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**Working group “Integrated Plant Protection in Fruit Crops”
Subgroup “Pome Fruit Diseases”**

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on Pome Fruit Diseases**

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Foreword

Dear Colleagues,
on behalf of the IOBC-WPRS Working group “Integrated Plant Protection in Fruit Crops” Sub Group “Pome Fruit Diseases”, we would like to recall that the 11th International IOBC-WPRS Workshop on Pome Fruit Diseases took place from 26 to 30 June 2017 in Jūrmala, Latvia.

The workshop takes place approximately every 3 years and aims to bring together the latest advances in research of pome fruit diseases, to provide an opportunity for exchange of information and ideas and to stimulate common research and collaboration.

We are pleased to announce that we had 50 participants, who had either oral or poster presentations during the workshop. The delegates attending the conference arrived from over 20 countries and represented universities, research institutes, industry and advisory services.

We thank all those who attended the workshop and wish you all successful meetings in your future career.

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A systems approach to managing lenticel decay in pome fruit in South Africa

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Abstract: Lenticel decay, caused by the pathogen *Neofabraea alba*, can be a major cause of postharvest losses to the apple industry of South Africa. This pathogen is considered a phytosanitary risk to a number of countries importing apples, and risk mitigation steps are required by these countries. Systems approach to phytosanitary risk mitigation measures have been defined as follows: "The integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests" (FAO, 2007), or alternatively: "The integration of pre and postharvest practices, from the production of a commodity to its distribution and end use, that cumulatively meet predetermined requirements for quarantine security" (Aluja & Mangan, 2008). Because of its sporadic occurrence and latency, producers do not routinely apply preharvest fungicides to manage lenticel decay in South Africa. Currently there are no postharvest fungicides registered in South Africa specifically targeting *Neofabraea alba*. The aim of this study was to design a systems approach to managing latent pathogens which cause lenticel decay in pome fruit, using *N. alba* as a case study. Pre and postharvest factors were considered in this systems approach. Preharvest factors included orchard sanitation, weed management, pollinators, fungicide application, rain events, picking bin type, and fruit maturity. Postharvest factors considered were cultivar type, storage duration, fungicide application in drench, fungicide application on packline, sanitation of drench solution, sanitation of packline, and shipping conditions. Data collected from orchards with either very high or low incidences of lenticel decay from Witzenberg Valley and Grabouw, seasons 2011-2013, were compared in order to establish the interaction and interdependence of the factors being considered, and their importance as factors in the systems approach. The following risk mitigation steps were considered for lenticel decay in pome fruit: Registration of all production units (PUCs); recording of all control measures; removal of fruit from orchard floor; application of fungicide in first cover spray (especially if there are late spring rains); preharvest fungicide application one month before harvest (repeat if rain event follows application); monitoring of inoculum levels on pollinators; postharvest fungicide application as drench before storage or during packing; controlled atmosphere storage with low oxygen (1%). It is also advised that retention samples of stored fruit from all registered production units be held at 18-21 °C for 30 days for evaluation of lenticel decay incidence.

Key words: lenticel decay, *Neofabraea alba*, systems approach, risk mitigation

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Apple scab control with colloidal sulphur

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Abstract: Contact fungicides play a major part in apple scab control in IPM production. Along with their positive they also show negative characteristics – higher phytotoxicity and rainfastness. In the Agricultural University Plovdiv in Bulgaria a new technology for production of colloidal sulphur based on lime sulphur was developed in 1960's. Such an innovation was in response to the need of more sophisticated products than copper and wettable sulphur. The main idea was to reduce the size of the product particles and achieve better activity. In 2016, a field trial was performed in a planting with apple cv. Red Delicious to evaluate the efficacy of colloidal sulphur compared to lime sulphur. The products were sprayed every week in two doses; 5 and 10 l/ha. Both products provided significantly different control in leaves and fruit compared to the untreated control. The level of control in leaves was low in all the treated variants and ranged from 52 to 63% severity. In fruit, however, the severity in the treatment with the higher dose rate of colloidal sulphur was 11% compared with 22% in lime sulphur at the same dose and 79% in the untreated control. The trial has shown that all products cannot completely prevent heavy infections without additional curative or "germinating spores window"-spray at the doses tested. Colloidal sulphur showed better results than lime sulphur in fruit protection and should be further evaluated.

Key words: apple scab, IPM, lime sulphur, alternative methods



Alternative products for the control of apple powdery mildew (*Podosphaera leucotricha*)

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Abstract: Apple powdery mildew (*Podosphaera leucotricha*) is one of the most important diseases on apple in the UK. Most cultivars are susceptible and a season long spray programme of 10-15 sprays is required to protect shoots and buds and prevent high levels of overwintering mildew. In many orchards, control of powdery mildew is difficult due to the limited range of fungicides available and reduced sensitivity of mildew to triazole fungicides, due to heavy reliance on these products (myclobutanil and penconazole) for control. The biofungicides evaluated in previous trials were only partially effective. The use of plant strengtheners/biostimulants or elicitors offers an alternative approach. These are usually based on plant extracts or nutrients and regular use increases the resistance of the plant to disease. There may also be other effects on yield and fruit quality. In a project funded by the Agriculture and Horticulture Development Board, the effects of a range of alternative chemicals on powdery mildew, yield and fruit quality were evaluated in small plot orchard trials on cv. Gala in 2015 and 2016. Products were evaluated with and without a reduced fungicide programme. Several potential products were identified which will be further evaluated in 2017. Eventually, promising products will be incorporated into orchard spray programmes and evaluated in apple orchards on commercial farms.



Low-residue production: an innovative and consumer-friendly fruit production

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Abstract: Pesticides are necessary to reduce yield and quality losses and to ensure a long-term profitable fruit production. However, in Switzerland and several other European countries, consumers and retailers are demanding a large reduction of pesticide residues on fruit to minimize the risk to human health and the environmental impact. Fruit growers need research results and advice to establish sustainable production systems that reduce the use and the residues of pesticides. Agroscope tested and evaluated a low-residue (LR) plant protection strategy that allows the production of residue-free apples. With regard to the incidence of apple scab and powdery mildew, the LR strategy was comparable to the integrated production (IP) strategy and superior to the organic production (OP) strategy. Losses of fruit during storage due to bull's eye rot (*Neofabraea* spp.) were a weakness of the LR and OP strategies. With regard to economical sustainability, the new LR strategy was linked to higher production risk and lower profit than the IP and OP strategies. A price premium for LR production, which is justified by environmental advantages, could have a positive effect on the economical sustainability of the LR strategy. Future long-term experiments in model orchards should evaluate how to combine and optimize the effects of robust or resistant varieties, different cultivation systems, weather influences (hail net, rain shelter, etc.) and an LR pest management system.

Key words: apple crop protection, low pesticide residue, innovative integrated pest management, fruit production, economic evaluation

Introduction

Fruit production involves a high input of plant protection products (Spycher & Daniel, 2013). Especially the application of pesticides helps to reduce yield and quality losses and allows long-term profitable fruit production. However, consumers and retailers in Switzerland and several other European countries demand that producers reduce or eliminate pesticide residues on fruit and minimize the number of applied pesticides to reduce the risks to human health and the environment by 50% (Bundesrat, 2017). To achieve this goal and nevertheless be able to produce a high-quality product, fruit growers need information and advice on designing and operating innovative, sustainable and reliable production systems (Gölles *et al.*, 2015). Therefore, Agroscope developed and tested during a five-year period a low-residue (LR) crop protection strategy for apples. The objective was to produce quality fruit without detectable residues and with yields comparable to those of the integrated production (IP) strategy. The LR strategy was compared with the IP and organic production (OP) strategies in an apple orchard in Wädenswil (Switzerland) with a total surface of 1.05 ha. The level of plant

protection, the produced fruit quality and yield, and attributes of economic sustainability were evaluated to compare all three strategies (Gölles *et al.*, 2015).

Material and methods

In 2008, a trial was started to investigate possibilities of minimizing fungicide residues suitable for professional apple production. Three crop protection strategies were compared: IP, OP and LR. The new LR strategy is a combination of the IP and OP strategies: In the first half of the season (bud break to approximately mid-June), the trees were treated according to IP standards to achieve an optimal control of apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). After mid-June, the application switched to authorized OP fungicides to minimize residues detectable on fruits at harvest (Gölles *et al.*, 2015). In Figure 1 «Description of production systems», implemented crop protection, fertilizing, thinning and other measures are described in detail.

	bud break	pre bloom	bloom	post bloom	summer	final treat.		
	scab primary season (ascospores)			scab secondary season (conidia)				
IP	1x Delan	2x Anilino-Pyrimidine	2x Qol + Captan	2x DMI + Captan	4 - 6x Captan	3 weeks	harvest hot water treatment	
LR	1x Delan	2x Anilino-Pyrimidine	1x DMI + Captan	5 - 6x Bicarbonate + S	2 - 3x Acid clay + S	1x Bicar bonat 8 days		
OP	1x Copper	3 - 4x Acid clay + S		5 - 6x Bicarbonate + S	2 - 3x Acid clay + S	1x Bicar bonat 8 days		
	Golden Del. only							
	thinnig	fertilization	fire blight	codling moth	other pests	weeds		
IP	chemical thinnig + handish	according to Swiss IP-guidelines	Streptomycin according to Maryblight	hail net + exclusion netting (barrier for bees)	exclusion netting + mating disruption	1 - 2x insecticides (IP)	1 - 2x herbicides (IP)	
LR			Myco-Sin according to Maryblight			1 - 2x insecticides (OP)	mechanical weeding (Ladurner)	
OP	mechanical thinnig (Darwin) + handish	according to Swiss OP-guidelines	Myco-Sin according to Maryblight					

Figure 1. Description of production systems, IP = integrated production, LR = low-residue production, OP = organic production.

The trial was performed on the variety ‘Golden Delicious’ (0.30 ha) and the scab resistant (Vf gene) varieties ‘Ariane’, ‘Otava’ and ‘Topaz’ (0.75 ha) in Switzerland (Wädenswil). The trial was run from 2008 until 2013. Because the LR strategy was optimized after 2008, only the years 2009 to 2013 were included in the evaluation. The size of the individual plots was chosen to enable customary production. The whole plantation was protected by a hail net and an exclusion netting (sides and headland), which was installed to prevent the intrusion of insects. Additionally, pheromone dispensers were applied on the entire area to distract codling moths (*Cydia pomonella*). Pest control, thinning, fertilization

and weed control were carried out equally in the LR and IP strategies. The OP strategy was conducted according to the guidelines of Swiss organic farming. Control trees received no pesticide treatments. For evaluation, data on the occurrence of diseases, pest infestation, labour time, used machines, physiological damages, yield and fruit quality were collected.

After harvest, apples of all strategies and varieties were graded according to Swiss guidelines (first-class fruit: graded by size, colour and parasitic and physiological damages after storage), and a random sample of 100 kg apples was stored for seven months under controlled atmosphere (1 °C, 1.5% CO₂, 1.5% O₂). Afterwards, fruits were examined for storage rots and physiological disorders. Samples from the LR and IP strategies were tested for pesticide residues (sampling: 1 kg apples at harvest; analysis: multi-method of UFAG Laboratories, Switzerland).

The economic sustainability of the different strategies was calculated only for the cultivars ‘Golden Delicious’ and ‘Topaz’ with data from the trial (plant protection and fertilization costs, labour and machine time) as an input of the economic calculation model Arbokost (Agroscope, 2014). Machinery unit costs were calculated according to the Swiss machine costs catalogue of Agroscope (Gazzarin & Lips, 2012). Costs for labour were defined according to Swiss Fruit Association standards. The profit was calculated by using the packout, i. e. the first-class fruit, and the growers-indication prices for first-class apples (Agridea, 2011; 2013). The same price (IP grower price) was used for the IP and LR strategies, whereas the grower price for organic apples was used for the OP strategy. The family income was calculated by using the outputs of the model Arbokost.

Results

The disease incidence of leaf scab and fruit scab on ‘Golden Delicious’ at harvest, averaged for all years (2009-2013), was below 0.5% with the IP and below 1% with the LR strategy. It was remarkably higher, up to 25%, with the OP strategy. Similar differences between the three strategies were found for powdery mildew. The results of the disease incidences are shown in Figure 2. Furthermore, the pest incidence was low in all varieties, in all strategies and all years (Figure 3). The biggest losses due to storage diseases were detected with the OP strategy (Figure 4). Bull’s eye rot (*Neofabraea* sp.) caused the biggest stored-fruit losses in all strategies. Especially the varieties ‘Otava’ and ‘Topaz’ were highly susceptible to this fungal disease (Gölles *et al.*, 2014). In both varieties, no statistical differences in storage rots could be detected between the untreated control, the OP strategy and the LR strategy, with losses ranging from 14 (LR) to 35% (control). With the IP strategy, storage rot losses of about 5% occurred for these varieties. In contrast, the variety ‘Ariane’ was very robust to storage rots [2 (IP) to 15% (control)]. ‘Golden Delicious’ showed similar losses with the IP and LR strategies (8 and 12%, respectively), but higher losses with the OP strategy (45%). The losses in this variety were mainly due to infestation with fruit scab.

In IP samples, residues of one or two pesticides could be detected, whereas no residues were found in LR samples. However, all residues in IP samples were within legal limits.

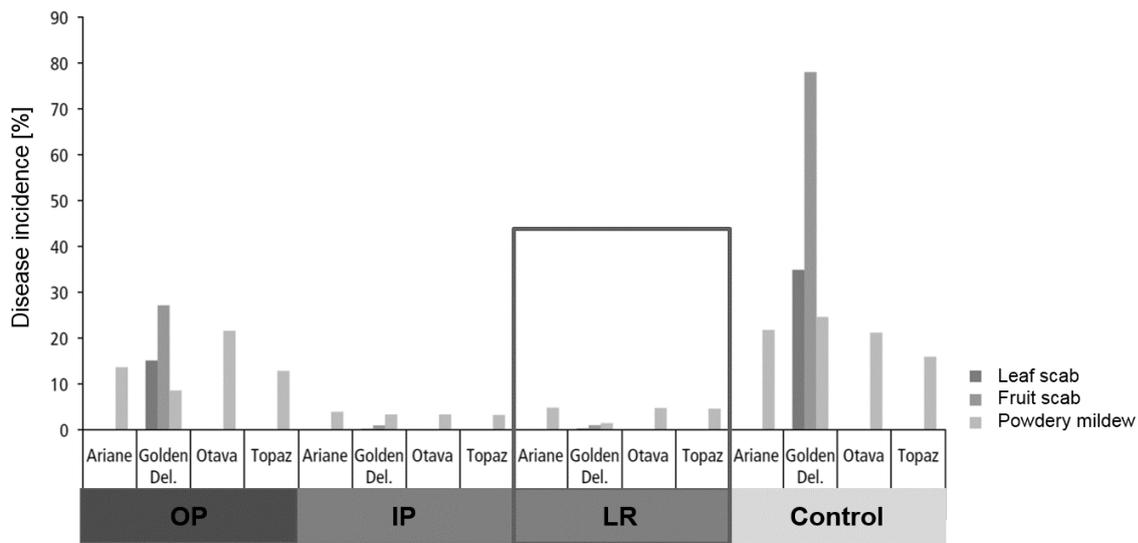


Figure 2. Apple scab and powdery mildew incidence (mean value of 2009-2013), OP = organic production, IP = integrated production, LR = low-residue production, control = untreated.

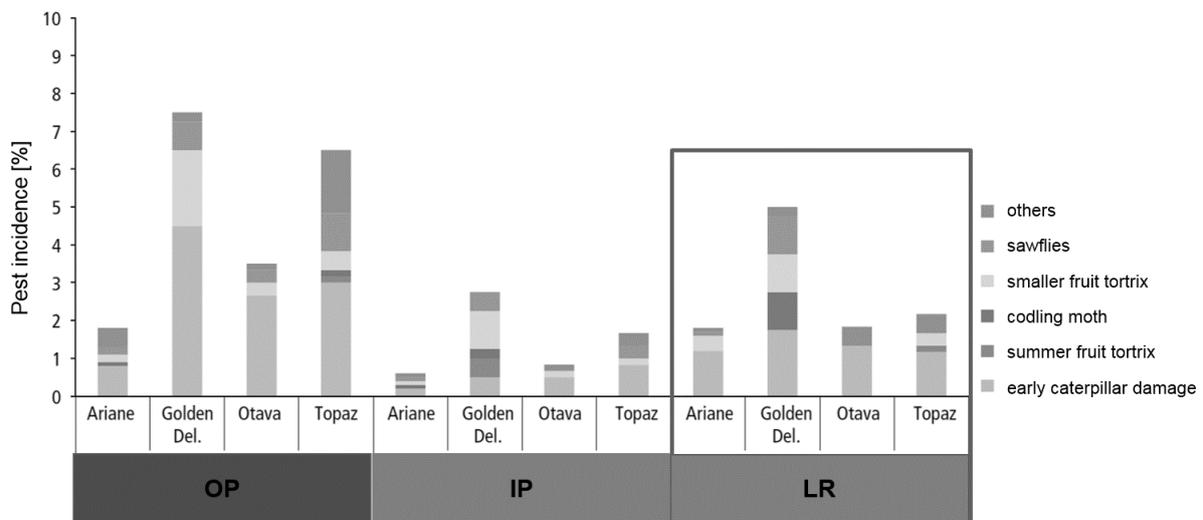


Figure 3. Fruit damages from pest incidence (mean value of 2009-2013), OP = organic production, IP = integrated production, LR = low-residue production.

The IP plots achieved, averaged for all varieties and years, higher yields (38,032 kg/ha) than the LR plots (37,103 kg/ha) and the OP plots (20,657 kg/ha). The results after storage showed that production with the IP strategy had a higher average packout (77%) than production with the LR (68%) or OP strategy (62%). In particular, organic ‘Golden Delicious’ achieved on average a very low packout (38%), whereas ‘Ariane’, ‘Otava’ and ‘Topaz’ had about 70% packout.

