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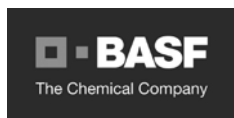
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Preface

EuroBlight Workshop St Petersburg, Russia 9-12 October 2011

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 13th Workshop was hosted by N.I. Vavilov Research Institute of Plant Industry, St Petersburg, Russia. The Workshop was attended by 91 persons from 20 European countries, Russia, Israel, Chile, Argentine and United States of America. Representatives from all countries presented information on the late blight epidemic in 2010 and 2011 and recent research results regarding integrated control, decision support systems, resistance of varieties and population biology of the late blight pathogen in potatoes. Since early blight is an increasing problem in Europe 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. 15. The current and previous Proceedings are also available on the EuroBlight website www.EuroBlight.net.

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Table of contents

| | |
|---|----|
| PAPERS | 9 |
| The development and control of Late Blight (<i>Phytophthora infestans</i>) in Europe in 2010 and 2011 Jens Grønbech Hansen, Björn Anderson, Ruairidh Bain & Alison Lees, Faye Ritchie, Guntis Gulbis, Steven Kildea, Louise Cooke, Ludovic Dubois & Catherine Chatot, Alexey Filippov, Asko Hannukkala, Hans Hausladen, Ervin Hausvater, Jan Heldak & Peter Vrabcek, Arne Hermansen & Ragnhild Nærstad, Jozefa Kapsa, Mati Koppel, Tomke Musa, Antanas Ronis, Huub Schepers & Kees Vogelaar, Pieter van Haverbeke | 11 |
| The population structure of <i>Phytophthora infestans</i> in the Netherlands during the years 2000 - 2009 Trudy van den Bosch, Ying Li, Bert Evenhuis, Marieke Förch, Theo van der Lee and Geert Kessel | 31 |
| Making Sense of <i>Phytophthora infestans</i> diversity at national and international scales David E. L. Cooke, Alison K. Lees, Poul Lassen & Jens Grønbech Hansen..... | 37 |
| Fortuna et al. Status and perspectives of GM approaches to fight late blight Thorsten Storck, Timo Böhme & Holger Schultheiss | 45 |
| Late blight resistance of <i>Solanum</i> species and potato hybrids: the evidence from coupled phytopathological and molecular study Elena Rogozina, Maria Patrikeeva, Maria Kuznetsova, Svetlana Spiglazova, Irina Kozlovkaya, Tatiana Smetanina, Artem Pankin, Maria Beketova, Ekaterina Sokolova, Elena Kinash, Polina Drobyanzina, Kenneth Deahl, Richard Jones and Emil Khavkin | 49 |
| Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes H.J. Kim, H.R. Lee, K.R. Jo, S.M.M. Mortazavian, D.J. Huigen, A. Evenhuis, G.T.J. Kessel, R.G.F. Visser, E. Jacobsen, J.H. Vossen | 55 |
| Characterization of <i>Phytophthora infestans</i> populations in North America from the 2009-2011 late blight epidemics K. L. Deahl..... | 57 |
| Analysis of correlation between soil moisture and late blight occurrence Jeanette Jung, Beate Tschöpe & Benno Kleinhenz | 59 |
| Analysis of volunteer density under the influence of cropping practices: a contribution to the modelling of primary inoculum of <i>Phytophthora infestans</i> in potato crops T. Rakotonindraina, R. Corbiere, C. Chatot, V. Pinchon, L. Dubois, F. Arousseau, J.E. Chauvin & J.N. Aubertot | 67 |

| | |
|---|-----|
| Ongoing changes in the Irish potato late blight population L.R. Cooke, S. Kildea, J. Mehenni-Ciz, L. Quinn, G. Little, F. Hutton, F. M. Perez, K. L. Deahl & D. Griffin..... | 75 |
| Strengthening Management of New Late Blight Genotypes in the North Central USA Gary Secor, Viviana Rivera, Asunta Thompson & Neil Gudmestad..... | 81 |
| An IPM2.0 Control strategy for Potato late blight based on cultivar resistance and monitoring of virulence in the local <i>P. infestans</i> population G.J.T. Kessel, A. Evenhuis, G.B.M. Van den Bosch, G.A. Hoekzema, L. Bosman, C.G. Topper, L.J. Esselink, M.P.E. van Gent-Peltzer, T.A.J. van der Lee and H.T.A.M. Schepers | 87 |
| Early outbreak of potato late blight in Denmark 2011 Bent Nielsen, Bødker, Lars & Hansen, G. Jens..... | 93 |
| Disease-orientated threshold values as tool for effective early blight control Juergen Leiminger & Hans Hausladen | 99 |
| Host-pathogen interaction between <i>Alternaria</i> species and <i>S.tuberosum</i> under different conditions Józefa S. Kapsa, Jerzy Osowski | 107 |
| Genetic structure of <i>Alternaria solani</i> - a new approach Eva Edin, Firuz Odilbekov, Larisa Garkava-Gustavsson & Erland Liljeroth | 113 |
| Ranman Top, again a step forwards in late blight control Johan Desnouck, Jos Testers, Clement Versmissen | 115 |
| Recommendations and field performance of Initium® based products against <i>Phytophthora infestans</i> in potato Vanessa Tegge, Tobia Erven, Eric Kiers, Marja Kruts, Angus Murray, Horst-Dieters Brix | 119 |
| Revus Top, A new product for the control of <i>P. infestans</i> and <i>Alternaria</i> in potatoes in Europe Jan Bouwman, Caroline Strypenstein, Frank Meier-Runge & Frederico Gonzalez | 123 |
| Report of the Fungicide Subgroup meeting on 11 & 12 October 2011: Discussion of potato blight fungicides, their properties and ratings Ruairidh A. Bain | 131 |
| Development of foliar late blight (<i>Phytophthora infestans</i>) in relation to cultivar resistance and fungicide dose on potato Ruairidh A. Bain, Faye Ritchie, Alison Lees, Chris Dyer and Adrian Roberts | 139 |
| Characterization of <i>Phytophthora infestans</i> population in Chile I. Acuña, B. Sagredo, M. Gutiérrez, C. Sandoval, A. Fahrenkrog, G. Secor, V. Rivera and S. Mancilla | 145 |

| | |
|---|-----|
| Molecular Identification of the Species Composition of Russian Isolates of Pathogens, Causing Early Blight of Potato and Tomato Sergey N. Elansky, Marina A. Pobedinskaya, Lyudmila YU. Kokaeva, Natalia V. Statsyuk & Alina V. Alexandrova | 151 |
| China-blight — A Web Based DSS on Potato Late Blight Management in China Tongle Hu, Jiehua Zhu and Keqiang Cao | 157 |
| Competition between genotypes of <i>Phytophthora infestans</i> Allison Chapman, Alison K Lees, David E L Cooke, Louise R Cooke | 165 |
| POSTERS | 169 |
| Target enrichment and next generation sequencing as tools to facilitate cloning of R genes from <i>Solanum</i> species Kamil Witek, Jadwiga Śliwka, Walter Verweij, Florian Jupe, Henryka Jakuczun, Ingo Hein, Ewa Zimnoch-Guzowska and Jonathan D. G. Jones | 171 |
| Assessment of foliar and tuber resistance in <i>Solanum neoantipoviczii</i> Buk. × <i>S. phureja</i> Juz. et Buk. hybrid populations using different isolates of <i>Phytophthora infestans</i> Nadezhda Zoteyeva | 173 |
| The adaptation of MAS for late blight resistance evaluation of potato breeding material Ilze Skrabule, Nadezhda Zoteyeva, Ieva Mezaka, Daiga Vilcane, Guna Usele | 179 |
| LEAFY intron 2-based markers of wild <i>Solanum</i> genomes for introgression breeding Polina E. Drobyazina and Emile E. Khavkin | 187 |
| Zoospore production in relation to temperature for current <i>P. infestans</i> genotypes Ruairidh Bain and Claire Convery | 193 |
| Glycoalkaloid content in potato tubers with different levels of resistance to <i>Phytophthora infestans</i> Ulrika Carlson-Nilsson, Nadezhda Zoteyeva & Fredrik Reslow | 195 |
| Genotypic variation of <i>Phytophthora infestans</i> populations in Argentina M. Florencia Lucca and Marcelo A. Huarte | 201 |
| Are simple <i>Phytophthora infestans</i> races really that simple? Artem A. Pankin, Elena A. Kinash, Irina N. Kozlovskaya, Maria A. Kuznetsova, Emil E. Khavkin | 205 |
| Phenotypic characteristics of Belgian populations of <i>Phytophthora infestans</i> (2005-2010) Vincent César, Véronique Labbe, Laurent Laguesse and Jean-Louis Rolot | 213 |

| | |
|--|-----|
| Distribution of Mating Types and Resistance to Metalaxyl of <i>Phytophthora infestans</i> in Germany | |
| Juliane Schmitt & Benno Kleinhenz | 215 |
| <i>Phytophthora infestans</i> 13_A2, diagnostic and monitoring in 2009 and 2010 | |
| M.P. Latorse, Y. Tarrionte, V. Brozek, H. Yadja, S. Veloso, D. Cooke | 223 |
| Changes in <i>Phytophthora infestans</i> aggressiveness as a result of repeated reproduction on different potato cultivars | |
| Svetlana Spiglazova, Maria Kuznetsova, Tatiana Smetanina and Alexey Filippov | 225 |
| Experiences of <i>Alternaria</i> Disease Forecasting in the UK | |
| Howard Hinds..... | 229 |
| Decision support systems for late blight integrated management in the southern Chile. | |
| Rodrigo Bravo, Ivette Acuña, Juan Inostroza & Dagoberto Villarroel..... | 231 |
| Simulator for the comparison of fungicides, cultivar resistance, and Decision Support Systems in the control of the late and early blight of potato | |
| A.N. Rogozhin and A.V. Filippov | 237 |
| Cryopreservation of <i>Alternaria solani</i> and <i>Phytophthora infestans</i> | |
| Christoph Andreas Braun & Anne Suty-Heinze..... | 239 |
| Postinfection Activity of Early Blight Fungicides | |
| Hans Hausladen, Birgit Adolf..... | 241 |
| Fungicide Resistance of Russian <i>Phytophthora infestans</i> strains | |
| Marina A. Pobedinskaya, Sergey N. Elansky, Natalia V. Statsyuk & Mikhail P. Plyakhnevich . | 243 |
| Criteria to choose fungicides to control potato foliar diseases | |
| S. Duvauchelle, J.F. Ricateau | 249 |
| Aggressiveness of different <i>Phytophthora infestans</i> isolates from Germany 2010 | |
| Sophia Gottschaller, Tongle Hu, Hans Hausladen | 251 |
| Early blight diagnostics in potato: Diagnostics: difficulties and digitalisation | |
| Jan Spoelder & Lo Turkensteen..... | 253 |
| Early Blight: Pathogenicity and fungicidal control of <i>Alternaria solani</i> and <i>Alternaria alternata</i> | |
| T. Erven, J. Philippi, V. Tegge and G. Stammler | 255 |
| Recombination between recently occurring A1 and A2 isolates of <i>Phytophthora infestans</i> in Ireland | |
| M. Nyongesa, D.S. Shaw, D. Wright, L.R. Cooke, S. Kildea, D. Griffin, K.L. Deahl & E. Mullins | 257 |

PAPERS

The development and control of Late Blight (*Phytophthora infestans*) in Europe in 2010 and 2011

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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, 2010 and 2011.

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.

METHODS

The country profiles have the following structure and content:

Summary

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

Early outbreaks of potato late blight

- Select the date of first observation of late blight in covered or very early planted potatoes

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

Weather conditions and late blight development

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

Use of fungicides and control strategies

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

Organic potatoes

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

Tuber blight

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

Alternaria spp.

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

Characteristics of *Phytophthora infestans*

- Write a short text (max 100 words) about pathogen characteristics. In the country reports graphs for mating type distribution and virulence pathotypes are automatically included based on available

data from the Eucablight database.

Use of cultivars

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

Use of DSS

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

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

THE DEVELOPMENT AND CONTROL OF *PHYTOPHTHORA INFESTANS* IN EUROPE IN 2010 AND 2011

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 2010 and 2011 is shown for all European countries on maps in Fig. 1-4. The same data are combined into marker plots per year in Fig. 5 and 6. The weather based risk at selected stations in Europe is shown in Fig. 7. The level of tuber blight attack is given in Fig. 8 and problems with tuber blight is shown in Fig. 9.

Belgium 2011

The spring months of March and April were very dry, and although late blight lesions had already been found early in the season on a dump pile (April 15), there was little or no risk for spread of the disease. This was also the case during a sunny, dry month of May, and disease pressure remained very low during the critical phase of emergence and rapid leaf growth of the ware potatoes. Late blight could develop somewhat during several consecutive infection periods in June, but it was not until the second half of July that weather conditions became very favourable for the disease. Late blight attacks in fields were observed from the last week of July, and continued to increase until the end of the growing season, as the weather conditions remained unfavourable.

Belgium 2010

A significant number of frost days during the winter of 2009/2010 most probably led to the destruction of a large proportion of the remaining (volunteer) potato tubers in the field. Average planting date for ware potatoes (mostly cv. Bintje) was around 20 April. Early development of the late blight disease was hampered by spells of dry, sunny weather in May. Very few early attacks were observed or reported. A dry, hot and sunny month of June further reduced disease pressure to a very low level. Wide spraying intervals, even in susceptible varieties, were applied. The return of rainy weather conditions in the second half of July quickly led to an increase in the disease pressure; short application intervals were necessary throughout the rest of the season, which remained excessively wet. Some late blight attacks were observed from the beginning of August, with a strong expansion towards the end of this month. Wet harvest conditions caused a lot of problems with bacterial rot and meant a difficult start of the storage season.

Czech Republic 2011

In 2011, the weather conditions were very favourable for the development of potato late blight.

Rainfall in May, June and August was near the normal in the main production region; however, in July it reached 160 – 180 % of the normal. The spread of foliage blight was intensive and the level of tuber infection was also severe. The first more important outbreaks in the potato production region were observed in the second decade of July; however, epidemic late blight spreading was very rapid and non-treated crops were completely destroyed in 2 – 4 weeks, based on locality and varietal susceptibility. The first important infection period was between the 18th and 26th June. In July very favourable conditions were recorded for late spreading between the 10th and 21st July and then between the 26th and 30th July. Infection period in August lasted for the whole first half of the month. Intensive rainfall and continuously wet soil supported tuber infection as well. Intensive infection pressure thoroughly verified efficacy of applied fungicides. The highest efficacy was recorded for fungicides Infinito (fluopicolide, propamocarb hydrochloride), Revus (mandipropamid), Ranman (cyazofamid), Altima (fluazinam), Consento (fenamidone, propamocarb hydrochloride) and Acrobat WG (dimethomorph, mancozeb). In the efficacy of systemic fungicides based on phenylamides pathogen resistance was highly expressed. However, potato late blight mostly did not cause important yield loss, since almost ideal conditions for crop growth and development resulted in obtaining of high yields prior to epidemic onset. Problems in ware potatoes were caused by tuber infection in most cases and consequences are expected in stores, especially in crops when haulm was not killed in time. Most of the early potatoes were harvested before epidemics occurred in the region.

Czech Republic 2010

The year 2010 was characterized by the intensive disease infection pressure, severe tuber infection and specific development due to very variable weather course. The situation was also complicated by diverse age of crops in the main potato production region, as the planting period was prolonged until the beginning of June due to unfavourable weather. Rainy weather in May and the first half of June initiated first outbreaks of potato late blight. However, further disease development was completely stopped due to warm and dry weather conditions from mid-June to mid-July. The drought period was followed by rainy weather that lasted until the end of the growing season. Late blight epidemics highly affected potato crops during August. Due to high rainfall tubers were directly infected from the onset of disease epidemics and a considerable amount of these decayed in the soil prior to harvest. Lower number of infected tubers was loaded into stores and storage loss was lower than initially expected. In the early potato production region epidemics developed prior to harvest, i.e. beginning June level of infections were extraordinary high for this region and tubers were also attacked. Considering fungicides the highest efficacy was found for Ranman (cyazofamid), Casoar (chlorothalonil, propamocarb hydrochloride), Altima (fluazinam), Infinito (fluopicolide, propamocarb hydrochloride) and Revus (mandipropamid). A gradual increase in fungicide resistance was observed for phenylamide-containing fungicides.

Denmark 2011

The date of crop emergence was relatively early, 12-20 May. Exactly during this period and especially in the South and Central Denmark, weather was wet and blight favourable and this resulted in early attacks from oospores in many fields – more than the in any of the previous 16 years. This was only recognized 3-4 weeks later when heavy attacks in many fields were reported in the Danish late blight surveillance network. Subsequently, weather conditions were blight favourable throughout the season. Despite the widespread early attacks, a survey on 900 ha in the South of Denmark showed that about 80 % of this area was without any blight symptoms and only 2 % had severe attacks (Pedersen unpublished). Most popular fungicides were Dithane, Ranman, Revus and Ridomil. The yield in 2011 was medium and the level of tuber blight was low. This might be due to widespread use of Ranman during the late season controlling tuber blight. The Blue 13 pathotype was found

in Denmark in 2011, but Ridomil generally was effective in 2011. Usage of the Danish DSS dose model in 2011 resulted only in a minor reduction in fungicide use in a season characterised by long periods with high infection pressure. The field test of a revised model with even lower fungicide inputs used at low disease pressure showed that it was possible in 2011 to reduce the fungicide use by 26% and still having a good control of potato late blight. Attacks of *Alternaria* was late and at low levels in 2011.

Denmark 2010

Crop emergence was normal 20-25 May. Indications of oospores were only found on a trial site and in a few other fields with narrow crop rotation. First attacks were recorded on 8 June. Attacks in more than five fields were reported on 7 July. June was unfavourable for late blight and July and August had several dry spells making blight control with fungicides relatively easy. Even Dithane is used widespread because it is cheap the use of Ranman/Revus control strategies is increasingly used in Denmark. Ridomil obtained a good effect. Yield was relatively high and tuber blight was only a problem in few fields due to a rainy period and blight favourable conditions in September. *Alternaria* was observed early in the season and caused some problems. Attacks of *Alternaria* seems to be an increasing problem in Denmark.

England & Wales 2011

Planting progress was generally good in England and Wales in 2011. Weather conditions conducive to late blight development were reported from mid-June and throughout July and August. There were 36 incidents of late blight reported on Potato Council-funded late blight maps in GB in England and Wales until mid-September. Most reported outbreaks were in crops, with one infection reported on a volunteer and one on an outgrade pile. Fourteen incidents were reported in July, 14 in August and 8 in September and, overall, control of late blight was good. Control of late blight in England and Wales was good in 2011, with no incidents until mid-July and weather conditions did not affect timing of fungicide applications. According to the UK pesticide usage survey report 235, the active ingredients used on the largest areas were fluazinam, mancozeb/cymoxanil, cyazofamid, mandipropamid and fluopicolide/propanocarb-hydrochloride.

England & Wales 2010

Planting was delayed in March due to heavy rain and snow across much of England and Wales, however, most of the crop was in by the last week of May. Weather conditions conducive to late blight development were reported mid-June, and throughout most of July and August. There were 67 confirmed incidents of late blight in England and Wales in 2010. Most reported outbreaks were in crops with the occasional infection reported on outgrade piles and volunteers. Only 1% of incidents were reported in June, with 80% reported across July and August and the remainder in September. Growers used a range of fungicides which were often applied as tank mixes. Control of late blight was good overall. Conditions during harvest were variable, with rain interrupting lifting and in some instances lifting was delayed due to poor conditions earlier in the season that delayed the cereal harvest. Most fungicides were applied at seven day intervals unless risk was considered to be low for extended periods. *Alternaria* is not considered to be a major disease of potatoes in England and Wales, although occasional outbreaks are reported, particularly on susceptible varieties such as cv. Markies. First symptoms in reported cases were reported mid- to late July in 2010.

Estonia 2011

Due to late spring there was only short time difference between planting, development and late blight infection of early and maincrop potatoes. After the very low incidence of late blight in previous 2010 year, the late blight established very late in 2011. The dry weather in first half of the 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 the 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. Rain events covering whole Estonia from 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 the DSS saved 1-2 fungicide applications in average.

Estonia 2010

The late blight established early in 2010. First infections were recorded at the end of May in covered field of early potatoes and at the end of June in open fields. The established infection stopped in following hot and dry weather. Late blight established again on late varieties in September just before the harvest. It was year of lowest late blight incidence since 2002. Weather conditions were more favourable for *Alternaria*, causing medium level of attacks. Leaf blotch of potato caused by *Botrytis cinerea* damaged the potato plants in the same level as did *Alternaria*. Early blight needed more fungicide input for effective control than late blight. There was low incidence of tuber blight. *Alternaria* caused more tuber infection than Phytophthora, especially in sandy soils.

Finland 2011

The potato late blight development in Finland in 2011 was highly determined by exceptionally warm weather accompanied by very local heavy thunder storms with excessive precipitation. The first blight observations were made at the first week of July, which has been very constant within past ten years. At the sites with high precipitation the progress of late blight epidemics was faster than ever recorded earlier during the 2000s. At sites with low precipitation no blight at all was detected. The use of fungicides as well was dependent on the blight risk induced by the local thunders. Tuber blight at the sites with high precipitation and failure in leaf blight control seems to be more devastating than normally in Finland. In 2011, probably due to warm but relatively rainy summer, *Alternaria* at some sites and cultivars was very common and also injured some crops very seriously. Also in the late blight control the intensive use of mancozeb-products has been replaced by more modern products with no or low efficacy against *Alternaria*.

Finland 2010

In 2010 there was practically no potato late blight in Finland. Due to the dry and warm weather conditions farmers normally sprayed 3 – 5 times.

France 2011

In Brittany (along the Channel coast), early crops (plastic covered and under tunnels) were planted by the end of January and most of the crops remained covered until end of March. The first outbreak of late blight occurred in a tunnel in the last days of March, followed by an outbreak in an open field in the first days of April. These very early crops were ready for harvest and tuber damage was not recorded at harvest. In the seed production areas, planting of the crops was almost completed by mid April (2-3 weeks earlier than usual) with unexpected dry and mild conditions. Climatic conditions of April and May were mild to warm and unusually dry, not conducive at all for large late blight development however sporadic outbreaks occurred on volunteers and cull piles late May. Few rains early June contributed to the first outbreaks in conventional fields which had no further favourable spreading conditions, afterwards. After a continuous low risk period from April to end

of June, the climatic situation completely changed, turning into rain and mild to cold temperatures. Crops were close to haulm killing but spraying program had to be adjusted following high risk periods. Late maturing crops had to be protected thoroughly in order to maintain high quality tubers at harvest. For the ware potatoes in the north of France, planting was earlier than usual, and late blight appeared in April during warm and humid conditions. The spring 2011 was marked by a particularly dry climate. During June, the lack of water and the high temperatures stressed the maturation of tubers and reduced the tuberisation from 10 to 30 % according to the varieties and the sectors. The return of rains in mid July strongly favoured the establishment of late blight. Weather was continuously conducive for blight development and tuber quality was affected. The incidence of *Alternaria* was severe in the Picardie region and disease appeared as early as end of June.

France 2010

Very early potato planting occurred mid February in Brittany, along the Channel coasts, mostly under plastic covers and tunnels. From March until end of May, the climatic conditions were not too conducive for late blight development though several late blight outbreaks were recorded for covered crops. In seed potato growing areas (Brittany), climatic conditions have been fairly cold and dry after planting and the first outbreak of late blight occurred early June. A low risk period followed in July and high risk periods occurred again at the haulm killing period, in August. Good tuber quality was guaranteed by a comprehensive spraying program. In the Northern potato growing areas (ware and processing), planting occurred from mid April until mid-May, in cold and humid conditions. These climatic conditions have limited late blight outbreaks though volunteers were growing with no protection against late blight as well as waste piles. Due to heavy rain falls from mid June, the first blight outbreaks occurred on waste piles (Picardie region) and gardens (Nord Pas de Calais region). Blight pressure was very low during July and, with the use of the DSS, some sprayings have been saved. A high risk period persisted in August when further disease dispersal has affected some commercial crops later by the end July. Overall harvested crops were blight free with very few exceptions.

Germany 2011

Planting of potatoes in Germany took place during end of March and mid of April. It is a normal planting date. Very warm and dry conditions in April and May resulted in an early emergence of potato plants (beginning of May= 10-14 days earlier than normal). The first outbreak of late blight in potatoes was in the beginning of June (04. June) – very late. The weather conditions for the development of late blight was low in the North and high in the South. The number of fungicide treatments was normal in 2011. All kind of products were used. The outbreak of early blight was normal (2-4 weeks after the crop emergence). The start of the early blight epidemic depends on the cultivar, crop emergence (plant age), weather condition and inoculum. In some regions there was a very early start of the epidemic. Therefore in most regions early blight has been a destructive disease and caused yield losses due to premature defoliation. Fungicide used to control early blight: Mancozeb-containing products, Ortiva (Azoxystrobin), Signum (Boscalid + F500).

Germany 2010

Planting of potatoes in Germany took place during end of March and mid of April. It is a normal planting date. The first outbreak of late blight in potatoes was in the mid of May in the early potato growing area (11.May). In the first week of June late blight was observed in the Southern potato growing region. The weather conditions for the development of late blight was low in the North and high in the South. The number of fungicide treatments was normal in 2010. All kind of products were used. The outbreak of early blight was normal (2-4 weeks after the crop emergence). The start of the early blight epidemic depends on the cultivar, crop emergence (plant age), weather condition

and inoculum. Therefore in some regions (north, east and west) early blight has been a destructive disease and caused yield losses due to premature defoliation. Fungicide used to control early blight: Mancozeb-containing products, Ortiva (Azoxystrobin), Signum (Boscalid + F500). There are two decision support systems, Phytophthora-Model Weihenstephan and ISIP for the control of late blight running in Germany. The information of the DSS's are also on the internet (www.krautfaeule.de; www.isip.de). The majority of the potato growers are directly informed by fax or e-mail. In many regions the state advisory services inform the farmers by telephone or fax.

Republic of Ireland 2011

Favourable weather conditions during April resulted in relatively early planting of the potato crop during 2011. Although wet weather conditions prevailed during May, June and July temperatures were below normal. No early outbreaks of late blight were reported, with the first outbreak recorded on the 8th of July in Co. Kerry (South-West of the country and away from the major potato production regions). Further outbreaks were not reported until late July / early October. In general routine fungicide applications were made at seven day intervals and little problems with disease control were reported. No major outbreaks of *Alternaria* were reported. There are limited information on individual use of DSS. Country-wide forecasting provided by Met Eireann using Effective Blight Hours based upon Bourke's rules were issued through national media outlets. *Phytophthora infestans* characterisations are ongoing.

Latvia 2011

Crop emergence was completed by the end of May. Cool weather conditions (average temperature of 8-10 °C) delayed the crop growth and development of late blight, therefore the first warning of the development of late blight was received on the 26th of June when the temperature and humidity conditions were favourable for the development of the disease. Also the first warning of the development of *Alternaria solani* was received in this period. In June 95 mm of rainfall was recorded in western part of Latvia, but in the northern part - 135 mm of rainfall. The first protective application of fungicide (systemic or translaminar + contact) was made before the infectious period. Temperature and humidity conditions were favourable for the development of both diseases. The second warning of the development of late blight was received on the 3th of July. The second protective application with fungicide (systemic or translaminar + contact) was made. The first symptoms of *Phytophthora infestans* were recorded on the 8th of July on unprotected crops. In July the infection pressure on unprotected crops was very high due to frequent precipitation and optimal temperatures. In July 136 mm of rainfall was recorded in western part of Latvia with average temperature of 15 – 19 °C for the most of July. The following applications in July were made with translaminar + contact fungicides and in August - with contact fungicides, mostly with mancozeb and fluazinam. Unprotected crops and those that were the most susceptible were totally killed in two weeks in the beginning of August. *Phytophthora infestans* and *Alternaria solani* progressed also in August. 101 mm of rainfall was recorded in northern part of Latvia in August and weather conditions were favourable for the development of tuber blight. The use of fungicides resulted in excellent control in all farms when the first protective application with systemic + contact fungicide was made at the end of June/ beginning of July. Control of late blight was very good to moderate in the 2010 season.

Lithuania 2011

The spring in 2011, was warm and dry in most of Lithuania therefore resulting in good conditions for planting. The first blight report came 11-12 June from an uncovered field where early potato cultivars were planted. This is later than normal. Until mid July there were only sporadic reports of blight in Lithuania. Good conditions for late blight development on leaves were recorded on the end of August and on the beginning of September. Late blight infections on tubers did not overcome

incidence in previous years as was on the controlled level. The most farmers sprayed normally starting at the end of June or first half of July and sprayed 4 – 6 times. In Lithuania, 2011, *Alternaria spp* was very rare and infection took place only in a limited number of fields, where disease severity remained at low levels.

Lithuania 2010

April was warm enough as usual. Average air temperature was 7.3 degree which is by 1.3 degree higher compared with long-term average. Amount of rain was higher by 16.3 % compared with long-term average. Air temperature in May was a little higher than usual but this month was very rainy. Total amount of the rain amounted nearly by double compared with long-term average. Therefore the planting of potato crop was delayed by nearly 3 weeks compared with usual practice. Most of the crop was planted on the second part of May. From June till August higher than usual, average air temperature prevailed. Very hot months were July and August when average air temperature was by 4.0 and 3.1 degree higher compared with long term-average, respectively. Also, during the same period, the amount of rain was exceeded. In June and July the amount of rain was higher by 13.7 % and 91.4 %, respectively. In September, air temperature and amount of rain was very close to long-term average. Rainy weather conditions are favourable for late blight occurrence and development but during the growing period heavy rains altered with very high air temperature, therefore disease occurred quite late (at the beginning of August). Heavy rain squeezed the soil and growing of potatoes was aggravated. Soil cultivation between rows was impossible due to the dense foliage. Therefore yield of potato tubers was not high enough as it was expected.

The Netherlands 2011

Just like 2007, spring 2011 in the Netherlands was extremely dry. In the southern part of the country it stayed dry till half June. The precipitation deficit (calculation starting at April first) ranged from 150 to 210 mm. From that time the weather changed and July was a wet month with precipitation amounts ranging from 100 mm in the eastern parts of the country till 200 mm in the coastal regions. This resulted in an early average planting date (first week of April) of the potatoes. Until the first week of June there has hardly been Late Blight all over the country. In May there was a report of Blight in a field under cover in the South Eastern part of the country. The disease pressure of Blight stayed rather low during the season till the beginning of August. The weather conditions were favourable for many days in July. But, the growers were able to keep their crops free of attack by spraying regularly. Most organic crops became infected during the last decade of this month. Fungicides used in 2011 differs not very much of the use in 2010. There is a shift towards the use of Revus, Valbon, and Orvego at the expense of Curzate M and Acrobat for the first 4-6 sprays. In the middle part of the season we see a lot of growers applying Infinito. In August there was a comprehensive use of Ranman and still a considerable share for Shirlan, although this product is decreasing because of disappointing experiences in August 2010.

Northern Ireland 2011

Generally crops were planted in fairly good conditions, but subsequent growth was slow due to unusually cool, dull weather during June, July and August. Blight was first reported on 18 June and was subsequently found in some crops in all potato-growing parts of Northern Ireland, but was more frequent in the north-west with less infection found in Co. Down (south and east). Overall, there were fewer reports of blight than the average, probably because of good fungicide programmes, rather poor growing conditions and weather which was not particularly favourable to blight until August. Fungicide usage was about average (seed crops received between 4 and 15 applications, with the average being 9-10); fluazinam was the most widely used active ingredient and growers also made substantial use of fluopicolide+propamocarb, dimethomorph+mancozeb, cymoxanil+mancozeb,

cyazofamid, mandipropamid and mancozeb. Blight was generally well-controlled in foliage and there were few reports of tuber blight.

Northern Ireland 2010

Crops were planted in good conditions. The weather in April, May and June was unusually dry (c. 50% of average rainfall) and some crops showed signs of stress in June and no late blight was seen. Although July was wetter than the average, the first report of blight was not received until 19 July, the latest of any year since monitoring started in 1981. Few outbreaks were reported (only 10 sites) and overall blight was well controlled. Blight fungicide usage was also therefore less than in recent years.

Norway 2011

High soil humidity in the first part of the growing season and more late blight favourable days in June than normal caused 2-3 weeks earlier infections than normal in the main crop. Late blight was also found relative early in Nordland, but was not found further north of Norway in 2011. Infected seed tubers were probably the main cause of primary infections. During July and August there were more days favourable for late blight than normal and this also continued into September. Regular fungicide treatments kept late blight under control, but about 50 % of the fields had leaf blight in early August, but at low levels. The precipitation was very high during the whole season. Potentially there will be more tuber blight than normal. On average about one more fungicide treatment was used than normal. Typically one treatment with Ridomil or Tyfon is used early and then Ranman or Revus. More than 70 % 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.

Norway 2010

Late blight started to develop at about the same time as normal. In the main potato growing areas there were many late blight favourable days in July and August. A lot of fields had some late blight attacks, but most farmers were able to spray their fields without getting heavy losses. The level of visible tuber blight was relatively low, but probably more latent infection than normal was present. The number of treatments was about the same as normal for the last years. Late blight was found late in the season in Nordland County but not further north in Norway in 2010.

Poland 2011

In Poland the beginning of growing season 2011 with very cold and dry weather conditions during April delayed planting of the majority of potato crops for a few days, with planting continuing in mid May. Weather conditions in May and first decade of June were also dry and unfavourable for late blight attacks. First outbreak of potato late blight was recorded at the beginning of June, on 6.06.2011. Higher rainfalls, after 10th June caused the majority of late blight attacks in observed potato fields around Poland during forthcoming two weeks. Late blight attacks in more than 5 conventional, normally planted potato fields were reported on 10th June. Weather conditions in the next days favoured the development of the disease. High precipitation in July and August extended high late blight pressure. The result of "blight year" was a high level of tuber blight in yield. Farmers applied 3-13 fungicide sprays in order to control late blight.

Poland 2010

In 2010, planting of potatoes in Poland took place mainly during April, at a normal planting

time. Unfavourable weather conditions after planting (high humidity and cold) resulted in late emergence of potato plants and were not conducive of late blight appearance (too cold for the late blight development). In southern and central parts of the country first outbreaks of late blight in potatoes were reported at the end of May and at the beginning of June in covered and early planted potato crops, at a very early growth stage of potato plants (BBCH= 24-37). Late blight attacks in more than 5 conventional, normally planted potato fields were reported on 21st June. In northern regions by 20th June only a few outbreaks of late blight were recorded in crops with foliar and stem symptoms. Following approximately two weeks of extreme disease pressure in early August, more severe outbreaks were reported in northern potato region. The development of the disease stopped in July because of very dry and warm weather. Very intensive development of late blight was reported again in August (after 10th), leading to the complete destruction of plants during 7-10 days on unprotected plots. Farmers applied 1-11 sprays to control late.

Russian Federation 2011

The most severe late blight development was registered in the Kaliningrad region. The first late blight attack was reported on June 23. The disease became widespread after July 20. To August 1, the foliage was destroyed on the unprotected potato fields. Due to heavy rains shortly before and during the harvesting, the disease became a big problem in the most of farms. A moderate development of the disease was observed in the northwestern (Leningrad, Pskov, and Novgorod), western (Bryansk), and some northern regions. Other parts of the European Russia demonstrated rather weak or even suppressed late blight development. A severe or moderate early blight attack was reported for some potato cultivars, growing on unprotected fields or fields, protected only against the late blight, of the central and southern regions of the country. The most popular fungicides were Tanos, Shirlan, Infinito, Ridomil Gold MZ, and Acrobat MZ. The average number of sprayings is about 3-4; the total number of treatments ranges between 2 and 11. The owners of allotment gardens did not use any fungicides. A small number of farms used such DSSes as Plant Plus (Dacom) or VNIIFBlight for the late blight control.

Russian Federation 2010

The severe, but late development of the potato late blight was observed in the Kaliningrad region and some districts of the Leningrad region. In the case of other regions of the European part of Russia, a long period of a hot and dry weather caused very unfavorable conditions of the *P. infestans* development. However, in spite of this fact, in the central regions (for example, in the Moscow region) we observed a strong infection of potato tubers (cv. Santé): 14% on the non-treated fields and 2% in the case of a twofold treatment with the Bravo fungicide. A strong early blight infection was registered in the Vologda region (cv. Udacha; foliar infection). Average number of fungicide applications: 3-4 (max 7-9) per season. The most popular fungicides were Tanos, Shirlan, Infinito, and Ridomil Gold MZ. The owners of allotment gardens did not use any fungicides.

Scotland 2011

One hundred and forty-five confirmed outbreaks in Scotland were reported on the Potato Council-funded blight outbreak maps up until the 16th of September. The progression of crop outbreaks (134 in number) was 0% in May, 0.8% in June, another 37.3% in July, 59.0% more in August and 3.0% more up to the 16th of September. There were five confirmed outbreaks on dumps of potatoes (7, 12 and 25 July, 10 and 18 August) and six outbreaks on volunteers (12 July and 5, 9, 17, 18 and 25 August).

Scotland 2010

Thirty-two confirmed outbreaks were reported on the Potato Council-funded blight outbreak maps

up until 20 September. The progression of crop outbreaks was 0% in May, 5.3% in June, another 15.8% in July, 57.9% more in August and a further 21.1% in September (up to the 20th). There was one confirmed outbreak on a dump of potatoes (20th of September) and one outbreak on volunteers (10 August). The most widely used fungicides in 2010, in declining order, were cymoxanil, fluazinam, cyazofamid, mandipropamid, mancozeb + cymoxanil, amisulbron, benthiovalicarb + adjuvant, fluopicolide + propamocarb, famoxadone + cymoxanil, fenamidone + propamocarb, dimethomorph + mancozeb and zoxamide + mancozeb.

Slovakia 2011

Compared to 2010, above-standard rainfalls were recorded but use of pesticides was possible and disease was adequate managed. Strong damaged vestures were not recorded. Therefore, much higher yields are expected compared to the last year. High rainfalls that support the blight development were approximately from half of June till the end of July. Locally this season started 2 weeks earlier, eventually lasted longer for other 2 weeks till the half of August. In comparison to the last year, there were rainfalls less intensive but the conditions for blight development were reached and lasted continuously about 7-11 weeks.

Slovakia 2010

With regard to a very long duration of suitable conditions for spreading of Late Blight, its occurrences were recorded relatively early, with a fast follow development. Damages of vestures were high, including economic losses. It was among other things caused by large floods and long-term impossibility to enter into vestures. Some of vestures were damaged so much, that there were no crops at all, or we were unable to harvest such a crop caused by long-term flood.

Sweden 2011

The spring was warm and dry in most of Sweden 2011 resulting in good conditions for planting. The first blight reports in 2011 came 3rd of June from a covered early potato field on the South west coast. The blight pressure was low in the beginning of the season in 2011 but switched to very high in the beginning of July. The weather in south Sweden was very favourably for blight in July and August. Many reports of late blight attacks from both organic and conventional potato fields. 2011 is according to experienced advisers the worst blight year in 30 years. In North Sweden the early season was very dry and no reports of blight attacks but August was rainy. This resulted in late blight reports in September as far north as from above latitude 65 N. 2011 can be considered as a year with big difficulties to control late blight and in addition very bad harvest conditions.

Sweden 2010

The spring was warm and dry in most of Sweden 2010 resulting in good conditions for planting. In 2010, the first blight report came 1 June from an covered early potato field on the South west. The weather was very dry in the early season, resulting in only sporadic reports of blight in south and mid Sweden. The first reports came from these areas came in late July. 2010 can be considered as a year with relatively small problems with late blight

Switzerland 2011

In 2011 late blight epidemic pressure was low. Until May it was very dry in Switzerland and during April in almost all parts of Switzerland no MISPs (main infection and sporulation period) were registered. A first late blight attack was observed early on May 9 in a covered potato field in the south-western part of Switzerland. As this is geographically an "isolated" region, this attack was not important for the other potato growing regions. On May 19 and June 14 two other late blight attacks were observed. In June some MISPs were registered, but as these were only single events (except canton TI), fungicides could be applied without problems. During July and August, weather

conditions were very favourable for the development of late blight and late blight spread over the potato growing regions, but at a low pressure. 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 very low (44) compared to former years (2010: 75, 2009: 95, 2008: 224).

Switzerland 2010

Winter of 2009/2010 was particularly long and cold. Last snowfall in the lowland was recorded at the end of March and many potatoes were planted only in April. Compared to former years, the first late blight attack was observed rather late on 27 May in a covered potato field (2009: 30.4.; 2008: 19.5.). During the first two weeks of May and June, the weather based infection risk was very favourable for the development of late blight. Several days with continuous main infection and sporulation periods (MISP) were registered for all weather stations. Therefore late blight could spread in all potato growing regions. However, during the second part of June until July 22nd it was hot and dry. Hence, late blight epidemic pressure was strongly reduced. With a heavy thunderstorm, this dry weather period ended and since then weather conditions were again favourable for the development of late blight. Nevertheless, late blight could only recover slowly and only a few new late blight attacks were observed. In summary, the spread of late blight started late and fast, but the hot and dry weather from June 22 until July 22 stopped the epidemic and therefore late blight epidemic was weaker than the year before. Tuber blight was found in fields with tardy late blight attacks, but in summary pressure of tuber blight was low.

EARLY ATTACKS OF LATE BLIGHT

In North-West Europe in countries like the Netherlands, Belgium, France and the UK, early attacks of late blight is often found on dump piles. In 2010 and 2011 spread from dumps was not a big problem: “As is usually the case, early inoculum was found on dump piles. The spread of the disease from these early sources however was very much hampered by the dry and sunny weather in the first half of the growing season, as was the case in the previous season 2010”.

In 2010 early attacks were found in most of Europe between 25 May and 8 June. Late blight was found very late in the Netherlands (20 July) and in Northern Ireland (19 July), Fig. 1 and Fig. 5. Attacks were found widespread in Mid June in central Europe, first half of July in North-East Europe and late July and August in Northern Ireland, Scotland and Russia (Fig. 2 and Fig. 5).

In 2011 very early attacks were found in April in France and in May in Switzerland. In most other countries late blight attacks were recorded in the first half of June (Fig. 3.). Widespread attacks in conventional fields were relatively late compared to 2010, except for Denmark where wet and blight favourable weather conditions late May and early June resulted in early attacks from oospores in many fields – more than in any of the previous 16 years in the country (Fig. 6). Early attacks from oospores were mentioned as possible source of inoculums in the reports from Denmark, Sweden, Estonia and Poland.

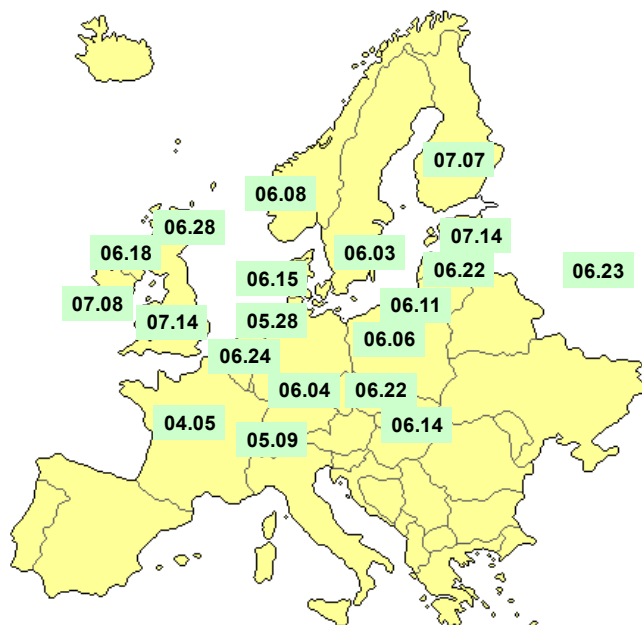
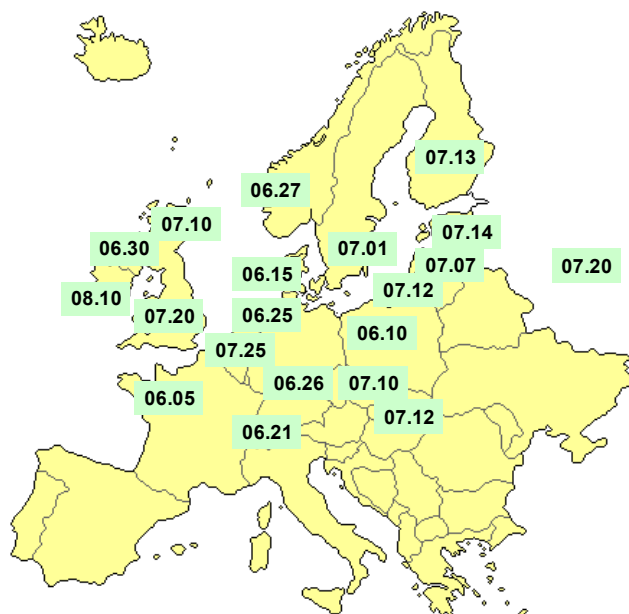


Figure 3. Date of first observation of late blight in covered or very early planted potatoes, 2011



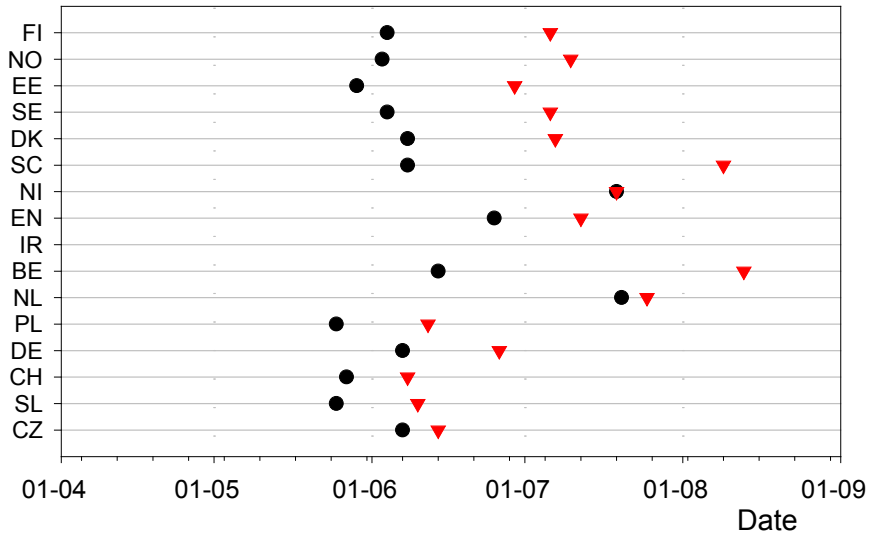


Figure 5. Date of first observation of late blight in covered or very early planted potatoes (black dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (red triangles), 2010

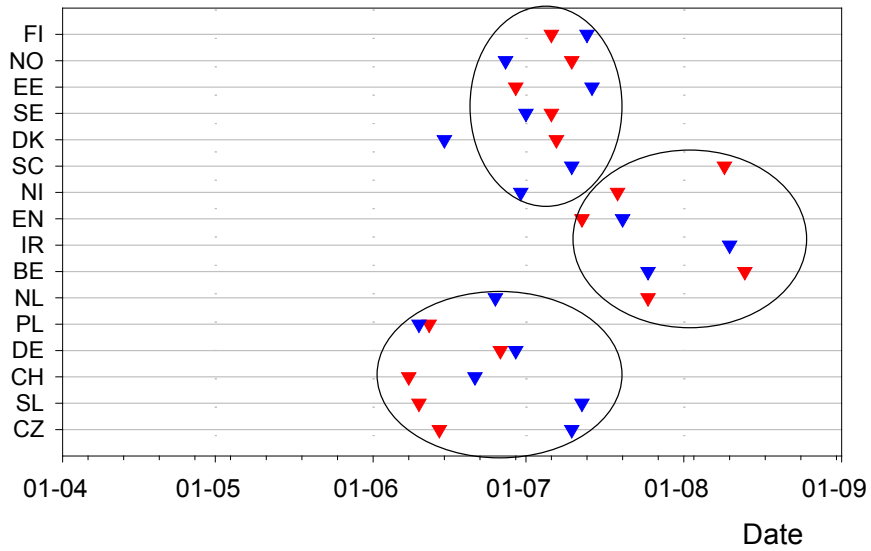


Figure 6. Date when first infections were reported in more than 5 conventional, normally planted potato fields in 2010 (red triangles) and 2011 (blue triangles) respectively.

WEATHER BASED RISK OF LATE BLIGHT DEVELOPMENT IN 2011

The weather based risk of late blight, given as infection pressure (0-20 = low; 20 – 40 = Medium and >40 = high) is given in Fig. 7. These calculations can be found on www.euroblight.net where outputs from several late blight sub-models can be compared for the period 2006-2011 across selected stations in Europe.



Figure 7. Infection pressure calculated at selected stations in Europe from 15 May-15 September, 2011

TUBER BLIGHT IN 2010 AND 2011

The level of tuber blight was low to medium in 2010, probably due to a combination of effective leaf blight control and favourable weather conditions during harvest (Fig. 8). For 2011, several countries have reported low incidence of tuber blight (not shown). The situation in 2010, reflect the situation in other countries too: “Although the weather conditions throughout the harvesting period were very wet and difficult, the level of tuber blight was less than could be expected. Fungicides with emphasis on tuber protection had been applied with (very) short intervals towards the end of the season, and the level of late blight attacks in the foliage was much lower than in previous years.”



Figure 8. The level of tuber blight attacks (low, medium or high) in 2010 compared to normal

ALTERNARIA 2010 AND 2011

The level of attack of *Alternaria Spp.* is shown for 2011 in Fig 9. A similar - but in Central and East Europe less severe situation - was the case in 2010. *Alternaria Spp.* seems to be a minor problem in North/West Europe. Some countries stress that attacks of *Alternaria Spp.* is an increasing problem, but severe attacks are only sporadic e.g. in Estonia, 2011, the conditions were more favourable for development of early blight than for late blight. The infection was at medium level. Also a shift in type of fungicide may change effective control i.e. in Finland "*Alternaria* has not so far been a problem. In 2010 some *Alternaria* lesions were found at the end of season. In 2011 probably due to warm but relatively rainy summer *Alternaria* at some sites and cultivars was very common and also injured some crops very seriously. Also in the late blight control the intensive use of mancozeb-products has been replaced by more modern products with no or low efficacy against *Alternaria*. In the Netherlands growers are now used to apply the *Alternaria* products (Amistar, Signum) two or three times during the months July and August.



Figure 9. Problems with *Alternaria*, 2011 in three classes compared to normal

USE OF DSSS

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 weakly published in farmer's newspapers. A lot of farmers have learned due to PhytoPRE to mind the critical facts/periods of late blight. In Estonia, a new network consisting of 13 iMetos stations was established in collaboration of Jõgeva PBI and farmers cooperative Talukartul for DSS in late blight control. A NegFry model provided through platform fieldclimate.com was used for fungicide timing. 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, growers can register and have free access to Blightwatch (www.blightwatch.co.uk) which gives the weather-based risk based on Smith Periods. Many growers are applying fungicides regularly, with a maximum interval of 7 days between applications, regardless of risk. DSSs providing information on a more localized scale are available to users on a subscription basis and include Forecast Extra. In Belgium, more than 2000 potato growers receive advice on late blight control from one of the two warning services, depending on the region. A network of more than 70 automatic weather stations collects the necessary meteorological data. The disease models used are historically based on the Guntz-Divoux model, but have been adapted and modified in the course of the past 20 years based on field trials and observations, new pathogen data etc. In the region of Flanders, extensions and sub models have been added, leading to a much more quantitative disease model. Additionally, the model has been integrated with GIS software and linked with a late blight attacks monitoring service. Advices are updated several times per week and communicated via internet, e-mail, fax or post. A separate advice for organic growers is available, pointing out critical days for preventative applications with copper fungicides. 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 Programm. The use of DDS's hasn't changed a lot during the last years. 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 analagous to Smith Periods and can also sign up to receive Blight Warnings by SMS. Warnings of Infection Periods are also given on the Blightline recorded phone message and via local radio. Growers can also access Blightwatch (<http://www.blightwatch.co.uk>) based on Smith Periods. DSS e.g. Plant-Plus are mainly used by pre-packing suppliers to supermarkets to provide justification for fungicide applications. In Poland, generally, farmers start with chemical control after high amount of precipitation in the region or based on information from Main Inspectorate of Plant Health and Seed Inspection web site or based on Negative prognosis. Some farmers use the NegFry DSS (mainly in wielkopolskie voivodship). In Russia, a small number of Russian farmers used the Plant Plus (Dacom) and VNIIFBlight DSSs.

The population structure of *Phytophthora infestans* in the Netherlands during the years 2000 - 2009

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SUMMARY

652 *Phytophthora infestans* isolates were collected from commercial potato fields in the Netherlands during a ten year period, 2000 – 2009. Population diversity was assessed using twelve highly informative microsatellite markers. The results describe a population structured in three major groups but also reveal the complexity of the Dutch *P. infestans* population with over 322 unique genotypes as well as the increasing importance of clonal lineages, their dynamics during the potato growing season and their ongoing stepwise displacement over the years. The results also emphasize the importance of the sexual cycle in generating genetic diversity as well as the importance of the asexual cycle as the booster and dispersal mechanism for successful genotypes.

KEYWORDS

Phytophthora infestans, late blight, microsatellites, SSR, population genetics

INTRODUCTION

In the Netherlands the total area under potato cultivation amounts to 165.000 ha and annually yields 7.9 million Mg of potato representing a value of about M€790. The number of fungicide applications varies between 10 and 16 per season. Costs for potato late blight control (chemicals, application and losses) amount to 125M€ per year, almost 16% of the total farm gate price (Haverkort *et al.* 2008). From these figures it is clear that farmers, the potato industry, consumers and the environment could greatly benefit from more efficient and environmentally friendly ways to control late blight through e.g. the introduction and durable exploitation of host plant resistance. *P. infestans* however is renowned for its capacity for adaptation under selection pressure exerted by e.g. cultivation of resistant cultivars. One of the prerequisites for durable management of late blight therefore is a thorough up to date knowledge on local *P. infestans* characteristics and high level understanding of population dynamics in order to avoid unnecessary erosion of resistance and development of fungicide resistance.

P. infestans is heterothallic and both, A1 and A2, mating types are required for completion of the

sexual cycle. Sexual reproduction results in large genetic variation in the offspring and may lead to increased and more rapid evolution of the pathogen. Before the 1980's, both mating types were only found in the highland of central Mexico which is the presumed center of origin of *P. infestans* (Flier *et al.* 2003, Fry *et al.* 1993). At that time, outside central Mexico only the A1 mating type was detected. In the early 1980's, A2 mating type isolates appeared in Europe (Frinking *et al.* 1987, Hohl and Iselin 1984, Tantijs *et al.* 1986). Nowadays, the A2 mating type is present all over Europe, South- and North American and Asia (Ann *et al.* 1998, Cooke *et al.* 1995, Ghimire *et al.* 2001, Wiem *et al.* 2006). As a result the population structure of *P. infestans* around the globe has undergone major changes over the past 20 years. The predominant 'old' populations of *P. infestans* in Europe, the Americas and Asia were displaced by "new" populations (Drenth *et al.* 1994, Fry *et al.* 1993, Spielman *et al.* 1991).

In the Netherlands only the A1 mating type was found prior to the 1980's and all isolates grouped in a single (US1) clonal lineage (Drenth *et al.* 1994), that was also found in many other parts of the world (Drenth *et al.* 1993). During the 1980's, following a renewed global migration of both (A1 and A2) mating types, a new *P. infestans* population rapidly displaced the US1 clonal lineage (Drenth *et al.* 1993, Spielman *et al.* 1991). Members of the old US1 clonal lineage were not detected in the Netherlands ever since. One of the driving forces behind this displacement may have been the higher levels of aggressiveness and fitness in the new population as compared to the old population (Flier and Turkensteen 1999).

More than two decades into this displacement process, investigators from the UK reported that a single *P. infestans* genotype with A2 mating type, EU13_A2 or "Blue_13" is dominant in the UK (Lees *et al.* 2009). "Blue_13's" dominant position was hypothesized to have emerged from superior levels of fitness in combination with resistance to the frequently used metalaxyl and a blue 13 favorable choice of commonly grown cultivars (White and Shaw 2009).

On the premise that understanding of the population genetics of plant pathogens will contribute to the development of more durable future disease management strategies, the objective of this study was to describe and analyze the dynamics of the Dutch *P. infestans* population over the course of a decade between 2000 to 2009. For this purpose, individual *P. infestans* isolates were characterized using a recently developed and standardized set of twelve highly informative microsatellite markers.

MATERIALS AND METHODS

Sampling areas were categorized according to geographical location and type of potato cultivation. The North East (NE) of the Netherlands is characterized by starch potato crops grown on sandy and peat soils. The North and North West (NW), as well as the Central (C) area is dominated by ware and seed potatoes grown on clay soils. The South West (SW) is also characterized by ware and seed potatoes grown mainly on clay soils. The South East (SE) is characterized by ware potato crops on sandy soils.

Infected leaves were predominantly sampled from production fields but also from allotment gardens, potato dumps and volunteer potato plants. The samples were generally collected between the 1st of April and the end of September. Location, sampling date and cultivar were recorded.

Infected potato leaflets containing a single lesion were hand-picked and positioned upside down in a Petri dish containing 1.5% water agar. A small tissue sample from the edge of the lesion was then placed under a potato disc inside an otherwise empty Petri dish and incubated for one week at 15°C. Mycelium emerging from the top of the tuber slice was transferred to ampicillin containing pea agar (PA). All isolates were maintained in liquid nitrogen as a part of the *P. infestans* collection at Plant Research International, the Netherlands. A total of 652 isolates were obtained.

Mating types were determined by confronting the individual isolates with an A1 (isolate VK98014) or A2 (isolate EC3425) tester isolate on PA. Plates were incubated in the dark for 14-21 days at 18°C.

After mycelial contact between both colonies was established, the contact zone was monitored for the presence of oospores. When oospores were found in the Petri dish with the A1 tester isolate the unknown isolate was classified to have the A2 mating type and vice versa.

Isolates were grown in liquid pea broth. After 3-4 days of incubation at 20°C in dark, mycelium was collected for freeze drying and subsequent DNA extraction. Genomic DNA was isolated from 20mg of lyophilized mycelium using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). Elution was done with 200µl ultra-pure water. DNA extracts were stored at -20°C until further use.

Mitochondrial haplotypes were determined using the PCR-RFLP method of Griffith & Shaw (23). Restriction digestions of the amplified regions P2 (MspI) and P4 (EcoRI) allowed for differentiation of the four mitochondrial (mtDNA) haplotypes Ia, Ib, IIa and IIb.

The Euroblight set of twelve microsatellite markers was used. Amplifications were run in a PTC200 thermocycler (MJ Research, Waltham, Massachusetts, USA), with an initial denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20 seconds, 58°C for 90 seconds, and 72°C for 60 seconds, and a final extension at 72°C for 20 min. Electrophoresis and visualization of SSRs was performed on a Herolab type RH-5 geldoc system and ABI3730 DNA analyser (Applied Biosystems). 5 µl PCR product was mixed with 1/6 volume of gel loading buffer (Orange G loading buffer) and separated on 3% agarose gel. For ABI3730 analysis, the PCR products were diluted 1000 times and one µl of diluted SSR product was added to 9µl of deionized formamide containing 0.045µl of GeneScan-500LIZ standard (Applied Biosystems). Capillary electrophoreses of the mixture was done on an automated ABI3730 according to the manufacturer's instructions. SSR allele sizing was performed and scored using GeneMapper v3.7 software (Applied Biosystems).

GENETIC DATA ANALYSIS

Basic measures of genetic diversity - to analyze the variation in microsatellite loci, the observed number of alleles (n_a), effective number of alleles (n_e) and Shannon's Information index (I) per locus in all populations were estimated using POPGENE. The significance of deviations from the Hardy-Weinberg equilibrium (HWE), using Bonferroni corrections, was determined using exact P values estimated using GENEPOP version 4.0 and the Markov chain algorithm with 10,000 dememorization steps, 100 batches and 1,000 iterations. GENEPOP 4.0 was also used to calculate the observed heterozygosity (H_o), expected heterozygosity (H_e), the polymorphism information content (PIC) value and the level of linkage disequilibrium (LD) to determine the extent of distortion from independent segregation of loci. To examine the distribution of genetic variation among and within populations analysis of molecular variance (AMOVA) using Arlequin version 3.5 was employed. Arlequin 3.5 was also used to perform two versions of the neutral tests, the Ewens-Watterson test and the Ewens-Watterson-Slatkin test to check whether an actual allele frequencies deviates significantly from a probability distribution for allele frequencies under the infinite-alleles model in a neutrally evolving population. Bottleneck software version 1.2.02 was employed to test the bottleneck hypothesis under a two-phased model of mutation (TPM).

The spatial genetic structure was analyzed using the Bayesian clustering program STRUCTURE 2.2. The range for the number of clusters (K) was specified from 1-15. For each run, we examined the output for consistency of clustering assignments and checked parameters for convergence. To infer K values and determine the best level of structure supported by the data, a more formal method developed by Evanno *et al.* (2005), calculates the ΔK statistic, the modal value of which can be a useful ad hoc indicator for the level of uppermost hierarchical structure (Evanno *et al.* 2005). To perform ΔK calculations, we randomly assigned the likelihood from each of 5 Structure runs from each K into one of 5 groups, each containing a single likelihood from each K. Within each of these 5 groups, we then calculated the necessary differences from $\ln'(K)$ and $|\ln''(K)|$. $|\ln''(K)|$ was averaged over the 5 groups, and divided by the standard deviation of the likelihood for the ultimate

calculation of ΔK . To validate the genetic substructure, principle component analysis (PCA) using NTSYS software was conducted to construct plots of the most significant axes for grouping pattern verification.

RESULTS

Isolates were collected from 207 different locations comprising the five Dutch potato-growing areas. A total of 652 *P. infestans* isolates were analyzed. The twelve microsatellite loci revealed very high PIC value with an average of 0.534 and different allele frequencies in the total collection. A total of 75 alleles were detected over 12 loci and the average number of alleles per locus was 6.25. SSR markers revealed 28 rare alleles with a frequency lower than 0.05.

The mean expected (H_e) and observed (H_o) heterozygosity were 0.524 and 0.577. Locus D13 had the highest observed number of alleles (17 alleles), but its effective number of alleles (1.872) was under the mean number (2.436) for the whole population. G11 is the most informative locus of the 12 SSRs, with the highest value for the Shannon's Information index ($I=2.006$). Six loci were not in HWE. After clone-correction (elimination of fully identical genotypes) all of the loci displaying heterozygote excess were still significantly different from HWE (Table 2).

The complete isolate collection shows a high genetic diversity with 311 unique genotypes among 652 isolates. To examine the distribution of genetic variation, among and within populations as defined by the five geographical areas, the data were analyzed by AMOVA. As a result, 95% of the variance was attributed to the regional stratum (within regions) whereas the remaining 5% was attributed to the national stratum (between regions) strongly indicating the absence of separate regional populations.

For the structure analysis, clone corrected data were used. Thus, the genetic structure behind 358 isolates was analysed using the correction for Structure 2.0 outputs as described by Evanno *et al.* (2005). For all K, memberships were consistent between all runs. The first ΔK peak for $K=3$ corresponds to the presence of three main groups.

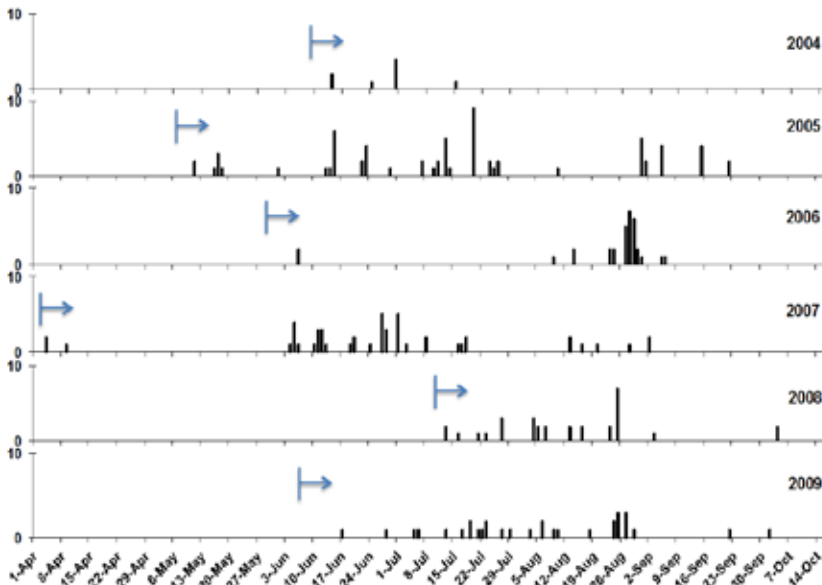


Figure 1. Isolation dates and numbers for genotype GS-001_A2 or EU13-A2 during the period 2004 – 2009.

Arrows indicate the first date *P. infestans* was retrieved in the season.

The AMOVA for $K=3$ indicated that 25% of the variance was attributed to variation between the three groups, 75% of the variance was due to variation within groups. Pair-wise estimates of F_{ST} indicated a high degree of differentiation between the three groups with values ranging from 0.18 between group 2 and 3 to 0.42 between group 1 and 2. Based on a plot against the first two dimensions from the principle coordinate analysis, the three groups detected by the structure analysis also separate from each other.

Within the three large groups identified above, three genotypes are dominant on the various temporal and spatial scales. One dominant genotype, called GS-001_A2 (A2 and Ia) was retrieved 144 times from isolates collected between 2004 and 2009 covering all five sampling regions and representing 22.1% of the 652 samples. GS-001_A2 has the same SSR genotype profile as the EU13-A2 (or Blue-13) clonal lineage previously reported by Lees *et al.* (2009). A second dominant genotype called GS-008_A1 (A1 and Ia) was retrieved 43 times between 2000 and 2009 whereas genotype GS-005_A1 (identical to the previously reported SSR genotype EU6_A1 or pink 6 (Lees *et al.* 2009)) was found 15 times at low frequency during this survey in 2002, 2005, 2006, 2007 and 2008. Apart from these three dominant genotypes, other much smaller clonal lineages with less than 15 isolates were found in multiple years and multiple regions. The vast majority of genotypes was however only found once. Figure 1 illustrates the dynamics of the GS-001_A2 or EU13-A2 during the years 2004 - 2009.

