> , 2002)
<i>R2/Rpi-blb3</i>	<i>blb, hou, pnt, hjt, ehr</i>	<i>R2/Rpi-blb3</i> <i>Rpi-abpt</i> <i>R2-like</i> (Lokossou <i>et al.</i> , 2010) <i>Rpi-edn1.1</i> <i>Rpi-hjt1.1, Rpi-hjt1.2, Rpi-hjt1.3</i> <i>Rpi-snk1.1, Rpi-snk1.2</i> (Champouret, 2010)	<i>tub, dms, edn, snk</i> <i>hjt, blb, vrn</i> (Champouret, 2010; Lokossou <i>et al.</i> , 2010)	<i>R2, Rpi-blb3, Rpi-abpt, R2-like</i> (Lokossou <i>et al.</i> , 2010)	<i>R2 Rpi-abpt R2-like</i> (Lokossou <i>et al.</i> , 2010)
<i>R3a</i>	<i>blb, hou, dms, cph, ehr, pld, mcd, plt, sto</i>	<i>R3a</i> (Huang <i>et al.</i> , 2005) <i>Rpi-sto2</i> (Champouret, 2010)	<i>tub, dms</i> (Liu, Halterman, 2006) <i>sto</i> (Champouret, 2010; Sokolova <i>et al.</i> , 2011) <i>blb, cph, hou, plt</i> (Sokolova <i>et al.</i> , 2011)	<i>Rpi-sto2, dms, sem, mcd, sto, cph, ehr</i> (Champouret, 2010)	
<i>R3b</i>	<i>blb, hou, dms, cph, hjt, jam, pnt, ver, sto, plt</i>	<i>R3b</i> (Li <i>et al.</i> , 2011)	<i>dms</i> (Lokossou <i>et al.</i> , 2010)	-	
<i>RB/Rpi-blb1</i>	<i>blb, hou, dms, cph, hjt, jam, pnt, pld, ber, sto, plt, mcd</i>	<i>RB/Rpi-blb1</i> (van der Vossen <i>et al.</i> , 2003; Song <i>et al.</i> , 2003) <i>Rpi-bt1</i> (Oosumi <i>et al.</i> , 2009) <i>RB ver</i> (Liu, Halterman, 2006) <i>Rpi-sto1, Rpi-pta1, Rpi-plt1</i> (Wang <i>et al.</i> , 2008)	<i>blb, sto, ver, plt</i> (Wang <i>et al.</i> , 2008; Liu, Halterman, 2006; van der Vossen <i>et al.</i> , 2003; Song <i>et al.</i> , 2003; Oosumi <i>et al.</i> , 2009; Vleeshouwers <i>et al.</i> , 2008)		<i>RG3</i> (van der Vossen <i>et al.</i> , 2003), <i>RB</i> (Song <i>et al.</i> , 2003; Vleeshouwers <i>et al.</i> , 2008) <i>Rpi-bt1</i> (Oosumi <i>et al.</i> , 2009) <i>RBver</i> (Liu, Halterman, 2006)

ber - *S. berthaultii*, *cph (ehr)*- *S. cardiophyllum*=*S. ehrenbergii*, *dms* - *S. demissum*, *edn* - *S. edinense*, *hou*- *S. hougasii*, *hjt* - *S. hjertingii*, *jam* - *S. jamesii*, *tbr* -*S. tuberosum*, *mcd* – *S. microdontum*, *pnt* - *S. pinnatisectum*, *plt* - *S. polytrichon* = *S. stoloniferum*, *pld* - *S. polyadenium*, *sem* – *S. semidemissum*, *snk* - *S. schenckii*, *sto* - *S. stoloniferum*, *tbr* - *S. tuberosum*, *vrn* – *S. vernei*, *ver* - *S. verrucosum*.

CONCLUSIONS

The present results confirm the previously known phytopathological data on the presence of *R1*, *R2*, *R3a* and *R3b* genes, initially identified in *S. demissum*, beyond the series *Demissa*. Structural homologues of the *RB/Rpi-blb1* gene, which was initially identified in genome B of *S. bulbocastanum* and *S. stoloniferum*, were found in many species comprising genomes A and B. The presence of markers RB-226 and RB-820 consistently indicates the functional gene *Rpi-blb1*. These results imply that the markers described in this communication can be used to search for new *R* genes homologues in genetic collections and to estimate their breeding potential.

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Characterization of Russian *Phytophthora infestans* populations: DNA fingerprinting and SSR analysis

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SUMMARY

The microsatellite analysis of 86 *Phytophthora infestans* isolates collected in 2008-2011 from potato and tomato fields of six regions of Russian Federation (Moscow, Nizhni Novgorod, Leningrad, Kostroma, Smolensk, and Astrakhan) has been first carried out using 12 common SSR primers. In addition, the RG57 fingerprinting of 74 isolates from the same collection has been performed along with the mtDNA haplotyping, allozyme analysis (*Pep* and *Gpi* loci), and determination of the mating type and metalaxyl resistance. The total number of revealed RG57 genotypes is 25 varying from 1 to 11 per population; their comparison with the data obtained for the Russian populations of 1997-1998 reveal one common genotype. The total number of SSR multilocus genotypes (MLGs) makes 47; the number of MLGs per population varied from 2 to 16. In both cases, the Moscow "potato" population was the most diverse (11 and 16 RG57 and SSR MLGs, respectively). According to the SSR study, both "tomato" populations are the most different to other populations; populations from the central Russia are the most similar. The obtained data revise our earlier ideas about the uniformity of some of the populations studied and are planned to be added to the Eucablight database.

KEYWORDS

late blight, potato, tomato, RG57 fingerprinting, SSR

INTRODUCTION

Potato is one of the most important crops in Russia. The average annual consumption of potato per a person in Russia makes 120-130 kg; thus, this crop remains a „second bread“ for many Russian people and significantly influences the food safety of Russia, which is considered as an important player in the field of the potato production (the annual potato production in Russia makes more than 30 mln. tons).

Potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) De Bary, is the most devastating potato disease able to significantly reduce the crop productivity during epiphytoties. In addition, tuber infection negatively influences the storage quality; as a result, total yield losses caused by this pathogen can reach 60%. In the XIX century the late blight epiphytosity caused the famous Great Famine in Ireland resulted in the death of approximately one million and the emigration of a million more people; as a result, the population of Ireland fell by 20-25% (Ross, 2002). In the last decades, new agrotechnical and disease control methods, such as the use of certified seeds, breeding programs, crop rotation, and highly-effective fungicides, significantly decreased potato yield losses caused by late blight. At the same time, due to high pathogen variability and an introduction of new strains by a potato shipment from central Mexico to Europe, the European population of *P. infestans* has undergone significant changes (Fry *et al.*, 1993; Gisi and Cohen, 1996). During 1980-1985 the „old“ pathogen population was almost completely replaced by a new one, which included earlier unknown clones (Spielman *et al.*, 1991; Fry *et al.*, 1992) and the „new“ mating type (A2), earlier observed only in the Central Mexico (Fry *et al.*, 1991). New populations could also reproduce sexually thus increasing the population diversity and provided the generation of oospores, able to overwinter on plant debris in the soil.

An increased epidemiological potential of *P. infestans* resulted in a sharp decrease in the crop protection efficiency. To develop new efficient late blight control strategies, it is necessary to know the features of the pathogen populations, their genotypic structure, and to forecast possible changes of these parameters in the future.

The purpose of this study was to extend the previous characterization of Russian *P. infestans* populations, sampled in 2008-2011 and characterized by a set of common markers (Statsyuk *et al.*, 2010), using such methods as the RG57 fingerprinting and SSR analysis.

MATERIALS AND METHODS

***P. infestans* isolates.** A set of 100 isolates, collected from commercial potato (*P*) and tomato (*T*) fields of the Leningrad, Moscow, Nizhni Novgorod, Astrakhan, Kostroma, and Smolensk regions and stored in the State Collection of Phytopathogenic Microorganisms of the All-Russian Research Institute of Phytopathology (ARRIP), was sent to US to replenish plant pathogen collections of the USDA-ARS Genetic Improvement of Fruits and Vegetables Laboratory (GIFVL, Beltsville, MD) and USDA-ARS Horticultural Crops Research Laboratory (HCRL, Corvallis, OR). Among 100 isolates sent, 74 and 86 were successfully restored in the GIFVL and HCRL, respectively. Within the framework of a joint research project, two first authors of this paper visited the GIFVL and HCRL, respectively, to perform the RG57 and SSR genotyping of the isolates. The SSR analysis was performed at the HCRL, whereas the RG57 fingerprinting was performed at the GIFVL. In addition, some other phenotypic and genotypic characteristics of the studied set of isolates, including the mating type, mtDNA haplotype, genotypes at two allozyme loci, and metalaxyl sensitivity, were repeatedly determined in the GIFVL.

Allozyme analysis. Genotypes at two loci, *Gpi* (glucose-6-phosphate isomerase, GPI) and *Pep* (peptidase, PEP) were determined using cellulose acetate gel electrophoresis as recommended by Helena Laboratories inc. (Hebert, Beaton, 1993) with some modifications. The genotypes of unknown isolates were determined by comparison with reference isolates of the US-1 (*Gpi* 86/100, *Pep* 92/100) and US-8 (*Gpi* 100/111/122, *Pep* 100/100) types.

Mitochondrial DNA haplotype identification was carried out by PCR-RFLP according to the common procedure (Griffith & Shaw, 1998).

Mating type determination. Isolates were grown on rye agar with known reference strains of the A1 (US940501) and A2 (US 940480) mating types. Two plates were used for each pairing; 4-mm mycelial plugs of the tested and reference isolates were placed approximately 20-30 mm apart. The plates were incubated at 15°C in the darkness for 7-10 days, and then microscoped for the presence of oospores where the two colonies interacted. If the tested isolate generated oospores only with the A1 or A2 isolate, it was referred to the A2 or A1 type, respectively.

Metalaxyl sensitivity assessment. The metalaxyl resistance test was performed according to the methodology used by Shattock (1988) and Deahl and Demuth (1993). Rye B agar plugs with mycelium obtained from the edge of colonies of each isolate of *P. infestans*, previously kept in darkness at 18°C for 6 days, were placed simultaneously on rye B agar enriched with 10 mg/l of metalaxyl and rye B agar plates without fungicide (control); each plate contained 3 plugs with mycelium. A US940501 strain of *P. infestans*, previously described as sensitive to metalaxyl, was used as a reference strain. The *in vitro* growth of each strain was measured after an incubation period of 6-10 days at 18°C using the following equation:

$$PC = (CS_{met}/CS_{contr}) \cdot 100\%,$$

where PC is the percentage of growth and CS_{met} and CS_{contr} represent the average colony size on the metalaxyl-containing and control medium, respectively (the average diameter of a colony (mm) minus 5 mm (diameter of a mycelium-containing agar plug)). An isolate was qualified as metalaxyl-resistant (R) if the PC value was equal to or greater than 60%, as moderately resistant (MR) – if this value was between 10 and 60%, and as sensitive (S) – if this value was below 10 %.

RG57 fingerprinting. DNA fingerprinting using the moderately repetitive probe RG57 was carried out on a subset of 75 isolates using the method described by Goodwin *et al.* (1992) with some modifications. The RG57 insert was PCR amplified with the oligonucleotide primers M13 (forward and reverse). The product was purified using the GeneJET PCR Purification Kit (Fermentas) and labeled with the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). *P. infestans* DNA was extracted using the DNeasy Plant Mini Kit (Quiagen) and transferred to a positively charged nylon membrane (Roche). The manufacturer's instructions for the above-mentioned kits were followed for membrane transfer, Southern hybridization, and nucleic acid detection. The presence (1) or absence (0) of bands 1-25 was scored.

SSR genotyping. A subset of 87 isolates was studied using simple sequence repeat (SSR) analysis. The SSR analysis was conducted using the protocol from EUCABlight found at <http://www.eucablight.org/EucaBlight.asp> and developed by Dr David Cooke (James Hutton Institute) using previously published primer combinations (Knapova and Gisi, 2002; Lees *et al.*, 2006). Fluorescently labeled primers for a 12-plex assay, decided on between Dr David Cooke (James Hutton Institute, Dundee, UK) and Dr Theo van der Lee (Plant Research Institute, Wageningen, The Netherlands) and used in this study, were the following: D13, G11, Pi04, Pi4B, Pi63, Pi70, SSR2, SSR3 (Pi02), SSR4, SSR6b, SSR8, and SSR11.

PCR products were sized using capillary electrophoresis on a 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) using the internal size-standard LIZ 500 (Applied Biosystems). Results were analyzed using GeneMapper 3.7 software packages (Applied Biosystems). Genotypes were determined by comparing of fragment sizes with isolates previously genotyped. Genotyping was replicated for a subset of isolates with independent DNA extractions, PCR, and sizing of fragments. Reproducibility of novel allele sizes was confirmed.

Data treatment. Population structure was studied by analyzing allele frequencies, gene diversity, genetic distance, clonality and genetic differentiation. A multilocus genotype (MLG) was constructed for each isolate by combining data for all 12 SSR loci (Goss *et al.*, 2009).