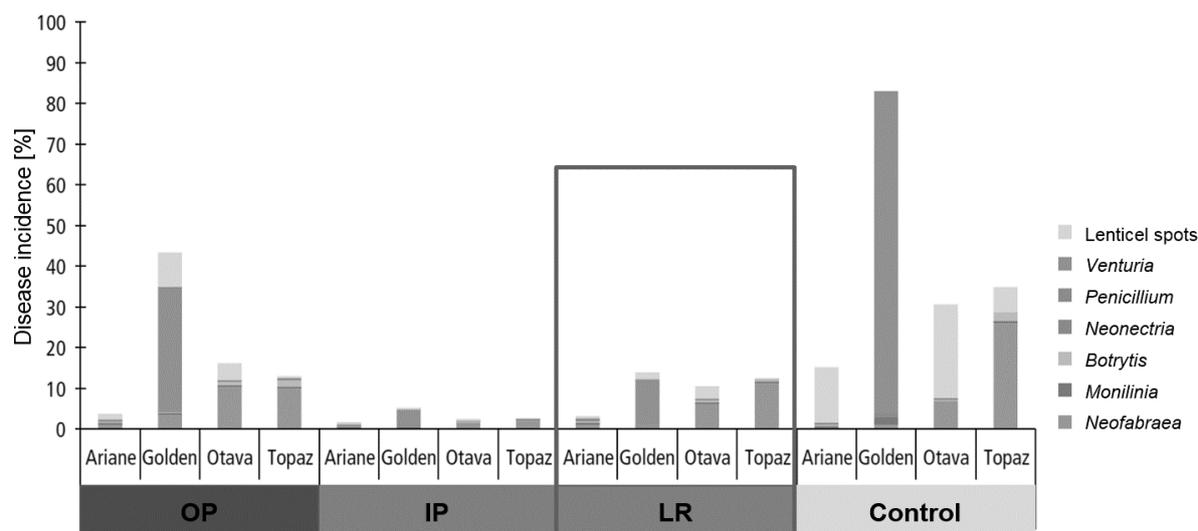


Figure 4. Storage rot incidence after 7-8 months of controlled atmosphere storage (mean value of 2009-2013), OP = organic production, IP = integrated production, LR = low-residue production, control = untreated.

The evaluation of the economic sustainability (Table 1) shows that the OP strategy had higher profitability but also higher production risk than the IP strategy. The newly developed LR strategy had slightly lower profitability and more production risk than the IP strategy.

Table 1. Calculated economic attributes for ‘Golden Delicious’ and ‘Topaz’ (2009-2012), LR = low-residue production, IP = integrated production, OP = organic production.

		LR	IP	OP
Profitability	Family income (€/h)	15.50	15.00	20.00
	Production costs first class (€/kg)	1.44	1.18	2.11
	Net income (€/ha)	-9,914	-7,230	627
Production risk	Income variability (€/h)	17.00	9.00	22.00
	Probability of dramatic yield loss	13%	0%	50%

Discussion

With this trial, the Agroscope research team was able to develop a new crop protection strategy for production of residue-free Swiss apples. With the chosen plant protection, fertilizer and thinning programs in the LR strategy, it was possible, even with susceptible cultivars such as ‘Golden Delicious’, to reach a yield comparable to that of the IP strategy. However, the packout for ‘Golden Delicious’ was about 10% and for ‘Topaz’ even 20% lower in the LR than the IP strategy. All varieties showed significant losses due to storage diseases (mainly bull’s eye rot) in the LR and OP strategies. ‘Ariane’ was the most robust variety in all strategies.

The incidence of bull's eye rot could be reduced with post-harvest hot water treatments (Good *et al.*, 2012). However, this energy- and capital-intensive measure results in economic disadvantages for the LR strategy compared with the established integrated production because minimizing residues gives no price premium.

The low packout for the LR strategy adversely affects the profitability. In contrast to the OP strategy, the LR strategy receives no price premium to compensate the lower yield and the higher production risk. A price premium for low-residue production might be justified by environmental advantages.

The development and the evaluation of improved LR strategies are continuing with different scab-resistant varieties and an optimized LR crop protection management such as reduction of applied herbicides and insecticides. By choosing the most adapted cultivar or variety, growers should be able to increase the packout after storage and minimize the years with dramatic packout loss. The positive consequences on the income variability could lead to a better economic sustainability.

Acknowledgements

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Integrated management of bull's eye rot (*Neofabraea* spp.) of 'Cripps Pink' apple in Emilia-Romagna region (Northern Italy): fungicide treatments and agronomic approaches

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Abstract: *Neofabraea vagabunda* represents one of the most important pathogens affecting apple during cold storage and distribution. However, several epidemiological aspects are still not completely elucidated. The introduction of apple cultivars characterized by a high susceptibility and at the same time with a high commercial value, such as those of the Cripps[®] group, makes the development of appropriate and effective control measures more urgent. An integrated management of bull's eye rot needs a holistic approach. Since 2010 a wide monitoring of diseases in packinghouses has been activated, aimed to highlight the real extent and the economic impact of the problem and to raise awareness of farmers. At the same time by means of several field trials in orchards with high inoculum potential the efficacy of active ingredients such as pyraclostrobin, pyrimethanil, boscalid, fludioxonil has been evaluated. The development of bull's eye rot symptoms were evaluated in relation to the harvest time and the length of cold storage. The possibility to control the disease with postharvest fungicide treatments and the use of 1-Methylcyclopropene were also assayed. The results will be discussed.

Key words: lenticel rot, susceptibility, management, fungicide, postharvest



Orchard specific management of scab (*Venturia inaequalis*) on apple

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Abstract: Scab, caused by *Venturia inaequalis*, is one of the major fungal diseases in Belgian fruit production, and over 50% of the pesticides used in apple cultivation are used for its control. As such, a good management of the primary infection moments caused by ascospores at the beginning of the season is essential. An accurate warning system in the spring is, therefore, an ideal strategy. The current scab warning system of the Research Station of Fruit Cultivation (pcfruit vzw) is based on: (1) evaluation of ascospore releases from heavily infected scab leaves and (2) climatological infection risks. As such, the warning system is considered very valuable and allows fruit growers to control their applied treatments, products and dosages. However, the use of a worst case does not take the initial inoculum pressure of a specific orchard into account. Consequently, the warning system provides potentially biased information concerning the intensity of scab infections which leads to maximal treatment on each possible infection moment. Too many treatments (or at the wrong times) is cost and time expensive, and accelerates the occurrence of resistant species. We have developed a method to artificially induce ascospore releases followed by molecular assessment resulting in a good estimation of the initial scab at a specific orchard, prior to the season. Moreover, this technique is also being used to monitor the ascospore release during the season in individual orchards. Such information is critical to better advice fruit growers in their use of pesticides to control scab infections. This will result in a better, more orchard-specific scab control strategy that might result in a reduction of treatments, which then affects the residues on apples and might lead to a significant decrease in costs for the fruit grower.

Key words: scab, apple, management, ascospores, initial inoculum pressure

Introduction

The most common fungal disease in Belgian apple fruit production is scab, which is caused by *Venturia inaequalis*. Over 50% of the pesticides are used for its control, and a perfect management strategy of apple scab remains the ultimate goal. A good control of the primary infection moments, caused by ascospores originating from overwintering infected leaves of the previous season, reduces secondary infection risks (via conidia) in the summer. At the moment, most Belgian growers rely on the current scab warning system of the Research Station of Fruit Cultivation (pcfruit npo) which is based on a worst case scenario: ascospore releases from heavily infected scab leaves are evaluated during rainy periods. The warning

system is additionally based on the climatological infection risks calculated by weather data gathered by the agrometeorological network in Flanders. This information combined with predictions from the RIMpro scab model (Trapman, 1994) is considered very valuable and allows fruit growers to control their applied treatments, products and dosages. This approach, however, has a major drawback as it does not take the inoculum of an orchard into account. Starting from a worst case scenario (pcfruit vzw) combined with RIMpro, which is based on a predetermined inoculum, leads to potentially biased information resulting in maximal treatment on each possible infection moment. Too many treatments (or at the wrong times) is cost and time expensive, and accelerates the occurrence of resistant species. An accurate warning system that takes the initial inoculum into account is, therefore, an ideal strategy to reduce primary infections as well as regulate the amount of treatments. In this project we developed and optimized a method to artificially induce ascospore releases in order to provide valuable information prior to the beginning of the season.

Material and methods

In 2014 and 2015, apple leaves were collected from different orchards with a low to high degree of scab infected leaves and fruits. In order to rapidly allow ascospores to ripen, leaves were transferred from outside to a greenhouse, during the middle of the winter (mid-February). The greenhouse was set at 20 °C and ascospores were allowed to ripen for two additional weeks. All leaf samples were then stored at -20 °C and thawed prior to analysis. From each orchard approximately 10 leaves were positioned on a mesh that was attached to the upper side of a funnel which in turn was connected to a vacuum pump. At the base of the funnel, a 0.5 ml tube was positioned in order to capture the released spores. After wetting the leaves once, the tube containing the spores was renewed each half hour, and spores were microscopically counted.

Results and discussion

Our results revealed that 80% of the spores were released during the first two hours after the simulated rain event and over 90% of the spores were released within the first three hours. Our results further confirmed that leaves from a highly infected orchard had the highest amount of released spores and leaves from the least infected orchard consequently had the lowest amount of spores. Similar results were obtained from different replicates and over different years. Using a funnel does, however, have some drawbacks. Even combining four funnels, to improve the amount of leaves that could be tested simultaneously, only a maximum of approximately 40 leaves from a single orchard was used for the forced ascospore release and thus provide information on the initial inoculum. Using such a low amount of leaves can create a bias depending on which leaves were selected. Moreover, the technique is rather laborious as all the leaves need to be positioned face down on the mesh, and spores need to be counted microscopically. Therefore, in 2016-2017, we optimized this method by using a spore-trap (Rotorod) which was positioned above approximately 300 leaves, thus increasing the amount of leaves by near ten-fold. Moreover, this technique can easily be used to test up to a 1000 leaves simultaneously from a single orchard, improving the reliability of the forced ascospore release. The downside of using a large amount of leaves in combination with a rotorod is the huge amount of spores that are captured on the rods, leading to time-consuming counting efforts. Therefore, a highly sensitive quantitative PCR was developed,

allowing the analysis of spore-saturated rods and multiple samples simultaneously. We further improved our technique by allowing spores to ripen for three weeks at 20 °C and wetting the leaves twice, once at the beginning of the experiment and a second time after 90 minutes. The use of a rotorod above sampled leaves from individual orchards was also used to monitor the ascospore release during the season (outside). Over the last three consecutive years we observed a good correlation between the forced release and actual ascospore release during the season. As such, using the ‘forced release method’ allows us to gain insight in the initial inoculum in an orchard, which allows better advice to fruit growers in their use of pesticides to control scab infections. This will result in a better, more orchard-specific scab control strategy, which might result in a reduction of treatments, which then affects the fungicide residues in apples and might lead to a significant decrease in cost for the fruit grower.

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A mechanical barrier against apple scab

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Abstract: In the frame of the French Ecophyto Plan aimed to reduce the number of treatments in agriculture, protecting apples against rain with plastic covers on the top of the trees was tried from 2010 by Ctifl at its site in Lanxade in the South-West of France. Different types of rain cover were studied. The effectiveness was very high on cvs. Braeburn and Gala, reaching 100% on fruits, without any additional use of fungicides, even in presence of some scab on the shoots. In a newly planted orchard, with a more susceptible variety, an outbreak of scab occurred under the covers. The different possible causes were analyzed, through measuring climatic data, trapping spores and running the apple scab warning program RIMpro. The microclimate under the rain covers seemed to be favorable to powdery mildew, woolly aphids and unfavorable to storage diseases. The system may be improved with a few fungicide treatments to avoid powdery mildew and during the most severe scab infections.

Key words: *Venturia inaequalis*, alternative control, rain covers

Introduction

Protecting apples against rain with plastic covers on the top of the trees was initiated by the French technical research Institute for fruits and vegetables (Ctifl) in 2010 in the south west of France. The aim is to limit apple scab contaminations, to reduce the use of pesticides and to limit residues on fruit. There were few references about this technique in apple growing: the only one was an experience reported in British Columbia (Mitham, 2008), of growing apples under plastic tunnels, but not focused on reducing diseases. The principle of rain cover, and the material, is directly inspired from the type of protection used in cherry production to reduce the incidence of skin cracking caused by rain.

Material and methods

Different types of rain covers and systems were studied (Table 1). The first design was a system with two plastic strips (commercialized by the French company Filpack) fixed on a cable tightened on the top of the orchard poles, and positioned under the hail-net; the second one was similar to the first for the fixation, but the cover was made by combining the plastic strips with hail-net, specially supplied by Filpack; the third one is the Voен system (VOEN Vöhringer GmbH & Co., Berg, Germany), mainly marketed for cherry rain protection.

For each system, four elementary plots were defined in the involved rows. Apple scab infection was observed on 100 shoots and 200 fruit per plot. Other pests and diseases (mainly aphids, powdery mildew, sooty blotch and flyspeck) were also assessed in elementary plots. Harvest data (yield, quality) were collected from specific other elementary plots.

Table 1. Different type of rain covers used in Ctifl trials from 2010 until now, and related orchards.

	Filpack [®] rain covers under hail-net			Filpack [®] rain covers combined with hail-net		Voen system
	2010-2014	2011-2013	From 2014	2011-2013	2014-2017	From 2014
Apple variety	Braeburn	Gala Brookfield [®]	Pink Lady [®] Rosy Glow	Gala Brookfield [®]	Gala Brookfield [®]	Pink Lady [®] Rosy Glow
Training system	Axis	Axis	Aximum	Fruit wall	Fruit wall	Aximum
Width of plastic strips	1.40 m	1.60 m	1.40 m	0.70 m	1.40 m	2.50 m
Height of the pole, fixing the roof	4 m	4 m	4 m	4.50 m	4 m	4 m
Nb of rows	3	4	10	4	4	6
Surface	800 m ²	1000 m ²	1260 m ²	1000 m ²	1000 m ²	760 m ²

Climate data were monitored by Watchdog sensors, positioned in the middle of the design, on a row: temperature, relative humidity and leaf wetness. Control measures were performed with the same device, in a neighboring part out of covered rows. To explain the outbreak of scab under covers, the warning scab model RIMpro was used, running with a specific interface for Watchdog export files.

A 7-day volumetric spore trap (Burkard Ltd, UK) was placed in the middle of the covered area, and ascospore flights were monitored during the most important infection period (April-May).

Results and discussion

Apple scab

Scab incidence in the Braeburn and Gala orchards was very low under covers without any fungicide use in the different systems tried from 2010 to 2014, compared to the untreated (and uncovered) control (Figure 1).

In the new orchard with the variety Pink Lady[®] Rosy Glow, an outbreak of scab occurred since the first year in 2015, under the covers, but only leaves were infected. It started from the borders and could be explained by the direction of wind during great infection periods (ascospore ejection peaks and high RIM value). In 2016, scab incidence was higher under the cover, with scab on leaves and fruits, but a lower degree was observed under the Voen roof (Figure 2). This orchard was more exposed to western winds than the Braeburn / Gala one.

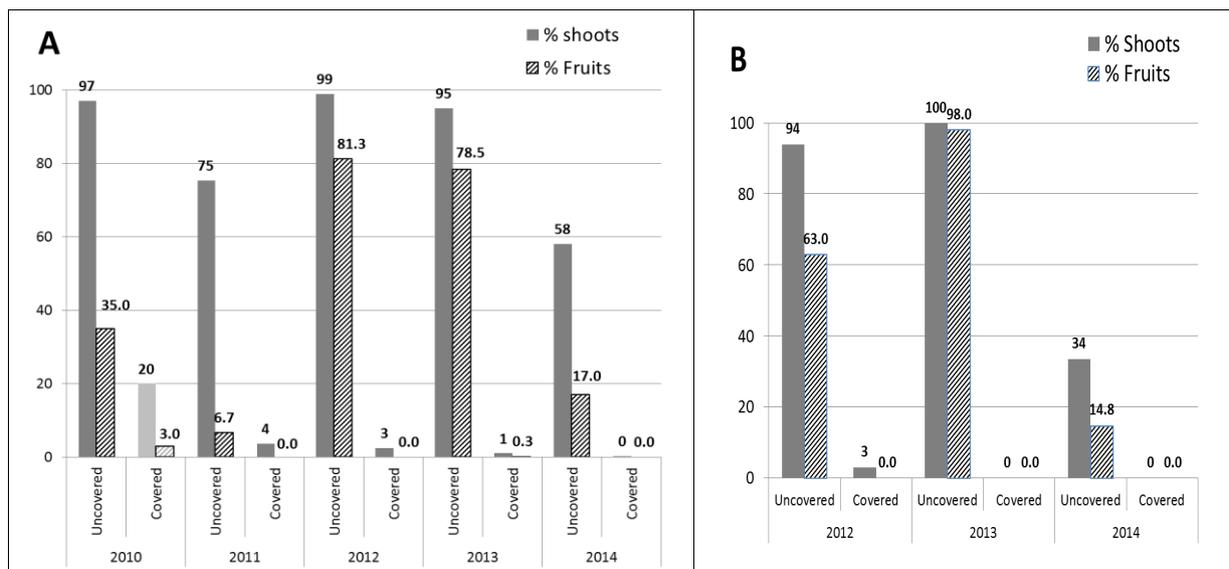


Figure 1. Incidence of apple scab on shoots and fruits, on Braeburn (A) and Gala (B), in covered and uncovered rows, without any fungicide treatment. In 2010, the first design presented flawed joints on the assembling point on the cable, resulting in scab (20% on shoots, 3% on fruits): this difficulty was then corrected.

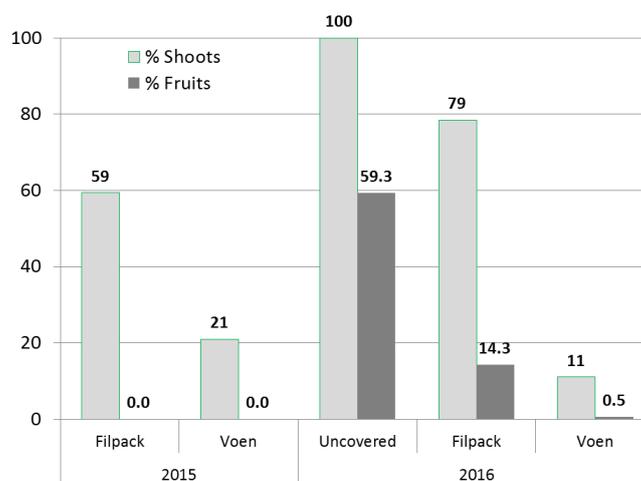


Figure 2. Scab incidence in the Pink Lady[®] orchard, under covers and outside (untreated control plot only in 2016). Differences between Filpack and Voen are significant.