Both A1 and A2 mating types were found in all years and all regions. Three haplotypes (Ia, IIa and Ib) were found in all years and all regions (Fig.3). Isolates with identical SSR genotypes also shared the same mitochondrial haplotype and the same mating type, further establishing clonality of these isolates.

The genetic structure of Dutch isolates was found to be of intermediate complexity, including multiple, closely related genotypes.

DISCUSSION

This paper aimed to describe and analyze 10 years of *P. infestans* population dynamics in the Netherlands. On the premise that a better understanding of *P. infestans* population dynamics contributes to more durable forms of disease management, the overall population structure, occurrence, dynamics and displacement of clonal lineages were investigated. The Dutch *P. infestans* population was found to be structured in three major groups, each containing one or more clonal lineages in a well (groups 2 and 3) or less (group 1) developed sub-structure. Clonal lineages emerging from an ongoing displacement process were found to be an important population feature. AMOVA revealed that the Dutch *P. infestans* population should be considered a national population rather than a meta-population consisting of several regional populations.

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Making Sense of *Phytophthora infestans* diversity at national and international scales

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SUMMARY

In this paper we present the status of investigations into the genetic diversity of *Phytophthora infestans* populations in Europe and an update on the means of storing, collating and interpreting such information in a central database. Simple Sequence Repeat markers (SSR), also termed microsatellites, remain the method of choice for examining population diversity as they are rapid, robust and resolve population structure at a level appropriate for identifying and discriminating clonal lineages and exposing the signature of sexual recombination in highly diverse populations. SSR analysis has progressed from 3 panels of 3-5 multiplexed markers to a single multiplex assay in which 12 markers are run in a single PCR. Frequent discovery of more than two alleles at a single SSR locus in some *P. infestans* populations suggests changes in chromosome complements. These different ploidy levels within a population have proved challenging for most methods of population genetic analysis but recent publications provide a way forward and are discussed. Several comprehensive national studies on *P. infestans* diversity are underway within Europe and, combined with some international comparisons will clarify the recent evolutionary history of this damaging pathogen. SSRs are considered selectively neutral markers. There is interest in examining sequence diversity in effector genes that are under selection pressure, in order to understand the evolution of *P. infestans* virulence/pathogenicity. In combination with neutral SSR data, effector sequences will provide a different perspective on pathogen diversity at local and international scales. The Eucablight database that was developed in the 'Eucablight' EU funded Concerted Action project is also evolving and we briefly describe its status as it merges with the larger and more flexible 'cropproblem' database that holds data for the Wheat Rust Toolbox which forms part of the Global Cereal Rust Monitoring System.

KEYWORDS

Microsatellites, Simple Sequence repeats, ploidy, effectors, population biology, database

BACKGROUND

We have reported previously the rationale for examining *P. infestans* populations and the benefits of a centralised database to store information in a standard format (Cooke *et al.* 2007b; Cooke *et al.* 2009; Cooke and Lees 2004; Hansen *et al.* 2007). Global populations of *P. infestans* are

characterised by the migration and domination of clonal lineages in many regions (e.g. Fry *et al.* 2009) with evidence of sexual recombination in others such as Mexico (Goodwin *et al.*, 1992; Grunwald and Flier 2005) and Scandinavia (Brurberg *et al.* 1999; Brurberg *et al.*, 2011; Widmark *et al.* 2007). Well documented cases of host resistance breakdown and fungicide resistance are testament to the challenges of managing potato blight. The success of integrated control strategies depends on our understanding of the pathogen diversity at local, national and international scales and the mechanisms and rates of pathogen evolution. The theoretical advantages of planned host resistance deployment strategies have been demonstrated (Skelsey *et al.* 2010) but their success hinges on understanding the stability of such sources of resistance in the face of a diverse repertoire of rapidly evolving pathogen effectors (Haas *et al.* 2009).

UPDATES ON PROGRESS

SSR markers and their analysis

SSR markers have been developed by at least three research groups (Knapova *et al.* 2001; Lees *et al.* 2006 ; Li *et al.* 2010) and used in various combinations (Knapova and Gisi 2002; protocols in www.eucablight.org). Multiplexing (the amplification of more than one locus in a single PCR mix) has been applied to increase the efficiency of the approach. Not all markers are equally informative (i.e. the polymorphism information content or PIC score varies) or easy to score due to levels of stutter (e.g. Brownstein *et al.* 1996). This has led to different numbers of markers being applied in different studies (e.g. Brurberg *et al.* 2011). A collaboration between Wageningen University and The James Hutton Institute has resulted in a multiplex assay which includes 12 of the most informative and easy-to-score markers (Li *et al.* 2012). Step-by-step protocols will be published in the near future on the Euroblight web site (www.eucablight.net). This 12-plex assay has been used on several thousand isolates in the UK and the Netherlands and is proving a rapid and efficient means of genotyping and identifying lineages according to their multi-locus genotype (MLG).

In a diploid organism, such as *P. infestans*, a specific SSR locus may be homozygous, in which case PCR amplification generates a single SSR allelic peak, or heterozygous resulting in two peaks on the electropherogram (Fig. 1). The occurrence of more than two peaks has been observed in our studies and is reported in the literature (Lees *et al.* 2006; Brurberg *et al.* 2011). Differences in ploidy have been observed in *P. infestans* (e.g. Tooley and Therrien 1991; Whittaker *et al.* 1991) and are consistent with these observations (Fig 1). Most methods for population genetic analysis are designed for examining diploid or haploid populations but analysis of other levels of ploidy and, in particular, populations comprising mixtures of isolates of different ploidies have proved challenging. However, a recent method (Bruvo *et al.* 2004) has been implemented in R as the package POLYSAT (Clark and Jaseniuk 2011). POLYSAT is capable of appropriately interpreting SSR data in populations of mixed ploidy to generate Bruvo genetic distances, principal co-ordinate analysis and F-statistics and will improve the interpretation of such *P. infestans* populations.

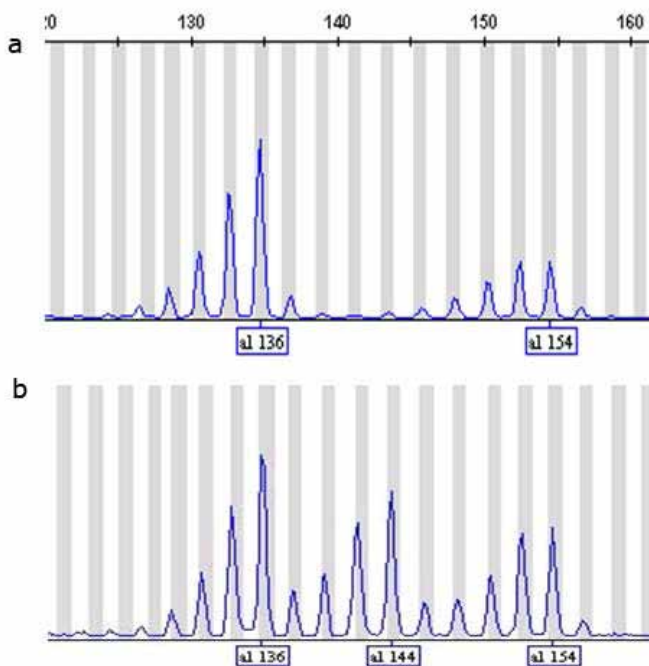


Figure 1. SSR peaks at the D13 locus of two isolates of *P. infestans* genotype 13_A2. In this marker, prominent but consistent stutter peaks form within each cluster, the largest being scored. a) An isolate with alleles 136 bp and 154 bp in which the height of the 136 bp peak is consistent with it being present in two copies compared to the lower 154bp peak. b) A different isolate of the same clonal lineage (identical at all other loci) but with an additional 144 bp allele that is consistent with an 8bp (4 dinucleotide) mutation of one of the 136 bp alleles. Note the peak height ratios are closer to 1:1:1 than in a).

Current studies on pathogen diversity

The Eucablight database currently holds data on over 25,000 isolates, with SSR data for 5,776 isolates from fifteen European countries. This is a comprehensive resource but a combination of some notable absences in the geographic coverage of Europe and the problem of variable ploidy, discussed above, has hindered an analysis of the evolutionary history on a pan-European scale. Such issues should soon be resolved as many comprehensive datasets now fill these gaps. Examples include; the Netherlands (van den Bosch *et al.* 2012), Nordic regions (Brurberg *et al.* 2011; Grönberg *et al.* 2010), Northern Ireland and the Republic of Ireland (Kildea *et al.*, 2010) France (Montarry *et al.* 2010; R. Corbiere pers. comm.), Poland (Chmielarz *et al.* 2010), Hungary (J. Bakonyi and Z. Nagy pers. comm.) as well as data generated at The James Hutton Institute on isolates provide by BayerCropScience and Syngenta as a part of their monitoring programmes. The inclusion of data from studies on isolates from North, Central and South America (F. Martin, M. Coffey, N. Grunwald and D. Cooke pers. comm.) will add a new dimension, placing the European population in a global perspective.

Effector sequencing

SSR markers as discussed above are highly polymorphic, making them a powerful tool for a relatively rapid and affordable view of population diversity and isolate discrimination even within clonal lineages; for example in *P. ramorum* clones, (Goss *et al.* 2009). For examining population

diversity at a slightly coarser phylogenetic resolution i.e. looking back further into the evolutionary history of the species, DNA sequence data has advantages (see Cooke and Lees 2004). Examples include sequencing selectively neutral genes to examine the genealogical history of *P. infestans* (Gómez-Alpizar *et al.* 2007) and as support for *P. andina* being a hybrid of *P. infestans* and another, as yet undescribed, taxa (Goss *et al.* 2011). Rapid advances in the discovery of effector genes, the products of which affect host cell function to allow pathogen infection, are providing opportunities to explore the evolutionary drivers of pathogenicity in *P. infestans*. The genes responsible for virulence against known resistances in the R gene differential series have now been identified (reviewed in Vleeshouwers *et al.* 2011) within a group of over 500 effectors with a distinct RXLR motif in the *P. infestans* genome (Haas *et al.* 2009). Sequencing a panel of these effectors amongst isolates of different MLG, defined by SSR markers, is underway at The James Hutton Institute. To date, the RXLR allelic diversity and MLG correspond closely. Avr2 provides an interesting example in which the R2 gene is ‘defeated’ by two mechanisms in different MLGs. In isolates of many MLGs the Avr2 gene is present and recognized by R2 resulting in resistance. The non-synonymous (replacement) single nucleotide polymorphisms (SNPs) reported did not affect this recognition. In isolates of other MLGs the Avr2 gene were deleted from the genome and R2 thus ‘defeated’. An alternative mechanism of overcoming R2 was also noted in isolates that were able to silence expression of the Avr2 gene (Gilroy *et al.* 2010). Such studies of the sequence diversity provides a means of directly examining the evolution of pathogenicity and, in the longer term, supports strategies for selecting and deploying host resistance that is more likely to be durable (Vleeshouwers *et al.* 2011).

The evolving Eucablight database

A major achievement of the EU concerted action Eucablight project was databases of *P. infestans* pathogen diversity and potato host resistance coupled with analysis tools and a web-based interface to summarise and view the data (www.eucablight.org). These databases and interfaces were planned by the project partners and implemented in 2003 at the University of Aarhus (Lassen and Hansen 2005; Hansen *et al.* 2007). Updates to the datasets of both host and pathogen databases have continued since the formal Eucablight project completion date in 2006. The web interface performs calculations and displays updates ‘on the fly’ so that summaries of all current data may be viewed. Both datasets are a valuable resource but it is recognised that improvements to the data upload facilities and the web interface for displaying the results are required for the databases to reach their full potential. Database and internet technology has evolved since these were designed and, as a result of additional funding, the team at the University of Aarhus has designed and built a new ‘crop problem’ database. This database is based on.NET technology and has a broader scope to track changes in cereal pathogens within the ‘Eurowheat’ project (www.eurowheat.org; Jørgensen *et al.* 2010) and the rust initiative (rustspore.cimmyt.org, www.wheatrust.org; Hodson *et al.* 2011). Powerful mapping tools are now exploited to present wheat rust population data geographically. The Eucablight database will be migrated to this new format and alternative means of uploading data from the projects mentioned above will facilitate its expansion. The addition of data from other continents is also planned.

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Fortuna et al.

Status and perspectives of GM approaches to fight late blight

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SUMMARY

The development of potatoes resistant to *Phytophthora infestans* via introduction of resistance (R) genes through genetic modification (GM) offers significant advantages over traditional breeding approaches. The different GM approaches of Wageningen University (the DuRPh project), of the Sainsbury Laboratory at the John Innes Centre in Norwich, and the development of Fortuna at BASF Plant Science Company, Limburgerhof, Germany are outlined.

The Phytophthora resistant potato Fortuna was developed by Agrobacterium-mediated transformation of the potato variety (var.) Fontane with two resistance (R) genes, *Rpi-blb1* and *Rpi-blb2*, isolated from *Solanum bulbocastanum*. These genes encode BLB1 and BLB2 proteins, respectively, and impart plant resistance to late blight disease caused by *P. infestans*. Apart from the late blight resistance trait, Fortuna displays the same agronomic and tuber quality characteristics as its mother variety Fontane.

KEYWORDS

Phytophthora infestans, genetic modification, resistance genes, R-genes, *Solanum bulbocastanum*, Fortuna

INTRODUCTION

Potato late blight caused by the oomycete *Phytophthora infestans* is the most devastating potato disease. This fungus thrives best under wet and cold weather conditions and, if untreated, is able to destroy entire harvests. It is estimated to account for a destruction of 15% of the global potato production (International Potato Center, CIP). Most potato farmers in the European Union (EU) protect their crop against this disease through frequent (up to 16) fungicide applications per growing season (Haverkort *et al.*, 2008). The protection against late blight causes by far the highest costs and requires the biggest efforts of the potato farmers as compared to the protection against other pests and diseases. Annual costs of potato late blight, i.e. of plant protection and damage in the EU are estimated to amount to about one billion Euro (Haverkort *et al.*, 2008). Due to its huge genome with a lot of repetitive sequences, its short generation cycle and its ability to mate, *Phytophthora infestans* is evolving rapidly and has a strong ability to develop resistances to fungicides.

An alternate defence strategy via the development of late blight resistant cultivars therefore represents a major goal in potato breeding. The introgression of R-genes (resistance genes) from wild potato

species resistant to late blight represents a major path since the 1930ies (Turner, 2008). However, for different reasons this approach so far has yielded only limited success:

- (1) To cross *Solanum tuberosum* with wild potato species is often difficult and requires in some cases bridge crosses involving a third *Solanum* species.
- (2) Wild potato species are typically small plantlets with low agronomic performance. In classical breeding some of these characteristics are typically co-segregating with the desired trait, rendering the progeny non-competitive based on their poor agronomical properties.
- (3) Oftentimes race-specific R-genes have been introgressed that, in contrast to the broad-spectrum R-genes, proved not to confer durable resistance.
- (4) Classical breeding methods typically yielded progeny containing single R-genes, which, especially when race-specific, have been overcome quickly by the evolving pathogen.

MATERIALS AND METHODS

Several test systems exist to test the resistance of potato cultivars against late blight either in the field (natural or artificial infection), the greenhouse, and or on isolated potato leaves in the detached leaf assay (Wang *et al.*, 2008, El-Kharbotly *et al.*, 1994). However, observations on effects made either via the detached leaf assay or on greenhouse grown plants need to be verified and validated in a field experiment, before valid conclusions can be drawn on the occurrence of resistance and its relevance for potato cultivation. For resistance conferred by the *Rpi-blb2* gene, resistance typically cannot be proven by the detached leaf assays beyond doubt and field evaluation is crucial (van der Vossen *et al.*, 2005).

RESULTS

Fortuna

The Phytophthora resistant potato Fortuna has been developed by introducing two R-genes, *Rpi-blb1* (van der Vossen, 2003) and *Rpi-blb2* (van der Vossen, 2005), from the wild potato species *Solanum bulbocastanum* into the broadly used European processing potato variety Fontane with the help of genetic modification.

To generate Fortuna, the *Rpi-blb1* and the *Rpi-blb2* genes were combined under control of their native regulatory elements. The resulting vector construct contained the genomic sequence of the *Rpi-blb1* gene under control of the native *Rpi-blb1* promoter and *Rpi-blb1* terminator, all derived from *S. bulbocastanum*, in combination with the genomic sequence of the *Rpi-blb2* gene under control of the native *Rpi-blb2* promoter and *Rpi-blb2* terminator all from *S. bulbocastanum*. The broadly used commercial potato variety Fontane was transformed with the *Rpi-blb1*:*Rpi-blb2* double gene construct by using conventional Agrobacterium-mediated transformation using imidazoline resistance as selection marker.

The performance of the late blight resistance trait in Fortuna was tested in field trials at locations representative for potato cultivation, such as The Netherlands, Germany, United Kingdom, Czech Republic, and Sweden. In the absence of natural infection, and in order to further increase disease pressure, artificial infection using mixed local isolates of *Phytophthora infestans* were applied in the field. In over five years of testing *Phytophthora infestans* resistant potato lines in the field, late blight disease on potato lines expressing the combination of the *Rpi-blb1* and *Rpi-blb2* genes was never observed.

In addition, the resistance of potato lines expressing the combination of the *Rpi-blb1* and *Rpi-blb2*

genes was tested in the greenhouse against a large collection of *Phytophthora infestans* isolates from all over the EU. Until today no isolate tested was able to overcome the resistance of Fortuna.

COMPARISON OF THE MAJOR GM APPROACHES TOWARD LATE BLIGHT RESISTANCE IN EUROPE

DuRPh project

The DuRPh (Durable resistance against Phytophthora) project is run by Wageningen University and Research Center, funded by the Dutch natural gas resources (national investment in innovation). The project focusses on cloning and analyzing from wild potatoes, testing R-genes in field trials, and developing resistance management strategies.

First field trials with Phytophthora resistant GM potatoes were performed in 2007, the trials are located at different sites in the Netherlands and at one site in Belgium. In 2011 the following R-genes were tested in the field trials: R3a, Sto1, Blb3, *Vnt1* in single gene constructs and in double and triple stacks.

DuRPh employs cisgenic, marker-free genetic modification, and fosters the concept of dynamic cultivars to increase the sustainability of R gene mediated resistance.

In order to offer farmers access to DuRPh results requires the following steps: Variety development through breeders of the DuRPh consortium, the target varieties need to be decided by consortium members. Currently it is not clear by when such varieties could be launched, since the development of such varieties by the DuRPh consortium would require a change in the existing GM regulations, which would decrease the safety assessment requirements for cisgenic crops. As with all approaches, market acceptance will be required for a successful launch.

The Sainsbury Laboratory's approach

The project at the Sainsbury Laboratory is funded by the UK's Biotechnology & Biological Sciences Research Council (BBSRC). Its scope encompasses cloning and analyzing of R-genes from wild potato species and testing of these genes in potato field trials.

First field trials took place in 2010 at one site in the UK. In 2011 the *Vnt1* and *Mcq1* genes were tested in the field. The genes are introduced by standard transformation methods using an NPT2 resistance marker conferring kanamycin resistance to transformed cells in tissue culture.

The envisioned path to the farmer requires either the joint development of varieties in a public/private partnership or the outlicensing of R-genes to third parties. Goal in a public/private partnership would be to use the varieties Maris Piper and Desiree. The time of market launch can only be predicted once a commercial partner has been identified.

The BASF Plant Science approach

The project is funded by BASF SE. It builds upon existing potato fungicide knowledge and focusses on the in-licensing of R-genes, the development of Phytophthora resistant varieties, the conduction of regulatory safety studies, and the striving for regulatory approval and commercialization.

Field trials with Fortuna were performed since 2006 at over 20 sites in Germany, the Netherlands, Belgium, the United Kingdom, Czech Republic, and Sweden.

BASF Plant Science employs transgenic modification using an imidazoline herbicide resistance marker for selection of transformants. This marker does not have a field effect.

In order to bring Fortuna to the market a regulatory approval for cultivation in the EU is required as well as global import approvals.

On 31.10.2011 BASF Plant Science applied for EU approval for Fortuna.

On 16.01.2012 BASF Plant Science announced that it will stop all its GM crop developments targeted for the European market due to lack of acceptance for GM technology in Europe. This comprises also a stop of the Fortuna project. However, the company will further pursue the regulatory approval of Fortuna.

CONCLUSIONS

Genetic modification allows developing potato varieties which contain broad and durable resistance to *Phytophthora infestans* and possess all features of competitive cultivars. However, to create a solution which can be offered to the farmers some significant hurdles need to be taken, including a regulatory approval and acceptance in the market place.

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Late blight resistance of *Solanum* species and potato hybrids: the evidence from coupled phytopathological and molecular study

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SUMMARY

The panel of wild *Solanum* species and potato hybrid clones was studied by coupled phytopathological and SCAR-marker analyses. Late blight (LB) resistance was assessed in individual plants under the field conditions and in the laboratory, with the detached-leaf test. The novel lines derived from previously unexplored sources of LB resistance among the Bolivian diploid species *S. alandiae* manifested a successful field resistance in the trials through two epidemic years. SCAR markers were developed using the R1, R3a and RB gene sequences. By screening wild *Solanum* species and clones of potato hybrids after natural and artificial infestation by *P. infestans*, we established that the presence of SCAR markers R1-1205 was significantly related to LB resistance. This evidence suggests the practicability of employing this marker as a breeding tool for early prediction of LB resistance in a wide range of *Solanum* germplasms.

KEYWORDS

Phytophthora infestans, wild tuber-bearing *Solanum* species, clones of interspecific potato hybrids, R-genes, SCAR-markers

INTRODUCTION

The most efficient and ecologically sustainable way to thwart the global threat of LB is to breed new potato varieties manifesting durable resistance to a wide range of *Phytophthora infestans* races. Initial programs of breeding for potato LB resistance were based on germplasm introgression from *Solanum demissum*. Nowadays, breeders try to incorporate germplasms of other *Solanum* species recognized as promising sources of LB resistance. Robust and reliable DNA markers greatly facilitate mapping and

isolation of new LB resistance genes and help identify and track them in the germplasm collections.

MATERIALS AND METHODS

Seeds and microtubers of wild *Solanum* species were obtained from the Vavilov Institute of Plant Industry, Russia (VIR), the Centre for Genetic Resources, the Netherlands (CGN) and NRSP-6 Potato Genebank, USA (PI), and potato tubers, from VIR and the Institute of Potato Husbandry, Russia. The plant genotypes used in this study belong to 213 accessions representing 21 wild tuber-bearing *Solanum* species and 26 potato interspecific hybrid clones which incorporate germplasm of *S. bulbocastanum*, *S. verrucosum*, *S. stoloniferum*, *S. polytrichon*, *S. pinnatisectum*, *S. acaule*, *S. alandiae*, *S. spegazzinii*, *S. microdontum*, *S. berthaultii*, *S. andigenum*, *S. rybinii*, *S. phureja* and demissoid cultivars. To check the response to LB in individual plants, the field and detached-leaf trials were conducted (for the protocols see Rogozina *et al.*, 2010). We developed SCAR markers for the genes R1, R3 of *S. demissum* and RB of *S. bulbocastanum* and for the corresponding germplasms (for markers design, amplification and cloning see Khavkin *et al.*, 2010).

RESULTS AND DISCUSSION

Field trials

Conducive weather conditions enabled rapid establishment of LB in both years (2008-2009) of trial, so that susceptible standards (cv. Bintje, accessions of *S. kurtzianum*, *S. acaule*) were defoliated finally by the end of July. In 2009, disease progress was much slower than in 2008; nevertheless, some cultivars previously considered resistant (Nevsky, Najada) were noticeable affected. Field trials demonstrated that *Solanum* species and hybrid clones showed a range of variability for infestation. Among *Solanum* species, individuals of *S. stoloniferum* and *S. pinnatisectum* showed higher levels of foliar resistance, the individuals of *S. demissum*, *S. bulbocastanum* and *S. polyadenium* showed marked differences in the LB lesions, the response of *S. jamesii* and *S. cardiophyllum* individuals depended on particular year conditions, whereas the individuals of *S. fendleri*, *S. brachystotrichum* and *S. stenophyllidum* were susceptible. The factorial analysis indicated that both factors, plant species and the year of testing, significantly affected the final defoliation of tested plants, and these two factors did not interact.

Potato hybrid clones and varieties were divided into two groups according to average AUDPC values in three-year trial (2007-2009). The first group included 12 hybrid clones with high levels of resistance (not more than 20% foliar infection by the end of growth period). The second group included rest of hybrid clones and cvs. Peterburgsky and Najada wherein the extent of the damage was more severe and variable. These genotypes manifested intermediate resistance (20% to 50% foliar infestation depending on the year, Fig.1). Potato hybrid clones and varieties demonstrated the same rank order of LB resistance across three trial years indicating stability of this trait. The growth period of potato hybrid clones and varieties was significantly shorter than that of wild *Solanum* species. Potato crop was removed by the end of August to early September. Wild *Solanum* grew for another month and therefore were more affected with LB.

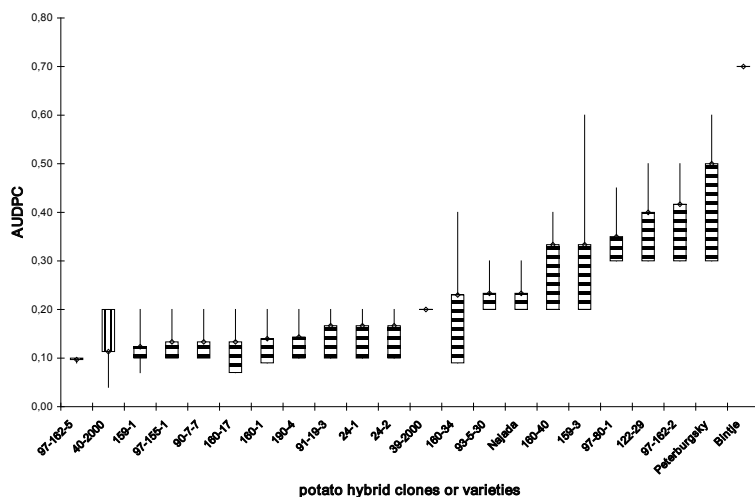


Figure 1. $rAUDPC$ values for potato hybrid clones and varieties across the three-years trial

Detached-leaf trials

Reaction of some *Solanum* genotypes to artificial infection by *P. infestans* was quite remarkable. Leaves of *S. polyadenium* were not affected at all or manifested only point necroses, leaves of *S. verrucosum* showed large necrotic lesions, and leaves of individual plants of *S. demissum*, *S. stoloniferum* and *S. bulbocastanum* variable responses to infestation similar to those observed with whole plants in the field trial. Leaves of all individuals of *S. stenophyllidum* and *S. fendlerii* produced large necrotic spots with sporulation indicating high susceptibility to *P. infestans*. Leaves of most potato hybrid clones showed necrotic reactions without sporulation indicating the horizontal type of resistance. A single clone 97-162-5 was not damaged by artificial infestation; such evidence matches the durability of its LB resistance in the field (Fig.1).

Marker analysis

The specificity of markers based on the R-gene sequences was verified with the cultivars reportedly free of wild *Solanum* germplasm and potato cultivars comprising the germplasm of *S. demissum*. All markers reliably discerned cultivated potato from wild *Solanum* species (Sokolova *et al.* 2011). Markers specific for the R-genes introgressed from *S. demissum* were found in not only *S. demissum* genotypes and cultivars reportedly originating from *S. demissum*. The marker R1-1205 was present in the accessions of *Solanum iopetalum*, *S. polytrichon*, and *S. stoloniferum*. The marker R3-1380 was present in the accessions of *S. hougasii*, *S. stoloniferum*, *S. cardiophyllum*, *S. ehrenbergii* and *S. bulbocastanum* (Table 1). Our study demonstrates the presence of R-like sequences in genotypes of a wide range of *Solanum* species. RB-like sequences were exposed in an especially wide range of *Solanum* species section Petota (Pankin *et al.* 2010).

Comparison of the data obtained by phytopathological tests and marker analyses for wild *Solanum* shows that the presence of the marker for the genes R1 and R3a initially characterized in *S. demissum* in most cases matched the evidence for LB resistance. The agreement was higher for R1-1205 than for R3-1380 (Table 1). The association between the presence of R1-1205 marker and LB resistance was significant according to Pearson chi-square test: $\chi^2 = 6,63 < 3,84$. The association between the presence of R3-1380 and LB resistance was not significant: $\chi^2 = 1,63 > 3,84$.

Table 1. The presence of markers for R-genes in *Solanum* genotypes with diverse reactions to *P. infestans*

| <i>Solanum</i> species | Number of tested accessions | Including resistant accessions | Presence of SCAR markers in resistant accessions * | | Including susceptible accessions | Presence of SCAR markers in susceptible accessions * | |
|----------------------------|-----------------------------|--------------------------------|--|---------|----------------------------------|--|---------|
| | | | R1-1205 | R3-1380 | | R1-1205 | R3-1380 |
| <i>S. verrucosum</i> | 7 | 0 | | | 7 | 0 | 0 |
| <i>S. demissum</i> | 20 | 17 | 10 | 1 | 3 | 0 | 0 |
| <i>S. iopetalum</i> | 1 | 1 | 1 | 0 | 0 | | |
| <i>S. bougasii</i> | 2 | 2 | 0 | 1 | 0 | | |
| <i>S. fendlerii</i> | 4 | 0 | | | 4 | 0 | 0 |
| <i>S. hjertingii</i> | 3 | 1 | 0 | 0 | 2 | 0 | 0 |
| <i>S. papita</i> | 3 | 2 | 0 | 0 | 1 | 0 | 0 |
| <i>S. polytrichon</i> | 8 | 2 | 1 | 0 | 6 | 0 | 1 |
| <i>S. stoloniferum</i> | 20 | 5 | 1 | 1 | 15 | 3 | 2 |
| <i>S. brachystotrichum</i> | 3 | 0 | | | 3 | 0 | 0 |
| <i>S. jamesii</i> | 8 | 3 | 0 | 0 | 5 | 0 | 0 |
| <i>S. pinnatisectum</i> | 4 | 2 | 0 | 0 | 2 | 0 | 0 |
| <i>S. tarnii</i> | 3 | 2 | 0 | 0 | 1 | 0 | 0 |
| <i>S. polyadenium</i> | 6 | 6 | 0 | 0 | 0 | | |
| <i>S. cardiophyllum</i> | 4 | 3 | 0 | 2 | 1 | 0 | 0 |
| <i>S. ehrenbergii</i> | 11 | 2 | 0 | 0 | 9 | 0 | 2 |
| <i>S. bulbocastanum</i> | 18 | 16 | 0 | 5 | 2 | 0 | 0 |
| Total | 125 | 64 | 13 | 10 | 61 | 3 | 5 |

*Note: the number of genotypes comprising the marker

To link the presence of the R-genes to LB resistance scores in hybrid clones, we used the non-parametric Mann-Whitney test. The statistical analysis demonstrates highly significant association ($z=-1,97, p=0,04$) between the presence of R1-1205 marker and LB resistance. The frequency of R1-1205 was notably higher in the first group of hybrid clones with stable LB resistance (Fig.1, Table 2). The association of high LB resistance with the presence of two other gene markers was not that evident.

None of analyzed markers were detected in two hybrid clones under study: 24-2 and 97-155-1. Both these clones possess high LB resistance; therefore, we presume that R-genes other than R1, R3a and RB or some yet unidentified QTLs for LB resistance contribute to their sustainability in the field. Our study demonstrates the suitability of novel lines derived from the Bolivian diploid species *S. alandiae* for use as a source of LB resistance.

It is noteworthy that, opposite to the evidence discussed above, we failed to relate LB resistance of wild *Solanum* species to the presence of R-genes recognized with the simple races of *P. infestans*, apparently because of more complicated virulence patterns of these isolates. To illustrate, we found that these races comprised the IpiO gene alleles encoding the effectors recognized by Rpi-blb1/RB gene from *S. bulbocastanum* and *S. stoloniferum* (Pankin *et al.*, this volume).

The association of particular *Solanum* germplasms in hybrid clones with the presence of the markers for R1 and R3a was found in most cases. Markers specific for the R-genes introgressed from *S. demissum* were found most often due to pedigree of all hybrid clones tracing back to demissoid cultivars. SCAR markers for RB were found in hybrid clones comprising the germplasm of *S. bulbocastanum* and in hybrid clones free of such germplasm (Table 2). In the latter case, some of these hybrids were derived from *S. stoloniferum* germplasm, which is known to contain the Rpi-blb1 ortholog.

Table 2. The presence of markers for R-genes in potato interspecific hybrid clones

| Hybrid clones | Wild <i>Solanum</i> species in potato pedigrees ¹ | Presence of SCAR-markers ² | | |
|---------------|--|---------------------------------------|---------|--------|
| | | R1-1205 | R3-1380 | RB-638 |
| 24-1 | <i>aln, dms</i> | 1 | 0 | 0 |
| 24-2 | <i>aln, dms</i> | 0 | 0 | 0 |
| 190-4 | <i>and, dms</i> | 1 | 0 | 1 |
| 97-155-1 | <i>and, dms</i> | 0 | 0 | 0 |
| 160-1 | <i>and, dms</i> | 1 | 0 | 0 |
| 160-17 | <i>and, dms</i> | 1 | 0 | 0 |
| 160-34 | <i>and, dms</i> | 0 | 0 | 1 |
| 160-36 | <i>and, dms</i> | 0 | 0 | 0 |
| 160-40 | <i>and, dms</i> | 0 | 0 | 1 |
| 89-1-12 | <i>and, sto, dms</i> | 1 | 0 | 0 |
| 90-7-7 | <i>and, sto, dms</i> | 1 | 0 | 1 |
| 159-1 | <i>and, sto, dms</i> | 1 | 0 | 0 |
| 159-3 | <i>and, sto, dms</i> | 1 | 0 | 0 |
| 159-31 | <i>and, sto, dms</i> | 0 | 1 | 0 |
| 97-162-2 | <i>and, sto, dms</i> | 0 | 0 | 0 |
| 97-162-5 | <i>and, sto, dms</i> | 1 | 0 | 0 |
| 97-80-1 | <i>and, vrn, dms</i> | 1 | 0 | 0 |
| 122-29 | <i>and, spg, mcd</i> | 0 | 0 | 0 |
| 91-19-3 | <i>and, blb, sto, acl, dms</i> | 0 | 1 | 1 |
| 93-5-30 | <i>and, phu, blb, sto, acl, dms</i> | 0 | 0 | 0 |
| 11-1 | <i>and, ber, dms</i> | 0 | 0 | 1 |
| 11-2 | <i>and, ryb, ber, dms</i> | 1 | 0 | 0 |
| 12-2 | <i>and, ryb, sto, ber, dms</i> | 0 | 0 | 0 |
| 13-1 | <i>and, ryb, sto, mcd, dms</i> | 1 | 0 | 0 |
| 39-2000 | <i>smp, dms</i> | n.d. | n.d. | n.d. |
| 40-2000 | <i>smp, plt, dms</i> | 1 | 0 | 1 |

¹ *acl* – *S. acaule*, *aln* – *S. alandiae*, *and* – *S. andigenum*, *ber* – *S. berthaultii*, *blb* – *S. bulbocastanum*, *dms* – *S. demissum*, *plt* – *S. polytrichon*, *phu* – *S. phureja*, *sml* – *S. simplicifolium*, *sto* – *S. stoloniferum*, *vrn* – *S. vernei*

² n.d. –no data

CONCLUSION

Our results indicate that R genes for LB resistance or their structural homologues are universally distributed across wild *Solanum* section Petota and in the progenies of crosses between wild species and cultivated potato. The presence of R1-1205 marker in LB resistant material with diverse background indicates the practicability of this marker as a breeding tool for early prediction of LB resistance in a wide range of *Solanum* germplasms.

ACKNOWLEDGMENTS

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Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes

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SUMMARY

Phytophthora infestans (Pi) is the causal agent of late blight in potato. The Mexican species *Solanum demissum* is well known as a good resistance source. Among the 11 R gene differentials, which were introgressed from *S. demissum*, especially R8 and R9 differentials showed broad spectrum resistance both under laboratory and under field conditions. In order to gather more information about the resistance of the R8 and R9 differentials, F1 and BC1 populations were made by crossing Mastenbroek (Ma) R8 and R9 clones to susceptible plants. Parents and offspring plants were examined for their pathogen recognition specificities using agroinfiltration with known Avr genes, detached leaf assays (DLA) with selected isolates, and gene-specific markers. An important observation was the discrepancy between DLA and field trial results for Pi isolate IPO-C in all F1 and BC1 populations, so therefore also field trial results were included in our characterization. It was shown that in MaR8 and MaR9, respectively, at least four (R3a, R3b, R4, and R8) and seven (R1, Rpi-abpt1, R3a, R3b, R4, R8, R9) R genes were present. Analysis of MaR8 and MaR9 offspring plants, that contained different combinations of multiple resistance genes, showed that R gene stacking contributed to the Pi recognition spectrum. Also, using a Pi virulence monitoring system in the field, it was shown that stacking of multiple R genes strongly delayed the onset of late blight symptoms. The contribution of R8 to this delay was remarkable since a plant that contained only the R8 resistance gene still conferred a delay similar to plants with multiple resistance genes, like, e.g., cv Sarpo Mira. Using this “de-stacking” approach, many R gene combinations can be made and tested in order to select broad spectrum R gene stacks that potentially provide enhanced durability for future application in new late blight resistant varieties.

Part of this work was presented in the Euroblight workshop under the title “Durability of resistance to late blight associated with stacking of R-genes” by Evenhuis, *et al.*. A full version of the manuscript can be viewed on www.springerlink.com/content/52x881847733m058/

Characterization of *Phytophthora infestans* populations in North America from the 2009-2011 late blight epidemics

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Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of potato and tomato worldwide and also attacks a range of other Solanaceous hosts. Late blight epidemics in the United States in 2009 were severe due to widespread inoculum and weather that was conducive to disease.

We collected and cultured more than 140 isolates of the pathogen from the 2009 late blight outbreaks on potato and tomato along the eastern seaboard from Florida to Maine and from several Midwestern states including Indiana, Wisconsin and North Dakota. We had previously isolated the pathogen from tomato late blight outbreaks in the southeastern and northeastern US. Our objectives were to (i) identify the genotype that caused the major disease outbreaks in the eastern North America in 2009-2011; (ii) examine the phenotypic and genotypic structure of *P. infestans* populations in eastern North America from 2009-2011 and compare them to genotypes identified from previous years.

Table 1. Summary of genotype characterization of *Phytophthora infestans* in North America from the 2009-2011 late blight epidemics

| Genotype | Host | Mating type | Allozyme genotype | | Sensitivity to mefenoxam | RG57 RFLP |
|-------------------------------------|------------|-------------|-------------------|---------|--------------------------|-------------------------------------|
| | | | Gpi | Pep | | |
| US-8 | Potato | A2 | 100/111/122 | 100/100 | R/I | 1,5,10,13,14,16,20,21,23,24,25 |
| <u>2009- 10 ISOLATES</u> | | | | | | |
| US-21 | Tomato | A2 | 100/122 | 100/100 | R/I/S | 1,5,10,13,14,18,20,21,24,25 |
| US-22 | Tom/Potato | A2 | 100/122 | 100/100 | S/I | 1,5,13,14,16,20,21,24,25 |
| US-23 | Tom/Potato | A1 | 100/100 | 100/100 | S/I | 1,2,5,6,10,13,14,17,20,21,24,24a,25 |
| US-24 | Tom/Potato | A1 | 100/100/111 | 100/100 | I | 1,3,5,7,10,13,14,16,20,21,23,24,25 |
| <u>2011 ISOLATES</u> | | | | | | |
| US-23 | Tom/Potato | A1 | 100/100 | 100/100 | S/I | 1,2,5,6,10,13,14,17,20,21,24,24a,25 |
| US-24 | Tom/Potato | A1 | 100/100/111 | 100/100 | I | 1,3,5,7,10,13,14,16,20,21,23,24,25 |

Characterization of isolates from 2009 showed the emergence of new A1 and A2 genotypes on both potato and tomato. These new genotypes have been found to persist and were identified again in isolates from 2010 and 2011, indicating that they are fit and capable of surviving over the winter. The new genotypes have been designated US-21, a tomato-specific A2 genotype, US-22, an A2 genotype which has moved from tomato to potato, US-23, an A1 genotype found on both potato and tomato, and US-24, an A1 genotype specific to potato. US-22 has been shown to have been spread on tomato transplants.

The disease led to millions of dollars of lost income to growers in many areas of the northeastern US and some organic tomato growers abandoned production altogether. The pathogen has reemerged as a significant disease threat to the organic tomato industry in the US where management options are limited.

Understanding phenotypic and genotypic variation in populations of *P. infestans* is clearly a significant factor in the planning of effective and durable control strategies but *P. infestans* populations continue to be in a state of flux and late blight management remains a significant challenge to the potato and tomato industry.

Analysis of correlation between soil moisture and late blight occurrence

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SUMMARY

During a 3-year project the relation between soil moisture and first appearance of *Phytophthora infestans* was characterised. The aim was to integrate a soil moisture module in the prediction model SIMBLIGHT1 (Kleinhenz *et al.* 2007), which calculates the first appearance of *P. infestans* in potato fields.

Potato tubers, which are infected with *P. infestans*, are able to release sporangia and zoospores in the ambient soil, when temperature and soil moisture are optimal for the fungus. Soil-borne infections can increase the risk of an early appearance of *P. infestans* in field (Adler 2000; Bäßler 2005). In a field trial inoculated potato tubers were buried next to healthy tubers. In this way the possibility for soil-borne infections of healthy potato plants were tested. Additionally monitoring data of first occurrence of late blight in Germany of the years 2006 to 2010 were analysed taking into account the influence of soil moisture.

The results showed that no explicit correlation between soil moisture and an early appearance of *P. infestans* could be detected.

KEYWORDS

Phytophthora infestans, soil moisture, zoospore infections, latent infected tubers, SIMBLIGHT1

INTRODUCTION

The prediction model SIMBLIGHT1 (Kleinhenz *et al.* 2007), which calculates the first appearance of *Phytophthora infestans* in potato fields, was developed by ZEPP (Central Institution for Decision Support Systems in Crop Protection) and is used by the GCPS (Governmental Crop Protection Services) in Germany. SIMBLIGHT1 calculates a higher risk for an early outbreak of *P. infestans* if there had been a four day long period of totally water saturated soil between planting and 7 days after emergence.

Former studies showed, that a correlation between high soil moisture after planting and early occurrence of *P. infestans* can be assumed. Adler (2000) found out, that latent infected potato tubers became more important on primary infections of late blight because of modern storages. In her opinion this fact leads to an earlier outbreak of sprout infections in years with wet springs. She called for focusing on soil-borne infections between planting and emergence.

Similar postulations were brought up by Bäßler (2005). In his experiments on the influence of

soil type and soil moisture on primary infections he recommended a soil module for prediction models, to describe the correlation between soil type, soil moisture and latent infected sprouts. The main aspects of his experiments showed an increase of latent infections of sprouts according to the duration of irrigation and the heaviness of soil. He postulated to specify the influence of soil physical parameters. Boyd (1980) argued that primary infections are caused from contaminated soil to leaves and not from soil-borne infected sprouts. His experiments produced more infected potato plants on the flat 3 % and 5.3 % respectively than on ridges where he had less than 1 % infected sprouts. The aim of this study was to specify the relation between soil characteristics and first appearance of *P. infestans* to integrate a soil moisture module in SIMBLIGHT1.

HYPOTHESES

In this study the influence of soil moisture on the first occurrence of late blight was investigated. Therefore the analyses were focused on the incubation period of *P. infestans*. In field trials soil-borne infections from infected tubers to healthy sprouts were taken into account.

Potato tubers, which are infected with *P. infestans*, are able to release sporangia and zoospores in the ambient soil, when temperature and soil moisture are optimal for the fungus (Zan 1962; Lacey 1967; Sato 1980; Adler 2000; Porter 2005).

As described in literature sporangia formation of *Phytophthora spp.* requires damp soil around 150 hPa (Macdonald and Duniway 1978). This soil moisture is characterised by the interval of field capacity. At field capacity the soil moisture tension is between 60 and 300 hPa.

After spores formation on the surface of an infected tuber indirect germination is required, because only zoospores have the possibility to be transported by soil water through soil pores (Porter *et al.* 2005). As written by Macdonald and Duniway (1978) for indirect germination of *Phytophthora spp.* a water potential from up to 25 hPa is required. In addition zoospore movement through soil pores is only possible when soil moisture is above field capacity, which means soil saturation is required.

Therefore it seems that in years with high soil moisture on the fields, the possibility for movements of zoospores exists. This process increases the risk of an early appearance of *P. infestans* in field. The effect of soil moisture on potato tuber infections due to *P. infestans* was assessed in a field experiment.

MATERIAL AND METHODS

Field trials 2010 and 2011

Based on the hypotheses a field trial was carried out in 2010 and 2011 (Figure 1). Inoculated potato tubers were buried next to healthy tubers in a field. The trial was surrounded by a crop not susceptible to *P. infestans* and in a potato free growing area to avoid *P. infestans* infections from outside the trial plot.

During planting contact between the inoculated and the healthy tubers was avoided. Additionally sensors for the measurement of soil moisture and soil temperature were installed. Afterwards the potato field was divided into three plots. Each plot consisted of 10 rows with a length of 4 meters planted with potatoes. Inoculated tubers were not buried near the borders of each plot to avoid infections between the plots. The three plots were irrigated for a different amount of days and at different times respectively; one was without irrigation, one with irrigation before emergence and one with irrigation after emergence. Additionally the half of each plot was covered with a foil to reduce the effects of precipitation. In the irrigated plots 20 litres of water per square meter were given every day.

The assessment of visual late blight symptoms was done twice a week after emergence. 20 sprouts per plot from the plants of the formerly healthy tubers were sampled at BBCH 65. They were used for

PCR-detection (Keil 2007; Judelson and Tolley 2000) of latent infections with *P. infestans*.

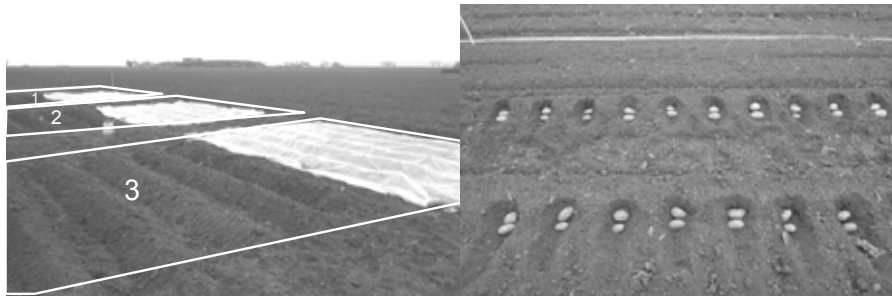


Figure 1: Design of field trial (left) and planting of the inoculated tubers next to the healthy tubers (right)

Figure 2 shows the dates of the field experiments in 2010 and 2011. The planting date was in both years in the middle of April. In 2010 the irrigation was reduced from the scheduled four days to two and one day respectively, because of continuous precipitation. In 2010 the sprouts for PCR-detection were sampled at the end of June and in 2011 at the beginning of June.

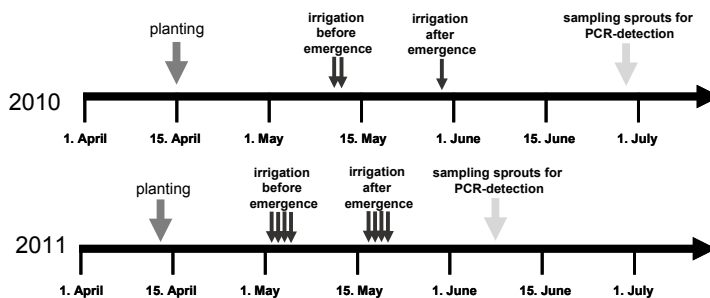


Figure 2: Dates of the field trials in 2010 and 2011

Analyses of monitoring data

To determine the influence of soil moisture on the date of the first occurrence of late blight in field, analyses with late blight monitoring data from Germany of the years 2006 to 2010 were done. Within this monitoring the parameters crop prevalence (high/low) and soil moisture (high/low) were assessed. Analyses concerning the variability of first late blight occurrences were carried out according to four groups

- low soil moisture and low crop prevalence (1)
- low soil moisture and high crop prevalence (2)
- high soil moisture and low crop prevalence (3)
- high soil moisture and high crop prevalence (4)

In total an amount of 510 data sets were split into the four different groups. Statistical differences between the four groups and significant differences respectively were tested by Tukey-test with a confidence interval of 95 %.

RESULTS

Field trial 2010

The irrigation led to a different amount of days above field capacity in the different plots. In Figure 3 the number of days above field capacity between planting and 7 days after emergence is shown. This is the relevant period for the influence of soil moisture in the prediction model SIMBLIGHT1. In addition Figure 3 shows the number of days above field capacity in the total period until the sampling of the sprouts for PCR-detection. On a second vertical axis the percentage of latent infected sprouts of the 20 samples is shown.

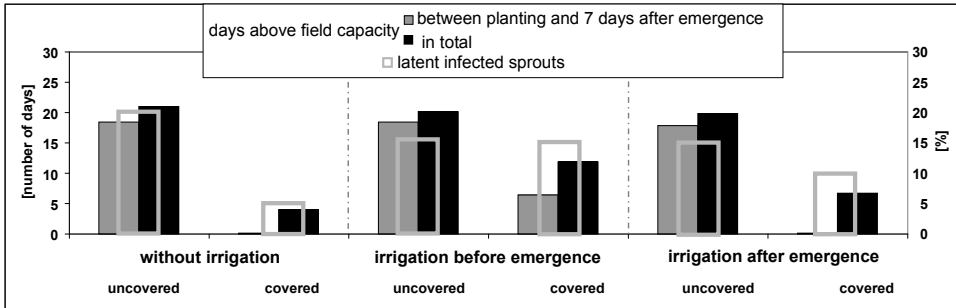


Figure 3: Number of days above field capacity between planting and 7 days after emergence, number of days above field capacity in the total period of the experiment and percentage of latent infected sprouts per plot in 2010

The correlation of the number of days above field capacity between planting and 7 days after emergence with the percentage of latent infected sprouts showed that both parameters are positively correlated (Figure 4). The coefficient of determination is about 0.67. The coefficient of determination is even better, if the percentage of latent infected sprouts was correlated with the number of days above field capacity between planting and the sampling of the sprouts (Figure 5). Until harvesting at the beginning of August no visual late blight symptoms in any plot could be observed.

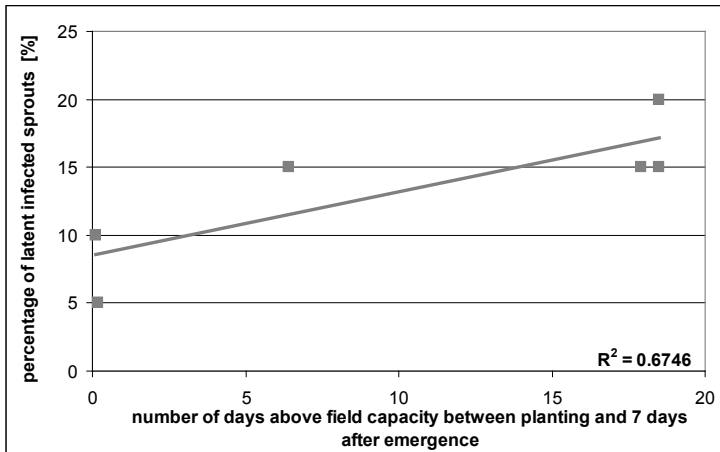


Figure 4: Correlation of the values number of days above field capacity between planting and 7 days after emergence and percentage of latent infected sprouts in each plot

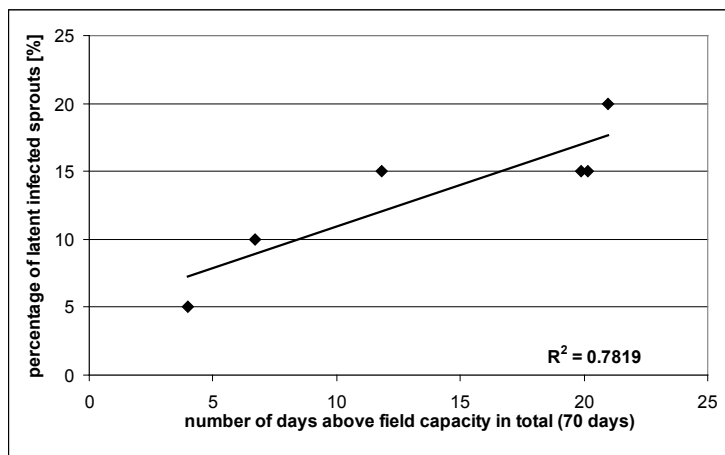


Figure 5: Correlation of the values number of days above field capacity in total and percentage of latent infected sprouts in each plot

Field trial 2011

Figure 6 shows the number of days above field capacity between planting and 7 days after emergence, the number of days above field capacity until the sampling of the sprouts for PCR-detection and the percentage of latent infected sprouts per plot. In 2011 there had been a clearly lower amount of days above field capacity because of very warm and dry weather conditions. The days above field capacity were only due to irrigation in this year. Out of the 120 sampled sprouts for PCR-detection of *P. infestans* only one showed a positive result. For this reason no statistical analysis could be done. Also in 2011 no visual late blight symptoms until harvesting were found.

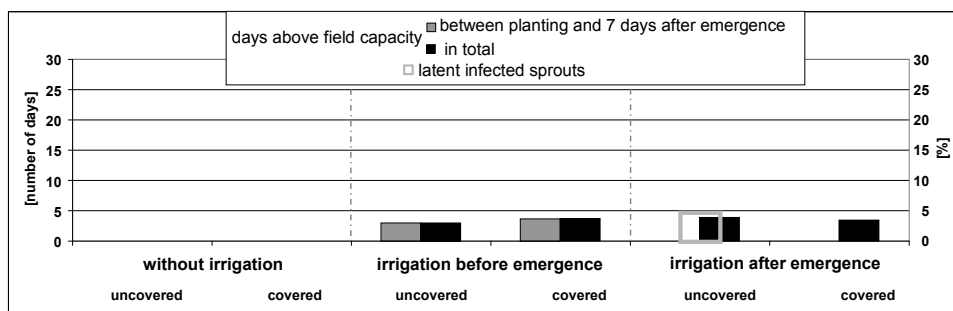


Figure 6: Number of days above field capacity between planting and 7 days after emergence, number of days above field capacity in the total period of the experiment and percentage of latent infected sprouts per plot in 2011

Analyses of monitoring data

The box plots in Figure 7 show the variability of the date of first late blight occurrence in field in the years 2006 to 2010 within the four defined groups. It can be seen that, except the fourth group, the variability of the whiskers has a nearly identical range. The average of the date of first occurrence lies within 7 days.

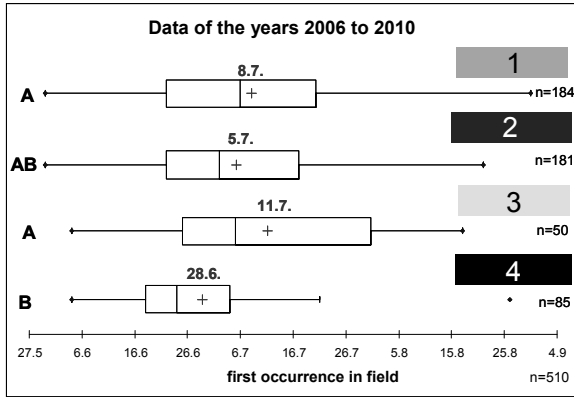


Figure 7: Box plots of the variability of the date of first late blight occurrence in field in the years 2006 to 2010 within the four defined groups (low soil moisture and low crop prevalence (1), low soil moisture and high crop prevalence (2), high soil moisture and low crop prevalence (3) and high soil moisture and high crop prevalence (4) and significant different groups as a result of Tukey-test (confidence interval of 95 %)

In Tukey-test the influence of crop prevalence and soil moisture on the date of first occurrence of *P. infestans* in field was determined. For that purpose each of the four groups was tested versus another to find out significant differences between the groups. To find out significant differences depending on soil moisture, the different groups of soil moisture within the same group of crop prevalence had to be tested. The result was that no significant differences in the date of the first occurrence of late blight relating to soil moisture could be found. Significant differences only occurred under the influence of crop prevalence.

| Contrast | | | Significance |
|----------|--------|---|--------------|
| 1 | versus | 3 | no |
| 2 | versus | 4 | no |
| 3 | versus | 4 | yes |
| 3 | versus | 2 | no |
| 1 | versus | 4 | yes |
| 1 | versus | 2 | no |

Figure 8: Results of the significant differences out of Tukey-test concerning the four different groups (low soil moisture and low crop prevalence (1), low soil moisture and high crop prevalence (2), high soil moisture and low crop prevalence (3) and high soil moisture and high crop prevalence (4))

DISCUSSION

Out of the results no explicit correlation between soil moisture and the appearance of *P. infestans* in field could be found. A correlation between high soil moisture and latent infected sprouts could be detected in the field trial in 2010. Despite a high percentage of latent infections from up to 20 %, no visual symptoms of *P. infestans* occurred until harvesting. This is one reason why it is difficult to transfer results based on latent infections to practical field results.

In the field trial in 2011 no correlation between days above field capacity and infected sprouts could be found. One reason could be the dry weather conditions in spring, which could have led to unfavourable conditions for sporangia formation on the tubers surface. Whereas in 2010 all the required processes for a soil borne infection were given, in 2011 caused by the warm and dry weather no conditions for sporangia formation were reached. One possibility for the missing infections of

the plants from the healthy tubers could be that there had been no sporangia for zoospore release at the time of irrigation.

A correlation between high soil moisture and the date of the first late blight occurrence in field could not be found neither in the analysis of the monitoring data nor in the field trials. Instead a correlation between high crop prevalence and the date of first late blight occurrence could be found. Out of this results it could be possible that the effect of soil moisture on the date of the first occurrence of late blight is low. Analyses which led to a high effect of soil moisture on the first occurrence of *P. infestans* are often related to latent infections and not to visual symptoms in field. The correlation between soil moisture and latent infections could be proved in field in 2010. The results suggest that latent infections are not correlated to an earlier outbreak of late blight. The outbreak of *P. infestans* in field must be related to other suitable conditions for the fungus. It is possible that high soil moisture could lead to an intense distribution of zoospores in the soil and in relation to this to a high percentage of latent infections. But statistical analyses revealed that high soil moisture has no influence on the date of the first occurrence of late blight in the field.

CONCLUSIONS

Out of the results no explicit correlation between soil moisture and an early appearance of *P. infestans* could be found. High soil moisture could lead to an intense distribution of zoospores in soil resulting in a high percentage of latent infections, but high soil moisture has no influence on the date of first occurrence of late blight in the field. For this reason the integration of a soil moisture module in model SIMBLIGHT1 predicting first occurrence of late blight has no practical use.

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Analysis of volunteer density under the influence of cropping practices: a contribution to the modelling of primary inoculum of *Phytophthora infestans* in potato crops

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SUMMARY

In order to improve current potato late blight risk assessment, potato volunteer distribution and densities have been studied in different agricultural environments. Preliminary mathematical approach helped designing sample size for field collecting data; the quadrat method was adopted with variable sample sizes. Large set of data have been collected in two contrasting potato producing regions. Only volunteer densities as influenced by cropping practices (waste piles or volunteers as weeds) and climatic conditions are presented in this report. High densities are found on waste piles whereas volunteer as weeds are dispersed in decreasing densities according the ability of the crop to cover the soil: important density in artichokes, moderate in cereals (wheat, barley) and low in ray-grass. Further implementation of the observed data will help simulate primary inoculum production and optimize predictive quality of current decision support systems, given the fact that any potato volunteer occurrence, even in its lowest expression, is a key factor to the setting of late blight epidemics in the vicinity of a growing potato crop.

KEYWORDS

Phytophthora infestans, *Solanum tuberosum*, modelling, potato volunteer, primary inoculum, cropping practices.

INTRODUCTION

In most parts of Europe where potatoes are grown extensively, volunteer potato plants may grow from left-over and over-wintering tubers; they are becoming a major concern for crop management and

they should be considered as weeds. They are in fact the result of a combination of different factors such as unsuitable harvesting machinery, harvest of too small sized tubers, that are maintained in the soil with sprouting ability thanks to mild winter temperatures or to following crops that do not fully cover the soil so to prevent potatoes from further development etc..., short rotation and climate change amplifying the situation (Cooke *et al.*, 2011). It is clear that excess of volunteers jeopardizes the phytosanitary status in any potato growing area by acting as a reservoir or an uncontrolled host for most pests and diseases of the potato, including late blight (LB).

In the development of Decision Support Systems, namely DSSs, that are aimed to integrate all factors which might contribute to an optimal control of potato late blight, the calibration of epidemiological models for the actual beginning of the LB epidemics, ie the primary source(s) of inoculum, is becoming a real challenge.

After more than a decade of development of potato late blight DSSs (MilPV and Mileos®) across French potato producing areas, the necessary improvement of the LB risk assessment relies upon a more accurate knowledge of primary source(s) of inoculum and its potential regional variability.

As in many European climatic conducive environments where potato is grown, the very initial source of inoculum to the epidemics of late blight is still subject to hypothesis: latent infested seeds, randomly dispersed and uncontrolled infested volunteers or aggregated on waste piles, soil-borne oospores: the respective share of these different potential sources of inoculum still has to be demonstrated. Up to date, there is no evidence for oospore formation under French cropping conditions, thus this study concentrates on volunteers as major primary sources of *P. infestans*.

This paper reports on the preliminary investigations and data collections that have been carried out in 2010 and 2011. The aim of the study is to build up a sub-model based on observed volunteer data in very contrasting potato growing areas as i) North-western Brittany for early potato crops and seed producing areas and ii) the Central Northern part of France where large acreages are devoted to fresh market and processing potato productions. After describing the different cropping practices in the two different environments and designing a preliminary sampling strategy, field surveys for potato volunteers have been carried out. Data analysis is presented and first steps to further integration of the data into sub-models will be investigated.

MATERIALS AND METHODS

Determination of the number of quadrats to be observed prior to assessing volunteer density with a given accuracy level

In order to determine the best strategy for getting references on volunteer density under different cropping conditions (open field or waste pile) with an acceptable level of confidence and for a limited labor cost, a preliminary simulation test has to be carried out. The quadrat method (count of individuals per area of 1 square meter) is chosen to estimate the potato volunteer density and the number of replicates, ie size of the observed area, has to be determined by simulation. Fields with a given volunteer density were simulated, assuming that potato volunteer stems were spatially and randomly distributed. By simulation, such fields were sampled several times, from 1 to n quadrats (n being the maximum number of blocks equal to the total area of the field). This set of data is repeated for volunteer densities, d_i , ranging from 0.1 to 5 and for 300 simulated experiments. The number of quadrats to be observed was identified according the estimated average (field or pile) volunteer density associated to the required level of accuracy.

Description of sampled units: geographical unit and cropping practice, choice of variables

Two different potato producing areas were selected for volunteer data collection. They reflect two of

the most prevalent cropping practices related to diverse climatic conditions.

In the Brittany region

This part of the country has an oceanic climate in most coastal areas, favourable to early crop productions, occasionally with plastic covers. Winters are mild (a few days per year with negative temperatures) and average temperatures and relative humidity are moderate all year round. Volunteer sampling occurred in May 2010 and May 2011, in two different agricultural environments and approximately ten fields were surveyed each year, in each location.

In the coastal areas, early potato production is prevalent. All fields had early potato crops in the preceding year; half of them were cultivated with artichoke (near Paimpol) at the time of sampling, the others were not yet cultivated (near Saint Pol de Léon) at the time of sampling (bare soil). The most important trait in this cropping practice is the very short rotation for the potato with intercrops, as artichoke or cauliflower, which last for complete soil cover.

Further inland as Brest and Landivisiau, fields were dedicated –the preceding year- to the production of certified potato seeds. The technical rules for such production require a rotation of minimum four years between potato crops (three years without potatoes). When sampled, the fields were covered respectively with winter wheat (18 fields), maize (7), barley (5) and ray-grass (3).

In the Picardy and Nord Pas-de-Calais regions

This part of the country has a more continental climate with harsh and cold winters and warm summers, suitable for maincrop potato production. All types of potato production are present: fresh consumption, processing (French fries and starch) and certified seed. Fields have a large size (average size: 10 ha). Scattered waste piles have been sampled in May 2011, and 34 piles in total were fully observed.

Volunteer density assessment and measured variables

For each field (or waste pile) to be sampled and, in order to assess the volunteer density, the chosen number of the randomly distributed quadrat counts (1 m²) was validated according the above-mentioned method. In each quadrat, the number of emerged stems from volunteer plants was recorded. Field and pile sizes were assessed and recorded. Cropping practices (cultivar, type of rotation and intercropping crops, soil type, harvesting techniques etc...) of the farmers were recorded (data not shown in this report). At the time of sampling, the phytosanitary status of the volunteer plants was recorded, namely for the presence of blighted volunteers.

RESULTS AND DISCUSSION

Determination of the number of quadrats to be observed prior to assessing volunteer density in a given environment

As a result of the simulation (Fig. 1), two sets of graphs are available for decision making. For a given volunteer density ranging from 0.5 to 5, the coefficient of variation of the assessed density (y axis) is varying according the number of replicates (x axis) contributing to the information. For practical reasons and feasibility when the expected volunteer density averages were 5 (or more) stems per square meter, then 20 replicated counts, distributed at random in a given area, provide an acceptable coefficient of variation of 10 %; more replicates would not improve the accuracy (Graph A1, Fig. 1). This number of replicates was applied for volunteer density assessment on waste piles.