Nei's gene diversity *H_{et}*, also referred to as heterozygosity (Nei, 1978; Nei, 1987), was calculated to adjust for selfing. Nei's gene diversity is particularly useful given that it is applicable to organisms of different ploidy levels or reproductive systems including selfing (Hedrick, 2000). *H_{et}* was calculated in TFPGA (Tools for Population Genetic Analyses, version 1.3; Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ) and adjusted for selfing by multiplication with $(n-1)/n$ where *n* equals sample size (Nei, 1987).

Cluster analysis was based on allele frequencies observed for populations or populations. Trees were constructed using the unweighted pair-group method of averages (UPGMA) algorithm from Nei's unbiased genetic distance matrix (Nei, 1978). Statistical support for phenogram branches was obtained using 1,000 bootstrapped samples using TFPGA.

Discriminant analysis of principal components (DAPC) was performed using the adegenet 1.3-4 package (Jombart, 2008; Jombart *et al.*, 2010).

RESULTS AND DISCUSSION

Mating type and metalaxyl resistance. The data on the mating type distribution are shown in Tables 1 and 2. The A1 mating type predominated in the Moscow *P*, Kostroma *P*, Astrakhan *T*, and Leningrad *P* populations; the last three of them were represented by only this mating type. The Moscow *T* and Smolensk *P* populations were represented by the A2 mating type (excepting one Smolensk isolate belonging to the A1 mating type). Finally, the Nizhnii Novgorod *P* population contained equal number of A1 and A2 isolates.

The data on the metalaxyl resistance (MR) of the examined populations are shown on Fig. 1. Susceptible isolates predominated in five populations, two of which (Moscow *T* and Kostroma *P* populations) were represented by only these isolates. Resistant and moderately resistant isolates predominated in two *P* populations (Nizhnii Novgorod and Smolensk regions); in addition, moderately resistant isolates formed a half of the studied sampling from the Leningrad region.

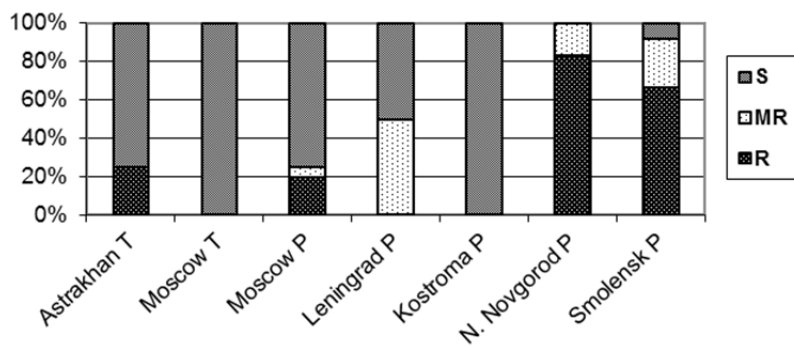


Figure 1. Frequency of *P. infestans* strains with different metalaxyl resistance levels in different regions of European Russia. *S*, susceptible strains, *MR*, moderately resistant strains, *R*, resistant strains; *P* and *T* mean "potato" and "tomato" populations, respectively

Allozyme analysis and mtDNA genotypes. The results of the analysis for the *Gpi* locus showed that all isolates tested were homozygous (100/100), excepting one *P* isolate, collected in 2010 in the Moscow region, which has the genotype 86/86. These data correspond to the earlier performed study shown a very low variability of this marker in Russian populations of *P. infestans* (Elansky, S.N. *et al.*, 2001). The vast majority of isolates were also homozygous at the *Pep1* locus (100/100) excepting two *P* isolates collected in the Moscow region in 2008 and 2010 and having the *Pep* 92/100 genotype that also corresponds to our earlier studies.

Like in the previous studies, only two mtDNA haplotypes (Ia and IIa) were revealed among the tested isolates (Table 1). Haplotype Ia predominated for the most of the studied populations; at least four of them (Leningrad *P*, Astrakhan *T*, Smolensk *P*, and Moscow *T* populations) included only this genotype.

RG57 analysis. According to the worldwide data, a total of 25 bands can be detected by the RG57 analysis of *P. infestans* isolates. In the case of the analyzed Russian isolates, five of these bands were not detected (bands 4, 11, 12, 15, and 18), and five were present in all isolates (bands 1, 13, 14, 24, and 25). The remaining 15 loci (bands 2, 3, 5, 6, 7, 8, 9, 10, 16, 17, 19, 20, 21, 22, and 23) were polymorphic.

Each revealed genotype was designated by a code, using **RU** for Russia followed by two letters designating the region of collection, one letter designating the host plant (**P** and **T** for potato and tomato, respectively) and then a genotype number.

The total number of different RG57 genotypes revealed among 74 samples was 25. The total number of multilocal genotypes (MLGs) determined on the basis of the RG57 fingerprints, *Pep1* and *Gpi* loci, mtDNA haplotype, and mating type, was 29 (Table 1). The maximum genotypic diversity was observed for the Moscow *P* population (14 MLGs/20 samples) followed by the Kostroma *P* (4 MLGs/6 samples) and Nizhnii Novgorod *P* (4 MLGs/6 samples) populations. In the case of the Smolensk *P* population, the majority of isolates had the same MLG (RU_SM-P1); two other genotypes were represented by one isolate each. The Leningrad *P* population included two genotypes, RU_LE-P1 and RU_LE-P2. All isolates belonging to the RU_LE-P2 genotype were metalaxyl-sensitive, whereas all but one isolates, belonging to the RU_LE-P1 genotype, were moderately resistant. In the case of the Astrakhan *T* population, two genotypes were revealed; the dominating RU_AS-T1 genotype was represented by only metalaxyl-sensitive isolates, whereas the second genotype (RU_AS-T2) included only resistant isolates. It is interesting that the RU_AS-T2 and RU_LE-P1 MLGs were found to be identical, in spite of their origin from distant regions and different host plants. Finally, the Moscow *T* population was represented by only one genotype and was uniform concerning all other above-mentioned characteristics.

Table 1. RG57-based multilocal genotypes (RG57-MLGs) and origin of Russian *P. infestans* populations, 2008-2011

RG57-MLG name	Number of isolates	RG57 fingerprint	Mating type	mtDNA haplotype	PEP genotype	Metalaxyl sensitivity
Moscow region, potato (20 isolates)						
RU_MO-P1	3	110 000 000 100 110 100 011 001 1	A1	Ia	100/100	S
RU_MO-P1_1	1	110 000 000 100 110 100 011 001 1	A1	IIa	100/100	S
RU_MO-P2	2	101 010 100 100 110 100 011 001 1	A2	Ia	100/100	R
RU_MO-P2_1	1	101 010 100 100 110 100 011 001 1	A2	IIa	100/100	R
RU_MO-P3	1	111 000 100 100 110 100 011 001 1	A1	Ia	92/100	S
RU_MO-P4	1	101 010 101 100 110 000 011 001 1	A1	IIa	100/100	R
RU_MO-P5	1	111 000 101 100 110 100 011 001 1	A2	Ia	100/100	S
RU_MO-P6	2	101 010 101 100 110 010 011 001 1	A1	Ia	100/100	S
RU_MO-P7	1	111 011 010 100 110 100 011 001 1	A1	IIa	100/100	S
RU_MO-P8	1	110 011 010 100 110 100 001 101 1	A1	IIa	100/100	MR
RU_MO-P9	1	111 010 100 100 110 000 011 001 1	A2	Ia	100/100	S
RU_MO-P10*	1	110 010 000 100 110 100 011 001 1	A1	Ia	92/100	S
RU_MO-P10_1	2	110 010 000 100 110 100 011 001 1	A1	Ia	100/100	S
RU_MO-P11	2	110 010 011 100 110 100 011 001 1	A2	Ia	100/100	S
Moscow region, tomato (9 isolates)						
RU_MO-T1	9	110 011 000 000 110 000 001 001 1	A2	Ia	100/100	S
Kostroma region, potato (6 isolates)						
RU_KO-P1	1	111 011 101 100 110 100 011 001 1	A1	IIa	100/100	S
RU_KO-P2	2	110 010 001 100 110 000 011 011 1	A1	Ia	100/100	S
RU_KO-P3	2	110 010 100 100 110 100 011 001 1	A1	IIa	100/100	S
RU_KO-P4	1	110 010 000 100 110 100 011 001 1	A1	Ia	100/100	S
Leningrad region, potato (13 isolates)						
RU_LE-P1**	8	110 011 000 100 110 000 011 001 1	A1	Ia	100/100	S
RU_LE-P2	5	100 010 100 100 110 000 111 001 1	A1	Ia	100/100	S, MR
Nizhni Novgorod region, potato (6 isolates)						
RU_NN-P1	2	100 011 000 100 110 100 111 001 1	A1	Ia	100/100	MR, R
RU_NN-P1_1	2	100 011 000 100 110 100 111 001 1	A2	Ia	100/100	R
RU_NN-P2	1	110 010 001 100 110 100 010 001 1	A1	IIa	100/100	R
RU_NN-P3	1	100 010 001 100 110 100 001 001 1	A2	IIa	100/100	R
Smolensk region, potato (12 isolates)						
RU_SM-P1	10	111 011 100 100 110 100 001 001 1	A2	Ia	100/100	MR, R
RU_SM-P2	1	111 011 101 100 110 100 001 101 1	A2	Ia	100/100	R
RU_SM-P3	1	100 010 000 100 110 100 011 001 1	A1	Ia	100/100	R
Astrakhan region, tomato (8 isolates)						
RU_AS-T1	6	110 011 000 100 110 100 011 001 1	A1	Ia	100/100	S
RU_AS-T2**	2	110 011 000 100 110 000 011 001 1	A1	Ia	100/100	R

*This MLG is also characterized by the *Gpi* 86/86 genotype; all other MLGs have the *Gpi* 100/100 genotype.

** These two MLGs (RU_LE-P1 and RU_AS-T2) are identical, though originate from different regions and host plants.

A comparison of the obtained data with the results of the earlier RG57 data on Russian *P. infestans* populations of 1997-1998 (Elansky, S.N. *et al.*, 2001) revealed only two coincidences, both for the Moscow region: RU_MO-P2 and RU_MO-P2_1 MLGs, collected in 2008 and differing only in their mtDNA haplotypes (Ia and IIa, respectively), corresponded to the MO-12 and MO-6 MLGs revealed in the earlier study. In addition, comparing the data obtained for the Moscow region, one can see that in both studies this region is characterized by a very high genotypic diversity of isolates: 23 unique genotypes were revealed among 27 isolates in the previous study, and 11 genotypes out of 20 isolates were revealed in the present study. Such high diversity can be explained by the fact that the Moscow region represents one of the largest potato-growing regions and the largest importer of a potato seed material in Russia that provides a high probability of the introduction of new *P. infestans* genotypes with infected seed potato.

A comparison of our data with the most common RG57 genotypes of US and European *P. infestans* populations showed a very low coincidence. Genotypes RU_NN-P1 and RU_NN-P1_1 have a RG57 pattern identical to that of the genotype NL-140 from Netherlands (Zwankhuizen, 1998). The genotype RU_MO-P4 is identical to the genotype NL-146 (Netherlands, Zwankhuizen, 1998). The genotype RU_SM-P3, which differs from other Smolensk genotypes in a number of characteristics, was similar to the genotypes RF008 (UK, Day *et al.*, 2004), NI-1a (Northern Ireland, Cooke *et al.*, 2006) and US-18 (US, Wangsomboondee *et al.*, 2002); however, in the last case it has a different mating type. The genotype RU-SM-P1, representing the majority of Smolensk isolates was similar to the genotype NL-72 (Netherlands, Zwankhuizen, 1998), but has another mating type; the same situation was found in the case of the RU_MO-P2 and RF060 (UK, Day *et al.*, 2004) genotypes.

SSR genotyping. According to the obtained results, Russian *P. infestans* populations are moderately diverse showing 2-19 multilocus genotypes per a region (Table 2). The total number of detected MLGs was 47; no any coinciding genotypes were revealed between different regions and host plants. A comparison of the obtained results with the data on the basic clonal lineages in Europe (D.E.L. Cooke, personal communications) did not reveal any coincidences.

Among the most representative MLGs, the largest one (MLG1) was revealed in the Smolensk *P* population (12 out of 13 samples) followed by the MLG2 (Moscow *T* population, 10 out of 14 samples), and MLG3 (Leningrad *P* population, 5 out of 14 samples). Four MLGs were represented by 3-4 samples. All other MLGs were represented by 1-2 samples.

Table 2. Number of multilocus genotypes in Russian *P. infestans* populations determined by the SSR analysis

Region	Host	Sample size	MLG (SSR)	A1:A2	Het
Astrakhan	Tomato	8	8	9:0	0.405
Kostroma	Potato	6	6	10:1	0.561
Leningrad	Potato	14	7	15:0	0.493
Moscow	(Total)	38	18	15:23	0.513
	Potato	24	16	15:9	
	Tomato	14	2	0:14	
Nizhnii Novgorod	Potato	8	6	9:3	0.589
Smolensk	Potato	13	2	1:13	0.291

MLG (SSR) = multilocus genotype based on the SSR analysis.

Het = unbiased average heterozygosity based on SSR analysis.