To understand the possible causes, several data were collected and the results are summarised below:

- Rain collectors show that some rain is passing through the cover, mainly during windy days; the Filpack[®] system is more susceptible, because the strips are moving with the wind and can let the rain to flow at the junction points on the cable. This rain can wet the leaves below, mainly in the lower part of the tree (related to incidence angle).

- Leaf wetness is recorded under the cover, on the rainy days, due to the higher relative humidity, the circulation of wet air from outside. The values are varying, often lower than the values outside. Leaf wetness depends on the rain cover type and on the rain and wind conditions (quantity, intensity, frequency and direction).
- Temperature is 1 to 2 °C above the outside data, increasing scab risk.
- Only very few ascospores were trapped under the rain cover. However, the Burkard spore trap is only collecting the spores flying in the lower part of the orchard. A hypothesis could be an introduction of spores by the rain flooding from the roof.
- Scab risk, calculated by the model RIMpro, shows that there could be more infection periods and higher RIM values under rain cover than outside. In 2016, the number of RIM values exceeding 300 was three outside and the maximum RIM was 835, four under Filpack[®] with 2 values above 1000, and three under Voen roof with 1 above 1000.

In the Voen system, we observed one period without scab risk, around April 15th, although the same period was credited to a 1400 RIM value under Filpack[®]. In this period the leaf wetness was lower under Voen roof than under Filpack[®] cover. Nevertheless interpretation is difficult since the Watchdog LW sensor provides intensity data (values ranging from 1 to 15) and not duration data as requested by the infection curves of scab; the interface used in RIMpro takes this parameter into account but needs to define a threshold value for LW data.

Other pests and diseases

Postharvest diseases were monitored only with Pink Lady[®] by 6-7 months storage in regular atmosphere. In 2015, apples were harvested on October 27th and stored 7 months. No lenticel rot was observed in the batches from covered areas without any pre-harvest treatments (6 boxes = replicates). In 2016, apples were harvested on November 28th and stored 6 months. The results were better than the chemical reference (2 sprayings of boscalid + pyrachlostrobine; Figure 3).

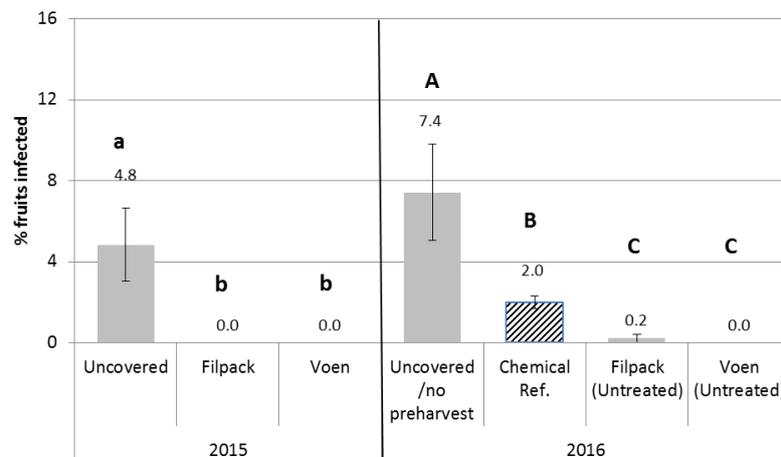


Figure 3. Incidence of rain covers on infection by *Neofabraea vagabunda*, the causal agent of lenticel rot, after 7 months of storage in 2015 and 6 months in 2016. The same letter above the columns means no significant difference at $P = 0.05$.

Wound pathogens such as *Monilia fructigena* or *Botrytis cinerea* also seemed to be reduced by the rain covers.

The microclimate under the rain covers seemed to be favorable to powdery mildew. In 2016, five applications of sulphur and two with a DMI were needed to maintain the level of powdery mildew under the threshold of 5% on the shoots.

Conclusion and perspectives

This experience has been developed in France as experimental design by some growers and experimental stations, and in Europe (Bertelsen & Lindhard Pedersen, 2014). A working group, representing 14 sites in France, was created to help and give support to them. To avoid powdery mildew, and scab infections, further trials are developed using plant defense inducers like phosphites, and sulphur. Plastic covers modify the behavior of the trees, because they receive less light, and irrigation/fertilization management is more complicated. The global management should be improved to reduce the extra costs of this technique that remains expensive. This technique is not suitable in too windy regions.

Acknowledgements

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Sensitivity and resistance mitigation strategies for next-generation SDHI fungicides in the apple scab pathogen *Venturia inaequalis*

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Abstract: The succinate dehydrogenase inhibitors (SDHI) are a class of broad spectrum, single-site fungicides that interfere with fungal respiration in the mitochondrion. Recent registration of several SDHIs for apple scab management prompted investigations into the sensitivity of *Venturia inaequalis* and resistance management practices. Isolates of *V. inaequalis* with no prior exposure to single-site fungicides were collected from Northeastern U.S. apple orchards from 2012-2016 to determine the effective concentration at which conidial germination and mycelial growth were inhibited by 50% (EC₅₀). Conidial germination mean EC₅₀ values for penthiopyrad, fluopyram, benzovindiflupyr, and fluxapyroxad were 0.086, 0.176, 0.0016, and 0.028 µg/ml, respectively. These values were significantly lower compared to those obtained for mycelial growth. Linear correlation revealed a significant and positive correlation in sensitivity between fluopyram and penthiopyrad ($P \leq 0.0001$, $r = 0.66$) and fluopyram and benzovindiflupyr ($P = 0.0014$, $r = 0.52$). In order to identify and characterize the *V. inaequalis* *sdh* genes, genomic sequencing was performed using the Illumina MiSeq platform. No mutations commonly associated with SDHI resistance were recovered from field isolates in plots receiving applications of SDHIs at high or low rates, or SDHIs applied with other fungicides. Results from this study provide a basis for phenotypic and genotypic monitoring of SDHI resistance in populations of *V. inaequalis*.

Key words: apple scab, succinate dehydrogenase inhibitor, *sdh* genes



Evaluation of fungicides and application practices for the management of *Glomerella* leaf spot and fruit rot in North Carolina

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Abstract: *Glomerella* leaf spot (GLS) and fruit rot (GFR) caused by fungi in the *Colletotrichum gloeosporioides* species complex is the most devastating fungal disease of apple in North Carolina. With few management options and a paucity of GLS-related research in the U.S., apple growers currently rely on excessive summer fungicide programs to manage the disease. To help producers more effectively manage *Glomerella* during the growing season and post-harvest, field experiments investigating single-site fungicide efficacy and application timing were conducted in a 'Gala' research orchard located in Mills River, NC in 2016. Fungicides with different modes of action (Inspire Super, difenoconazole + cyprodinil; Merivon, pyraclostrobin + fluxapyroxad; Sercadis, fluxapyroxad; and Cabrio EG, pyraclostrobin) were applied in non-rotational programs from petal fall-8th cover. The incidence of GLS, pre-harvest GFR, and post-harvest GFR was significantly lower for programs in which Cabrio EG or Merivon were applied. The Merivon and Cabrio EG programs also resulted in a significantly lower incidence of scaffold defoliation due to GLS. In a separate field experiment, the timing of Merivon applications for GFR management was investigated. While no significant differences in GFR incidence prior to harvest were observed between programs with differing Merivon timings, Merivon applications at petal fall and 1st cover resulted in significantly lower fruit rot incidence 14 days post-harvest.



Optimal use of bicarbonate to control scab

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Abstract: Multiple sprays are needed to control apple scab (*Venturia inaequalis*) in organic fruit growing. In the Netherlands copper and lime sulfur are not allowed anymore. Therefore growers heavily rely on sulfur. It was found in several trials that potassium bicarbonate was effective against scab which made it an interesting product besides sulfur. However, some growers had problems with low efficacy or phytotoxicity. In order to overcome this problem several small scale experiments were done to know more about the mode of action of potassium bicarbonate. Trials were done on small potted M9 rootstocks. Rootstocks were infected by conidiospores of apple scab and placed at 20 °C at 100% RH. After about 400 degree hours potassium bicarbonate was applied in different ways. Potassium bicarbonate is a salt and can only chemical react when it is in ion form. Therefore some growers prefer to spray on wet leaves. In the trials the way of application was tested by spraying bicarbonate on dry or wet leaves. Overall no difference was found between the two application methods. Because higher doses of bicarbonate increase efficacy, spraying on wet leaves cause dilution with the chance of lower efficacy. Spraying on wet leaves can also result in higher concentration at the tips of the leaves. SEM photo's revealed damage of the cuticula of the leaves. On the other hand spraying bicarbonate during fast drying conditions might negatively influence the efficacy. It is advised to spray bicarbonate on dry leaves under slow drying conditions.

Key words: Fruit, disease, Vitisan, Armicarb



Rainfastness of lime sulphur and other inorganic fungicides used for scab control in apple and pear production

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Abstract: The aim of this study was to determine, under controlled and field conditions, the rainfastness of new fungicide formulations available for organic apple and pear farming. The rainfastness was assessed by both (i) analysing the content of residual chemical elements present on the leaves subjected to various amounts of rain (from 0 to 30 mm), using an atomic emission spectrometer (ICP-AES) under controlled and field conditions, and (ii) measuring scab control effectiveness on inoculated seedlings following various amounts of artificial rain (from 0 to 30 mm) applied on the leaves under controlled environment. The field study in apple orchards showed that with protective applications of lime sulphur (2%), copper hydroxide (0.1%), wettable sulphur (1%), and potassium bicarbonate (1%) subjected to 33 mm of precipitation, leaves still contained 61, 48, 36, 43%, respectively, elemental sulphur, copper or potassium ions applied initially. This means that among sulphur formulations, lime sulphur showed significantly higher retention on leaves subjected to rain. A greenhouse study on seedlings showed that protective applications on dry leaves of lime sulphur (2%), wettable sulphur (1%), copper hydroxide (0.1%) and potassium bicarbonate (1%) significantly reduced pear scab severity, with more than 96% effectiveness on susceptible cultivars under high scab pressure. Rainfastness of lime sulphur appeared to be lower compared to wettable sulphur, copper and bicarbonate, providing scab-intensity control effectiveness of 85, 52, 28, 6%, and scab-severity control effectiveness of 92, 64, 75, 27%, respectively, after an application of 30 mm of artificial rain. Other formulations, such as Heliosoufre, Heliocuire and Armicarb were also tested in this study.

Key words: fungicide, lime sulphur, organic farming, pear scab, potassium bicarbonate, rainfastness

Introduction

Apple and pear scab, caused by *Venturia inaequalis* and *Venturia pirina*, are the most significant pome fruit diseases, causing economic losses in many production areas. Its significance is indicated by the fact that up to 15-20 fungicide treatments per season are applied in these crops, mostly to control scab. In organic apple and pear growing, scab control is mainly based on the protective use of copper or wettable sulphur. As the use of copper is subject to European restrictions and wettable sulphur has some phytotoxic effects on the main cultivated pear cultivars, accurate control strategies are needed. Simulation models based on weather data reliably predict infection periods, but the success of these control strategies strongly depends on the availability of efficient curative fungicides. Lime sulphur has already been shown to be useful as a curative application for apple scab control (Holb *et al.*, 2003),

but has sometimes also shown phytotoxic effects. In several trials under controlled and field conditions, potassium bicarbonate has been demonstrated as an effective control agent against apple scab, including curative action in some cases (Jamar *et al.*, 2010, Van Hemelrijck *et al.*, 2012).

The phytosanitary products usually used against fungal pathogens in organic farming are mainly contact fungicides, acting on the upper leaf surfaces, and are not likely to be systemic or curative. They are therefore easily washed off by small amounts of rain. Today, there is a lack of quantitative information regarding the behaviour of alternative fungicides and the levels required to fully protect the apple and pear trees against scab (Hall *et al.*, 1997; Xu *et al.*, 2008; Hunsche *et al.*, 2011). Usually, for copper and sulphur compounds, 20 mm of precipitation is the threshold recommended by commercial companies before the treatment application has to be renewed. A general rule of thumb that is often used is that 5 mm of rain removes about 50% of the protectant fungicide residue and over 5 mm of rain will remove most of the spray residue (Kunz & Hinze, 2014; 2016). However, newer “sticky” formulations and fungicides applied with spreader-stickers may be more resistant to wash-off by rain. Also, fungicides and formulations differ a lot in their ability to adhere to plant surfaces. Therefore research is needed to describe the effect of rain on wash-off for specific products. While the main inorganic fungicides have been used for a long time, very few studies have focused on their rainfastness (Jamar *et al.*, 2017).

The aim of this study was to determine, under controlled and field conditions, the rainfastness of new fungicide formulations available for organic apple and pear farming, with a view to adapting the timing of treatment applications and reducing the amount of fungicide applications according to the climatic conditions.

Material and methods

Fungicide application

The chemicals tested included wettable sulphur (Thiovit jet, 80%, Syngenta Agro, France), liquid sulphur (Heliosoufre, 80%, Action Pin, France), copper hydroxide (Ko-Plus 40, 40%, Dupont, USA and Heliocuvivre, 40%, Action Pin, France), lime sulphur (Curatio®, calcium polysulphide, 23% of elemental sulphur, Biofa AG, Germany), and potassium bicarbonate (Armicarb APC-09CD, 85%, Helena Chemical Company, USA and BioUltra, 85%, Sigma Aldrich, Belgium). The chemicals were applied at the following doses: potassium bicarbonate 1%, sulphur 1%, lime sulphur 2%, copper 0.1%. A single spray of fungicide was applied before rain, using the same volume of treatments for each experimental object (350 l/ha in orchard).

Rainfastness of chemicals under controlled conditions

In a first set of experiments, chemicals were sprayed once onto apple seedling leaves 18 h before artificial rain applications. Apple seedlings were subjected to 0, 8, 16 and 24 mm of simulated rain. For each treatment, five repetitions were achieved, each repetition including 40 seedlings. The rainfastness was assessed by analysing the content of residual chemical elements present on the leaves subjected to various amount of rain using an atomic emission spectrometer (ICP-AES).

Rainfastness of chemicals under field conditions

In a second set of experiment, chemicals were applied on cv. Pinova in an experimental orchard in Gembloux, Belgium, on May 28th. A Munckhof tunnel sprayer machine was successfully used for treatment applications. The leaves were taken on May 28th after 0 mm of rain, May 29th after 14 mm of rain, May 30th after 20 mm of rain, on June 01st after 27 mm of rain and on June 02nd after 33 mm of rain. The leaves were taken randomly between 1.2 and 1.8 m height, on the right and left part of the tree rows, on 48 trees for each repetition (3 × 48 trees per treatment). For each treatment, three repetitions were achieved and each analysed sample was composed of 96 leaves. The rainfastness was assessed by analysing the content of residual chemical elements present on the leaves using an atomic emission spectrometer.

Rainfastness impact on scab control

In a third set of experiments, chemicals were sprayed once onto pear seedling leaves 18 h before artificial rain applications. Pear seedlings were subjected to 0, 5, 10, 15, 20, 25 and 30 mm of simulated rain. After that, pear seedlings were inoculated with scab conidia. This artificial inoculation was scheduled for completion 24 h after the protective treatments. The plants were then incubated for 48 h in a dew chamber at 100% relative humidity. They were then placed in greenhouse for 3 weeks to promote plant and disease development. There were 45 plants (3 replicates of 15 seedlings) per treatment × rain; 1575 plants were therefore used for the experiment. Disease incidence (proportion of infected leaves) and severity (leaf area infected) were assessed 21 days after inoculation.

Results and discussion

Rainfastness of chemicals under controlled conditions

For protective applications of lime sulphur 2 % ('Curatio'), sulphur 1% ('Héliosoufre', 'Thiovit Jet'), copper 0.1% ('Heliocouvre', 'Kocide WG', 'Bordeaux mixture') and potassium bicarbonates 1% ('Armicarb', 'Sigma BioUltra'), apple seedling leaves subjected to 24 mm of rain still contain, respectively 52, 36, 10% of S; 45, 33, 33% of Cu; 50, 5% of K applied initially (Figure 1).

Rainfastness of chemicals under field conditions

For protective applications of lime sulphur 2% ('Curatio'), sulphur 1% ('Héliosoufre', 'Thiovit Jet'), copper 0.1% ('Heliocouvre', 'Kocide WG', 'Bordeaux mixture') and potassium bicarbonates 1% ('Armicarb'), apple orchard leaves subjected to 33 mm of rain still contained, respectively 61, 30, 36% of S; 44, 48% of Cu; 43% of K applied initially (Figure 2). Copper content of leaves treated with one application of copper hydroxide (0.1%) and subjected to 33 mm of precipitation, was still five times higher than untreated leaves, suggesting copper accumulation in the leaf, following successive treatment applications in the orchard.