Whereas, when the expected volunteer densities are 3 or below 3 per square meter, the number of replicates allowing an acceptable coefficient of variation less than 20 %, is 40 as it can be deduced from the graphs (Fig. 1). Thus, in open fields, 40 replicates were assessed when volunteer density was

three or less per square meter, and 30 replicates when density was five or more. In both cases, the size of replicated quadrat assessment was a fair compromise between accuracy and required time to score each field and waste pile.

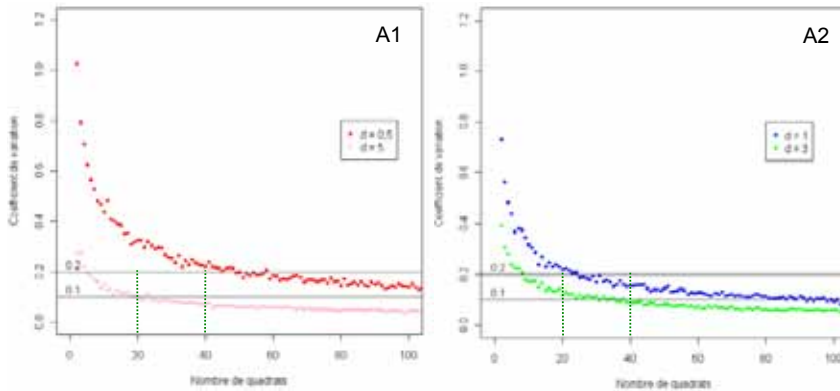


Figure 1 Simulated graphs for sample size according volunteer densities.
 If $0,1 \leq d < 0,5$ then for $nb = 40$ $cv \approx 40 \%$ if $1,0 \leq d < 3,0$ then for $nb = 30$ $cv \approx 15 \%$
 if $0,5 \leq d < 1,0$ then for $nb = 40$ $cv \approx 20 \%$ if $d \geq 3$ then for $nb = 20$ $cv \approx 10 \%$
 d : volunteer density (m^{-2}) nb : number of sampled quadrats cv : coef. of variation on the assessed volunteer density

Assessment of potato volunteer density in different agroecological environments

In the Brittany region

In all sampled fields and in both years, potato volunteers were present. Highest densities (ca 6 stems per m^2) were recorded in the coastal areas where early potatoes alternate with artichoke productions (Fig 2). Cropping practices linked to this specialized production are highly favourable to the development of any tubers left in the soil: stem cuttings are planted one meter apart leaving uncovered soil where potatoes can easily grow. There is no chemical weed control only mechanical. In the inland sampled fields, mostly in the certified seed areas, cereal crops as barley or ray-grasses are more efficient for limiting volunteer growth (Fig 3). On average, the volunteer densities are contained below 2 stems per m^2 . In winter wheat and maize, volunteer density is intermediate, with a mean of three stems per square meter (Fig 3). Lower density of volunteers was noticed in fields near Brest and Landivisiau, but field areas were greater, compared to those of St Pol and Paimpol (Fig. 2). LB epidemics occurred later in the season in 2010 and 2011, and only one blighted plant was recorded in May 2011, in a wheat field, near Landivisiau.

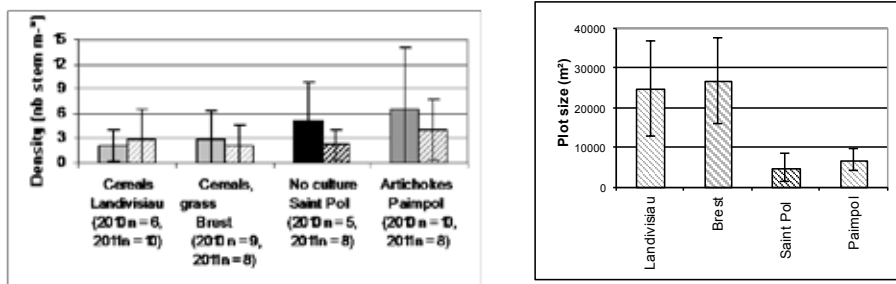


Figure 2 Left: Volunteer potato density (number of stems per m²) in fields (Brittany) as a function of sampling year and location; plain columns: density assessed in 2010 (from May 25th to 28th) and shaded columns: density assessed in 2011 (May 16th to 19th). Right: Average field area at the four different locations.

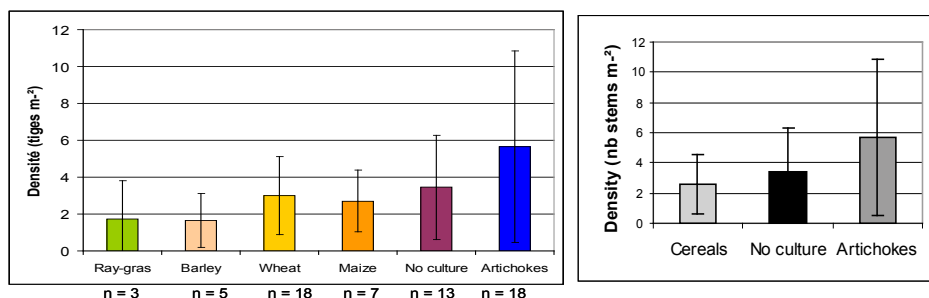


Figure 3 Left: Volunteer density in fields (Brittany) as a function of the following crop (average density, 2010 and 2011). Right: Volunteer density in fields where cereal crops (wheat, barley and maize) and artichokes are grown and fields that are left uncultivated at the time of sampling (May).

In the Picardy and Nord Pas-de-Calais regions

Large ware and processing production areas were studied in these two Northern Regions, Picardy (near Amiens) and Nord Pas-de-Calais (near Lille and Dunkerque). Waste piles are a common cultural practice among potato producing growers. However, they tend to be found in very isolated corners; nevertheless, there are always found in the middle of potato growing areas and could play an important role as primary *P. infestans* source. Thanks to local extension technicians, a set of 34 piles was identified for both regions and scored in 2011. After the sampling period, these piles were actively controlled as it is mandatory requested.

For each pile, the projected area was assessed (Fig 4, right). The largest mean size is observed in Nord Pas de Calais (175 m²), some piles exceeding 750 m² found in the vicinity of packing industries. According the visual estimation of volunteer densities (above 10 stems per m²), only 20 quadrats per pile were scored in order to assess the actual density with an expected 10 % level of accuracy. At the time of sampling, tubers on the piles were actively emerging and all stages of development were present. Only one pile (in Nord Pas de Calais) was found to be blight infested, this as a result of a very warm but dry spring. However, most piles were infested by large and actively evolving populations of Colorado beetles (*Leptinotarsa decemlineata*). In both regions, average volunteer densities were very high, above 90 stems per m² and irrespective of the projected area.

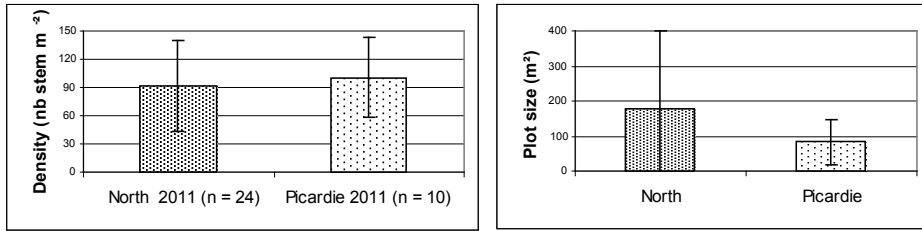


Figure 4 Left: Volunteer densities on waste piles (number of stems per m²), in Nord Pas-de-Calais and Picardie regions, assessed from May 2nd to 5th, 2011. Right: Mean pile projected area at the two locations.

CONCLUSION AND PERSPECTIVES

This paper reports on preliminary analysis on a large collection of field data with the objective to assess, with optimal accuracy, the primary sources of *P. infestans* in agricultural areas where potato crops are prevalent. Volunteers were found in all fields of Brittany where potatoes were grown on the previous year, but density varies according to year, crop and fields for the same crop. They are less abundant in dense crops (grass, barley, wheat) and in maize which is treated with herbicides, hormones than in artichokes.

Prior to data collecting, a mathematical tool had to be designed in order to help deciding sample size as a function of potato volunteer density. The concept of quadrat counting associated with differential levels of replicates proved to be useful for optimal spatial sampling associated with labour and time constraint.

The field data are original because they represent a large variation between regions and within regions, from one field to the other. Traditional cropping practices do influence the source of *P. infestans* primary inoculum. Waste potato piles are more prevalent in the Northern parts and they potentially represent a large source of primary inoculum. They are however spatially distributed in an aggregated manner. On the contrary, dispersed volunteer plants are randomly distributed in the field where a potato crop was grown the year before, they are more prevalent in the oceanic part of Brittany and the nature of the succeeding crop influences the volunteer density, grasses as opposed to artichokes, for example.

This set of data will enable the authors to validate a dynamic model which is currently under construction, to simulate the incidence of volunteer density as a function of potato cultivar, climate and cropping practices. This model, coupled with an epidemiological model, will help design integrated management strategies of potato late blight into the conceptual framework of the SIPPOM model, i.e simulator for integrated pathogen population management, (Lô-Pelzer *et al.*, 2010a and 2010b; Rakotonindraina *et al.*, 2010 and 2012).

Ultimately, this volunteer density simulator could help design control strategies for other pests and diseases of the potato, namely Colorado beetles, quarantine cyst nematodes or soil-borne pathogens, for which epidemics are closely related to volunteer dynamics.

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Ongoing changes in the Irish potato late blight population

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SUMMARY

As part of a continuing all-Ireland late blight project, the 2010 and 2011 Irish *Phytophthora infestans* populations were characterised phenotypically and genotypically. The 2010 season was not conducive to late blight and only 51 viable single lesion isolates were obtained, all from Northern Ireland, but in 2011 more favourable weather allowed collection of nearly 200 isolates from across the island of Ireland. In 2010, over 70% of isolates characterised were A2 mating type and over 80% were metalaxyl-resistant. In contrast, in 2011, overall only 16% of isolates were A2 and only 30% were metalaxyl-resistant. SSR analysis showed that all A2 isolates were the 13_A2 Blue 13 genotype, while the A1 isolates belonged mainly to the older genotype 8_A1. The highly clonal structure of the Irish *P. infestans* population was confirmed using mitochondrial haplotyping, allozyme genotyping and RG57 fingerprinting.

KEYWORDS

Phytophthora infestans, Ireland, mating type, metalaxyl resistance

INTRODUCTION

The 'new' *Phytophthora infestans* population arrived in Ireland in the early 1980s (O'Sullivan & Dowley, 1991; Cooke *et al.*, 1995) and by the mid-1990s it was dominated by two A1 clonal lineages NI-1/IE-1 and NI-2/IE-2 with the A2 mating type present at a low frequency (Carlisle *et al.*, 2001; Griffin *et al.*, 2002). This situation continued up to 2007 with very few A2 isolates detected after 1995 and phenylamide resistance present, but manageable with an anti-resistance strategy. However, the major changes in the *P. infestans* population structure in Great Britain which began in 2005 when the genotype 13_A2, also referred to as 'Blue 13' was first detected (Cooke *et al.*, 2007) were a cause for concern. In 2007, this genotype was first detected in Northern Ireland (Cooke *et al.*, 2009) and an all-Ireland project on potato late blight was initiated. Genotypic and phenotypic characterisation of *P. infestans* isolates from throughout the island of Ireland in 2008 and 2009 showed the presence of 'Blue 13' in both years and by 2009 it was the dominant genotype (Kildea *et al.*, 2010) It was detected mainly in the eastern counties, with limited numbers in the north-west and none in the south-east. Here we report results of population characterisation in 2010 and 2011.

MATERIALS & METHODS

*Collection, isolation and storage of *Phytophthora infestans**

Surveys of the Irish *P. infestans* population were carried out during the 2010 and 2011 growing seasons. Blighted potato leaf material was collected mainly from commercial crops by members of the Seed Certification Division of the Irish Department of Agriculture, Food and the Marine (DAFM; formerly the Department of Agriculture, Fisheries and Food, DAFF), the Northern Ireland Department of Agriculture and Rural Development (DARD) Quality Assurance Branch Potato Inspectors and Teagasc potato advisors. 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.* (2009). The sensitivity of isolates to the fungicide metalaxyl was determined using a floating leaf disk assay (Kildea *et al.*, 2010).

The isolates were genotyped by SSR analysis (Kildea *et al.*, 2010) using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) and in accordance with the protocol developed by EUCABLIGHT. Genotypes were determined by comparing fragment sizes with isolates previously genotyped (kindly supplied by D.E.L. Cooke, James Hutton Institute).

Selected isolates were also subjected to mitochondrial haplotyping (after Griffith & Shaw, 1998), allozyme genotyping (Carlisle *et al.*, 2001) and DNA fingerprinting using the moderately repetitive probe RG57 (Goodwin *et al.*, 1992) as described by Cooke *et al.* (2006).

RESULTS

2010

Very few outbreaks of late blight occurred in Ireland in 2010, probably because unusually dry weather early in the season prevented the development of primary infections and secondary spread. Ten outbreaks were sampled in Northern Ireland and yielded 51 isolates, of which 33 were successfully cultured and tested for mating type. However, the majority of these isolates (71% of those metalaxyl sensitivity tested and 58% of those mating typed) were obtained from a single site, AFBI Crossnacreevy, where blight differentials and selected cultivars were grown unsprayed for monitoring. In the Republic of Ireland, only six outbreaks were sampled and due to viability problems, no isolates were characterised.

In Northern Ireland, the frequency of the A2 mating type was 73% in 2010 (compared with 56% in 2009) and as in previous years it was more frequent in Co. Down (south-east) than in the north-west (Fig. 1). However, the sampling was biased because 80% of A2 isolates were from AFBI Crossnacreevy in Co. Down. Metalaxyl-resistant isolates dominated the population (82%), particularly in Co. Down (Fig. 1). All A2 isolates tested were metalaxyl-resistant, whereas only one A1 isolate was resistant.

2011

The weather in 2011 was more conducive to late blight. In Northern Ireland, 27 outbreaks were sampled and 100 isolates were obtained (the majority from outbreaks in the north-west) and in the Republic of Ireland 13 outbreaks were sampled and 91 isolates obtained.

In both Northern Ireland and the Republic of Ireland there was a dramatic change in mating type frequencies; of isolates tested for mating type, only 10% and 22% of isolates from Northern Ireland and the Republic of Ireland, respectively, proved to be A2 (Fig.2). The A2 isolates were from only five sites in Northern Ireland and three sites in the Republic of Ireland.

Eighty-six isolates from Northern Ireland were tested for metalaxyl sensitivity: only 33% proved to be resistant and no isolates with intermediate sensitivity were detected. The proportion of metalaxyl-resistant isolates was higher in Co. Down (56%) than in the north-western counties (Antrim, Londonderry, Tyrone; 18%; Fig. 2). Seventy-two isolates from the Republic of Ireland were tested for metalaxyl sensitivity: only 26% proved to be resistant, but 19% were intermediate (Fig. 2). All of the A2 isolates tested from Northern Ireland tested for metalaxyl sensitivity proved to be resistant, but of the 19 A2 isolates from the Republic of Ireland tested, five were resistant, 13 were intermediate and one was sensitive. Of the A1 isolates tested, eight out of 62 from Northern Ireland were resistant and 14 out of 53 from the Republic of Ireland were resistant and one was intermediate.

SSR analysis of a collection of isolates from Northern Ireland (2010 and 2011) and the Republic of Ireland (2011) detected only three genotypes, 5_A1, 8_A1 and 13_A2 (Blue 13). Genotype 6_A1 (Pink 6), which had been identified in 2009 (Kildea *et al.*, 2010), was not detected in 2010-2011. The majority of A1 isolates in both Northern Ireland and the Republic of Ireland belonged to the 8_A1 genotype. All A2 isolates analysed were 13_A2.

Mitochondrial haplotyping, allozyme genotyping and RG57 fingerprinting all confirmed the limited number of multilocus genotypes detected by SSR. The 5_A1 isolates were mtDNA Ia; *Gpi*, *Pep 100/100*, *100/100* with a common RG57 fingerprint. All 8_A1 isolates were mtDNA IIa, *Gpi*, *Pep 100/100*, *100/100* and all shared a common RG57 fingerprint. Similarly all 13_A2 SSR isolates were mtDNA Ia; *Gpi*, *Pep 100/100*, *96/96* and these also shared common RG57 fingerprints (a variant lacking one band was detected in a few isolates).

DISCUSSION

The Irish *P. infestans* population is continuing to undergo dramatic changes. All characterisation techniques employed in this study support the view that the population remains highly clonal and there is no current evidence of recombination, although the potential for this exists (Nyongesa *et al.*, 2012). Only three multi-locus genotypes were detected in 2010-2011 and two of these (8_A1, 5_A1) are genotypes present in the Irish population since at least the mid-1990s. The most notable feature was the marked decline in the incidence of the Blue 13 (13_A2) genotype which had dominated the population in 2009 and (in Northern Ireland) 2010, but in 2011 was only detected in 10% of Northern Ireland and 22% of Republic of Ireland isolates from a very limited number of sites. A decline has also occurred in Great Britain (Cooke, D.E.L., personal communication). The other 'new' genotype Pink 6 (6_A1), which had been found at a few sites in 2009, was not detected at all in 2010-2011. This finding is in marked contrast to the situation in Great Britain, where, as the frequency of Blue 13 declined in 2010 and 2011, there was a concomitant increase in Pink 6 (Cooke, D.E.L., personal communication). In the island of Ireland, the decline in Blue 13 was associated with an increase in the genotype 8_A1, which as NI-1 and IE-1 was the commonest type in the mid-1990s. There was thus a loss of both 'new' genotypes from the population in 2011, which may possibly be related to the very dry weather and low incidence of blight in 2010. The decline in Blue 13 also resulted in the population becoming predominantly metalaxyl sensitive, so that use of phenylamide-containing formulations might once again be an option for control of late blight in Ireland.

ACKNOWLEDGEMENTS

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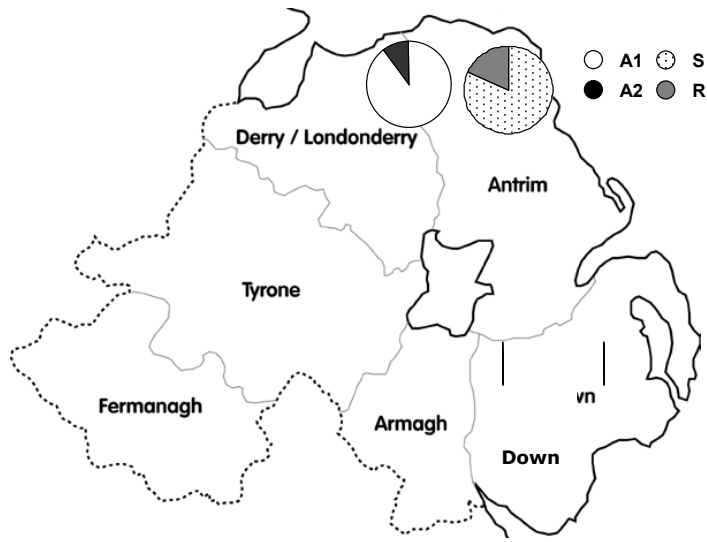


Fig 1. Mating type and metalaxyl sensitivity of isolates in Northern Ireland, 2010.

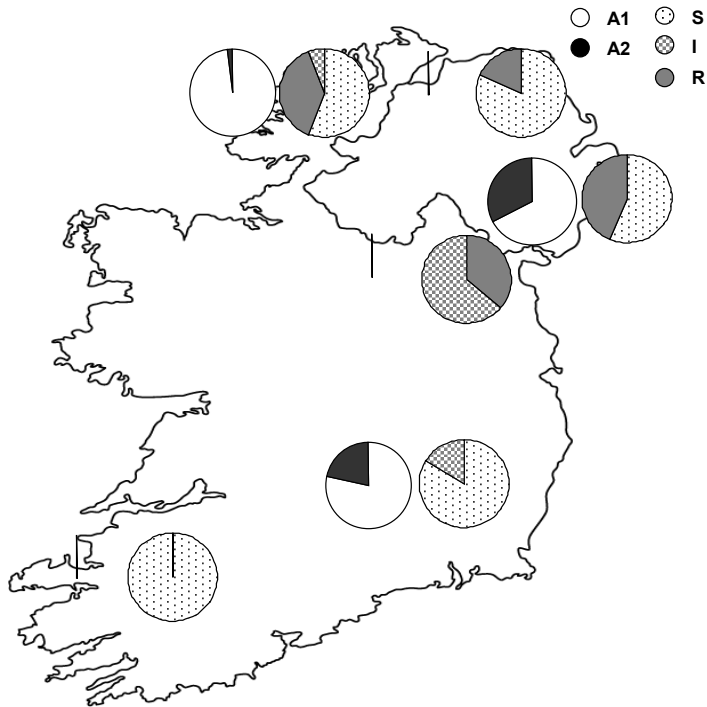


Fig 2. Mating type and metalaxyl sensitivity of *Phytophthora infestans* isolates in the Republic of Ireland and Northern Ireland, 2011.

Strengthening Management of New Late Blight Genotypes in the North Central USA

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SUMMARY

Due to the appearance of a new *Phytophthora infestans* genotype, US24, a regional program was implemented in the north central region of the USA to strengthen late blight management. The new genotype first appeared in 2009 and replaced the US8 genotype in which had been dominant since 1995. Our 2011 management plan was an integrated approach with multiple components that included grower and industry education, early detection, late blight forecasting, fungicide trials and development of resistant varieties. The participants included growers, potato commodity groups, allied industries, government agencies, and universities. Late blight disease appeared late in the growing season and was not found in sentinel plots or retail tomatoes in commercial outlets. The disease became widespread in the region, and growers applied protective fungicides early in the season and generally continued on a 5-7 day schedule to maintain control. Special attention was given to organic, home gardeners and local markets to manage late blight. In 2011, 321 families of 544 families created (59%), included late blight resistance breeding. Despite our collective efforts to control late blight, some late blight infected tubers were found in seed tubers in storage, and it is anticipated that late blight may be present early in the 2012 growing season. The need for a seed treatment to reduce the spread of late blight during seed cutting was identified as a need by the industry. Continue vigilance and teamwork will be necessary to manage this community disease in 2012.

KEYWORDS

Phytophthora infestans, US24 genotype, forecasting, resistant varieties, USA late blight history

INTRODUCTION

Late blight is a periodic, rather than endemic disease in the north central region of the USA, in part due to the occurrence of cool, wet weather favoring disease epidemics. Late blight was generally absent, or at very low levels in the north central region of the USA, during the five year period of 2003-2008, but there was a resurgence of late blight by new genotypes of *Phytophthora infestans* in the US and Canada, and in our region, beginning in 2009. Because of the importance of potato production in our region, and the appearance of new genotypes, we began a regional program to strengthen and improve our regional late blight management program. Potatoes are an important crop in our region. In 2009, ND planted 83,000 acres of potatoes with a value of 175 million

dollars; MN planted 47,000 acres of potatoes with a value of 160 million dollars. The program we implemented was conducted in cooperation with the potato commodity groups in the states of North Dakota (ND), Minnesota (MN), and the province of Manitoba (MB), the state governments of ND and MN, and the entire potato industry of ND, MN and MB - growers, processors, allied industries. This report tells the history of our late blight from 2009-2011, our accelerated plan for late blight management, and the outcomes of our work.

LATE BLIGHT HISTORY IN THE NORTH CENTRAL REGION OF THE USA

From 1850-1991 late blight was sporadic and caused by the old A1 US1 genotype. In 1992 new genotypes were introduced from Mexico and a mixture of genotypes and mating types were found from 1993-1995. In 1995 the US8 A2 genotype became dominant and prevailed until 2008. The US8 genotype was more aggressive, caused more tuber rot, was able to survive more adverse weather conditions and was resistant to the fungicide mefenoxam, which was used for late blight control prior to the arrival of the US8 genotype. US8 was a complex race with many virulence genes. Unexpectedly in 2009, three new genotypes of Pi, US22, US23 and US24, were discovered throughout the US and our region. The overall objective of this project is to evaluate the impact of these new genotypes in our region and strengthen our management plan to maintain control of late blight.

Late blight in 2009

There was a localized outbreak of late blight in our region found in mid-August which was the first significant late blight in five years. In ND it was limited to one county in processing potatoes and garden tomatoes. Due to the widespread epidemic of late blight on the east coast, several premium fungicides were scarce, including Gavel, Revus Top, Curzate, Ranman. The late blight was caused by a genotype new to our region, US24 A1. There were few storage issues due to this new genotype.

Late blight in 2010

Late blight was found on tomatoes in retail stores in Manitoba in early June and detected in ND potato field June 24, the earliest it has been found since 1994. It was reported in MB potato fields June 30 and in MN potato fields July 21, and became widespread in region during the season. The genotype was identified as US24 A1 in ND, both US23 and US24 in MB, and US24 in Montana (MT), an important seed producing state bordering on western ND. This was the first report of late blight in MT in many years. Late blight severity values accumulated early and continued to climb all season, and the early appearance late blight caught growers by surprise. The early appearance suggested seed-borne or cull pile source(s) of inoculum from the previous year. An organic field in central ND acted as a source of inoculum in mid-season, became a political problem that was eventually resolved. Many conventional growers near the organic field wanted the field condemned. There was a shortage of some fungicides during the season and tuber infection was found at harvest and in storage. Based on our 2010 observations, we knew we needed to get ready for late blight in 2011.

Late blight in 2011

Early in 2011 we held several meetings and told the growers they would have late blight this year. We emphasized that late blight is a community disease and the industry needed to work together to manage late blight. To this end, we strengthened a unified multi-state late blight management plan for 2011 for our regions. The participants included growers, potato commodity groups, allied industries, government agencies, and universities.

MATERIALS AND METHODS

Our 2011 management plan was an integrated approach with multiple components that included:

- Grower and industry education
- Early detection
- Late blight forecasting
- Fungicide trials
- Development of late blight resistant varieties

Grower and industry education

Several meetings were held to educate growers about the understanding and managing late blight. University educators spoke at the Manitoba Potato Days January 26, coordinated a late blight symposium at the International Crop Expo in Grand Forks, ND February 17, spoke at the MN Area II Potato Growers meeting March 1, and at a grower only meeting April 21 in Hoople, ND. A late blight update was given at the NPPGA field day meetings August 25. For these meetings, lunch is always served, and additional literature and sources of information are distributed. In cooperation with the Departments of Agriculture in ND and MN and North Dakota State University, we wrote and electronically distributed to wide audiences, a Plant Disease Alert for organic growers, home gardeners and farmers markets entitled “Late Blight: A Plant Disease That Impacts the Community” (PP1565, NDSU Extension Service). Part of the plan was to urge growers to communicate late blight findings with extension and regulatory personnel in order to know location of infected fields, and to submit samples to our lab for verification and typing. Some growers are reluctant to submit samples, as they feel it will affect seed sales or marketing.

Early detection

Sentinel plots, blocks of 50 potato plants with no fungicide applications were planted early to act as a biological trap and early notification for any late blight in the area. Ten locations in ND, including Potato Research Farm, NDSU campus, consultant offices and field sites, fungicide distributor sites, grower fields, were planted with the late blight susceptible variety Red LaSoda. Additional sites were planted in MB. The sites were planted early and monitored frequently. If and when late blight found, potato plants are destroyed and late blight reported on the NDSU Blightline. A retired extension potato specialist (Duane Preston) was hired to monitor sentinel plots, to monitor retail tomato plants for late blight at large retail stores, and to scout fields for late blight.

Late blight forecasting

The North Dakota State University Blightline operated from June 1 – August 30 to forecast weather conditions favorable for late blight, forecast presence of late blight in the field, and provide fungicide recommendations. The Blightline was operated by the authors, and is the main source for late blight information for the region. It uses data from 29 North Dakota Agricultural Weather Network weather stations to forecast late blight severity and favorability. A new report is issued every Monday, Wednesday, and Friday with late blight locations and disease control recommendations. Reports are available by phone, email, and the NDSU website.

Fungicide efficacy trials

Trials are planted at the NDSU Prosper research station near Fargo annually to evaluate efficacy of registered, experimental and biological fungicides for control of late blight. These trials are inoculated with a mixture of three current late blight genotypes collected in the region. Parallel trials were planted in the Manitoba production area. Fungicide treatments in 2011 included 5-10 “real life” grower fungicide programs that producers actually use. Both foliar and tuber late blight infection are rated in the trials. A non-field late blight seed treatment trial was conducted. Cut seed

of the late blight susceptible cultivar Red LaSoda was tumbled with a mixture of late blight infected seed and lab prepared inoculum. The trial consisted of 18 fungicide treatments selected on the basis of foliar late blight efficacy. The rates for seed treatment were estimated based on foliar rates. The trial was designed as 4 replications with 25 seed pieces/replication. After inoculation and treatment, the seed was stored four weeks in paper bags at 12°C, and evaluated for late blight.

Development of late blight resistant varieties

As part of the NDSU potato breeding program, headed by Dr. Asunta Thompson, a dedicated crossing block with late blight resistant parents was established in 2000 to develop late blight resistant varieties. In 2011, approximately 9000 seedlings from 90 crosses were tested using a detached leaf assay procedure in the new NDSU greenhouse. From each cross, 100 seedlings were tested to identify families with high levels of late blight resistance for field selection. Selections are made in the field based on agronomic traits from families with highest levels of resistance. We continued to search for new late blight resistant parents to use in this program in future years; an example is the Patagonia variety from Chile, which may be a new source of resistance. We also plant field trials at the NDSU Prosper site of new and advanced selections from the NDSU potato breeding program for late blight resistance, and the National Late Blight Trial to test advanced selections from multiple US potato breeding programs for resistance to late blight.

RESULTS AND DISCUSSION

What happened in 2011?

Due to prolonged wet weather, there was widespread late planting of potatoes and other crops. Surprisingly, based on the presence of inoculum in the seed and the wet conditions, late blight did not appear until the end of July, about 60 days after planting. Only the US24 A1 genotype was found. It appears that this new genotype has replaced the aggressive US8 A2 genotype that was prevalent from 1995-2008. The US24 genotype is moderately resistant to the fungicide mefenoxam. US8 was resistant to mefenoxam. There was a widely scattered, low incidence of late blight August – September; some fields with high levels. Because we do not have good commercially acceptable varieties with resistance to late blight, growers sprayed early and often until August. Producers applied fungicides on a 5-7 day schedule full season, which was very costly. The months of August and September were unusually dry, with virtually no rain, and growers stopped fungicide applications to save money and mistakenly thought the late blight would not persist. Unfortunately, the late blight did persist resulting in some tuber infection end of the season.

Education results

Education to the growers and industry very well received, good attendance, lots of questions – growers want to learn. Approximately 40 samples of late blight were received from growers, agronomists and consultants. Many phone calls were received to answer questions about late blight and to provide management recommendations.

Early detection results

No late blight was found in either the sentinel plots or retail tomato plants. It may be better to use larger blocks of sentinel plots to provide longer periods of wetness for infection in order for sentinel plots to work.

Late blight forecasting results

The NDSU Blightline was an effective communication device with the industry and was widely

used. This year, most growers began spraying fungicides early before threshold severity value of 15 was reached and continued on a 5-7 day regular schedule. Early fungicide application was due to several factors, including a high threat of late blight, seed borne late blight, favorable weather, large acreages that take several days to spray, and because growers do not want to get behind if late blight appears. Because we have few resistant varieties, frequent fungicide applications are necessary.

Fungicide efficacy trial results

Fungicide trials were planted, but the trials were lost to flooding due to excessive rain. Differences between experimental seed treatment fungicides for control of seed-borne late blight were found, and the results communicated to the industry. Several fungicides were identified that reduced the spread of late blight during seed cutting and handling, including zoxamide, phosphorous acid, ametoctradin, mancozeb dust, fluopicolide and mandipropamide. The results were communicated to the industry, and it is anticipated that these trials will be continued, as there is great interests in this trial from all parts of the potato industry.

Development of resistant varieties results

Overall, only 5% of families tested had >60% of the 100 seedlings with resistance to late blight in detached leaf assays, as defined by a reading of 0 or 1. In 2011, 321 families of 544 families created (59%), included late blight resistance breeding.

Due to excessive flooding, the late blight resistance field trials were lost in 2011. New genotypes affect cultivar resistance, and continuous screening will be necessary to develop resistant varieties.

CONCLUSION

Late blight is a serious and continuing disease and finds a way to cause disease despite our best integrated management strategies and tactics we develop for control. Using this grant as a pattern, we anticipate continuing to actively manage late blight in future years to collectively reduce losses in our region.

ACKNOWLEDGEMENTS

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An IPM2.0 Control strategy for Potato late blight based on cultivar resistance and monitoring of virulence in the local *P. infestans* population

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KEYWORDS

Phytophthora infestans, potato late blight, host plant resistance, Integrated Pest Management. IPM 2.0.

INTRODUCTION

In the Netherlands the total area under potato cultivation amounts to approximately 165.000 ha, annually yielding 7.9 million Mg of potato representing a value of about M€790. Potato late blight, caused by the oomycete *Phytophthora infestans*, is the major problem during potato cultivation in the Netherlands requiring an annual input of around 1400 tons of active ingredient. The number of fungicide applications for potato late blight control varies between 10 and 16 per season inferring a cost (chemicals, application and losses) of 125M€ per year, almost 16% of the total farm gate price (Haverkort *et al.* 2008).

From these figures it is clear that farmers, the potato industry, consumers and the environment could greatly benefit from more efficient and environmentally friendly ways to control late blight through e.g. the introduction and durable exploitation of host plant resistance. *P. infestans* however is renowned for its ability to adapt under the selection pressure exerted by e.g. the cultivation of resistant cultivars. In the past, newly introduced resistance was generally quickly overcome rendering the budget, time and effort spent to create it useless.

Traditionally, potato late blight control heavily relies on frequent (calendar based) applications of fungicides supported by preventive cultural measures such as the use of healthy seeds and the timely destruction of primary sources of inoculum. Cultivation of resistant cultivars is currently limited, mostly due to an overwhelming demand for a limited number of commercially very successful cultivars that are also very susceptible to late blight.

Integrated pest management (IPM) is a broad based ecological approach to structural agricultural pest and disease control that integrates pesticides into a management system incorporating a wide range of practices for economic control of a pest. In general, IPM builds on:

1. Acceptable pest levels with an emphasis on control.
2. Preventive cultural practices, including host plant resistance.
3. Monitoring: regular observation of the crop and the pathogen is the cornerstone of IPM.
4. Mechanical control.
5. Biological controls.
6. Responsible use of pesticides.

Additionally, basic epidemiology relates emergence of a disease to the disease tetrahedron (Figure 1, Zadoks and Schein 1979). Potato late blight (or any other plant disease) epidemics can only develop when a host is present, the pathogen is present and the environment ((micro) climate) is supportive for epidemic development. “Man” in turn is influenced by the pathogen, the host and the environment but also has the capacity to influence these three factors himself to (attempt to) prevent disease development.

When we compare currently applied potato late blight (PLB) control strategies with the theory comprised in IPM and the disease tetrahedron, inconsistencies become apparent. Calendar based spray schedules only consider presence of the host. When the host is present (in a certain growth stage), sprays are applied according to schedule assuming the pathogen is also always present (in sufficient numbers) and the environment is suitable for epidemic development. By definition, this results in more sprays than strictly necessary and a sub-optimal spray schedule due to sub-optimal spray timing.

Decision support systems (DSS's) (e.g. Dacom, Agrovision, NegFry, Phytopre, Simphyt etc.) introduced the concept of preventive fungicide applications directly preceding a predicted infection event. DSS's thus take into account the presence of the host and a conducive environment supporting epidemic development before spray advice is issued. This results in “spraying when necessary” and optimally timed spray applications although it is still assumed the pathogen is simply always present in sufficient numbers.

Here we set out to develop and test a more complete PLB IPM control strategy that uses host plant resistance as the backbone for PLB control, aims to deliver perfect PLB control AND prevent *P. infestans* from breaking the resistance while using as little chemical input as possible. This paper thus aims to introduce and evaluate the next level of IPM (IPM2.0) for potato late blight control allowing for a much more durable exploitation of host plant resistance, cheaper PLB control and a strongly reduced burden on the environment.

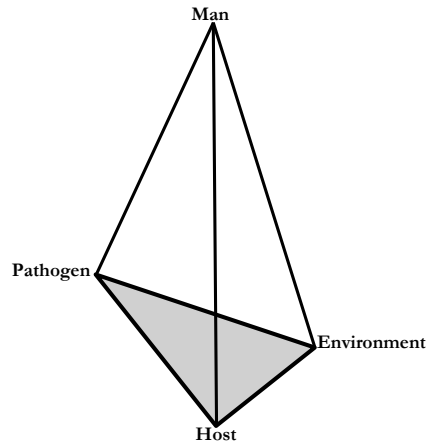


Figure 1. The disease tetrahedron. The base symbolizes the interaction of host, pathogen and environment. Man has various effects on each of these, important to the control and development of plant disease epidemics. (Zadoks and Schein, 1979).

MATERIALS AND METHODS

An IPM2.0 control strategy for potato late blight

The control strategy developed and tested uses host plant resistance as the back bone of the control strategy. Resistant cultivars are NOT sprayed as long as virulence to the R-gene(s) they contain is absent from the local *P. infestans* population. For this purpose, the local *P. infestans* population is continuously monitored for virulence against the R-gene(s) used during the entire growing season. If virulence against the R-gene(s) used is detected, the non-spray strategy is abandoned and replaced by a low input control strategy. Spray advice is then calculated on a daily basis using historic and forecasted weather to predict potential infection events. Preventive fungicide applications are recommended prior to predicted infection events if the residual protection from the previous spray application is insufficient. The dose rate of preventive fungicides depends on the resistance level of the cultivar: 25% dose rate on highly resistant (HR) cultivars, 50% dose rate on medium resistant cultivars (MR) and 100% dose rate on susceptible (S) cultivars. Furthermore, this resistance level based dose rate can be further reduced depending on the predicted fungicide degradation during the predicted critical period. If a critical period is predicted to last two days during which only half of the dose rate applied will be degraded, only half of the resistance level based dose rate will be applied. Last, when a spray advice is issued for the resistant cultivars the Distance Weighted Infection Pressure (DWIP) is calculated according to Skelsey *et al.* (2008). When the DWIP value is below the threshold, atmospheric conditions are unsuitable for viable aerial transport of sporangia and the spray advice is cancelled.

Host resistance thus truly is the backbone of the control strategy, only supplemented with (preventive) fungicide applications when necessary. Curative fungicides are applied when the calculations show that the crop is not sufficiently protected and the critical period has started 0.5 day or more ago.

Summarizing:

- A preventive fungicide application is necessary when:
 - infection event is predicted in the near future AND
 - the protection level from the previous spray is insufficient AND
 - virulence for the R-gene or R-gene combination used is present in the local *P. infestans* population.
- Reduced dose rates of protectant fungicides are used:
 - on crops with intermediate (50% reduction) or high levels (75% reduction) of resistance AND
 - prior to relatively short predicted infection events on all levels of resistance
- Scheduled sprays are delayed by 1 day on resistant cultivars when the Distance Weighted Infection Pressure (DWIP, Skelsey *et al.*, 2008) is below the threshold.

Field experiments

Field experiments were carried out in 2010 and 2011 in Lelystad and Valthermond, the Netherlands. Randomized block experiments with 6 cultivars and 4 replicates were established each year at both locations. Plots measured 8 * 12m. Cultivars included were: Bintje (S), Sante (MR), Bionica (HR), Chc containing clone (HR) and a Sto1 containing clone (HR) in Lelystad and Starga (S), Sante (MR), Bionica (HR), Chc containing clone (HR) and a Sto1 containing clone (HR) in Valthermond. Plots in this randomized block experiment were sprayed according to the advice calculated as described above.

Monitoring plots

Scattered across both farms, 20 – 45 unsprayed monitoring plots, each containing six plants of each genotype included in the spray experiment were planted. Monitoring plots were not sprayed at all during the entire growing season.

Monitoring of virulence

Monitoring plots were checked at weekly intervals during the growing season. *P. infestans* lesions were counted and sampled followed by removal and destruction of the remaining lesions. A maximum of two lesions per genotype, plot and date were sampled, collected in separate Petri dishes containing 15 ml 1.5% water agar and sent to the laboratory by courier.

In the lab, the lesion samples from the field experiments were analyzed for Sto1 virulence using a TaqMan PCR designed to amplify the Avrblb1/ipiO class I region Li *et al.* (2012). *P. infestans* isolates lacking class I ipiO were shown to be virulent on *Rpi-blb1* (Champouret *et al.*, 2009).

Lesion counts and PCR results were used as indicators for the presence or absence of specific virulences in the local *P. infestans* population.

RESULTS

First lesions on Bintje, Sante, Bionica, Chc1-clone and the Sto1-clone in Lelystad were found on respectively 18 July, 25 July, 1 August, 1 August and 8 August 2011. In Valthermond, first lesions on Starga, Sante, Bionica, Chc1-clone and the Sto1-clone were found respectively on 18 July, 25 July, 1 August, 8 August and 15 August 2011. The PCR assay for Sto1 virulence detected Sto1 virulence in a *P. infestans* sample originating from Bintje in Lelystad on 25 July and in a *P. infestans* sample originating from the Sto1-clone in Valthermond on 15 August. Both first findings of Sto1 virulence triggered a change in the control strategy from non-spraying on the Sto1-clone to a low input spray strategy. Results regarding the fungicide input and infection levels in the 2011 spray experiment are given in Figure 2. The fungicide input in Figure 2 is expressed as “full dose rate equivalents”. Infection is expressed as severity (% infected foliage). From Figure 2 we can see that in both locations the fungicide input dramatically decreases with increasing levels of resistance (left to right in the graphs). The level of control in Lelystad was excellent with *P. infestans* completely absent. In Valthermond, infection occurred in the susceptible and medium resistant cultivars, not in the HR cultivar Bionica and the HR Sto-1 clone. Chc1 plots in Valthermond were marginally infected. Apparently, the level of protection of a HR cultivar under a low input PLB control strategy is higher than the level of protection of an S or MR cultivar under a much higher fungicide input strategy.

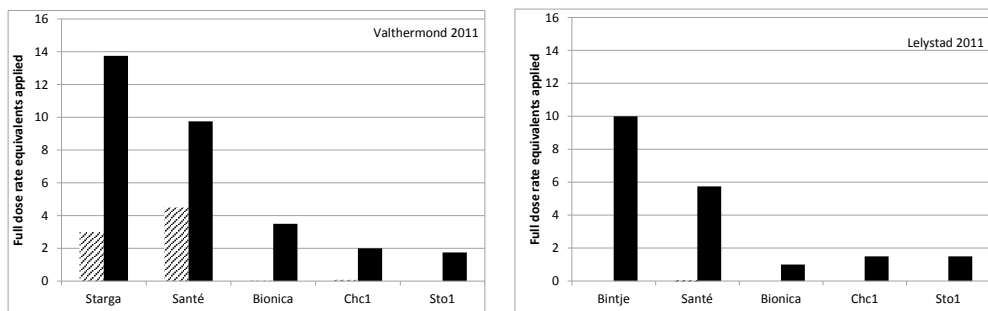


Figure 2. Results from the 2011 field experiments, fungicides input (■, full dose rate equivalents) and infection (% severity).

DISCUSSION

This study was initiated within the Dutch Umbrellaplan Phytophthora to help enhance the level of sustainability of potato cultivation. The fungicides used to control potato late blight pose a major burden on the environment. Reduction of this environmental burden would already greatly contribute to enhanced sustainability. As a result a more complete PLB IPM control strategy was designed and tested that uses host plant resistance as the backbone for PLB control, aims to deliver perfect PLB control AND prevent *P. infestans* from breaking the resistance while using as little chemical input as possible. The results are encouraging, demonstrating the possibilities high levels of host plant resistance have to offer with a reduction of the fungicide input of 80% or more. At the same time caution is necessary as *P. infestans* is well known for its ability to adapt under the selection pressure exerted by e.g. newly introduced resistance and fungicides.

Ideally, the R-gene content of cultivars grown under this IPM2.0 control strategy should be designed in such a way that it is very difficult to overcome in the first place, even for *P. infestans*. This means R-genes should be stacked in sufficient numbers and cultivars containing a single R-gene should be avoided since it can and will serve as a stepping stone for *P. infestans* to overcome stacks of R-genes. The switch from a non-spraying strategy to a low input strategy could then be made when e.g. all but one R-gene have been overcome. Monitoring the local *P. infestans* population thus remains of key importance to success.

Also, application of reduced dose rates hold a risk of exposing *P. infestans* to sub-lethal concentrations of active ingredients. This however only holds in a curative or eradicator application of a fungicide when active infections are exposed to sub-lethal concentrations of A.i.'s. In a preventive application, *P. infestans* is not actively growing in the crop and prevention of infection using a combination of host plant resistance (e.g. expressed as a very low infection efficiency) supplemented with reduced dose rates of fungicides is the goal. The overall resulting protection level of host plant resistance plus the fungicide application should at least be equal to the protection level resulting from a fungicide applied in the recommended dose rate on a susceptible crop. Results from the 2011 field experiment in Valthermond demonstrated that the protection level of a HR cultivar sprayed with reduced dose rates of preventive fungicides can even be higher than those on a susceptible cultivar sprayed with the recommended dose rate.

This paper thus demonstrates the viability of the next level of IPM2.0 control strategy for potato late blight allowing for a much more durable exploitation of host plant resistance, cheaper PLB control, a strongly reduced burden on the environment and a more durable growing system as a whole.

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Early outbreak of potato late blight in Denmark 2011

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SUMMARY

In 2011, the weather in Denmark was relatively warm and dry in April and first half of May. From mid May the weather changed, and there were many days with precipitation in the second half of May. Potato emergence was early (already from mid May) and in many places the fields were very wet. Oospores are expected to have played a significant role as source of primary inoculum and the first infections from soil borne oospores probably took place in the second half of May. More wide spread attacks appeared in the beginning of June and the first recorded outbreaks of late blight were in South and Mid Jutland on the 15th June. From these foci there were a secondary spread to many fields. Late blight was finally controlled but with a relatively high fungicide input. Field trials with the Danish Dose Model were continued in 2011. In the dose model the fungicide input is adjusted to the actual need, and it was possible to achieve a safe and economically profitable control of late blight. However, in a season like 2011, characterised by a prolonged and high infection pressure, there was only a minor reduction in fungicide use using the general model. However, using a revised model (Dose Model 2) with even lower fungicide inputs at low disease pressure it was possible to reduce the fungicide use by 26% and still having a good control of late blight.

KEYWORDS

Potato late blight, *Phytophthora infestans*, oospore infection, early outbreak, dose model, reduction in fungicide input

INTRODUCTION

The importance of oospores as soil-borne inoculum is documented in the Nordic countries (Anderson *et al.*, 1998; Hannukkala *et al.*, 2007) as well as in many European countries (Cooke *et al.*, 2011). Danish and Finnish studies of the correlation between crop rotation and early late blight infections (Bødker *et al.*, 2006; Hannukkala *et al.*, 2007) and investigations from The Netherlands show that oospores remain viable for 3 and 4 years in clay and sandy soils (Turkensteen *et al.*, 2000). Both mating types are present in the Nordic late blight populations and the high genetic variation present indicates sexual recombination (Letinen *et al.*, 2008; Letinen *et al.*, 2009). However, in general oospores play a minor role as source of primary inoculum compared to tuber borne infections (Cooke *et al.*, 2011). In Denmark, oospores seem to play a role in connection with early disease outbreak in some years. Since 1995, in 5 out of 16 years indications of infections from oospores were found in the Danish late blight monitoring network (1995, 1997, 2001, 2003 and

2005; not published). In 2011, reports again showed cases of oospores as source of primary inoculum of significant importance.

DISEASE SITUATION 2011

In 2011, the weather in Denmark was relatively warm and dry in April and first half of May. From mid May, the weather changed and there were many days with rain in the second half of May (Fig. 1). Potato emergence was early (already from mid May) and in many places the fields were very wet. The first unnoticed oospore infections took place mid May in some of these fields.

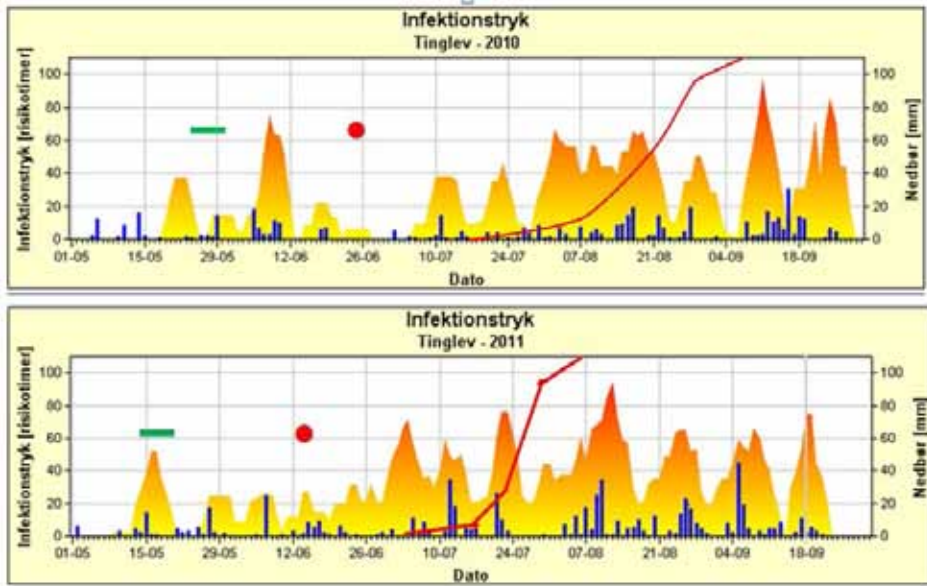


Fig. 1. Infection pressure for potato late blight (left axis) and precipitation (mm, right axis) at Tinglev (Southern Jutland) in 2010 and 2011. Green bar (mid May) indicates potato emergence, red dot indicates the first recorded outbreak of late blight and the red curves show development of late blight in untreated field plots at Jynde vad Field Station (10 km from Tinglev). Data on infection pressure from www.euroblight.net.

The first recorded outbreak of late blight in 2011 was seen in South and Mid Jutland 15 June (Fig. 1). From these fields, there was a secondary spread of late blight to neighbouring fields and since the weather condition was favourable for infections from late June blight developed in many fields. Figure 1 shows the important difference between the seasons 2010 and 2011. In 2011, the weather was characterised by a high and continuous infection pressure from Mid-late June until harvest. In 2011, the fungicide applications were in general 1-2 weeks too late in many fields and often the applications were performed on established infections. However, later in the season, blight was finally controlled but with a relatively high fungicide input (30%-50% more applications and in some fields up to 100% more applications). In many fields, one application of metalaxyl (Ridomil Gold) effectively stopped further development of the disease.

In 2011, the tuber yield was on average with a high variation. Despite the high incidence of fields with late blight, the frequency of fields and tubers with tuber blight were very low.

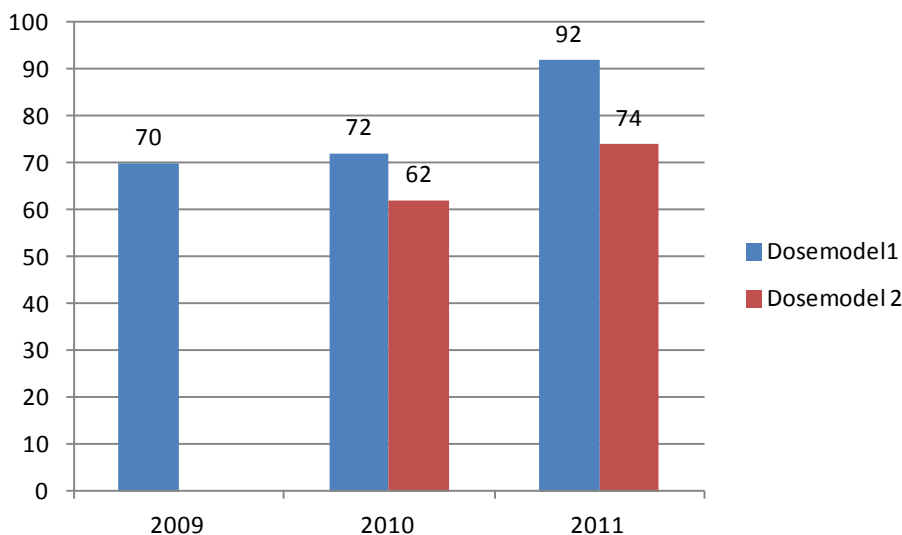


Fig. 2. Fungicide use in dose models relative to standard, full dose (fungicide use measured as relative treatment frequency index where standard full dose is 100, approximately 12-13 sprayings). Trials in starch and ware potatoes 2009 (6 trials), 2010 (9 trials) and 2011(3 trials). Dose Model 1: dose x interval depends on presence of late blight in the area, infection pressure and variety (Nielsen *et al.*, 2010). Recommend lower dose especially in beginning of season. Dose Model 2: In principle same as Dose Model 1 but reduces doses further and possibilities for longer intervals early season. Dose models gave same control of late blight as standard full dose and same economy as standard Dithane NT.

REDUCED FUNGICIDE INPUT MODELS

In the Danish experimental Risk Dependent Dose Model, the fungicide input is adjusted according to the actual need. Prior to periods with a high risk of infection, the dose model uses full dosage of the most efficient fungicides. By contrast, in periods with a low risk, the dose model recommends reduced dosage or no sprayings. All sprays are carried out at weekly intervals. By optimising the spray applications according to need, there is thus a possibility of cheaper disease control in potatoes while still having an effective control (Nielsen, 2004; Nielsen *et al.*, 2010). In the dose model, the potential risk for infection (infection pressure) and dose depend on variety resistance and how close to the potato field late blight has been observed (www.euroblight.net).

Potato late blight occurred relatively late in the trials 2009-2010 and did not develop until the end of July. Under these conditions, the trials with reduced doses show that it is possible to reduce the application of effective fungicides (Revus and Ranman) by up to 30% (treatment index, Fig. 2). In 2011, late blight developed earlier in the trials but it was only 1-3 weeks earlier than in 2009-2010. The important factor was widely the continuous and high infection pressure throughout the season from late June to September (Fig. 1). Under these circumstances, it was only possible to use reduced dosages in the beginning of the season. Use of the general model (Dose Model 1 as in 2009 and 2010) gave in 2011 a good control of late blight but the reduction in fungicide use was only 8% compared with routine spraying (Fig. 2). However, using a model with lower fungicide inputs at low disease pressure (Dose Model 2) it was possible in 2011 to reduce the fungicide use by 26% and still having good control of potato late blight (Fig 2).

CONCLUSION

In 2011, potato emergence was early and oospores played a significant role as source of primary inoculum for infections events in the second half of May. The first outbreaks of late blight were recorded in South and Mid Jutland on 15 June and from these fields there was a secondary spread of late blight. Later in the season, blight was kept under control by a relatively high level of fungicide input. In many fields, one application of metalaxyl (Ridomil Gold) effectively stopped further spread of the disease.

Using a dose model where fungicide input is adjusted to the actual need, it is possible to achieve a safe and economically profitable control of potato late blight. The fungicide reduction potential depends on the weather conditions and duration of periods with high infection pressure. Field trials in 2011 using the dose model showed only a minor reduction in fungicide use in a season with a prolonged and high infection pressure. However, using an experimental model with lower fungicide inputs at low disease pressure it was possible to reduce the fungicide use by 26% and still having good control of potato late blight. In the seasons 2009-2010, where the epidemics started later and where the disease pressure was lower, the general dose model used 30% less fungicide than standard routine spraying and with good control of late blight.

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Disease-orientated threshold values as tool for effective early blight control

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SUMMARY

Potato early blight is a major disease of potatoes. Integrated and targeted early blight control represents a growing challenge for agriculture. Epidemics of early blight caused by *Alternaria solani* and *Alternaria alternata* can cause significant economic damage to potato, if not adequately controlled. Nevertheless, early blight is not an insoluble problem. We developed a disease threshold-based framework to define the optimal timing of fungicide application and to reduce the number of applications. Efficiency of fungicide application against EB could be improved, if treatments were carried out at pivotal times in the epidemic. Fungicide treatments have been adapted to the actual early blight epidemic in the field. Increases in disease incidence or severity were the basis for the initiation and subsequent applications of fungicides. A three-time application with azoxystrobin provided adequate disease control. Targeted applications of fungicides reduced the number of sprayings required to protect starch yield. Using fungicide application thresholds based on disease progress can help to effectively manage early blight.

KEYWORDS

Early blight, disease control, threshold values, fungicide termination, strobilurines

INTRODUCTION

Early blight (EB) can be found in many potato growing regions of the world (Rotem, 1994), and belongs to one of the most common and widespread diseases in potatoes. Due to its high adaptability, EB has the potential to become a serious threat for potato cultivation. Next to the widespread potato disease late blight (LB) caused by *Phytophthora infestans*, EB has become a noticeable problem for German potato production within the last years. A rapid increase in disease severity has been observed for German potato growing areas (Leiminger, 2009). EB is caused by *Alternaria solani* and *A. alternata*, which is also the causal agent for brown spot. EB mainly affects potato foliage and leads to leaf necrosis and premature defoliation. Symptoms include characteristic concentric rings that appear dark and sunken and become papery. Lesions enlarge, coalesce and cause leaf death (Pscheidt, 1985).

Because of its increasing economic importance, EB is in the focus of future integrated pest management strategies. EB is difficult to control because of its capacity to produce huge amounts

of secondary inoculum (Campo Arana *et al.*, 2007; Pasche *et al.*, 2004). In order to suppress EB and to prevent the losses it causes, potato fields are intensively sprayed with fungicides (Horsfield *et al.*, 2010). Fungicides of various chemical groups are currently used in Germany to control EB in potatoes. Until recently, only protectant fungicides were available for the suppression of *Alternaria* species. Since 2007 and 2008, respectively, strobilurine fungicides like azoxystrobin or boscalid in mixture with pyraclostrobin have been registered for control of EB. Because of its improved efficacy against EB these active ingredients have a considerable influence on the course of disease progress of EB. However, optimization of fungicide use on potatoes for the management of EB is still a considerable challenge. In order to control EB properly, many farmers use fungicides frequently from early in the growing season until vine desiccation (Campo Arana *et al.*, 2007; Gent and Schwartz, 2004; Shtienberg *et al.*, 1989). As fungicides are applied regardless of existing disease levels or disease-favourable weather conditions, most fungicide strategies may result in superfluous or ineffective fungicide applications.

The aim of this work was to incorporate a reduced fungicide strategy into EB management and to combine methods to reduce fungicide use in potato. Criteria to optimize the timing of fungicide applications against EB have not yet been established for potatoes in Germany, nor have studies examined the effectiveness of varying threshold values on control of EB disease. Therefore, spraying strategies were evaluated according to thresholds based on disease progress. For this, fungicide treatments were adapted to the actual epidemic in the field. Targeted applications at particular times of the disease progress led to effective control of EB and protection of starch yield. This may allow for a reduction in the number of sprayings per season, and will thus benefit producer, consumer and environment.

MATERIALS AND METHODS

Fungicide field trials

Field trials were carried out in 2005 through 2007. Experimental plots were situated within a commercial potato field, which was naturally infected by EB. Trials were carried out using the potato cultivar Kuras (Europlant Pflanzenzucht GmbH, Lüneburg, Germany), which is a late maturing starch cultivar and highly susceptible to EB. Plant density was 40,000 plants ha⁻¹. Trials were designed as a randomized complete block and were replicated four times. Field plots consisted of four rows (0.75 m between rows) and were 8 m long (24 m²). In 2009, control thresholds were evaluated for practical use. Therefore, selected thresholds were tested at different locations (Aiterhofen and Laberweinting, Bavaria, Germany) in terms of their functionality and practicality. Fungicide treatments were carried out by the farmers themselves, using their in-house equipment. Plot size for each variant was 1,000 m².

Assessment of tuber yield

Beside the quantification of EB disease, potato yield as well as starch content was recorded for all trials and variants. Tuber yield ha⁻¹ was determined from the two rows in the centre of each plot. For this, tubers were dug out, collected by hand and weighed on site. Before the starch content was measured, harvested tubers were stored for four weeks. The starch yield was calculated as the economically relevant measure for starch potatoes. The percentage of yield increase between threshold-treated and EB non-treated (EB control) plots was assessed. Site-specific yield losses were evaluated by comparing the EB non-treated control with treatments applied according to thresholds and healthy controls (weekly fungicide treatments).

Disease rating

Disease progress was observed weekly from potato emergence until vine kill. In each of the replications, 10 plants per plot were monitored for disease progress of EB or other diseases (e.g. LB). For the ratings, each potato plant was divided into three levels (lower, middle and upper leaf section) in order to follow disease development. For each leaf level, one leaflet was examined to determine the percentage of necrotic leaf area. The disease severity per plant was calculated as a mean value. At the end of the season, severity values were plotted against time, and the area under disease progress curve (AUDPC) was calculated for each treatment. The height of AUDPC values, which reflected the intensity of the EB epidemic, was used to assess the individual control thresholds.

Implementation of control threshold values

Thresholds correspond to certain stages of the disease progress and were assessed as disease incidence (DI), or disease severity (DS). They formed the basis for the timing of fungicide sprays, in order to optimize the control of EB. Treatments were carried out after pre-defined threshold values have been exceeded. Applications at early stages of disease development were compared to sprays during the progress of an EB epidemic. As variants of a threshold-based disease control, different (consecutive) stages of EB development were selected.

| Threshold value | definition |
|-------------------------|--|
| 50% DI _p | 50% of plants showing EB symptoms at any leaf section |
| 100% DI _p | 100% of plants showing EB symptoms at any leaf section |
| 100% DI _{m.l.} | 100% of plants showing EB symptoms at the middle leaf (m.l.) section |
| 1% DS _{u.l.} | 1% of the upper leaf (u.l.) area necrotized by EB |

EB-specific treatments were applied either alone, at one specific threshold, or as a combination of thresholds, which resulted in fungicide double (two EB-specific applications) or triple (three EB-specific applications) treatments. Therefore, additional applications were chosen at designated stages of disease development. Thresholds were compared to EB untreated plots in terms of their effect on disease control and reduction of yield loss. Fungicide trials included a fully untreated control, an LB-free variant with unrestricted development of EB (EB control), and treated plots for comparing disease severity and yield response. Additionally, fixed date double treatments, applied six and seven weeks after crop emergence, and non-threshold-based weekly applications of azoxystrobin or mancozeb were used for comparison. Threshold values were evaluated with regard to their efficiency of disease control and reduction of yield loss.

Evaluations of selected thresholds were conducted with the active ingredient azoxystrobin. Azoxystrobin was applied according to recommended dose rates with 125 g active ingredient (a.i.) ha⁻¹ (Ortiva® 0.5 l ha⁻¹). The active ingredient mancozeb was integrated as a standard treatment and was applied at a dose of 1,350 g A.i. ha⁻¹ (Dithane Neo Tec® 1.8 kg ha⁻¹). Next to threshold-specific treatments, field trials included variants with weekly fungicide treatments of either mancozeb or azoxystrobin in order to determine the site-specific yield potential. To prevent the development of LB, the fungicide Ranman® (400 g cyazofamid l-1) was applied as a cover spray at a dose of 0.2 l ha⁻¹ every 8 to 10 days. As the disease progress of EB was not affected by the use of Ranman®, EB was allowed to develop naturally during the course of the growing season. All fungicides were applied with a portable overhead backpack-sprayer, using air-mix nozzles (Lechler, air-mix 110-0.4) at a pressure of 190 kpa. The respective fungicides were sprayed in a water volume equivalent to 400 l ha⁻¹ and did not contain any additional spreader, sticker or adjuvant.

RESULTS AND DISCUSSION

Disease progress

EB epidemic was monitored over the years. Based on disease observations, EB appeared as primary foliar disease in potatoes. Heavy EB epidemics occurred in all years of investigation. Initial disease symptoms appeared on lower leaf sections, thus leading to inconspicuous disease onset. As the season progressed, EB symptoms rapidly enlarged and spread onto higher leaf levels. Secondary spread of EB was observed in all years starting from the end of July or beginning of August. A stronger increase in disease severity was predominately observed for leaves from the middle and upper leaf sections. Rapid increase in leaf necrosis weakened potato foliage and reduced photosynthetic area. In EB untreated plots, potato plants were premature defoliated. Here, disease severity increased from less than 5% to more than 90% within 5 weeks, resulting in yield losses. Our investigations showed that EB disease development followed a gradual upward progression. First symptoms were obvious on lower leaves. As disease progressed, EB heavily infected leaves from the middle and upper leaf section (Figure 1), resulting in premature defoliation.

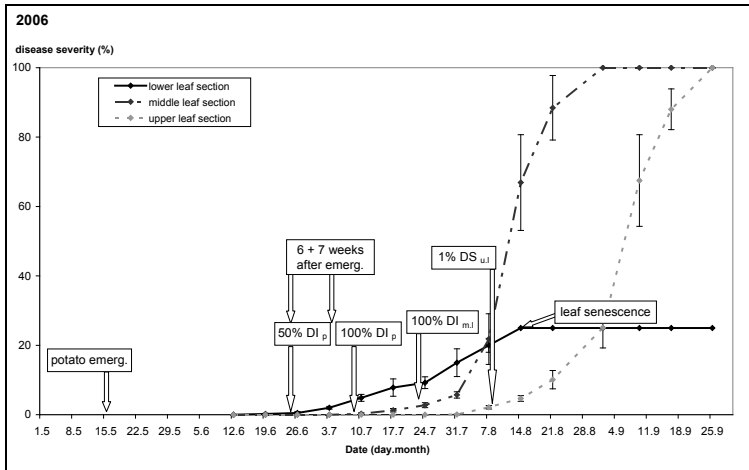


Figure 1 EB disease progress within different leaf layers, and termination of specific threshold, e.g. 2006, cultivar Kuras

Evaluation of threshold values

The control of EB significantly improved foliage health. Threshold values based on certain stages of disease development were highly effective in controlling EB. However, early initial application of fungicides was pivotal for effective disease control and resulted in statistically different levels of effectiveness of EB control. Single treatments with azoxystrobin at early stages of disease progress (50% DI_p or 100% DI_p) significantly reduced leaf blight compared to the untreated EB control. The effect of the time of spray initiation and subsequent fungicide application could clearly be seen throughout the years. Fungicide double treatments, which started early in the course of disease progress, resulted in improved disease control. The adaptation of disease control according to leaf section-specific thresholds was highly effective. Mainly those thresholds achieved satisfying EB control, which were initiated before disease onset or at only marginal disease severities at the specific leaf levels (50% DI_p + 100% DI_{m.l.} or 100% DI_p + 1% DS_{u.l.}). In comparison to treatments, which were applied regardless of the existing disease pressure (6 + 7 weeks after crop emergence), threshold-based treatments resulted in improved disease control.