The maximum number of alleles (6) was detected for the D13, G11, and SSR4 loci (Table 3). The minimum number of alleles (2) was detected for the SSR2, SSR6b, and Pi70 loci; the last one was the most monomorphic, since 81 out of 86 isolates tested had the same genotype.

Table 3. Alleles detected for the used SSR markers

Marker	Detected alleles	Marker	Detected alleles
G11	142, 154, 156, 160, 162, 206	Pi63	270, 273, 279
D13	Null, 118, 136, 152, 154, 156	SSR11	331, 341, 356
SSR4	285, 289, 291, 293, 295, 297	SSR8	260, 264, 266
Pi04	160, 166, 168, 170	Pi70	192, 195
SSR3	258, 264, 266, 268	SSR2	173, 175
Pi4B	205, 213, 217	SSR6b	242, 244

The dendrogram constructed using the UPGMA algorithm from Nei's unbiased genetic distance matrix showed that the tomato growing area in Astrakhan is most different from the other regions where either tomatoes and potatoes or just potatoes are grown (Fig. 2). The Moscow *T* population also was well differentiated from all *P* populations. Populations in central Russia were the most similar based on genetic distance.

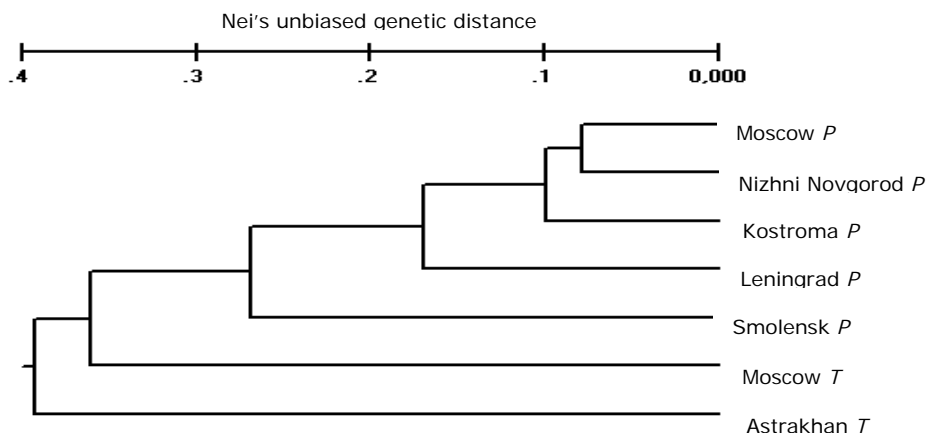


Figure 2. Dendrogram of Nei's unbiased genetic distance (Nei, 1978) for Russian populations of *Phytophthora infestans*. Shown are bootstrap support values greater than 50% based on 1,000 samples. Names of populations are in bold if more than 3 isolates were observed in a cluster from that population

The discriminant analysis of principal components (DAPC) showed that the populations studied are well differentiated by region and crop; the *T* populations are clustered separately (Fig. 3).

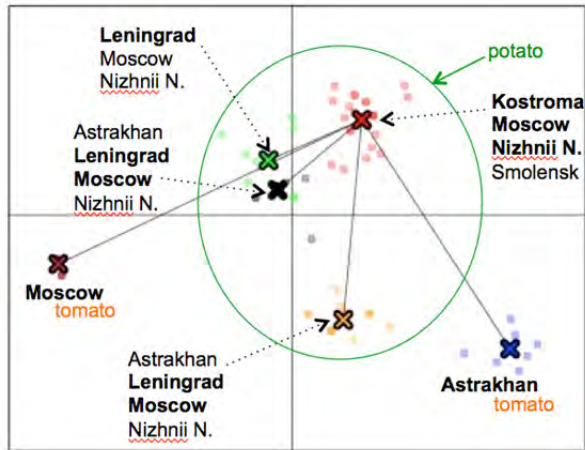


Figure 3. A scatter plot showing the first two principal components of a discriminant analysis of principal components (DAPC) used to infer population structure. The plot shows individual strains based on multilocus genotypes determined by SSR analysis. The crosses show the center of a cluster and the lines denote distances of a minimum spanning tree based on the squared distances between populations within the space

CONCLUSIONS

The obtained molecular data revise our earlier ideas about the uniformity of some of the studied populations. For example, according to the earlier obtained phenotypical and genotypical data (Statsyuk *et al.*, 2010), Astrakhan *T* and Leningrad *P* populations were considered to be uniform; however, new data revealed they include different genotypes.

The analysis of the SSR data showed that both *T* populations are the most different to other Russian populations; a good differentiation of genotypes by region and crop was revealed.

Both RG57 and SSR data showed the majority of the genotypes revealed are unique; in both cases, a high diversity level was confirmed for the Moscow region representing the largest potato seed importer in Russia. Only several revealed RG57 genotypes coincided with those revealed in Europe; all others seem to be unique for Russia. In the case of SSR genotypes, the comparison with the European data did not reveal any coincidences.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this paper (RG57 and SSR results for all isolates tested) can be found online at the ResearchGate page of the first author:

https://www.researchgate.net/profile/Natalia_Statsyuk/?ev=hdr_xprf

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Does *Phytophthora infestans* exhibit host specialisation on tomato in Great Britain?

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SUMMARY

A study was carried out with the aim of establishing whether *Phytophthora infestans* exhibits host specificity on tomato in Great Britain. Samples of *P. infestans* were collected from tomato crops throughout Great Britain, and genotyped using an 11 SSR marker set adapted from that published by Li *et al.* (2013). Significant differences were observed between the samples collected from tomato and published data for *P. infestans* genotypes collected from British potato crops, with fewer 6_A1 and more 23_A1 and Miscellaneous genotypes on tomato. Further work is needed to establish the reasons for this.

KEYWORDS

Phytophthora infestans, host specificity, tomato, SSR, Great Britain, UK, 23_A1

INTRODUCTION

In Great Britain, *P. infestans* infects potato (*Solanum tuberosum*) and tomato (*S. esculentum*). It is widely assumed that *P. infestans* overwinters on infected potato tubers, and then spreads to tomato during the summer. The British *P. infestans* population on potato comprises of many genetically and pathotypically distinct clonal lineages of both A1 and A2 mating types (Cooke *et al.*, 2007). Various examples of host specialisation have been observed worldwide (e.g. Goodwin *et al.*, 1992; Oyarzun *et al.*, 1998; Suassuna, Maffia & Mizubuti, 2004), but no empirical studies have been carried out in Great Britain. It was hypothesised that the summer tomato-host *P. infestans* population would comprise of a subset of the potato-host population, but may have a different frequency distribution of genotypes.

MATERIALS AND METHODS

Sample Collection

Tomato-derived *P. infestans* samples were collected from throughout Great Britain by appealing to readers of Kitchen Garden magazine and members of the charity Garden Organic to send samples from infected plants by post.

Fragment Analysis

DNA was extracted using Qiagen DNeasy Plant Mini DNA extraction kits (Qiagen no. 69106). SSR genotyping was conducted with eleven dye-labelled SSR markers (adapted from Li *et al.*, 2013) amplified in two multiplex PCR reactions. Marker SSR4 was not used as it did not amplify sufficiently strongly to score reliably, and further work is needed to optimise the adapted panels. Fragment analysis was carried out using a Beckman-Coulter CEQ8000 DNA analyser, with 13_A2, 6_A1, and 23_A1, 1_A1, 2_A1 and 8_A1 samples supplied by the James Hutton Institute included in the analysis as reference isolates. Scoring was carried out manually, designating the fragments as alleles defined in Li *et al.* (2013).

Statistical Analysis

The R applications POLYSAT and APE (R Project, 2013) were used to calculate Bruvo Distances (Bruvo *et al.*, 2004) between samples and to cluster similar genotypes. The SSR profiles were compared with published SSR profiles of common *P. infestans* clonal lineages (Li *et al.*, 2013) to ascertain if samples belonged to known clonal lineages. A Chi-Squared goodness of fit test was used to compare the frequency distribution of clonal lineages making up the tomato-host sample with published data for the national potato-host population from both years.

RESULTS AND DISCUSSION

DNA was obtained from 17 tomato-derived *P. infestans* isolates collected in 2011, and from 36 collected in 2012. Genotypes 13_A2 and 23_A1 were dominant amongst the samples collected, with unique Miscellaneous isolates making up much of the remainder. Two distinct groups of genetically similar isolates (separated by a Bruvo distance of less than 0.1) were identified, but they did not closely resemble any of the common genotype fingerprints published by Li *et al.* (2013).

The population of tomato isolates from both years appears strikingly different in composition from the national population of (mainly) potato isolates collected as part of the "Fight against Blight" (British Potato Council, 2013), shown in *Fig. 1*. 6_A1 genotypes make up 80% and 60% of all potato isolates nationally in 2011 and 2012, but only 6% of tomato isolates in 2011 (one isolate) and none in 2012.

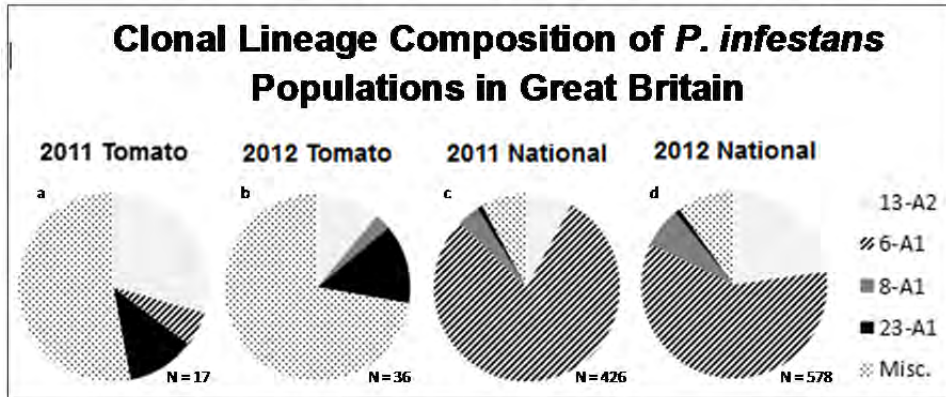


Figure 1. Genotypic composition of tomato-host *P. infestans* sample in 2011 (a) and 2012 (b) and the composition of the national samples (mostly potato-host) held by the James Hutton Institute (c, d) (Cooke, 2013). Chi-square tests were carried out after pooling 23_A1, 8_A1, and Misc. in order to achieve adequate minimum expected values. 2011 $\chi^2 = 50.04$, $p < 0.001$. 2012 $\chi^2 = 101.6$, $p < 0.001$

In both years, the composition of tomato population differed significantly from that of the national (potato) population (2011: $\chi^2 = 50.04$, $p < 0.001$; 2012: $\chi^2 = 101.6$, $p < 0.001$). 8_A1 and 23_A1 counts were integrated into the "Misc." counts in order to satisfy minimum expected value requirements of the Chi-squared test.

Specific genotypes such as 6_A1 and 23_A1 differed in frequency between the two hosts, and this may be explained by differential virulence (Legard, Lee & Fry, 1995) and rate of growth and sporulation (Suassuna, Maffia & Mizubuti, 2004) on different hosts. 23_A1 is uncommon on potato, but appears to be one of the most frequent genotypes on tomato. Reasons why the numerous diverse "Misc." isolates were so much more prevalent on tomato than potato are unclear. However, it has been reported anecdotally (Shaw, 2013) that "Misc." isolates tend to be more common in allotments and home gardens (the source of the tomato isolates used here) than in agricultural settings from which most of the potato isolates were derived.

CONCLUSIONS

The results of this study are limited in scope, but do suggest that the *P. infestans* population on tomato in Great Britain is distinct from the total national population in its genotypic composition. Further work is needed to establish the reality and cause of this difference. Genotype 23_A1 may be an important genotype on tomato, and further work to investigate the phenotypes of tomato-derived isolates may establish if and why this is the case.

ACKNOWLEDGEMENTS

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Early Blight: Pathogenicity of *Alternaria solani* and *Alternaria alternata* and fungicidal activity

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Early Blight: Pathogenicity of *Alternaria solani* and *Alternaria alternata* and fungicidal activity



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Introduction

In recent years, several studies were conducted within BASF to understand the Early blight disease complex in potato and tomato. An important key aspect of these studies was the question about the pathogenicity of *Alternaria solani* and *Alternaria alternata*. Furthermore, the fungicidal activity of different compounds was evaluated under different conditions.

Trial objectives

- The pathogenicity tests were conducted with different strains of *A. solani* and *A. alternata* in the greenhouse and under field conditions. The pathogenicity was evaluated on tomato as well as on potato. The results shown in this poster summarize the results of a field pathogenicity test in potato.
- The fungicidal efficacy was evaluated in greenhouse as well as in field test. The results below give a summary about field trials with natural infection on different locations and years.