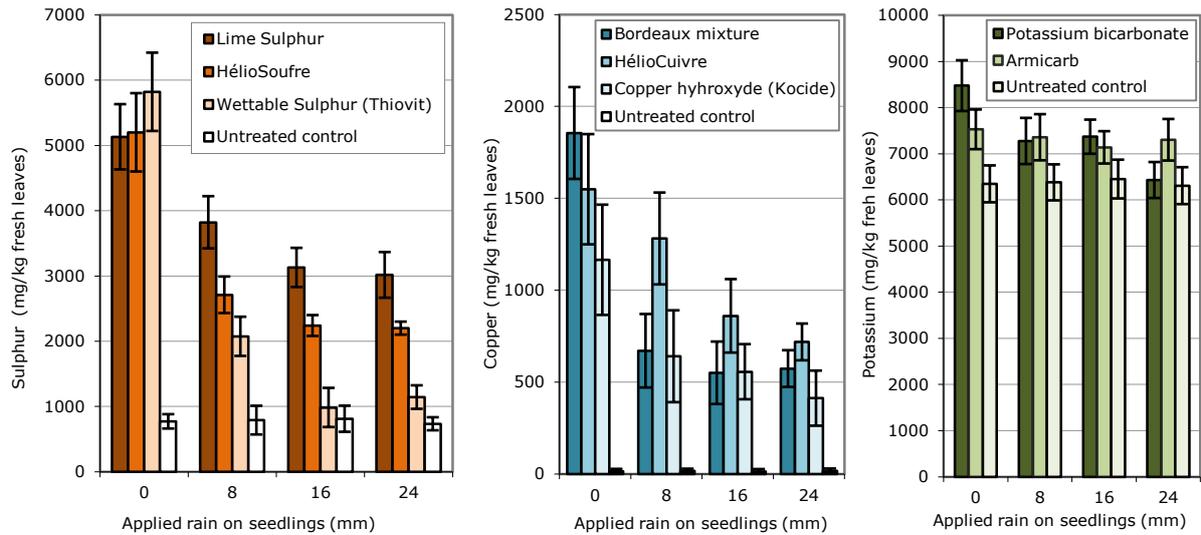


Figure 1. Content of residual chemical elements present on apple seedling leaves treated with protective applications of chemicals and subjected to 0 to 24 mm of artificial rain. Vertical bars represent the standard error (n = 5).

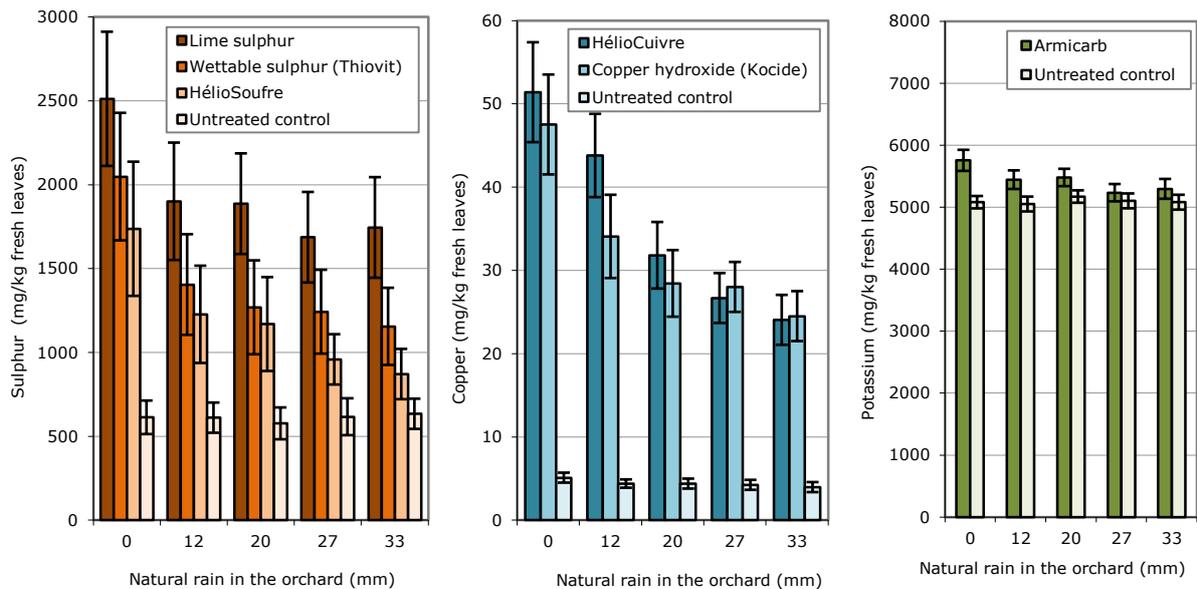


Figure 2. Content of residual chemical elements present on apple orchard leaves treated with protective applications of chemicals and subjected to 0 to 33 mm of natural rain. Vertical bars represent the standard error (n = 3).

Rainfastness impact on scab control

Among sulphur formulations, lime sulphur showed significantly higher pear scab control in comparison with wettable sulphur formulations whatever the rainfall application rate (Figure 3). This indicates that the adhesion capacity of the wettable sulphur formulation was lower than for lime sulphur, since both formulations have the same effectiveness under protective applications without rain.

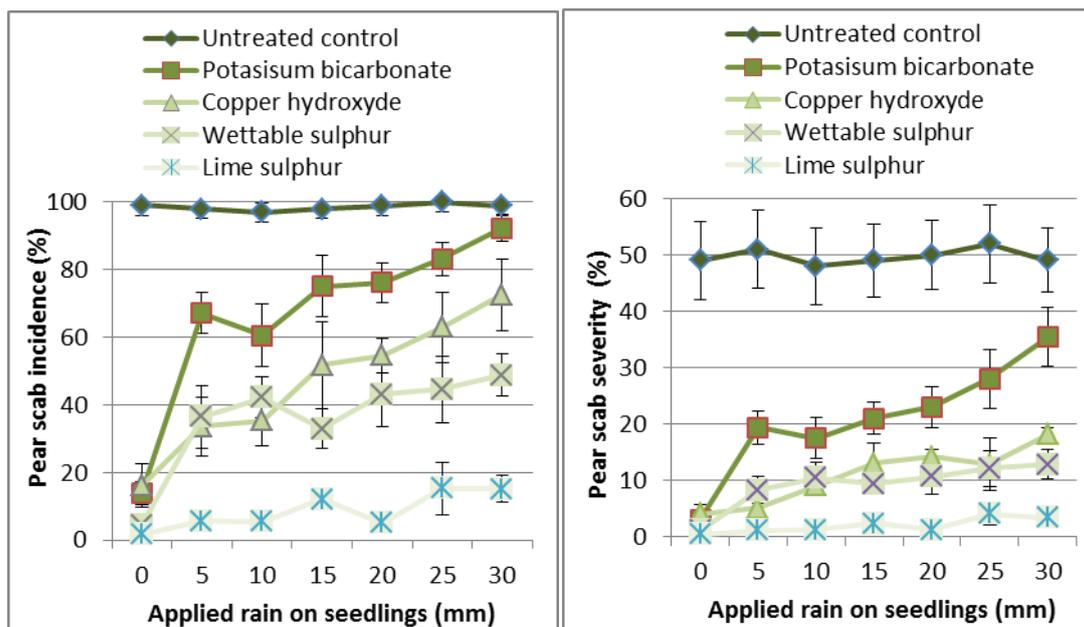


Figure 3. Scab-intensity and scab-severity control effectiveness of chemicals following protective applications on pear seedling leaves subjected to 0 to 30 mm artificial rain. Vertical bars represent the standard error ($n = 6$).

After 30 mm of rainfall, lime sulphur showed better behaviour, showing 85 and 92% effectiveness for scab incidence and scab severity control, respectively. The artificial rainfalls of 5 mm were sufficient to significantly reduce the scab control effectiveness of copper and wettable sulphur. However, after 30 mm of rainfall, copper and sulphur controlled scab severity with 64 and 75% effectiveness, respectively. Concerning potassium bicarbonate, 5 mm of artificial rainfall was sufficient to reduce significantly the control of scab incidence and severity, from 85 and 92% to 32% and 60% effectiveness rates, respectively.

This study showed a great difference of retention between formulations. Among sulphur formulations subjected to rain, lime sulphur shows significantly higher resistance to wash-off by rain as well as higher scab-severity control effectiveness, compared with other sulphur formulations. Regarding sulphur compounds, rainfastness of Heliosoufre is lower compared to wettable sulphur under controlled conditions but not under field conditions. Chemical analysis showed that past effect of copper is significantly higher with Heliocuire than with Kocide WG. Results suggest that copper could be accumulated in the leaf following successive treatments. Bicarbonates show high susceptibility to rainfastness, already before 5 mm of rain.

Acknowledgements

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Development of direct and indirect methods to control *Marssonina coronaria* on apple

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Abstract: The fungus *Marssonina coronaria* causes early leaf fall on apple cultivars of different varieties and is found in European apple growing regions since 2010. In Europe, mainly organically managed orchards or untreated traditional orchards are affected so far, contrary to other regions of the world. The Kompetenzzentrum Obstbau Bodensee (Germany) and the FiBL Research Institute for Organic Agriculture (Switzerland) work together in an INTERREG-funded project to investigate *Marssonina coronaria* from 2016 to 2019. Thus, there are only one-year observations available yet.

The research covers three areas: biology of the fungus / control methods / susceptibility of different apple varieties. Both outdoor and in-vitro-methods are used, including the following:

- First appearance and further development of symptoms are monitored on untreated trees in the field.
- Potted apple trees are put into the field to detect possible time frames of infection in the field.
- PCR-analysis of different parts of the tree to detect overwintering spore material.
- Outdoor and in-vitro screening of various plant protection agents permitted in organic production.
- Possible effects of phytosanitary treatments.
- Monitoring of the susceptibility of different old and new apple varieties to the fungus.



The effectiveness of fungicides and warning systems in controlling *Botryosphaeria dothidea* in apple fruit rot in southern Brazil

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Abstract: White rot causes up to 30% losses in apple orchards with frequent outbreaks in hot and humid summers. The current study reports the results of a warning system applied to white rot outbreaks, as well as the effectiveness of fungicides in controlling the disease. The epidemiological warnings were recorded in the 2002 to 2003 and 2003 to 2004 periods. During the first season, warnings were issued whenever there was inoculum available and two consecutive days of rainfall occurred, and in the second season after 5 mm of rain. In the 2016 to 2017 period, spraying was done when white rot risk warnings were predicted according to the weather forecast information. To compare fungicide effects, fungicides were sprayed on cv. Fuji apple trees before and after harvest, as well as before and/or after inoculation with *Botryosphaeria dothidea*. The warning system under the 5 mm of rainfall condition and the preventive treatment were equally efficient in managing the disease. Fungicides belonging to the benzimidazole group, sprayed at the pre-harvest stage, showed the most efficient disease control. The most effective products sprayed at the post-harvest stage were thiophanate-methyl, mancozeb and calcium chloride.

Key words: summer apple rot, disease control, chemical control

Introduction

White rot (*Botryosphaeria dothidea*) may lead to more than 30% of fruit loss in cv. Fuji apples during hot and rainy summer years in the Rio Grande do Sul State in Brazil. Fungicides belonging to the benzimidazole group have been recommended for the curative chemical control of latent lesions caused by *Botryosphaeria dothidea*. Parker & Sutton (1993 a) developed a model, under laboratory conditions, to analyze the occurrence of white rot epidemics; however, the model was not yet validated in the field.

Disease management recommendations comprise spraying apple trees with contact fungicides such as captan, folpet and chlorothalonil, as well as with systemic fungicides belonging to the benzimidazole group such as benomyl and carbendazim, from late spring on. In Brazil, it is also recommended using dithianon and fluazinam in apple trees (Jones & Aldwinckle, 1990; Boneti *et al.*, 1999). Parker & Sutton (1993 b) found in laboratory condition that SBI fungicides had an eradicating effect on the control of *B. dothidea*, whereas Kim & Uhm (2002) showed that the preventive spraying of SBI fungicides in South Korea orchards, approximately 1 month before harvest, reduced the losses caused by the disease.

Calcium chloride is used as calcium source for apple trees cultivated in all producing countries. This compound is known for its efficacy in controlling several apple pathogens through germination inhibition (Stošić *et al.*, 2014). Biggs (2004) found that CaCl_2 shows different effects on *B. dothidea* isolates and on rot development.

Recent studies have shown that *B. dothidea* germination may be stopped when there are moisture interruption periods up to 1 hour long (Kim *et al.*, 2016). Accordingly, the authors recommend using just contact fungicides in early warning systems.

The *Sisalert* System was developed in the Vacaria Region in 2001 to 2002 in order to support the decisions made by producers concerning the rational use of fungicides to manage the main diseases affecting the crop. The ease of incorporating new modules, Internet availability and security aspects are among the advantages presented by such systems (Fernandes *et al.*, 2001). The aim of the current study was to validate the model by Parker & Sutton (1993 a), under Vacaria conditions, Rio Grande do Sul State, by using the *Sisalert* system database, as well as to characterize the fungicide action on the infection process.

Material and methods

Assessing the preventive treatments and warning systems

Two experiments were conducted in 8-year-old cv. Fuji/EM7 apple trees infected with *B. dothidea* in order to compare chemical control strategies in Vacaria, RS state, Brazil. The model activation was when conidia and/or ascospores were first detected in the pruning branches left under the canopy of apple trees from the last week of July. The fungicides were sprayed with the aid of a 15 l Jacto knapsack mist blower sprayer (J.10 nozzle) in the first and second experiments and with an airblast sprayer in the third one. The present study recorded the effect of fungicides registered in Brazil in 2002 to be used in apple trees, as well as that of the potassium phosphite fertilizer.

Experiment 1. Comparison between fungicides used as preventive measure and along with the warning system in the 2002 to 2003 period

The warnings were based on the model by Parker & Sutton (1993a) and considered severe and moderate epidemic conditions for spraying. The pre-condition was two rainfall events in a 48-hour period. The model by Parker & Sutton (1993a) for apple infection is described as follows:

$$Y = -0.1546 + 0.0123T + 0.0329W - 0.00169 W^2 + 0.0000225W^3 - 0.00153(TW^2) + 0.000111(TW^2) - 0.00000151(TW^3),$$

wherein Y is the proportion of infected apples; T is the temperature; and W is the leaf wetness expressed in hours.

Spraying was performed during the 2002-2003 period in the preventive evaluations (Captan + thiophanate-methyl; famoxadone; fluazinam and chorothalonil), and warning systems with a 10-day protection, or when there was rainfall equal to or higher than 50 mm within this interval. The experiment followed a completely randomized design, with four repetitions, with four trees/plot. The incidence of latent infections was assessed at harvest. Table 1 describes the treatments.

Table 1. White rot incidence in fruits from apple trees preventively treated with fungicides, according to the warning system, or with 2 rainfall events as pre-condition, Vacaria, RS (2002-2003).

Fungicidal treatment Active ingredient/commercial product	Active ingredient/ 100 l water	White rot at harvest	Latent white rot infection
Control	-	8.9 ab ¹	10.4 a
Warning with two rainfall events as precondition. (Captan + thiophanate-methyl) (Captan + /Cercobin)	150+ 49	8.4 abc	6.9 ab
Mancozeb + Famoxadone/Midas	78 +7.8	8.4 abc	9.5 a
Mancozeb + Famoxadone/Midas	100 +10	8.6 abc	6.9 ab
Fluazinam/Frownicide	25	6.4 abc	5.1 ab
Chlorothalonil/Bravonil	160	10.9 a	5.4 ab
Captan/Captan	120	1.3 bc	0.7 b
Captan/Captan + thiophanate-methyl/ Cercobin	150+ 49	1.2 c	1.2 b

¹ Means of 4 replicates comprising one plant each. Data followed by equal letters do not differ from each other (Tukey, $p < 0.05$).

Experiment 2. Assessing the warning system and the preventive treatment to control apple white rot in the 2003-2004 period

Cv. Fuji apples trees were sprayed with Captan, 0.15% when 25 mm of accumulated rainfall was reached or when moderate or severe infection periods occurred (Parker & Sutton, 1993 a), after a rain of at least 5 mm rain. The experiment followed a completely randomized design, with three replicates and plots planted with 15 plants. Fifteen apples were collected from each plot for detecting of latent infections. Two boxes with 100 fruits were kept in cold storage during 135 days to record white rot incidence.

Experiment 3. Assessing the epidemic forecasting system

Spraying was conducted according to the weather forecast information to deliver white rot risk warnings and compare with calendar spraying system in two plots. Disease scouting was conducted on a monthly basis in 5 groups of 5 apple trees. Fifteen groups of 20 fruits/plot were collected at harvest to record disease symptoms and to investigate latent infections by incubating fruits in polyethylene bags at 20-23 °C.

Effects of pre-harvest fungicides on *B. dothidea* control in apples inoculated under controlled conditions

The experiment was conducted in a cv. Fuji apple orchard. Products used were kresoxim-methyl (0.01%), calcium chloride (0.078 and 0.156%), chelated calcium (0.3%), calcium nitrate (0.3%), dithianon (0.035%), chlorothalonil (0.106%), thiabendazole (0.065%), mancozeb (0.24%), captan (0.12%), folpet (0.12%), thiophanate-methyl (0.07%) or benomyl (0.02 and 0.03%), and they were applied six times, at 10-day intervals. Three-tree plots were used in a completely randomized block design. Ten fruits were harvested from each plot 24 hours after the last treatment. Then, they were inoculated with *B. dothidea*, according to a

method described by Parker & Sutton (1993), and incubated at 30 °C. The number of fruits affected by white rot was recorded 10 days later.

Assessing the protective and curative effects of fungicides on the incidence of white rot in apples inoculated with *Botryosphaeria dothidea*

After 4 months of cold storage, cv. Fuji apples were inoculated with *B. dothidea* conidia using the method described by Parker & Sutton (1993 b). The apples were sprayed with thiophanate-methyl (0.063%), carbendazim (0.03%), benomyl (0.03%), dithianon (0.035%), folpet (0.125%), captan (0.125%), mancozeb (0.2%), chlorothalonil (0.106%) or calcium chloride (0.16%) and inoculated to assess the protective effect of each treatment. For the curative test, apples were inoculated, incubated at 30 °C for 72 hours, sprayed with the fungicides, and stored for 12 days in a humid chamber at 30 °C. Calcium chloride (0.078%) was only evaluated as a protectant and potassium phosphite (0.06% P₂O₅) as a curative compound. Three repetitions of 10 fruits were used in each treatment. The number of fruits affected by white rot was recorded.

Results and discussion

By comparing the two systems tested in the 2002-2003 period, which used fungicides as preventive measure, as well as captan and thiophanate-methyl according to the warning conditions set at the precondition of 2-rainfall events, the sprays were performed on 12/13th, 12/26th, 1/6th, 1/18th, 1/28th, 2/3rd, 2/14th, 2/21st, 3/5th and on 3/17th in the preventive system; and on 1/16th and 1/26th when the warning system was used. The apples were harvested on 4/14th. There was much rainfall during the entire period, a fact that sometimes made it impossible to protect the apple trees at the expected intervals and, certainly, increased the disease pressure in the period (Table 1).