Already two to three disease-orientated fungicide applications resulted in a significant reduction in disease severity. Among the threshold-based applications, a triple application of azoxystrobin (50% DIp + 100% DI m.l. + 1% DSu.l.) against EB was more effective than double treatments. Here, applications of fungicides according to EB development provided adequate disease control and reduced the number of sprayings compared to unspecific treatments. Our data indicate that proper timing of initial treatments may be of importance (Leiminger and Hausladen, 2012). As EB disease development followed a gradual upward progression in the three-year trials, fungicide sprays should be recommended at early stages of the disease because most of the inoculum for infection of the upper leaves is likely to be formed on lower leaves. The use of fungicides with different modes of action had strong influence on the AUDPC. Applications of azoxystrobin led to a reduction in foliar disease severity and likewise to a delayed disease progress compared to mancozeb. Although weekly treatments with mancozeb led to a reduced disease severity of EB, it was not able to reach the disease control of azoxystrobin.

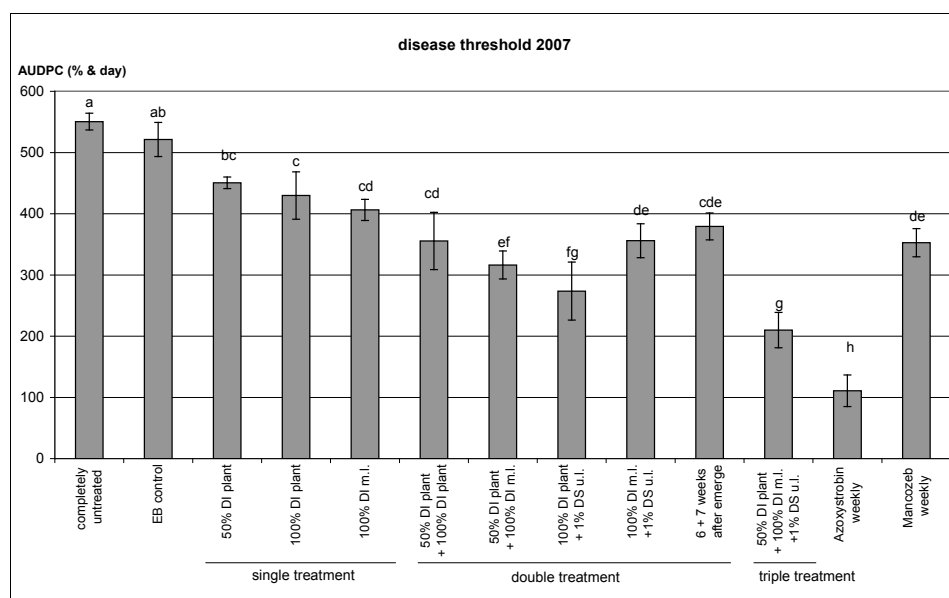


Figure 2 Expression of early blight AUDPC values as a function of fungicide treatments at different control thresholds, cultivar Kurus, 2007

Yield assessment

The estimation of potato yield (2005 to 2007) showed that control of EB resulted in increase of starch yield. According to AUDPC data, most fungicide treatments suppressed foliar blight compared to the EB untreated plots. However, timing of treatments was crucial for the achievement of high starch yields (Fig. 3). In accordance with the results of the disease ratings, the fully untreated control (EB+LB control) generated the lowest yield over all years because of simultaneous infections with *P. infestans* and *Alternaria* species. Single EB treatments did not result in significant yield increase. Distinct differences in potato starch yield depending on threshold-based fungicide applications were observed in 2006 and 2007. When azoxystrobin was applied twice, treatments resulting in particularly increased yield were those applied at thresholds 100% DIp or 100% DI m.l. + 1% DSu.l. Triple applications of azoxystrobin at disease thresholds 50% DIp + 100% DI m.l. + 1% DSu.l. allowed for high starch yields that were similar to those reached after weekly application of

the fungicide. In contrast, applications, which were carried out at fixed time points independently of disease progress (“6 + 7 weeks after crop emergence”), were not significantly different to EB untreated controls or to weekly treatments with mancozeb. The data show that the timing of fungicide treatments influences progress of EB as well as yield. Fungicide applications, which were not adapted to actual disease development, tended to result in lower starch yields. An increase in starch yield was especially evident after application at thresholds, which also reduced AUDPCs according to lower disease severity of EB.

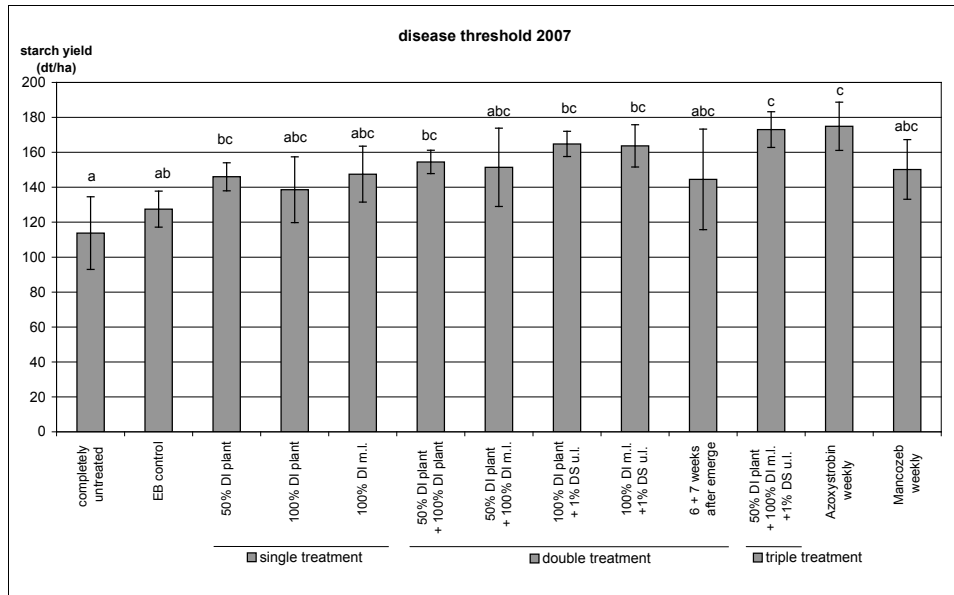


Figure 3 Comparison of starch yields as a function of fungicide treatments at different early blight control thresholds, Kuras, 2007

Practicality of thresholds

According to commercial field trials, the use of disease threshold values was examined in 2009 at different locations. Functionality and practicality of selected thresholds was tested. Differentiated EB epidemics could be observed for the various locations (Figure 4a). Site- as well as cultivar-specific factors had a considerable influence on the development of EB. Due to the distinct development of *Alternaria* species and the corresponding increase in EB, a high disease level (AUDPC) was achieved at both sites Aiterhofen and Laberweinting. At the location Aiterhofen, EB developed quite early, whereby a considerable increase in leaf necrosis was already visible during the course of August. Likewise, at Laberweinting, an increase in EB disease, albeit with a time lag, resulted in the complete destruction of photosynthetically active leaves until the beginning of September.

Depending on the investigated disease thresholds, effective EB control was achieved, if initial applications were carried out at relatively low levels of disease manifestation. Already one single treatment at an early stage of disease progress (50% DIp) significantly reduced EB disease compared to the untreated EB control at both locations.

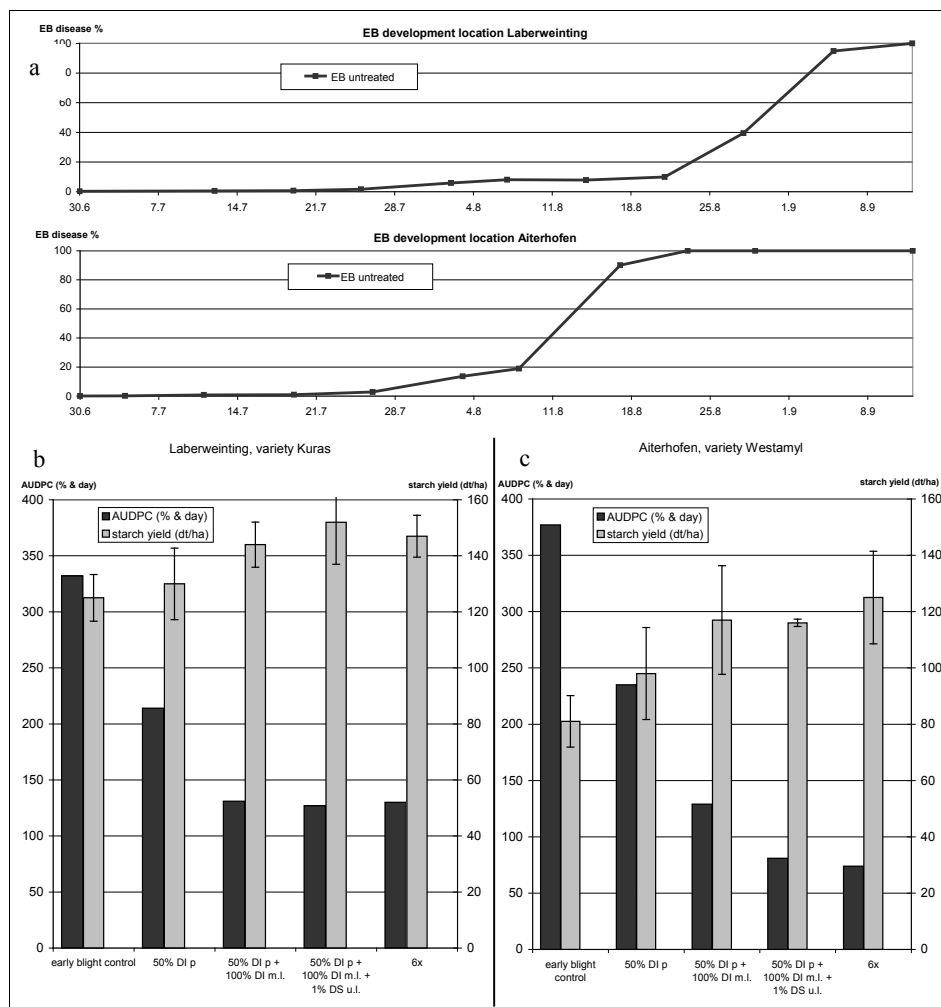


Figure 4a) development of EB disease at different locations (Laberweinting, Aiterhofen), AUDPC value as well as starch yield according to threshold specific EB treatment at b) location Laberweinting and c) location Aiterhofen, 2009

To prevent an uncontrolled increase in EB disease, follow up treatments were tailored according to the specific pathogen development on the plant. Effectiveness of additional treatments was more evident, if EB disease developed strongly. It could be revealed that at the site Aiterhofen, where heavy EB epidemics occurred, a three-fold application according to threshold values was as effective as multiple treatments, which were not adapted to disease development (Figure 4c). Due to the delayed disease progress at Laberweinting, already a two-fold application achieved the best disease control (Figure 4b). Further treatments did not result in improved EB control. Adequate EB control prolonged maintenance of green leaf area as was evident by a reduction in AUPDC values, which likely explains the increase in starch yield. Using disease-orientated thresholds, yield significantly increased over all years. It could be shown that timing of treatments was crucial for the achievement of high starch yields. Targeted applications at particular times of the disease progress led to effective control of EB and protection of starch yield (Leiminger and Hausladen, 2012). At both locations, triple applications of azoxystrobin at disease thresholds 50% DIp + 100% DI m.l. + 1% DS u.l. allowed for high starch yields that were similar to those reached after a six-fold application of

azoxystrobin. Our experiments show, that frequencies of fungicide treatment can be reduced. EB could be effectively controlled, if fungicide application was adapted to the type of epidemic, given that treatments were carried out at pivotal times in the epidemic (Leiminger and Hausladen, 2012). Already two to three fungicide applications based on disease thresholds can protect developing parts of the plant from secondary inoculum. Therefore, the consideration and suppression of EB development on the middle and upper leaf levels is important for a successful EB control. In contrast, calendar-based and “late-initiation” methods were less effective in limiting EB.

SUMMARY

Investigation on EB progress demonstrated the importance of fungicide use for the control of EB in the production of potatoes. Depending on the application frequency of specific EB fungicides and the control threshold used, EB disease could be prevented, and starch yield was safeguarded. Ineffective EB control allowed leaf necrosis, which resulted in reduced green leaf area and premature defoliation. Results show that an effective EB control should start at early disease onset in order to prevent of secondary invasion of the pathogen.

At locations with high disease levels, EB could be efficiently controlled by few fungicide treatments, if they were carried out according to the site-specific disease progress. Especially at locations with less EB disease, non-specific treatments could be prevented, and EB could be controlled by only few fungicide applications. The implementation of control thresholds helped to improve EB control and to prevent yield losses. By this, EB treatments could be restricted to the most necessary. Minimizing the number of applications of a particular fungicide is one of the most effective ways to reduce the risk of fungicide resistance. The development of disease-orientated threshold values as criteria for timing of fungicide applications can be seen as an important tool for farmers to reduce EB epidemics.

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Host-pathogen interaction between *Alternaria* species and *S. tuberosum* under different conditions

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SUMMARY

Under favorable for the early blight development conditions, the *Alternaria* fungus species (*A.alternata* and *A.solani*) differed in their pathogenicity towards potato leaves and tubers. *A.alternata* isolates are characterized by the higher aggressiveness especially towards the leaves, whereas isolates of *A. solani* are more aggressive towards tissue of potato tubers. On the basis of a leaf reaction for *A.alternata* and *A.solani* infection, tested cultivars were divided into 3 groups: resistant, medium sensitive and sensitive. Only a part of them showed clear-cut reaction for both of the *Alternaria* species. A few cultivars revealed quite a dissimilar reaction for *A.alternata* and *A.solani* species.

KEYWORDS

potato, early blight, *Alternaria alternata*, *Alternaria solani*, cultivar resistance.

INTRODUCTION

Recent years showed an increase in the importance of a fungal disease called early blight. Early blight, that is caused by two species of genus *Alternaria* (*A.solani* and *A.alternata*), occurs worldwide on potato crops, particularly in the regions with high temperature and alternating periods of dry weather and high humidity and/or irrigated potato soils, light-textured, sandy, low in organic matter (Gudmestad and Pasche 2007). *A.solani* and *A.alternata* – causal agents of the early blight are more and more risk-important pathogens on potato crops. The early blight occurs in all potato production areas, but there is a significant impact on the tuber yield and the quality only in warm, wet conditions in the early season, which favours a rapid disease development. Quantity share of both species varies and is dependent on the climate / on the weather conditions (Hausladen and Leiminger 2007, Kapsa 2007).

In Polish climatic conditions high regional losses caused by early blight – up to 45%, were recorded. However, most of these high losses were associated with cultivars with recognized susceptibility to this disease (Kapsa and Osowski 2004).

The causal agents of the early blight are typical examples of a necrotrophic organism, when a pathogen infects weaker and older plants (Rotem 1966). Potato plants infected with some viruses are more susceptible to the early blight infection (Hooker 1990).

Genetic resistance of cultivars to pathogens is one of the major factors taken into consideration at the assessment of usefulness of a cultivar for cultivation at severe infection pressure of pathogen or for determining the number of chemical treatments.

The aim of the studies carried out in Bonin in the years 2003-2005 was to estimate the level of susceptibility of different potato cultivars to two species of *Alternaria* fungus and the correlation between the leaf and tuber resistance of the tested cultivars.

MATERIALS AND METHODS

Studies on the leaf and tuber resistance of selected potato cultivars to 2 pathogens: *Alternaria alternata* and *Alternaria solani* were carried out under laboratory conditions. Thirty six selected potato cultivars, which differed in the earliness (from very early to late), were examined during summer (on the leaf resistance) and after the harvest (on the tuber resistance). The aggressiveness of the pathogens was assessed using a method with artificially inoculated detached leaves and sliced tubers („sandwich method”).

From each examined cultivar, 10 leaves from the same stem segment and 10 tubers were collected for the tests. Plant material was placed in a cuvette on moistened filter paper and infected with inoculum of *A.alternata* and *A.solani* at the concentration 100 spores / 1 mm³. One droplet of inoculum was placed with a pipette on 10 detached leaves and between 10 pairs of tuber slices.

The inoculated plant material was incubated for 7-9 days (leaves) and 15-20 days (tubers) at the temperature of 18°C, RH 100%, under 16-hour artificial light (2000 luxes). Criteria for evaluation were as follows:

- percentage of successful infections and size of lesions,
- percentage of changed leaf surface (yellowing tissue, chlorosis, necrosis),
- percentage of upper tuber slice surface covered with mycelium.

Collected results were analyzed statistically with the use of the analysis of variance and the index of correlation.

RESULTS AND DISCUSSION

The experimental results and analysis of variance revealed significant differences in the susceptibility of leaves and stems of examined potato cultivars to *Alternaria* infection, depending on the fungus species which was used for inoculation. *Alternaria alternata* was more aggressive towards potato leaves, developing 2-3 times greater changes on the leaf tissue (tab.1). The same reaction was observed among all tested cultivars in all maturity groups. The mean level of leaf infection caused by *A. alternata* was assessed for 12,7%, whereas caused by *A.solani* was 5,3%

Table 1. Average level of infection caused by *Alternaria* spp. on leaves (%)

| Pathogen | Maturity | | |
|---------------------|------------------------|-------------------|-----------------------|
| | First early+early (13) | Medium early (10) | Medium late+late (11) |
| <i>A. alternata</i> | 13,6 | 12,3 | 12,2 |
| <i>A. solani</i> | 5,7 | 4,1 | 6,2 |

Opposite reaction was observed after the tuber inoculation (tab.2). *A.solani* species seemed to be very aggressive towards the flesh of the tested potato tubers, causing really greater changes in the tuber tissue. An average level of infection caused by *A.alternata* was assessed for 4,2% whereas caused by *A.solani* was 32,7%.

Table 2. Average level of infection (%) caused by *Alternaria* spp. on tubers

| Pathogen | Maturity | | |
|---------------------|------------------------|-------------------|-----------------------|
| | First early+early (13) | Medium early (10) | Medium late+late (11) |
| <i>A. alternata</i> | 2,1 | 4,3 | 6,1 |
| <i>A. solani</i> | 35,5 | 32,5 | 30,1 |

Very early and early cultivars.

Among fourteen of the very early and early cultivars, a few of them - Rosalind, Inovator, Lady Claire, Korona and Felka showed the most sensitive reaction of the leaves for *A. alternata* infection (tab.1, fig. 1). Symptoms of a changed leaf surface caused by *A. solani* were much weaker, when compared to the *A. alternata* infection; the most sensitive reaction was observed on cultivars Lady Claire, Korona and Lord.

A different tuber reaction was also observed after *A. alternata* and *A. solani* inoculation. Tuber tissue of cultivars Gracja, Lady Claire, Bard and Rosalind was the most sensitive for *A. solani* infection. The reaction of the all cultivars' tubers was very weak after inoculation with *A. alternata* (tab.2, fig. 1). Only tubers of Dorota and Molli reacted more clearly for *A. alternata* pathogen.

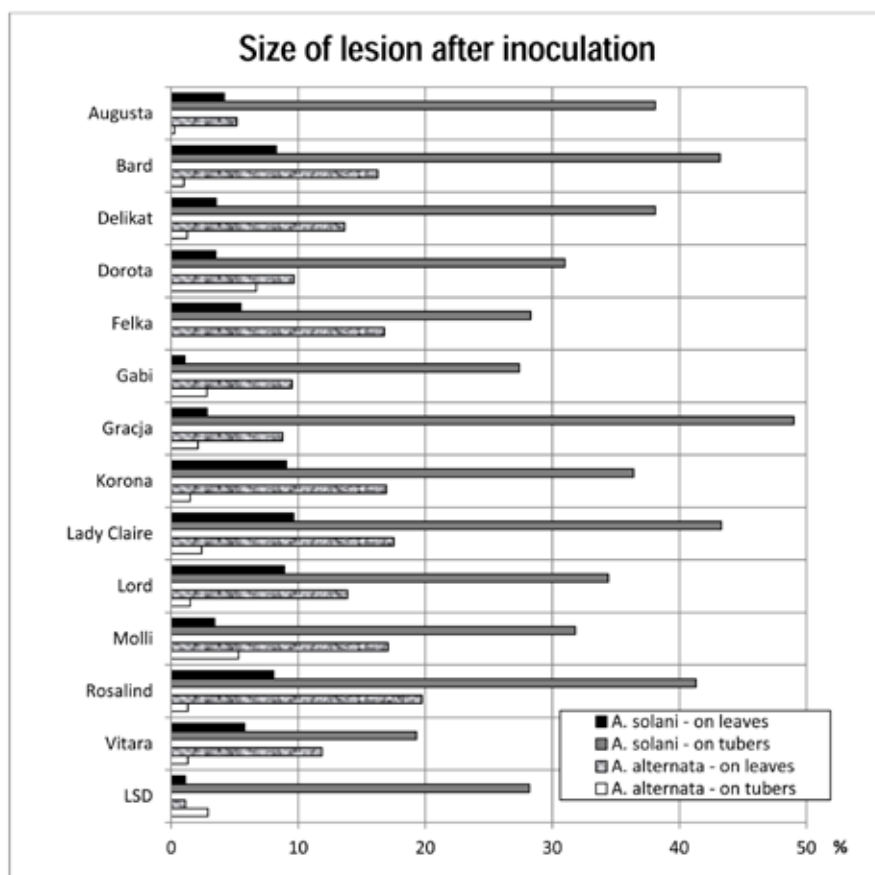


Figure 1. Comparison of leaf and tuber reactions of very early and early potato cultivars to *Alternaria* infection

Medium early cultivars.

Similar reaction for *Alternaria* species infection was observed in the group of medium early cultivars (tab. 2). Generally, stronger reaction of the leaves was observed after inoculation of *A.alternata* compared with *A.solani*. On the other hand, tubers of the all tested cultivars were more sensitive to *A. solani* infection.

Leaves of cultivars Asterix, Andromeda, Romula and Cycloon reacted very strongly on *A.alternata* infection whereas cultivars Asterix and Victoria were the most sensitive to *A.solani* infection. Tubers of Asterix, Andromeda and Pirol were very susceptible to *A.solani* infection. Andromeda, Zebra and Pirol were more sensitive to *A.alternata* than the rest of the tested cultivars.

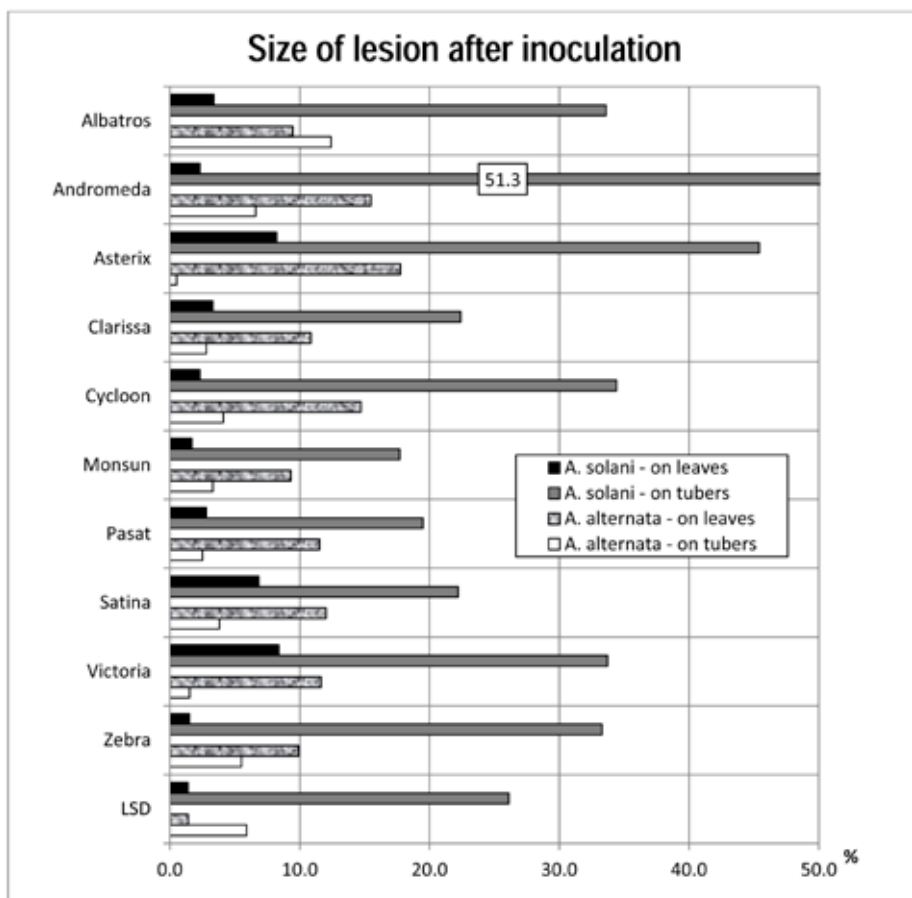


Figure 2. Comparison of leaf and tuber reactions of medium early potato cultivars to *Alternaria* infection

Medium late and late cultivars.

The biggest differentiation of leaf reactions to *A.alternata* was observed among eleven of the tested potato cultivars in this group of maturity. Cultivars Danusia, Fianna and Skawa were significantly more susceptible than the others. Differences of leaf reaction of tested cultivars in the group for *A.solani* infection was not so clear. Nevertheless, significant differences of tuber susceptibility to both of the pathogens were observed (fig.3).

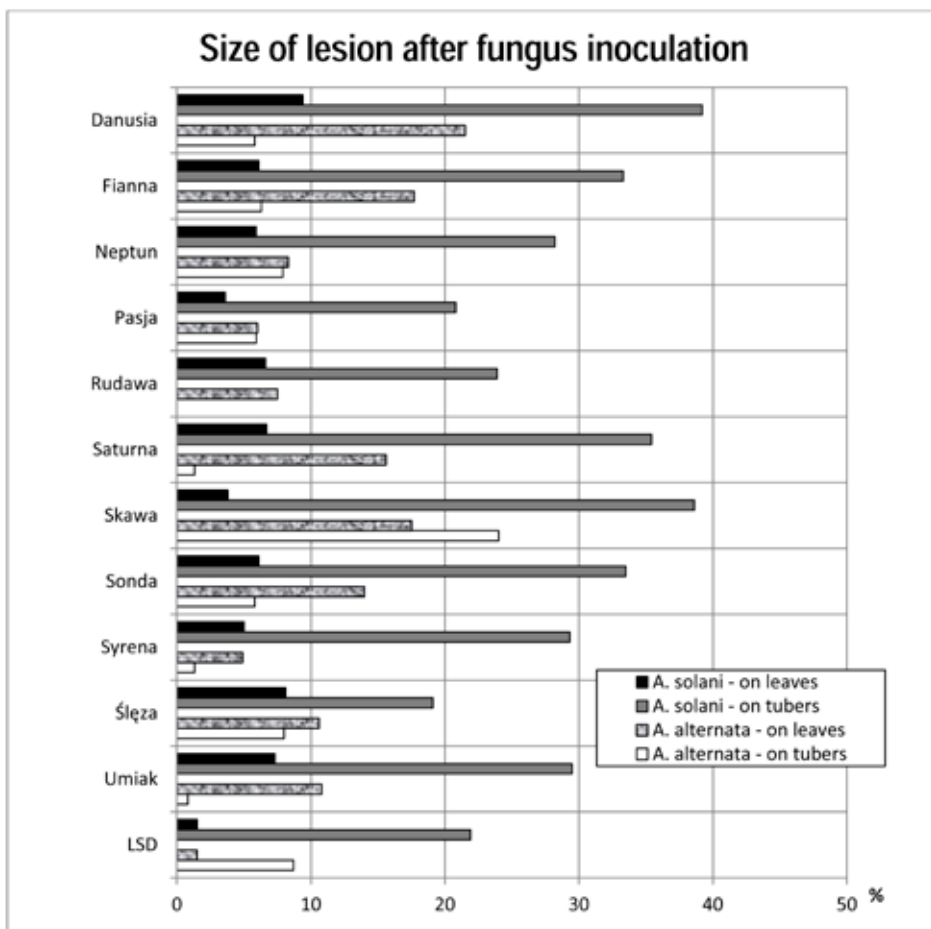


Figure 3. Comparison of leaf and tuber reactions of medium late and late potato cultivars to *Alternaria* infection

Laboratory tests carried out on 36 potato cultivars showed different reactions of the leaves and tubers to fungus *Alternaria*, depending on the pathogen species used for inoculation. Only some of the tested cultivars showed a similar leaf reaction to *A.alternata* and *A.solani* (tab.3). Resistant to both species were the cultivars Augusta, Dorota, Gabi, Gracja, Albatros, Clarissa, Monsun, Zebra, Pasja and Syrena. Medium sensitive cultivars were Lord, Vitara, Satina, Victoria, Sonda, Ślęza and Umiak. A few tested cultivars (Innovator, Molli, Andromeda and Skawa) showed quite different leaf reactions – susceptibility to the *A.alternata* infection and the resistance to *A.solani*.

Table 3. Level of susceptibility of different potato cultivars to *A.alternata* and *A.solani* (on the basis of leaf reaction)

| Very early + early | | | Medium early | | | Medium late + late | | |
|--------------------|-------------------|---------------|--------------|-------------------|---------------|--------------------|-------------------|---------------|
| Cultivar | <i>Alternaria</i> | | Cultivar | <i>Alternaria</i> | | Cultivar | <i>Alternaria</i> | |
| | <i>alternata</i> | <i>solani</i> | | <i>alternata</i> | <i>solani</i> | | <i>alternata</i> | <i>solani</i> |
| Augusta | +++ | +++ | Albatros | +++ | +++ | Danusia | + | ++ |
| Bard | + | ++ | Andromeda | + | +++ | Fianna | + | ++ |
| Delikat | ++ | +++ | Asterix | + | ++ | Neptun | +++ | ++ |
| Dorota | +++ | +++ | Clarissa | +++ | +++ | Pasja | +++ | +++ |
| Felka | + | ++ | Cycloon | ++ | +++ | Rudawa | +++ | ++ |
| Gabi | +++ | +++ | Monsoon | +++ | +++ | Saturna | + | ++ |
| Gracja | +++ | +++ | Pasat | ++ | +++ | Skawa | + | +++ |
| Innowator | + | +++ | Romula | + | ++ | Sonda | ++ | ++ |
| Korona | + | ++ | Satina | ++ | ++ | Syrena | +++ | +++ |
| L.Claire | + | ++ | Victoria | ++ | ++ | Śleza | ++ | ++ |
| Lord | ++ | ++ | Zebra | +++ | +++ | Umiak | ++ | ++ |
| Moli | + | +++ | | | | | | |
| Rosalind | + | +++ | | | | | | |
| Vitara | ++ | ++ | | | | | | |

| | | |
|----------|-----|------------------|
| ratings: | +++ | resistant |
| | ++ | medium sensitive |
| | + | sensitive |

The process of profiling the susceptibility of the chosen potato cultivars to *Alternaria* showed different leaf and tuber reactions. The variations in the reactions depended on the fungus species that was used in the inoculation of the plant material. The further research in this area demands answering a basic question – if characteristics of the resistance of new potato clones should be based on the inoculation of leaves and tubers with a single species of the fungus or with a mixture of both species?

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Genetic structure of *Alternaria solani* - a new approach

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SUMMARY

The diagnostic PCR analyses of lesions with similar symptoms to early blight collected during two years (2009-2010), revealed that *Alternaria solani* probably is the major causal agent to early blight in Sweden. *Alternaria alternata* was identified in one single sample in 2009 and in the following year, 69 out of 360 samples contained *A. alternata*, of which the vast majority co-occurred with *A. solani*. In 2011 the epidemic of early blight started in the second half of July in ecologically cultivated fields and in the beginning of August in the conventional fields. The incidence of early blight was intense during September despite one or commonly two applications of fungicides based on strobilurins. In order to determine if the population had become tolerant toward strobilurins the PCR products of the samples with confirmed *A. solani* were sequenced. None of the substitutions at position 129 associated with loss of sensitivity toward strobilurins were observed.

During the collections in mid September 2011 scattered lesions with similar symptoms to early blight were found on *Solanum nigrum* in two different fields. These lesions are to be analysed together with the samples from the collections of 2011.

The genetic structure of the population *A. solani* has been primarily analysed in Germany (Leiminger *et al.*, 2010), South Africa (van der Waals *et al.*, 2004), USA (Weir *et al.*, 1998) and Brazil (Lourenzo *et al.*, 2011). The studies revealed that populations of *A. solani* had large variation both within and among fields, which is unusual for an asexually reproducing fungus. Analysis of the genetic structure may give valuable information about the range of dispersal of the conidia between areas.

The genetic structure of the Swedish and Tajikistani populations will be analysed by 8 SSRs developed for *A. alternata* (Tran-Dinh and Hocking, 2006) and *A. dauci* (Benichou *et al.*, 2009), which have shown to work on *A. solani* too. However, the method needs to be optimised for best results. Preliminary results from the Swedish population, using DNA from mycelium and lesions, show that there is some genetic diversity even within fields.

KEYWORDS

Alternaria solani, early blight, fungicide sensitivity, genetic structure, SSR

ACKNOWLEDGEMENTS

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Ranman Top, again a step forwards in late blight control

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INTRODUCTION

'Ranman' is a contact fungicide already on the European market at a rate of 0,2 l/ha of Ranman A (active ingredient) and 0,15 l/ha of Ranman B (surfactant). 'Ranman A+B' was registered in the UK in 2000 on potato and in France in October 2001 on potato, tomato and in December 2005 on melon, cucumber and gherkins. Registrations were obtained in other European countries since 2002 (The Netherlands, Belgium, Germany, etc.).

'RANMAN TOP' is a new formulation based on cyazofamid with 160 g ai/l; it is an SC formulation. The dose rate is 0.5 l/ha (80 gai/ha).

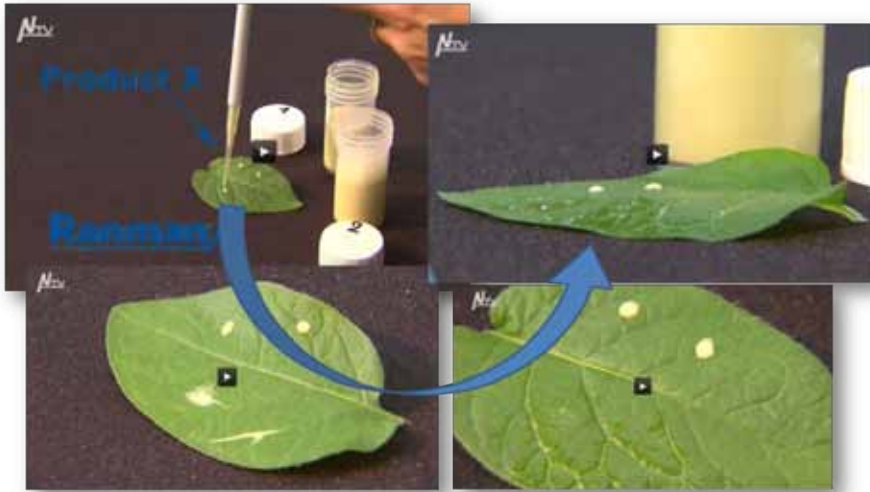
'RANMAN TOP' is RTU formulation with the adjuvant built-in. The EU first registration in 2009 was in the UK. Today we obtain in the most EU countries a registration for use in potatoes, tomatoes, melons and cucumbers,...(depending the country) The maximum number of applications on potato is generally 6 applications in Europe with an interval between two applications of generally seven days.

'RANMAN TOP' can be applied from the beginning till the end of the season (no restriction on potato).

Its active ingredient, named cyazofamid, is a representative of a chemical class based on the cyanoimidazole group. The active substance is specific to the inhibition of Oomycete diseases, specifically late blight of potato (*Phytophthora infestans*) and downy mildew of grape (*Plasmopara viticola*). Cyazofamid inhibits mitochondrial respiration of the fungus at 1 micron M, and its activity was specific to Oomycete fungi at the level of mitochondria. Cyazofamid works by inhibiting complex III on the mitochondrial electron transport system. It is suggested to bind the Qi centre site on cytochrome b.

SPREADING EFFECT

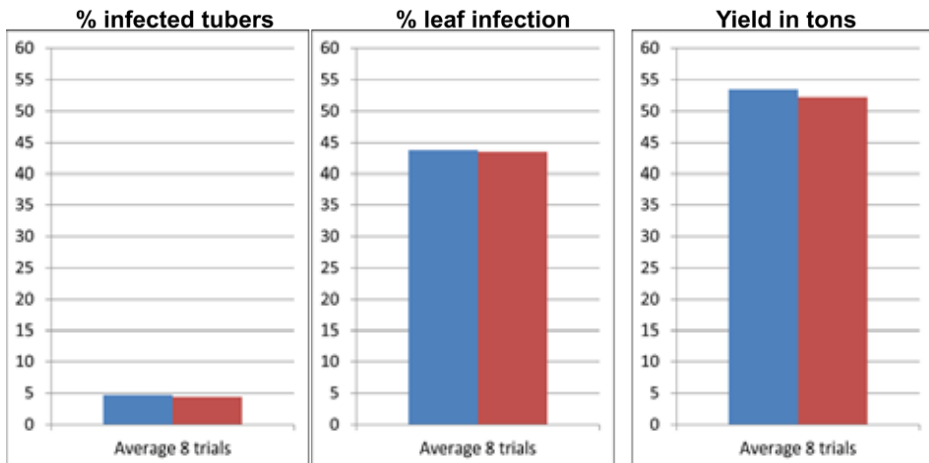
Ranman Top has a unique distribution on the leaf. The spreading effect of the adjuvant, build in the Ranman Top formulation, ensures that the active ingredient is perfectly distributed on the plant. The good distribution allows that the active ingredient penetrates also in the smallest parts to ensure the necessary protection. This is unique to Ranman. This feature can also explain why Ranman as a contact fungicide is able to protect the new growth. The good distribution also gives the advantage that the potato grower can spray with lower volumes of water per hectare, a good spraying technique remains of great importance.



EFFICACY WITH RANMAN TOP

'RANMAN TOP' is an excellent fungicide to be used in potato against *Phytophthora infestans*. This compound is similar to 'Ranman twin pack' (= 'Ranman' A+B).

Trials have shown that independently of climatic and crop conditions in North-West Europe, Ranman Top can achieve a level of efficacy equal to or superior than standard products used as a comparison. Beside the excellent efficacy the product is completely selective to the treated crops.



- Performance: 'RANMAN TOP' at 0.5 l/ha (80 g ai/ha active ingredient) consistently achieved significantly better or similar foliar control versus the standards used in the majority of the trials. 'RANMAN TOP' at 0.5 l/ha gave very similar foliar control versus 'Ranman A+B'. This also holds true, when subsets of trials, selected for certain criteria are compared: whatever the disease level in the untreated plots, the arrival of disease (early or late), the disease pressure (high, medium or low), the variety (Bintje or other varieties) and the method of contamination (artificial or natural).

- Yields: The treatments generally achieved an increase in yield compared to the untreated plots. 'RANMAN TOP' at 0.5 l/ha achieved a 76% mean increase (on 15 trials) in yield compared to 'Dithane' at 2.1 kg/ha.
- Tuber blight: 'RANMAN TOP' will strongly reduce the percentage of blight affected tubers at harvest and after storage. 'RANMAN TOP' is also similar in its efficacy to 'Ranman TP'.
- Rainfastness: 'RANMAN TOP' formulation demonstrated good rain fastness. There is no need to renew the protective spray cover immediately after a significant rainfall event.

The applications should be made "preventive", before conditions favorable to blight development occur. The first application of 'RANMAN TOP' should be made as soon as a blight warning is issued or when conditions are favorable for infection. Under conditions of repeated infection periods, applications at 7 day intervals will be recommended.

Recommendations and field performance of Initium® based products against *Phytophthora infestans* in potato

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SUMMARY

In this paper the results of Orvego® (Initium + dimethomorph) and Orvego Duo® (Initium + mancozeb) from trials conducted between 2009 and 2011 in the Netherlands, Germany and the United Kingdom are summarized. Orvego and Orvego Duo were tested in season-long sprayed efficacy trials and in spray-programmes. Additionally, the effects of Orvego and Orvego Duo on the protection of new growth were evaluated.

The results confirm the excellent efficacy of both products against *Phytophthora infestans* including the capability to protect growing leaves.

KEYWORDS

Phytophthora infestans, potato late blight, new growth, Initium, Orvego, Orvego Duo

INTRODUCTION

Late blight and downy mildews are devastating diseases of several crops world-wide and play an important economic role in commercial food production. Economic losses by *Phytophthora infestans* in potatoes were estimated in developing countries alone to be more than 2,7 billion \$ (CIP, Centro Internacional de la Papa, Lima [Peru]). Initium is a fungicide developed by BASF with high activity against these Oomycete pathogens. Since its discovery in 2004, Initium has undergone detailed evaluation in laboratory tests and in extensive global field testing programmes. It is remarkable for its high intrinsic efficacy against different infectious stages of peronosporomycetes. An additional key benefit is its strong adsorption to the leaf wax layer, due to molecule structure and the low water solubility in combination with the high log POW. This specific characteristic of the molecule results in very good rainfastness. However under periods with dew and leaf wetness, Initium has the capability for redistribution on the leaf. Due to this effect Initium can protect growing leaves. An excellent toxicological and ecotoxicological profile completes the requirements of a modern fungicide. During the EuroBlight Workshop 2010 in Arras, Initium and Initium containing products had been presented in detail. Respectively details can be found in the Proceedings N° 14. In this paper an overview of the trials conducted between 2009 and 2011 is given.

MATERIAL AND METHODS

Two Initium containing products are marketed in North-Europe for the control of *Phytophthora infestans*.

Orvego Duo combines the modern active ingredient Initium with mancozeb, which is the key multi-side active ingredient in potatoes. The excellent preventive efficacy against *Phytophthora infestans* is supplemented with a side effect against *Alternaria spp.* The target dose rate is 2.5 kg/ha.

Orvego contains in a liquid formulation besides Initium the locally systemic active ingredient dimethomorph. This ensures very good control of several stages in the life cycle of *Phytophthora infestans*, which provides preventive, curative and antisporent efficacy. The target dose rate is 0.8 l/ha.

Both products have been shown to have very good rainfastness and due to their good miscibility with other plant protection products flexible usage in spray programmes is possible.

In 2009 to 2011 Orvego and Orvego Duo were tested not only in season-long sprayed efficacy trials, but also in practical spray programme trials. Furthermore specific trials, e.g. protection of growing leaves and rainfastness, were performed.

Season-long sprayed efficacy trials and spray-programme trials

The trials to evaluate the efficacy of Orvego Duo and Orvego were performed as field trials according to GEP and different EPPO guidelines (especially EPPO PP 1/2 (4)). Each trial had 4 replicates; the trial design was set up as a randomized block design. The application timings were chosen according to the weather conditions and the disease development; in general the application interval was 5-10 days. Disease assessments were performed before each application and additionally according to disease pressure. Orvego Duo was compared to the active ingredients fluazinam and mandipropamid. Orvego was positioned in spray-programme trials in the first block and was compared to spray-programmes with competitor products containing mandipropamid and cymoxanil + mancozeb.

Protection of New Growth

Different trials had been conducted to evaluate the efficacy of Orvego and Orvego Duo on growing leaves. Exemplarily the results of a trial conducted in 2010 on the request of BASF by PPO Lelystad are presented in this paper.

For this trial potato plants had been grown under field trial conditions. During the phase of active growth 4 different leaf sizes were marked before the test fungicides were applied. Seven days after the application the marked leaves were collected and brought to the greenhouse. There they had been inoculated with the spore suspension (strain 98014). Once symptoms became visible assessments were made.

RESULTS AND DISCUSSION

Season-long sprayed efficacy trials

Figure 1 shows a summary of 14 field trials conducted between 2009 and 2011 in different regions of Germany.

All trials selected for the summary were performed under conditions of very high disease pressure, indicated by an average attack of 89 % in the untreated plots. Orvego Duo at the target rate of 2.5 kg/ha provided an excellent level of efficacy, comparable with mandipropamid. In comparison to fluazinam, Orvego Duo performed significantly better.

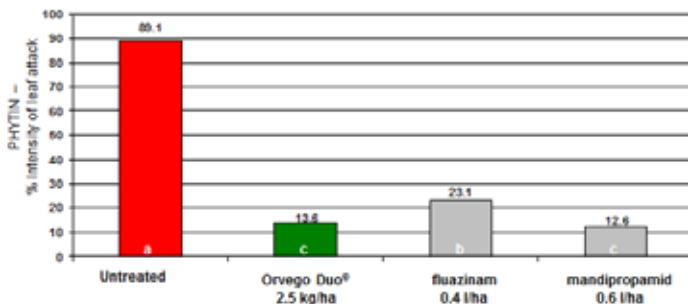


Figure 1: Summary of 14 season-long sprayed field trials (Germany, 2009-2011) Spray-programme trials

In 2010 and 2011 several spray-programme trials were conducted in the Netherlands. In these trials Orvego was always sprayed in the early application block, according to the intended positioning. The results can be compared to spray-programmes with mandipropamid and cymoxanil + mancozeb in the early block. In the second block all treatments were sprayed with Acrobat® (dimethomorph + mancozeb) and in the third block cyazofamid was used.

The assessments, which were relevant for the performance evaluation of the products in the early block, were made at the end of July/beginning of August. Summarizing all trial results, the average disease attack in untreated plots reached only moderate levels at this assessment timing. Therefore in summary no significant differences between the products in the early block could be detected. Both the reference products and Orvego provided a very good level of efficacy.

Looking at individual trial results, significant differences can be identified. For example figure 2 presents the result of a spray-programme trial, conducted in 2011 in the Netherlands. Infection rows in the trial had been inoculated mid-June. Therefore high disease pressure was already reported for the assessment done on the 25th of July (10 days after the last application in the first block). Orvego performed like mandipropamid at a very high efficacy level, while cymoxanil + mancozeb was significantly weaker.

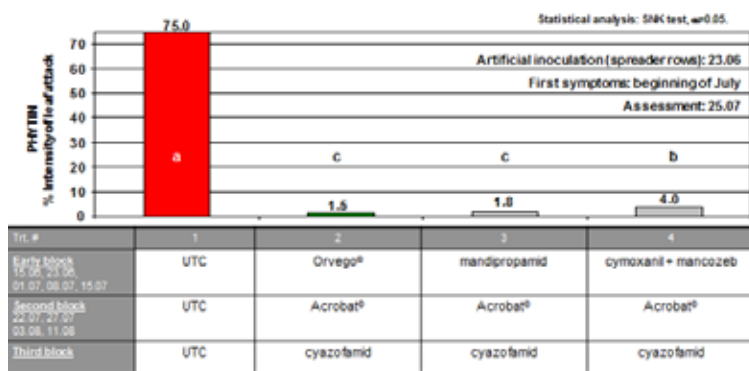


Figure 2: Comparison of Orvego, mandipropamid and cymoxanil + mancozeb positioned at the beginning of a spray-programme trial (assessments done 10 days after the last application in the first block)

Protection of new growth

Several trials were conducted to evaluate the efficacy of Orvego and Orvego Duo on growing leaves.

The columns in figure 3 visualize the results of 3 trials conducted in 2010 by PPO Lelystad on the request of BASF. Even though some variation can be seen in the individual trials, it can be concluded that Orvego and Orvego Duo give clearly better protection of growing leaves than the contact active ingredient fluazinam. For Orvego Duo these results can be explained by the redistribution effects of Initium. For Orvego the locally systemic properties of dimethomorph can also be considered a critical factor.

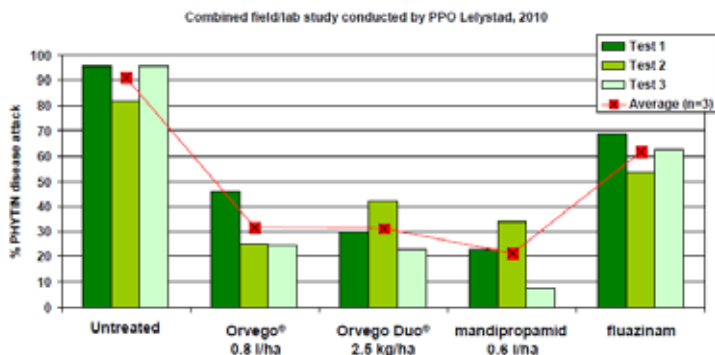


Figure 3: Results of 3 combined field / greenhouse studies evaluating the protection of growing leaves (PPO Lelystad, Netherlands, 2010)

CONCLUSION

Initium is an innovative fungicidal active ingredient developed by BASF. In numerous field trials both the Initium containing products, Orvego and Orvego Duo, have proven their high efficacy. The presented field trial results for season-long applications as well as for spray-programme applications underline the very good efficacy of Orvego and Orvego Duo against *Phytophthora infestans*. A specific characteristic of Initium products is the protection of growing leaves, which is related to the redistribution effects of Initium.

Due to good miscibility with other plant protection products, Orvego and Orvego Duo can be flexibly positioned in different spray programmes according to their strengths. For Orvego Duo a side effect against *Alternaria spp.* can be considered.

ACKNOWLEDGEMENTS

The authors would like to thank all colleagues who have contributed to the development of Orvego and Orvego Duo.

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Revus Top

A new product for the control of *P. infestans* and *Alternaria* in potatoes in Europe

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SUMMARY

Revus Top is a new fungicide developed by Syngenta Crop Protection for the control of *Phytophthora infestans* and *Alternaria spp.* in potatoes. The compound contains mandipropamid and difenoconazol from which the last one is a new active ingredient for the control of *Alternaria* in potatoes in the North of Europe. Efficacy trials against late and early blight were carried out in Austria, Belgium, the Netherlands, Czech Republic, Denmark, Germany, France, Sweden, United Kingdom and Switzerland between 2008 and 2010. The results show that Revus Top is very effective to control both diseases in a rate of 0.6 liter per hectare when compared to current standard compounds. The compound is safe for crop yield, quality and propagation material.

KEYWORDS

Phytophthora infestans, *Alternaria spp.*, potatoes.

INTRODUCTION

Targets of the product

Late blight (*Phytophthora infestans*) is one of the world's most devastating crop diseases. The disease can rapidly cause severe foliar damage, leading to very significant loss of tuber production. Additionally infection of the tubers at any time – either in the field or in subsequent storage – will lead directly to the loss of edible yield.

The “early blight of potato”, caused by two fungi of the genus *Alternaria*, can occur in susceptible varieties even before the “late blight of potato”, which is caused by *Phytophthora infestans*. Massive infestation becomes visible generally in late summer in depending on weather during the vegetation period. Damage caused by *Alternaria spp.* occur predominantly in dry and warm crop areas or in years with dry and warm weather during spring and early summer because of the higher temperature demands and the lower dependence on moisture of the pest organism. Damage occurs in form of

reduced yield as a result of reduced assimilation surface and early ripening of the crop. Connected to a reduced assimilation surface and early ripening is a reduced starch content resulting in total yield losses of up to 30%.

It is important to notice that any protection measure against Early Blight has to be combined with protection against Late Blight as in Europe Late Blight is the dominant, always occurring disease.

Composition of Revus Top

Revus Top is a suspension concentrate (SC) containing 250 grams per liter (g/l) mandipropamid and 250 grams per liter (g/l) difenoconazole for use on potatoes. Registered rate will be 0.6 liter per hectare (l/ha) with a maximum of three applications per season.

Mandipropamid is a highly effective fungicide against most foliar Oomycete pathogens. It belongs to the chemical class of the mandelamide fungicides and is the first compound of this class for commercial use. Mandipropamid has a high affinity to wax layers of plant surfaces. After the spray liquid reaches plant surfaces, the major part of the active ingredient is absorbed into the wax layer and is fully resistant to wash-off by rain as soon as the spray deposit has dried. A small amount of active ingredient penetrates into the plant tissue. Due to its high intrinsic activity, the amount taken up into the plant tissue is sufficient to stop mycelia growth inside the plant and to protect the opposite leaf surface by translaminar movement. These properties of mandipropamid ensure consistently excellent, long lasting disease control.

Mandipropamid is highly active against spore germination. It also inhibits mycelia growth and sporulation. Mandipropamid is best used as preventive spray against the target diseases but also provides curative activity during the incubation period.

The biochemical mode of action of mandipropamid and all other CAA fungicides has just recently been discovered and is the inhibition of cellulose biosynthesis. No direct effects of mandelamide on other metabolic processes such as respiration or synthesis of cell walls, proteins, sterols or amino acids were observed.

Mandipropamid has been registered against Late Blight (*Phytophthora infestans*) on potatoes as REVUS since 2006 in almost all EU countries. The formulation contains 250 g mandipropamid per liter and is applied at 0.6 l/ha which will deliver 150 g mandipropamid per hectare.

Difenoconazole is a translaminar fungicide with long-lasting preventative and curative broad-spectrum-control, including leaf spot diseases, powdery mildews, rusts and scab of annual and perennial crops. It is active against plant pathogens from the Deuteromycetes, Basidiomycetes and Ascomycetes.

Difenoconazole is from the triazole class of chemistry and its mode of action is similar to other triazoles. Its main biochemical mode of action is the inhibition of the sterol biosynthetic pathway of fungi, which stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes.

Taken up by the plant, difenoconazole acts on the fungal pathogen during penetration and haustoria formation. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes. Interference with sterol biosynthesis leads to disruption of membrane function, leakage of cytoplasmic contents and hyphal death.

Difenoconazole is currently registered and used in European countries for repeated applications at dose rates between 100 and 200 g ai/ha, depending on the country.

The active ingredient difenoconazol is new for *Alternaria* control in potatoes in North of Europe.

The combination of mandipropamid and difenoconazole in Revus Top will provide long residual foliar control against both important potato pathogens Late Blight (*Phytophthora infestans*) and Early Blight (*Alternaria spp.*).

EFFICACY RESULTS

Laboratory and glasshouse tests

Mandipropamid is highly active against *Phytophthora infestans* in potatoes and tomatoes, *Plasmopara viticola* in grapes and against *Pseudoperonospora cubensis* in cucurbits in greenhouse tests in whole plant assays. In Figure 1 results are shown from a comparison of mandipropamid with key competitive compounds against *Phytophthora infestans*. Applications made 1 day before (preventively) artificial inoculation. 5 days after inoculation (6 days after spraying) mandipropamid gives around 90 % control. These results show that mandipropamid provides clearly better preventive disease control than the reference standards cymoxanil or dimethomorph, when used at the same application rates. For field use the recommendations for cymoxanil is 100-150 g ai/ha and for dimethomorph 150-200 g ai/ha.

At equal application rates mandipropamid is also clearly more effective than the preventive standard mancozeb. For field use mancozeb is recommended at 1000-2000 g ai/ha.

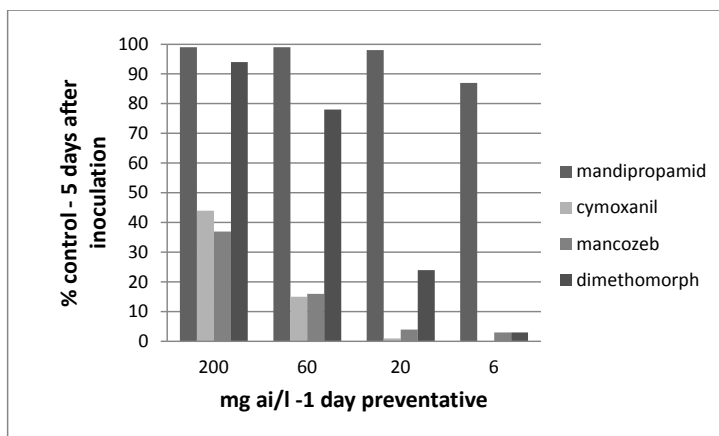


Figure 1. Comparison efficacy of several active ingredients against *Phytophthora infestans*

Difenoconazole is highly active against *Alternaria solani* in potatoes and tomatoes. Typical results from greenhouse screening tests with different difenoconazole rates against *Alternaria solani* in potatoes are summarized in Figures 2 and 3.

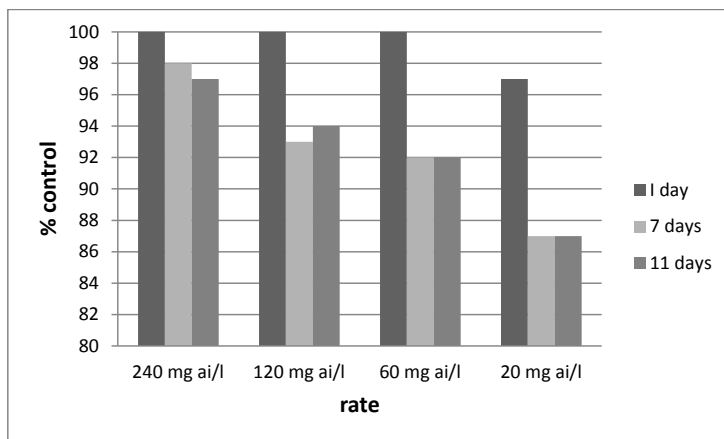


Figure 2. Difenconazol efficacy against *Alternaria solani*. Preventative spraying 1, 7 and 14 days before inoculation. Assessment 6 days after inoculation.

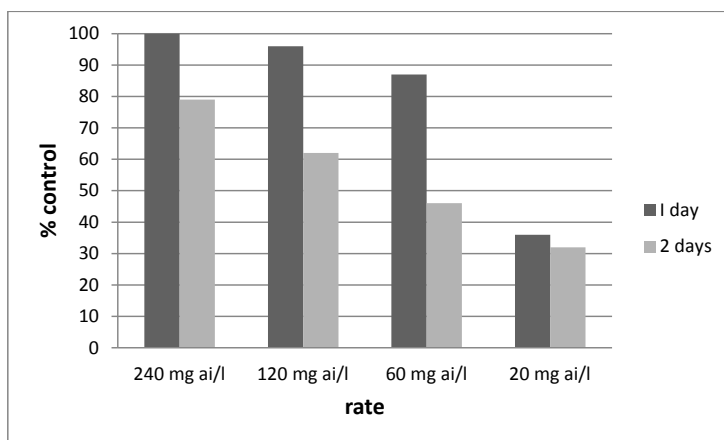


Figure 3. Difenconazol efficacy against *Alternaria solani*. Curative spraying 1 and 2 days after inoculation. Assessment 6 days after inoculation.

The results in Figures 2 and 3 show that difenoconazole provides a very good control of *Alternaria solani* by both preventative and curative action.

However, difenoconazole does not only provide activity against *A. solani* but also on the other *Alternaria* causing species *A. alternata*. Comparing the efficacy of difenoconazole on the two *Alternaria* species appearing on potatoes, *A. solani* and *A. alternata*, it has to be stated that the intrinsic activity against *A. solani* is somewhat higher than against *A. alternata* (Figure 4).

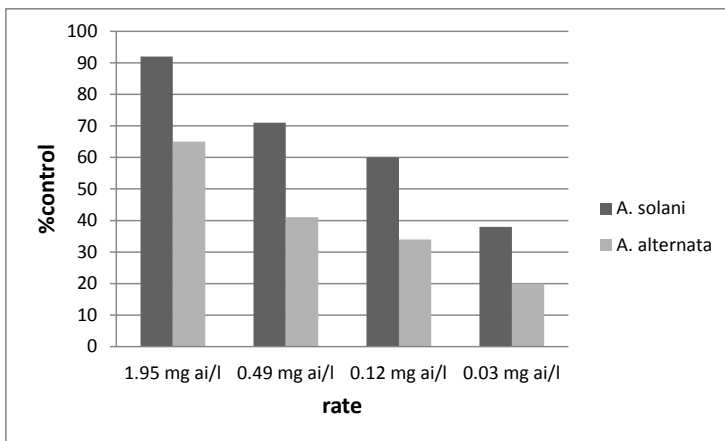


Figure 4. Difenconazole efficacy against *Alternaria solani* and *A. alternata*. (Petri dish tests)

Field tests

Phytophthora infestans

To investigate the efficacy of Revus Top in field potatoes against Late blight (*Phytophthora infestans*) 21 efficacy trials were carried out. These trials were carried out between 2008 and 2010 in Belgium, Czech Republic, Denmark, Germany, France, Ireland, The Netherlands, Sweden, Switzerland, and the United Kingdom. The objective was to confirm the performance of Revus Top at 0.6 l/ha (150 g mandipropamid +150 g difenoconazole /ha) in season long applications.

In all trials Revus Top at 0.6 l/ha was compared with the commercial reference product Revus at 0.6 l/ha. In average Revus Top showed an excellent control of Late Blight relative to the untreated (eff. = 87 %) and was in all cases equal or statistically superior (two cases) to the reference product Revus (average eff. = 84 %).

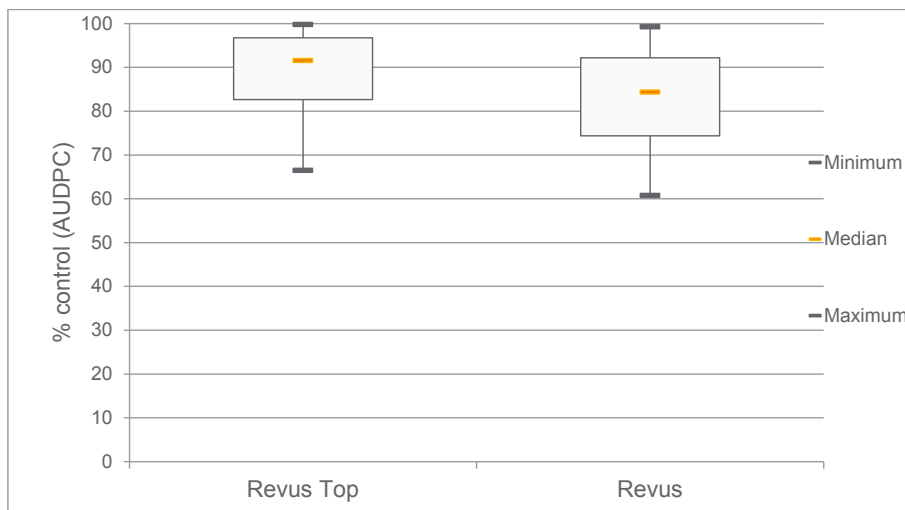


Figure 5. Efficacy of Revus Top and Revus against Late Blight (Box-Whisker, n = 21)

Revus is in the Euroblight ranking systems (updated January 2012) evaluated over the last couple of years as the strongest compound currently registered on the European market for the control of *Phytophthora infestans*. Revus Top is equal to better than Revus showing the strong performance of the formulations on this disease.

The strong performances of Revus Top is also experienced in a trial carried out in 2011 in the Benelux comparing Revus Top with a lot of standard compounds registered on the Dutch market, including Revus.

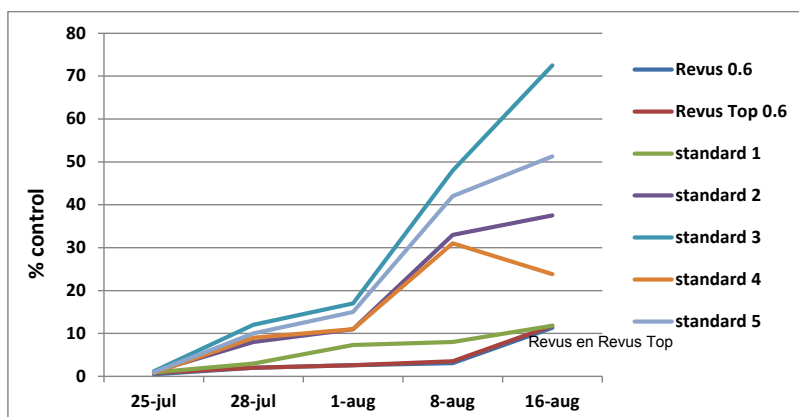


Figure 6. Trial in the Benelux in 2011 with Revus Top for the control of *P. infestans*.

***Alternaria* spp.**

13 Efficacy trials to check the efficacy of Revus Top against Early Blight (*Alternaria solani* and *A. alternata*) on potatoes are carried out between 2008 and 2010 in Austria, Germany, The Netherlands, Sweden and Switzerland. The objective was to confirm the performance of Revus Top at 0.6 l/ha (150 g mandipropamid +150 g difenoconazole /ha) in season long applications.

The results over three seasons demonstrated that the efficacy of Revus Top at the proposed label rate of 0.6 l/ha matches the efficacy of standard compounds. This rate should thus be considered to be effective against Early Blight (*Alternaria solani* and *A. alternata*) on potatoes.

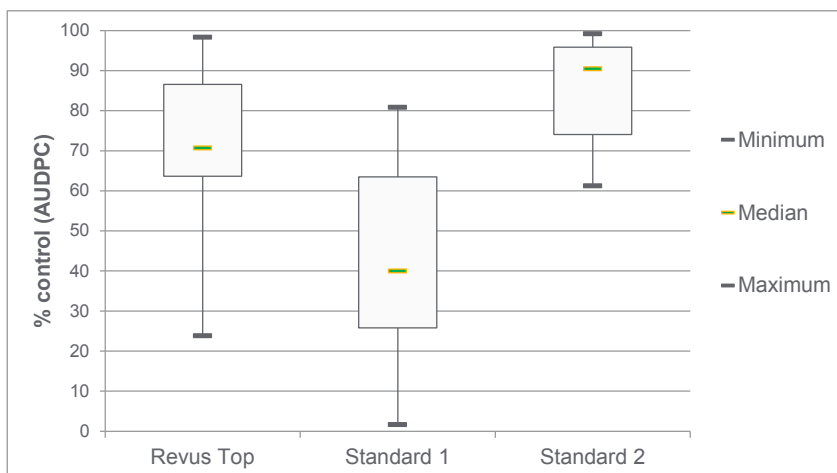


Figure 7. Comparison Revus Top with standard compounds against Early Blight (Box-Whisker, n 9)

Effects on yield and quality and other aspects

In 35 trials in 11 different countries with 19 different varieties as Amado, Bintje, Dali, Elkana, Felsina, Gala, Hermes, Kardal, Karnico, King Edward, Jumbo, Rooster and Secura no observation of phytotoxic effects were observed. No negative impact of Revus Top on yield, grading of the harvested product nor on propagation material were found.