Results and Conclusions

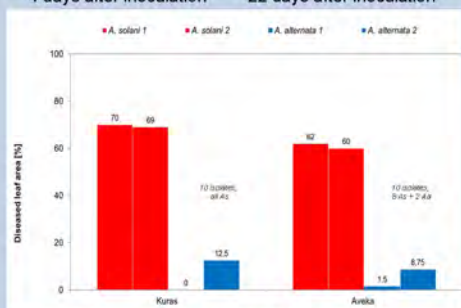


Figure 1: Assessment of a field trial in potato 22 days after inoculation with *A. solani* and *A. alternata*

- In the greenhouse, *A. solani* was highly virulent, *A. alternata* was not or only partially virulent on tomatoes and potatoes.
- In the field, *A. solani* inoculations resulted in disease symptoms already 4 days after inoculation and ended in a very strong disease attack 3 weeks after inoculation. *A. alternata* was in the same test not pathogenic.
- In a few plots inoculated with *A. alternata*, symptoms occurred. Isolates obtained from these lesions were identified as *A. solani*. This indicates that the symptoms were caused by a cross-contamination during the inoculation process with *A. solani*.

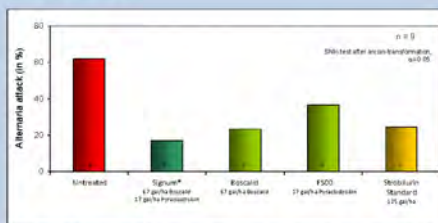


Figure 2: Summary of 9 field trials with natural infection conducted in NL and in DE

- Both active ingredients of Signum® (Boscalid and F500) contribute in the relevant field rates significantly to the efficacy against *Alternaria*.
- Therefore, an efficient disease and resistance management is provided by Signum®.

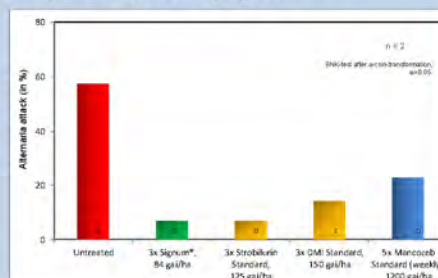


Figure 3: Summary of 2 field trials with natural infection conducted in DE

- Special *Alternaria* fungicides, e.g. Signum®, provide a significant stronger control compared to other products.
- Sprayed in a weekly interval, Mancozeb / Metiram based products contribute significantly to the *Alternaria* control. Therefore, Mancozeb / Metiram based fungicides provide a contribution to the *Alternaria* control strategy.

Evaluation of leaf treatment products to control late blight in organic potato production

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SUMMARY

A selection of copper-free products for the control of late blight (*Phytophthora infestans*) infections in organic potato production was tested in both laboratory and field trials. Among the most promising preparations was a commercial garlic product and a knotweed product, of which the former was almost as effective as copper in *in vitro* assays. Still, their efficacy in the field remains to be confirmed. A mixed or alternating application with copper might particularly show potential for some of the alternative products, and allow a further reduction in the usage of copper fungicides in organic potato production.

KEYWORDS

Phytophthora infestans, leaf infection, disease management, copper-free products

INTRODUCTION

Potato late blight (*Phytophthora infestans*) can cause severe losses in potato yield and quality in organic farming. Still, in organic production *P. infestans* can only be effectively controlled by the application of copper fungicides. Due to their accumulation in the soil and expected detrimental effects on the environment and non-target organisms, a reduction in the usage of copper fungicides is urgently required.

Within the course of a project aiming at the reduction and avoidance of copper in organic farming, trials are being performed investigating the use of different Cu-free products for the control of *P. infestans* leaf infections. The tests will allow the identification of potential alternatives replacing or amending copper based fungicides for leaf treatments in the future, and thus enable both a reduction of yield losses through late blight infection and minimizing the amount of sporangial inoculum deposited on the crop.

MATERIALS AND METHODS

We have tested several commercial and non-commercial organic products and preparations in both *in vitro* leaf assays and field trials. In total, 15 Cu-free alternative products were tested so far (Table 1).

In the *in vitro* abscised leaf assay, freshly picked leaves (single leaflets, cultivar: Agria) were inoculated approx. 2-3 hours after spray application of the product. Products were applied until runoff using the concentration given in Table 1. In our optimized setup a water agar plug kept a 50- μ l-droplet of a sporangial suspension (5 sporangia/ μ l) of *P. infestans* in place, ensuring equal infection pressure in all variants, even when leaf surface properties were altered by the product. Products and preparations were applied according to the manufacturer's recommendations or according to data given in the literature. Leaves were incubated at 15°C for 5 days and disease rated as number of leaves successfully infected and as % leaf area affected, according to an index by Lobato *et al.* (2008): 1 = no lesions; 2 = single spots; 3 = <5% ; 4 = 5–10%; 5 = 10–25%; 6 = 25–50%; 7 = 50–75%; 8 = 75–85%; 9 = 85–95%; 10 = 95–100% of the leaf area showing necrosis. Each test consisted of 10-15 leaflets per product. There were up to 29 independent tests for a particular product/ preparation.

In the field trials in 2012, *Bacillus*, chitosan and the citrus product were tested in two experimental fields. An untreated control and a copper variant (3 kg/ ha) were also included in the tests. Tests were designed in a fully randomized block design with four replications per variant, cultivar Agria or Nicola. Products were applied 6-8 times between June 12 and July 30 (BBCH 79) at the rates given in Table 1.

Table 1. Products tested in abscised leaf assay *in vitro* and in field test

Product type	Active ingredient (product name, if available)	Lab tests, product concentration	Field trial 2012, amount/ ha * application
Preparations based on microorganisms	<i>Aureobasidium pullulans</i> (BoniProtect)	0.1%	-
	<i>Aureobasidium pullulans</i> (BoniProtect forte)	0.1%	-
	<i>Pythium oligandrum</i> (Polyversum)	0.1%	-
	<i>Bacillus subtilis</i> (Serenade)	1%	3.0 l
Preparations based on plant extracts	garlic extract (non-commercial)	1%	-
	commercial garlic product (AMN BioVit)	1%	-
	knotweed product (Regalia Max)	0.25%	-
	commercial citrus extract (ViCare)	0.3%	1.2 l
Mineral substances	test product (n/a)	0.5%	-
	activated water + zeolith/ clioptilolith (Desanol)	1.6%	-
	dolomite clay suspension (DCS)	2%	-
	DCS + 10% calcium hydroxide	2%	-
Pure chemical substances	DCS + 20% calcium hydroxide	2%	-
	Sodium phosphonate (test product)	1%	-
	commercial chitosan (ChitoPlant)	0.1%	0.4 kg
	copper hydroxide (Cuprozin fl.)	0.4%	0.5 kg

RESULTS

Only few of the substances tested in our laboratory assays proved to be effective against *Phytophthora* leaf infections in the lab assay (Table 2). Among the most promising agents was a commercial garlic product which was almost as effective as the copper control with regard to disease incidence and severity. Sodium phosphonates, although highly effective, are currently not acceptable in organic farming due to residues problems. Chitosan and a knotweed product were of intermediate efficacy. Chitosan, however, was shown to be highly variable in its control capabilities. The citrus product also provided fairly good protection, but has been banned from the market in the meantime.

In the field tests, none of the alternative products alone provided sufficient protection for the crop. Both degree of leaf infections and potato yield data did not differ from the untreated controls, while copper hydroxide provided good control and significantly higher yields (data not shown). However, an alternating application of copper and an alternative product (equaling a Cu reduction by 1/3) was almost as effective as the application of copper alone at the full rate in terms of disease severity and yield.

Table 2. Results of the *in vitro* leaf assays of a total of 15 products + control and Cu: mean disease severity (leaf area affected) and disease incidence (number of leaves infected). * denotes significant difference from the control at $p \leq 0.05$ (Dunnnett's Multiple Comparison Test)

Product/ preparation	Number of tests performed	% leaf area affected (scale 1-10)	Number of leaves infected (%)
control	29	6.4	95.8
Cu hydroxide	24	1.2*	12.0*
garlic product	8	1.4*	20.8*
Na phosphonate	18	1.9*	26.3*
citrus product	10	2.6*	49.6*
garlic	6	3.7*	61.2*
knotweed product	14	4.2*	76.8
chitosan	21	4.3*	73.4*
<i>A. pullulans</i>	4	5.8	98.1
<i>A. pullulans</i> forte	4	5.9	100
<i>B. subtilis</i>	10	6.2	82.5
test product	12	6.3	96.5
<i>P. oligandrum</i>	7	6.5	92.7
'activated' water	3	6.8	100
DCS + 10	4	6.8	100
DCS	4	7.0	100
DCS + 20	4	7.6	100

DISCUSSION

The field trials performed in addition to the laboratory assays with a selection of Cu-free leaf treatment products in 2012 showed that even products that provided good protection in *in vitro* tests were not effective under field conditions. Thus, these products alone currently are unlikely to be a full substitute for copper in the medium or short term. Therefore, mixed or alternating

applications of copper and promising Cu-free products will be further tested during the course of the project. However, the promising commercial garlic product and the commercial knotweed product have not yet been tested in the field and are included in the field trials in 2013. In agreement with the manufacturer of the knotweed product, a mixture with a reduced amount of the copper product will also be tested.

Together with other agricultural and technical measures such as leaf removal or seed treatment foliar applications of copper and copper-free products can be part of a management strategy to reduce the extent of leaf infections and to minimize the deposition of sporangial inoculum on the soil surface and the potato crop in organic farming. Such tuber infestations are thought to be among the major pathways of *Phytophthora* inoculum onto the field, serving as starting points for subsequent late blight epidemics (Zellner *et al.*, 2011; Wharton *et al.*, 2012).

ACKNOWLEDGEMENT

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SCAR markers for the *RB/Rpi-blb1* gene of potato late blight resistance

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SUMMARY

The *RB/Rpi-blb1* gene was initially isolated from *Solanum bulbocastanum* and has been successfully employed in potato breeding for durable late blight (LB) resistance. Breeder-friendly DNA markers of this gene would considerably promote introgression of broad-spectrum LB resistance. Using the clonal collection of wild *Solanum* species from several series of section Petota, we compared the frequencies of the markers RB-226, Blb1-820 and RB-629 with the indices of LB resistance for particular clones. RB-226 and Blb1-820 were found only in genome B of *S. bulbocastanum* and *S. stoloniferum* and fairly well predicted LB resistance. In contrast, RB-1223 and its fragment RB-629 were widely distributed in section Petota, and RB-629 was a poor predictor of LB resistance.

KEYWORDS

P. infestans, *Solanum* species, late blight resistance, R genes, SCAR markers.

INTRODUCTION

Highly virulent forms of *P. infestans* have overcome LB resistance of many potato varieties, which until recently were considered highly resistant (Cooke *et al.*, 2012). The transfer of race-specific resistance genes (*R* genes) from wild *Solanum* species plays an important role in breeding new potato cultivars manifesting durable resistance to wide range of *P. infestans* races. A primary step towards such introgression breeding is to identify and evaluate the sources of LB resistance, including race-specific genes conferring resistance. To this end, we use SCAR (sequence-characterized amplified regions) markers, the fragments of *R* genes, which are highly specific and suitable for screening large samples of individual plants. The Mexican diploid species *Solanum bulbocastanum* is well known for its broad-spectrum LB resistance. Four different NBS-LRR genes for LB resistance have been characterized in this species: *RB/Rpi-blb1* and its paralogue *Rpi-bt1*, *Rpi-blb2* and *Rpi-blb3* (Vleeshouwers *et al.*, 2011). A functional orthologue of *Rpi-blb1* was found in the Mexican tetraploid species *S. stoloniferum*, the species currently

including *S. papita* and *S. polytrichon* (Vleeshouwers *et al.*, 2008; Wang *et al.*, 2008). The presence of *Rpi-blb1* in *S. stoloniferum* was suggested to result from common ancestry (Wang *et al.*, 2008).

MATERIALS AND METHODS

Plant materials

We used a clonal collection of wild *Solanum* species section Petota: *S. bulbocastanum*, *S. cardiophyllum*, *S. ehrenbergii*, *S. jamesii*, *S. stenophyllum* (genome B); *S. stoloniferum* and *S. polytrichon* (A1B); *S. verrucosum* (A1); *S. microdontum* and *S. berthaultii* (A3); *S. demissum* and *S. hougasii* (AD) and *S. pinnatisectum* (Api/B). To evaluate LB resistance, plants were grown in glasshouse; detached leaves were infected with a highly virulent complex race (genes R1-R11; compatibility type A1) isolated in the Moscow region and scored against the susceptible cultivar Santé as a control.

DNA extraction

Genomic DNA was isolated from young leaves using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA). DNA samples were quantified at 260 nm with a NanoPhotometer P 300 (IMPLEN, Germany).

PCR amplification

The PCR mix contained 10x PCR buffer, 2 mM MgCl₂, 100 ng of genomic DNA, 0.2 mM dNTP, 1 mM forward and 1 mM reverse primers, and 1 U of *Taq* DNA polymerase (Fermentas, Germany). PCR was run using the following programs: RB-226: one cycle of 7 min at 94°C; 35 cycles of 20 s at 94°C, 20 s at 50°C, 2 min at 72°C; one cycle of 5 min at 72°C; RB-629: one cycle of 3 min at 94°C; 35 cycles of 35 s at 94°C, 30 s at 65°C, 2 min at 72°C; one cycle of 5 min at 72°C; Blb1-820: one cycle of 3 min at 94°C; 35 cycles of 35 s at 94°C, 35 s at 62°C, 2 min at 72°C; one cycle of 5 min at 72°C; PCR products were separated by electrophoresis in 1% (w/v) agarose in 1x TAE buffer for 40 min at 6 V/cm and visualized under UV after staining with ethidium bromide.