The high-volume late-season rainfalls during the herein developed experiment favored the onset of white rot and caused considerable damage to apples (Table 2). There were approximately 20% total losses of apples in the assessed orchard. The warning system treatment, which was conditioned to 2 previous rainfall events and to 50 mm protection, did not control the disease. The incidence of white rot at harvest was reduced by the preventive treatments with Captan and Captan + thiophanate-methyl, which were applied every 10 days, or whenever the rainfall was higher than or equal to 50 mm.

The number of treatments in the assessed period was lower in the second experiment (Table 3), whose precondition relied on rainfall events higher than 5 mm. The number of treatments in the period was lower (Table 2) and the disease incidence was 2.2% in the warning areas and in the preventive control. The assessment of disease incidence after storage recorded 1.5% white rot in the warning areas, and in the preventive treatment, as well as 2.8% in untreated apples. Two sprays were conducted during the cycle in the warning areas, and five were conducted in the preventive treatment; Captan spraying was performed throughout the entire period (Table 1). There were warnings almost every week from December, when the disease-risk forecasting system was used; apple white rots reached 1.3% in the calendar system, and 0.6% in the forecasting system (Table 2).

Table 2. The effects of using the warning systems and preventive fungicide sprays on cv. Fuji apples affected by white rot in Southern Brazil.

Sprays	Experiment 2 2003-2004		Experiment 3 2016-2017	
	Number of Sprays	Disease Incidence	Number of Sprays	Disease Incidence
Preventive treatment	10	0.1%	8	1.3%
Warning system	2	0.1%	7	0.6%
Check	-	8.4 %	-	12.3 %

Table 3. Fungicide spraying on the conventional and warning areas to control white rot in apples (*Botryosphaeria dothidea*) in Vacaria, RS (2003-2004).

Sprayings in the warning system		Sprayings in the preventive system	
Dates/ Warning type	Dates/ Rainfalls (mm at intervals)	Dates/ Rainfalls (mm at intervals)	Fungicides and doses
Jan. 16 th / Moderate		Jan. 15 th	Mancozeb, 0.24% + Ca chloride, 0.12%
Jan. 26 th / Severe	April 26 th	Jan. 26 th /56.6	Dithianon, 0.049%
		Jan. 31 st /26.4	Captan, 0.15% + Ca chloride, 0.12%
		Feb. 12 th /18.2	Mancozeb, 0.24%+ Thiophanate-methyl, 0.049%,
		Feb. 27 th /38.4	Dithianon, 0.07%

Beresford & Manktelow (1994) applied the disease risk assessment associated with weather forecast to apple trees affected by scab in New Zealand, and they considered this as not being an acceptable option from the economic-return perspective. Results in the present study showed that this forecasting system is advantageous in Brazil to manage white rot of apples, and due to the great accuracy of the current forecast available in the country.

The control of Botryosphaeria dothidea through pre-harvest fungicides

Table 4 shows data on white rot incidence and severity in fungicide-treated plants. None of the fungicidal treatments controlled the disease when fruits were inoculated and refrigerated 1 day after the treatment. Calcium chloride (at the rate 0.156% and 0.078%) and kresoxim-methyl increased white rot incidence and severity. Non-visible damages caused by calcium chloride on apple may favor the onset of infection by the pathogen. The most efficient control was reached by the benzimidazole fungicides.

Table 4. White rot incidence and severity in Fuji apples treated with pre-harvest fungicides and calcium sources.

Treatments	Diseases fruits	Spots/fruit
Inoculated control	1.9 bcde	3.3 efg
Kresoxim-methyl, 0.01%	6.0 a ¹	38.6 a
Calcium chloride, 0.156	5.7 a	24.1 ab
Calcium chloride, 0.078%	5.6 a	11.2 cde
Chelated calcium, 0.3%	4.3 ab	15.4 bc
Calcium nitrate, 0.3%	4.1 ab	12.6 bcd
Dithianon, 0.035%	3.6 abc	4.5 defg
Chlorothalonil, 0.106%	2.6 bcd	5.2 cdefg
Thiabendazole, 0.060%	1.9 cde	6.9 cdef
Mancozeb, 0.240%	1.6 cde	5.3 cdefg
Captan, 0.120%	0.6 de	0.6 fg
Folpet, 0.120%	0.6 de	0.9 fg
Benomyl, 0.02%	0.6 de	0.5 fg
Thiophanate-methyl, 0.063%	0.3 e	0.2 g
Benomyl, 0.03%	0.3 e	0.5 fg

¹Mean of 3 repetitions comprising 8 fruits each. Means followed by equal letters do not differ from each other (Duncan, P = 0.05).

The chemical control of Botryosphaeria dothidea in inoculated apples

Results recorded for cv. Fuji (Table 5) showed that only folpet and chlorothalonil did not have a protective effect. Thiophanate-methyl, mancozeb and calcium chloride (0.3%) were the most effective products. The post-inoculation action was higher in treatments with potassium phosphite and mancozeb, and it was not recorded in treatments with folpet and carbendazim. Treatments applied 72 hours after inoculation should not necessarily be considered curative, given the difference in the period necessary for the pathogen to germinate (Sutton & Arautz, 1991).

For the first time, results showed the usefulness of the warning system applied to white rot in apple orchards, based on the model developed by Parker & Sutton (1993 a), on the inoculum availability in the orchard (Valdebenito-Sanhueza *et al.*, 2005) and on the precondition of 5 mm rainfall. The warning system enables farmers who practice crop control in the region of Vacaria (RS), to monitor the occurrence of the pathogen in their orchards and to use the most effective fungicides only when the risk of the white rot epidemic is detected.

Table 5. Protective and post inoculation effect of fungicides on *Botryosphaeria dothidea* infection in cv. Fuji apples, under laboratory conditions.

Treatment	Dose/100 l active ingredient	Preventive action Number of fruits with white rot/10 fruits	Post-inoculation action Number of fruits with white rot/10 fruits
Thiophanate-methyl	63	0.32 c ¹	0.57 bc
Carbendazim	30	0.97 bc	1.65 ab
Benomyl	30	0.66 bc	0.93 bc
Dithianon	35	0.91 bc	0.57 bc
Folpet	200	0.97 bc	1.60 ab
Captan	125	0.65 bc	1.0 bc
Mancozeb	240	0.0 c	0.0 c
Chlorothalonil	106.2	3.98 ab	0.3 bc
Calcium chloride	156	0.62 bc	- ²
Calcium chloride	78	0.0 c	-
K phosphite	60	-	0.0 c
Inoculated control	---	4.87 a	2.96 a

¹ Means of 3 repetitions comprising 10 fruits each. Data followed by equal letters do not differ from each other (Duncan, p<0.05).

² Non-assessed treatment.

Conclusions

- Using the warning system for white rot of apples (*Botryosphaeria dothidea*), with pre-conditions such as the presence of inoculum and rainfall equal to or greater than 5 mm, allows rationalizing the disease control.
- Fungicides such as thiophanate-methyl, captan, mancozeb and the fertilizer potassium phosphite show potential to be used in warning systems applied to white rot.

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Experience of apple tree sustainable plant protection in Lithuania

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Abstract: The research was carried out in 2011-2013 with apple cvs. Auksis, Alva, Connell Red, Ligol, Lodel, Rubin and Shampion. The aim of our investigation was to estimate the effects of a sustainable plant protection system on apple fruit quality. The sustainable plant protection system was based on the internet supported forecasting system iMETOS[®] sm. According to the rules of the sustainable plant protection system, plant protection products with the same active ingredients were used not more than two times, and the preharvest interval was 1.5 times longer than indicated on the product label. Plant protection products labelled as "Very toxic", and "Toxic" were not used.

Key words: apple scab, codling moth, forecasting system, fruit damage, preharvest interval

Introduction

Among the main problems of the Integrated Production of fruits, the control measures of pests and diseases, a lack of resistant/tolerant cultivars and poor pesticide availability were detected in Lithuania. Integrated production or quality assurance schemes are operated in many European countries (Poulsen *et al.*, 2009; Holb *et al.*, 2012; Beckerman *et al.*, 2015). The total production area of crops managed under integrated pest management (IPM) rules is enlarging year by year. However, there are noticed differences between country requirements for soil management, plant nutrition as well as crop protection and control procedures. Several investigations on IPM were carried out at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry (LRCAF). The first investigations of the warning equipment Metos D were initiated in 2001-2006 (Raudonis & Valiuškaitė, 2003) and from 2007 they were extended using an internet based system iMETOS[®] sm for prediction of infection risks of apple scab (Raudonis & Valiuškaitė, 2009). The scab warning system gave a possibility to optimise the use of fungicides against scab and to reduce spray applications per season. The forecasting models, new tree training systems, and innovative application equipment have been developed to improve treatment coverage, to mitigate pesticide drift and to reduce chemical residues in fruits (Damos *et al.*, 2015).

The sustainable plant protection system protects the environment and gives an income to fruit-growers and takes care of farmers and consumers health. The traditional IPM is not so efficient in comparison with the sustainable plant protection system. The aim of the current research was to estimate the effect of sustainable plant protection on apple fruit quality.

Material and methods

The experiment was carried out at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2011-2013. Apple trees of cvs. Auksis, Alva, Connell Red, Lodel, Ligol, Rubin and Shampion on rootstock P 60 were tested in a full bearing orchard. Planting distance was 4×1.25 m. Experimental plots with four apple trees in each were arranged randomly in four replicates. Fruit thinning was performed after June drop.

Forecasting system

The Sustainable plant protection system, based on the internet supported forecasting system iMETOS[®]sm (Pessl Instruments, Austria), was located at the experimental apple orchards. The iMETOS[®]sm system was equipped with sensors for registration and transmission of data on temperature, relative humidity, rainfall, leaf wetness and other data needed for prediction of apple scab infections. Fungicides were applied on scab sensitive varieties immediately after infection appeared. The same active ingredients of plant protection products were not used more than two times (Table 1), and the preharvest interval was 1.5 times longer than indicated on the label. Plant protection products labelled as "Very toxic" and "Toxic" were not used.

Assessments

Fruit scab damages were recorded during the harvest and assessed on random samples of 100 fruits in 0-2 point scale, where 0 = no scab symptom; 1 = 1-2 spots per fruit; 2 = more than 3 spots per fruit. Damage incidence was calculated according to the formula $P = n/N \cdot 100$. (P – incidence, %, n – number of damaged fruits, N – total number of investigated fruits).

Delta pheromone traps (Biobest, Belgium) were used to monitor codling moth flight activity and population density. Pest population density was expressed as a relative measure based on a mean number of insects captured per trap during a particular time interval (Tamošiūnas *et al.*, 2013; 2014). Insecticides were applied when the density of pests was reaching the threshold of harmfulness. Apple growth stages were characterised according to BBCH scale (Meier, 1997).

Statistical analysis

The experimental data were evaluated by general analysis of variance (ANOVA) for randomised block designs using the statistical program SAS (SAS Institute, Cary, N.C.).

Results and discussion

Minimizing the usage of pesticides in horticulture is an important condition in the integrated apple production. Apple scab was controlled by applications of fungicides based on the forecasting system iMETOS[®]sm (Pessl Instruments, Austria). This system records meteorological conditions and calculates apple scab infections at three levels: light, medium and high. Scab susceptible cultivars were sprayed when the risk of ascospore release or conidia light infection reached more than 70-80 %. Scab susceptible cvs. Alva, Ligol and Conell Red were sprayed twelve times (Table 1), other less susceptible cvs. Auksis, Rubin and Shampion were sprayed nine times, and cv. Lodel was sprayed seven times per season. Cv. Lodel is resistant to apple scab. However, fungicides treatments were applied on this cultivar to control incidence of mildew and fruit rot.

Table 1. Sustainable pest and disease control system in an apple orchard. Average 2011-2013.

Growth stage by BBCH	12 fungicide treatments cvs. Alva/ Ligol/ Conell Red	9 fungicide treatments cvs. Auksis/ Rubin/Shampion	7 fungicide treatments, cv. Lodel
03	copper hydroxide (2.5) ¹	–	–
07-09	copper hydroxide (0.75)	copper hydroxide (0.75)*	copper hydroxide (0.75)
10	cyprodinil (0.2)	cyprodinil (0.2)	kresoxim methyl (0.2)
	thiamethoxam (0.2)	thiamethoxam (0.2)	thiamethoxam (0.2)
57	captan (2.0)	–	–
59	cyprodinil (0.3)	cyprodinil (0.3)	cyprodinil (0.3)
69	difenoconazole (0.2)	difenoconazole (0.2)	cyprodinil (0.3)
	mancozeb (2.0)	mancozeb (2.0)	–
	pheromone traps for insects	pheromone traps for insects	pheromone traps for insects
71	spirodiclofen (0.4)	spirodiclofen (0.4)	spirodiclofen (0.4)
	kresoxim methyl (0.2)	kresoxim methyl (0.2)	kresoxim methyl (0.2)
	dithianon (0.5)	dithianon (0.5)	dithianon (0.5)
	acetamiprid (0.2)	acetamiprid (0.2)	acetamiprid (0.2)
73	difenoconazole (0.2)	difenoconazole (0.2)	–
	mancozeb (2.0)	mancozeb (2.0)	
75	kresoxim methyl (0.2)	kresoxim methyl (0.2)	deltamethrin (0.25)
	deltamethrin (0.25)	deltamethrin (0.25)	
76	captan (2.0)	captan (2.0)	captan (2.0)
77	trifoxystrobin (0.1)	–	
81	trifoxystrobin (0.15)	trifoxystrobin (0.15)	trifoxystrobin (0.15)
	dithianon (0.5)	dithianon (0.5)	dithianon (0.5)

¹Numbers in brackets represent the rate of product sprayed in kg or l/ha

When scab lesions are present on fruits, such production is not marketable. The influence of sustainable plant protection system on fruit quality is provided in Figure 1. A reduced pesticide program did not guarantee total scab control; therefore, damaged fruits should be thinned manually. On average, cvs. Ligol, Alva and Rubin had the highest part of damaged fruits (13-19%), when sustainable plant protection system was efficient for cvs. Lodel and Auksis (0-1.7% of damaged fruits)

Results of our investigation showed that fruits of all cultivars were well protected against diseases and pests. Some studies indicated highly variable damage levels by secondary apple pests like apple sawfly (Tamošiūnas *et al.*, 2013; 2014; 2015) or green apple aphids (Raudonis *et al.*, 2010) on different apple cultivars in the same orchard, what was successfully controlled in our experiment applying the sustainable plant protection system.

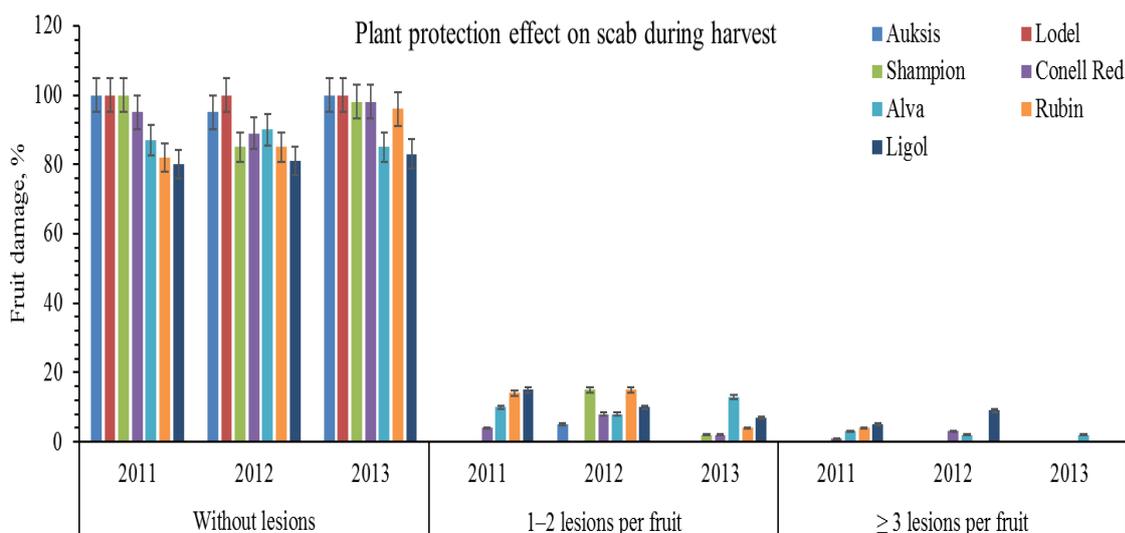


Figure 1. Effect of the sustainable plant protection system on fruit damages (%) by scab at harvest.

Apple codling moth adult's activity was present during all seasons of investigations. The cumulative population density increased until last week of June and reached its peak on July 4. Trap catches varied between cultivars during the period of the study. The highest mean trap catches were observed on cvs. Lodel and Alva (Figure 2) and stable the lowest mean trap catches showed cvs. Rubin, Champion and Conell Red.

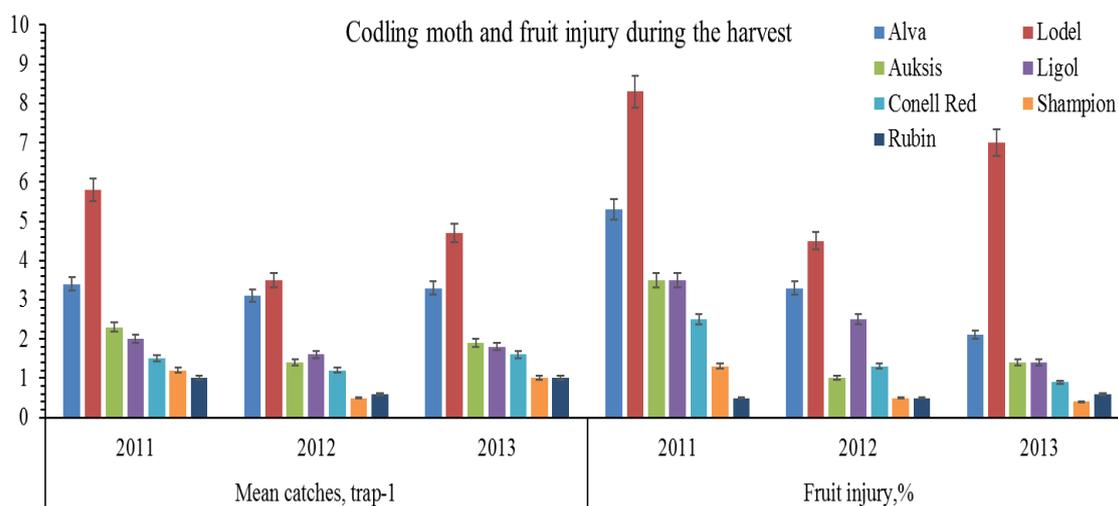


Figure 2. Annual mean trap catches (\pm SE) of codling moth and fruit ($n = 100$) injury during harvest.