Revus Top is not harmful to honeybees and safe to *Aphidius rhopalosiphi*, *Typhlodromus pyri*, earthworms and soil micro-organisms.

Revus Top has no restrictions on following crops nor on adjacent crops.

The maximal number of three registered applications for Revus Top are regarded as sufficient to minimize selection, no additional anti-resistance strategies are deemed necessary.

Report of the Fungicide Subgroup meeting on 11 & 12 October 2011: Discussion of potato blight fungicides, their properties and ratings

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CHAIRMAN: Huub Schepers

On Tuesday 11 October the following presentations were made to the subgroup.

| | |
|--|--|
| Schirring A, Wannigen & Tafforeau | Infinito - tuber blight control experiences in the period 2006 -2010 |
| Desnoux J, Testers J & Versmissen C | Another step forward in blight control with Ranman Top |
| Kiers E, Erven T, Tegge V | Recommendations and field performance of INITIUM® based products against <i>P. infestans</i> in potatoes |
| Bouwman J <i>et al.</i> | Revus Top – A new product for the control of <i>P. infestans</i> and <i>Alternaria</i> in potatoes in Europe |
| Leiminger J, Adolf B & Hausladen H | Sensitivity of German <i>A. solani</i> isolates against QoI fungicides |

On Wednesday 12 October 34 delegates attended the discussion session of the Fungicide Subgroup meeting. The following areas were discussed.

- 1.1 Inclusion of tank mixes in the fungicide efficacy tables
- 1.2 Tuber blight efficacy ratings calculated from trial results
- 1.3 Contribution by EuroBlight experts to Country Specific Guidelines for Integrated Potato Protection in Europe
- 1.4 CropLife Foundation
- 1.5 Other

1.LATE BLIGHT

DISCUSSION AND AGREEMENTS REACHED

1.1 Inclusion of tank mixes in the fungicide efficacy tables

At the meeting of the Fungicide Subgroup in Arras in 2010 it was agreed that fungicide ratings based on trial results would be updated in advance of EuroBlight workshops except where there was a serious problem that required to be resolved by the Fungicide Subgroup. In this event the problem would be discussed and resolved at the next meeting of the Fungicide Subgroup. Such a situation arose with the possible inclusion of tank mixes in the efficacy tables, specifically with the test case of the Nufarm fungicide Canvas (Shinkon in the UK)(amisulbrom).

A rating for 0.5 l/ha Canvas + 2.0 kg/ha mancozeb product was included in the draft report of the EuroBlight leaf blight trials 2010. This was criticised mainly because the tank mix does not match the dose rates recommended in practice by Nufarm in Germany and the UK. In these countries the recommendation is 0.3 l/ha + 1.75 kg/ha. Canvas is registered at 0.5 l/ha in the UK and Germany without mention of a tank mix partner on the label. However, it was stated at the meeting that Canvas would soon be registered in Benelux at 0.5 l/ha and tank mixing with a partner fungicide will be mentioned on label.

The following points were discussed and decisions reached.

1.1.1 Should a rating for a tank mix be included in the EuroBlight table or should ratings only be given to straight products and formulated mixtures?

It was agreed that tank mixes could be rated and included. However, certain conditions have to be met.

To be included in the EuroBlight table the tank mix has to be registered in at least one country in Europe, i.e. the tank mix is included on the product label. (AGREED)

The product label has to mention the specific tank mix partner. (AGREED) The label for Canvas only refers to a tank mix partner in general terms but Nufarm will only recommend mancozeb as the tank mix partner.

Tank mixes are to be included in the B Table (provisional) because of the lack of information on efficacy in commercial practice. (AGREED)

Tank mixes should be tested and rated only if there is no corresponding formulated product. (NOT AGREED)

The formulation of the tank mix partner needs to be specified on the product label. (NOT AGREED)

1.1.2 Should a rating be included in the table for lower rates of the tank mix partners than those tested?

The highest label rates of tank mix partners should be tested because this is consistent with EuroBlight testing the highest label rates of formulated mixtures.(AGREED) Also, biological dossier data, in which the tank mix was tested at the highest rates, will back up data from the six EuroBlight trials. The tank mix of 0.5 l/ha Canvas + 2.0 kg/ha mancozeb product was tested in registration trials.

Tables should have dose rates included. (AGREED) This will clearly distinguish tank mixes from formulated products.

1.1.3 Should ratings for a straight product and/or a ready formulated product be included if there is a rating for the tank mix?

The inclusion in the EuroBlight table of a rating for straight Canvas was discussed. Nufarm does not support this because the company does not recommend straight Canvas anymore.

The decision to test one partner of the tank mix alone, in this case amisulbrom, and/or the ready formulated product, in this case amisulbrom plus mancozeb, to generate EuroBlight ratings is a decision for the fungicide company alone. (AGREED)

1.2 Tuber blight efficacy ratings calculated from trial results

The first combined report will include results from all 3 years of testing, 2009 to 2011, because of low incidences of tuber blight in some trials. The trials-based ratings will be released before the next EuroBlight meeting to be held in spring 2013.

It was agreed that all data from the dedicated trials are to be submitted to Bert Evenhuis by 28th February 2012 to allow earlier circulation of the combined report.

1.3 Contribution by EuroBlight experts to Country Specific Guidelines for Integrated Potato Protection in Europe

Huub Schepers proposed that volunteer EuroBlight experts should contribute to harmonised Country Specific Guidelines for Integrated Potato Protection in Europe. National experts were requested to make EuroBlight information and expertise available to National Action Plan committees. Harmonisation was considered essential to ensure that Europe was co-ordinated.

Europe-wide harmonisation of *P. infestans* population monitoring was discussed. The discussion covered testing at a few centres only, genotyping, fungicide insensitivity testing, phenotyping and the inclusion of samples from the fungicide companies.

1.4 Crop Life Foundation

It was agreed that there should be a link to the website of CropLife Foundation.

1.5 Other

The table with provisional ratings for late blight fungicides (Table B) and the table with efficacy ratings for early blight (*Alternaria*) control need to be more easily accessible on the EuroBlight website.

BASF proposed harmonised varietal resistance testing, with a table to complement the fungicide efficacy tables.

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

The ratings given in Table A are for late blight fungicides currently registered in several EU countries and are based on the label recommendations for commercially available products containing one or two active ingredients as a co-formulated mixture. The ratings are NOT for the active ingredients themselves. Table 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 the one for leaf blight, were endorsed by the Fungicides Subgroup at the St Petersburg workshop in 2011 and are based on field experiments and experience of the performance of products when used in commercial conditions. Ratings for leaf blight were calculated from the results of 19 EuroBlight field trials during 2006-2011, and only compounds included in a minimum of six of these trials are rated for leaf blight. The scale for leaf blight is a 2-5 scale, to one decimal place. All other ratings are on a 0 to +++ scale, using (+) to indicate half marks. The ratings are intended as a guide only and will be amended in future if new information becomes available. Tables A and B are available on the EuroBlight website, www.euroblight.net/Fungicide/FungicideComparison.asp These tables on the website 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 efficacy only) or are the consensus view of the Fungicide Subgroup and are based on information from field experiments or minimal practical experience of a product and will be amended at future workshops, as new information becomes available and the body of experience in commercial use increases.

DEFINITIONS AND DISCLAIMER (REPRODUCED FROM THE TALLINN 2005 PROCEEDINGS)

PHENYLAMIDE RESISTANCE

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

NEW GROWTH

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

PROTECTANT ACTIVITY

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

CURATIVE ACTIVITY

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

ANTISPORULANT ACTIVITY

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

STEM BLIGHT CONTROL

Effective for the control of stem infection either by direct contact or via systemic activity.

TUBER BLIGHT CONTROL

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

DISCLAIMER

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

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

2. EARLY BLIGHT (*Alternaria solani* and *Alternaria alternata*)

At present there is only an A table for early blight fungicide efficacy because there are currently no products in the provisional category. It was stated at the St Petersburg meeting that there are two new candidates that could be considered for the B table, i.e. Revus Top (mandipropamid + difenoconazole) and also a new coded product from Gowan.

It was confirmed again at the meeting in St Petersburg that in the *Alternaria* table one column to cover the efficacy of fungicides against both *A. solani* and *A. alternata* was currently still appropriate because of insufficient information on fungicide activity against the individual species.

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

| Product [Dose rate (l or kg/ha)] ¹ | Effectiveness | | | Mode of Action | | | Rainfastness | Mobility in the plant |
|---|--------------------------|------------|--------------------|----------------|------------|----------|--------------------|-------------------------|
| | Leaf Blight ² | New growth | Stem blight | Tuber blight | Protectant | Curative | | |
| copper | ? | ? | + | + | ++(+) | 0 | 0 | contact |
| dithiocarbamates (2.0) ³ | 2.0 | ? | + | 0 | ++ | 0 | 0 | contact |
| chlorothalonil | | ? | (+) | 0 | ++ | 0 | 0 | contact |
| cyazoflamid (0.2 + 0.15) | 3.8 | ++ | + | +++ | +++ | 0 | 0 | contact |
| fluzinam (0.4) | 2.9 | ? | + | ++(+) | +++ | 0 | 0 | contact |
| zoxamide+mancozeb (1.8) | 2.8 | ? | +5 | ++ | +++ | 0 | 0 | contact+translaminar |
| famoxadone+cymoxanil | | ? | ++(+) | N/A | ++ | ++ | + | translaminar+contact |
| mandipropamid (0.6) | 4.0 | ++ | ++(+) | ++5 | +++ | +6 | ++(+) | translaminar+contact |
| benthiavalicarb+mancozeb (2.0) | 3.7 | ? | ++(+) ⁵ | ++(+) | +++ | ++(+) | + | translaminar+contact |
| cymoxanil+mancozeb | | ? | ++(+) | 0 | ++ | ++ | + | translaminar+contact |
| cymoxanil+metiram | | ? | ++(+) | 0 | ++ | ++ | + | translaminar+contact |
| cymoxanil+copper | | ? | ++(+) | 0 | ++ | ++ | + | translaminar+contact |
| dimethomorph+mancozeb (2.0) | 3.0 | ? | ++(+) | ++ | +++ | + | ++ | translaminar+contact |
| fenamidone+mancozeb (1.5) | 2.6 | ? | ++(+) ⁵ | ++ | +++ | 0 | ++(+) ⁵ | translaminar+contact |
| benalaxyl+mancozeb ⁴ | | ++ | ++ | N/A | +++ | ++(+) | +++ | systemic+contact |
| metalaxyl-M+mancozeb ⁴ | | ++ | ++ | N/A | +++ | ++(+) | +++ | systemic+contact |
| metalaxyl-M+fluzinam ⁴ | | ++ | ++ | N/A | +++ | ++(+) | +++ | systemic+contact |
| propamocarb-HCl+mancozeb | | ++(+) | ++ | ++ | +++ | ++ | +++ | systemic+contact |
| propamocarb-HCl+chlorothalonil (2.7) | 3.4 | ++(+) | ++ | ++ | +++ | ++ | +++ | systemic+translaminar |
| propamocarb-HCl+fenamidone (2.0) | 2.5 | ++(+) | ++ | ++ | +++ | ++ | +++ | systemic + translaminar |
| propamocarb-HCl+flupyridifluro (1.6) | 3.8 | ++ | ++ | +++ | +++ | ++ | ++(+) | systemic + translaminar |

¹The scores of individual products are based on the label recommendation and are not additive for mixtures of active ingredients. Inclusion of a product in the list is not indicative of its registration status either in the EU or elsewhere in Europe. ²Based on European field trials in 2006-2011. ³ Includes maneb, mancozeb, propineb and metiram. ⁴ See text for comments on phenylamide resistance. ⁵ Based on limited data. ⁶ In some trials there were indications that the rating was ++(+).

Key to ratings: 0 = no effect; + = reasonable effect; ++ = good effect; +++ = very good effect; N/A = not recommended for control of tuber blight; ? = no experience in trials and/or field conditions. The scale for leaf blight is a 2-5 scale (2 = least effective, 5 = most effective).

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

Late Blight Table B. Provisional ratings¹ for the effectiveness of new fungicide products for the control of *P. infestans* in Europe. These ratings are the opinion of the Fungicides Sub-Group at the St Petersburg blight workshop, 2011 and are based on field experiments and not experience in commercial potato production.

| Product [Dose rate (l or kg/ha)] | Effectiveness | | | Mode of Action | | | Rainfastness | Mobility in the plant contact + contact | | |
|-----------------------------------|--------------------------|------------|-------------|--------------------|------------|--------------------|--------------|--|----------------|------------------------|
| | Leaf blight ³ | New growth | Stem blight | Tuber blight | Protectant | Curative | | | Anti-sporulant | |
| amisulbrom + mancozeb (0.5 + 2.0) | 4.5 | ? | + | ++(+) ² | ++(+) | 0 | 0 | ? | +++ | contact + contact |
| irinium + mancozeb (2.5) | 3.6 | ? | ? | ? | ++ | 0 | 0 | ? | +++ | contact + contact |
| propamocarb + cyproconazole (2.0) | ? | ? | ? | ? | + | ++(+) ³ | ++(+) | ? | ? | systemic + transaminar |

¹ The ratings for individual products are based on the label recommendation and are NOT additive for mixtures of active ingredients. Inclusion of a product is NOT indicative of its registration status either in the EU or elsewhere in Europe. ² Based on limited data, an efficacy greater than ++(+)³ was observed in some trials. ³ Calculated from Euroblight trials. ⁴ Observations from some field trials indicated that both new growth and stem blight efficacy were ++. ⁵ In some trials the curative activity was +++.

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

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 ¹ |
|-------------------------------|-----------------------|
| azoxystrobin | +++ |
| fluazinam | (+) |
| metiram/mancozeb ² | ++ |
| propineb | ++ |
| chlorothalonil | +(+) |
| famoxadone+cymoxanil | ++ |
| fenamidone+mancozeb | ++ |
| or propamocarb ³ | |
| zoxamide+mancozeb | ++(+) |
| pyraclostrobin + boscalid | +++ |

¹ **Key to ratings :** 0 = no effect ; + = some effect; ++ =reasonable effect ; +++ = good effect ; ++++ very good effect

² This rating applies to products containing mancozeb when used at the highest dose rates (>1500g/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*. ³ In some trials there were indications that the rating was ++(+).

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

Development of foliar late blight (*Phytophthora infestans*) in relation to cultivar resistance and fungicide dose on potato

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SUMMARY

In Britain the shift in the late blight population towards more aggressive and virulent *Phytophthora infestans* genotypes, including 13_A2 and 6_A1, is well documented. Genotype 13_A2 is now used to screen varieties for resistance to late blight and, consequently, disease pressure is considerably greater than before as the protocol remains the same. There is an optimum inoculum density range below or above which discrimination between varieties is diminished, therefore, there is an argument for managing the inoculum density in trials testing cultivar resistance to 13_A2. Of the many ways in which inoculum density could be managed, e.g. isolation of the cultivar screen from other trials that are a potent source of inoculum or a reduced ratio of susceptible infectors to test cultivars or larger plots of test cultivars, this paper examined the impact of a fungicide programme.

Experiments consistently showed that cultivar differences, between AUDPCs, were greater for fungicide treated plots compared with untreated plots. In the integrated control trials carried out in 2009, 2010 and 2011 at 2 sites, the difference between the more resistant Cara and the susceptible King Edward decreased progressively with decreasing fungicide dose. In 2010, two experiments with 19 cultivars showed discrimination between cultivars to be improved where a full- or half-rate fungicide programme was applied to the plots and the resistance ranking orders obtained for untreated and fungicide-treated plots were not significantly different. Additional experiments are required to confirm these findings.

The reduced resistance of some cultivars, associated with the change in *P. infestans* population, is a setback to implementing integrated control, but there remain substantial differences between cultivars and these are large enough to be exploited.

KEYWORDS

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

INTRODUCTION

There is increasing pressure from EU legislation for member states to promote lower pesticide inputs and incorporate non-chemical approaches into crop disease management practices including for the control of late blight (*Phytophthora infestans*) on potato. Cultivar resistance offers the potential to reduce fungicide inputs, whilst still achieving adequate disease control. Reduced fungicide inputs have been shown in previous studies to successfully reduce foliar blight severity when used on potato cultivars with good foliar blight resistance (Fry, 1978; Nielsen 2004; Kirk *et al.* 2001 & 2005; Kessel *et al.* 2006; Naerstad *et al.* 2007). In GB, there has been a shift in the late blight population towards more aggressive and virulent *P. infestans* genotypes including 13_A2 and 6_A1. As a result, the resistance ratings of several cultivars have been downgraded, for example Lady Balfour, a cultivar with a resistance rating of 7 and originally developed for the organic market, was downgraded to a resistance rating of 4 (BVDB, 2012). A key part of integrated control based on cultivar resistance is sufficiently large differences in foliar resistance between varieties. Previous trials in GB have shown that cultivar differences tended to be smaller in untreated compared with fungicide-treated plots, suggesting that cultivar resistance in conjunction with reduced fungicide inputs could give greater separation of cultivars (Bain *et al.*, 2008). At present, 99% of the potato cultivars grown in GB have a cultivar resistance rating of 5 or below.

This work was carried out as part of a government and industry funded Sustainable Arable LINK project which aims to deliver robust information to the GB industry on the use of integrated late blight control. One of the objectives was to test whether the downgrading of cultivar resistance ratings will affect the use of cultivar resistance for integrated control and whether the use of fungicides improves discrimination between cultivars in high disease pressure situations.

MATERIALS AND METHODS

Integrated control trials

In 2009, 2010 and 2011 at SAC, Auchincruive Estate, Ayrshire, Scotland and Cilcennin, near Aberystwyth, Ceredigion, Wales, six trials were conducted to determine the effectiveness of 24 integrated control treatments incorporating fungicide dose and cultivar resistance to control foliar late blight during the stable canopy phase. The trials were laid out in a randomised split plot design with 4 replicates. Each sub-plot 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. All plots were over sprayed with propamocarb-HCL + chlorothalonil (as Merlin; 2.5 L/ha) during rapid canopy growth at 7 or 10 day intervals depending on early season risk as soon as plants started to meet within the rows. Three fungicides were tested (Infinito, Revus and Shirlan) at 7-day intervals and also 10-day intervals during stable canopy at 0, 25, 50, 75 and 100% of recommended dose on each of 4 application dates to cvs King Edward (foliar resistance rating 3) and Cara (5). Dithane NT (1.7 Kg/ha) or an alternative mancozeb product at an equivalent rate was applied for the remainder of the season once test treatment applications were completed.

Discrimination between 19 cultivars

In 2010, a separate experiment was included at both the above sites, with three fungicide treatments applied to 19 cultivars with cultivar resistance ratings from 2 to 8 (Table 1). The trial was laid out in a randomised split plot design with three replicates. Three treatments: two fungicide programmes, Shirlan (0.4 or 0.2 L/ha) plus an untreated control were included and applied as main plot treatments, with the cultivars included as sub-plots (Table 2). Plots at both sites 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 single rows of King Edward acted as spreader rows and were left untreated at Cilcennin but were fungicide treated at Auchincruive.

Table 1. The 19 cultivars included in the cultivar x fungicide dose trial and their foliar resistance ratings in 2010 and 2011

| Resistance rating | Varietal resistance ratings in 2010 | Varietal resistance ratings in 2011 |
|-------------------|-------------------------------------|---|
| 2 | Harmony | |
| 3 | Hermes, King Edward, Russet Burbank | Hermes, King Edward, Russet Burbank |
| 4 | Maris Piper, Rooster, Saturna | Cosmos, Lady Balfour, Maris Piper, Orla, Pentland Dell, Rooster, Sante, Saturna |
| 5 | Claret, Navan, Valor | Cara, Claret, Druid, Harmony, Navan, Slaney, Valor, |
| 6 | Cosmos, Pentland Dell, Axona | Axona |
| 7 | Cara, Sante, Slaney | |
| 8 | Druid, Lady Balfour, Orla | |

Table 2. Fungicides, rates and intervals applied to all 19 cultivars in the cultivar x fungicide dose trial

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

The sites were inoculated on 12, 12 and 3 July (Cilcennin) and 7, 12 and 8 July (Auchincruive) in 2009, 2010 and 2011 respectively using an appropriate *P. infestans* isolate of 13_A2, representative of the GB population. 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.

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 were used to calculate the Area under the Disease Progress Curve (AUDPC) and converted to the relative AUDPC (rAUDPC) prior to an analysis of variance (ANOVA) where appropriate. To test whether there was an interaction between fungicide application and cultivar resistance rating, the rAUDPCs from both sites were subjected to an over trials ANOVA following log transformation.

RESULTS AND DISCUSSION

Integrated control trials

The 13_A2 genotype of *P. infestans* dominated at both sites, with the exception of Cilcennin in 2011 where only 6_A1 was identified in the trial area. To determine whether there were differences in foliar blight development between fungicide treated and untreated plots, the AUDPCs for the different fungicide products were averaged to give a single figure for fungicide dose at each site/year. The AUDPCs were then used to compare whether these were similar for Cara (5) and King Edward (3) when untreated and following fungicide treatment. This was done by expressing the AUDPC for Cara as a percentage of that of King Edward for each fungicide dose (Fig. 1). At Cilcennin in 2009, 2010 and 2011 disease progress on untreated King Edward and Cara was similar, with the AUDPC for Cara between 77.3% and 90% that of King Edward across the 3 years. There was greater distinction between the two cultivars when left untreated at Auchincruive over the 3 years, however, this was still high with the AUDPC for Cara between 44.1% and 64.8% of King Edward. Following fungicide application and regardless of dose, the AUDPC of the more resistant cultivar Cara was proportionally much lower than on the more susceptible King Edward than the two varieties left untreated. For example, where full rate fungicides had been applied, the AUDPC for

Cara was between 27.6% and 56.6% of the AUDPC for King Edward at Cilcennin. This was more pronounced at Auchincruive, where the AUDPC for Cara was between 8.4% and 40.7% of the more susceptible variety. In all six trials, the AUDPC for Cara as a percentage of K Edward decreased progressively with increasing fungicide dose (Fig. 1).

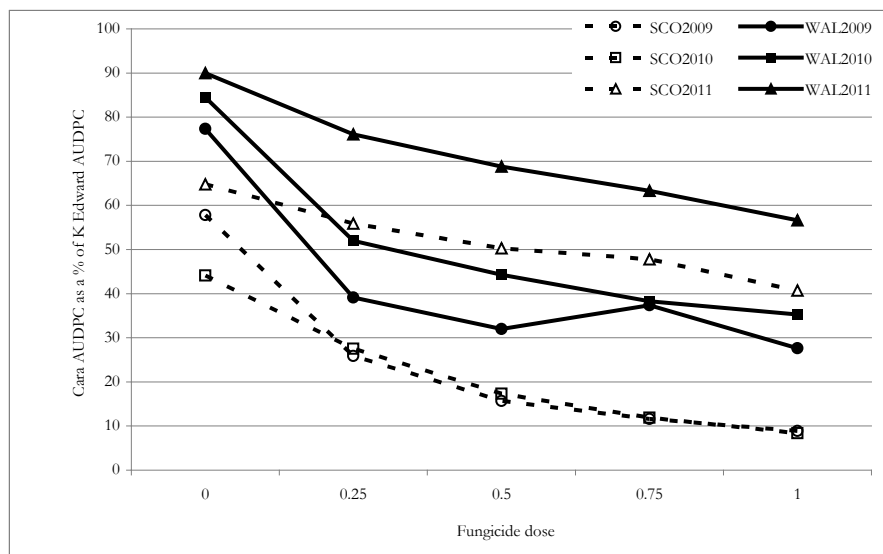


Figure 1. Change in AUDPC of the more resistant Cara expressed as a percentage of AUDPC of the susceptible King Edward with increasing fungicide dose in six field experiments at Cilcennin (WAL) and Auchincruive (SCO). Fungicide dose is the proportion of the recommended UK label rate, applied four times

Discrimination between 19 cultivars

A similar effect of fungicide treatment was also seen in the 2010 trials with 19 varieties at Cilcennin and Auchincruive (Fig. 2). The Mean Squares and F-statistics, following log transformation of rAUDPCs, from the over-trial ANOVAs are shown in Table 3. Varietal discrimination (F-statistic for variety) was significant for the half- and full-rate treatments only. However, further experiments are required to confirm this finding. It is clear that the variety mean squares (a measure of between variety variability) were much larger for the half- and full-rate treatments. This is not reflected in the F-statistics; the extent of this difference is reduced because the residual is smaller for untreated varieties and may well represent greater consistency. At both sites the disease pressure was very high and this should be taken into consideration when interpreting the results.

Table 3. ANOVAs for each level of fungicide (log transform of rAUDPC)

| Source | df | No fungicide | | Half-rate | | Full-rate | |
|----------|----|--------------|--------|-----------|--------|-----------|--------|
| | | MS | F-stat | MS | F-stat | MS | F-stat |
| Trial | 1 | 0.964 | 50.2 | 0.003 | 0.1 | 0.000 | 0.0 |
| Variety | 18 | 0.081 | 4.2 | 0.371 | 8.1* | 0.596 | 6.7* |
| Residual | 18 | 0.019 | | 0.046 | | 0.089 | |

*significant at the 5% level

There is no evidence of a treatment-by-variety interaction over the two trials (F-stat 0.41), implying that the broad ranking of varieties is similar regardless of whether left untreated or fungicide treated.

Genotypes of the new population of *P. infestans* in GB, such as 13_A2, are both more virulent (Lees *et al.*, 2011) and more aggressive. When 13_A2 is used in cultivar resistance screening trials the decline in resistance of cultivars is a combination of resistance genes being overcome and the greater aggressiveness of new genotypes compressing differences between cultivars. It is not straightforward to quantify the relative contribution of these two effects to a general decline in cultivar resistance.

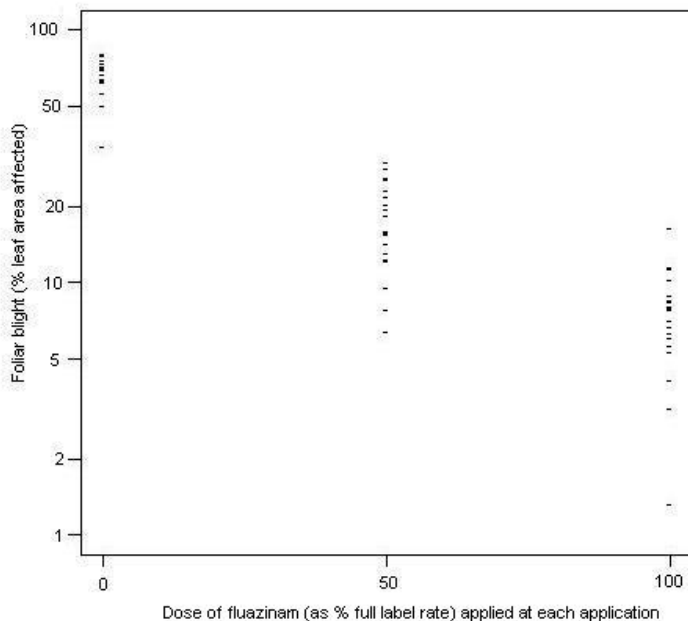


Figure 2. Variation in foliar blight between 19 cultivars left untreated (0), or treated with 50% or 100% of the recommended rate of fluazinam (as Shirlan) in 2010

Results from any trial evaluating cultivar resistance to 13_A2 clearly need to provide an accurate assessment of the current relative resistances of different cultivars. However, data presented in this paper suggest that the contribution of more resistant cultivars to disease control is consistently relatively lower where plants are unprotected. As a consequence of the EU Sustainable Use Directive 2009/128/EC there is increased interest in exploiting cultivar resistance in integrated control. Results presented here suggest that the contribution of cultivar resistance in commercial potato growing, in which a very high percentage of the national crop is protected by fungicide, may be underestimated by resistance ratings obtained in screening trials using the more aggressive genotypes without managing (in most cases reducing) inoculum density. Currently, plots in cultivar screening trials are untreated. Further studies are required to allow resistance ratings obtained from trials using genotypes from the new population to accurately inform the true contribution of foliar resistance for control of *P. infestans* in commercial crops.

It is reassuring that the resistance ranking orders obtained for untreated and fungicide-treated plots were not significantly different. However, additional experiments are again required to confirm this result. Although reduced resistance in some cultivars, associated with the change in *P. infestans* population, is a setback to implementing integrated control, there remain substantial differences between cultivars and these can be exploited.

ACKNOWLEDGEMENTS

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Characterization of *Phytophthora infestans* population in Chile

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SUMMARY

Today, Late blight is the most important disease in potato in Chile, being an epiphythotic disease since 2005, showing high incidence and severity, causing more than 50% of yield reduction, depending of the season weather conditions. Studies performed since 2003 to 2011 on *P. infestans* population in southern Chile revealed genetic changes, although still it is an A1 mating type. But until the season 2004-05 the population was susceptible to metalaxil (<3 ppm EC50), today, the population is highly resistant to metalaxil (> 100ppm EC50), shows pathotypes highly complex and has different SSR patterns than the previous population. Moreover, the mitochondrial DNA analyses showed different haplotypes, while the first collection was Ib, the current is Ia.

KEYWORDS

Late blight, *Phytophthora infestans*, characterization of pathogen population

INTRODUCTION

Phytophthora infestans (Mont.) De Bary, the causal agent of late blight, like the potato, its host, has been able to adapt to different climates and latitudes (Garlick *et al.*, 2002). New biotypes have arisen in the last decades making the control more difficult (Fry and Goodwin, 1997).

P. infestans is heterotalic, with two mating groups A1 and A2. The group A1 was predominant worldwide. The group A2 was reported only in Mexico until late 80's. At the end of the 20th century *P. infestans* migrated from Mexico, thus increasing the genetic diversity of populations of this pathogen in most of the continents. The first new genotypes were detected in Europe, then in South America, Africa and Asia and finally USA and Canada. This group has becoming predominant and more aggressive (Stevenson *et al.*, 2001). In South America the group A2 has being described in Argentina, Bolivia, Brazil, Ecuador, and Uruguay (Adler *et al.*, 2002; Crissman and Lizárraga, 1999).

The first reports of disease caused by *P. infestans* in Chile are from the 50's and it is thought that it came from Argentina (Anónimo, 1951). This had a great impact on the potato crop where most varieties being cultivated in that period almost disappeared, what actually happened to the red

Corahila potato variety. Since then few studies have been done to characterize the late blight population. All the isolates described in a study done by INIA and NDSU in the northern Chile were found to be the group A1/US1 of several pathotypes of low aggressiveness. The isolates were highly resistant to Mefenoxam (> 300 ppm), because of the continuous use of Ridomil (Secor, 2003). Previously, Fernandez (1979) studied *P. infestans* virulence in southern Chile populations, describing complex pathotypes able to infect even five plant differentials.

Today, Late blight is the most important disease in potato in Chile, being an epiphytotic disease, showing high incidence and severity, causing more than 50% of yield reduction, depending of the season weather conditions. Since 2003, The Agricultural Research Institute of Chile (INIA), associated with public and private institutions, are performing studies to Implement an Integrated pest management for late blight based on a forecasting system (Acuña, *et al.*, 2009). One of the main objective of this project is to characterize and monitor the *Phytophthora infestans* population associated to the potato crop in southern Chile

MATERIALS AND METHODS

P. infestans monitoring has been performed through the seasons 2003-04 until 2010-11 in the potato production area of southern Chile determining mating types, avirulence genes, metalaxil resistance, DNA polymorphism (SSR) and mitochondrial haplotypes.

Collection of 250 *P. infestans* isolates were performed during years 2003 to 2005 and 259 during 2006 to 2011, from lesions on potato plants and tubers, at southern Chile from Araucania region (parallel 39° S) and Chiloe island (parallel 43°S).

A piece of leaflet or tuber with symptoms was put under a potato slices of Yagana cultivar. Then, the samples were incubated at 18°C for 7 days. After that, four pieces of infected tissue were transferred to a Petri plate containing rye B agar, amended with antibiotics (Ampicillin) (Forbes, 1997). The isolates were incubated during 4 to 7 days at 18°C in darkness. *P. infestans* isolates were transferred to Rye B media and maintained at 18°C in darkness.

Because A2 mating type has not been described in Chile, the isolates were evaluated for mating type at the Agricultural National Service (SAG) in Santiago, Chile. The test was conducted by placing an agar plug containing mycelium on the edge of two rye B agar plate pairing with a similar size agar plug of known A1 and A2, one for each plate. After 15 days at 15°C, the plates were examined for oospore production (Tooley *et al.*, 1989, Miller *et al.*, 1998 and Dorrance *et al.*, 1999).

In vitro metalaxyl sensitivity was assessed by comparing *P. infestans* radial growth on Rye B media amended with 5 different concentrations of metalaxil to a growth on metalaxil free control (Deahl, 1993). The test for each isolate and metalaxil concentration (0, 0.1, 1.0, 10, 100 ug/ml) was performed with 5 mm plug of a 10 days old growing colony placed in the center of a 9 cm Petri dish with the corresponding amended media. After 10 days of incubation at 18°C on darkness, two perpendicular measurements were taken for each plate. The percentage of relative growth on amended media versus control was scored and the EC50 was calculated as described by Miller *et al.* (1998).

The virulence assay was carried out by inoculation of detached leaflets of a differential set of plants with the 11 known major R genes for resistance. Craigs Royal cultivar was used for Race R0. Differentials were obtained from NDSU, originally from the Scottish Crop Research Institute, Scotland. Race determination was based on compatible host-pathogen reaction seven days after inoculation with a 2×10^4 zoospores/ml (Miller *et al.*, 1998). Lesions were read on a scale 0=no symptoms, 1= hypersensitive reaction, 2= necrosis no sporulating, 3= necrosis and sporulating lesion. A DNA polymorphism among isolates was established using the SSR Pi02, Pi04, Pi16, Pi26, Pi33, Pi56, Pi66, and Pi70. Primer sequences and PCR protocol for SSR were facilitated for Dr. David Cooke from the SCRI. Amplified PCR products were separated in standard DNA sequencing PAGE and silver stained method was used to visualize the DNA fragments.

RESULTS AND DISCUSSION

Results sent by the SAG laboratory described all the isolates as A1 mating type for all the evaluated crop seasons.

However, the *P. infestans* population until the season 2004-05 was susceptible to metalaxil with an EC50 less than 3ppm, highly complex pathotypes, but mainly with 2, 4 and 5 avirulence genes. Moreover, the most frequent genes were 10 and 11, present in 93% of the population (Acuña *et al.*, 2007). However, the 2006 to 2011 collection was metalaxil resistance with an EC50 over than 100 ppm and a relative growth on Rye B media amended with 10 ppm of metalaxil over a 60% (Figure 1). It shows complex pathotypes with mainly 7, 8 and 9 avirulence genes and with high frequency of R1, R3, R5, R7, R8, R10 and R11 (Figure 2 and Table 1).

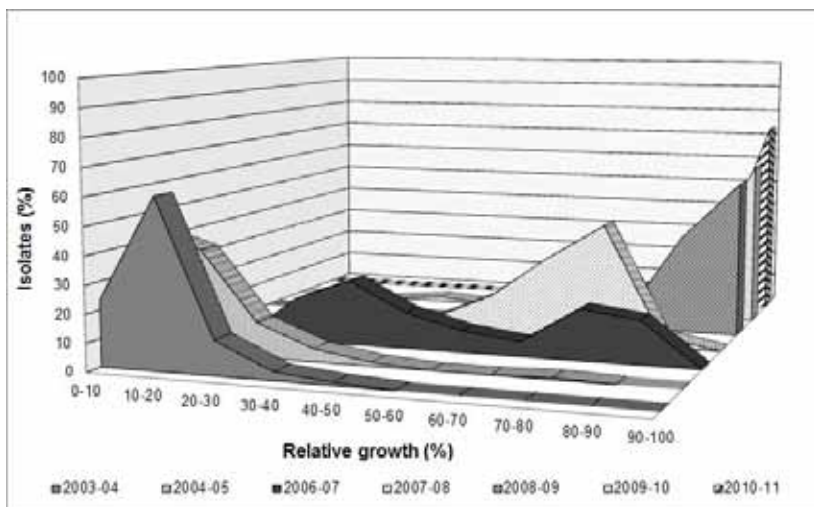


Figure 1. Relative growth of *P. infestans* isolates collected since potato growing season 2003 to 2011, cultured on Rye B media compared to isolates grown on Rye B media amended with 10 ppm of metalaxil.

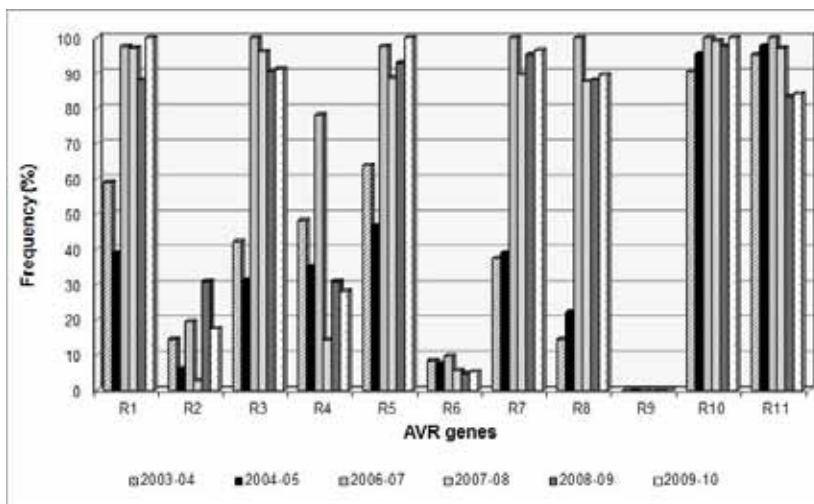


Figure 2. Avr gene frequency in the *P. infestans* population since 2003 to 2011 from the southern Chile.

Table 1. Pathotype complexity of *P. infestans* population for the season 2003 to 2011 at southern Chile.

| AVR genes per isolates | Isolates per season (%) | | | | | |
|------------------------|-------------------------|---------|---------|---------|---------|---------|
| | 2003-04 | 2004-05 | 2006-07 | 2007-08 | 2008-09 | 2009-10 |
| 1 | 3,6 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| 2 | 14,5 | 22,9 | 0,0 | 0,0 | 2,4 | 0,0 |
| 3 | 8,4 | 13,0 | 0,0 | 3,8 | 2,4 | 0,0 |
| 4 | 16,9 | 20,6 | 0,0 | 1,9 | 2,4 | 0,0 |
| 5 | 25,3 | 16,0 | 0,0 | 2,9 | 2,4 | 1,8 |
| 6 | 13,3 | 18,3 | 2,4 | 13,3 | 9,5 | 14,0 |
| 7 | 4,8 | 6,1 | 22,0 | 62,9 | 42,9 | 52,6 |
| 8 | 10,8 | 1,5 | 51,2 | 13,3 | 31,0 | 29,8 |
| 9 | 2,4 | 1,5 | 19,5 | 1,0 | 7,1 | 1,8 |
| 10 | 0,0 | 0,0 | 4,9 | 1,0 | 0,0 | 0,0 |
| 11 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |

Additionally, this last population shows different SSR pattern than the previous population. According SSR pattern, 5 genotypes were distinguished during the collecting seasons 2003 to 2005, but one of them showed 70% of the frequency (Acuña *et al.*, 2010). Moreover, at the collection 2006 to 2011, two new genotypes were present, one of them with more than 90% of the frequency (Figure 3). This last genotype was described for the first time during the season 2005/06, where only one isolated was found. Today it is the more predominant. Among the genotypes most polymorphisms were detected at Pi02 and Pi16 loci.

Additionally, the mitochondrial DNA analysis revealed a new haplotypes since 2006, then the previous population was Ib and the new one is Ia (Figure 4) (Acuña *et al.*, 2011).

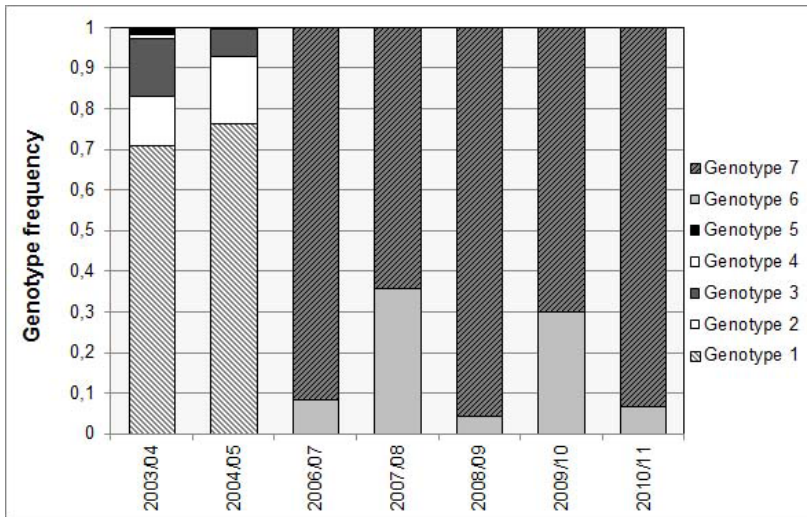


Figure 3. Genotype frequency of *P. infestans* population in southern Chile using SSR analysis.

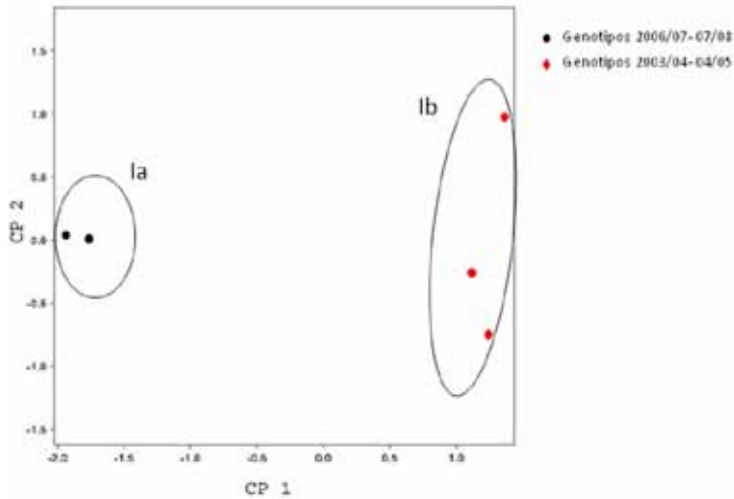


Figure 4. Mitochondrial DNA analysis for *P. infestans* isolates from the season 2003 to 2008 at southern Chile.

CONCLUSIONS

The epidemiology of late blight on potato in Chile has changed since 2005, being an epiphythotic disease when the weather conditions are favorable for the disease. The main possible reason is that the *P. infestans* population is showing genetic changes, although still it is an A1 mating type. But until the season 2004-05 the population was susceptible to metalaxil (<3 ppm EC50), today, the population is highly resistant to metalaxil (> 100ppm EC50), shows pathotypes highly complex and has different SSR patterns than the previous population. Moreover, the mitochondrial DNA analyses showed different haplotypes, while the first collection (2003-05) was Ib, the current is Ia (2006-08)

These results showed a change in population characteristic, due to, probably, an introduction of a new population or a selective pressure, because of the management or weather conditions, on a previous population in low frequency, associated to cultivated or wild *Solanums*. Future steps in this research will include *P. infestans* isolates associated to other *Solanum* species in Chile, both cultivated and wild, and considering other worldwide databases information.

ACKNOWLEDGEMENTS

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Molecular Identification of the Species Composition of Russian Isolates of Pathogens, Causing Early Blight of Potato and Tomato

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SUMMARY

A comparative study of the genome structure has been carried out for seven large-spore and 25 small-spore strains, isolated in 2007-2010 from infected tomato and potato plants, growing in seven distant regions of the European part of Russia and Russian Far East. To make a comparison, a sequence of ribosomal genes and intergenic regions of the nuclear rDNA has been selected. The tested strains have been divided into three groups. The first one includes isolates, which were morphologically determined as *A. alternata*, *A. tenuissima*, and *A. arborescens*. These isolates do not have any differences in the structure of the studied DNA region. The second group includes large-spore isolates, classified as *A. solani*. Some strains of this group differ from others in small single-nucleotide substitutions. No any differences between large-spore strains, isolated from potato and tomato, have been observed. The third group includes the only *A. infectoria* isolate, collected in 2010 in the Kostroma region.

KEYWORDS

early blight, potato protection, *Alternaria solani*, *Alternaria alternata*

INTRODUCTION

Early blight is a dangerous potato and tomato disease, typical in the whole area of cultivation of these crops. This disease is able to result in the significant yield decrease under favorable weather conditions.

Among early blight agents in Russia, there can be some species from the genus *Alternaria*, including one large-spore species *Alternaria solani* Sorauer and several small-spore species, such as *A. alternata* (Fr.) Keissl., *A. tenuissima* (Kunze) Wiltshire, *A. infectoria* E.G. Simmons, and *A. arborescens* E.G. Simmons (Orina *et al.*, 2010).

Different species can have their own biological features and differ in such important characteristics as the aggressiveness, virulence toward different potato and tomato cultivars, fungicide resistance, toxigenicity, optimum growth temperature, and winter survivability (Ivanyuk *et al.*, 2005).

The identification of species by their morphological characteristics is connected with some problems, related to the dependence of the morphology of conidiogenic structures on the medium composition, temperature, lighting mode, etc. Large-spore strains often do not form any conidial fruiting on a nutrient medium. Therefore, morphology-independent features, such as those, based on the genome structure analysis, are especially important for this type of studies.

The purpose of our study was to investigate the species composition of early blight agents, isolated on the territory of Russia, using both morphological and molecular markers.

MATERIALS AND METHODS

Samples

In our study we analyzed early blight agents, isolated in 2007-2010 from infected potato and tomato plants in the Leningrad, Moscow, Astrakhan, and Kostroma regions, Mariy El Republic, Tatarstan (Fig. 1), the Stavropol Territory, and Primorye. Isolates of *A. solani* and *A. arborescens* from the Primorye and some strains of small-spore species from the Leningrad region were kindly provided by our colleagues from the Laboratory of Mycology and Phytopathology of the All-Russian Research Institute of Plant Protection.



Figure 1. Location of sampling sites in the European part of Russia. 1, Leningrad region; 2, Moscow region; 3, Kostroma region; 4, Mariy El Republic; 5, Tatarstan; 6, the Stavropol Territory; 7, Astrakhan region.

Strain isolation

Isolation of strains into pure culture was carried out using wet chambers. After the appearance of fruiting on the surface of an infected sample, the sample was microscopied; using a sharp sterile preparation needle, we transferred conidia onto wort agar medium, supplemented with penicillin (1000 µg/ml), and incubated until the colony diameter reached 4-5 cm. Then a piece of mycelium from the edge of the colony was transferred onto a Petri dish with nutrient medium.

Species identification

Identification of species was performed according to morphological criteria (Simmons, 2007). Isolates were grown on Petri dishes with potato carrot agar (PCA) under fluorescent lamps at 25°C. Colonies were microscopied after 7-10 days of incubation to register the features of the formation of conidial chains and the morphology of spores.

DNA isolation

DNA isolation from the studied *Alternaria* isolates was carried out by the chlorophorm deproteinization using a CTAB buffer.

PCR reaction

The amplification was carried out in a Biometra T1 thermocycler using the following scheme: initial denaturation at 95°C for 3 min, then the denaturation at 94°C for 40 s, the annealing temperature shown in Table 1 for 40 s, elongation at 72°C for 60 s, and a final elongation at 72°C for 3 min. The number of amplification cycles was 25. To amplify specific DNA regions, we used primers listed in Table 1.

The final volume of a PCR reaction mixture was 25 µl and included 2.5 µl of 10x PCR buffer (Helicon, Russia), 2 µl of 25 mM MgCl₂, 0,5 µl of Taq polymerase (5 U/ µl), 2 µl of dNTP mix (0,2 mM of each dNTP), 0,4 µl of each primer, and 1 µl of sample DNA. The rest of volume represented deionized water.

Table 1. List of primers used in the study

| Name | DNA sequence | Reference | Annealing temperature, °C |
|---|-------------------------------|-----------------------------------|---------------------------|
| ITS5-ITS4 region of nuclear ribosomal genes | | | |
| ITS5 | 5'-GGAAGTAAAAGTCGTAACAAGG-3' | White et al., 1990 | 58 |
| ITS4 | 5'-TCCCTCCGCTTATTGATATGC-3' | White et al., 1990 | |
| Large subunit region of mitochondrial ribosomal genes (mt LSU) | | | |
| ML1 | 5'-GTACTTTTGCATAATGGGTCAGC-3' | White et al., 1990 | 52 |
| MLR1 | 5'-GCCCTTCCGAGAGCAAATAC-3' | Peever et al., 2004 | |
| Reverse primer for small-spore species; is used together with the ITS 5 primer | | | |
| MR | 5' – GACCTTGGCTGATAGAGAGTG | Designed by authors of this study | 50 |

Sequencing

After the electrophoretic separation in 1.2% agarose (TBE buffer), the band with the PCR product was cut out of the gel, and the DNA fragment was isolated using a “Cytokin” kit. DNA sequencing was performed by the Eurogene company with the use of a BigDye® Terminator v3.1 Cycle Sequencing kit; the subsequent analysis of the reaction products was carried out using an Applied Biosystems 3730 xl DNA sequencer. The primers used for the amplification of the studied region, were also used as the sequencing primers. The reading of each sequence was carried out twice using the forward and reverse primers. All DNA sequences, obtained during the sequencing, were combined into a database, used for the reconstruction of taxonomic relations between studied species. The reconstruction was carried out by a maximum-likelihood method using a Mega software.

RESULTS AND DISCUSSION

To identify the species composition using the genome structure, we took DNA samples from 7 large-spore and 25 small-spore isolates, collected from potato and tomato plants in different regions of Russia. For the comparative study we selected a nuclear rDNA sequence, confined by ITS5 and ITS4 primers (Fig. 2). This region is well studied in many species and is widely used for the analysis of taxonomic relations.

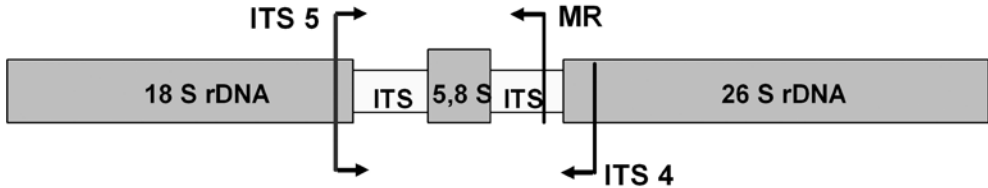


Figure 2. The arrangement of genes and intergene sequences in the nuclear rDNA region and the localization of ITS5, ITS4, and MR primers within this region.

As a result of our study, we determined nucleotide sequences of the studied region, which lengths were equal to 595 bp (small-spore isolates), 60 bp (*A. solani*), and 627 bp (*A. infectoria*), including the sequences of ITS5 and ITS4 primers.

According to the obtained results, the studied strains were divided into three groups (Fig. 3).

The first group included all studied small-spore isolates, excepting *A. infectoria* and including typical strains of *A. arborescens* and *A. tenuissima* (the sequences were taken from the Genbank).

The second group contained all studied large-spore isolates, including typical strains of *A. tomatophyla* and *A. solani*. However, *A. tomatophyla* strain significantly differed from other strains of this group. It seems that the studied large-spore strains, isolated from tomato plants, represent rather *A. solani* than *A. tomatophyla* (it is also confirmed by the results of the morphological diagnostics), though this supposition contradicts the opinion of Simmons (Simmons, 2007), who postulates the absence of *A. solani* on tomato plants. *A. solani* strains, isolated from tomato and potato plants on the Far East, demonstrated some minor differences comparing to other samples.

The third group included one *A. infectoria* isolate from the Kostroma region, one typical isolate from US, kindly provided by our colleagues from the Laboratory of Mycology and Phytopathology of the All-Russian Research Institute of Plant Protection, and also the sequence of strain from the Genbank.

The studied isolates were also identified by their morphological characteristics. As a result of this part of our study, we identified strains of four small-spore (*A. alternata*, *A. tenuissima*, *A. arborescens*, and *A. infectoria*) and one large-spore (*A. solani*) species (Fig. 3). According to the genome structure, *A. solani* and *A. infectoria* were referred to the corresponding separate groups. Morphological species *A. alternata*, *A. tenuissima*, and *A. arborescens* did not differ in their genome structure and, therefore, were included into the same group with the typical *A. arborescens* and *A. tenuissima*.

The dendrogram, based on the data of the mtLSU sequencing, allowed us to divide the analyzed isolates into two groups. The first group consisted of small-spore strains, including *A. infectoria*; the second group consisted of *A. solani*. Within the groups, the strains were almost identical, whereas the groups significantly differed from each other. The typical *A. alternata* and *A. infectoria* strains were included into the group, containing small-spore strains.

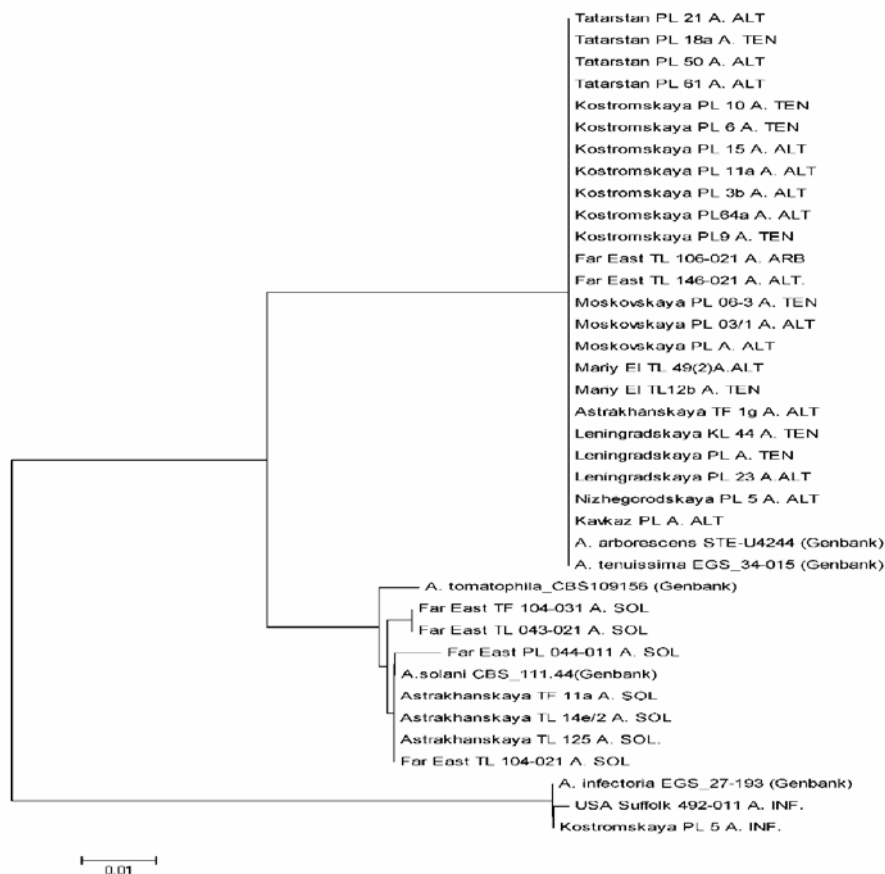


Figure 3. Dendrogram, obtained by the maximum-likelihood method for the structure of the ITS5-ITS4 region. Species identification according to the morphological study: A.ALT, *A. alternate*; A.TEN, *A. tenuissima*; A.ARB, *A. arborescens*; A.SOL, *A. solani*; A.INF, *A. infectoria*. Plant parts, from which the strains were isolated: PL, potato leaves; TL, tomato leaves; TF, tomato fruits.

Thus, the analysis of the *Alternaria* genome showed the studied strains are divided into three clades: *A. solani*, *A. infectoria*, and small-spore strains. The revealed differences between the sequences of these species, confined by ITS5 and ITS4 primers, allowed us to design a specific MR primer for the selective amplification of the corresponding genome regions of small-spore *Alternaria* species (Table 1).

The amplification of DNA of a small-spore strain using the ITS5-MR primer pair resulted in a specific 505-bp PCR product (including primer sequences). At the same time, the amplification of DNA of *A. infectoria*, *A. solani*, and other fungi, often isolated from potato leaves (genera *Bipolaris*, *Fusarium*, etc.), did not result in the formation of a PCR product (Fig. 4).

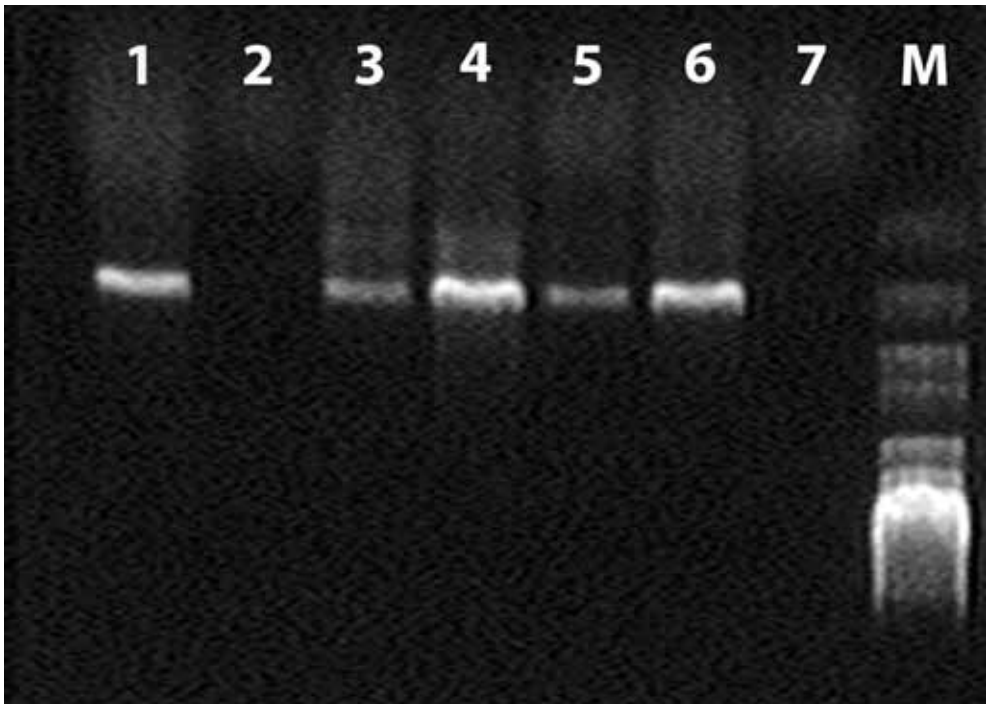


Figure 4. Specific amplification of *Alternaria* genome fragments using ITS5/MR primer pair. 1, control (*A. alternate*); 2, *A. solani*; 3–6, various small-spore isolates; 7, *A. infectoria*; M, 1-kb molecular weight marker.

The designed primer can be used for the specific amplification of DNA of small-spore *Alternaria* species, providing their successful identification in the case of any problems with their morphological identification.

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China-blight — A Web Based DSS on Potato Late Blight Management in China

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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. This system is composed of the three sub-systems of “Real-time distribution of potato late blight in China”, “Infection risk of late blight pathogen based on measured as well as forecasted weather data” and “A farm based simple DSS for the chemical control on potato late blight”. Besides, knowledge information as well as services such as “Control methods on late blight”, “Resistances of cultivars”, “Fungicides database”, “Other pests on potatoes”, “Questions & experiences exchange” and “Electronic record for field practices of users” also included. The three main function of “China-blight” were described and the work need to be done in the near future was also discussed here.

KEY WORDS

Potato late blight, *Phytophthora infestans*, Monitoring and warning system, Decision support system (DSS)

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 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. 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. This system is composed of the three sub-systems of “Real-time distribution of potato late blight in China”, “Infection risk of late blight pathogen based on measured as well as forecasted weather data” and “A farm based simple DSS for the chemical control on potato late blight”. Besides, knowledge information as well as services such as “Control methods on late blight”, “Resistances of cultivars”, “Fungicides database”, “Other pests on potatoes”, “Questions & experiences exchange” and “Electronic record for field practices of users”

also included. The three sub-systems of “China-blight” were described and the main work in the near future of its use was also discussed below.

THE MAIN FUNCTION OF “CHINA-BLIGHT”

Potato late blight monitoring

One of the main functions of “China-blight” is monitoring attacks of potato late blight in China during the current growing season. The data and working flow showed in Fig. 1, when end users (farmers or local advisors, etc.) find late blight attacks in their fields or areas, they can report to “China-blight” via internet (www.china-blight.net) or send SMS to a noted mobile phone number (in case of the end uses have no internet access) and the person in charge of the system running will report to “China-blight”. As while as the system receive these “late blight attacks reports”, it can put the red dots on to the nation and regional map according to the location of the attacks (see red dots in A and B in Fig. 1). At the same time the detailed information about these attacks will be put into the “list of late blight attacks”. All the leaflets in Fig. 1 can be updated automatically in real time.

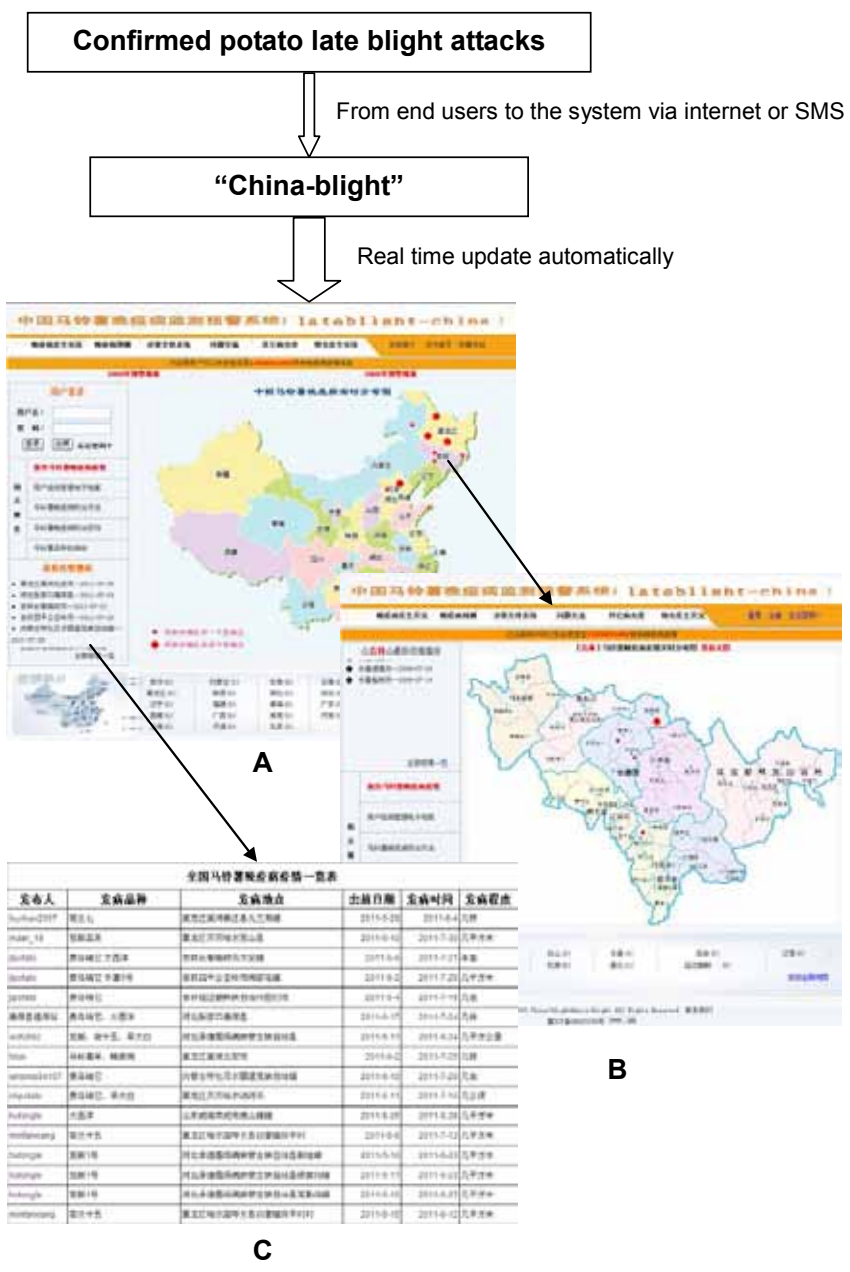


Figure 1. Data and working flow of potato late blight monitoring in “China-blight”. A and B, real time distribution of late blight (red dots on the maps, small dot means only one attack of late blight in the county and big one means more than one attacks in the county) in national and regional scale. C, list of the detailed information of late blight attacks.

Weather data based infection risk assessment of *P. infestans* on potatoes

“Weather data based infection risk of potato late blight” is another main function of “China-blight”. From 2008 to 2010, there is no possibility to get measured hourly weather data so only “Infection risk of potato late blight for the coming 2 days” (Fig. 2) was published and updated daily during the main growing season, the infection risk assessment based on the weather forecast of National Meteorological Center of CMA (www.weather.gov.cn). In 2011 measured hourly weather data (Temperature, Relative humidity and Precipitation) for some selected locations can be obtained from National Meteorological Center of CMA (www.weather.gov.cn), so weather data based infection risk (Fig. 3) was also published and updated every morning from mid of May to the end of August 2011, the model in use was MISP model (Cao *et al.* 1996).