SCAR markers

To screen the clonal collection of wild *Solanum* species, we used two SCAR markers: RB-226 (Colton *et al.*, 2006) and Blb1-820 (Wang *et al.*, 2008) recognizing the LRR region of *Rpi-blb1*. Two other markers, RB-629 (Beketova *et al.*, 2007) and RB-1223 (Pankin *et al.*, 2011), represent the CC region of the gene (Table 1, Fig. 1).

Table 1. SCAR markers recognizing gene *RB/Rpi-blb1* and its structural homologues

SCAR markers	Position on the prototype gene (AY336128), bp	References
RB-226	3143-3368	Colton <i>et al.</i> , 2006
Blb1-820	2547-3143	Wang <i>et al.</i> , 2008
RB-629	595-1223	Pankin <i>et al.</i> , 2011
RB-1223	1 - 1223	Pankin <i>et al.</i> , 2011

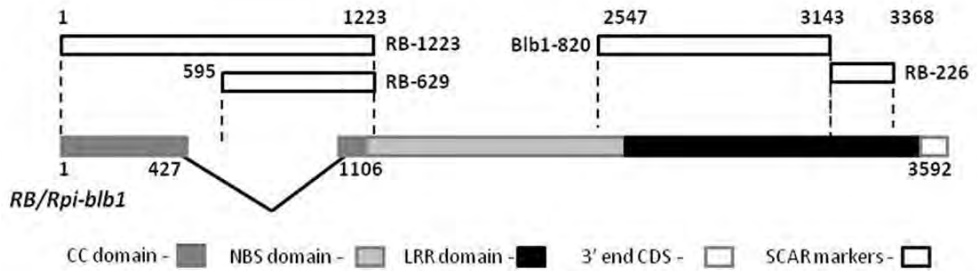


Figure 1. Markers recognizing the functional gene *RB/Rpi-blb1* and its structural homologues.

RESULTS AND DISCUSSION

We compared the evidence on the presence/absence (1/0) of SCAR markers for the *RB/Rpi-blb1* gene with LB resistance of *Solanum* clones (Table 2).

Table 2. Three markers of the gene *RB/Rpi-blb1* in *Solanum* species

Series*	Genome**	Functional <i>RB</i> gene	Species, accessions, clones	RB-629	RB-226	Bib1-820	LB resistance, points***
BUL	B	<i>Rpi-blb1</i> , <i>Rpi-bt1</i>	<i>S. bulbocastanum</i> VIR24866, CD-76-1	1	1	1	9
	B	Same	<i>S. bulbocastanum</i> PI255516, CD-75-1	1	1	1	9
	B	Same	<i>S. bulbocastanum</i> VIR24866, CD-76-3	0	0	0	9
	B	Same	<i>S. bulbocastanum</i> VIR21266, S-137	1	1	1	9
	B	Same	<i>S. bulbocastanum</i> VIR23181, 511-1	0	0	0	1
	B	Same	<i>S. bulbocastanum</i> VIR23181, 511-2	0	0	0	1
	B	Same	<i>S. bulbocastanum</i> VIR19981, 431	0	0	0	9
	B	Same	<i>S. bulbocastanum</i> PI24866, CD-76-5	1	1	1	9
	B	Same	<i>S. bulbocastanum</i> VIR24866, CD-76-3	0	0	0	9
	B	Same	<i>S. bulbocastanum</i> VIR21274, 509-1	0	0	0	9
	B	Same	<i>S. bulbocastanum</i> VIR21274, 509-3	0	0	0	7
	B	Same	<i>S. bulbocastanum</i> VIR21266, 432-1	1	1	0	7
LON	A1B	<i>Rpi-blb1</i>	<i>S. stoloniferum</i> PI275248, CD-360-5	1	1	1	9
	A1B	Same	<i>S. stoloniferum</i> PI255525, CD-356-1	1	0	1	2
	A1B	Same	<i>S. stoloniferum</i> PI255525, CD-356-2	1	0	0	2
	A1B	Same	<i>S. stoloniferum</i> VIR23652, PI195169, D-481	1	0	0	9
	A1B	Same	<i>S. stoloniferum</i> PI365401, CD-362-1	1	1	1	8
	A1B	Same	<i>S. stoloniferum</i> VIR24263, D-99	1	0	0	3
	A1B	Same	<i>S. stoloniferum</i> PI255534, D-482	1	1	1	9
	A1B	Same	<i>S. stoloniferum</i> VIR20106, D-479	1	0	1	8

	A1B	Same	<i>S. polytrichon</i> VIR24463, D-98	0	0	1	4
	A1B	Same	<i>S. polytrichon</i> VIR24462, D-46	1	0	1	5
	A1B	Same	<i>S. polytrichon</i> VIR24298, 318	0	0	0	2
	A1B	Same	<i>S. polytrichon</i> VIR24410, 319	0	0	0	7
PIN	B	-	<i>S. cardiophyllum</i> VIR21301, PI279272, 403	0	0	0	8
	B	-	<i>S. cardiophyllum</i> ehr. VIR23277, PI251725, S-123	0	0	0	4
	B	-	<i>S. cardiophyllum</i> VIR18225, PI274213, S-122	0	0	0	5
	B	-	<i>S. cardiophyllum</i> PI347759, D-609	0	0	0	5
	B	-	<i>S. cardiophyllum</i> VIR24373, PI275213, 425	0	0	0	8
	B	-	<i>S. cardiophyllum</i> VIR23276, PI186548, 411	0	0	0	3
	B	-	<i>S. cardiophyllum</i> VIR24375, PI283062, 426	0	0	0	8
	B	-	<i>S. cardiophyllum</i> VIR18224, D-11	0	0	0	8
	B	-	<i>S. ehrenbergii</i> VIR24373, PI275213i, D-629	0	0	0	2
	B	-	<i>S. ehrenbergii</i> PI275216i, D-616	1	0	0	4
	B	-	<i>S. ehrenbergii</i> PI255520, D-625	0	0	0	4
	B	-	<i>S. ehrenbergii</i> PI275216, D-610	0	0	0	4
	B?	-	<i>S. jamesii</i> PI275265, CD-210-1	0	0	0	7
	B?	-	<i>S. jamesii</i> PI275265, CD-210-2	1	0	0	6
	B?	-	<i>S. stenophyllidium</i> PI24255, D-574	1	0	0	3
	B?	-	<i>S. stenophyllidium</i> PI255530, D-573	1	0	0	2
	Api/B?	-	<i>S. pinnatisectum</i> VIR24239, D-564	0	0	0	9
	Api/B?	-	<i>S. pinnatisectum</i> VIR21955, D-560	1	0	0	5
DEM	A1?D?	-	<i>S. demissum</i> VIR18487, Och14156, CD-130-2	1	0	0	9
	A1?D?	-	<i>S. demissum</i> PI161167, CD-142-2	1	0	0	6
	A1?D?	-	<i>S. demissum</i> VIR15174, S-98	0	0	0	4
	A1?D?	-	<i>S. hougasii</i> VIR24389, CD-188-2	1	0	0	7
TUB	A1	RBver	<i>S. verrucosum</i> PI275260, CD-410	0	0	0	7
	A1	Same	<i>S. verrucosum</i> PI161173, CD-407-1	0	0	0	8
	A1	Same	<i>S. verrucosum</i> VIR24313, PI365404, CD-401-1	0	0	0	5
	A1	Same	<i>S. verrucosum</i> VIR24991, PI195171, CD-408-1	0	0	0	7
	A3	-	<i>S. microdontum</i> VIR5399, D-262-09	1	0	1	9
	A3	-	<i>S. microdontum</i> VIR12658, D-264-09	0	0	0	9
	A3	-	<i>S. berthaultii</i> PI473331, CD-38-2	0	0	0	9

*Series of the section *Petota* Dumort.: BUL – *Bulbocastana* (Rydb.)Hawkes; LON – *Longipedicellata* Buk.; PIN – *Pinnatisecta/Cardiophylla* (Rydb.)Hawkes; DEM – *Demissa* Buk.; TUB – *Tuberosa* (Rydb.)Hawkes; **Matsubayashi, 1991; Hawkes, 1990: ***Detached-leaf assays.

The markers RB-226 and Blb1-820 are specific for *Rpi-blb1*. These markers were found only in *S. bulbocastanum*, *S. stoloniferum* and *S. polytrichon* - and were absent from other *Solanum* species comprising genome B, such as *S. cardiophyllum*, *S. ehrenbergii* and *S. pinnatisectum*. These data confirm the evidence by Wang *et al.* (2008), who found the marker Blb1-820 only in *S. bulbocastanum* and *S. stoloniferum*, and do not fit into the concept that genome B of Pinnatisecta species was the progenitor of genome B in tetraploid Longipedicellata species, such as *S. stoloniferum* (Pendinen *et al.*, 2008). Further experiments will certainly help resolve this contradiction.

The presence of the functional gene *RB/Rpi-blb1* in *S. stoloniferum* was demonstrated by several independent methods (Sokolova *et al.*, 2011; Vleeshouwers *et al.*, 2011). The markers RB-226 and Blb1-820 do not recognize the functional paralogues of *Rpi-blb1*: *Rpi-bt1* and *RBver*. In contrast, two other markers, RB-1223 and its fragment RB-629, were widely distributed in genomes A and B screened in over 100 genotypes of *Solanum* species section *Petota* (Pankin *et al.*, 2011; Fig. 2). However, only few of these *Solanum* accessions manifested high LB resistance. All these accessions are genome B species, except genome A species *S. verrucosum*, with several accessions comprising the functional gene *RBver* (Liu and Halterman, 2006).

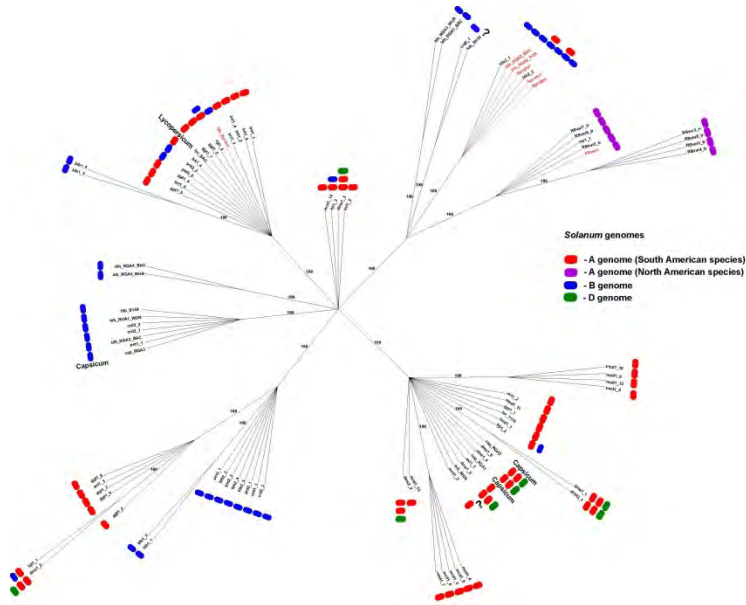


Figure 1. Maximum likelihood phylogenetic tree (strict consensus tree) of the fragments of genes of the RB family corresponding to the CC domain. *Solanum* species: *S. avilesii* (avl); *S. berthaultii* (brt); *S. bulbocastanum* (blb); *S. cardiophyllum* (crd); *S. demissum* (dms); *S. hjertingii* (hjt); *S. microdontum* (mcd); *S. microdontum* ssp. *gigantophyllum* (ggt); *S. papita* (pta); *S. pinnatisectum* (pnt); *S. stoloniferum* (sto); *S. tarjense* (tar); *S. tuberosum* (tub); *S. verrucosum* (ver). Bootstrapping was performed using rapid bootstrap algorithm (Stamatakis *et al.*, 2008), and values are shown at the nodes. Coloured branches correspond to seven tentative groups of the RB homologues established upon the combined evidence from the analysis of CC domain and intron sequences. Numbers from 1 to 35 correspond to the intron subgroups. Already characterised genes conferring resistance to *P. infestans* are denoted by the "+" symbol

In the series comprising 24 clones of *S. bulbocastanum*, *S. stoloniferum* and *S. polytrichon*, the markers RB-226 and Blb1-820 are fairly closely related to LB resistance (the Spearman's correlation coefficient of 0.426 is significant at the 5% level). Of special interest are several exceptions in this pattern. Four clones of *S. bulbocastanum* and two of *S. stoloniferum* were devoid of the markers RB-226 and Blb1-820, yet they were highly resistant to LB. Their resistance may depend on the presence of other *R* genes identified in these two species, such as *Rpi-blb2*, *Rpi-blb3* and *R3a* (Vleeshouwers *et al.*, 2011; Sokolova *et al.*, in press). High resistance of several genome B accessions, such as *S. cardiophyllum* and *S. pinnatisectum*, lacking *Rpi-blb1* markers indicates that other *R* genes are probably active in these species. By reasonable assumption, the same genes could also function in resistant clones of *S. bulbocastanum* and *S. stoloniferum* along with *Rpi-blb1*. The ambiguous pattern presented in Table 2 invites further inquiry.