The investigated sustainable plant protection system has the requirement for 1.5 times longer preharvest interval than indicated on the product label. Obtained results showed that actual preharvest intervals after the last application exceeded indicated from 1.5 to 39.5 days (Figure 3). Results of our study proved that apple scab could be effectively managed with

reduced plant protection system and provides a good example for growers enabling a significant reduction of pesticide applications in apple orchards. Fruits were intensively colored, and all tested cultivars fulfilled Extra class requirements.

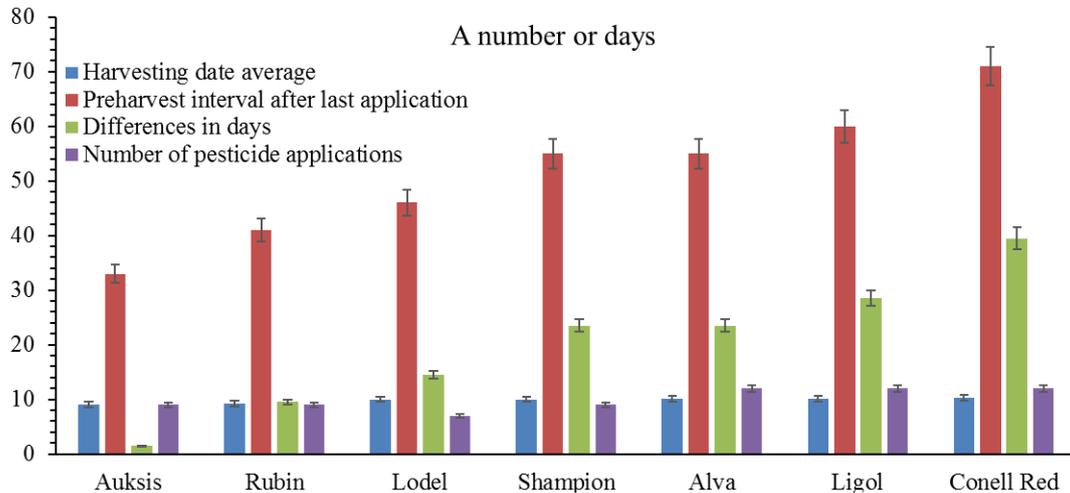


Figure 3. The difference between indicated preharvest interval and interval after the last application in the sustainable protection system, a number of days. Average 2011-2013.

In order to obtain the reduction of fungicides without any significant crop damage, the control of apple scab should be based on registration of climatic data, scouting of biotic parameters, infection risks and simulation disease models, resistance management for modern fungicides, efficient available packet of plant protection products, and risk profile of available products.

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Spray application in 3D-Crops – unexploited potentials beyond spray drift reduction

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Abstract: Releasing pesticides into the environment may cause contaminations of non-target objects, including human settlement structures and objects with potentially negative effects on non-target organisms. In 3D-crops the use of drift reducing nozzles on air assisted sprayers is recommended as a key technique for drift reduction. Drift reduction by the sole use of big droplets represents an end of the pipe technique, since very little attention has been given to the processes before and during droplet release as there are effects of droplet size, air distribution, fan speed and forward speed on spray cover formation, pesticide consumption, work rate, spray drift, fuel consumption and noise emission. During works for reducing spray drift of small droplet nozzles, rectangular air distribution, canopy adapted fan speed and forward speed and small droplets increased work rate and pesticide deposition efficiency, compensating significant dose rate reductions and enormously reduced spray drift, fuel consumption and noise emissions. The prerequisite are fans with a low vertical angle of the air stream and a rectangular air distribution adjusted at an air test bench to allow the adaptation of the air stream to the canopy at any forward speed, keeping most of the droplets inside the canopy. Officially recognized drift reduction is obtained by fans with cross flow characteristics and two air induction nozzles at the two top most nozzle positions and a model for canopy adapted dosing and application.

Key words: 3D-crops, air distribution, droplet size, canopy adapted spray application, pesticide consumption, spray drift reduction



Effective spray drift reduction in fruit growing by the use of coarse droplet spray applications

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Abstract: Use of excessive airflow and fine spray nozzles is usually responsible for spray drift during spray applications in orchards. The reduction of the emission of plant protection products to the environment is an important issue. For a long time, fruit growers have been used to spray with fine droplet spectra which were assumed to be most effective and to reduce spots and visible residues on the fruits. In the Netherlands, priority is given to introduce low drift nozzles (coarse droplets) in fruit growing, because: (i) low drift nozzles can be used on every (already in use) orchard sprayer; (ii) low drift nozzles do not require high investment costs from the grower; and (iii) introduction of low drift nozzles for orchard spraying can be fast. In practice, fruit growers were reluctant to change from fine droplet applications to coarse droplet applications because of (i) clogging of this type of nozzles, (ii) fear of reduced biological efficacy, and (iii) fear of increased visible and measurable PPP-residues on fruits. Trials and projects carried out over a number of years, showed that using coarse droplet applications result in similar biological efficacy compared to conventional nozzles for all important pests and diseases in pome fruit orchards. No differences exist in average residue levels or visible residue between fine and coarse droplet applications. Practical problems, such as clogging of nozzles and relatively high spray volumes were solved in cooperation with fruit growers, advisors, and nozzle and sprayer manufacturers.

Key words: spray drift reduction, orchard sprayers

The reduction of the emission of plant protection products (PPP) to the environment is an important issue when applying agrochemicals in fruit growing. In the Netherlands, spray free and crop free buffer zones were introduced, to mainly minimize the risk of spray drift (Water Pollution Act, Plant Protection Act). In general, applications of fungicides in fruit growing in the Netherlands are carried out with low spray volumes. The most often used nozzles are of the size 0050 (lilac) and 0067 (olive green), producing a very fine spray quality (Southcombe *et al.*, 1997). In the Netherlands, the most commonly used sprayers are cross flow fan sprayers. A minority of the fruit growers use axial fan sprayers or multi-fan sprayers. Therefore, the reference spraying machine for spray drift measurements in orchard spraying is a cross flow fan sprayer, equipped with Albuz ATR lilac hollow cone nozzles (spray pressure 7 bar, generating fine droplets), and a spray volume of approximately 200 l/ha.

So far, several drift reducing measures have been accepted in the Netherlands by water quality control organizations and the Board for the Authorization of Pesticides (CTGB), e. g. presence of a windbreak, the use of a tunnel sprayer or drift reducing venturi type nozzles with one-sided spraying of the outer tree rows. With reduced air assistance and low drift nozzles it is possible to achieve very high drift reductions, even at short distance from the

orchard. Recently, a nozzle classification system for drift reduction in orchard spraying has been developed (Zande *et al.*, 2012). Some of the drift reduction methods have marked disadvantages for the fruit grower, such as high investment costs (tunnel sprayer) or the loss of cropping area (windbreak). More advanced systems, such as the CASA system showed the potential of sprayer models equipped with wind sensor and GPS navigation systems, which enable real time adjustment of application parameters, such as airflow and spray quality, to reduce the negative environmental impact of spray applications in orchards (Doruchowski *et al.*, 2012). Prototypes of Canopy Density Sprayers are recently introduced at a practical farm level in the Netherlands (Nieuwenhuizen & Van de Zande, 2012).

New strategies have to be developed to retain chemicals for crop protection and a clean environment. New developments have shown great perspectives for multiple row orchard sprayers. Because of increased work capacity, the use of these types of sprayers has increased much in the Netherlands in the recent years. It has been proven that multiple row sprayers reduce spray drift significantly (Wenneker *et al.*, 2014). This is due to a system that sprays tree rows from both sides at the same time, in contrast to standard orchard sprayers that spray the tree row only from one side. It is assumed that spray deposits are improved when spraying with multiple row sprayers. Therefore, doses of agrochemicals can be reduced, without reducing biological efficacy, implicitly reducing emission to the environment while maintaining high levels of spray drift reduction. Further research is therefore necessary to assess spray deposition in the tree canopy (Wenneker *et al.*, 2016).

The results show that very high drift reductions can be achieved by a multiple row sprayer equipped with drift reducing nozzles. It is therefore advised to set up additional spray drift reduction classes of 97.5% and 99% in the spray drift reduction classification system. The evaluation of the latest drift figures in orchard spraying in the Netherlands, and measurements of surface water quality parameters, show that the current legislation and measures are insufficient. This could also have implications for approval of pesticides in fruit growing. To meet the national and European objectives regarding surface water quality, a reduction of chemical input is also required. Further research is therefore required to assess spray deposition in the tree canopy when spraying with multiple row sprayers. It is assumed that spray depositions are improved with these types of sprayers, and dose can be reduced accordingly, without reducing biological efficacy.

In addition, airborne spray drift can be very high when using Albus ATR lilac (Fine Spray quality). These airborne drift values are significantly higher than spray depositions at soil surface at the same distance (Michielsen *et al.*, 2005). The use of low drift nozzles and a multiple row sprayer results in very low airborne drift figures. This implies that reducing drift to the soil and surface water would also reduce drift exposure to residents and bystanders.

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***Colletotrichum* species causing the postharvest problem of bitter rot on apple in Belgium: from pathogen to host**

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Abstract: Worldwide, *Colletotrichum* spp. have been identified as detrimental pathogens in apple production, causing the postharvest disease bitter rot. However, until now, *Colletotrichum* spp. on apple were not yet known to be present and problematic in Belgium. Although, in surrounding European countries there are increasing numbers of first reports concerning problems of bitter rot on apple (Munda, 2014; Munir, 2015; Weneker *et al.*, 2015; Børve and Stensvand, 2015; Nodet *et al.*, 2016). Since postharvest diseases can cause considerable fruit damage and fruit growers are continuously trying to reduce these losses during and after storage, a better knowledge on the presence of the pathogen, pathogenicity and the pathogen-fruit interactions is essential to assess the problem more in detail. In this research, the presence (morphologically and molecularly) and pathogenicity of *Colletotrichum* spp. postharvest on apple fruit in Belgium was assessed. The study was performed by sampling isolates from necrotic lesions of 21 different apple cultivars from three different commercial orchards in Belgium. Isolates from strawberry were added to compare and evaluate for possible cross-infections. Seven different species, *C. fiorinae*, *C. kahawae*, *C. salicis*, *C. rhombiforme*, *C. acutatum*, *C. nymphaea* and *C. godetiae*, were identified on apple and strawberry in Belgium from 99 isolates, based on multigene sanger sequencing results from six gene sequences (*ITS*, *GAPDH*, *ACT*, *HIS3*, *TUB2* and *CHS-1*). Some of the species were found for the first time on apple: *C. kahawae* and *C. rhombiforme*. A selection of 14 isolates (covering the seven species) was used to compare morphological characteristics *in vitro* (growth rate, colony colour, spore shape and size) and evaluate the pathogenicity. Conclusions could be made that morphologically it is difficult to separate *Colletotrichum* species, in contrast with the molecular tool, where a clear distinction between the species was obtained. Pathogenicity tests were performed on cvs. Pinova and Nicoter. Artificial infection was performed by injecting a 5 µl drop of spore solution (5.0×10^5 conidia/ml) in the wound, while control fruits were injected with 5 µl sterile distilled water. Fruits were placed in a dark incubation room at 22 °C and a constant relative humidity of 95%. The development of necrotic lesions was examined by measuring two perpendicular diameters of each lesion at 4, 8, 12 and 15 days post infection (dpi). Results showed that there was a clear difference in pathogenicity between isolates and that even an isolate from strawberry easily can infect an apple. We also considered to focus towards a better understanding of the differences in susceptibility of apple fruits and cultivars for *Colletotrichum* species. Artificial inoculation experiments showed that cv. Nicoter apple fruit

are less susceptible than cv. Pinova apple fruit and storage time of fruit (5 to 20 weeks) in a cold room (1 °C) have an effect on the lesion development in time. The susceptibility of fruits increases during ripening (and storage), resulting in a faster growth of the lesion in time. Previous studies about the presence of *Colletotrichum* spp. in Belgium are not available, since the causal agents of apple bitter rot have not been studied. These data provide a first broad study about the *Colletotrichum* species in Belgian orchards. The effect of ripening on the susceptibility of apple fruit is an important factor that will be investigated further.

Key words: *Colletotrichum*, *Malus x domestica*, pathogenicity, sanger sequencing

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Non-conventional methods for postharvest management of bull's eye rot of apple

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Abstract: Bull's eye rot caused by *Neofabraea* spp. represents one of the main postharvest pathogens affecting 'Cripps Pink' apple during storage. The aim of the work was to investigate two non-conventional methods: fruit sorting with DA-meter and hot water treatment (HWT) for disease control. Immediately after harvest, 'Cripps Pink' apples, harvested from two different orchards, were sorted by DA-meter, and divided in two classes according to their chlorophyll content, expressed as Index of Absorbance Difference (I_{AD}): class H ($I_{AD} > 0.8$) with high chlorophyll content and class L ($I_{AD} < 0.75$) with a chlorophyll content lower than class H. Control fruit were represented by apples not sorted by the DA-meter. Other apples, derived from four different orchards, were treated by dipping in water at 45 °C for 10 min using a prototype machine for HWT, for semi-commercial trials. Control apples were represented by apples dipped in water at 14-15 °C. All apples were stored for 4 months at 0 °C and then evaluated for bull's eye rot incidence. Among I_{AD} , the results showed that apple of class H were significantly less susceptible to bull's eye rot than apple of class L, especially after 90 days of storage. In addition, also HWT reduced the bull's eye rot incidence, with efficacy ranging from 62.7 to 85.3% compared to the controls, depending on year of trial and harvest time. The evaluation of the integration of these two methods for a further improvement of bull's eye rot control is in progress.

Key words: Cripps Pink, DA-meter, hot water machine, *Neofabraea* spp.

Introduction

Bull's eye rot caused by *Neofabraea* spp., previously known as 'Gloeosporium rot', is one of the most frequent and damaging diseases occurring in stored pome fruit worldwide (Cunnington, 2004; Michalecka *et al.*, 2016; Soto-Alvear *et al.*, 2013). The incidence of this disease varies considerably: in humid areas, with abundant rain and persistent fog, close to harvest, the economic losses can be as high as 40% for 'Golden Delicious' apples (Sholberg & Haag, 1996) and over 50% for 'Bosc' pears (Lennox *et al.*, 2004). In addition, 'Cripps Pink' apple, a highly regarded cultivar, is extremely susceptible to bull's eye rot and after extended storage, the disease incidence may exceed 70% (Cameldi *et al.*, 2016).

Partial control of bull's eye rot can be achieved by repeated applications of fungicides such as captan, phenyl pyrroles and strobilurines during the two months preceding harvest (Giraud & Bompeix, 2012). However, the use of fungicides on apples during late-season and the postharvest phase is under scrutiny by the European Commission, because of the increasing demand for fruit without pesticide residues (Poulsen *et al.*, 2009).

1-methylcyclopropene (1-MCP), a synthetic cyclic olefin that blocks an ethylene-binding receptor (Sisler and Serek, 1997), has been used on harvested apples to extend fruit firmness in storage and marketing (Saftner *et al.*, 2003). A reduction of bull's eye rot by a 1-MCP treatment was observed on 'Elstar' apples stored for 6 months under controlled atmosphere (Maxim & Weber, 2001) and on 'Cripps Pink' apples after 5 months of storage at 2 °C (Cameldi *et al.*, 2016). Previous results suggest an integrated approach with a combination of two fungicide preharvest treatments and a postharvest 1-MPC treatment. However, besides conventional means, the control of storage diseases by methods alternative to chemical products is also in progress, including the use of hot water treatment (Usall *et al.*, 2016) and a non-destructive technique able to evaluate fruit maturity. Among this non-destructive technique, recently a hand-held instrument (DA-meter) developed from vis/NIR spectroscopy has been introduced to obtain fast and reliable information about the internal characteristics of many fruit species including apple (Nyasordzi *et al.*, 2013). The instrument provides an Index of Absorbance Difference (I_{AD}) (Ziosi *et al.*, 2008), and this enables to quantify indirectly the chlorophyll content. When the I_{AD} value is close to 0, only a small amount of chlorophyll is present in fruit peel, and the more advanced is the fruit maturity. It is known that *Neofabraea* spp. infect fruit in the orchard, but the symptoms of decay appear only at complete fruit ripeness, generally after 3 months in storage. Therefore, it could be crucial to evaluate immediately after harvest fruit propensity to show bull's eye rot symptoms, to adopt an adequate postharvest management of fruits.

The aims of the present study were to investigate two non-conventional methods: (i) hot water treatment (HWT), and (ii) fruit sorting with DA-meter for the control of bull's eye rot of 'Cripps Pink' apples.

Material and methods

Fruit

'Cripps Pink' apples (*Malus domestica* Borkh.) were harvested from an orchard located in the Emilia Romagna Region (Italy). Trees were under-tree irrigated; insects and weeds were controlled following the recommendations commonly used for Italian integrated production applied after planting. No fungicides were used in the orchard after August. Fruit were harvested on two dates in a period from 2 to 20 November depending on the year.