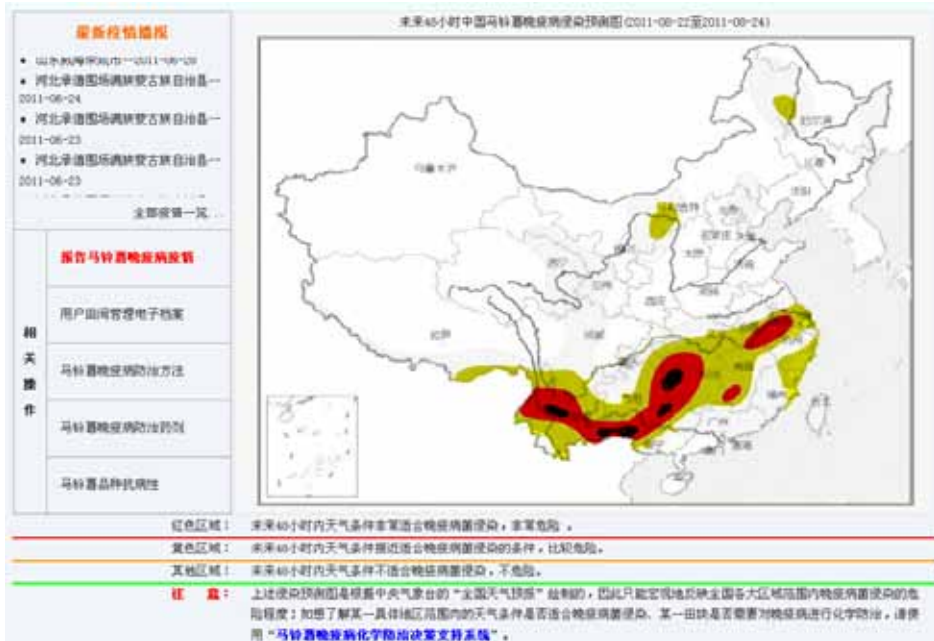


Figure 2. The China-blight leaflet “Infection risk of potato late blight for the coming two days in the national level” on Aug. 22 2011. Red color means “highly risk”, yellow color means “risk” and, no color means “no risk”. This map drawn by hand based on the weather forecast of National Meteorological Center of CMA (www.weather.gov.cn) and updated daily during mid of May to end of August annually.

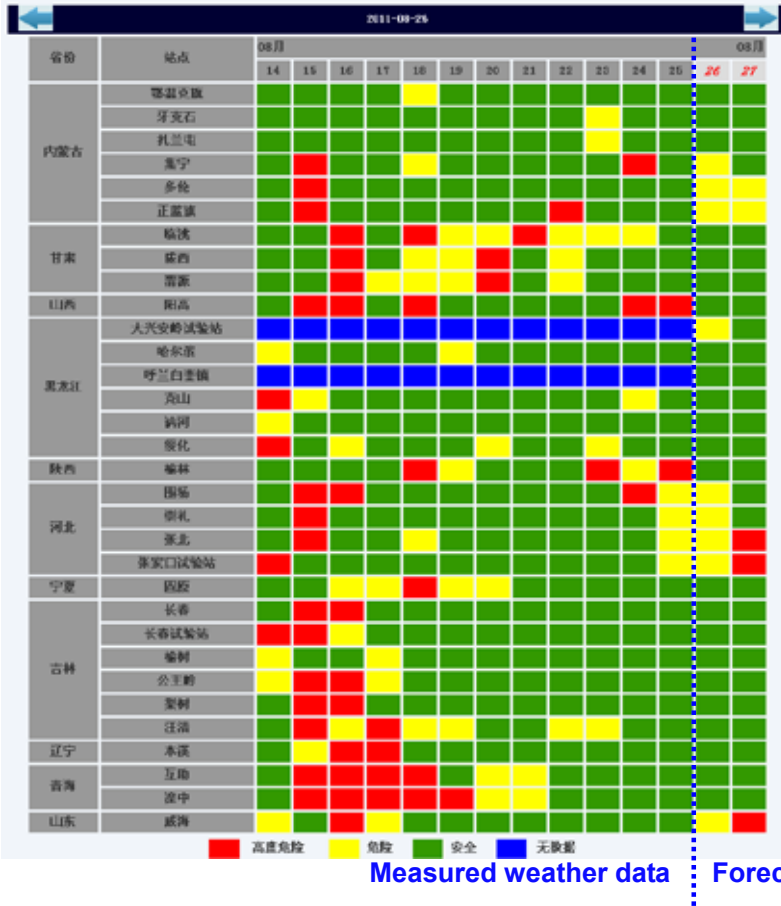


Figure 3. The China-blight leaflet “Weather data based infection risk of potato late blight” for the selected locations on Aug. 26 2011. Red color means “highly risk”, yellow color means “risk”, green color means “no risk” and, blue color means short of weather data input. Hourly weather data (Temperature, Relative humidity and Precipitation) came from National Meteorological Center of CMA (www.weather.gov.cn) or onset weather data loggers located in the experimental stations of the potato industry of China. Data updated daily during mid of May to end of August annually.

A farm based simple DSS for fungicide spray against potato late blight

The third main function of “China-blight” is “A farm based simple DSS for fungicide spray against potato late blight”. As shown in Fig. 4, it is a questionnaire based simple DSS in order to assist farmers to decide when to spray fungicide against potato late blight during growing season. After a farmer answered the question 1 to 5 a suggestion will be given by the system about whether a spray is necessary or not, when to spray and which kind of fungicide should be use in terms of mode of action (Fig. 5).

中国马铃薯晚疫病监测预警系统 (lateblight-china)

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 感病 抗病 不确定

3、您的地块及邻近地块晚疫病发生情况：
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4、近期天气情况：

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| 明天： | <input type="radio"/> | <input type="radio"/> |
| 后天： | <input type="radio"/> | <input type="radio"/> |
| 第四天： | <input type="radio"/> | <input type="radio"/> |
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5、最近一次针对晚疫病的喷药距今天：
 超过14天 7-14天 7天之内

Figure 4. The China-blight leaflet “A farm base simple DSS for fungicide spray against potato late blight”. It is a questionnaire based simple DSS. 1, growth stage of your crop, 2, resistant level of your cultivar, 3, the late blight situation in your own and neighboring fields, 4, precipitation in the coming days and, 5, the time of your last spray against late blight.



Figure 5. The China-blight suggestion leaflet of the “farm base simple DSS for fungicide spray against potato late blight”. The suggestion in this case (words in red color) means “The infection risk of late blight is very high in the coming days, we suggest you spray fungicide with protective and curative activity as soon as possible (please pay attention to change the fungicide from your former sprays in case of fungicide resistance of *P. infestans*).

DISCUSSION

Since China is a big country and different regions have different meteorological characteristics, in different areas the occurrence and epidemics of potato late blight are also quite different. So the way for “China-blight” is still long and full of challenges. The planned work of “China-blight” in the near future include 1), validate of different late blight control strategies in different regions in China, 2), setup and test different DSS for chemical spray against late blight in different regions, and 3), cooperate with more farms and local advisors in the main potato producing areas.

ACKNOWLEDGEMENTS

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Competition between genotypes of *Phytophthora infestans*

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SUMMARY

Dramatic changes to the GB population of *Phytophthora infestans*, the cause of potato late blight, have resulted in a new set of highly aggressive genotypes dominating the population in Great Britain. One genotype in particular, genotype 13_A2, is an aggressive genotype that can overcome the resistance of some previously resistant potato cultivars and since 2005 has become dominant in the population. The field study reported here investigates the competitive ability of 13_A2 compared with three other genotypes (6_A1, 7_A1 and 8_A1) on the potato cultivars Cara (moderately resistant) and Maris Piper (susceptible). Genotype 13_A2 was found to be the dominant genotype in most plots within the trial. Cara was more resistant to all genotypes compared with Maris Piper.

KEYWORDS

Phytophthora infestans, genotype 13_A2, genotype 6_A1, aggressiveness, competition

INTRODUCTION

For the fifteen years up to 2005, the A2 mating type of *Phytophthora infestans* comprised a low proportion of the population with around 90% of the UK population being the A1 mating type. Since then, there has been a dramatic increase in the A2 mating type with a large proportion being of the genotype 13_A2, sometimes also referred to as 'Blue 13'. Genotype 13_A2 was first identified in 2005 and has dominated the population since 2007 (Cooke *et al.*, 2010). Within genotype 13_A2 there are variants: between 2003 and 2009 13_A2_1 was the dominant variant but it has declined in frequency and now 13_A2_2 dominates in Scottish *P. infestans* populations. 13_A2 is a highly aggressive genotype and can overcome the resistance of some previously resistant potato cultivars. Competition between genotypes may be a factor that has contributed to the dominance of genotype 13_A2 as this cannot be explained by aggressiveness alone. In 2007 a field study showed that 13_A2 dominated when co-inoculated with other genotypes in field plots (Cooke *et al.*, 2010), but in a 2010 aggressiveness study 13_A2 was not always the most aggressive genotype on detached leaflets (Chapman, A., unpublished data). A direct competitive interaction may be occurring if aggressiveness alone does not account for dominance (Young, 2007). There could be inhibitory effects that would give one genotype a competitive advantage over other genotypes (Young, 2007).

METHODS

The field trial was planted on the 4th May 2011 at the Agri-Food and Biosciences Institute, Belfast, Northern Ireland. The two potato cultivars used were Cara and Maris Piper with foliar late blight resistance ratings of 5 and 4 (on a 1-9 scale of increasing resistance) respectively. Each plot was surrounded by a guard row of the potato cultivar Sárpo Mira. Three inoculation treatments were used in the trial: the top left corner plant (Plant 1) of each plot was inoculated with genotype 13_A2 and the bottom right corner plant (Plant 16) was inoculated with genotype 6_A1, 7_A1 or 8_A1. Inoculation took place on the 29th June 2011. A fully randomised block design was used with four replicate blocks each containing six plots of 16 plants. A unit plot consisted of four rows of four plants. Plots were monitored daily until the first signs of infection were found on the inoculated plants and then disease assessments for every plant took place every 3 to 5 days using the ADAS blight assessment key (Anonymous, 1976). Up to four leaflets with single lesions were collected from each plant depending on the number of lesions present and the lesions were sampled onto a Whatman FTA card (Whatman FTATM Classic Card, Cat No. WB120205, GE Healthcare UK Limited) for storage according to the manufacturer's instructions until they were genotyped using SSR analysis (Lees *et al.* 2006).

RESULTS

Cultivar

On a whole plot basis there was a highly significant difference in percentage foliar blight between cultivars from 27 days post inoculation onwards (27 days: $P=0.006$, 32 days: $P<0.001$, 36 days: $P<0.001$). After 27 days, disease was clearly progressing rapidly in Maris Piper, increasing from 17.7% foliar blight (after 27 days) to 35% (after 36 days), whereas on Cara disease increased only slightly from 10.3% (after 27 days) to 12.3% (after 36 days) (Figure 1). Thirty-six days into the epidemic Maris Piper (35%) had nearly three times more foliar blight than Cara (12%).

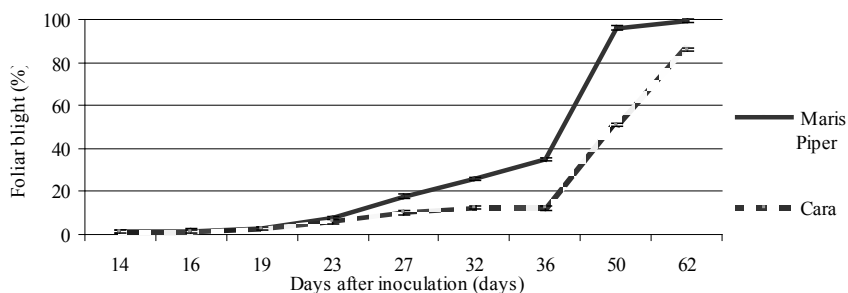


Figure 1: Average disease severity for all plots. Values for both cultivars

Inoculation treatments

No significant effect of treatment was observed on Cara, with percentage foliar blight scores of 12.3% for all treatments. Conversely, Maris Piper showed percentage foliar blight scores of 44%, 31% and 30% for the treatments 13_A2+6_A1, 13_A2+7_A1 and 13_A2+8_A1, respectively with treatment 13_A2+6_A1 having a significantly larger percentage foliar blight score ($P<0.001$).

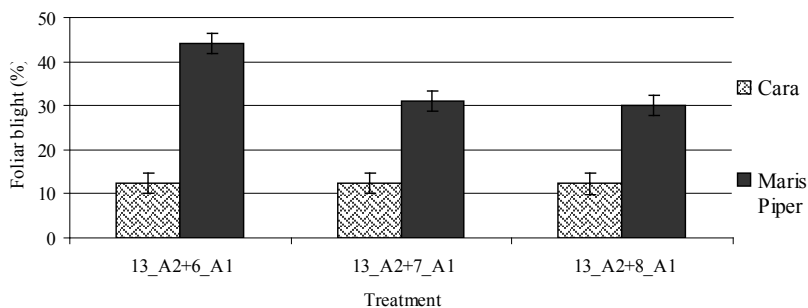


Figure 2: Average disease severity for each treatment on potato cultivars Cara and Maris Piper

Genotyping

In total, 994 samples from individual blight lesions were collected and genotyped. Across all the plots genotype 13_A2 was predominant but only by 3.4%. 13_A2 contributed 49.7% of the samples (this percentage has been weighted to account for the fact that there was three times more 13_A2 inoculum initially introduced into the field). Genotype 6_A1 had the second largest disease incidence at 46.3% with genotypes 7_A1 and 8_A1 at 2.1% and 1.9%, respectively. 13_A2 completely dominated the plots when it was paired with 7_A1 and 8_A1. 13_A2 also caused the largest percentage of lesions when comparing treatments. In the treatment 13_A2+6_A1, 60.9% of the lesions sampled were caused by 13_A2 and 39.1% by 6_A1 (Figure 3).

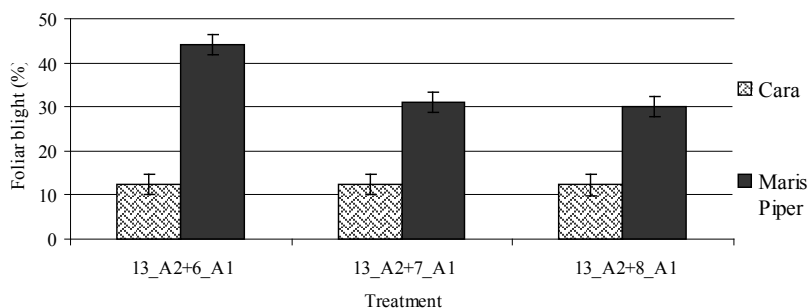


Figure 2: Average disease severity for each treatment on potato cultivars Cara and Maris Piper

CONCLUSIONS

The focus of this study was to investigate the competitive ability of *P. infestans* genotypes based on their spread through plots from inoculated plants in a field trial. The study showed a significant difference in foliar blight susceptibility between Cara and Maris Piper with Cara being more resistant to all of the genotypes tested compared with Maris Piper. Genotype 13_A2 was the most prevalent genotype: it was found in each plot hence its dominance could be due to the rate at which genotype 13_A2 spreads from plant to plant.

ACKNOWLEDGEMENTS

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POSTERS

Target enrichment and next generation sequencing as tools to facilitate cloning of R genes from Solanum species

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Target enrichment and next generation sequencing as tools to facilitate cloning of *R* genes from *Solanum* species

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Late blight caused by oomycete pathogen *P. infestans* is the most destructive disease in cultivated potato. Since *P. infestans* is known to quickly overcome resistance genes used in breeding programs, there is a constant necessity to identify and clone novel *Rpi* genes (*Rpi*-Resistance to *P. infestans*). Classical map-based cloning is a laborious and time-consuming effort; therefore we are developing a technique which combines target enrichment and next generation sequencing to accelerate cloning of new *Rpi* genes. This technique allows to avoid classical polymorphism discovery for fine-mapping of *Rpi* genes. Ideally, our approach will allow to 'land' on the gene (cluster of genes) conferring resistance. Here we present the developed pipeline, discuss current troubleshooting and communicate potential of the approach. This newly developed technique should be applicable to facilitate cloning not only *Rpi*, but also other *R* genes from *Solanaceae* which are of NB-LRR type. Briefly, samples consisting of combined 50 susceptible (BS) and 50 resistant (BR) individuals are first enriched for NB-LRR genes using Agilent SureSelect with probes designed against 470 NB-LRR genes predicted from sequenced doubled monoploid potato genome (DM). Such enriched sample is sequenced using Illumina GA2 platform. Next, obtained data are analysed using various bioinformatic tools. Predicted SNP/InDels are confirmed by Sanger sequencing and fine-mapped using segregating populations.

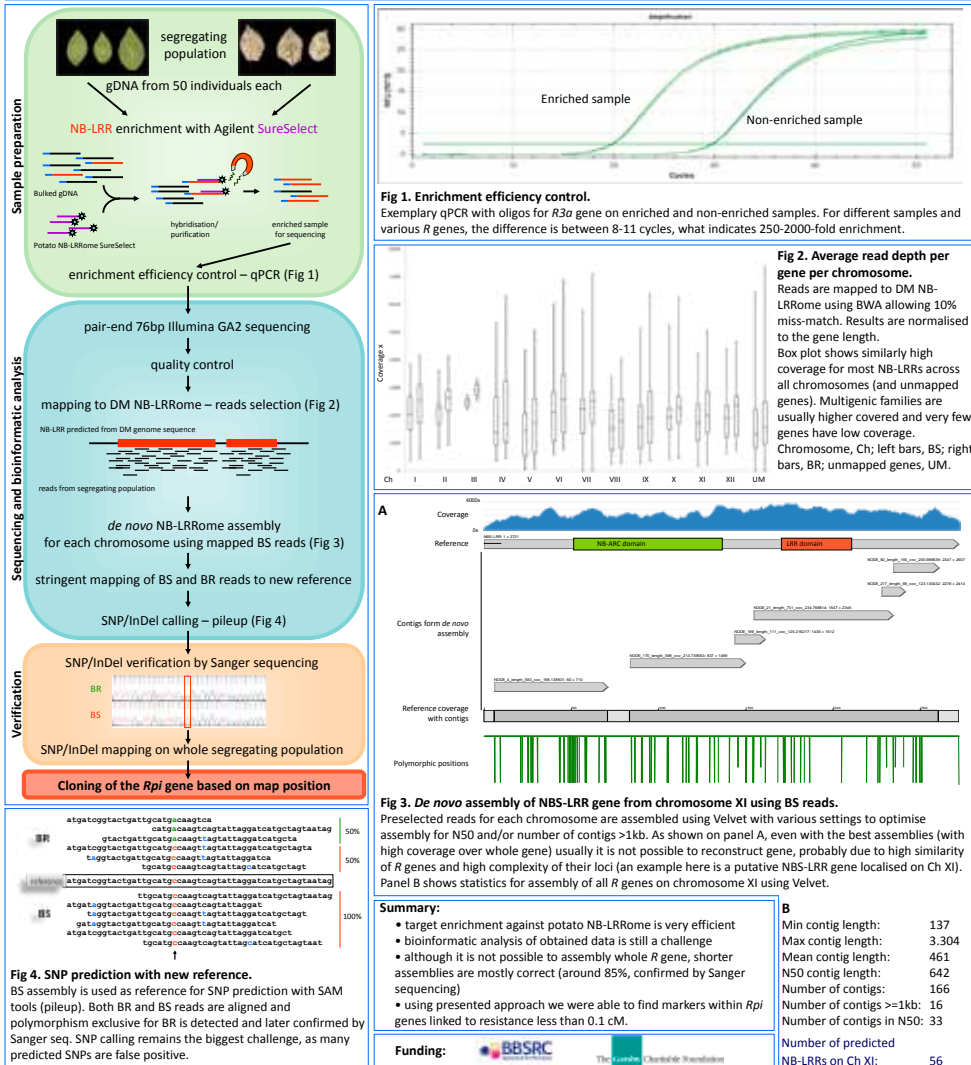


Fig 4. SNP prediction with new reference.
BS assembly is used as reference for SNP prediction with SAM tools (pileup). Both BR and BS reads are aligned and polymorphism exclusive for BR is detected and later confirmed by Sanger seq. SNP calling remains the biggest challenge, as many predicted SNPs are false positive.

**Assessment of foliar and tuber resistance in
Solanum neoantipoviczii Buk. × *S. phureja* Juz. et Buk.
hybrid populations using different isolates
of *Phytophthora infestans***

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SUMMARY

In an evaluation of resistance to *Phytophthora infestans* in wild potato species from VIR's collection performed in late 90-ies at IHAR-Mlochow Research Center (IHAR-Mlochow), Poland the accession *Solanum neoantipoviczii* Buk. k-8505 was found highly leaf and tuber resistant. In 1999 it was used in direct cross with an accession of *S. phureja* Juz. et Buk DB 254 from SCRI (UK) collection. Resistance to *P. infestans* in the hybrid obtained were assessed in several tests using two (leaflet tests) and three (tuber tests) isolates of *P. infestans* differing by phenotype. Foliar and tuber resistance evaluation were performed in 2001 at IHAR-Mlochow and in 2008, 2010 at Swedish University of Agricultural Sciences (SLU-Alnarp), Sweden. In all tests hybrid progenies were found segregating for foliar and tuber resistance. Lower tuber resistance was observed after application of more virulent isolates. In each test genotypes combining both leaf and tuber resistance were found.

KEYWORDS

Phytophthora infestans, resistance, potato hybrid

INTRODUCTION

Breeding achievements using large scale approaches have not been able to significantly decrease yield losses caused by late blight. New, more virulent *Phytophthora infestans* strains have evolved which overcome the genetic resistance that has been introgressed by conventional breeding from wild and cultivated potato species into commercial varieties. The most effective and environmentally friendly way is the incorporation of genes for resistance to *P. infestans* from newly found natural sources able to cross with cultivated potatoes.

The R genes from *Solanum demissum* Lindl. have been introgressed into potato cultivars. However, their durability proved to be a problem due to the rapid appearance of compatible races of the pathogen after market introduction (Wastie 1991). Several other wild species of the genus *Solanum* beside *S. demissum* are also being considered as possible sources of resistance to late blight (Hermesen and Ramanna 1973). Recently new sources of R-gene resistance have been found in number of wild

potato species (Gebhardt and Valkonen 2001).

The accession of *S. neoantipoviczii* maintained in VIR's collection was identified as foliar and tuber resistant on evaluation carried out in the late 90-ies (Zoteyeva *et al.* 2004). Mexican wild potato species *S. neoantipoviczii* was described and specified as an independent species by Russian taxonomist Bukasov. This species is closely related to *S. stoloniferum* Schlecht. and mentioned in Hawkes's monography as its synonym. (Hawkes, 1990).

MATERIAL AND METHODS

Plant material

Two F1 populations of the interspecific hybrid *S. neoantipovichii* × *S. phureja* were evaluated in 2001 at IHAR-Mlochow, Poland and in 2008 at SLU-Alnarp, Sweden. Clones obtained from the seedlings evaluated in 2008 were tested in 2010.

Pathogen material

Three isolates of *P. infestans* were used in tuber and two ones in leaflet tests. The 2001 leaflet test and one of tuber tests were done using isolate MP-324 (1.2.3.4.6.7.10.11.) from IHAR-Mlochow collection of pathogens. The other isolate applied in the 2001 tuber test was American US-8 (1.2.3.4.5.6.7.10.11.) also maintained at IHAR-Mlochow. In the tests performed at SLU-Alnarp Swedish isolate SE 03058 (1.3.4.7.10.11.) was used for leaf and tuber inoculation. The virulence of isolates was examined using a set (R1-R11) of Black's differentials maintained in IHAR-Mlochow's collection of pathogens.

Leaf and tuber resistance assessment

For foliar resistance evaluation three leaflets from each plant in two replications have been inoculated with a drop of inoculum (20 µl) of *P. infestans*. Two isolates of *P. infestans* were used for inoculation: MP-324 and SE 03058. The reading of disease symptoms was done after 6 days of incubation at 16°C. Leaflet disease rating was recorded using 1-9 grade scale, where 9 is the most resistant. General score criteria was a combination of a percent of affected leaf area and mycelia development intensity (Zarzycka 2001). Cultivars Irys (S) and cv. Bintje (S) as well as cultivar Meduza (R) and selection from *S. guerreroense* (R) were used as controls (Table 1).

For tuber resistance evaluation the original method of inoculation of decapitated tubers was applied (Zoteyeva and Zimnoch-Guzowska 2004). Three *P. infestans* isolates were used for inoculum preparation: MP-324, US-8 and SE 03058. From five to 10 tubers per each plant were inoculated. Lesion sizes were scored using grade scale 1-9 where 9 is the most resistant. For mycelia growth 0 - 3 grade scale where 0 means lack of mycelium growth and 3 very intense mycelium growth was used. Cultivars and breeding lines (6 tubers of each) with different resistance levels were included as standards in each test (Table 1). In all tests the inoculum concentration comprised 20000 zoospores /ml.

For statistical analyses the MINITAB 15 Statistical Software was used.

Table 1. Resistance of *S. neointipoviczii* × *S. phureja* hybrid progenies in laboratory tests using different isolates of *Phytophthora infestans*

| <i>P. infestans</i> isolate | Number of tested plants/tubers | Accession tested | Resistance, grade | |
|-----------------------------|--------------------------------|--------------------|-------------------|-----------|
| | | | average | min - max |
| leaflet tests | | | | |
| 2001 (MP -324) | 26 | nan* x phu | 8.6 | 5.2 – 9.0 |
| 2010 (SE 03058) | 20 | nan x phu | 6.9 | 4.0 – 9.0 |
| Controls: | | | | |
| 2001 (MP 324) | | cv. Irys (S**) | 1,0 | 1.0 – 1.0 |
| “ “ | | cv. Meduza (R***) | 6,2 | 5.0 – 7.0 |
| 2010 (SE 03058) | | cv. Bintje (S) | 1.4 | 1.0 – 2.0 |
| “ “ | | grr-08-3 (R) | 8.7 | 7.0 – 9.0 |
| tuber tests | | | | |
| 2001 (MP-324) | 26/251 | nan x phu | 5.3 | 3.0 – 7.5 |
| 2001 (US-8) | 20/160 | nan x phu | 4.7 | 2.8 – 6.5 |
| 2008 (SE 03058) | 18/78 | nan x phu | 7.6 | 6.2 – 9.0 |
| 2010 (SE 03058) | 20/90 | nan x phu | 6.2 | 7.8 – 3.8 |
| Controls: | | | | |
| 2001 (MP 324) | | cv. Irys (S) | 2.8 | 2.2 – 3.4 |
| “ “ | | cv. Meduza (R) | 6.5 | 5.0 – 7.0 |
| 2001 (US-8) | | cv. Irys (S) | 2.3 | 1.0 – 3.0 |
| “ “ | | cv. Meduza (R) | 6.1 | 5.0 – 7.8 |
| 2008 (SE 03058) | | cv. Bintje (S) | 3.1 | 3,0 – 3.6 |
| “ “ | | cv. Matilda (R) | 6.8 | 6.0 – 7.4 |
| 2010 (SE 03058) | | cv. Bintje (S) | 2.9 | 2.0 – 4.0 |
| “ “ | | cv. Matilda (R) | 6.4 | 5.0 – 9.0 |

*) – potato species name abbreviations (nan = *S. neointipoviczii*, phu = *S. phureja*)

***) – susceptible

****) – resistant

RESULTS AND DISCUSSION

The F1 hybrid population assessed in leaflet (using the isolate MP-324 (1.2.3.4.6.7.10.11.)) and tuber (using the isolates MP-324 and US-8 (1.2.3.4.5.6.7.10.11.)) tests was found slightly segregated for leaf resistance shown high percent of highly resistant plants but much stronger segregated for tuber resistance (Table 1). In leaflet test resistance was scored on average 8.6 grades for two replications. Resistance in 24 out of 26 tested plants in both replications was scored on average grades from 8 to 9. The infection pressure in this test was sufficient as could be observed by the reaction of the controls scored with grade 1 (susceptible) and grade 6.2 (resistant). Another population of this hybrid was obtained from original seeds at SLU-Alnarp in 2008. Twenty clones from this population were evaluated in leaflet test in 2010 using an aggressive isolate SE 03058 (1.3.4.7.10.11.). In this test resistance of hybrid progenies was lower than in 2001 when Polish isolate (1.2.3.4.6.7.10.11.) was applied. To classify populations by resistance plants were divided into three groups: resistant (R, grades from 7 to 9), moderately resistant (MR, grades from 5 to 6) and susceptible (S, grades from 1 to 4). Distribution by resistance levels within populations evaluated in 2001 and 2008 tests resulted in: 24R : 2MR : 0S (2001) and 13R : 6MR : 1S (2008). The results obtained showed reduced share of

resistant plants in population tested in 2008. Regardless less virulent isolate applied, the resistance of hybrid progenies tested in 2010 was lower. Nevertheless, about half of the population expressed resistance.

Table 2. Resistance of *S. neointipoviczii* × *S. phureja* progenies in tuber test applying two isolates of *Phytophthora infestans*

| <i>P. infestans</i> Isolate | Nr of plant | Resistance grade | | <i>P. infestans</i> isolate | Nr of plant | Resistance grade | |
|--------------------------------|-------------|------------------|-----------------|--------------------------------|-------------|------------------|-----------------|
| | | Lesion size | Mycelium growth | | | Lesion size | Mycelium growth |
| MP-324 | 1 | 6.4 | 0.4 | US-8 | 1 | 5.2 | 1.4 |
| MP-324 | 2 | 5.3 | 1 | US-8 | 2 | 5.0 | 1 |
| MP-324 | 3 | 5.1 | 0.8 | US-8 | 3 | 6.0 | 1 |
| MP-324 | 4 | 3.8 | 1.4 | US-8 | 4 | 4.2 | 1.2 |
| MP-324 | 5 | 4.5 | 1 | US-8 | 5 | 3.7 | 1.8 |
| MP-324 | 6 | 6.3 | 0.6 | US-8 | 6 | 6.0 | 0.8 |
| MP-324 | 7 | 5.3 | 0.6 | US-8 | 7 | 4.3 | 1.2 |
| MP-324 | 8 | 3.1 | 2 | | 8 | non tested | |
| MP-324 | 9 | 5.7 | 0 | US-8 | 9 | 5.9 | 1.2 |
| MP-324 | 10 | 5.9 | 0.4 | US-8 | 10 | 5.9 | 1.2 |
| MP-324 | 11 | 5.1 | 1 | US-8 | 11 | 3.7 | 1.4 |
| MP-324 | 12 | 5.3 | 0.2 | | 12 | non tested | |
| MP-324 | 13 | 5.6 | 0.4 | US-8 | 13 | 4.7 | 1 |
| MP-324 | 14 | 7.1 | 0 | US-8 | 14 | 6.5 | 0.6 |
| MP-324 | 15 | 7.5 | 0 | US-8 | 15 | 4.5 | 2.5 |
| MP-324 | 16 | 4.6 | 0.8 | US-8 | 16 | 4.7 | 1.4 |
| MP-324 | 17 | 6.1 | 0.2 | | 17 | non tested | |
| MP-324 | 18 | 5.4 | 0.6 | US-8 | 18 | 3.3 | 1.6 |
| MP-324 | 19 | 6.0 | 0.4 | US-8 | 19 | 5.5 | 1.6 |
| MP-324 | 20 | 4.9 | 0.2 | US-8 | 20 | 4.3 | 0.8 |
| MP-324 | 21 | 3 | 1.6 | US-8 | 21 | 2.8 | 2.2 |
| MP-324 | 22 | 4.9 | 2 | US-8 | 22 | 4.4 | 1.8 |
| MP-324 | 23 | 5.7 | 0.2 | | 23 | non tested | |
| MP-324 | 24 | 5.6 | 0 | | 24 | non tested | |
| MP-324 | 25 | 5.3 | 1 | | 25 | non tested | |
| MP-324 | 26 | 4.1 | 3 | US-8 | 26 | 4.1 | 3 |
| | Controls: | | | | | | |
| MP-324 | Irys (S) | 2.8 | 1.2 | US-8 | Irys | 2.3 | 1.8 |
| MP-324 | Meduza (R) | 6.5 | 0.6 | US-8 | Meduza | 6.1 | 1.5 |

In tuber test performed at IHAR-Mlochow two isolates MP-324 (1.2.3.4.6.7.10.11.) and US-8 (1.2.3.4.5.6.7.10.11.) were applied. The difference in virulence between these two isolates was a lack of gene for virulence 5 in isolate MP-324 compared to isolate US-8. Results of the tests showed tuber resistance ranged from 3.0 to 7.5 grades after inoculation with isolate MP-324 and from 2.8 to 6.5 grades after inoculation with isolate US-8 (Table 2). To classify plants for tuber resistance the ones scored on average: from 1 to 4.9 grades were found as susceptible (S), from 5 to 6.4 grades as moderately resistant (MR) and up to 6.5 grades as resistant (R). The reaction of tubers of the standard cultivars inoculated with isolates MP-324 or US-8 showed sufficient infection pressure (Table 2). Tuber resistance of the susceptible cultivar Irys was scored on average 2.8 and 2.3 grades after inoculation with isolates MP-324 and US-8, respectively. The disease scores on tubers of resistant control (cultivar Meduza) were, respectively, 6.5 and 6.1 grades on average. The distribution of the resistance levels within 26 plants when tubers were inoculated with isolate MP-324 was 2R:16MR:8S. Twenty plants inoculated with isolate US-8 segregated for tuber resistance in proportion 1R:7MR:12S. The application of isolate US-8 resulted higher disease development on inoculated tubers regarding lesion sizes and mycelia growth: the lesion sizes score were 4.6 grades (US-8) and 5.3 grades (MP-324) grades on average and mycelia growth score were 0.8 grades (US-8) and 1.5 grades (MP-324) on average.

Statistical analyse using Minitab program showed that the resistance levels of hybrid progenies inoculated with different *P. infestans* isolates were differed significantly. Grouping Information using

Tukey Method showed that with mean values of MP-324 = 5,26 and Us-8 = 4,60 the resistance scores of lesion size were significantly different ($p < 0.0001$). A mean values of MP-324 = 0.84 and of US-8 = 1.45 showed significant differences for mycelium growth in tests with the use of isolates MP-324 or US-8 ($p < 0.0001$).

While leaf resistance of hybrid progenies was lower in test using the Swedish isolate SE 03058 compared to the test where the Polish isolate MP-324 was used, tuber resistance was higher in both tests performed at SLU-Alnarp. Resistance in 18 seedlings tested in 2008 was scored on average 7.6 grades and ranged from 6.2 to 9.0 grades (Table 1). Distribution by resistance levels in this test was 15R:3MR:0S.

Clones obtained from 20 seedlings in 2008 were tested in 2010. In this test resistance occurred to be lower and was scored on average 6.2 grades and ranged from 4.3 to 7.8 grades. Plant distribution within the population regarding resistance levels was 6R:11MR:3S. In 2010 resistance was scored with lower grade on average due to higher number of moderately resistant plants and lower number of resistant ones compared to 2008 when tuber susceptible plants were not found at all.

A comparison of tuber test results where the isolates of *P. infestans* containing 8 (MP-324), 9 (US-8) and 6 (SE 03058) genes for virulence showed higher resistance in the tests where isolate containing lower number of genes for virulence were used. The use in the same test of two highly virulent isolates differing by the presence/absence of only one virulence gene (out of the range R1 – R11) resulted in significantly different tuber resistance score values.

In all tests performed hybrid populations were found segregating for foliar and tuber resistance. In each test the genotypes with combined foliar and tuber resistance were found.

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The adaptation of MAS for late blight resistance evaluation of potato breeding material

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SUMMARY

The goal of this study was to determine contribution of race-specific resistance conferred by genes R1 and R3 to general resistance of potato breeding clones to late blight (agent *Phytophthora infestans*). For this purpose the assessment of breeding clones for resistance to late blight of leaf and tubers and field resistance as well their screening for presence of resistant alleles of R1 and R3 genes were performed. No influence of resistance genes on reduction of level of foliar damage in field and leaf resistance was observed. A significant influence of presence of R3 gene on tuber resistance to late blight was found. The significant difference was detected between tuber resistance level of clones with detected presence of resistant allele of R3 gene and clones with detected presence of susceptible alleles of both tested genes and clones with detected resistant allele of R1 gene ($p < 0.05$)

KEYWORDS

Phytophthora infestans, MAS, potato breeding, resistance.

INTRODUCTION

Late blight caused by *Phytophthora infestans* is still challenging potato fields around the globe. Disease mediated by the R genes is one of the cognate effectors that is introduced into the plant cell by the pathogen and induces resistance. R-genes (R1-R11) in potatoes are remaining to be the valuable sources for development of new cultivars resistant to late blight, but potatoes containing these R genes are only effective in preventing the development of late blight. Another application on the resistance is to be the optimal selection of R genes identified by monitoring of *P. infestans* populations in each area where the potatoes are grown (Visser *et al.*, 2008). A marker assisted selection (MAS) is to be the effective tool in use for resistance level improvement in breeding programme. Classical selection of resistant progenies is difficult due to appearance of new pathogenic pathotypes and due to expensive and time consuming field evaluation. Since 1980s molecular markers are being widely used as a principal tool for plant breeding. In several studies molecular markers linked to late blight resistance genes have been found (Leonards-Schippers *et al.*, 1992; Gebhardt and Valkonen, 2001; El-Kharbotly *et al.* 1994; 1996; Li *et al.*, 1998; Ewing *et al.*, 2000; Huang *et al.*, 2004). *P. infestans* genes virulent to R1 and R3 were detected among most common in late blight European populations (Andrivon *et al.*, 1994; Lebreton *et al.*, 1998; Lehtinen *et al.*, 2008; Lebecka *et al.*, 2007) including North-Western Region of Russia and Estonia (Runno-Paurson *et al.*, 2009; Zoteyeva and

Patrikeeva, 2010) neighbouring with Latvia.

The association of resistance genes R1 and R3 presence in genotypes and high late blight resistance was observed in previous research (Khavkin *et al.*, 2010). The R genes contribute some resistance; combining resistance genes with high levels of field resistance would be a desirable goal for breeding programme (Bradshaw, 2009).

The goal of this study was to determine contribution of genes for race-specific resistance to *P. infestans* R1 and R3 to general resistance to late blight of potato genotypes. For this purpose breeding clones were assessed for resistance to late blight of leaf and tubers and field resistance as well as screened for presence of resistant alleles of R1 and R3 genes by molecular markers.

MATERIAL AND METHODS

Description of field growing conditions

The soil type was sod-podzolic (PVv), loamy sand. Organic matter content in soil was 24 - 27 mg kg⁻¹, pHKCl was 5.5 – 5.7, availability of K and P in soil was high. Fertiliser N – 50-60, P – 100, K – 100 kg ha⁻¹ was used. The fungicides for restriction fungal diseases were used two times in July each year. In 2010 the air temperature in the second part of vegetation was 3-5°C higher than perennial data (PD). In 2011 air temperature was similar to PD. The precipitation exceeded PD by 24 – 31% in 2010. The July was dry in 2011 (precipitation only 85% of PD), but precipitation in second decade of August exceeded PD by 109 %.

Plant and infection materials

Third, fourth and fifth generations of breeding clones (total number 463) were assessed for late blight resistance in field in 2010, for selected clones assessment was continued in 2011. Ten clones involved in field assessment were screened by molecular markers. 100 clones were evaluated for leaf resistance to *P. infestans* and 38 clones were evaluated for tuber resistance. Screening with molecular markers was performed in 69 potato clones undergoing leaf tests and in 28 clones undergoing tuber tests. Complex evaluation of all assessments was performed for 16 potato clones.

The isolates of *P. infestans* were sampled from infected potato plants grown in field. The virulence factors in sampled isolates were studied using a set of Black's differential genotypes R1 – R11 offered by IHAR-Mlochow Research Center, Poland. For inoculum preparation the mixture of two isolates expressing nine and six genes for virulence (1.2.3.4.(5).6.7.10.11 and 1.3.4.7.8.10.11.) were used.

Field observation

Observations were performed from the beginning of July to the end of August once in 7-10 days. The disease development on foliage was assessed as percentage of foliage area damaged by *P. infestans* infection. Diseases damages on foliage for each clone was set to grade scale (assessment key for foliar late blight of the Dutch Plant Protection Service): grade 1 – when 90-100% damaged area out of total foliar area, grade 9 - less than 10 % of total foliar area was damaged (Bus *et al.*, 1995).

Marker assisted selection (MAS)

Clones involved in field observations were tested with molecular markers for presence of resistant alleles of R1 and R3 genes. Resistant allele of R1 gene was detected with marker 76-2S according to protocol developed by Ballvora *et al.* (2002) and resistant allele of R3 was detected with marker RT-R3a_L01 according to protocol of Huang *et al.* (2005).

Leaf and tuber tests

The leaflets were collected from plants grown in the field in the beginning of flowering. Inoculum

applied comprised 20000 sporangia ml⁻¹. Symptoms were observed on 6th day after inoculation using grade scale 1-9, where grade 9 is highest resistance, no disease symptoms observed (Zarzycka, 2001).

Tuber test was performed approximately two months after harvesting. Method of tuber testing described by Zarzycka (2001) was applied using the same *P. infestans* isolates and inoculum concentration as in leaflet tests.

Analysis of variance was done using Minitab 15. The average assessment values were compared using T-test.

RESULTS AND DISCUSSION

Potato clones field (foliar) resistance assessment in field conditions depending on presence of resistance genes

Percentage of foliage damaged by disease was assessed and ranged from 0% to 70 % in 2010, and from 5% to 100% in 2011. The first symptoms of late blight were recorded in the first dates of July in both years. The intensive disease invasion was observed in mid of August after several rainfalls. The infection of *P. infestans* was higher during 2011 than 2010, when climatic factors of second part of growing season were more favourable for disease development.

P. infestans isolates sampled from the field in 2010 were complex and showed large spectrum of genes for virulence on the leaflets of R-1 - R11 Black's differential genotypes. From six (1.3.4.7.10.11.) to ten (1.2.3.4.(5).6.7.(8).10.11.) genes for virulence were detected in tested isolates.

The presence of resistant allele of R1 gene was detected for 8 %, and the presence of resistant allele of R3 gene in 27 % of tested clones. Presence of resistant alleles of both genes was detected in 1.9 % of breeding clones. The source of genes for resistance found in breeding clones were parental varieties, mostly containing resistance genes derived from *Solanum demissum* Lindl.

Presence of resistant allele of R1 gene was almost equal in all groups of clones classified by the level of disease damage (Table 1). In more clones resistant allele of R3 gene was detected compared to resistant allele of R1 gene. In groups of clones characterized by disease development from 0% to 30 % and from 31% to 50% presence of resistant allele of this gene was detected for similar amount of clones as other groups of clones with higher diseases development level. There was not observed dependence of field (foliar) resistance to late blight on detected presence of resistant alleles of tested genes. There was not found significant difference between diseases damages level between groups with detected presence of resistant or susceptible alleles of tested genes ($p>0.05$).

Table 1. Distribution of clones differed by diseases damages level into three groups: with detected presence of resistant to P. infestans alleles of R1 or R3 genes and with detected presence of susceptible alleles of genes, Priekuli, 2011

| Disease damaged area from total foliage,% | Number of tested clones | Percentage of total number of clones, % | | |
|---|-------------------------|---|---|--|
| | | Presence of resistant allele of R1 gene | Presence of resistant allele of R3 gene | Presence of susceptible alleles of R1 and R3 genes |
| 0% - 30.0% | 20 | 10 | 25 | 65 |
| 31% - 50.0% | 21 | 10 | 29 | 61 |
| 51 % - 70.0 % | 39 | 6 | 28 | 66 |

The analyses of pedigree of tested potato clones revealed that one third of clones with low diseases damage (less than 30 %) were progenies of variety 'Zarevo'. High resistance level to *P. infestans* of variety 'Zarevo' was commonly reported. In late 90's this cultivar was identified as one of the most resistant to *P. infestans* among European cultivars (Douches *et al.*, 1997, Bisognin *et al.* 2002). The variety is an interspecific hybrid of crosses of *S. tuberosum* L. with germplasm of *S. leptophyes* Bitt., *S. demissum* Lindl. and *S. andigenum* Juz. et Buk. (Swiezynski *et al.*, 1997).

Evaluation of leaf and tuber resistance

High and relatively high leaf resistance to *P. infestans* (resistance score up to 5) was detected for 10 % of tested clones. The presence of one of resistance allele of tested genes were detected for only two out of seven resistant clones, R1 for one and R3 for other. Among clones expressed susceptibility in leaf tests (resistance score less than 5) in about one third of clones' presence of resistant allele of genes have been detected. For one clone presence of resistant alleles of both genes was detected. The presence of resistant allele of R1 gene was detected in 5 clones and presence of resistant allele of R3 gene in 15 ones in this group. There was not found significant difference between leaf resistance assessment between groups with detected presence of resistant or susceptible alleles of tested genes ($p>0.05$).

High tuber resistance (resistance score up to 5 grade on average) was found for 42 % of tested clones. Only in one of 12 clones' resistant alleles of both genes was detected. In group of clones with high tuber resistance the presence of resistant allele of R1 gene was detected in two and presence of resistant allele of R3 gene in seven clones. In clones with low tuber resistance presence of resistant allele of R1 gene was detected in 4 clones and presence of resistant allele in R3 gene for 3 clones. Distribution of clones with resistant or susceptible R1 or R3 genes alleles within whole amount of tested clones is shown in figure 1. The proportions of clones with detected presence of resistant allele of R1 or R3 genes and clones with detected susceptible alleles within the groups of clones with high and low tuber resistance differed. However, within the group of clones with high tuber resistance to *P. infestans* the share of those with detected presence of resistant allele of R3 gene was much higher than in group of clones with low tubers resistance. Share of clones with detected resistant allele of R1 gene was larger in group of clones with low tuber resistance then in group with high tuber resistant. In evaluated clones presence of resistant allele of gene R3 significantly ($p=0.012$) influenced tuber resistance. If the influence of each gene was analysed separately, a significant influence of resistance gene was detected for R3 only ($p=0.001$) and not in case of R1 ($p>0.05$). Comparing clones with presence of resistant allele of R3 gene with clones with presence resistant allele of R1 gene significant difference was found ($p=0.039$)

No significant differences between resistance levels of group with detected presence of resistant allele of R1 gene with group of clones with detected presence of susceptible alleles of tested genes ($p>0.05$) were found.

One of breeding clones (nr.322 on Figure 2.) was found to be the most resistant (resistance score 7.8) but neither presence of resistant allele of R1 nor presence of resistant allele of R3 was detected.

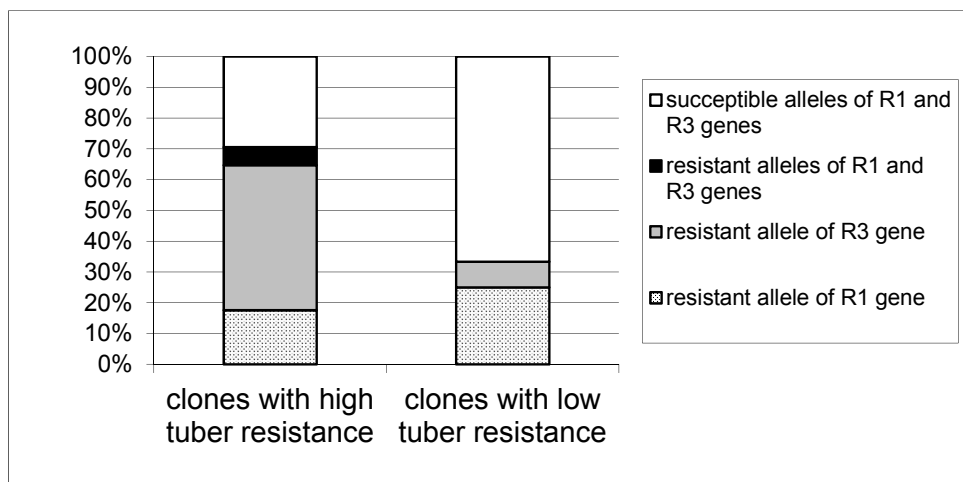


Figure 1. Distribution of clones with detected presence of resistant allele of R1 gene, with detected presence of resistant allele of R3 gene allele and with susceptible those genes alleles, within the group of clones with low tuber resistance and clones with high tuber resistance.



Figure 2. Potato breeding clones with different tuber resistance levels.

Complex evaluation of potato clones for tested traits

Comparing groups of potato clones with resistance or susceptibility detected by molecular markers, the slightly increased field resistance (comparing grade of disease damages) in 2011 was noted for groups in which resistance was declared (Table 2.). The average leaf resistance grade was higher for both groups with detected presence of resistant alleles of R1 and R3 genes compared to group with susceptible plants. Comparison of mean values using T-tests showed that the difference was

not significant with confidence level 95 %. The group of plants with resistant allele of R3 gene had higher resistance grade on average than group with susceptibility, but difference was not significant ($p>0.05$). The average tuber resistance grade for potato clones with resistant allele of R1 gene was lower than potato clones with susceptible allele, but difference was not significant, comparing mean values using T-test ($p>0.05$).

Table 2. The evaluation of potato clones general resistance to late blight in field conditions, leaflet and tuber tests (grade scale 1 – 9, where 9 is the most resistant or less damages)

| Potato clones | Number of tested clones | Average grade of disease damage | | Average grade of leaf resistance | Average grade of tuber resistance |
|--|-------------------------|---|------|----------------------------------|-----------------------------------|
| | | 2010 | 2011 | | |
| | | Clones with detected presence of susceptible alleles of R1 and R3 genes | 6 | | |
| Clones with detected presence of resistant allele of R1 gene | 4 | 8.8 | 7.3 | 4.1 | 5.0 |
| Clones with detected presence or resistant allele of R3 gene | 6 | 8.8 | 6.3 | 5.2 | 5.6 |

$t_{stat} < t_{crit}, p>0.05$

For evaluation of race specific genes R1 and R3 impact on general resistance to late blight more data have to be obtained. In our research influence of R1 and R3 gene resistant alleles' presence on foliage resistance in field and leaf resistance in laboratory was not observed. Data obtained partly confirm the data reported by Umaerus and Umaerus (1994) postulating that the presence of resistance R genes could improve genotypes for tuber resistance to late blight. Presence of resistant allele of gene R3 gene in evaluated clones significantly ($p<0.05$) influenced tuber resistance. If compare with clones with detected presence of susceptible alleles of both genes or resistant allele of R1 gene Stacking of multiple resistance (R) genes is considered to be one of the most promising approaches to provide durable resistance to potato late blight (Li *et al.* 2011) Recent data show that stacking of multiple genes as in variety 'Sarpö Mira' (R3a, R3b, R4, Rpi-smira1 and Rpi-smira2) provide durable resistance (Rietman, 2011). The future work should be focused on stacking additional genes to R1 and R3 to obtain higher resistance level.

As a result of the analysis of the data obtained in 2010 and 2011 seasons, the potato clones combining foliar, leaf and tuber resistance to *P. infestans* were identified.

CONCLUSIONS

No significant influence of resistant alleles of R1 and R3 gene on foliar (field observations) and leaf (leaflet tests) resistance levels was found.

The significant difference was found between resistance level of clones with detected presence of resistant allele of R3 gene and clones with detected presence of susceptible alleles of both tested genes and clones with detected presence of R1 gene.

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LEAFY intron 2-based markers of wild *Solanum* genomes for introgression breeding

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SUMMARY

SCAR (sequence characterized amplified region) markers developed from polymorphic sequences of FLORICAULA/LEAFY intron 2 (FLint2) discern genomes A, B and D and subgenomes A1-A3 in tuber-bearing *Solanum* species. Screening 125 *Solanum* accessions representing 26 species from six series of section Petota produced the evidence mostly consistent with the established genome classification in wild *Solanum* species (Hawkes, 1990; Matsubayashi, 1991). Potato hybrids with several *Solanum* species listed in their pedigrees mostly retained FLint2 markers of introgressed wild germplasms.

KEYWORDS

FLORICAULA/LEAFY, *Solanum* genomes, taxonomy of section Petota, FLint2-derived markers

INTRODUCTION

Many wild tuber-bearing *Solanum* species (section Petota) are important sources of late blight resistance transferred to the cultivated potato by sexual and somatic hybridization or by genetic engineering, and DNA markers considerably facilitate introgression of resistance genes (Simko *et al.*, 2007). In particular, these markers would assist monitoring the transfer of alien germplasm and its further loss from the hybrids as the latter go through backcrosses and are finally maintained as registered potato varieties.

FLORICAULA/LEAFY is a nuclear-encoded homeotic gene in control of several key morphogenetic processes, including floral transition (Moyroud *et al.*, 2010). The gene contains two introns, and the polymorphisms of intron 2 (FLint2) are widely employed by plant molecular taxonomists (for recent references see Peng *et al.*, 2010; Zheng *et al.*, 2011). Smith and Baum (2006) were first to use FLint2 for the systematics of Solanaceae (Physaleae). Earlier we described manifest inter- and intraspecific polymorphisms of FLint2 in *S. demissum* and *S. tuberosum ssp. tuberosum* (Drobyazina and Khavkin, 2007). Here we report a set of FLint2-derived SCAR markers, which discriminate between genomes A, B and D in the section Petota.

MATERIALS AND METHODS

Seeds and microtubers of wild *Solanum* species were obtained from the Vavilov Institute of Plant Industry, Russia (VIR), The Centre for Genetic Resources, the Netherlands (CGN), and NRSP-6 Potato Genebank, USA (PI), and potato tubers, from VIR and the Institute of Potato Husbandry, Russia. Genomic DNA was isolated from green leaves of individual plants by a modified STAB method (Doyle and Doyle, 1987) or with AxyPrep™ Multisource Genomic DNA Miniprep kit (www.axxygenbio.com/products). Conservative primers for gene regions within exons 2 and 3 (Fig. 1) described previously (Drobyazina and Khavkin, 2007) were used to amplify FLint2 sequences. Standard protocols were employed for PCR amplification and cloning gene fragments. The programs BLAST 2.2.26 (blast.ncbi.nlm.nih.gov/Blast.cgi) and WU-BLAST (www.ebi.ac.uk/Tools/sss/wublast/nucleotide.html) were used for mining databases. For phylogenetic analysis, we used the matrix of pair distances and the Neighbor Joining clustering algorithm with the MEGA5 software (Tamura *et al.*, 2011).

RESULTS AND DISCUSSION

First, we obtained 32 FLint2 sequences from nine *Solanum* species representing five series of the section Petota (Table 1). The intraspecific homology varied from 79 to 100 %, with most variable FLint2 sequences found in polyploid *S. demissum* and *S. stoloniferum* and practically identical sequences, in such diploid species as *S. verrucosum* and *S. bulbocastanum*. The phylogenetic analysis separated the cloned FLint2 sequences into several discernable clusters presumably corresponding to different *Solanum* genomes and subgenomes.



Figure 1. Primers (LFYex2-F/LFYex3-R) flanking FLORICAULA/LEAFY intron 2 (FLint2) against the prototype gene sequence (Genbank accession number EU371047).

| Ploidy | Species | Genbank accession numbers | Homology, % |
|-----------|--|---|-------------|
| | <i>S. bulbocastanum</i> | JQ617270, JQ617271 | 94 |
| | <i>S. cardiophyllum</i> | JQ617268, JQ617269, JQ617276 | 89-100 |
| Diploid | <i>S. ehrenbergii</i> | JQ617264, JQ617265, JQ617272, JQ617273 | 95-98 |
| | <i>S. microdontum</i> | JQ617256- JQ617261 | 100 |
| | <i>S. pinnatisectum</i> | * | 99 |
| | <i>S. verrucosum</i> | JQ617266, JQ617267, JQ617274, JQ617275 | 97-100 |
| | <i>S. stoloniferum</i> (4n) | JQ617262, JQ617263, JQ617277- JQ617279 | 80-97 |
| Polyploid | <i>S. tuberosum</i> ssp. <i>tuberosum</i> (4n) | DQ266895, DQ256076, DQ285574- DQ285576, | 82-93 |
| | <i>S. demissum</i> (6n) | DQ256075, DQ266893, DQ266894 | 79-89 |

* Two sequences under registration

Table 1. The intraspecific homology of FLint2 sequences.

To verify FLint2 markers of *Solanum* genomes, we screened 125 accessions representing 26 species from six series of section Petota (Table 2). The data thus obtained are mostly consistent with the established genome classification in wild *Solanum* species section Petota (Hawkes, 1990; Matsubayashi, 1991).

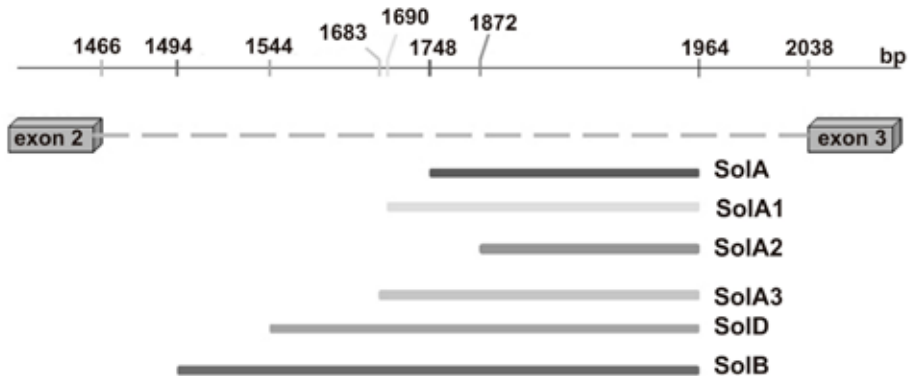


Figure 2. Markers of *Solanum* genomes and subgenomes derived from FLint2 sequences. Marker positions are shown regarding the prototype gene EU371047.

The genome A cluster included FLint2 sequences from *S. verrucosum* (A), *S. tuberosum* ssp. *tuberosum* (AA), and *S. microdontum* (A). One of two FLint2 variants from *S. stoloniferum* and one of three FLint2 sequences cloned from *S. demissum* also belonged to genome A cluster. In addition, our analysis distinctly discriminated between genomes A in *S. verrucosum* and *S. microdontum*, the species corresponding to putative subseries *tuberosa1* and *tuberosa3* (Hawkes, 1990). *S. stoloniferum* contained genome A variant found in *S. verrucosum*, whereas *S. tuberosum* apparently comprised both these variants. The genome B cluster embraced FLint2 sequences from *S. bulbocastanum*, *S. cardiophyllum* and *S. ehrenbergii* and one of two FLint2 variants found in *S. stoloniferum*. Genomes of *S. cardiophyllum*, *S. ehrenbergii* and *S. pinnatisectum* (the latter was designated by Matsubayashi, 1991, as ApiApi) were in fact closer to genome B than to genome A. FLint2 polymorphisms definitely separated the *pinnatisectum* genome from two former genomes B. This evidence seems to support the existence of the characteristic genome Bpi. Finally, two of three FLint2 variants from *S. demissum* clustered separately from all other species; presumably, they correspond to genome D. FLint2 sequences from *S. bulbocastanum* were evidently discriminated from *S. cardiophyllum* and *S. ehrenbergii*, and FLint2 sequences from *S. stoloniferum* clustered with two latter rather than with the former species. These observations support the conclusions by Spooner and his associates (Pendinen *et al.*, 2008; Spooner *et al.*, 2008; Rodriguez and Spooner, 2009) made previously on the basis of other polymorphic fragments of wild *Solanum* genomes. They are contrary to the suggestion that *S. bulbocastanum* provided genome B germplasm to *S. stoloniferum*; the latter presumption is based on the identity of the RB/Rpi-*blb1* gene sequences in two species (Wang *et al.*, 2008).

Table 2. Genomes and subgenomes in *Solanum* species section *Petota* tentatively discerned with FLint2 markers

| Series* | Species | Ploidy | Genomes after** | | Genomes and subgenomes*** |
|--|---|--------|-----------------|-------------|---------------------------|
| | | | 1 | 2 | |
| TUB | <i>S. berthaultii</i> Hawkes | 2n | | A1A1 | A3 |
| | <i>S. brevicaule</i> Bitter | 2n-4n | | | A1A2A3D |
| | <i>S. immite</i> Dunal | 2n | | A1A1 | A2 |
| | <i>S. kurtzianum</i> Bitter&Wittm. | 2n | | | A1 |
| | <i>S. marinasense</i> Vargas | 2n | | | A2 |
| | <i>S. microdontum</i> Bitter | 2n | | A1A1 | A3 |
| | <i>S. phureja</i> Juz.&Bukasov | 2n | | | A1 A2 |
| | <i>S. spegazzinii</i> Bitter | 2n | | | A1 A2 |
| | <i>S. vernei</i> Bitter&Wittm. | 2n | | | A1 A2 |
| | <i>S. verrucosum</i> Schldtl. | 2n | AA | A1A1 | A1 |
| | <i>S. tuberosum</i> ssp. <i>andigenum</i> (Juz.&Bukasov)Hawkes | 4n | | | A1A3 |
| <i>S. tuberosum</i> ssp. <i>tuberosum</i> L. | 4n | | | A1A2 / A1A3 | |
| LON | <i>S. fendleri</i> A. Gray | 4n | | | A1B |
| | <i>S. hjertingii</i> Hawkes | 4n | | | A1B |
| | <i>S. stoloniferum</i> Schldtl. | 4n | AABB | A4A4BB | A1B |
| DEM | <i>S. brachycarpum</i> Correll | 6n | | | A1A3 |
| | <i>S. demissum</i> Lindl. | 6n | AADDDdDd | A1A4[B,C,D] | A1D |
| | <i>S. hougassii</i> Correll | 6n | | | A1B |
| | <i>S. iopetalum</i> (Bitter)Hawkes | 6n | | | A1 |
| BUL | <i>S. bulbocastanum</i> Dunal | 2n | AbAb | BB | B |
| PIN | <i>S. cardiophyllum</i> Lindl./ <i>S. ehrenbergii</i> (Bitter)Rydb. | 2n | ApiApi | | B |
| | <i>S. jamesii</i> Torr. | 2n | ApiApi | | B |
| | <i>S. pinnatisectum</i> Dunal | 2n | ApiApi | | B (Bpi?) |
| | <i>S. tarnii</i> Hawkes&Hjert. | 2n | | | B |

*Series in the section *Petota*: TUB – *Tuberosa* (Rydb.)Hawkes; LON – *Longipedicellata* Buk.; DEM – *Demissa* Buk.; BUL – *Bulbocastana* (Rydb.)Hawkes; PIN – *Pinnatisecta/Cardiophylla* (Rydb.)Hawkes; **As classified by (1) Matsubayashi, 1991, and (2) Hawkes, 1990: ***Molecular evidence from FLint2 polymorphisms.

FLint2 polymorphic sequences were further employed to develop SCAR markers discerning different *Solanum* genomes A, B and D. Positions of these markers are schematically presented in Fig. 2. We also managed to discriminate between three variants of genome A (tentative subgenomes A1, A2 and A3) corresponding to *tuberosa* 1, *tuberosa* 2 and *tuberosa* 3 subseries as suggested by Hawkes (1990). Subgenome A2 was detected in the species belonging both to the *tuberosa* 2 subseries (*S. brevicaule*, *S. immite*, *S. marinasense*) and to the *tuberosa* 3 subseries (*S. phureja*, *S. spegazzinii* and *S. vernei*). Two subspecies of tetraploid *S. tuberosum* include subgenomes A1A2 or A1A3. All analyzed species from the series *Longipedicellata* comprised subgenome A1. This evidence supports the cytogenetic observations by Pendinen *et al.* (2008) who presumed that *S. verrucosum* was the donor of genome A for *S. stoloniferum*. A separate subcluster characteristic for *S. demissum* seems to correspond to genome D; meanwhile other *Demissa* species (*S. brachycarpum*, *S. iopetalum* and *S. hougassii*) lacked the putative FLint2 marker of genome D. At the same time, *S. hougassii* contained the genome B marker.

Are FLint2 markers of wild *Solanum* genomes maintained in the registered potato cultivars following

several crosses and backcrosses? When the genome-specific FLint2 markers described above were used to screen potato hybrids highly resistant to late blight and combining several wild *Solanum* germplasms (strictly speaking, with several *Solanum* species listed in their pedigrees), most hybrids retained FLint2 markers of genomes A1, A3 and D, and some, genome B.

CONCLUSIONS

SCAR markers for *Solanum* genomes were developed from the polymorphic sequences of FLORICAULA/LEAFY intron 2 (FLint2). Screening 26 species representing six series of the tuber-bearing section Petota demonstrated that these markers presumably discerned genomes A, B and D and in particular subgenomes A1-A3. Potato hybrids with several *Solanum* species listed in their pedigrees mostly retained FLint2 markers of introgressed genomes.

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**Zoospore production in relation to temperature for current
P. infestans genotypes**

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Zoospore production in relation to temperature for current *P. infestans* genotypes



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Introduction

The production of zoospores is a key process in tuber infection by the blight pathogen. This work investigated the optimum temperature for zoospore production by two new genotypes of *P. infestans* compared with six established ones. Earlier work at James Hutton Institute, funded by the Potato Council, demonstrated that the new genotype 13_A2 had a clear competitive advantage in foliar aggressiveness at the relatively low temperature of 13 °C. The work reported here investigated whether this genotype also has a different optimum temperature for zoospore production compared with established genotypes.



Methods

Indirect germination (the germination of sporangia to produce zoospores) was assessed at eight temperatures, i.e. 4, 6, 8, 10, 12, 14, 16 and 18 °C. These covered the range prevailing later in the growing season when the risk of tuber infection is higher. Two new genotypes of *P. infestans*, 6_A1 and 13_A2, were compared with six genotypes that had been detected in the GB population for many years, i.e. 1_A1, 2_A1, 3_A2, 7_A1, 8_A1 and 10_A2. Isolates were collected in 2006 to 2008. Standardised suspensions of sporangia of two isolates of each genotype were incubated for 24 hours and the incidence of indirect germination recorded.