CONCLUSIONS

Rpi-blb1 is found only in genome B of *S. bulbocastanum* and *S. stoloniferum*. In these species, the markers derived from the LRR region of *Rpi-blb1* seem to predict LB resistance.

ACKNOWLEDGEMENTS

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Zoxamide sensitivity in European *Phytophthora infestans* isolates, 2003-2012

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SUMMARY

Zoxamide has been registered for potato late blight control in Europe (as 'Electis' and 'Aderio', both co-formulations with mancozeb) since 2001. In support of its European registration, sensitivity testing of isolates of *Phytophthora infestans* was initiated in 1997; results for isolates collected between 1997 and 2000 were previously reported. Between 2003 and 2012, 130 isolates of *P. infestans* collected from potato field trial sites in France, Germany, Greece, the Netherlands, Poland, Romania and the UK, and 20 characterised isolates from potato crops in the Channel Island of Jersey, Northern Ireland and Ireland (collected in 2005, 2008-2009 and 2009, respectively) were tested for their sensitivity to zoxamide *in vitro* using a poisoned agar technique. Isolates varied in sensitivity (expressed as EC₅₀ for mycelial growth on agar) having EC₅₀ values ranging from 2 to 80 µg zoxamide/l; over 90% of isolates had EC₅₀ values <50 µg/l and all were inhibited by 125 µg/l (0.125 mg/l). There was no evidence that any isolates had reduced sensitivity to zoxamide and no association was found between zoxamide treatment or non-treatment of sampled crops and zoxamide sensitivity of the *P. infestans* isolates obtained from them. There was no consistent trend in zoxamide sensitivity over years, although there was some indication that isolates from the island of Ireland and from Scotland and Wales were less sensitive than those from elsewhere in Europe. There was no association between zoxamide sensitivity and phenylamide resistance or mating type. Six isolates of the 13_A2 (Blue 13) genotype had similar sensitivities to isolates of other genotypes from the same geographical area. Thus, there is to date no evidence of reduced sensitivity to zoxamide in 12 years of its use to control potato late blight in Europe.

KEYWORDS

potato late blight, zoxamide, RH-7281, fungicide resistance

INTRODUCTION

The benzamide fungicide, zoxamide (RH-7281, Zoxium™), has been registered in Europe for the control of potato late blight since 2001. It is non-systemic and specifically active against Oomycetes, inhibiting β-tubulin assembly by binding covalently during mitosis (Young and

Slawecki, 2001). It was developed by Rohm & Haas, subsequently Dow AgroSciences, but is now owned by Gowan. For potato blight control, zoxamide is marketed only in co-formulations with mancozeb (e.g. 'Electis', 'Aderio'). Zoxamide is rapidly rainfast and zoxamide + mancozeb formulations have good protectant activity against late blight in field trials (e.g. Bradshaw and Schepers, 2000; Bain, 2012). Under EU Directive 93/71/EC, for the purposes of registration of a new pesticide, "Laboratory data and where it exists, field information relating to the occurrence and development of resistance or cross resistance in populations of harmful organisms to the active substance(s), or to related active substances, must be provided". To support zoxamide's European registration, Rohm & Haas initiated sensitivity testing of *Phytophthora infestans* isolates in 1997; this has been continued by Dow and Gowan up to the present. Because zoxamide is effectively non-systemic, a test relying on the uptake by leaf discs of the fungicide from solution (as used for phenylamides) was not appropriate. Initially, a protocol was developed which used potato leaf discs from glasshouse-grown plants sprayed with a series of concentrations of zoxamide and inoculated with *P. infestans*. However, this *in vivo* test did not always give consistent results, probably because of the difficulty of applying the fungicide uniformly to leaf discs and because of differences in the physiological condition of potatoes leaves grown at different times of the year. An *in vitro* poisoned agar plate method based on that initially developed by Dow AgroSciences was therefore used to test selected isolates from the year 2000 (Cooke *et al.*, 2002) and for all isolates after 2000.

Results for zoxamide sensitivity testing of *P. infestans* isolates collected 1997-2000 were reported by Cooke *et al.* (2002); the vast majority of these were derived from European field trials and where possible isolates were obtained from both zoxamide-treated and non-zoxamide-treated plots. Isolates varied in sensitivity; some laboratory isolates never exposed to zoxamide in the field were among the least sensitive tested, so the range of sensitivity was considered to reflect the natural variation in the pathogen population. Isolates from zoxamide-treated plots were no less sensitive than those from non-zoxamide-treated plots and there was no association between phenylamide resistance and zoxamide sensitivity. Here results of tests on isolates 2003-2012 using the *in vitro* method are reported.

MATERIALS & METHODS

Sources of isolates of Phytophthora infestans used in the study

Samples of blighted potato foliage (or tubers in the case of Scotland, 2005) were collected from Dow/Gowan field trial sites from seven European countries (within the UK isolates were obtained from England, N. Ireland, Scotland and Wales) between 2003 and 2012 and submitted to AFBI Newforge. Details of location, cultivar, trial protocol and disease severity were recorded. Where possible, samples were obtained from both zoxamide-treated plots and those receiving other fungicides or untreated. *P. infestans* was isolated onto antibiotic rye agar, obtaining up to four isolates per sample as described by Cooke *et al.* (2002). Some additional characterised isolates collected from surveys of *P. infestans* populations on commercial crops were included. A total of 150 isolates was used in the tests.

Determination of mating type, phenylamide resistance and genotype

For selected isolates, mating type and sensitivity to the phenylamide metalaxyl were determined (Cooke *et al.*, 2006). Ten isolates from the surveys in Northern Ireland and Ireland had been SSR genotyped (Kildea *et al.*, 2010).

Zoxamide sensitivity in vitro

Isolates were tested for zoxamide sensitivity using an *in vitro* poisoned plate protocol (modified from one supplied by Dr David Young, Dow AgroSciences). Aliquots of solutions of technical grade zoxamide in acetone were added to carrot agar (Erselius and Shaw, 1982) to give final concentrations in agar of 0, 0.98, 3.9, 15.6, 62.5 and 125 µg zoxamide/litre and 0.05% acetone. The agar was poured into 9 cm Petri plates, which were inoculated centrally with mycelial plugs (c. 6 mm diameter) cut from actively growing cultures of the appropriate test isolates (three replicate plates/concentration including the control). The plates were incubated at 18-20°C and the diameters of the mycelial growth zones measured after 7 days. EC₅₀ values (the concentrations inhibiting radial mycelial growth by 50%) were derived from log-probability plots.

RESULTS

Isolates of Phytophthora infestans

A total of 130 isolates of *P. infestans* was obtained from field trials in seven countries, these represented every year between 2003 and 2012 except 2007 and 2010 when testing was not carried out (Table 1). In addition, 20 characterised isolates collected during surveys of *P. infestans* populations on commercial crops were included, these were from the Channel Island of Jersey (2005, 5 isolates), N. Ireland (2008, 2009, 9 isolates) and Ireland (2009, 6 isolates). All isolates were from potato foliage, except for 11 derived from infected tubers from a trial in Scotland in 2005. Forty-five isolates were derived from known zoxamide-treated potato plots and 76 were from known non-zoxamide-treated potato plots or crops; the treatments used on the remaining plots/crops from which isolates (29) were derived were not known. Isolates were obtained from 12 different potato cultivars (Table 2).

Table 1. Number of *Phytophthora infestans* isolates from each country tested for sensitivity to zoxamide

Country of origin	Total number of isolates (number from known zoxamide-treated, number from known non-zoxamide-treated potatoes)								
	2003	2004	2005	2006	2008	2009	2011	2012	Total
France	2 (1, 1)	6 (3, 3)	4 (1, 3)	1 (1, 0)	0	8 (5, 3)	9 (1, 8)	4 (2, 2)	34 (14, 20)
Germany	1 (0, 0)	1 (1, 0)	3 (1, 1)	0	0	0	0	1 (1, 0)	6 (3, 1)
Greece	6 (3, 3)	2 (1, 1)	4 (0, 0)	0	0	0	0	0	12 (4, 4)
Ireland	0	0	0	0	0	6* (0, 0)	0	0	6 (0, 0)
Netherlands	0	2 (0, 0)	1 (0, 1)	1 (0, 0)	0	1 (0, 0)	0	0	5 (0, 1)
Poland	4 (0, 1)	0	0	0	0	0	0	0	4 (0, 1)
Romania	0	2 (1, 1)	2 (0, 0)	0	0	0	0	0	4 (1, 1)
UK England	2 (1, 1)	0	5 (3, 2)	22 (8, 8)	0	0	0	16 (5, 11)	45 (17, 22)
UK Scotland	0	0	11 (3, 8)	3 (0, 2)	0	0	0	0	14 (3, 10)
UK Wales	0	0	0	0	0	2 (1, 1)	2 (0, 2)	0	4 (1, 3)
UK N. Ireland	2 (2, 0)	0	0	0	6* (0, 6)	3* (0, 2)	0	0	11 (2, 8)
UK Jersey	0	0	5* (0, 5)	0	0	0	0	0	5 (0, 5)
Total	17 (7, 6)	13 (6, 5)	35 (8, 20)	27 (9, 10)	6 (0, 6)	20 (6, 6)	11 (1, 10)	21 (8, 13)	150 (45, 76)

* isolates which were collected as part of *P. infestans* population studies (all other isolates were from Dow or Gowan field trial sites).

Table 2. Potato cultivars from which *Phytophthora infestans* isolates were obtained for zoxamide sensitivity testing

Country	Cultivar(s) sampled from trial sites	Cultivar(s) sampled in surveys
France	Bintje	
Germany	Bintje, Linda, Fontane	
Greece	Agria, Spouda (<i>sic</i>)	
Ireland	not applicable	Orla, Rooster
Netherlands	Bintje	
Poland	Rywal	
Romania	Santé	
Great Britain (England, Scotland, Wales)	Désirée, King Edward, Maris Piper,	
N. Ireland	Up-to-Date	Dunbar Standard, Home Guard, Kerr's Pink, King Edward, Lady Claire, Maris Piper, Sagitta, Sárpo Mira
Jersey (Channel Islands)	not applicable	Jersey Royal

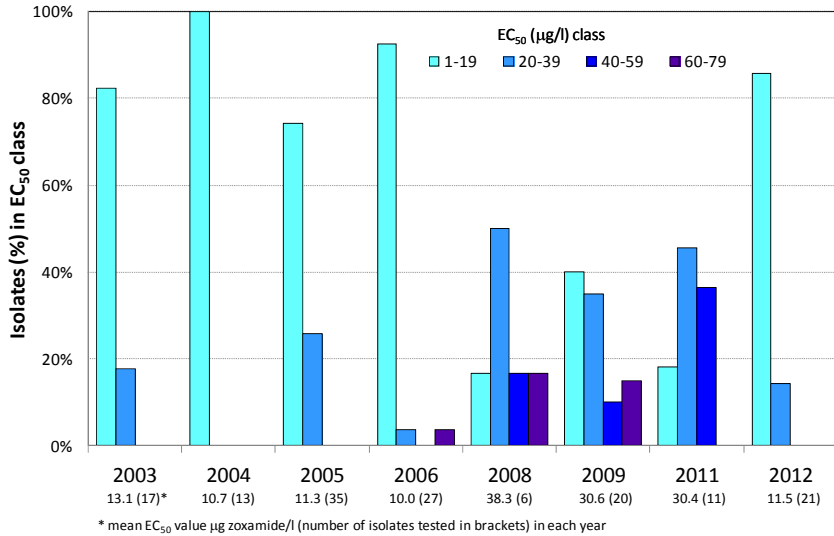


Figure 1. Sensitivity to zoaxamide of European isolates of *Phytophthora infestans*: proportion of isolates in sensitivity classes, 2003-2012

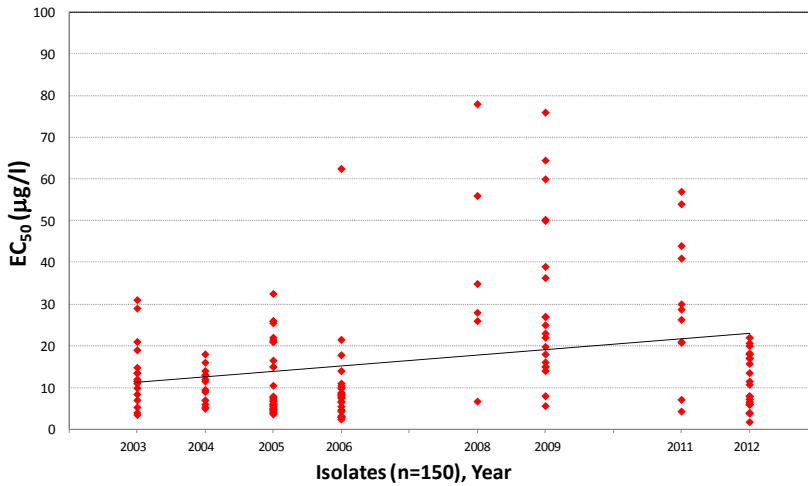


Figure 2. Sensitivity to zoaxamide of European isolates of *Phytophthora infestans*, 2003-2012

Zoaxamide sensitivity *in vitro*

The 150 isolates tested *in vitro* had EC₅₀ values ranging from 1.8 – 78 µg/l; over 90% of isolates had EC₅₀ values <50 µg/l and all isolates were inhibited by 125 µg/l (Figure 1). There was no consistent trend over years (Figure 2). Although there was a wider range of sensitivities in some

years than others (cf. 2008, 2009, 2011 v. 2006, 2012), this appeared to reflect the regions from which isolates had been obtained (see below).