Influence of hot water treatment on bull's eye rot in naturally infected fruit

At harvest, fruits were placed in bins containing 400-450 fruits. A dipping machine (Xeda International, S. Andiol, France) was used for performing the heat treatment. The machine consisted of a tank containing 450 l of water, heated by electric resistance elements placed in the water and regulated by a thermostat with temperature stability of ± 1 °C. The machine has a working capacity of 5 bins per hour. The treatment parameters were set at 45 °C for 10 min. Moreover, the machine was provided with a lid covering the bin, to ensure that all fruit remained entirely submerged throughout the treatment. A digital thermometer automatically recorded the temperature of the bath. Control fruit were treated in the same machine before water heating at 14-15 °C. After treatment, fruit were stored at 0 °C, and the disease incidence was recorded after 4 and 6 months of storage. The sample unit was represented by a bin divided vertically in four parts (replicates) with two plastic sheets. The experiment was conducted in the same orchard, with the same methodology for two consecutive years.

I_{AD} classification

Fruit from the same orchard used for HW treatment trials were used for I_{AD} classification. Immediately, after harvest, in order to obtain two different I_{AD} classes, apples were analyzed using a portable DA-Meter (TR-Turoni, Forlì, Italy) for an indirect determination of chlorophyll content. Apple fruits with an $I_{AD} > 0.8$ were classified in the high (H) class, corresponding to apples less ripe than fruit with an $I_{AD} < 0.75$ that were classified in the low (L) class. In order to avoid an overlapping of samples, apples with the I_{AD} of 0.75 and 0.80 were not selected for the trial. Selected fruits were stored at 0 °C and at high humidity (> 90%) and bull's eye rot incidence was assessed after 150 days of storage. Sample unit was represented by three replicates of 75 fruit each for each I_{AD} class.

Results and discussion

Effect of hot water treatment on bull's eye rot in naturally infected fruits

The results show that the date of harvest influences the appearance of bull's eye rot in 'Cripps Pink' apples, in fact in fruit harvested 14 days after the first harvest, the disease incidence increased with 40% in the first year and with 29.4% in the second year (Table 1). Probably the fruit maturity plays an important role in disease development as observed in organic 'Aroma' apple, where the incidence of total fruit rot, including bull's eye rot, increased significantly in fruit harvested one week after the commercial date (Borve *et al.*, 2013). In addition, the use of HWT consistently reduced the development of bull's eye rot symptoms. The effectiveness of treatment was almost the same between the harvests in the first year of experimentation (62.7% and 65.2% reduction of bull's eye rot incidence for the first and the second harvest, respectively), while, in the second year, the reduction of disease was higher in the first harvest (85.3%) than in the second one (67.3%). Fruit treated with hot water did not show phytotoxic effects, and quality parameters were not significantly different from untreated fruits (data not reported). A main advantage of HWT in comparison to other non-conventional methods for fruit disease control, such as biological control agents or natural compounds, is the feasible use of this technique (HWT does not require any registration from the European Community) and its complete safety for humans and environment (residue-free). On other hand, a main issue of this control strategy could be the length of the treatment; ten minutes might be an obstacle for the practical application of HWT in apple packinghouses, therefore, research based on the use of higher temperatures and shorter length of treatment are in progress. However, apples are more sensitive to high temperatures than other fruit species, such as peach, which can be dipped in water at 60 °C without detrimental effects. For this reason, the reduction of duration of treatment by use of higher temperature could involve the risk of appearance of heat scald damage on fruit surface. In conclusion, HWT remains a valid approach for postharvest disease control, and appears to be especially recommended for organic apple production, where use of synthetic products is not allowed.

I_{AD} classification

The classification with DA-meter showed the possibility of sorting asymptomatic fruit, immediately after harvest, into two I_{AD} classes: L with an I_{AD} value lower than 0.75 and H with I_{AD} values higher than 0.80. During storage, apples of the two classes showed significant differences in bull's eye rot incidence (Figure 1). In particular, the apples of L class ($I_{AD} < 0.75$) were more susceptible to disease compared to fruit of H class ($I_{AD} > 0.80$). The more marked difference of disease incidence between the two IA classes was observed after 90 days of storage (-83.1% in the fruits of H class compared to fruits of L class). After

120 days this difference was lower (-59.5%) and at the end of storage (after 150 days), the fruits of H class (I_{AD} values > 0.80) showed 32.7% less infections compared to the fruit of L class.

Table 1. Effect of hot water treatment (HWT, 45 °C for 10 min) on bull's eye rot incidence of 'Cripps Pink' apple, naturally infected, after 150 days of storage at 0 °C plus 10 days of shelf-life¹

	First year		Second year	
	First harvest	Second harvest	First harvest	Second harvest
Control	49.1a	83a	42.9a	60.8a
HWT	18.3b	28.9b	6.3b	19.9b

¹First and second harvest are shown for each year. First harvest was performed every year in the first week of November and the second harvest after 14 days. In the same column, data followed by different letters are significantly different for least significant difference test ($P < 0.05$).

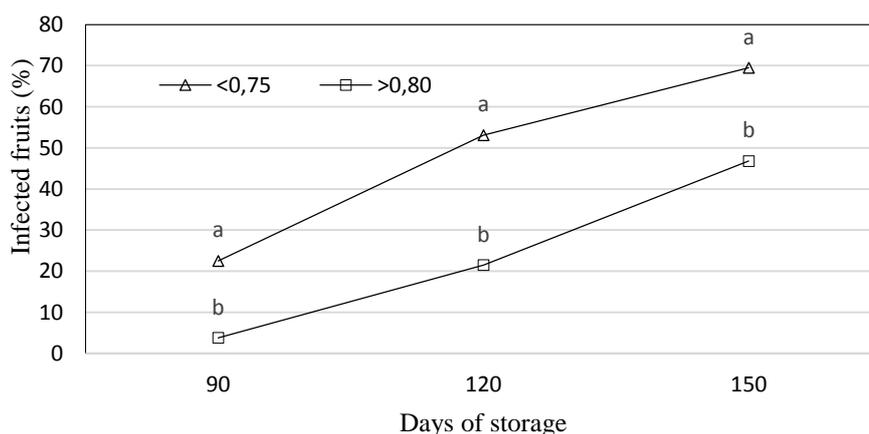


Figure 1. Influence of I_{AD} value on bull's eye rot of apple. Fruit were stored at 0 °C and evaluated for disease incidences after 90, 120 and 150 days of storage. Each value represents the mean of 225 fruits. Within the same evaluation time, different letters represent significant difference for least significant difference test ($P < 0.05$).

Overall, our results show that bull's eye rot is a disease related to apple maturity stage and storage. Accordingly, Guidarelli *et al.* (2011) observed that *Colletotrichum acutatum*, the cause of another latent infection (antrachnose), arrests its growth in immature strawberry. In relation to this, the DA-meter, a non-destructive instrument for the measurement of fruit maturity, may be a useful tool to predict fruit susceptibility to bull's eye rot and improve apple postharvest management. By inserting DA-meter in sorting lines, fruit may be divided in lots immediately after harvest and intended to different marketing (i. e. long/short storage duration) on the basis on their I_{AD} class. Moreover the possibility to integrate this technique with HW treatment is in progress, in view of the postharvest apple management without the use of chemical fungicides.

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***Neofabraea* spp. causing apple bull's eye rot: Identification and characterization of some Italian isolates**

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Abstract: Bull's eye rot, one of the main postharvest diseases of apples, can be caused by four species belonging to *Neofabraea* (*N. vagabunda*, *N. malicorticis*, *N. perennans* and *N. kienholzii*). To verify the current causal agents of apple bull's eye rot in Italy, a pathogen monitoring of stored apples showing bull's eye rot symptoms was carried out from 2014 to 2016 in Emilia-Romagna in Italy. The molecular identification of 42 isolates of *Neofabraea* by DNA sequencing of the β -tubulin region, demonstrated the prevalence of *N. vagabunda*, however, the presence of *N. malicorticis* was also reported for the first time in Italy. *In vitro* assays were carried out to test the nutritional and temperature requirements of the isolates. The use of relatively low temperature (< 20 °C) was considered fundamental to favor the conidial production. The culture at 15 °C on tomato agar for 14 days provided a rapid and a reliable method to allow steady growth and favor the conidial production of *N. vagabunda* and *N. malicorticis*. A biological and morphological characterization of the isolates was performed *in vitro* on tomato agar, and the pathogenicity of the isolates was tested on 'Cripps Pink' apples. For the first time, the presence of two morphotypes of *N. vagabunda* was recorded. In addition, the alkalizing ability of the pathogen was demonstrated *in vitro* and in apple infected tissue.

Key words: host alkalization, molecular identification, morphotypes, *Neofabraea malicorticis*, *Neofabraea vagabunda*, sporulation

Introduction

Bull's eye rot is one of the most common and damaging diseases of apples, bringing notable economic losses during storage and the supply chain, especially in highly susceptible cultivars, such as Cripps Pink (Pink Lady[®]) (Neri *et al.*, 2009; Soto-Alverar *et al.*, 2003; Cameldi *et al.*, 2016 a, 2017). The disease can be caused by various fungal species belonging to the genus *Neofabraea* (*N. vagabunda*, *N. malicorticis*, *N. perennans* and *N. kienholzii*), which infect fruit in the orchard, but remain quiescent for some months (usually for two-three months) after harvest before symptoms appear. Historically, bull's eye rot has been attributed to a species of *Neofabraea* on the basis of the geographic area of decayed fruit and/or disease symptoms *in planta*. In Italy, as elsewhere in Southern Europe, bull's eye rot has assumingly been attributed to *N. vagabunda*, especially on the basis of absence of cankers on the apple trees, which are associated with *N. perennans* and *N. malicorticis*. Recent molecular studies demonstrated that: i) different *Neofabraea* species can occur together in the same area,

ii) *N. vagabunda* is prevalent in many apple producing areas and is common in sites where bull's eye rot has been attributed solely to *N. malicorticis* and *N. perennans*, iii) similarity or overlap in morphologies of conidia occurring among *Neofabraea* species make the identification of causal agents of bull's eye rot based on conidia morphology uncertain (Garipey *et al.*, 2005; Soto-Alverar *et al.*, 2003; Spotts *et al.*, 2009; Michalecka, *et al.*, 2016; Pešicová *et al.*, 2017).

Despite the economic importance of bull's eye rot, many aspects regarding the biology of *Neofabraea* species have not been studied in detail, and in particular the *in vitro* description available in literature for *N. vagabunda* is based on very few isolates. Some of the reasons of this are the slow growth of *Neofabraea* species and the difficulty of obtaining a sufficient number of conidia in artificial media (Olsson, 1965; Neri *et al.*, 2009; Spotts *et al.*, 2009; Rooney-Latham, *et al.*, 2013). Moreover, only a few studies investigated factors involved in the bull's eye rot transformation from a quiescent to an active infection during fruit storage (Lattanzio *et al.*, 2001).

As extended abstract of two previous studies (Cameldi *et al.*, 2016 b; Cameldi *et al.*, 2017), we report: i) the identification of the species of *Neofabraea* currently occurring in the Emilia-Romagna Region of Italy, ii) the biological and morphological characterization of the *Neofabraea* isolates collected *in vitro*, and iii) the pathogenicity of *Neofabraea* isolates and their ability to modify the host pH.

Material and methods

Apple fruit with typical bull's eye rot symptoms were collected over three years during cold storage. Putative *Neofabraea* isolates were established and maintained as monoconidial cultures on tomato agar (TA: 500 g of commercial Italian tomato puree, 15 g of Oxoid agar technical and 500 ml of distilled water), which proved to be the best media for both growth and conidial production of *Neofabraea* in assays on different culture media. The molecular identification of the isolates was carried out by the amplification of a fragment of the *tub2* gene. The PCR products were sequenced with both forward and reverse primers, and sequences were submitted to the GenBank database. To assay the effect of temperature on colony growth and formation of conidia, mycelial plugs of 10 isolates of *N. vagabunda* were incubated on TA Petri dishes at temperatures ranging from 0 to 30 °C. A morphological characterization *in vitro* of all *Neofabraea* isolates was performed after 14 days of incubation at 15 °C on TA, and the pathogenicity of the isolates collected in 2014 and 2015 was recorded in wound-inoculated 'Cripps Pink' apples. The changes of pH induced by *N. vagabunda* growth were also evaluated in *in vitro* and *in vivo* assays.

Results and discussion

Based on sequence analysis and sequence assembly, 41 putative *Neofabraea* isolates were identified as *N. vagabunda* and one as *N. malicorticis*. These results documented for the first time the presence of *N. vagabunda* as the prevalent causal agent of bull's eye rot in Italy on DNA-based assay evidence. However, the presence of *N. malicorticis* was reported for the first time in apples. In agreement with recent molecular studies, which detected the occurrence of *N. perennans* and *N. kienholtzii* in a minority of samples in other European areas (Michalecka *et al.*, 2016; Pešicová *et al.*, 2017; Wenneker *et al.*, 2017), the results of our studies suggest that other less frequent species of *Neofabraea* could cause bull's eye rot

besides *N. vagabunda* also in Italy. Moreover, two single-nucleotide polymorphisms were detected in some isolates of *N. vagabunda* by the *tub2* gene fragment amplification, indicating that some genetic diversity could exist within populations of this species.

The temperature markedly influenced colony growth, morphology and conidia production of the *N. vagabunda* isolates. In particular, the incubation at 20 °C allowed the greatest mycelial growth, while a drastic decline of growth and sporulation was observed at 30 °C. More abundant production of conidia per surface unit (conidia/mm²/ml) was recorded in most isolates at lower temperatures (0-15 °C) than at higher (20-25 °C). The higher sporulation observed in our study at low temperature could be involved in the increased susceptibility to bull's eye rot of late harvested apples. Conversely, the sparse conidial production observed at 25-30 °C could explain the low level of *Neofabraea* spp. inoculum in the orchard during summer (Henriquez *et al.*, 2006). Overall, the incubation for 14 days on TA at 15 °C led to steady mycelial growth and the most abundant conidial production per colony both in *N. vagabunda* and *N. malicorticis*, and was considered a quick and reliable method to induce abundant conidia production of *Neofabraea* for *in vitro* assays and artificial inoculations of fruit.

For the first time, the presence of two *N. vagabunda* morphotypes was detected. The largest group of isolates, grouped in morphotype I, formed colonies with more visible development of aerial mycelia compared to morphotype II, and morphotype I produced typically straight conidia, shorter than the typically long curved conidia of morphotype II. On the other hand, the isolates belonging to morphotype II showed an earlier formation of conidiomata on the agar surface and produced conidia more abundantly after 14 days of incubation at 15 °C. Large and erumpent conidiomata were visible on the surface of *N. vagabunda* isolates grouped in morphotype I after a longer period of incubation (about 75 days at 15 °C), while smaller and more abundant conidiomata covered the colony surface of *N. vagabunda* isolates grouped in morphotype II. A more marked cold-tolerant character was also observed for the isolates belonging to morphotype I than those belonging to morphotype II. Some similarities regarding shape and size of conidia and conidiomata were observed *in vitro* between *N. vagabunda* morphotype I and the isolate of *N. malicorticis*, confirming the difficulty of identification of the causal agents of bull's eye rot based on these traits. However, the formation of more abundant floccose mycelium, radially arranged in synnema-like hyphal clusters, seemed to be a distinctive trait of the isolate of *N. malicorticis*.

The pathogenicity of all the isolates of *N. vagabunda* and *N. malicorticis* was demonstrated by artificial inoculation on 'Cripps Pink' fruit. Some differences in disease symptomology between the two morphotypes of *N. vagabunda* were recorded 4-6 months after inoculation, with the development of conidiomata on fruit lesions. Most isolates grouped in morphotype I formed large and erumpent conidiomata and some of them developed mycelium growing over the lesion or conidiomata, conversely, all isolates grouped in morphotype II showed small subepidermic conidiomata.

The alkalizing ability of *N. vagabunda* was recorded for the first time in *in vitro* assays. In addition, more than 80% of *N. vagabunda* isolates caused an increase of pH in the decayed tissue of apple fruit compared to sound tissue, with a delta pH between healthy and infected tissue areas ranging from 0.1 and 0.8. These results may indicate that the transition of *N. vagabunda* from quiescence to necrotrophic colonization in apples could involve the secretion of alkalizing compounds, acting as pH and pathogenicity modulators.

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Prevalence and management of two emerging postharvest diseases of apple in Washington State

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Abstract: *Lambertella corni-maris* and *Phacidiopycnis washingtonensis*, the causal agents of yellow rot and speck rot, respectively, are two postharvest pathogens of apple reported recently in Washington State. This study was aimed to assess their prevalence and distribution in packinghouses and evaluate the efficacy of existing fungicides for their control. A statewide survey was conducted among 160 grower lots (50 fruit per lot) distributed throughout central Washington between February and June of 2016. Pure cultures of all pathogens were made on Petri plates containing potato dextrose agar (PDA). Purified cultures of all isolates were made on PDA, and pathogens were identified using key morphological and molecular traits. Yellow rot was found in 50 lots (31.3%) of the 160 surveyed, with frequencies ranging from 2 to 38% of total decay. Speck rot was found in 44 lots (27.5%), at frequencies ranging from 1.7 to 68%. Isolates of *L. corni-maris* and *P. washingtonensis* were tested for sensitivity to three postharvest fungicides including thiabendazole, pyrimethanil and fludioxonil. Effective concentrations necessary to inhibit 50% growth (EC₅₀ values) were determined *in vitro* and were lower than 1 µg/ml for fludioxonil and pyrimethanil for *P. washingtonensis* and for fludioxonil in *L. corni-maris*. Mean EC₅₀ values were the highest for thiabendazole for both pathogens, whereas the highest variability in sensitivity within the populations was seen for pyrimethanil.

Key words: Control, *Lambertella*, *Phacidiopycnis*, postharvest

Introduction

Phacidiopycnis rot has long been known to cause sporadic disease on pome fruit in Europe (Giraud *et al.*, 2001) but was found to be more preponderant in Washington State in 2005 (Xiao *et al.*, 2005; Kim & Xiao, 2006). The species was identified as *Phacidiopycnis washingtonensis* and has been known since to cause speck rot. The latter occurred in about 20% of the surveyed lots in 2005 in Central Washington and accounted for 3% of total decay (Kim & Xiao, 2006). Lately, it has been reported on persimmon in Italy (Garibaldi *et al.*, 2010), on pacific Madrone in the U.S. Pacific Northwest (Elliot *et al.*, 2014), and on apple in Germany, Denmark, and Chile (Weber, 2011; Diaz *et al.*, 2016). In the U.S. PNW region, the fungus has been reported to survive on crab apple on which it causes twig dieback (Xiao *et al.*, 2005). Inoculum released from infected crab apple trees infects commercial apples in the orchard through water splash and insects (Sikdar *et al.*, 2013; Xiao *et al.*, 2009). Infections remain latent until later in storage when typical symptoms of speck rot can be observed. The decayed tissue is firm to spongy with light brown lesions easily confused with gray mold or

Sphaeropsis rot. At later stages, infected areas turn black, and under high humidity abundant white pycnidia can be visible (Giraud *et al.*, 2001; Kim & Xiao, 2006).