Results and conclusions

Almost all previous reports stated an optimum temperature range for zoospore production between 10 and 16 °C, i.e. 12 to 13 °C (Crosier, 1934), 12 to 16 °C (Bohnen, 1963), 12 °C (Yamamoto & Tanino, 1961) and 10 to 12 °C (Schrodter & Ullrich, 1967). In the study reported here the overall optimum temperature for zoospore production by the eight genotypes was 4 °C but the values for 6 and 8 °C were not significantly lower. Shaw *et al.* (2006) observed a similar optimum temperature range

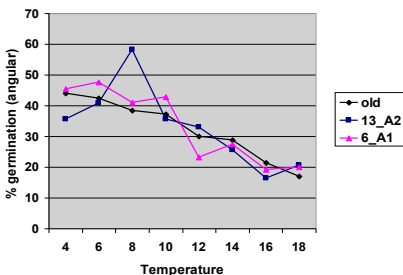


Fig. 1 Indirect germination (%) of sporangia of six old and two new genotypes (13_A2 and 6_A1) in relation to temperature ($P=0.05$, LSD 7.2)

| Genotype | Temperature (°C) | | | | | | | |
|----------|------------------|------|------|------|----|----|----|----|
| | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 |
| 1_A1 | 42.4 | 45.0 | | 43.0 | | | | |
| 2_A1 | | | 47.6 | | | | | |
| 3_A2 | 43.9 | | 47.3 | | | | | |
| 6_A1 | 45.4 | 47.5 | 41.0 | 42.8 | | | | |
| 7_A1 | | 46.6 | | | | | | |
| 8_A1 | 56.7 | 62.5 | | | | | | |
| 10_A2 | 53.4 | | | | | | | |
| 13_A2 | | | 58.3 | | | | | |

Table 1 Optimal incidences (%) of indirect germination of sporangia at different temperatures (angular transformed data). Values shown for the same genotype are not significantly different.

of 5 to 9 °C for 11 isolates collected and tested on leaves in 2004 and 2005, prior to the new genotypes 13_A2 and 6_A1 dominating the GB population.

In the experiment reported here, at most temperatures the difference in indirect germination between the new and old genotypes was small (Fig. 1). However, at 8 °C the incidence of indirect germination was significantly greater for 13_A2 than the established genotypes. For some genotypes there was a clear optimum temperature, e.g. as already stated 8 °C for 13_A2, but for others the optimum was a wide range, e.g. 6_A1 produced most zoospores between 4 and 10 °C (Table 1).

In conclusion this study suggests that the optimum temperature range for zoospore production is not substantially different for new compared with old genotypes. Differences between recent and much earlier experiments are probably due to differences in methodology.

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Glycoalkaloid content in potato tubers with different levels of resistance to *Phytophthora infestans*

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SUMMARY

In this paper the results of investigation of relationship between potato plant tuber resistance and glycoalkaloid content are reported. The plant material was represented by *Tuberosum* type breeding lines and potato cultivars as well as by accessions of four *Solanum* species and two interspecific hybrids. Within *Tuberosum* accessions tuber resistant, moderately resistant and susceptible clones were registered. Wild species accessions and interspecific hybrid populations were segregating for resistance. Glycoalkaloid content data was also characterized by a wide range of values. The α -solanine and α -chaconine content varied between different types of potatoes (*Tuberosum* contra wild species) or between groups with different resistance levels. Spearman analyses showed that in potato species accessions as well in interspecific hybrids there were no significant correlations between resistance and α -solanine as well as α -chaconine contents. A significant correlation (Spearman's rho=0.224, p=0.03) was however observed between lesion size and α -chaconine in the *Tuberosum* material.

KEYWORDS

α -solanin, α -chaconine, TGA, late blight

INTRODUCTION

Many Solanaceae family members synthesize steroidal glycoalkaloids; to date there are over 90 described in the literature with diverse chemical structures. α -chaconine and α -solanine are the most abundant (95%) in most commercial potatoes and are often referred to as Total GlycoAlkaloids (TGA). The average ratio of α -solanine to α -chaconine is 40:60, but deviations have been reported. Glycolakaloid content varies depending on tissue as well as between species and cultivars and expression is determined by both genetic and environmental factors (Friedman 2006; Friedman and McDonald 1997; Maga 1994). Many of the *Solanum* L. species of interest to potato breeders may contain levels in excess of 200 mg/kg (fresh weight) which is the standard maximum level allowed by international health regulations.

It has been assumed that glycoalkaloids are a part of the natural defence against some pests as well as diseases. The situations in Colorado Potato Beetle (CPB) (Tingey, 1984; Deahl *et al.*, 1991), potato cyst nematode (Grassert and Lellbach, 1987), leafhopper (Sanford *et al.*, 1992) as well as in the diseases caused by *Erwinia carotovora* subsp. *atroseptica* (Andrивon *et al.*, 2003), *Rhizoctonia solani* (Morrow and Caruso, 1983) and *Phytophthora infestans* (Deahl *et al.*, 1973; Sarquis *et al.*, 2000; Andrивon *et al.*, 2003) have been investigated. Results showed a significant influence of glycoalkaloid content on resistance to CPB (Deahl *et al.*, 1991). The steroidal aglycone leptine (a form of solanidine) found in *S. chacoense* was identified as responsible for resistance to CPB. To achieve resistance against CPB in potato cultivars, high-leptine genotypes of *S. chacoense* were integrated in a breeding program (Yencho *et al.*, 2000). No influence of glycoalkaloid content on tuber resistance to late blight was found in the study performed by Deahl *et al.* (1973). Total glycoalkaloid contents from blight-infected plants were not significantly different than TGA contents from healthy plants (Deahl *et al.*, 1973). Data obtained by Sarquis *et al.* (2000) showed that the correlation between tuber and foliage alkaloids is poor. In view of the observed field resistance to late blight, it was also concluded that tuber glycoalkaloid content might not be responsible for such resistance. The results of research performed by Andrивon *et al.* (2003) indicated that neither race-specific nor partial resistance to late blight and soft rot in the accessions used as progenitors of resistance depend on high α -solanine or α -chaconine concentrations. With the exception of low, but statistically significant, correlations between the concentration of α -solanine and late blight resistance in progenies derived from *S. vernei*, no consistent relationship between resistance to disease and concentrations of α -solanine and/or α -chaconine was observed (Andrивon *et al.* 2003).

Sources of resistance to *P. infestans* found in potato species are intensively used in potato breeding. The glycoalkaloid content in such plant material exceeds these in cultivated potatoes (Sarquis *et al.*, 2000). The composition of glycoalkaloids in potato hybrids derived from some wild species show, beside α -chaconine and α -solanine, the specific glycoalkaloid demissidine (Mattheij *et al.*, 1992). Tuber resistance to *P. infestans* has been found in a number of wild potato species (Zoteyeva, 2006). For breeding purposes it is therefore important to find and select breeding lines derived from wild potato species that possess traits for both resistance and low glycoalkaloid content.

The aims of our study were

- 1) to investigate if a relationship exists between the *P. infestans* tuber resistance components mycelium growth and lesion size on one hand and TGA, α -chaconin and α -solanin concentrations in tubers on the other hand
- 2) to study if the proportion of α -solanine to α -chaconine varies between different types of potatoes (*Tuberosum* contra wild species) or between groups with different resistance levels.

MATERIALS AND METHODS

The study was performed at Alnarp (southern Sweden), Swedish University of Agricultural Sciences University during two years (2008 and 2010).

Tubers of 12 *Tuberosum* breeding lines, 4 cultivars, 15 accessions from different *Solanum* species (*S. andigenum*, *S. neoantipoviczii*, *S. papita* and *S. ruiz-ceballosii*) and 12 interspecific hybrids (derived from crosses using *S. microdontum*, *S. neoantipoviczii*, *S. tarijense* and *S. phureja*) were evaluated for tuber resistance to *P. infestans* as well as for TGA, α -solanine and α -chaconine contents. The tubers originated from field grown plants.

Evaluation of tuber resistance

Tuber resistance to *P. infestans* was evaluated by inoculating tubers with an aggressive isolate (SE 03058, mating type A1, virulence genes 1.3.4.7.10.11). The inoculum comprised 20,000 sporangia/

ml. The incubation period lasted 6 days and the disease rating was performed on the 7th day after inoculation. Mycelium growth was scored using a scale 0–3 (0=lack of mycelium and 3=very abundant growth) and afterwards lesion sizes were scored on longitudinally cut tubers using a scale with grades 1 – 9 (1=up to 90% of diseased area and 9=highest resistance, no lesion).

Quantification of glycoalkaloid content

For TGA analysis, five tubers from each genotype were selected and rinsed in tap water. Each sample was finely diced (skin and cortex) and mixed. Then 20 g was homogenized with an Ultra Turrax homogenizer TP 18/10 with shaft 18-N (Janke & Kunkel KG, IKA-Werk, D-7813, Staufen, Germany) for 2 min with 100 ml water:acetic acid:ascorbic acid, 100:5:1 (vol/vol/wt). The volume was adjusted to 200 ml with the same solvent, clarified by centrifugation at 4°C at 10,000 rpm (Sorvall Evolution RC) for 10 min and filtered through 1F (Munktells). Ten ml of the supernatant was put onto a Sep-Pak C18 cartridge previously activated by acetonitrile in accordance with the method of Hellenäs and Branzell (1997), which was also used for the subsequent analytical procedure that was performed on HPLC. The α -solanine and α -chaconine (Sigma Chemical Co.) were used as standards. The concentrations were given in mg/kg fresh weight (FW).

Statistical analysis

Spearman 1-tailed analyses of correlation were performed with the computer program SPSS.

RESULTS AND DISCUSSION

Average TGA content found in tubers from accessions of wild species and interspecific hybrids from wild parents (838 mg/kg FW) highly exceeded the one in the *Tuberosum* group (135 mg/kg FW). None of the cultivars and only one of the breeding lines showed TGA values exceeding the maximum level allowed by the international health regulations whereas only four of the wild species and interspecific hybrids had acceptable TGA values. This shows the importance of regular analyses of TGA contents of wild accessions and their hybrids during the breeding process.

No significant correlations were found neither between TGA or α -solanine and the two evaluated resistant factors nor between α -chaconine and mycelium growth in the *Tuberosum* material. A significant correlation (Spearman's $\rho=0.224$, $p=0.03$) was however observed between lesion size and α -chaconine in this material (Table 1). In the wild potato species as well as in the interspecific hybrids no significant correlation were found between the resistance components (lesion size and mycelium growth) and α -chaconine or α -solanine.

The material was sorted in 3 resistance groups (HR=high resistance (grades 7.0-9.0), MR=moderate resistance (5.0 to 6.9) and S=susceptible (1.0 to 4.9)) depending on results in the tuber inoculation test (lesion size). The HR-group consisted of 5 breeding lines and 12 wild species accessions/interspecific hybrids. Five breeding lines and cv. Bintje were found susceptible together with 4 wild accessions/interspecific hybrids. The rest of the material, including cv. Asterix, Matilda and Superb, had moderate resistance levels.

All *Solanum* species accessions and interspecific hybrids strongly segregated for tuber resistance to *P. infestans*. Resistance in the accession of *S. andigenum* was scored with average grade 6.8 and ranged from 4.5 to 7.3. Average mycelium growth was 0.4 grades. Tubers of all tested plants of *S. neoantipoviczii* expressed resistance scored 7.7 on average and were lacking mycelium growth. Plants of *S. papita* segregated in the proportion 1S:1MR:2R. Their resistance was scored on average 5.7. The accession of *S. ruiz-ceballosii* segregated for resistance in equal proportions (1R:1MR:1S). The hybrid

Table 1. Correlations between late blight resistance components (lesion size and mycelium growth intensity) and α -chaconine, α -solanine and total glycoalkaloid content in tubers of potato accessions.

| Spearman's rho | | α -solanine | α -chaconine | TGA | Mycelia | Lesion |
|---------------------|-------------------------|--------------------|---------------------|----------|---------|-----------|
| α -solanine | Correlation Coefficient | 1,000 | ,848(**) | ,920(**) | -,120 | ,029 |
| | Sig. (1-tailed) | . | ,000 | ,000 | ,153 | ,404 |
| α -chaconine | Correlation Coefficient | | 1,000 | ,983(**) | -,181 | ,224(*) |
| | Sig. (1-tailed) | | . | ,000 | ,060 | ,027 |
| TGA | Correlation Coefficient | | | 1,000 | -,181 | ,181 |
| | Sig. (1-tailed) | | | . | ,060 | ,061 |
| Mycelia | Correlation Coefficient | | | | 1,000 | -,465(**) |
| | Sig. (1-tailed) | | | | . | ,000 |
| Lesion | Correlation Coefficient | | | | | 1,000 |
| | Sig. (1-tailed) | | | | | . |

between *S. microdontum* and *S. tarijense* showed a distribution for resistance 1R:1S with mycelium growth scored 0.9 on average. Lack of mycelium growth was noted on all tubers of another hybrid (*S. neoantipoviczii* \times *S. phureja*). Its tuber resistance was scored on average 7.1 and ranged from 6.0 to 8.4.

As noted in other studies, α -chaconine was the predominant glycoalkaloid in all cultivars and breeding lines in the *Tuberosum* group and in most of the accessions from the wild species/ interspecific hybrids when studied for the 2008 analyses. Only in six out of nine of the hybrids between *S. neoantipoviczii* and *S. phureja* as well as in one of the three accessions belonging to the species *S. neoantipoviczii* α -solanine was the predominant glycoalkaloid. For the rest, α -chaconine was predominant just as in the *Tuberosum* group. Also when average values were calculated for the three different resistance groups of the accessions from the wild species/interspecific hybrids α -chaconine was the predominant glycoalkaloid. The percentage of α -chaconine out of the total glycoalkaloid content were higher in the *Tuberosum* group compared to the group of wild species and interspecific hybrids (Fig. 1, 1–6). However, the different resistance groups showed slightly different proportions between the two glycoalkaloids and the domination of α -chaconine was most evident for the most sensitive genotypes in both groups.

The conclusions about the lack of relationship between glycoalkaloid content and tuber resistance to late blight have been made in investigations performed at different sites and time periods (Deahl *et al.*, 1973; Sarquis *et al.*, 2000). In the results of a study performed by Andrivon *et al.* (2003) the correlations between concentration of α -solanine and two late blight resistance components (incubation period and spore production per unit lesion area) was found. In opposite, in our study, the higher content of α -chaconine was found might be responsible for resistance. One explanation for the differences in results obtained might be genetically determined glycoalkaloid concentrations in potato (Kozukue *et al.*, 2008). Beside differences in genetic background of tested materials the

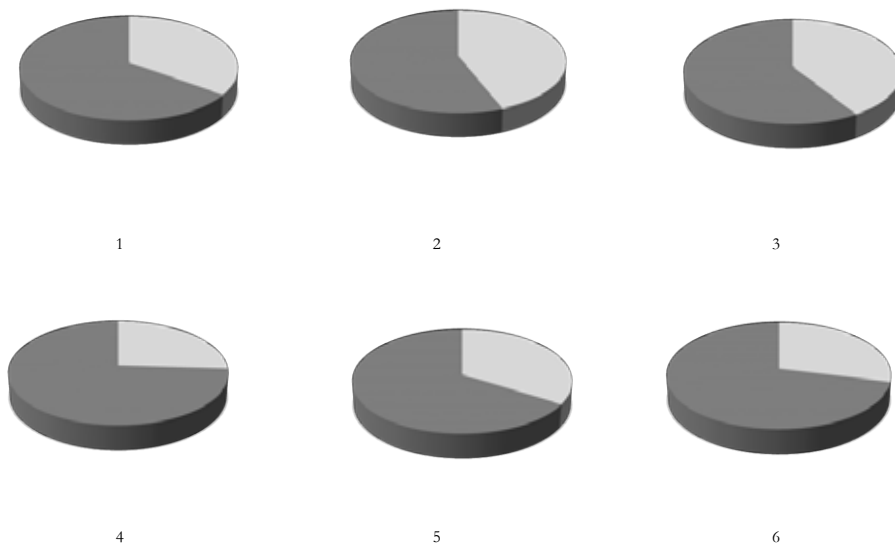


Figure 1. Ratio of α -solanine and α -chaconine in tubers from accessions originating from wild species and interspecific hybrids (1–3) and genotypes of *Tuberosum* type (4–6). S=1 and 4, MR=2 and 5 HR=3 and 6. Dark grey = α -chaconine, light grey = α -solanine.

glycoalkaloid content may be strongly affected by environmental factors, particularly by light and limiting temperature regimes (Dao and Friedman, 1994; Bowles *et al.*, 2006).

ACKNOWLEDGEMENTS

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Genotypic variation of *Phytophthora infestans* populations in Argentina

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KEYWORDS

Potato late blight, Argentina, population genetics, simple sequence repeats, mating type, mitochondrial DNA haplotype, genotyping

INTRODUCTION

Potato is an important staple crop in Argentina and their production fluctuates over the years, reaching in 2010 almost 2 million tonnes (FAOSTAT, 2012), for domestic market. Spunta is the most important potato variety with more than 70% of the national production for fresh market (Rodríguez Quijano, P., 1989; private and official estimates not published, 2011). Based on production data, recent statistics positioned Argentina in 2010 in the 30th place in the world and fourth in Latin America (FAOSTAT, 2012). Potato is grown almost continuously throughout the year, alternating between different production areas. 80% of production is concentrated in the Provinces of Buenos Aires, Córdoba and Santa Fe, the remainder being distributed between the Provinces of Mendoza and Tucumán and the rest of the country. The highest yield in the country was observed in the Southeast region of the Province of Buenos Aires (SEBAP).

Potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the greatest biotic limitation of production worldwide and is a major threat to food security and poverty reduction. It is responsible for global losses of over 5 billion US\$ (Anderson *et al.*, 2004; Pennisi, E., 2010).

The development of the disease is highly dependent on weather conditions (Agris, G. N., 2005). In SEBAP, where is concentrated the highest potato production, the weather conditions are conducive to the disease development, reporting losses during the period 1986-2005, without chemical control, over 40% for commercial tubers and over 35% in total yield (Mantecón, J. 2009).

In order to advance in the knowledge of the pathogen populations present in Argentina, we characterized by different phenotypic and genotypic markers, 87 isolates of *P. infestans* collected during 1992-1995, 1997-99 (Distel and Huarte, 2000) and 2009-10 and from different potato growing areas of the country.

MATERIALS AND METHODS

87 isolates of *P. infestans* were collected from the main potato areas of Argentina: SEBAP, Tafí del Valle (Tucumán) and Córdoba Province. The collection of isolates was done during three different periods: 1992-95 (45 isolates), 1997-99 (24 isolates) and 2009-10 (18 isolates).

P. infestans cultures obtained by isolation from late-blight lesions (from potato leaves, tubers and stems) were done on potato tuber slices and then transferred onto Rye A medium and Pea Broth medium (Perez and Forbes, 2008).

The isolates were examined for mating type, mitochondrial DNA haplotypes, specific virulence using standard techniques (Cooke *et al.*, 2003).

For genotyping of *P. infestans* isolates, DNA was extracted using a commercial kit and the multiplex amplification was performed as Lees *et al.* (2006) suggest with 12 microsatellites markers: SSR3, SSR11, SSR4, SSR6, Pi63, SSR2, PiG11, Pi70, Pi4B, SSR8, D13, Pi04. The isolates of 2009-10 and a subgroup of 1997-99 were genotyped. Peak size and quantification data generated using GeneMapper 2.7 (Applied Biosystems).

RESULTS AND DISCUSSION

Phytophthora infestans is a heterothallic organism that has two mating types A1 and A2. In relation to the population of *P. infestans* in Argentina, their structure varied significantly during these periods. For the isolates collected during 1992-95. Both mating types were detected with an average of 25% for A1 and 75% for A2 mating types (Distel and Huarte, 2000). For the following period the mixed population of A1 - A2 was displaced rapidly towards A2 mating type. This trend was apparently conserved during 2000 and 2003 (Andreu *et al.*, 2005). Isolates of *P. infestans* from Uruguay collected between 1998 and 1999 showed similar profile to that found in Argentinian *P. infestans* populations (Deahl *et al.*, 2003). On the other hand, newer isolates collected between 2009-10 potato crop season showed a new variation of the population of *P. infestans* towards predominant A1 mating type and with Ia Mitochondrial DNA haplotype. This trend is observed in preliminary studies conducted with *P. infestans* isolates from 2010-11 season.

On the other hand, diversity for virulence and complexity of races increased greatly during the mentioned periods (Table 1). Isolates collected throughout 1992-95 showed only 4 virulence factors (1, 4, 10, 11), increasing the number of factors to 6 during 1997-99 (1, 3, 4, 8, 10, 11). For the last period (2009-10), all virulence factors were present in at least one of the isolates tested (Fig. 1). Virulence factors 2, 5, 7 and 9 were only found in newer isolates. In addition, physiological races of *P. infestans* had increased the number of virulence factors in time. The distribution of races in the different potato growing areas revealed that Tafí del Valle (Tucumán Province) isolates had the highest number of virulence factors (10 virulence factors) and old isolates from Balcarce (SEBAP) had the lowest complexity (2 virulence factors).

Genotyping was performed in isolates collected during 2009-10 based on microsatellites to study genetic variation in pathogen populations and to identify the impacts of migration on the evolution of new strains. For the characterization some isolates collected during 1997-99 were included. Oldest isolates (1997-1999) showed a divergent profile from the new population, as well as on the type of mtDNA haplotype. Recent isolates showed Ia haplotype and the oldest ones showed IIa and Ia haplotypes.

Although more recent isolates have shown high homogeneity for most of SSR markers, the PiG11 marker allowed to clearly distinguished those isolates from the area of Tafí del Valle (Tucumán Province) from those collected in Córdoba and SEBAP in the same period.

Currently, the genotyping of new *P. infestans* isolates that were collected during the 2010-11 and 2011-12 season in the SEBAP is under analysis, in order to follow the dynamics of the *P. infestans* populations in details. All phenotypic and genotypic characterization tools are implemented to confirm the trend shown with the isolates collected in the last period, 2009-10. The information of changes occurred with *P. infestans* populations in Argentina will render a valuable tool to develop durable resistance to potato Late Blight.

CONCLUSIONS

The structure of *P. infestans* populations in Argentina have undergone significant changes over the last 20 years, showing variations in the predominant mating type populations, increasing the degree of genetic variability, the diversity of races and number of physiological factors virulence of the pathogen. The results of mitochondrial haplotype analysis indicated that oldest population of *P. infestans* was characterized by a combination of Ia and IIa haplotypes, and turned predominantly to Ia haplotype in the newer population. On the other hand, the mating type of the populations has also shown high dynamic performance, detecting both mating types in the period 1992-95, that was shifted to A2 during 1997-99. This trend appears to have remained during the early years of 2000. Recent isolates showed the predominance of type A1.

Genotyping of recently isolates, which included some isolates of the period 1997-99 as reference of this population, showed divergent profiles in both populations. The screening of isolates collected during 2010-11 and 2011-12 seasons is still to be finished, but preliminary results support that trend. Characterization of a much greater number of isolates will help to confirm the trend shown in the population in 2009-10 and also provide an updated picture of the structure of *P. infestans* population in Argentina.

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Table 1. Diversity of virulence factors present in *P. infestans* populations based on isolates collected during 1992-95, 1995-97 and 2009-10.

| Virulence factors | <i>P. infestans</i> isolates * | | | | | |
|-------------------|--------------------------------|---------|-----------|---------|-------------|--------|
| | 1992-95 | % | 1997-99 | % | 2009-10 | % |
| Present | 1 | 100,00% | 1, 10, 11 | 100,00% | 1, 3, 4, 11 | 88,89% |
| | 10 | 62,20% | 3 | 93,80% | 10 | 66,67% |
| | 11 | 55,60% | 8 y 4 | 81,30% | 2 | 55,56% |
| | 4 | 51,10% | | | 5 | 44,44% |
| | | | | | 6, 7, 8 | 22,22% |
| | | | | 9 | 11,11% | |
| Absent | 5 | 100,00% | 6 | 100,00% | | |
| | 6 | 100,00% | 9 | 100,00% | - | |
| | 8 | 100,00% | | | | |
| | 9 | 100,00% | | | | |

* (years of collection- % of individual virulence factor)

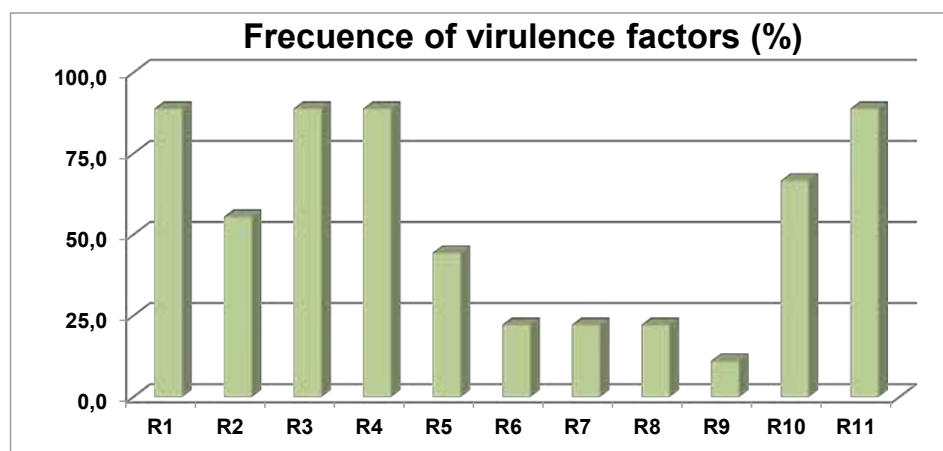


Figure 1. Average gene frequencies in *P. infestans* populations from 2009-10 season.

Are simple *Phytophthora infestans* races really that simple?

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SUMMARY

Allelic diversity of the IpiO gene encoding the effector recognised by Rpi-blb1/RB gene from *S. bulbocastanum* and *S. stoloniferum* was assessed by PCR screening and sequencing in 15 races (1, 3, 4, 10, 11, 1.2, 1.3, 1.4, 2.4, 3.4, 1.11, 1.2.3, 1.2.4, 1.3.4, and 1.2.3.4) maintained in the collection of the Institute of Phytopathology. IpiO locus was present in all these isolates. Phylogenetic analysis demonstrated that IpiO alleles from races 3 and 4 belonged to the classes I and II recognised by Rpi-blb1/RB. It follows that these “simple” races exhibit simple virulence patterns on potato comprising only R1-R11 genes from *S. demissum*. Growing interest in exploiting exotic *Solanum* germplasm in potato breeding is an incentive to reconsider and unambiguously redefine the terms “simple” and “complex” race.

KEYWORDS

Phytophthora infestans, *Solanum*, potato, late blight resistance, effectors, simple races

INTRODUCTION

Approaches to describe the diversity of P. infestans

Various methods and protocols were developed to identify, distinguish, and classify *P. infestans* isolates collected in the fields worldwide. The most prominent examples of such methods are pairing test (mating type A1, A2), response to metalaxyl (Sozzi *et al.*, 1992), polymorphism of isozymes (Goodwin *et al.*, 1995), RFLP markers mtDNA, marker RG57 (Goodwin *et al.*, 1991 and 1992), AFLP fingerprinting (Chen *et al.*, 2008), microsatellite analysis (Lees *et al.*, 2006), AVR profiling (Cárdenas *et al.*, 2011), and virulence spectra determined on potato differentials (“simple” and “complex” races). In our study, we critically analysed the concept of “simple” and “complex” races using current knowledge about the molecular basis of *P. infestans* - plant interactions.

Determinants of specificity in race-specific resistance

During attack on host plants, *P. infestans* secretes hundreds of molecules aimed at promoting infection by various mechanisms including reprogramming of plant immune system by interacting with proteins involved in the immune response. Such molecules are called effectors (Kamoun, 2006). The diversity of characterised effectors is classified according to the pathogen lifestyle, their allocation in plant tissues, and the presence of extremely conserved motifs, such as RXLR (Fig. 1).

Inside a cell, effectors can be directly or indirectly recognised by the sentinel receptors encoded by R genes - determinants of race-specific resistance (Vanderplank, 1963). All intracellular effectors characterised as triggering R gene-mediated immune response belong to the class of RXLR, also known as Avr (avirulence) effectors. The genome of *P. infestans* strain T30-4 contains 563 genes encoding proteins with unique RXLR motifs (Haas *et al.*, 2009). Eight RXLR effectors were identified as factors triggering immune response following their recognition by well-characterised *Solanum* R proteins (reviewed in Vleeshouwers *et al.*, 2011; Table 1).

From the pathogen side, allelic diversity of Avr effectors apparently determines the specificity of their recognition by R proteins. As little as a single amino acid change in Avr3a could circumvent its recognition by R3a. Surprisingly, such mutations were always associated with the loss of the primary (pathogenic) function of Avr3a in suppressing the plant cell death response induced by INF1 elicitor protein (Bos *et al.*, 2009).

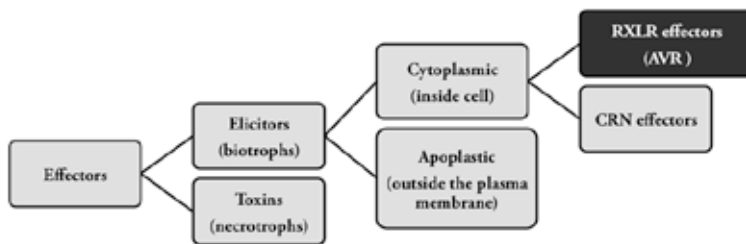


Figure 1. Diversity of effectors classified by the pathogen lifestyle, localisation, and family-specific conserved motifs. A family of RXLR Avr effectors cognate to the LB-related R gene products is highlighted in black.

Table 1. Functionally characterised R protein – Avr effector pairs (reviewed in Vleeshouwers *et al.*, 2011).

| <i>Solanum</i> species* | R protein | <i>P. infestans</i> effector |
|-------------------------|----------------------|------------------------------|
| dms | R1 | Avr1 |
| blb/dms | Rpi-blb3/R2 | Avr2 |
| dms | R3a | Avr3a |
| dms | R3b | Avr3b |
| dms | R4 | Avr4 |
| vnt | Rpi-vnt1.1 | Avr-vnt1 |
| blb/sto | RB/Rpi-blb1/Rpi-sto1 | IpiO1 |
| blb | Rpi-blb2 | Avr-blb2 |

*Abbreviations: dms – *S. demissum*, blb – *S. bulbocastanum*, sto – *S. stoloniferum*, vnt – *S. venturii*

MATERIAL AND METHODS

P. infestans races and DNA isolation

Genomic DNA was isolated from 15 races (1, 3, 4, 10, 11, 1.2, 1.3, 1.4, 2.4, 3.4, 1.11, 1.2.3, 1.2.4, 1.3.4, and 1.2.3.4) maintained in the Institute of Phytopathology using AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Biosciences) according to the manufacturer's recommendations. DNA concentration was measured using a UV/Vis NanoPhotometer P300 (IMPLEN), and DNA integrity was accessed using the agarose gel electrophoresis.

PCR amplification and cloning of IpiO

IpiO alleles were amplified from genomic DNA using the forward primer specific for the IpiO locus including the region of signal peptide (5'-CTTTCCGGCAATGCGTTTCGC-3') and the reverse primer 5'-CTATACGATGTCATAGCATGACAC-3' described in Champouret *et al.* (2009). PCR primers were optimised using the Oligonucleotide Properties Calculator (<http://www.basic.northwestern.edu/biotools>). The amplification reactions contained 1 µl of 10x PCR buffer, 100-150 ng of genomic DNA, 1 µl 2.5 mM dNTP, 10 pmol each of two primers, 1 U of either Pfu DNA polymerase (Fermentas) for cloning or Taq DNA polymerase (Syntol) for screening and sterile water to a volume of 10 µl. Reactions were run in an MJ PTC-200 thermocycler (Bio-Rad) using the following program: 3 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 65°C, 1 min at 72°C; 10 min at 72°C. PCR products were separated by electrophoresis in 1.5% w/v agarose and stained with ethidium bromide. Amplified fragments were eluted from the gel using QIAquick gel extraction kit (Qiagen), cloned using CloneJET™ PCR Cloning Kit (Fermentas), and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI 3730 DNA Analyzer (Applied Biosystems).

Phylogenetic analysis of IpiO sequences

DNA sequences of IpiO effector genes characterised by Champouret *et al.* (2009) were extracted from NCBI Genbank (accession numbers GQ371190 - GQ371200, GQ371202). For multiple alignment, we employed MAFFT (Kato *et al.*, 2002) algorithm implemented at GUIDANCE web server (<http://guidance.tau.ac.il>; Penn *et al.*, 2010). The alignment was manually curated and edited in BioEdit software (Hall, 1999). Maximum likelihood search was performed using RAxML algorithm with rapid bootstrap analysis (<http://phylobench.vital-it.ch/raxml-bb>; Stamatakis *et al.*, 2008) and the best scoring ML tree was analysed.

RESULTS AND DISCUSSION

What makes “simple” race simple?

The concept of “simple” and “complex” races was introduced by Black and associates who bred the set of 11 potato cultivars (so-called differentials) each presumably containing a single R gene introgressed from *S. demissum* (Black *et al.*, 1953). *P. infestans* isolates virulent only on one or two of these differentials were considered as “simple”, isolates with wider virulence spectra as “complex”. “Simple” races were widely used to map *S. demissum* R genes (Leonard-Schippers *et al.*, 1992) and to detect R genes in potato cultivars (Sokolova *et al.*, 2011). “Simple” races are frequently referred to as carrying one or two virulence factors (e.g. Ghimre *et al.*, 2001); but how does it fit the Avr-R gene model? We suggest that virulence factors as defined by classic phytopathology are products of mutated Avr genes evading recognition by R proteins. Therefore, we propose that “simple” races contain a single “broken” Avr gene, whereas other Avr effectors corresponding to *S. demissum* R1-R11 genes are intact.

The concept of “simple” race may need revising

We suggest revising the use of the term “simple” and “complex” races based on the following arguments.

According to the so-called “arms race” model, pathogens and plants are constantly co-evolving by the re-assortment of the virulence and defence factors (e.g. alleles of Avr and R genes; Bergelson *et al.*, 2001). Such co-evolution creates strong evolutionary forces that shape distribution of active and inactive alleles on both sides of the frontier. Recurrent selection of the “simple” races on the Black’s differentials imposes constant selective constraint only on Avr effectors cognate to *S. demissum* R receptors, whereas allele frequencies of other effector genes (>500) apparently serve the best interest

of the pathogen. Recently, wild *Solanum* species other than *S. demissum* attracted close attention of potato breeders and the corresponding R genes were isolated and successfully introduced into potato cultivars (Vleeshouwers *et al.*, 2011). Selection on differentials will not affect virulence spectra of “simple” races on these cultivars; therefore, these spectra will not characterise a particular “simple” race. In other words, “simple” races are simple only in relation to the potato cultivars comprising *S. demissum* R1-R11 genes. While in potato cultivars R genotyping with “simple” races is in line with the presence of particular R genes, the evidence for wild *Solanum* species is apparently misleading. The second argument is more philosophical. The term “simple” race would suggest that pathogen isolate characterised as “simple” may have less ability to infect or be less aggressive and vice versa for “complex” race isolates. Such presumption is not true. In fact, Montarry *et al.* (2010) showed that complexity of *P. infestans* race negatively correlated with pathogen fitness. Selection would favour less complex isolates with less virulent factors (and the higher numbers of intact Avr effectors). The trade-offs between acquiring more functional Avr effectors essential for fitness and the chance to be recognised by R protein would drive such selection. The evidence that mutation of Avr3a “hiding” this effector from R3a was also detrimental for the primary function of the effector in promoting infection fits this model (Bos *et al.*, 2006). Summing up, in contradiction with the semantics of their name, isolates with “simple” race are generally more fit and aggressive than “complex” race isolates.

Allelic diversity of IpiO effector gene in “simple” races

To verify our suggestions, we studied allelic diversity of the IpiO gene encoding the effector recognised by Rpi-blb1/RB gene from *S. bulbocastanum* and *S. stoloniferum* (Vleeshouwers *et al.*, 2008). To this end, we designed the forward primer specific for the IpiO locus and used the reverse primer described by Champouret *et al.* (2009). Screening 15 races (1, 3, 4, 10, 11, 1.2, 1.3, 1.4, 2.4, 3.4, 1.11, 1.2.3, 1.2.4, 1.3.4, and 1.2.3.4) maintained in the collection of the Institute of Phytopathology showed that IpiO was present in all these isolates. These results are in line with previous evidence that the diversity of IpiO locus results from allelic variation in nucleotide sequences rather than from the presence-absence polymorphism (Halterman *et al.*, 2010). Champouret *et al.* (2009) showed that the IpiO genes fell into three distinct classes. IpiO effectors of classes I and II were recognised by Rpi-blb1/RB, whereas IpiO class III did not trigger RB-mediated hypersensitive response. To classify IpiO alleles in “simple” races, we analysed ten clones of IpiO gene from races 3 and 4. Cluster analysis revealed that “simple” races 3 and 4 comprised alleles encoding IpiO variants avirulent on *Solanum* germplasm with active allele of Rpi-blb1/RB gene. Therefore, because virulence patterns of these “simple” races are not simple, they cannot be predicted by (in)compatibility reaction on potato differentials.

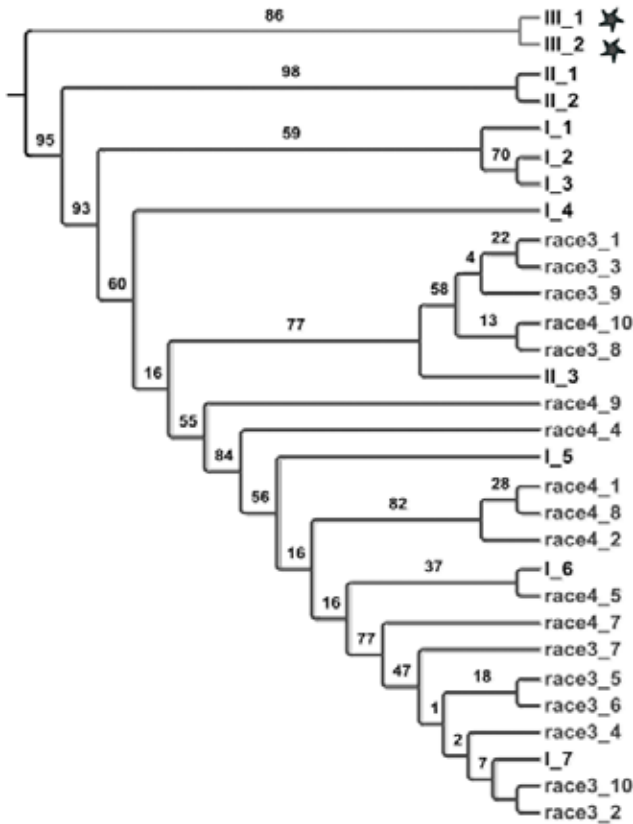


Fig. 2. Maximum likelihood cladogram of IpiO alleles from the “simple” and “complex” races of *P. infestans*. Star pictogram denotes the cluster of IpiO alleles of class III, which circumvents recognition by *Rpi-blb1/RB*. Alleles extracted from NCBI Genbank are I_1 – I_7, II_1 – II_3, and III_1, III_2 (accession numbers GQ371190 – GQ371200, GQ 371202). Other alleles are sequenced in this study. Bootstrap values are shown at the nodes.

CONCLUSIONS

We presume that the virulence factors described by classic phytopathologists are Avr effector genes, which avoided recognition by the corresponding R proteins due to the various mutations fixed by the evolutionary “arms race”.

“Simple” races comprise active alleles of effectors cognate to the products of R genes other than R1-R11 initially recognised in *S. demissum*. Therefore, the “simple” virulence pattern of these races would emerge only in potato plants containing R1-R11 genes introgressed from *S. demissum*.

Apparently, the terms “simple” and “complex” race should be reconsidered and clearly redefined by the research community to avoid possible ambiguities.

ACKNOWLEDGEMENTS

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Thirteenth EuroBlight workshop
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**Phenotypic characteristics of Belgian populations of
Phytophthora infestans (2005-2010)**

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Phenotypic characteristics of Belgian populations of *Phytophthora infestans* (2005-2010)

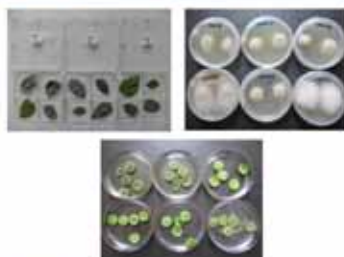


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Summary

A total of 216 isolates of *Phytophthora infestans* were collected in the South part of Belgium (Wallonia) in several potato fields, volunteers and dumps during 2005-2010. Most of isolates were tested for several phenotypic characteristics, such as mating type, virulence and sensitivity to metalaxyl. 55% of the Belgian isolates were A2 mating type. All 11 virulence factors were found among the tested isolates. 86% of the A2 isolates are resistant to metalaxyl and 94% of the A1 isolates are sensitive to metalaxyl. A2 isolates have more complex virulence profiles than A1.



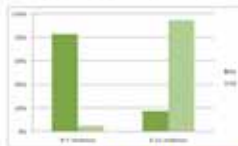
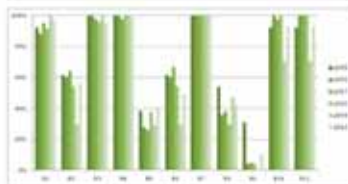
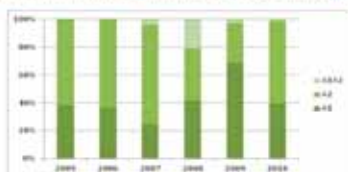
Materials and Methods

- The mating type was tested by growing isolates on rye agar with the known references strains of the A1 and A2 mating types. After 7-14 days incubation at 16°C, the presence or absence of oospores was recorded under a microscope
- Virulence pattern were determined using Black's differential set of potato clones, each having one of the R1-R11 pathotype-specific resistance genes.
- The floating potato leafdisc method (Cooke) was used to determine metalaxyl sensitivity. Isolates were tested on 0.1, 1, 10 and 100 mg metalaxyl/ml. Isolates sporulating on water containing 10 and 100 mg/ml were rated as resistant, those on 1 mg/ml were rated as moderately resistant and those that sporulated only on water and 0.1 mg/ml were rated as sensitive.

Results

Among the 215 tested isolates, 55% were A2 mating type, 41% were A1 mating type and 4% were self-fertile. From 2005 to 2010, A2 mating type were predominant except in 2009 where 60% of strains were A1.

All known virulence factors were found among tested isolates. Virulence against R7 was found in 100% of isolates. Virulence factors corresponding to genes R1, R3, R4, R10 and R11 were found in more than 90%. Virulence factors against R2, R5, R6 and R8 were found in between 30 to 60%. Virulence for R9 was rare, up to 10% of tested isolates with exception of R9 in 2005 (31%). The most frequency race were 1-3-4-7-10-11 (19%), avirulent to R9 (13%) and 1-2-3-4-6-7-10-11 (13%).



In total, 202 isolates were screened for resistance to metalaxyl. During the 5 years, 52% were resistant, 4% were moderately resistant and 44% were sensitive to metalaxyl. The association between metalaxyl resistance and mating type were significant. Of the A2 mating type strains, 85% were resistant and of the A1 mating type, 94% were sensitive.

A2 isolates have more complex virulence profiles than A1. Among 199 tested isolates, 83% of the A1 strains have 4 to 7 virulences and 95% of the A2 strains have 8 to 11 virulences.

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Distribution of Mating Types and Resistance to Metalaxyl of *Phytophthora infestans* in Germany

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SUMMARY

In 2010 a monitoring was carried out to characterise several German populations of the pathogen *Phytophthora infestans* (Mont.) de Bary based on mating type and sensitivity to metalaxyl. All tests were done by laboratory experiments. Mating types were determined in a pairing test. By using a floating leaf disc method the sensitivity to metalaxyl was evaluated. After first detecting mating type A2 in 1984 in Europe many trials were done to investigate the changes in the populations. Latest studies showed a high presence of A2 in European countries (Gisi *et al.*, 2011). The result of this work agrees with this observation. The proportion of A2, as well as the level of resistance, was proved to be still high in Germany. The frequency of mixed populations, found in the fields, was conspicuously high. It can be assumed that sexual reproduction of *P. infestans* takes place in many German production areas which causes high genetical variability. The analyses of a linkage between mating type and resistance to metalaxyl revealed no relationship between both traits.

KEYWORDS

Phytophthora infestans, late blight, mating type, metalaxyl resistance, monitoring

INTRODUCTION

Phytophthora infestans is a heterothallic species with two compatible mating types designated as A1 and A2. If both mating types come into physical contact it results in the formation of oospores (Drenth, 1994). Until the early 1980s the European populations were dominated by mating type A1. The epidemic spread was affected of asexual sporangia (Schulte, 2011). The exchange of crossing partners with same mating types is not possible. Thus, the pathogen is limited adaptable. Hohl and Iselin (1984) reported first data on A2 mating type occurrence in Switzerland. Two years later first observations of this type followed in Germany (Schöber and Rullich, 1986). In 1987, the detection of oospores in the field succeeded (Götz, 1991). By sexual recombination the late blight gains genetic variability and adaptability (Hausladen, 2007). Increasing aggressiveness (Krauthausen and Flier, 2005) and foreshortening of latency periods were reported for example of other implications (Hausladen, 2007). After detecting in 1986, several studies were carried out in Germany on the population structure of *P. infestans*. During 1986 and 1999 the proportion of A2 was in a range of 0% to 40%. In 2000 the ratio significantly changed to a proportion of about 70% of A2 (Bangemann,

2009). Subsequently, investigations were reduced and only carried out sporadically, but analysed samples from neighbouring European countries from 2006 to 2007 also showed a high presence of A2 up to 90% (Gisi *et al.*, 2011). Furthermore a simultaneous increase of metalaxyl resistance with the appearance of A2 was observed in many countries (Schöber-Butin, 2001).

The aim of this study was to resume the research and to get a current survey about the population structure of *P. infestans* in Germany. Therefore a monitoring was carried out in 2010 to collect isolates for characterisation by mating type determination and evaluation of their resistance to metalaxyl. Furthermore, it was studied if the two traits may be genetically linked.

MATERIAL AND METHODS

Origin of P. infestans isolates

37 isolates were collected from 14 locations out of six federal states of Germany with different potato cultivars (Table 1). The isolates were sampled at various dates during 2010. Up to five isolates were taken per monitoring field. At five locations only one isolate could be educed. The single-lesion isolates were maintained in-vitro as axenic cultures on pea agar medium and incubated at 15°C in darkness.

Table 1: Origin of German isolates collected in 2010

| Location | Number of isolates | Potato cultivar | Date of sampling |
|---------------|--------------------|-----------------|------------------|
| Hamm | 5 | Magda | 15. Jun. |
| Guntersblum | 1 | Magda | 16. Jun. |
| Hechtsheim 1 | 1 | n.s. | 21. Jun. |
| Gimbsheim | 5 | Pirol | 21. Jun. |
| Bretzenheim | 3 | Agria | 22. Jun. |
| Hechtsheim 2 | 1 | n.s. | 29. Jun. |
| Schwalmtal | 3 | n.s. | 11. Aug. |
| Oberlichtenau | 3 | Talent | 11. Aug. |
| Nemt | 2 | Ditta | 12. Aug. |
| Müncheberg | 1 | Ballerina | 14. Aug. |
| Neusustrum | 1 | Euronova | 18. Aug. |
| Lathen | 4 | Euronova | 18. Aug. |
| Puch | 2 | Agria | 18. Aug. |
| Straßmoos | 5 | Agria | 18. Aug. |

Mating type determination

The experiment was done following the protocol of Bakonyi and Cooke (2004). The mating type of each unknown isolate was determined by individually pairing them with known A1 and A2 tester strains (Plant Research International, Wageningen, NL). The isolates were placed in a distance of 3 cm apart in Petri dishes (4.5 cm in diameter) containing pea agar. The plates were incubated in the climatic chamber at 15°C and darkness. After ten days of incubation the combinations were microscopically observed. The presence

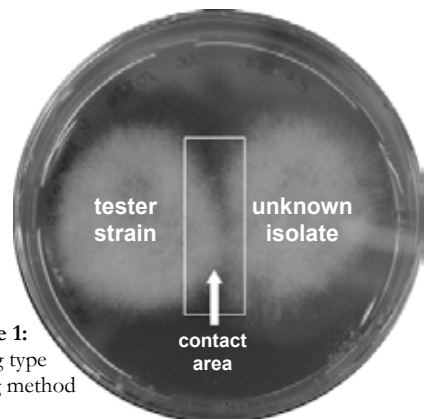


Figure 1: mating type testing method

or absence of oospores was recorded in the “contact area” where the mycelia of both mating partners crossed (Figure 1). If the contact area showed oospores, the unknown isolate was assigned to the complementary mating type of the tester strain’s type. The result was confirmed by an observation of exclusive sporangia formation in the other plate.

Metalaxyl sensitivity test

The sensitivity to metalaxyl of the isolates was assessed by using a floating leaf disc method (Sozzi *et al.*, 1992). It was analysed on potato leaf discs from six week old plants of the cultivar Laura, grown under glass. Petri dishes (9.5 cm in diameter) were filled with 15 ml fungicide solution (Fongamil Neu, Novartis Agro AG) at four concentrations (0.01, 0.1, 1.0 and 10.0 ppm) plus an untreated control consisting of distilled water. Ten leaf discs (10 mm in diameter) per dish were placed with the abaxial side upwards on the solution. For each isolate a suspension of sporangia (105/ml) was prepared by flooding two week old cultures with 6 ml sterile water (½ distilled water, ½ tap water). Sporangia colonies were gently scrapped off the surface and solutions were filtrated through cotton wool to separate mycelia. Leaf discs were inoculated by a 10 µl droplet of sporangia suspension centred on each disc. The test was incubated for ten days at 15°C and with 12-hour photoperiod. Leaf discs were examined for sporulation and diseased discs were counted in each Petri dish. Tested isolates were rated as sensitive when sporulation proceeded at 0 to 0.01 ppm, as intermediate when sporulation proceeded at 0.1 to 1.0 ppm and as resistant when the pathogen sporulated at 10.0 ppm. Incubation period and latency time were detected in the untreated control in 24-hour intervals.

RESULTS

Mating types

With a proportion of 64.9% the majority of all field isolates was determined as mating type A2. With 21.4%, a mixed population was found on every fifth field. By excluding the locations with only one extracted isolate per monitoring site the proportion of fields with a mixed population amounts to more than 33%.

Sensitivity to metalaxyl

In the untreated controls the isolates showed incubation periods (time between inoculation and appearance of first symptoms) less than 24 hours. Latency times (time between inoculation and appearance of first sporangiophores) were between 3-4 days. Eight isolates sporulated until the third day. Except the field of Guntersblum at least one isolate per location sporulated until the fourth day. With 51.6% the isolates (n=31) were mostly resistant to metalaxyl. Only 3.2% stopped sporulation

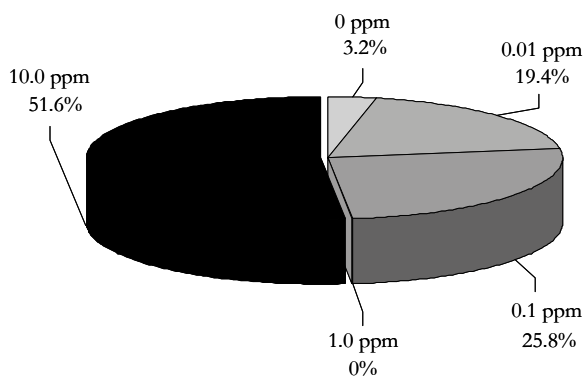


Figure 2:
Proportion of isolates with sporulation per concentration level

at 0.01 ppm and 19.4% stopped at 0.1 ppm. Thus, a proportion of 22.6% responded sensitive. A reduced effectiveness of metalaxyl was recorded at 25.8% of the isolates (Figure 2). All isolates of Gimbshheim and one isolate of Oberlichtenau showed no sporulation in all variants and were not evaluated. No site with a sample size of more than one showed only sensitive or intermediate isolates. Both sensitivity levels occurred always in common. Oberlichtenau was the only site where sensitivity occurred next to resistance.

Climatic conditions in 2010 were first favourable to late blight. Due to cool and humid weather, infections of *P. infestans* were found in June. High temperatures in July repressed late blight and sampling was not continued until August. By examining the two sampling periods a difference in the level of resistance becomes clear. Samples collected later in the season showed a higher level of resistance compared to samples collected early in the season. While 45.5% of the early sampled isolates responded sensitive to metalaxyl and as many isolates were intermediate, the isolates sampled in August were sensitive in only 10% and intermediate in 15% of the cases. Though, the resistance level increased from 9% in June to 75% in August (Figure 3).

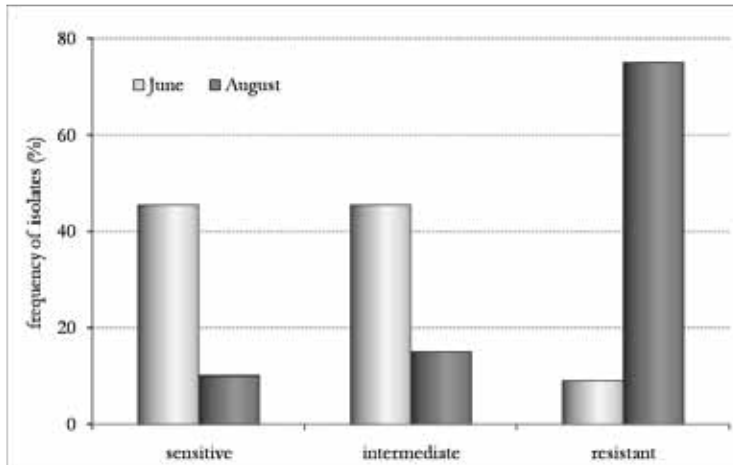


Figure 3:
Level of resistance depending on sampling time

The observation of mating types in relation to their sensitivity to metalaxyl showed that mating type A2 occurred preferably in conjunction with resistance. With 87.5% most of the resistant isolates were assigned to A2. Mating type A1 was resistant in 20% of the cases. Sensitive and intermediate A1 isolates were both found in 40% of the cases. Isolates with mating type A2 showed resistance in about 67% of the cases. Full efficacy of metalaxyl was observed at only 14% of the A2 isolates (Figure 4).

The geographical distribution did not show a regional concentration, neither regarding mating types, nor regarding sensitivity to metalaxyl.

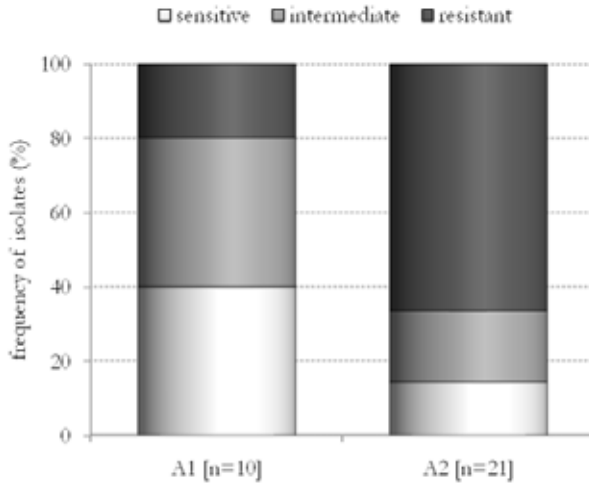


Figure 4. Level of resistance in conjunction with mating types.

DISCUSSION

After first detecting mating type A2 in 1984 in Europe, many trials were done to investigate the changes in the populations. Latest studies showed a high presence of A2 in European countries. From 2006 to 2007 the proportion of A2 was up to 90% in the neighbouring countries (Gisi *et al.*, 2011). The result of this work agrees with this observation. With 65%, the proportion of mating type A2 in the population proved to be high in Germany. Despite the low numbers of isolates per monitoring field (max. 5) mixed populations were found. The mating types occurred in a ration of 1:1 which leads to a significant chance of sexual reproduction in German potato production areas. The possibility of an occurrence of both mating types in a different relation cannot be ruled out in the remaining fields, especially not in those where only one isolate was won.

Leaf disc test showed latency periods between 3-4 days. Latency times of the old populations are indicated with 5-7 days (Hausladen, 2007). It can be assumed that the tested isolates belong to the new population. The level of resistance was high in 2010. However, the validity of this result strongly depends on the fungicide strategies which are used by the seed producers.

Caused by the fact, that *P. infestans*-isolates were collected in two different sampling periods, it was possible to observe an increasing level of resistance. Bangemann (2008) obtained a similar result in his investigations. Late samples showed barely a sensitive reaction, while the majority of isolates collected early in the season represented a high efficacy of metalaxyl. The variations in sensitivity during the season can be explained by a reduced hibernation ability of the resistant strains. Due to their higher vitality, the mycelium continues growing at low temperatures (in the storage) in the tissue of the tubers. By reaching and infecting the eyes, the tuber is prevented from germination in the following spring. Though, first infections of late blight are mostly initiated by sensitive strains. Increasing selection pressure during the season results the occurrence of resistant strains.

Mating type A2 mostly appeared in conjunction with resistance properties. However, the hypothesis of a dependency between mating type and resistance was disproved. Sensitive, intermediate and resistant isolates were observed in both mating types. Same observations were made by Gisi *et al.* (2011). Consequently the two traits are genetically unlinked.

CONCLUSIONS AND OUTLOOK

The results of monitoring the distribution of mating types and resistance to metalaxyl showed that mating type A2 occurred not only prevalent in German acreages, but also in combination with its mating partner A1. Added to the formation of sporangia, *P. infestans* most likely propagates by sexual reproduction. Despite the fact that more than half of all isolates reacted resistant to metalaxyl, the fungicide is still applicable in consideration of a strict resistance management strategy. The level of resistance increases significantly during the season. To prevent the formation of resistant strains, metalaxyl can be used prophylactic at the beginning of the season. Subsequently, other active substances should be applied to avoid the use of phenylamids.

To further observe the level of resistance and the development of the ratio of A1 to A2, the monitoring was continued in 2011. The data are currently in evaluation.

ACKNOWLEDGEMENTS

Thanks to Trudy van den Bosch from Plant Research International (Wageningen, NL) for providing the tester strains and for her help. Thanks to the employees of the Plant Protection Services for participation in the monitoring.

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Distribution of Mating Types and Resistance to Metalaxyl of *Phytophthora infestans* in Germany



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Introduction

P. infestans (Mont.) de Bary is a heterothallic organism with two mating types designated as **A1** and **A2**. The interaction of both mating types results in formation of oospores.

Until the early 1980s the European populations were dominated by mating type A1. In 1984 mating type A2 was first discovered in Switzerland. In 1986 first observations in Germany followed. Ever since, the proportion of A2 steadily increased in Europe. Simultaneously a rise in resistance to fungicides with the active ingredient metalaxyl was reported from many countries.

In 2010 a monitoring was carried out to determine the occurrence of mating type A2 and resistance to metalaxyl in Germany. The genetic linkage between the two traits was analyzed.

Materials and Methods



Figure 1: mating type testing method

Mating type determination

37 isolates were collected from 14 plots in 2010.

For mating type determination every isolate was paired separately with an A1 and an A2 tester strain on pea agar (Figure 1).

After ten days of incubation at 15°C in the dark, the zone of contact was checked for oospore production. If the contact zone showed oospores, the unknown isolate was assigned to the complementary mating type of the tester strain's type.

Metalaxyl sensitivity test

The sensitivity to metalaxyl was analysed by potato leaf discs. Petri dishes were filled with 15 ml fungicide solution at four concentrations (0.01, 0.1, 1, 10 ppm) plus an untreated control consisting of distilled water. Ten leaf discs per dish were placed upside down on the solution and inoculated by a 10 µl droplet of sporangia suspension (10⁵/ml). The test was incubated for ten days at 15°C with 12-hour photoperiod.

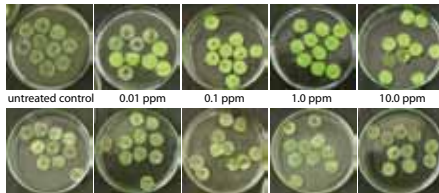


Figure 2: sensitive isolate (top) and resistant isolate (bottom) in leaf-disc test

Leaf discs were examined for sporulation and diseased discs were counted in each dish. Tested isolates were grouped into **sensitive** (sporulation at 0-0.01 ppm), **intermediate** (sporulation to 0.1-1 ppm) and **resistant** strains (sporulation to 10 ppm). Incubation period and latency time were detected in the untreated control in 24-hour intervals.

Results

Mating type

With a proportion of **64.9 %** the majority of all field isolates was determined as mating type A2. In **21.4 %** of the fields a mixed population was found.

Metalaxyl sensitivity

The isolates showed incubation periods less than 24 hours. Latency time was between 3-4 days. **51.6 %** of all isolates showed resistance to metalaxyl. Only **22.6 %** responded sensitive and **25.8 %** were rated intermediate (Figure 3). Mating type A2 occurred preferably in conjunction with resistance. **87.5 %** of the resistant isolates were assigned to A2.

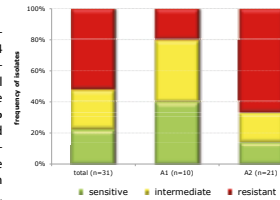
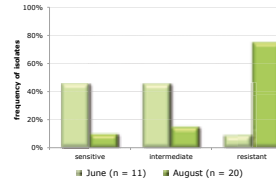


Figure 3: Resistance level of all isolates, A1- and A2-isolates

Samples collected later in the season showed a higher level of resistance than samples collected earlier in the season. (Figure 4).

Figure 4: Sensitivity to metalaxyl depending on sampling time



The geographical distribution did not show a regional concentration, neither regarding mating types, nor regarding sensitivity to metalaxyl.

- resistant
- intermediate
- sensitive
- not evaluable

Figure 5: Distribution of mating types and resistance to metalaxyl

Conclusion

The proportion of A2 in the population proved to be high in Germany. Despite the low number of isolates per field (max. 5), mixed populations were found. The mating types occurred in a **ratio of 1:1**, which leads to a significant chance of sexual reproduction. The possibility of an occurrence of both mating types in a different relation cannot be ruled out on the remaining fields.

Variations in sensitivity during the season can be explained by increasing selection pressure and a reduced hibernation ability of the resistant strains.

The hypothesis of a dependency between mating type and resistance was disproved. Sensitive and resistant isolates were observed in both mating types. Consequently the two traits are genetically unlinked.

***Phytophthora infestans* 13_A2, diagnostic and monitoring in 2009 and 2010**

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SUMMARY

Some tendency for the regression of the 13_A2 genotypes resistant to metalaxyl-M was observed from 2009 and 2010. This evolution could be the consequence of changes in the fungicides applications with the limitation or absence of metalaxyl-M selection pressure in some countries such as NDL. The competition with other more aggressive genotypes such as 6_A1 is not relevant in these limited random monitoring.

KEYWORDS

Potato late blight, *Phytophthora infestans*, monitoring sensitivity, fluopicolide, propamocarb, fenamidone, competitors, mefenoxam, genotyping, blue 13, sexual mating type A1/A2

INTRODUCTION

In order to answer EPPO, monitorings have been performed since 2001 with *Phytophthora infestans* covering all important European regions. Concurrently to the base line monitoring and sensitivity studies to optimize an effective anti resistance strategy for fluopicolide, fenamidone and propamocarb based products, molecular methodologies have been investigated since 2009 and conducted in parallel at the Scottish Institute (David Cooke) to characterise the genotype of the EU populations. Confirmation of mating types A1 & A2 and resistance to metalaxyl-M was also included in the characterisation of the strains.

MATERIALS AND METHODS

Sampling

Samples originated from France (Northern France, Champagne and Brittany), the Netherlands, Germany, UK, Belgium, Sweden, Baltics (EE, LT, LI) Poland and Italy were collected during in the season according the respective weather conditions.

Around ten leaves with potato late blight symptoms were sampled from each location. For the transport a sandwich with healthy leaves was made and each sample was then wrapped up in newspaper and posted in a paper bag to avoid rotting during transport.

The 108 samples collected in 2009 and the 120 in 2010 were tested for their sensitivity to metalaxyl M, fluopicolide, fenamidone and propamocarb. Fourty eight isolates out of 108 and fourty six out of

120 were respectively selected for genotyping and/or sexual mating type survey.

Molecular 13_A2 characterisation

An in house methodology was developed based on D. Cooke technology and primers to characterise 13_A2 (Blue 13) genotype directly from infected potato leaves to avoid fungal purification which takes time and is a human resources consumer task. The protocol is summarized in the poster.

A1/ A2 sexual mating type characterisation

Sexual mating type diagnostic is based on the observation of oospores when reference types A1 or A2 are confronting with the unknown strain:

Sporangia suspensions of *P. infestans* are calibrated for the two standards A1 and A2 strains and the unknown one. Then these suspensions are mixed by pair for all combinations and 10µl droplets of each are distributed on 30 potato leaf discs surviving on agar medium in Petri dishes. Petri dishes are then placed in a climatic chamber at 16°C and 90% humidity for 5 or 6 days.

After 5 or 6 days of incubation, discs are discolored by calcium hypochlorite during about 30 minutes until there's no more chlorophyll, washed in, at least, one water bath for 10 minutes and finally stained with calcofluor and observed under fluorescent microscope.

If oospores are visible in the positive standard confrontation (A1XA2) but not in the 2 negative ones (A1XA1 and A2XA2), the test is considered valid.

The presence of oospores on discs resulting from the mixture between the unknown strain and one of the A1 or A2 reference leads to the conclusion that the unknown strain is from the opposite sexual mating type. In the case oospores are formed in confrontation with the two different mating types, we could say that the two mating types are present in the population tested.

RESULTS

No shift of Potato Late blight sensitivity was detected in 2009 & 2010 monitoring in Europe for the 3 actives fluopicolide, fenamidone and propamocarb.

This monitoring was carried on to validate A2_13 genotypes which showed resistance to metalaxyl-M. We could observe that the resistance to mefenoxam is largely present in all the European countries as described for a long time. All the strains characterized for being 13_A2 genotype were exclusively resistant to metalaxyl-M.

There were isolates with the D13 alleles 136/154 that were NOT 13_A2. This illustrates the danger of relying on a single locus to test for clonal lineages. Only very few 6_A1 isolates were detected compared to the UK situation where it is mentioned more prevalent (D.E.L. Cooke com.).

Some tendency for the regression of the 13_A2 genotypes was observed from 63% of the metalaxyl-M resistant strains in 2009 to around 25% in 2010 random monitoring.