Considering the countries from which isolates were obtained, there were fewer than ten isolates each from Germany, the Netherlands, Poland and Romania, so mean sensitivities were not calculated for these. Considering the countries/regions from which ten or more isolates were obtained, there was a tendency for isolates from the island of Ireland to be slightly less sensitive than those from elsewhere, but they were still well within the normal sensitive range (Table 3). It was not considered appropriate to compare these statistically as different numbers of isolates were obtained in different years.

There was no evidence of any association between zoxamide sensitivity and zoxamide usage on the sampled crops, phenylamide resistance or mating type (Table 4). As above, it was not considered appropriate to compare these statistically as different numbers of isolates were obtained from different countries and in different years. Very limited information on zoxamide sensitivity and SSR genotype was obtained and this only for isolates from the island of Ireland collected in 2008 and 2009 (which tended to be less sensitive): the least sensitive isolate belonged to the 8_A1 genotype, while the mean sensitivity of the six 13_A2 (Blue 13) isolates tested was 29.4 µg/l (Table 4).

Table 3. Mean sensitivities of *Phytophthora infestans* isolates to zoxamide for countries/regions from which more than ten isolates were obtained

Country	Number of isolates	Years in which isolates were obtained	Mean zoxamide sensitivity (EC ₅₀ µg/l)
France	34	2003, 2004, 2005, 2006, 2009, 2011, 2012	17.3
Greece	12	2003, 2004, 2005	8.9
Great Britain (England, Scotland, Wales)	63	2003, 2006, 2009, 2011, 2012	14.0
Great Britain and Jersey	68	2003, 2005, 2006, 2009, 2011, 2012	14.0
Northern Ireland/Republic of Ireland	17	2003, 2008, 2009	34.8

Table 4. Mean sensitivities of *Phytophthora infestans* isolates grouped by zoxamide usage, phenylamide resistance and mating type

Isolate type	Number of isolates	Years and countries from which isolates were obtained	Mean zoxamide sensitivity (EC ₅₀ µg/l)
<u>Zoxamide treatment</u>			
Non-zoxamide-treated	76	2003, 2004, 2005, 2006, 2008, 2009, 2011, 2012 France, Germany, Greece, the Netherlands, Poland, Romania, UK	17.9
Zoxamide-treated	45	2003, 2004, 2005, 2006, 2009, 2011, 2012 France, Germany, Greece, Romania, UK	13.2
<u>Phenylamide resistance</u>			
Sensitive	39	2003, 2004, 2005, 2006, 2008, 2009 France, Germany, Greece, Ireland, the Netherlands, Poland, Romania, UK	20.3
Resistant	70	2003, 2004, 2005, 2006, 2008, 2009, 2011, 2012 France, Germany, Greece, Ireland, the Netherlands, Poland, UK	13.3
<u>Mating type</u>			
A1	92	2003, 2004, 2005, 2006, 2008, 2009, 2011, 2012 France, Germany, Greece, Ireland, the Netherlands, Poland, Romania, UK	17.3
A2	46	2003, 2004, 2005, 2006, 2008, 2009, 2012 France, Germany, Greece, Ireland, the Netherlands, Poland, Romania, UK	12.8
<u>Genotype</u>			
6_A1	1	2009, Ireland	15.0
8_A1	2	2008, 2009, N. Ireland	51.5
12_A1	1	2008, N. Ireland	34.9
13_A2	6	2008, 2009, N. Ireland, Ireland	29.4

DISCUSSION

Cooke *et al.* (2002) reported the sensitivity to zoxamide of 136 European isolates of *P. infestans* obtained from Rohm & Haas and Dow field trials between 1997 and 2000 and 33 laboratory isolates never exposed to zoxamide and concluded that the isolates varied in sensitivity as indicated by the concentration required to prevent sporulating growth on excised potato leaf discs sprayed with the fungicide. This was attributed to natural variation within the pathogen population, since the variation was as great among laboratory isolates never exposed to zoxamide in the field as among isolates collected from sites where zoxamide was applied. As noted above, the leaf disc test did not always give consistent results so the subsequent sensitivity studies reported here used an *in vitro* poisoned agar plate technique.

As in the previous study, the 150 isolates collected between 2003 and 2012 varied in their sensitivities to zoxamide: EC₅₀ values for mycelial growth on agar were between *c.* 2 and 80 µg/l; pathogen populations naturally vary in sensitivity to fungicides. There was no consistent trend for zoxamide sensitivity to become less over time. Only ten isolates had EC₅₀ values of 50 µg/l or more and all but one of these was from the UK (Northern Ireland, Scotland and Wales) or Ireland (the other one being from France). There was thus some evidence that the UK and Ireland *P. infestans* populations are slightly less sensitive to zoxamide than those from England and from mainland Europe, but all isolates were considered to be well within the normal sensitivity range. As in the previous study, there was no indication that isolates from zoxamide-treated plots (all within standard replicated field trials in the various countries) were any less sensitive than those from non-zoxamide-treated plots or crops; no isolates tested were associated with poor field performance by zoxamide formulations.

Limited additional characterisation of isolates indicated that there was no association between zoxamide sensitivity and phenylamide resistance: this was in agreement with the previous study and was to be expected since the mode of action of zoxamide (binding to β-tubulin, Young and Slaweki, 2001) is quite different from that of the phenylamides which inhibit RNA synthesis (Davidse, 2005). Similarly there was no association between zoxamide sensitivity and mating type, and the limited testing of isolates of known genotype indicated that isolates of the newer genotypes (6_A1, 13_A2) were, if anything, more sensitive than those of the older genotypes (8_A1, 12_A1). Associations between characters such as fungicide sensitivity and genotype can arise and be perpetuated in *P. infestans* in regions where it is predominantly reproducing asexually and has a strongly clonal population structure (e.g. genotype 13_A2 is invariably phenylamide-resistant), but were not seen in the present study.

Thus, in the 12 years during which formulations containing zoxamide have been marketed and used for the control of potato late blight in Europe, there is no evidence of reduced sensitivity to this fungicide. FRAC (Fungicide Resistance Action Committee) classifies zoxamide as having low to medium risk and advise that resistance management required: marketing zoxamide only in mixtures with a fungicide with a different mode of action (e.g. mancozeb, Young *et al.*, 2001) and monitoring pathogen sensitivity contribute to such management.

ACKNOWLEDGEMENTS

We thank Mark Wilson, Lisa Quinn and Patrick Nugent and several undergraduate students of Queen's University, Belfast for assistance in isolating, maintaining and testing *P. infestans* isolates.

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Performance of fungicide programmes based on 'Revus', 'Shirlan' and 'Dithane NT' in controlling potato blight in a Northern Ireland field trial, 2012

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Performance of fungicide programmes based on 'Revus', 'Shirlan' and 'Dithane NT' in controlling potato blight in a Northern Ireland field trial, 2012

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Introduced in the 1960s, mancozeb was one of the most popular fungicides for late blight control in Northern Ireland for many years, but its use declined after fluazinam was approved in 1994. Trials in Belfast in the 1990s found season-long application of fluazinam to be more effective in terms of foliar and particularly tuber blight control than programmes based on mancozeb. However, no comparisons involving mancozeb have been made since 1998. For the past 15 years, the standard programmes used in trials in Belfast have started with 2 or 3 applications of a systemic or translaminar (metalaxyl-M or mandipropamid) mixed with a protectant, followed by fluazinam. However, reports from The Netherlands suggesting a decline in the performance of fluazinam prompted re-evaluation of mancozeb in a programme starting with mandipropamid+fluazinam.

Field trial 2012

- Fully randomised block with 5 replicates
- Plots (yellow in diagram) 4 drills x 10 tubers
- Planted 21 May
- Fungicides applied 26 June - 30 August, 7-day intervals
- Unsprayed, infector drills (green) inoculated 2 July with 2011 isolates including 13_A2 and 8_A1
- Foliage blight assessed twice weekly
- Trial desiccated 6 and 13 September
- Harvested 9 October
- Yield and soft/blight assessed 16-17 October, 13-21 November 2012, 28-30 January 2013

Programmes reported here:

- mandipropamid + fluazinam (Revus + Shirlan, 150 + 100 g a.i./ha) x 2; fluazinam (Shirlan, 200 g a.i./ha) x 8
- mandipropamid + fluazinam (Revus + Shirlan, 150 + 100 g a.i./ha) x 2; mancozeb (Dithane NT, 1500 g a.i./ha) x 8

Growing conditions

Summer 2012 was cool and wet with little sunshine; growth was poor and blackleg was a problem.



Foliar blight

After inoculation, blight developed rapidly in the infector drills and they were dead by early August (above). Both fungicide programmes gave good control of foliar blight.



At the final assessment, the mancozeb programme had significantly less infection (after angular transformation).

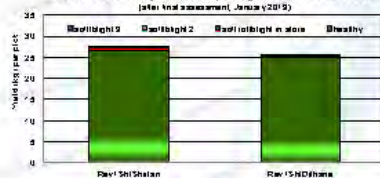
Yield and tuber blight

There were no significant differences between programmes in terms of yields, which were the poorest of recent years, nor in terms of the percentage rotted tubers in store. Very extensive soft rotting made it impossible to determine how much rotting was associated with blight.

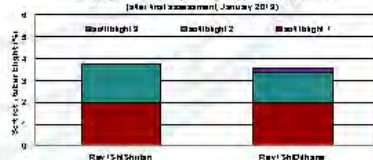
2012 Potato blight control trial: Newforge, AFBI



Yield assessments: Proportion of healthy and blighted tubers of marketable size



Tuber blight assessments: percentage blighted tubers by number



Conclusions

The performance of the programme including Dithane NT was encouraging as it was comparable to or better than the standard. This is the first comparison involving mancozeb since the appearance of new genotypes including 13_A2 in the Northern Ireland population. It would be worthwhile to repeat the trial in a year with more normal tuber blight development.

Acknowledgement Indofin is thanked for funding the Dithane NT programme.

Assessing the resistance of potato cultivar Sarpo Mira to Algerian isolates of *Phytophthora infestans*

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Assessing the resistance of potato cultivar Sarpò Mira to Algerian isolates of *Phytophthora infestans*

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Sarpò Mira cv. has been reported to retain foliar resistance to *Phytophthora infestans* populations even under high blight pressure conditions, for several years.

This resistance could however be eroded when challenged with new *P. infestans* genotypes, because of the pathogen ability to rapidly adapt and evolve.

Within Algerian populations from Western and Central coastal regions, most *P. infestans* isolates had the A2 mating-type, predominantly genotype 13_A2 (EU_13), although A1 mating-type isolates are also present on potato and tomato crops.

This study aims to examine the resistance of Sarpò Mira under natural and controlled conditions to current Algerian isolates.

Field resistance of Sarpò Mira cv. under natural blight infection in ENSA trials (Algiers)

In 2010 :

- all cvs (Spunta, Bintje, Désirée, Atlas, Kondor...) : totally defoliated by 17 days from first observed symptoms.
- Sarpò Mira : no symptom, fully resistant.

In 2011 :

- Sarpò Mira exhibited blight necrosis, but sporulation was more limited than on susceptible cvs, as Bintje.

Spunta Sarpò Mira - May 2010 Sarpò Mira - January 2011



Differential responses of Sarpò Mira to Algerian *P. infestans* isolates under controlled assay

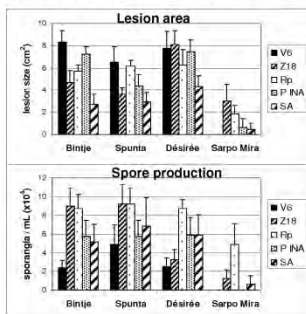
5 potato isolates tested (3 A2, 2 A1)

- V6, 13_A2 MLG, ENSA 2011, from Bintje
- Z 18, 13_A2, sampled in 2007 on Atlas
- SA, A2, ENSA 2011, sampled on Sarpò Mira

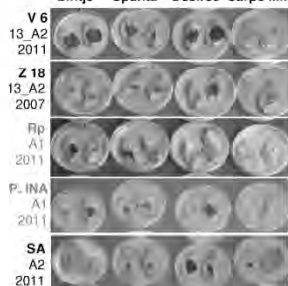
- Rp, A1, ENSA 2011, from Spunta
- P. INA, A1, ENSA 2011, from Timale, SSR MLG similar to tomato isolates MLGs (distinct from MLGs of potato isolates)

on Sarpò Mira and 3 reference cvs

- 8 leaflets / cv, inoculated with a 20- μ L droplet (5x10⁴ sp/mL); incubation at 20 °C
- Lesion size measured at 6 dpi
- Spore production noticed at 7 dpi (each leaflet washed in 5 mL of water)



Bintje Spunta Désirée Sarpò Mira



Sarpò Mira cv. showed a high level of resistance to three isolates (2 A2 and 1 A1) :

- V6 was not able to infect any Sarpò Mira leaflets.
- SA gave very small and limited sporulating lesions on Sarpò Mira.
- with P. INA, Sarpò Mira leaflets displayed small necrosis without sporulation, although this isolate was highly aggressive on the three susceptible cvs.