In parallel, A1 mating type seems to significantly progress.

CONCLUSIONS

No shift of Potato Late blight sensitivity was detected in 2009 and 2010 monitoring in Europe for actives fluopicolide, fenamidone and propamocarb.

Some tendency for the regression of the 13_A2 genotypes was observed from 75% of the metalaxyl resistant strains in 2009 to 60% in 2010.

In parallel, A1 mating type seems to significantly progress, may be due to some genotypes' aggressiveness such as A1- pink 6

Changes in *Phytophthora infestans* aggressiveness as a result of repeated reproduction on different potato cultivars

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INTRODUCTION

The aim of our study was to determine the level of influence of the cultivar resistance on the aggressiveness of *P. infestans* isolates. We supposed that the long-term passing of *P. infestans* isolates on the same potato cultivars can result in the change of their aggressiveness level. Such possibility was quite possible according to the results of the study of the aggressiveness of *P. infestans* strains, collected in different geographic regions. It has been found that isolates, collected on the fields with a long-term cultivation of the same potato cultivar, are more aggressive than those from the fields with the rotation of cultivars. Possibly, a long-term cultivation of the same potato cultivar results in the selection of the most aggressive strains of a *P. infestans* population. The mass reproduction of the same strain makes the population to be monoclonal, i.e., the process of accumulation of a single strain type, optimal for the survival and development on the certain potato cultivar, takes place. This process reduces the biodiversity, necessary for this pathogen to survive in the changing environment. In this case the population accumulates pathogen forms, which evolution is directed to the increase of the aggressiveness level.

MATERIALS AND METHODS

We selected potato cultivars differing in the level of their horizontal resistance (Table 1). Potato plants were grown in a greenhouse (winter-spring) or in the field. Detached leaves of each cultivar were infected under laboratory conditions.

Table 1. Potato cultivars used in the experiment

| Cultivar | Origin | Resistance level |
|------------|----------------|------------------------|
| Escort | Netherlands | Highly resistant |
| Udacha | Russia | Moderately susceptible |
| Lugovskoi | Russia | Moderately resistant |
| Sante | Netherlands | Moderately susceptible |
| Sarpo Mira | United Kingdom | Highly resistant |

As the infection material, we used *P. infestans* isolates, collected in different geographic regions and having the known characteristics (race composition, mating type, phenylamide resistance, and aggressiveness (Table 2).

Table 2. Characteristics of *P. infestans* strains used in the experiment

| Name | Origin, year of isolation | Race | Mating type | Phenylamide resistance | mtDNA haplotype | Aggressiveness level* |
|------------|---|---------------------|-------------|------------------------|-----------------|-----------------------|
| KBK-33.01 | Kislovodsk, the Stavropol Territory, 2001 | 1.3.4.7.10.11 | A1 | S | IIa | MA |
| KBB-42.01 | Kislovodsk, the Stavropol Territory, 2001 | 1.2.3.4.7.8.9.10.11 | A1 | S | Ia | MA |
| OPCKA-S14 | Ryazan region, 2000 | 1.3.4.7.10 | A2 | S | n/d | WA |
| Cx-40BB.03 | Sakhalin island, 2003 | 1.2.3.4.7.8.10.11 | A1 | R | n/d | HA |

*HA, highly aggressive; MA, moderately aggressive; WA, weakly aggressive (the level of aggressiveness was calculated in the year of isolation as an average score for the testing on 30 potato cultivars).

All selected isolates were propagated on the tuber slices and then re-isolated via the artificial infection of the detached potato leaves (cv. Sante).

To obtain *P. infestans* re-isolates, leaves with necrotic lesions, representing each studied strain-cultivar pair, were placed into the cut tubers (cv. Sante). After the sporulation, the obtained spores were used for the repeated infection of the detached leaves of the studied potato cultivars. The total number of generations was 20 for each potato cultivar. The experiment was performed within two vegetation seasons (10 generations during summer – autumn of the first year and 10 generations during spring – summer of the second year). During winter the isolates were maintained via the inoculation of potato tubers of the studied cultivars with the further storage at +7°C.

To determine the aggressiveness of initial isolates and re-isolates, an express-method was used (Filippov *et al.*, 2004). We determined the efficiency of the inoculum, the diameter of necrotic lesions, and the sporulation capacity. Basing on this data, we calculated the rated yield losses and converted them into scores using 1-9 score scale, where 9 means the highest level of a cultivar resistance (fig.1).

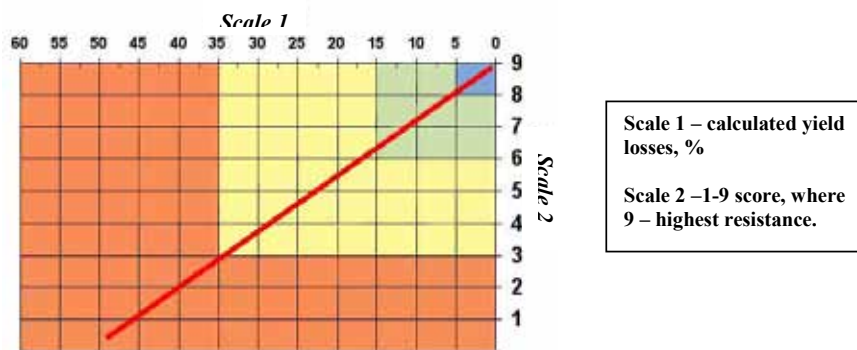


Figure.1. Scale for determination of potato cultivars resistance

RESULTS AND DISCUSSIONS

The results of our analysis are shown in Table 3.

Table 3. Characteristics of the aggressiveness of initial *P. infestans* isolates and their re-isolates

| Isolate | Cultivar | Resistance data | | | Initial isolate, (1-9 score) | Re-isolates (1-9 score) | Difference in score between initial isolates and re-isolates |
|-----------------|------------|-----------------|----|----|------------------------------|-------------------------|--|
| | | 1* | 2* | 3* | | | |
| KBB42 | Escort | + | + | + | 5,3 | 5 | +0,3 |
| | Udacha | + | + | + | 4 | 3 | +1 |
| | Lugovskoi | + | - | + | 5,3 | 5 | +0,3 |
| | Sante | + | + | + | 3,5 | 2 | +1,5 |
| | Sarpo Mira | + | + | + | 8 | 6,3 | +1,7 |
| KBK33 | Escort | + | - | 0 | 6,5 | 6,2 | +0,3 |
| | Udacha | + | + | + | 4 | 2,5 | +1,5 |
| | Lugovskoi | + | + | + | 6 | 5 | +1 |
| | Sante | + | + | + | 5 | 3 | +2 |
| | Sarpo Mira | + | 0 | 0 | 8 | 7 | +1 |
| Cx40BB | Escort | + | + | + | 8 | 5,5 | +2,5 |
| | Udacha | + | + | + | 3,5 | 2,5 | +1 |
| | Lugovskoi | + | + | + | 7 | 5 | +2 |
| | Sante | + | + | + | 3 | 2 | +1 |
| | Sarpo Mira | + | 0 | + | 7 | 5,5 | +1,5 |
| OPCKAS14 | Escort | + | - | 0 | 7,5 | 6,5 | +1 |
| | Udacha | + | + | + | 5 | 4 | +1 |
| | Lugovskoi | + | + | + | 8 | 4,5 | +3,5 |
| | Sante | + | + | 0 | 4,5 | 4 | +0,5 |
| | Sarpo Mira | + | 0 | 0 | 8,5 | 8 | +0,5 |

* 1 - effectiveness of infection; 2- diameter of necrotic lesions, cm; 3 - sporulation capacity
(+) - increase of score; (-) - decrease of score; (0) – without differences

According to the obtained results, the level of aggressiveness of re-isolates was significantly higher than in the initial isolates almost for all cultivars. The highest increase in the level of aggressiveness was observed for cvs. Udacha and Sante. In two cases we observed the significant increase of this parameter for the cv. Lugovskoi (infection with KBK33 and OPCKA S14 isolates); the same was observed twice for the cv. Escort (Cx40BB isolate).

The data obtained for each isolate were summarized for all used cultivars to determine how the level of aggressiveness increased for each isolate. One can see that all isolates, used in our experiment, became more aggressive. To determine which isolate is more dangerous for potato, we analyzed the growth of the level of aggressiveness comparing to the initial one (Fig. 2)

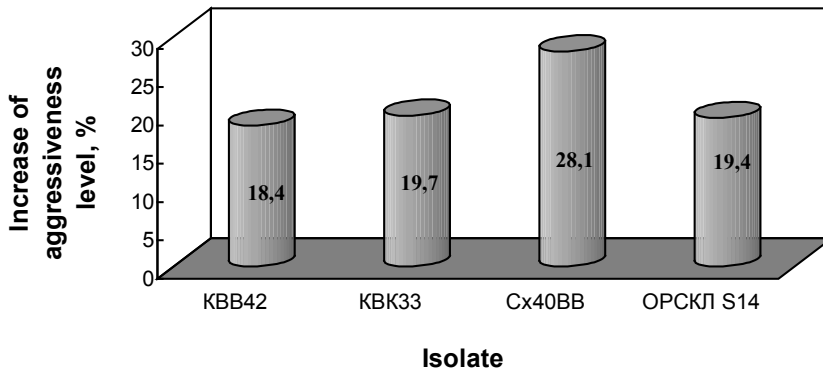


Figure 2. Increase in the level of aggressiveness of *P. infestans* isolates (% of initial value).

Thus, in the course of a two-year experiment, we showed that the long-term passaging of *P. infestans* isolates without any rotation of potato cultivars resulted in a significant growth of the level of aggressiveness of isolates regardless of their initial level of aggressiveness or the resistance level of cultivars.

ACKNOWLEDGEMENTS

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Thirteenth EuroBlight workshop
St. Petersburg (Russia), 9-12 October 2011

Experiences of *Alternaria* Disease Forecasting in the UK

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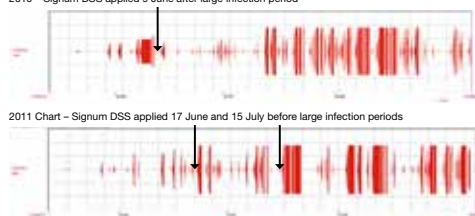
Introduction

In the last few years *Alternaria* has become more of an issue in UK potato crops (Florendine 2010), with severe infections being experienced in certain susceptible varieties, such as the processing cultivar Markies. Because not all late-blight fungicides have activity against *Alternaria*, products with specific activity to this disease have been recently introduced. However timing of application is important for these products (along with dual-activity fungicides) as control is through protectant action only. In 2010 and 2011, with support from BASF, *Alternaria* trials were carried out by SAC. Included was a Decision Support System (DSS) treatment, with the aim to evaluate the effect of application timing on disease control. The potato *Alternaria*-specific fungicide, Signum (Jilderda 2005), was used for the DSS treatment.



Infection 13 September 2011

Dacom *Alternaria* Disease Pressure Charts (infection periods in red)
2010 - Signum DSS applied 9 June after large infection period



Conclusions

In 2010 a high *Alternaria* risk period occurred early in the season around the 6 June, as a consequence infection in the crop was seen a few weeks later. Because of a late set-up of the DSS treatment, it was not applied until after this date (ref. 2010 chart above). The other programmes also did not start until mid June, which explains why there were few differences between treatments in this year.

Disease pressure in 2011, in contrast, occurred later with infection periods starting in late June. Two timings of the DSS treatment are highlighted above (2011 Chart) on 17 June and 15 July. These application dates were ahead of infection events. However, the standard 14 day programmes were not applied until after these dates. The difference in this timing is the most likely reason for improved disease control by the DSS treatment. This is evidence that such systems could prove to be useful tools for product timing and choice for *Alternaria* control in the UK.

Methods

The trials were set up in a commercial field, cv. Markies. Plot size was 4 rows x 7.5 metres, with 4 replicates in a randomised complete block design. In addition to untreated plots, there were infector strips (2.5m) between replicates. Because of low early disease pressure in 2011, the infector strips were inoculated with a mixture of *A. solani* on 22 June.

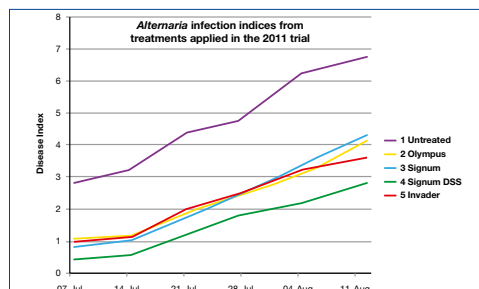
Treatments

- 2 **Olympus** (azoxystrobin/chlorothalonil) @ 1.0 l/ha x 3 then Dithane (mancozeb) @ 2.0 kg/ha in 2011
- 3 **Signum** (boscalid/pyraclostrobin) @ 0.25 kg/ha x 4
- 4 **Signum DSS** @ 0.25 kg/ha x 4 then Invader @ 2.4 kg/ha in 2011
- 5 **Invader** (dimethomorph/mancozeb) @ 2.4 kg/ha x 4 (2011 only)

Treatments 2, 3 and 5 were applied 4 times at a standard 14 day programme. The first timing of these treatments was made around 50% crop cover, in June. The DSS treatment timing was dictated by the Dacom *Alternaria solani* model (Hadders 2004), using local real time + forecast weather data and crop growth stage to calculate disease pressure.

Results

In both years all treatments gave significantly better *Alternaria* control than the untreated. In 2010 trial there were few differences between treatments, however, in 2011 the DSS treatment gave lower % plant infection and disease severity compared to other treatments, as expressed by a disease index in the chart below.



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Decision support systems for late blight integrated management in the southern Chile.

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SUMMARY

Potato is an important crop in Chile and represents a vital part of the agriculture. The major potato disease in Chile is late blight. In previous studies Blitecast was calibrated and used to evaluate chemical control strategies and the interaction with cultivar susceptibility and agronomic management season, with big success. With the results and the network of meteorological stations of INIA between the Bio-bio and the Los Lagos regions, has been implemented an late blight early warning system to support decisions of potato farmers in southern Chile, using tools of information and communications technologies such as an application that delivers information to farmers via SMS and e-mail. The early warning system of late blight is inserted in an integrated management strategies for disease control. The use of information technologies (SMS & email) for the late blight early warning system in southern Chile will allow farmers to have better support to decision making. It is expected to increase the number of system users by facilitating the access to information, specially farmers.

KEYWORDS

Early warning system, late blight, agrometeorological station network

INTRODUCTION

Potato is an important crop in Chile and represents a vital part of the agriculture and economy. The major potato disease in Chile is late blight, caused by *Phytophthora infestans*, which spreads fast and attacks vast areas if weather conditions are favorable. The disease can affect plants at any growing stage depending on inoculum and weather. An integrated disease management plan for disease control considers the knowledge of the genetic characteristics of the pathogen population, the relative susceptibility of the host and the proper timing of chemical controls based on weather conditions favorable for the disease. Since 2003, the Agricultural Research Institute of Chile (INIA), associated with public and private institutions, developed a study which main objective is to implement an integrated pest management for late blight based on a disease forecasting system. After studying different late blight models, the Blitecast was calibrated and used to evaluate chemical

control strategies and the interaction with cultivar susceptibility and agronomic management season, with big success. This forecast system is based in an automatic weather network, late blight model processor and web page information.

Since, wireless communication system begun to be important in rural areas of southern Chile, this season, the INIA forecasting system will use technologies such as Short Messages Service (SMS) and e-mail. With the use of this method the farmers will receive the late blight warning quickly and easily and therefore to make better management decisions. This new system will give access to those who did not have it for internet connectivity problems and also will improve the information access.

MATERIALS AND METHODS

The early warning system of late blight has three components, which allows the generation of information for the users and spread it readily and efficiently. These components include a network of automatic meteorological stations and corresponding database; a processing model, where estimates the conditions for the development of the late blight; and an information system, using tools of information and communications technologies. The model of the system can be seen in figure 1.

Meteorological station network

The network of INIA's meteorological stations are localized in various parts of the Chilean territory, however, for the early warning system of late blight, the data used comes from the stations that are located in the southern Chilean areas, between the Bio-bio and the Los Lagos regions. Here more than 73% of the surface is used for crop cultivation and produces 80% of the potatoes in Chile. This network is composed of 31 automatic meteorological stations, where temperature variables, relative humidity and precipitation on hourly basis were used as an input for the Blitecast model, which was validated under the conditions of Chile (Acuña *et al.* 2009).

Information technology

This system uses tools of information and communications technologies so the users can obtain quickly the warning information. The media used are the e-mails and the short messages service (SMS), which are sent through the system every day when an event of late blight, takes place.

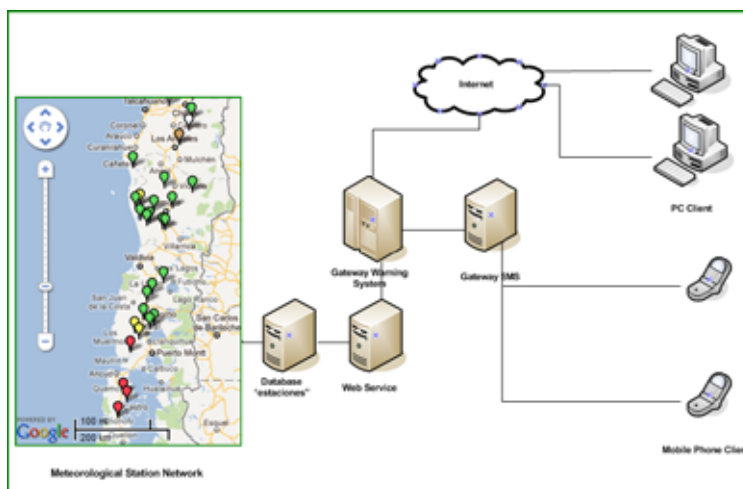


Figure 1. Late blight alert system structure.

RESULTS AND DISCUSSION

The early warning system of late blight is inserted in an integrated management strategies for disease control, minimizing the impact and damage on potatoes. Under these conditions, the web page of the early warning system is a site of information that offers a set of tools for the users that allows an improvement in the decision taking in the control of late blight in the south of Chile. These tools are:

The website

Corresponds to home page of the website (figure 2) and contains sections where users can find information about late blight and *Phytophthora infestans*. Here are present the following sections: Documents and photographs: corresponding to publications by INIA for the divulgation of different topics for the recognition of symptoms, integrated management, strategies evaluation, etc.



Figure2. Home page the late blight alert system web page (<http://tizon.inia.cl>)

The objective is that the users can know more about late blight and how the alert system can help them to take better decisions to control late blight.

News and events: facts and events are published and that can be of interest for the system users, like seminars, interviews, etc.

Late blight forecast system

It is where the web page is based, since it is where the information is generated from late blight daily development conditions. The information of the warning system is published in the website and the users can visualize them for the conditions present in all of the monitored sites, through a map where the automatic meteorological stations are located, using the Google Maps® technology (figure 3(a)). In the map, it is indicated with a color the conditions current according the meteorological data measured. The colors indicate the different condition category;

Green: There are NO conditions for the development of late blight.

Yellow: There are LOW conditions, must be alert for the conditions in the next few days.

Orange: Conditions are MID-LEVEL for the development of late blight.

Red: There are HIGH conditions for the development of late blight.

Another way to visualize the information is as indicated in figure 3 (b), where it is possible to observe the conditions for the development of late blight through out the season and for every meteorological station that is chosen. In the bottom axis it can be seen the dates from the start of the season and every color indicates an alert category in the same way as described before.



Figure 3 (a and b). Daily information display in <http://tizon.inia.cl>.

Through out these two ways the user can have a complete view on the meteorological conditions that have existed in the area where the crop is grown and that allows a correct decision on the crop protection.

Late blight warning messages

By the propagation characteristics of *P. infestans*, it is necessary that the information is delivered as soon as possible to the potato farmers, so this way it can be timely for the decision making. However, in the more rural sectors of Chile, the connectivity to internet is low, but the use of mobile phones is a communication medium that has a great coverage over the Chilean territory. For these reasons, this system has incorporated an application that delivers information to farmers via SMS. This service guarantees that the information can be sent directly to the users and they can see it any time of the day. For this, in registers of system, the farmers must enter their mobile phone number and the meteorological station of which receive late blight warning. If the users register his or her e-mail, they will receive the information also by this medium. Once completed all the steps, the system will send the information through SMS and/or e-mail to all the users according to the meteorological station to which they registered. An example of the message can be observed in table 1.

Table 1. Settings of some messages sent to the users through email and/or SMS.

| Alert Category | Message sent by email | Message sent by SMS |
|----------------|--|---|
| Yellow | Dear User: According to the data of the metrological station to which you are associated, there is a YELLOW ALERT. Check the crop the next few days and the state of the other meteorological stations of our network by visiting http://tizon.inia.cl . | YELLOW ALERT. Check the crop the next 3 days. Visit http://tizon.inia.cl . |
| Orange | Dear User: According to the data of the metrological station to which you are associated, there is an ORANGE ALERT. It is recommended to apply fungicides every 10 days. Check the state of the other meteorological stations of our network by visiting http://tizon.inia.cl . | ORANGE ALERT. There are médium conditions for the development of late blight. Visit http://tizon.inia.cl . |
| Red | Dear User: According to the data of the metrological station to which you are associated, there is an RED ALERT. It is recommended to apply fungicides every 7 days. Check the state of the other meteorological stations of our network by visiting http://tizon.inia.cl . | RED ALERT. There are high conditions for the development of late blight. Visit http://tizon.inia.cl . |

CONCLUSIONS

INIA late blight forecast system is working very successful since 2006. Today, the system shows a registration of 461 users, however, they have difficulties in access to information because of connectivity problems. The use of information technologies (SMS & email) for the late blight forecast system in southern Chile will allow farmers to have better support to decision making. It is expected to increase the number of system users by facilitating the access to information, specially farmers.

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Simulator for the comparison of fungicides, cultivar resistance, and Decision Support Systems in the control of the late and early blight of potato

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The presented simulator is based on the known van der Plank hypothesis (1968), which assumes a direct ratio between the area under the curve, describing the seasonal disease dynamics on the potato foliage, and the yield losses. According to our long-term field studies (Gurevich, Filippov, and

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

Tverskoy, 1977), this dependency can be expressed by the following equation: where ω is a yield loss (%), caused by a premature leaf decay, AUDPC is an area under the curve, describing the disease dynamics, and q represents 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 frost or desiccant or the harvesting is carried out before its natural death, then q is considered as a number of days, passed between the bud formation stage and the moment of the foliage death.

Fig. 1. Working window of the simulating program for the calculation of yield losses, caused by the development of late and early blight on the potato foliage (http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru).

It was found that the standard deviation of calculated yield losses from the actual losses was 9.8% for the set of 219 independent late blight dynamic curves.

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Thirteenth EuroBlight workshop
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Cryopreservation of *Alternaria solani* and *Phytophthora infestans*

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Cryopreservation of *Alternaria solani* and *Phytophthora infestans*



Christoph Andreas Braun & Anne Suty-Heinze

INTRODUCTION

Various fungi belonging to all taxonomic groups are routinely used in the disease control institutes of Bayer CropScience. The maintenance and mass production of this large variety of phytopathogenic fungal strains is essential for a high quality biological testing in the fungicide screening process. After an exhaustive comparison of different storage methods, cryopreservation of fungal material in the vapor phase of liquid nitrogen (-160°C) has been chosen to guarantee a reliable delivery of phytopathogenic fungal isolates for routine tests in the lab, greenhouse and field.

While conidia of most fungi (e.g. *Alternaria solani*) are relatively resistant to cellular injury during freezing and thawing, asexual bodies of many *Phytophthora* species (sporangia) are very susceptible to this kind of damage (Fig. 1). The objective of our work was to optimize the cryogenic storage procedure to define

conditions that increase viability of *Phytophthora infestans* sporangia for their direct use in the screening process.

MATERIALS AND METHODS

Experiments were performed with sporangia (and zoospores) of 15 different isolates of *P. infestans*. 21 cryoprotectants (penetrating & non-penetrating) in varying concentrations and compositions combined with nine different freezing methods and four different thawing temperatures were used. Viability of sporangia and zoospores were analyzed by germination assays on H₂O agar 14 days and three months after freezing. Disease development after inoculation of tomato plants was assessed to determine the effects of the storage conditions on pathogenicity.

RESULTS

Viability of both sporangia (Fig. 2) and

zoospores (data not shown) after storage varies depending on the isolate of *P. infestans*.

Moreover, the viability of *P. infestans* sporangia and zoospores could be significantly improved by the use of both cryoprotectants (Fig. 3) and controlled freezing. The most suitable cryoprotective agents were DMSO (15 %) and propylene glycol (12 %).

The highest recovery rates (45 % for sporangia and 67 % for zoospores) were obtained for samples of *P. infestans* frozen in DMSO (15 %) and controlled freezing. The most suitable cryoprotective agents were DMSO (15 %) and propylene glycol (12 %).

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Pathogenicity of stored sporangia and zoospores was confirmed in a bioassay with tomato plants (data not shown), even after 3 months of storage at -160°C.

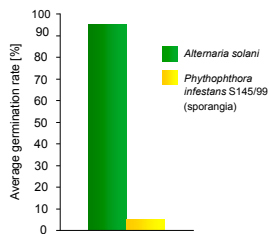


Fig. 1: Viability of conidia of *A. solani* (A) compared to sporangia of *P. infestans* S145/99 (B) after storage in liquid nitrogen for 14 days (cryoprotective agent: glycerol 10%).

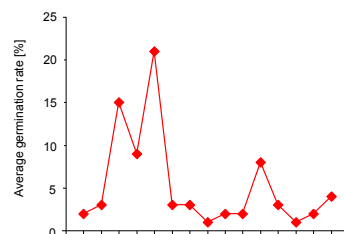
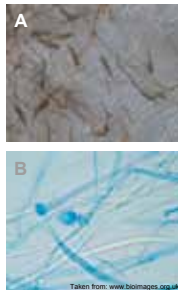


Fig. 2: Influence of different isolates on the viability of sporangia of *P. infestans* after storage in liquid nitrogen for 14 days (cryoprotective agent: glycerol 10%).

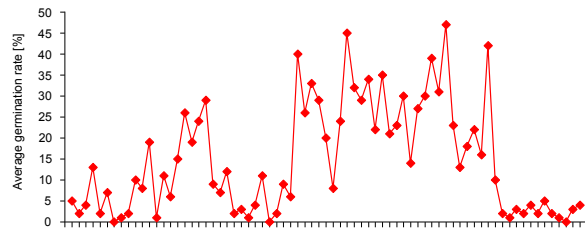


Fig. 3: Effect of different cryoprotective agents and freezing methods on the viability of sporangia of *P. infestans* S145/99 after storage in liquid nitrogen for 14 days.

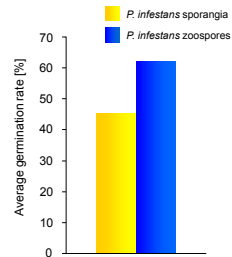


Fig. 4: Viability of sporangia compared to zoospores of *P. infestans* S145/99 after storage in liquid nitrogen for 14 days (cryoprotective agent: DMSO 15%).

CONCLUSION

Phytophthora infestans, the causal agent of late blight, is a problematic pathogen regarding storage due to its sensitive sporangia. The results of this work demonstrate that cryopreservation using DMSO (15 %) and controlled freezing represents an effective method for long-term storage of *P. infestans* inoculum. Cryopreservation thereby offers an unique solution to optimize the time of maintenance and production of the *P. infestans* inoculum used in the different screening tests.

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Postinfection Activity of Early Blight Fungicides

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Postinfection Activity of Early Blight Fungicides

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Introduction

The objective of this study was to compare the postinfectious activity of selected early blight fungicides. Fungicides are most effective if applications are carried out prior to infection (protective mode of action). But in practice, the early blight fungicide management mostly occurs curative, because the infection takes place before the first treatment is done. Two sets of different experiments were carried out. The first determined the effectiveness of selected fungicides in field trials. The second quantified the curative activity of selected fungicides applied 12 and 24 hours after inoculation in greenhouse experiments.

Materials and methods

Field trial

Field trials were carried out within 2009 and 2011 at Kirchheim near Munich. Trials were designed as a randomized complete block and were replicated four times. Potato trials were carried out using the variety Maxilla. Field trials were all naturally infected by early blight (EB). To prevent the development of late blight, the fungicide Ranman® (400 g cyazofamid/l) was applied as a cover spray at a dose of 0.2 l/ha every 8 to 10 days. As the disease progress of EB was not affected by the use of Ranman® early blight was allowed to develop naturally during the course of the growing season. Fungicide treatments (Tab. 1) were carried out once after exceeding the disease severity (% infected leaf area) of 25%.

Greenhouse trial

The trial was carried out using the cultivar Kuras grown in pots. The plants were inoculated by spraying an *Alternaria solani* spore suspension (5×10^4 sporangia per ml). The plant was incubated at 20°C and 12 hours photoperiod. 12 and 24 hours after inoculation plants were sprayed to runoff with selected fungicides (Tab. 1). Plants were again placed in an incubation chamber to provide symptom development.

Tab. 1: Fungicide features

| Fungicide | Active ingredient (a.i.) | Amount of a.i. (g/l, g/kg) | Dosage tested in the field (l,kg/ha) | Dosage tested in the greenhouse (l,kg/ha) |
|-----------------|---------------------------|----------------------------|--------------------------------------|---|
| Ortiva | Azoxystrobin | 250 | 0,5 | 0,25 |
| Signum | Boscalid + Pyraclostrobin | 267 + 67 | 0,25 | 0,125 |
| Cantus | Boscalid | 500 | | 0,25 |
| Dithane Neo Tec | Mancozeb | 750 | 2 | 1 |
| Score | Difenoconazol | 250 | | 0,25 |

Results

Field trial

The three tested fungicides (Ortiva, Signum, Dithane Neo Tec) showed no efficacy in controlling early blight in the field applied at a disease level of 25%. The disease development in the treated plots were not significantly different to the untreated control plot in all years.

Greenhouse trial

The study showed different curative effects of the tested fungicides (Fig. 1). Except for Dithane Neo Tec, all tested fungicides reduced the disease development by more than 90% applied 12 hours after inoculation. A reduction of the efficacy is shown for all tested fungicides after prolonging the time of the curative treatment from 12 to 24 hours.

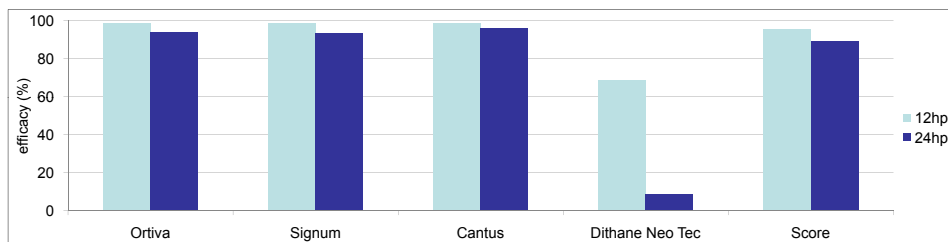


Fig 1: Efficacy of different fungicide treatments 12 and 24 hours after artificial inoculation (hpi) with *Alternaria solani* in a greenhouse trial.

Conclusion and outlook

The results of this study indicated that all tested fungicides have a curative activity under controlled conditions. But in the field under curative and eradication conditions a threshold value of 25% disease severity for the first early blight specific fungicide treatment was not functional.

In the future further trials are necessary to get a database for DSS modelling with the aim to optimise the control of early blight by the use of fungicides.

Fungicide Resistance of Russian *Phytophthora infestans* strains

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SUMMARY

P. infestans strains have been isolated in pure culture from blighted samples, collected in the Moscow, Leningrad, Astrakhan, Smolensk, and Kostroma regions, Mariy El Republic, and Belarus. The fungicide resistance of collected strains has been tested in Petri dishes with agar medium. The most efficient fungicides are azoxystrobin and dimethomorph. The efficiency of fluazinam, chlorothalonil, and mancozeb is also rather high. Therefore, the application of these fungicides in recommended dosages can provide a successful potato late blight control. The strains, highly resistant to metalaxyl, has been revealed in the Moscow and Smolensk regions, and also in Belarus.

KEYWORDS

Phytophthora infestans, late blight, fungicides

INTRODUCTION

Late blight is a dangerous disease of potato and tomato, which is typical for many countries and causing significant yield losses. The late blight control is complicated by extremely high variability of its agent, *Phytophthora infestans* (Mont.) de Bary, that results in the appearance of fungicide-resistant strains, highly aggressive to the earlier resistant potato cultivars.

The main way to control late blight is the chemical protection of crops, i.e. fungicidal treatments of fields. However, the efficient action of fungicides is possible only in the case if pathogen populations do not have or have a very low number of highly resistant strains. The assessment of the resistance of the pathogen to the used fungicides is carried out not too often, and such data are absent for many regions of Russia and Belarus.

In our study we assessed the resistance of late blight strains from Belarus and some distinct regions of the European part of Russia to several popular fungicides, such as metalaxyl (systemic fungicide), azoxystrobin (limited systemic fungicide), dimethomorph (translaminar fungicide), fluazinam, chlorothalonil, and mancozeb (contact fungicides).

MATERIALS AND METHODS

Collection of isolates

In our study we used *P. infestans* isolates, collected in 2007-2009 from tomato and potato fields in the Leningrad, Moscow, Astrakhan, Kostroma, and Smolensk regions, Mariy El Republic, and Belarus (Fig. 1, Table 1). One isolate was isolated from each leaf lesion.



Figure 1. Sampling site locations on the European part of Russia. 1, Leningrad region; 2, Moscow region; 3, Kostroma region; 4, Mariy El Republic; 5, Astrakhan region; 6, Belarus.

Table 1. Location and time of the sampling of blighted plants and the number of strains, isolated into the pure culture

| Sampling location | Sampling date | Fungicidal treatment | Host plant | Number of isolated strains |
|--|--------------------|----------------------|------------|----------------------------|
| Leningrad region, Belogorka village, fields of the Leningrad Research Institute of Agriculture | 07.2008 | – | PL* | 22 |
| Kostroma region, Minskoe village, fields of the Kostroma Research Institute of Agriculture | 08.2008 08.2009 | + | PL | 84 |
| Mariy El Republic Yoshkar-Ola outskirts, private gardens | 08.2007 | – | TF** | 89 |
| Moscow region, Lyubertsy district, fields of the All-Russian Potato Research Institute | 08.2008 | – | PL | 45 |
| Smolensk region, Safonovo district, Izdeshkovo village | 08.2009 | + | PL | 49 |
| Astrakhan region, Kamyzyak district | 08.2008 | + | TF | 33 |
| Belarus, different regions | 2006-2007 | + | PL*** | 78 |

*PL, potato leaves.

**TF, tomato fruits.

Isolation of strains into the pure culture

Isolation of strains into the pure culture was carried out using wet chambers. After the appearance of the fruiting on the surface of an infected sample, it was microscopied, and zoosporangia were collected using a microbiological needle with a piece of agar medium on its tip; during the collection of zoosporangia, the agar block did not touch the sample surface. Zoosporangia were put onto oat agar medium, supplemented with penicillin (1000 µg/ml) and incubated until the diameter of a colony reached 4-5 cm. Then a piece of mycelium from the edge of the colony was transferred into another Petri dish with the same medium.

Fungicide resistance assessment

The resistance of isolates to fungicides was assessed on oat agar medium, supplemented with the corresponding fungicide at various concentrations (0.1, 1, 10, 100, and 1000 µg/ml), and on the medium without any fungicide (control). The experiments were made in three repetitions. For each isolate we determined the EC₅₀ value, i.e. the concentration of a fungicide, causing a twofold delay in the colony growth rate.

RESULTS AND DISCUSSION

Fungicides significantly differ concerning the chance of appearance of fungicide-resistant strains in a pathogen population. An increase in the resistance to multi- and oligo-site fungicides occurs rather slowly and involves a step mutagenesis. Probably, due to this fact, the treatment of plants with mancozeb, fluazinam, chlorothalonil, and dimethomorph did not result in the appearance of highly resistant strains in a population. The analysis of a mancozeb resistance did not revealed any strains, which EC₅₀ level would exceed 31 µg/ml, though several populations included strains, which characteristics were close to this level (Table 2). Probably, this level represents a threshold value, and strains with a higher resistance level are nonviable or noncompetitive in agroecosystems.

The resistance of *P. infestans* strains to azoxystrobin should be discussed separately. In all studied populations we observed only highly susceptible isolates, though, according to other authors, the risk of development of the azoxystrobin resistance is considered to be rather high. Probably this fact is connected with a low level of application of the Quadris preparation in the European part of Russia, since this preparation is registered only for the treatment of tomato. We specially examined strains, isolated from commercial tomato fields in the Astrakhan region, where azoxystrobin and krezoxymethyl, another strobilurine fungicide, are widely used. However, in this case we also observed only highly susceptible isolates. On the other hand, in the case of potato fields in the European part of

Russia, farmers often use famoxadon and phenamidon; resistance to these preparations is crossed with that to azoxystrobin (Bartlett *et al.*, 2002). It seems that the development of the azoxystrobin resistance in *P. infestans* strains is a very rare event.

The analysis of a metalaxyl resistance showed that the most of the studied populations were represented by susceptible strains. The EC₅₀ levels of isolates, collected from the Astrakhan and Leningrad regions and Mariy El Republic, did not exceed 5 µg/ml. In the case of the Kostroma region, we did not observe any isolates, which EC₅₀ value would exceed 40 µg/ml, though in 2009 we specially collected samples from fields, treated with Ridomil Gold MC. Among 64 isolates, collected in different regions of Belarus from commercial potato fields, which are usually treated with phenylamide-containing fungicides, only five had the EC₅₀ level, exceeding 10 µg/ml; only two of them were highly resistant (EC₅₀ > 100 µg/ml). In the case of the Moscow region, samples were collected from an untreated field, surrounded by commercial potato fields. Among 31 examined isolate, 8 had EC₅₀ > 100 µg/ml. The highest percentage of highly resistant isolates was observed in the Smolensk population. Among 49 tested isolates, 28 were highly resistant (EC₅₀ > 100 µg/ml); only 5 isolates were susceptible (EC₅₀ < 10 µg/ml).

The appearance of metalaxyl-resistant strains in the Smolensk region probably was caused by either an increase in the resistance of initially susceptible strains, or the introduction of resistant strains from other potato fields. The second version seems to be rather unlikely, since we did not reveal any highly resistant isolates in the Kostroma region (the source of a seed material for the Smolensk region), even on the fields, treated with metalaxyl-containing preparations. In addition, the study of the allozyme structure of peptidase showed some differences between resistant strains from the Smolensk region and the strains, collected in 2008 in the Kostroma region. Therefore, resistant Smolensk strains have rather local origin.

A high genotypic diversity of *P. infestans* populations, observed in some regions of Russia (Amatkhanova *et al.*, 2004; Shein *et al.*, 2009; Elansky *et al.*, 2001, 2003), can provide the selection of highly resistant and aggressive strains in the case of intensive and wrong fungicidal treatments; the appearance of such strains is able to stultify the efficiency of a crop protection. First of all, it concerns the application of metalaxyl-containing preparations, since even if only 3% of resistant forms present in a population, it will be enough for the rapid increase in the number of resistant strains in the case of an uncontrolled metalaxyl application.

In general, according to the obtained results, the most of strains, composing Russian *P. infestans* populations, are susceptible to the most popular fungicides and, therefore, the resistance of even the most resistant isolates can be successfully overcome by the use of fungicides in the dosages, recommended for Russia and Belarus. The only exception is metalaxyl resistant isolates, which control with phenylamide-containing fungicides can be inefficient in the Moscow and Smolensk regions and also Belarus.

Table 2. Fungicide resistance of *P. infestans* strains from different regions of Russia and Belarus

| Fungi- cide | Sampling site | Number of tested isolates | EC ₅₀ variability, µg/ml | Average EC ₅₀ level, µg/ml | Number of strains with different EC ₅₀ levels | | | |
|----------------|-------------------|------------------------------|-------------------------------------|---|---|------|--------|------|
| | | | | | <1 | 1–10 | 10–100 | >100 |
| Marcozeb | Moscow region | 23 | 0,6 – 22,4 | 5,51 | 5 | 16 | 2 | 0 |
| | Leningrad region | 19 | 0,98 – 26 | 10,9 | 1 | 10 | 8 | 0 |
| | Smolensk region | —* | — | — | — | — | — | — |
| | Kostroma region | 25 | 0,5 – 25,6 | 6,48 | 3 | 20 | 2 | 0 |
| | Astrakhan region | 20 | 0,6 – 18,6 | 5,31 | 4 | 14 | 2 | 0 |
| | Mariy El Republic | 45 | 0,5 – 27 | 8,42 | 2 | 30 | 13 | 0 |
| Methalaxyl | Belarus | 57 | 0,64 – 30,8 | 10,2 | 6 | 31 | 20 | 0 |
| | Moscow region | 31 | 0,52 – 398 | 100,0 | 14 | 4 | 5 | 8 |
| | Leningrad region | 12 | 0,54 – 4,7 | 2,0 | 7 | 5 | 0 | 0 |
| | Smolensk region | 48 | 0,51 – 380,0 | 168,9 | 2 | 2 | 12 | 32 |
| | Kostroma region | 52 | 0,51 – 38,75 | 1,67 | 44 | 7 | 1 | 0 |
| | Astrakhan region | 25 | 0,51 – 2,85 | 0,85 | 22 | 3 | 0 | 0 |
| Azoxy-strobin | Mariy El Republic | 47 | 0,5 – 4 | 0,76 | 42 | 5 | 0 | 0 |
| | Belarus | 64 | 0,5 – 151,5 | 5,5 | 55 | 4 | 2 | 2 |
| | Moscow region | 15 | 0,05 – 0,07 | 0,05 | 15 | 0 | 0 | 0 |
| | Leningrad region | 10 | < 0,05 | < 0,05 | 10 | 0 | 0 | 0 |
| | Smolensk region | 10 | < 0,05 | < 0,05 | 10 | 0 | 0 | 0 |
| | Kostroma region | 14 | 0,05 – 0,07 | 0,05 | 14 | 0 | 0 | 0 |
| Chlorothalonil | Astrakhan region | 10 | < 0,05 | < 0,05 | 10 | 0 | 0 | 0 |
| | Mariy El Republic | 10 | < 0,05 | < 0,05 | 10 | 0 | 0 | 0 |
| | Belarus | 27 | < 0,05 | < 0,05 | 27 | 0 | 0 | 0 |
| | Moscow region | 8 | 0,51 – 0,79 | 0,6 | 8 | 0 | 0 | 0 |
| | Leningrad region | 5 | 0,53 – 5,5 | 3,0 | 2 | 3 | 0 | 0 |
| | Smolensk region | 8 | 0,64 – 4,95 | 2,17 | 4 | 4 | 0 | 0 |
| Fluazinam | Kostroma region | 6 | 0,81 – 11,5 | 4,48 | 3 | 2 | 1 | 0 |
| | Astrakhan region | 10 | 0,55 – 5,5 | 3,18 | 3 | 7 | 0 | 0 |
| | Mariy El Republic | 4 | 1,82 – 3,45 | 2,67 | 0 | 4 | 0 | 0 |
| | Belarus | 23 | 0,69 – 16,75 | 4,85 | 3 | 19 | 1 | 0 |
| | Moscow region | 8 | 0,53 – 5,24 | 2,03 | 4 | 4 | 0 | 0 |
| | Leningrad region | 5 | 0,59 – 5,34 | 2,36 | 2 | 3 | 0 | 0 |
| Dimethomorph | Smolensk region | 7 | 0,52 – 6,83 | 2,48 | 4 | 3 | 0 | 0 |
| | Kostroma region | 8 | 0,53 – 5,99 | 1,7 | 6 | 2 | 0 | 0 |
| | Astrakhan region | 9 | 0,75 – 4,26 | 1,92 | 5 | 4 | 0 | 0 |
| | Mariy El Republic | 4 | 4,21 – 8,43 | 5,51 | 0 | 4 | 0 | 0 |
| | Belarus | 21 | 0,5 – 30,12 | 6,98 | 3 | 14 | 4 | 0 |
| | Moscow region | 9 | 0,05 – 0,09 | 0,06 | 9 | 0 | 0 | 0 |
| Dimethomorph | Leningrad region | 5 | 0,05 – 0,07 | 0,06 | 5 | 0 | 0 | 0 |
| | Smolensk region | 7 | < 0,05 | < 0,05 | 7 | 0 | 0 | 0 |
| | Kostroma region | 7 | 0,05 – 0,06 | 0,05 | 7 | 0 | 0 | 0 |
| | Astrakhan region | 7 | 0,05 – 0,09 | 0,06 | 7 | 0 | 0 | 0 |
| | Mariy El Republic | 5 | 0,05 – 0,06 | 0,06 | 5 | 0 | 0 | 0 |
| | Belarus | 34 | < 0,05 | < 0,05 | 34 | 0 | 0 | 0 |

*Not studied.

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Criteria to choose fungicides to control potato foliar diseases

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CRITERIA TO CHOOSE FUNGICIDES TO CONTROL POTATO FOLIAR DISEASES

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CONTEXT & OBJECTIVES

Context: Potato foliar diseases are very important, particularly the light blight which is more and more aggressive and the early blight which is spreading widely. Fungicides are essential tools to control those diseases. To build up the Decision Support System (DSS) it is necessary to choose the best fungicide at the best moment. Moreover, the grower and the seller of phytosanitary products need to dispose immediately these fungicides.

Objective: A decision scale to choose fungicides is useful and can be used as a training tool. It could be a little DSS.

CRITERIA OF CHOICE

Criteria of choice are very numerous: what diseases to control, growth stages of the crop, disease pressure (plant and pathogene action, effectiveness), rainfall, fungicides resistance risks, regulation rules, quality/price balance, using easiness

How to choose the criteria:

1. The diseases: In France, the potato area in North West are concerned essentially by late blight, but in the central and East part the early blight is important and it is spreading also in the North West. So criteria 1 is *Phytophthora infestans*, and the second is *Alternaria sp*

2. The stages:



3. Priority of criteria by stage:

- **Crop emergence:** to avoid early contaminations, it is necessary to spray according to the pressure disease, but there only few leaves
 → criteria 1: cost of the product, criteria 2: disease pressure given by the DSS, BSV in France (sanitary bulletin), dumps observations.
- **Stem growth:** the target is no stem blight
 → **systemic, retroactive, and very effective** fungicide.
- **Foliage growth:** We have to protect all the growing leaves and particularly the « top bud or bunch »
 → criteria 1: **diffusant** fungicide, criteria 2: **efficacy** according to pressure disease, criteria 3: rainfall.
- **Foliage stabilisation:** The targets are to protect the leaves during rain or irrigation period and also the tubers
 → in a first period: criteria 1: **efficacy/pressure, rainfall**, criteria 2: **alternaria risk** (alternaria efficacy) criteria 3: efficacy against tuber blight (number of spores).
 → in a second part: criteria 1: efficacy against foliar and **tuber blight** (mobility and survival of the spores), rainfall, criteria 2: alternaria.
- **Senescence:** the most important point is the quality of the tuber
 → criteria 1: **tuber blight efficacy** (survival, mobility), criteria 2: cost/risk (rains on foliar symptoms)
- **Emergency treatment however the stage:** retroactive and antispurulant fungicide

EVALUATION OF FUNGICIDES ACCORDING TO GROWTH STAGES

How to obtain the fungicides informations: the EUROBLIGHT TABLE: fungicides comparison, Arvalis Institut du Végétal leaflet: « traitement en végétation contre le mildiou et l'alternariose », regional trials, price scales.

Examples of analyses on two stages (tab.1, tab.2):

Table 1: leaves growth stage

| Fungicide | Late blight | | | |
|-------------------|-------------|--------------|---------------|--------------|
| | Diffusant | Low pressure | High pressure | Rainfastness |
| Diméthomorphe | XXX | XXX | XX (X) | XXX |
| Benthiavaliacarbe | XXX | XXX | XX (X) | XXXX |
| Mandipropamid | XXX | XXX | XXX | XXX (X) |
| Fluopicolide | XXX | XXX | XXX | XXX (X) |
| Dithiocarbamate | X | XXX | X | X |
| Fluazinam | X | XXX | X | XX |
| Cyazofamide | XXX | XXX | XXX | XXX (X) |
| Cymoxanil | X | XXX | XX | XX |

Table 2: Stage: foliage stabilisation part 2

| Fungicide | Late blight | | | | Early blight | Cost |
|--------------------|--------------|---------------|--------------|--------------|--------------|--------|
| | Low pressure | High pressure | Rainfastness | Tuber blight | | |
| Diméthomorphe | XXX | XX (X) | XXX | XX | 0 | XX (X) |
| Benthiavaliacarbe | XXX | XX (X) | XX (X) | X (X) | 0 | XXX |
| Mandipropamid | XXX | XXX | XXX (X) | XXX | 0 | XX |
| Fluopicolide | XXX | XXX | XXX (X) | XXX | 0 | XXX |
| Dithiocarbamate | XX | X | X | X | XX | X |
| Fluazinam | XXX | XX | XX | XXX | 0 | XX |
| Cyazofamide | XXX | XXX | XXX | XXX | 0 | XX |
| Zoxamide | XXX | XX | XXX | XXX | XX | XX (X) |
| X (not registered) | 0 | 0 | 0 | 0 | XX (X) | XX |

LIST OF NECESSARY FUNGICIDES

Examples of decision:

- Production with good benefit in aera with high late blight risk: emergence: fluazinam, stem growth: fluopicolide, foliar growth: mandipropamid, stabilisation 1: mandipropamid, zoxamide, stabilisation 2 and senescence: cyazofamide and fluazinam
 → list: fluazinam, fluopicolide, mandipropamide, zoxamide, cyazofamide, and cymoxanil for emergency spray
- Production with intermediat benefit: dithiocarbamate, fluopicolide or mandipropamide or dimetomorphe, fluazinam, and cymoxanil
- Production with low benefit: dithiocarbamate, fluazinam, cymoxanil

NB: for each stage we have a table with regulation rules (number of sprays...), for some stages we have the risk of resistance of the two diseases

Thirteenth EuroBlight workshop
St. Petersburg (Russia), 9-12 October 2011

Aggressiveness of different *Phytophthora infestans* isolates from Germany 2010

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Aggressiveness of different *Phytophthora infestans* isolates from Germany 2010

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Introduction

The objective of this study was to compare the aggressiveness of *Phytophthora infestans* isolates collected from different regions of Germany in 2010. The aggressiveness index, based on latent period, spore production and necrosis development, was calculated according to the results of a bioassay.

Materials and methods

Isolates from single lesions were sampled from different regions. After storage the isolates were first cultured on tuber slices. After 3 to 7 days the sporulating mycelium was transferred to V8 agar petri dishes and incubated at 16°C. The inoculum was prepared by washing off the sporulating mycelium of two weeks old isolates, the concentration was adjusted to 1,2 to 3,8 x 10⁴ sporangia per ml. For the bioassay leaf discs of the cultivar Agra were placed in petri dishes containing water agar. Inoculation was performed by placing a 30 µl droplet of a sporangial suspension at the center of each leaf disc. The petri dishes were incubated at 16°C and 12 hours photoperiod. The assessments (necrosis development and sporulation) were carried out every 12 hours. 168 hours after inoculation the spore production per leaf disc was determined on 10 leaf discs which were 100% infected. Each experiment was independently repeated for at least two times.

Two different aggressiveness indices (AI) were calculated:

$$AI\ 1 = [\Delta \text{ necrotic development (d5-d4, mm}^2)] \times [\Delta \text{ sporulation (d6-d4) / 2, Sp mm}^{-2} \text{ d}^{-1}]$$

$$AI\ 2 = [\Delta \text{ necrotic development (d5-d4, mm}^2)] \times [\Delta \text{ sporulation(d6-d4) / 2, Sp mm}^{-2} \text{ d}^{-1}] \times [1 / \text{latent periode}]$$

Results

Tabelle 1 shows the calculated aggressiveness index (AI 1), final spore production (168 hpi) and latent period for 20 isolates from all over Germany. There was no correlation between aggressiveness index and spore production detectable. The latent period in the bioassay was between 48 and 76 hours. Figure 1 indicates the high correlation between the two different aggressiveness indices.

| Isolate | Aggressiveness Index (AI 1) | Sporangia concentration 168 hpi (sporangia/ml) | Latent period (hours) |
|---------|-----------------------------|--|-----------------------|
| 6 | 1194,5 | 30,4 x 10 ⁴ | 76 |
| 7 | 1065,8 | 42,4 x 10 ⁴ | 52 |
| 8 | 1942,5 | 33,8 x 10 ⁴ | 60 |
| 10 | 311,3 | 33,0 x 10 ⁴ | 72 |
| 11 | 1454,3 | 48,9 x 10 ⁴ | 66 |
| 12 | 1625,0 | 52,2 x 10 ⁴ | 54 |
| 15 | 1722,0 | 38,3 x 10 ⁴ | 54 |
| 17 | 1030,3 | 42,2 x 10 ⁴ | 64 |
| 18-2 | 1606,0 | 41,3 x 10 ⁴ | 48 |
| 18-3 | 1339,3 | 55,2 x 10 ⁴ | 60 |
| 19 | 713,7 | 45,6 x 10 ⁴ | 52 |
| 20 | 1068,5 | 52,4 x 10 ⁴ | 66 |
| 22 | 486,5 | 41,8 x 10 ⁴ | 68 |
| 23 | 1878,8 | 41,9 x 10 ⁴ | 48 |
| 24 | 641,0 | 35,1 x 10 ⁴ | 72 |
| 30 | 481,8 | 36,4 x 10 ⁴ | 72 |
| 31 | 518,2 | 40,3 x 10 ⁴ | 72 |
| 32 | 1909,8 | 39,2 x 10 ⁴ | 60 |
| 38 | 775,8 | 9,2 x 10 ⁴ | 60 |
| 57 | 619,8 | 18,8 x 10 ⁴ | 54 |

Tab. 1: Results of the bioassay: aggressiveness index (AI 1), final spore production (168 hpi) and latent period (hours).

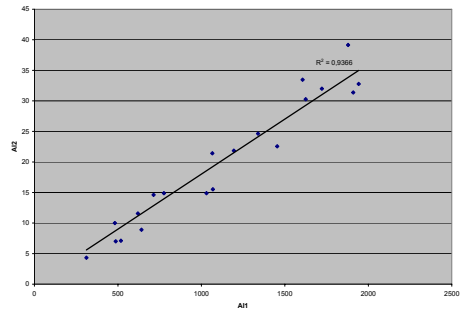


Fig 1: Correlation between the calculated aggressiveness index AI1 and AI2. There was a high correlation between both indices (R²=0,94).

Conclusions and outlook

In the present study the inclusion of the latent period into the aggressiveness index did not influence the rating of aggressiveness of the isolates. However, the latent period of the pathogen plays a key role in all DSS models. So far it is necessary to evaluate these data and update the DSS systems.

Thirteenth EuroBlight workshop
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Early blight diagnostics in potato: Diagnostics: difficulties and digitalisation

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Early blight diagnostics in potato: Diagnostics: difficulties and digitalisation

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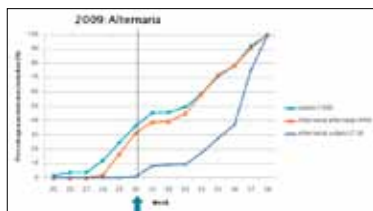
Early blight diagnostics

Find the early blight (*Alternaria solani*)!



Four potato leaflets, each showing similar symptoms. Which one, if any, is actual early blight? The correct answer is shown at the bottom of the poster!

Diagnosis of early blight has proven difficult, as demonstrated in 2009 and 2010: Early blight is often confused with ozone damage, which is potentially more damaging to yield than the blight.



Experts brought in early blight samples. It was not until week 30 when the first real early blight (*Alternaria solani*) appeared, indicated by the blue arrow. At this point over 35% of the total number of samples had falsely been identified as such in the field. Results in 2010 were similar to 2009.

The role of *Alternaria alternata* as a pathogen is currently debated: we have not been able to induce symptoms in lab- or fieldsituations and the fungus is mostly found as a saprophyte in lesions.

Results show that diagnostics of early blight can be difficult, resulting in unnecessary applications of fungicides. This is detrimental for the environment and the economic yield for farmers. It also leads to increased resistance development of *Alternaria* to a number fungicides.

More knowledge and a different approach are useful to correctly diagnose early blight.

Digital diagnostics

Current diagnostics is relatively slow and costly: time lost here is time gained for the disease to spread

Combining fielddata and image recognition software leads to faster diagnosis of crops with symptoms:



The project started by characterizing differences between symptoms caused by early blight and those caused by other, mostly physiological, diseases, such as ozone damage.

Image recognition software is not perfect and the human eye and brain are surprisingly complex and difficult to capture in "computer-language". Therefore, image recognition by itself is not enough and other data is needed to produce the correct diagnosis.

Cultivar, nutrients, temperature, humidity and other factors influence the occurrence of diseases. Data collected is obtained from farmers or sensors located in the field and combined with image recognition to produce the correct diagnosis.



Expansion to other diseases in other crops is planned in the coming years, as well as continuous testing and adjusting of our systems. Field trials will be used to obtain insight into symptom development and many samples will be taken from around the country and continent to further improve the reliability

A: mangrove exerts, B: ozone damage, C: early blight, D: ozone damage
Image "R" courtesy of Fabian Peizer



Thirteenth EuroBlight workshop
St. Petersburg (Russia), 9-12 October 2011

Early Blight: Pathogenicity and fungicidal control of *Alternaria solani* and *Alternaria alternata*

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Early Blight: Pathogenicity and fungicidal control of *Alternaria solani* and *Alternaria alternata*



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Introduction

Early blight of potato and tomato is caused by *Alternaria solani* and the involvement of *Alternaria alternata* in this disease is under discussion. Therefore greenhouse studies of tomatoes were performed to check differences in pathogenicity.

Early blight is mainly controlled by fungicide treatments, e.g. QoIs. Resistance to QoIs is mediated by mutations in their target gene, the cytochrome *b*. Because of the genetic structure of cytochrome *b* in *Alternaria solani*, the most important mutation, the G143A, did not occur so far. However, another mutation, the F129L has been reported. Sensitivity of isolates carrying the mutation F129L were compared with wild type isolates.

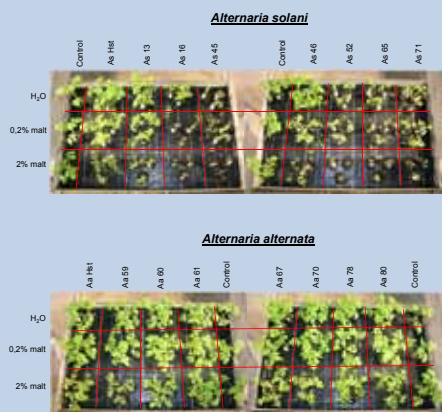
Material

- Comparison of the pathogenicity of 8 *Alternaria solani* and 8 *Alternaria alternata* isolates in the greenhouse
- Microtiter assays to evaluate the sensitivity of *Alternaria solani* and *Alternaria alternata* against pyraclostrobin (QoI), azoxystrobin (QoI), and metiram (dithiocarbamate) with a serial dilution of 30 - 10 - 3 - 1 - 0.3 - 0.1 - 0.03 - 0 ppm in YBA medium

Results

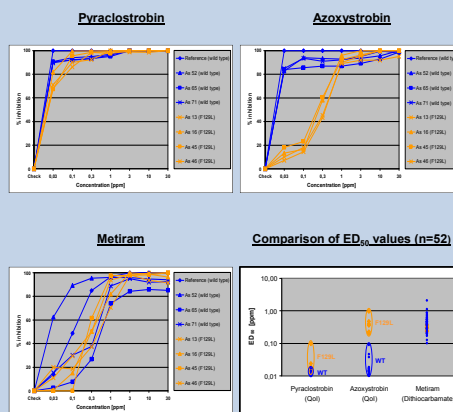
Alternaria solani is more pathogenic than *Alternaria alternata* on tomatoes in the greenhouse

Pathogenicity of different *Alternaria*-isolates, concentration of the conidial suspension about 5×10^7 conidia per ml in H₂O, 0.2% malt-extract solution, and 2% malt-extract solution, 6dpi



Isolates of *Alternaria solani* with F129L mutation are good controlled *in vitro* by pyraclostrobin

Microtiter assay with different isolates of *Alternaria solani*, inhibition after 5 days of inoculation



Conclusion

- Alternaria solani* is more pathogenic under greenhouse conditions than *Alternaria alternata*
- No G143A mutation in cytochrome *b* of *Alternaria solani* detected so far, but F129L
- Influence of F129L mutation on QoI efficacy is limited

**Recombination between recently occurring A1 and A2 isolates
of *Phytophthora infestans* in Ireland**

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Recombination between recently occurring A1 and A2 isolates of *Phytophthora infestans* in Ireland

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Background

Recent reports have shown a resurgence of the A2 mating type and occurrence of new genotypes of *P. infestans* in Ireland and the UK with novel SSR (simple sequence repeat) loci. These include aggressive isolates of 13_A2 ('Blue 13'), which exhibits phenylamide resistance and 6_A1 ('Pink 6'). The emergence of these strains has increased the risk of sexual recombination and the subsequent generation of complex genotypes. As the implications for bright control strategies are as yet unknown, it is important to determine whether novel recombinant progeny exhibit fungicide resistance and whether they have an ability to overcome established host resistance.

Objective

- Investigate the potential for genetic recombination between traditional (8_A1) and novel (13_A2 and 6_A1) isolates of *Phytophthora infestans* via two crosses: 13_A2 x 6_A1 and 13_A2 x 8_A1
- Characterise the F1 populations for mating type and metalaxyl sensitivity

Methodology

Oospores were harvested from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses on paired culture plates. A 1:0.5 (v/v) suspension of 2000 oospores/ml and cellulase from *Trichoderma reesei* 1,-4-(1,3:1,4)- β -glucan-4 glucanohydrolase, respectively was incubated overnight to digest residual mycelia. Oospore germination was completed on 0.5% water agar overlaid with rye agar. Pure cultures were raised from single colonies after 10 days (Fig. 1). The 13_A2 parental isolate was metalaxyl resistant in contrast to 6_A1 and 8_A1 which were sensitive. Mating type was tested on F1 progeny by pairing with tester isolates on carrot agar and metalaxyl sensitivity assessed using the floating leaf disc technique at concentrations of 0, 5 and 100 mg/L metalaxyl. Results were scored as resistant when progeny sporulated on leaf discs floated on 0, 5 and 100 mg/L, sensitive when sporulation occurred only on 0 mg/L and intermediate with sporulation on discs floated on 0 mg/L and 5 mg/L metalaxyl only. DNA was extracted from mycelia of each culture (Raeder & Broda 1985) and used for microsatellite genotyping using 10 markers (Lees *et al.*, 2006). Genetic similarities between F1 progenies were assessed using UPGMA and Jaccard Co-efficients in Free Tree with 1000 replications (Fig. 3).

Results

Twenty-four and 40 F1 progeny were isolated from the 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses, respectively. Corresponding segregation of mating type (A1:A2) was 13:11 and 24:16 from 13_A2 x 6_A1 and 13_A2 x 8_A1, respectively. The metalaxyl phenotypes (Fig. 2) of the F1 population segregated into 3:16:3 (sensitive:intermediate:resistant) and 6:25:5 for 13_A2 x 6_A1 and 13_A2 x 8_A1 pairings. There was segregation in 7 of the 10 SSR loci in F1 progenies with the occurrence of new alleles at Loci D13 and G11.

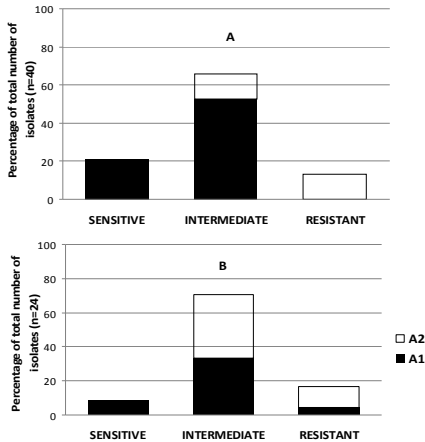


Fig. 2: Metalaxyl sensitivity and mating type profiles of recombinant progeny derived from 13_A2 x 8_A1 (A) and 13_A2 x 6_A1 (B) crossings. Data presented as % of total no. of isolates collected per cross.

Acknowledgements

This work is funded through the DAFM Research Stimulus Fund

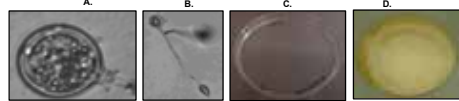


Fig 1: Images of digested oospore wall (A), germinating oospore post-cellulase treatment (B) single spore colony on 0.5% water agar with rye agar overlay (C) and pure culture from single colony (D).

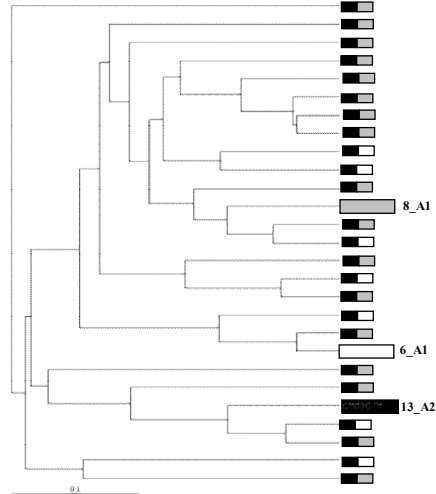


Fig. 3: Dendrogram illustrating genetic diversity within F1 populations from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses. Bar illustrates genetic distance. Colour bars show genetic contribution to progeny from each parent in respective crosses.

Conclusion

Isolates of the 13_A2 genotype will mate with isolates of the opposite mating types of 6_A1 and 8_A1 genotype, producing a segregating F1 population. The A1:A2 ratio from 13_A2 x 6_A1 and 13_A2 x 8_A1 crosses did not differ significantly from a 1:1 ratio. In both crosses, the segregation in metalaxyl response produced a low frequency of F1 progeny with sensitivity and resistance, and a high frequency of those with an intermediate phenotype. The F1 populations are currently being tested for haplotype and aggressiveness.

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