However, Sarpò Mira resistance was overcome by two isolates Z18 (A2) and Rp (A1) which had a great sporulation on the cultivar.

Different phenotypes were observed among the Algerian isolates. Aggressiveness of these isolates was not related to their mating-types, nor to SSR genotypes.

Large variations in pathogenic traits were noticed on each cultivar, according to the isolates.

The resistance of Sarpò Mira is due to at least five different genes (Rietman *et al.*, 2012). Our results show that variability exists within Algerian populations of *P. infestans* to this cultivar. Some isolates (like V6) are avirulent, while others, both from A1 and A2 mating types, are able to infect and sporulate on it, albeit with a low efficacy.

Therefore, if Sarpò Mira cv. currently retains a high level of resistance in Algeria, maximizing the potential durability of this resistance requires thorough monitoring of *P. infestans* populations, on both hosts potato and tomato, and a flexible deployment strategy.

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Advances in control of potato Late Blight in Argentina

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SUMMARY

In the southeast of Buenos Aires Province (SEBAP) in Argentina, an area with highest yields in the country, the weather conditions are very conducive for Late Blight (LB) development. Losses due to the absence of chemical control of LB during the last 20 years were in average 35.6 % for total yield. PROPAPA, the Potato Research Group of INTA Balcarce focusses their research in breeding for resistance, the introduction of DSS in potato growing regions of the country and *P. infestans* characterization in order to exam the population biology, epidemiology and management of the pathogen. Supported by a linkage project between Argentina and the Netherlands (WUR), we optimized the practical use of the DSS in Argentina and we developed tools for the characterization and monitoring of populations of *P. infestans*. During 2010 to 2013, we have established, optimized and successfully implemented Phytoalert®, a DSS for LB control in the SEBAP. This service offered to private growers and McCain Argentina SA Company allowed anticipating critical conditions for LB, performing preventive applications in many cases and reducing one or more sprays per season. During the 2011-12, Phytoalert® reduced 40% the cost of fungicides in high potato production fields. The monitoring network of LB increases annually the number of meteorological stations and the area covered by Phytoalert®. Phytoalert® has the potential to be applied in other potato regions of the country.

For the characterization and monitoring *P. infestans* populations in Argentina, over 130 isolates were collected from the main potato growing regions of Argentina during the last 20 years. The sampling covered three main periods: 1992-95, 1997-99 and 2009-13. The phenotypic and genotypic characterization was based on mating type, mitochondrial DNA haplotype, aloenzymes (GPI), RFLP analysis with probe RG-57 and a mefenoxam sensitivity assay. We also performed a genotyping wiht 12 highly informative microsatellite markers mainly with recent populations. The structure of *P. infestans* populations in Argentina has undergone significant changes over the last 20 years. The current isolates have shown the predominance of type A1, 1a haplotype and GPI 100/100 (Figure 3) and also high levels of resistance to mefenoxam. Genotyping showed divergence beetwen recent populations in relation to the old populations included in this study. New isolates showed high homology in their SSR´s pattern but could be distinguished isolates from different potato areas. Better knowledge of changes in *P. infestans* populations allows designing better strategies to control LB.

KEYWORDS

Phytophthora infestans, DSS, populations, genotyping, phenotypic characterization, Argentina

INTRODUCTION

Potato is an important staple crop in Argentina. The production fluctuates over the years, reaching in 2011 almost 2.12 million tons (FAOSTAT, 2013). Spunta is the most important potato variety with more than 70% of the national production addressed to fresh market (Private and official estimates not published, 2013).

Potato Late Blight (LB) caused by the oomycete *Phytophthora infestans* is the most important potato disease worldwide. In the southeast of Buenos Aires Province (SEBAP) in Argentina, where the country's highest yields have been reported, the weather conditions are conducive for the disease development.

According to Mantecón (2009), the average losses in SEBAP due to the absence of chemical control of LB during the period 1986-2005 was 41.8 % for commercial tubers and 35.6 % for total yield.

In order to explore productive alternatives that use lower levels of chemicals, PROPAPA, the Potato Research Group of INTA Balcarce focuses its current and future efforts in breeding for resistance, introduction of DSS in potato growing regions and characterization of *P. infestans* populations.

A cooperative work among INTA, Wageningen University Research (WUR) and Mc Cain Argentina S.A., introduced a Decision Support System (DSS), Phytoalert®, a guided control strategy in the Southeast of Buenos Aires Province (Kessel *et al*, 2010).

During 2010 to 2013, the DSS was oriented to highly technified potato growers in the SEBAP. Phytoalert® is based on the Simcast model including a weather forecast, specific fungicide doses depending on the varieties and an automated running of the system. Phytoalert® efficiently detected risk periods for LB to generate an optimal schedule of chemical control.

Tools for the characterization and monitoring *P. infestans* populations in Argentina to optimize the use of durable resistant varieties were also developed.

MATERIALS AND METHODS

Phytoalert®

Developed by INTA after three potato crop seasons (2007 to 2010) in the SEBAP, is based on SimCast Model (Fry *et al*, 1983) and integrates meteorological data (records in real time and forecast) with information on the disease cycle, the potato growth stage and chemical sprays applied on the field. Based on data recorded by the weather stations located in the fields, the system calculates risk units that build up to a critical threshold. The model uses two types of units: LB and Fungicide (FU) Units. When the system reaches the threshold, an advice of risk is sent together with a recommendation on chemical product to be applied.

The last three years a monitoring network of LB in the SEBAP has been established, including a service to private growers and to McCain Argentina SA, who is implementing Phytoalert® in their high production potato fields. The network increases annually the number of meteorological stations and the area under survey. During the last season (2012-13) the network had five weather stations of Tecmes SA (Pegasus PLUS) and Seedmech (iMetos version II), distributed in the SEBAP as shown in Figure 1.



Figure 1. Monitoring network of Late Blight with Phytoalert® during 2012-13 season in the South East of Buenos Aires Province

Advices were issued every two days via email and SMS, with a forecast risk in order to allowing management decisions for the control of LB. The advice included a list of fungicides classified on the mode of action and biological efficiency in order to facilitate the selection of the appropriate product. The model was also validated at Research Station of INTA Balcarce with other potato varieties of interest as Spunta, Calen INTA, Newen INTA and Pampeana INTA.

*Characterization of *P. infestans* populations in Argentina*

Over 130 isolates of *P. infestans* were collected from the main potato growing regions of Argentina during the last 20 years. The sampling focused on three main periods: 1992-95, 1997-99 and 2009-13. The main areas covered in this study include the provinces of Buenos Aires and Córdoba, which concentrate the highest potato production surface, as well as Tafi del Valle (Tucumán Province), a location where weather conditions are very favorable for the disease development. Most strains were isolated from potato infected leaves, stems and tubers. The isolates were collected from different potato varieties and clones. The phenotypic and genotypic characterization was based on: mating types, mitochondrial DNA haplotype, aloenzymes (GPI), RFLP analysis with probe RG-57 and a mefenoxam sensitivity assay. Genotyping was performed using 12 highly informative microsatellite markers (Lees *et al*, 2006; Li *et al*, 2012) mainly with the newest *P. infestans* population.

RESULTS AND DISCUSSION

Phytoalert®

Phytoalert® allowed anticipating critical conditions for LB, performing preventive applications in many cases. The application of an effective preventive strategy could avoid dramatic outbreaks of disease and reduce intensive chemical input to control potato LB. The effectiveness of Phytoalert® has reduced one or more sprays per season during 2010-13. During the 2011-12 season, Phytoalert® reduced the cost of fungicides in high potato production fields by 40%.

Phytoalert® can synchronize the chemical applications with periods with high risk reducing unnecessary sprays when the risk of infection was low. Extensive communication on how to use Phytoalert® was performed in field days and meetings (Figure 2).



Figure 2. Communicate How to use Phytoalert® during field days and meetings

Characterization of *P. infestans* populations in Argentina

The structure of *P. infestans* populations in Argentina has undergone significant changes over the last 20 years. Compared to the information reported until 2000 (Distel and Huarte, 2000) current isolates have shown the predominance of type A1, Ia haplotype and GPI 100/100 (Figure 3).

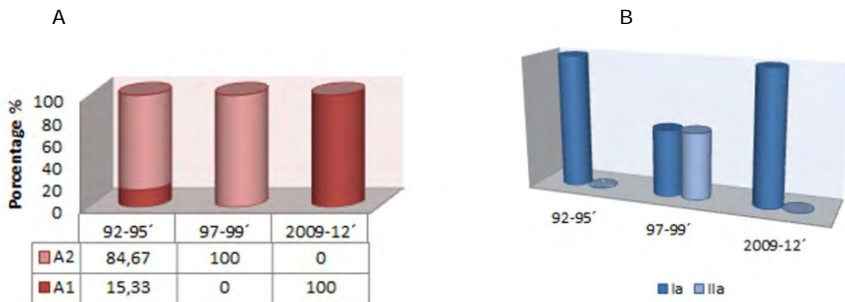


Figure 3. Characterization of *P. infestans* populations during the last 20 years. A) mating types B) mitochondrial DNA haplotypes

Meanwhile, isolates from 1997-99' have different SSR patterns compared to new isolates. Furthermore, genotyping showed divergence between recent populations with the oldest one included in this study (Figure 4). The most recent population showed high homology in their SSR's pattern and the PIG11 marker allowed to clearly distinguish the isolates from the area of Tafí del Valle (Tucumán Province) from those collected in Córdoba and Southeast of Buenos Aires Province in the same period.

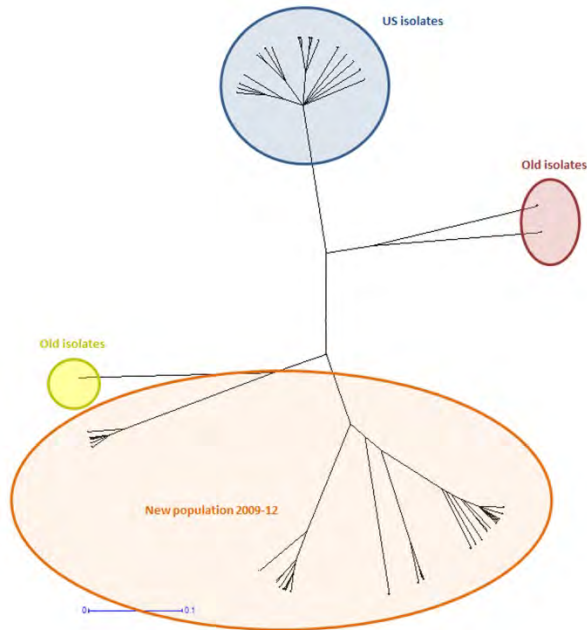


Figure 4. Genotyping of *P. infestans* populations. Tree based on the alleles at 12 SSR loci showing population diversity of *P. infestans* collected during 2009-12 compared with 3 old isolates (1995-97), and recent US populations

The mefenoxam sensitivity assay has shown high levels of resistance in the new population (2009-1) (Figure 5).

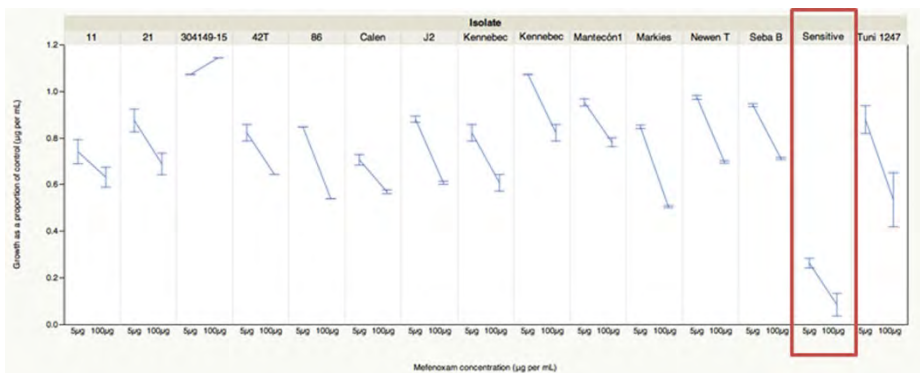


Figure 5. Mefenoxam sensitivity assay. Effects of mefenoxam concentration (0 µg, 5 µg and 100 µg) on colony growth in recent *P. infestans* populations. Susceptible control is shown in a red square

CONCLUSIONS

Tools for characterization and monitoring of *P. infestans* populations were developed to optimize the durable performance of potato varieties and fungicides. Phytoalert® was optimized and successfully implemented in the SEBAP during the last 3 years in the frame of a monitoring network of LB. The practical use of DSS in Argentina was supported by a cooperation project between Argentina and the Netherlands. Phytoalert ® enhanced the effectiveness of strategies to control LB without increasing the risk of an outbreak of the disease. Phytoalert ® generated an optimal spray schedule for potato late blight control and has the potential for application in other potato regions. Knowledge of population changes of *P. infestans* allows the design of better strategies to control LB.

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