Yellow rot, caused by *Lambertella corni-marisi* von Höhnelt (Wiseman *et al.*, 2015), is another emerging postharvest disease reported recently in Washington State. In 2005, yellow rot was found in 36% of packinghouses surveyed in central Washington at frequencies lower than 3% of total decay (Wiseman *et al.*, 2015). The fungus *L. corni-marisi* was previously reported on mummified apple fruit in Switzerland and pear fruit in Germany (Harrison and El Helaly, 1935). The fungus was found to cause disease on fruit crops such as pear, plum, quince, orange and lemon inoculated in lab conditions (Harrison & El Helaly, 1935). Interestingly, *L. corni-marisi* exhibited a strong antagonistic *in vitro* activity against mycelium of *B. cinerea* and several other fungi (Wood 1953). The fungus causes brown spongy lesions which may exhibit sporadic yellow-tan and thick mycelia.

Speck and yellow rots are quarantined in several countries, and still very little is known about their epidemiology and best management practices. Therefore, in an effort to improve knowledge about these two species, we initiated this study aimed to (i) evaluate the spread and preponderance of speck and yellow rots in Washington State and (ii) determine the *in vitro* sensitivity of *P. washingtonensis* and *L. corni-marisi* to three postharvest fungicides.

Material and methods

Survey of yellow and speck rots in central Washington

A total of 15 packinghouses and 160 grower lots were surveyed in major apple production areas in Washington State (Figure 1) from February to June of 2016. Fifty decayed fruit originating from each orchard were randomly collected on the packing line, laid on clamshells to avoid contacts between fruit, and transferred to the laboratory for further disease characterization. After a first visual symptom assessment, isolations were made by taking a 2 × 2 mm piece of flesh from the margin of decayed and healthy tissue inside the fruit and transferring it upside down to the center of a potato dextrose agar (PDA) plate acidified to pH 3.5 (APDA). Plates were incubated at 22 °C for 6 days, and a characterization of *L. corni-marisi* or *P. washingtonensis* isolates was conducted following standard lab procedures. Spray records of fungicides applied during the survey season were obtained from each packinghouse. Pure cultures of *L. corni-marisi* and *P. washingtonensis* were saved as mycelial plugs in 30% glycerol and stored at -80 °C.

Baseline sensitivity of L. corni-marisi or P. washingtonensis to three postharvest fungicides

Eighty isolates each of *L. corni-marisi* and *P. washingtonensis* were tested for their sensitivity *in vitro* to thianbendazole, pyrimethanil, and fludioxonil. Sensitivity was tested on potato dextrose agar for TBZ and FDL and on asparagine (ASA) agar for PYRI (Hilber and Schüepp, 1996). Besides the control plates (no fungicide), four different concentrations were tested for each fungicide (Table 1). Stock solutions of fungicides were made in sterile water and mixed with autoclaved molted media and poured into Petri plates. Fungicide tests were conducted using a mycelial growth inhibition assay. Plugs of growing colonies of each isolate were taken from the margin of each colony and transferred up-side-down onto plates amended with fungicides. Four replicate plates were used per isolate and fungicide concentration, and the test was conducted twice. Plates were incubated for 4 days at 21 °C and the radial growth recorded as the average value from the two runs and used to calculate percent inhibition relative to the control. For each isolate, percent inhibition was plotted against log fungicide concentrations to calculate the effective concentration necessary to inhibit 50% growth (EC₅₀).

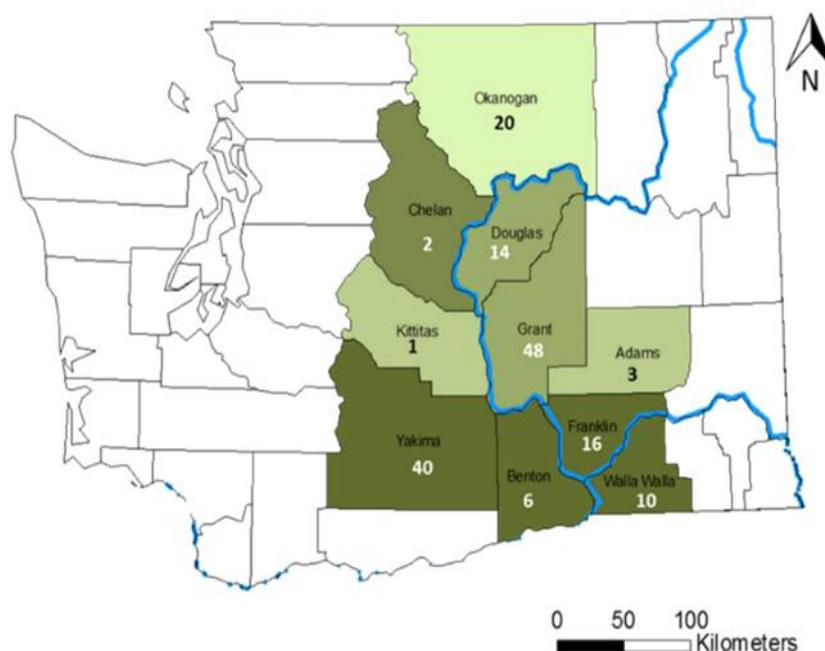


Figure 1. Geographic distribution of apple packinghouses surveyed in 2016 from central Washington. The number below each county name indicates the number of grower lots surveyed.

Table 1. Fungicides and concentrations used for *in vitro* tests of fungicide sensitivity in *Lambertella corni-marisi* and *Phacidiopycnis washingtonensis*

Active ingredient	Trade name	Medium	Concentrations ($\mu\text{g/ml}$)
Thiabendazole	Mertect	PDA	0, 0.01, 0.1, 1.0, 10.0
Pyrimethanil	Penbotec	ASA	0, 0.002, 0.02, 0.2, 2.0
Fludioxonil	Scholar	PDA	0, 0.001, 0.01, 0.1, 1.0

Results and discussion

Prevalence of yellow and speck rots in Washington apple packinghouses in 2016

Yellow rot (*L. corni-marisi*) was found in 31.3% of the lots surveyed, accounting for 5.7% of total decay. Frequency of yellow rot ranged from 0% to 38% among the lots in which the disease was detected (Figure 2). The highest incidence was found in Chelan County whereas the lowest incidence was in Yakima and Benton Counties (Figure 2). Speck rot (*P. washingtonensis*) was detected in 27.5% of lots surveyed with the highest frequency, showing an incidence between 1 and 25% (Figure 3). Countywise, the highest incidence was in Benton County (7%) versus 1% in Yakima and Douglas Counties (Figure 3).

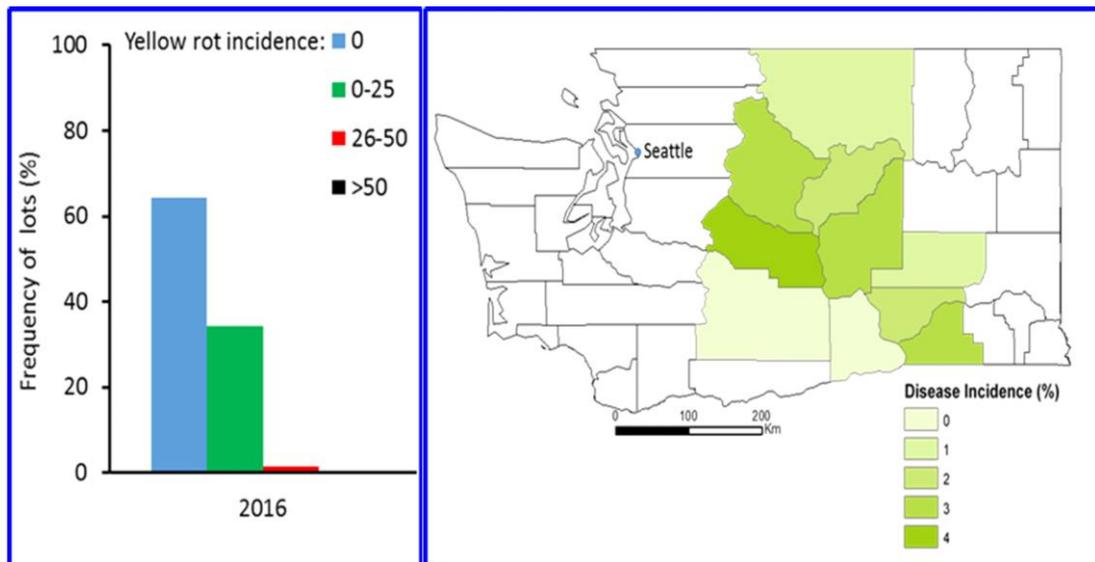


Figure 2. Frequency of grower lots showing yellow rot separated by incidence (left) and frequency distribution by county (right) in 2016.

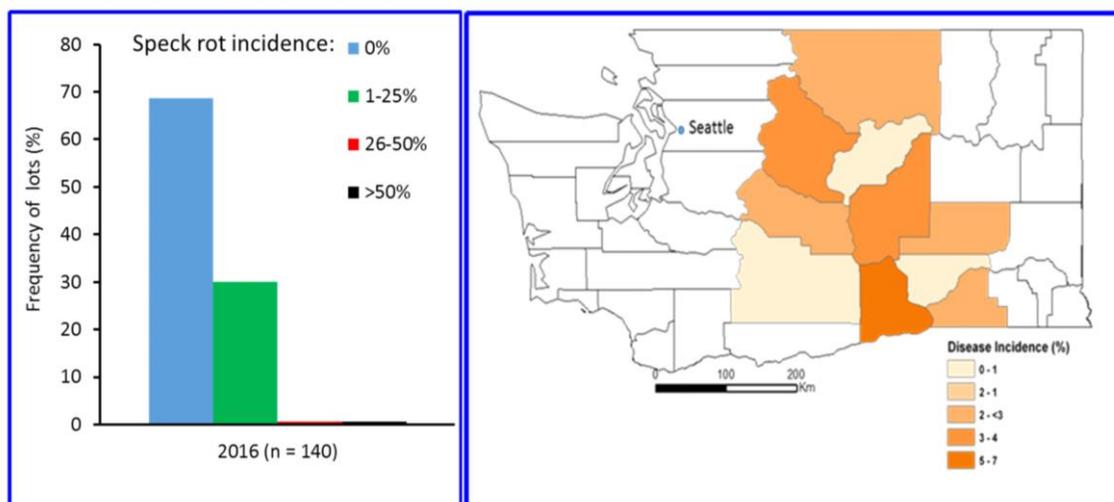


Figure 3. Frequency of grower lots showing speck rot separated by incidence (left) and frequency distribution by county (right) in 2016.

***In vitro* sensitivity of *L. corni-mar*is and *P. washingtonensis* to three postharvest fungicides**

Figure 4 (left) shows that for *L. corni-mar*is, the mean EC_{50} value was lower for fludioioxonil (0.23 $\mu\text{g/ml}$) followed by pyrimethanil (2.5 $\mu\text{g/ml}$) and thiabendazole (3.5 $\mu\text{g/ml}$). The variation factors were higher for pyrimethanil (140) followed by thiabendazole (18) and fludioioxonil (6). Figure 4 (right) indicates that for *P. washingtonensis*, the mean EC_{50} values for fludioioxonil, pyrimethanil and thiabendazole were 0.1, 0.3 and 0.81 $\mu\text{g/ml}$, respectively. Similarly, variation factors in *P. washingtonensis* were higher for pyrimethanil (1000) compared to 400 and 190 for thiabendazole and fludioioxonil, respectively.

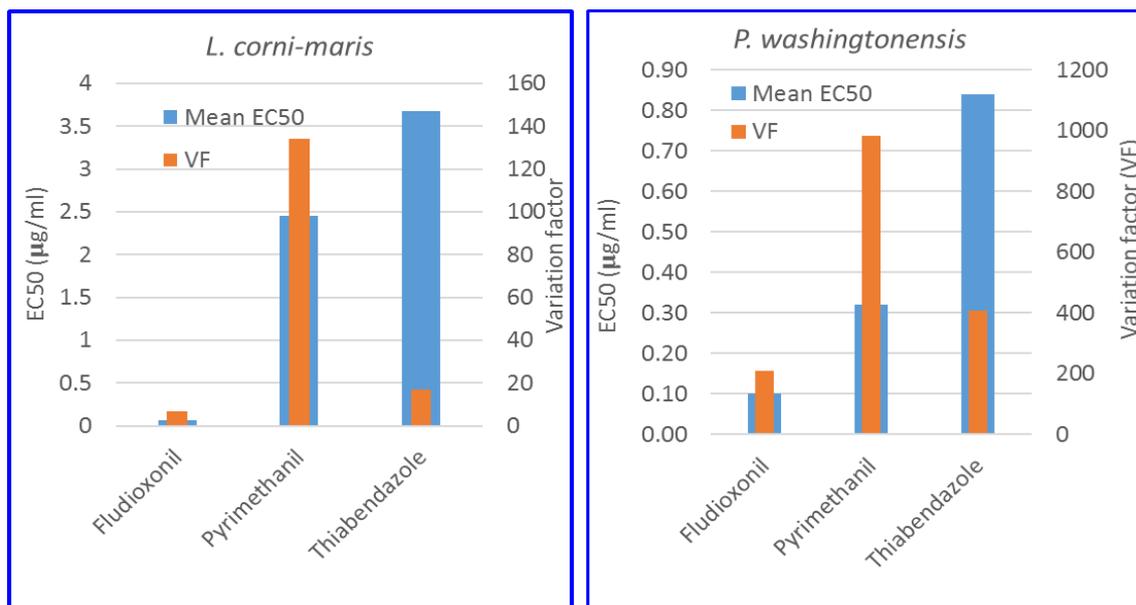


Figure 4. *In vitro* sensitivity of *L. corni-maridis* and *P. washingtonensis* to three postharvest fungicides. Sensitivity tests were done with 80 isolates of each fungus collected between 2005 and 2010. VF indicates a variation factor within the population for each fungicide.

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Mitigating the severity of shoot blight caused by *Erwinia amylovora*: Exploration of plant systemic resistance and low doses of prohexadione calcium

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Abstract: Fire blight, caused by *Erwinia amylovora*, is the most destructive bacterial disease to affect pome fruit production worldwide. Disease management strategies beyond the streptomycin bloom sprays need to be evaluated for mitigating shoot infection on dwarf trees during the season to prevent tree loss and spread of the disease. Two strategies to manage shoot blight are through application of materials containing plant defense activating compounds and using the plant growth regulator prohexadione calcium. In 2016 at the Penn State Fruit Research Center in Biglerville, PA, USA, we evaluated separately acibenzolar-s-methyl, 3.7% glycerol, and *Reynoutria sachalinensis* as foliar applications on greenhouse potted Gala/M.26 trees. When applied three weeks prior to shoot tip inoculation with *E. amylovora*, disease severity was reduced by at least 25% or more for each product compared to the control when evaluated two weeks after inoculation. During the same year, we evaluated field applications of prohexadione calcium at 2 oz/A and 4 oz/A on 5-year-old Crimson Crisp/M.9. Rates were applied at pink, petal fall, first and third covers; shoot tip inoculations occurred after the second treatment; data was collected early July. While both rates reduced shoot growth by 40-50%, fire blight severity was reduced 20-40% in the current year's growth and 15-30% in the previous year's growth. Both rates prevented fire blight from reaching the central leader. Results from 2017 greenhouse and field experiments will also be discussed.

Key words: fire blight, acibenzolar-s-methyl, glycerol, *Reynoutria sachalinensis*



Population dynamics of *Erwinia amylovora* on apple flower stigmas and effect of antibiotic treatment

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Abstract: Fire blight is caused by the bacterium *Erwinia amylovora*. Fire blight epidemics are typically initiated by blossom blight infections. Growth of *E. amylovora* to large population size on apple flower stigmas (10^6 to 10^7 cfu per flower) typically precedes extensive outbreaks of blossom blight. We inoculated stigmas of 1-, 3-, and 5-day old flowers of field grown 'Gala' and 'Fuji' trees with a marked strain of *E. amylovora* and monitored populations over a 5-day period. We observed rapid increases in population size, for example from 10^3 to 10^6 cells, within a single 24-hr period. As expected, older flowers did not support much *E. amylovora* growth. Apple growers in the U.S. have three antibiotic options to control fire blight: streptomycin, kasugamycin, and oxytetracycline. Streptomycin and kasugamycin are both bactericides while oxytetracycline is bacteriostatic. Since populations build over the course of bloom, the timing of when antibiotics are applied may aide in control of fire blight. We inoculated one day old flower stigmas with 1×10^3 cfu *E. amylovora*, and before or after inoculation, either streptomycin, kasugamycin, or tetracycline was applied to trees by air blast. Results indicated that streptomycin and kasugamycin suppressed bacterial populations when applied after inoculation. In contrast, oxytetracycline treatments initially suppressed populations, however, growth was recovered after 72 hrs.

Key words: *Erwinia amylovora*, fire blight, population dynamics, blossom blight, flower age, antibiotics

Introduction

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a devastating disease and a limiting factor to apple and pear production throughout most pome fruit growing regions of the world. The significance of fire blight includes both current season yield loss due to flower infections and systemic infections leading to rootstock blight and tree death. *E. amylovora* is capable of infecting flowers, fruits, vegetative shoots, woody tissues and rootstock crowns, leading to blossom, shoot, and rootstock blight symptoms. Young orchards of susceptible apple cultivars are particularly vulnerable to tree losses due to fire blight. Disease epidemics are stimulated by weather conditions that are conducive for infection which include warm and wet weather during bloom and extreme weather events such as hailstorms that can injure trees leaving a point of entry for infection by fire blight bacteria (i. e. trauma blight).

On apple, blossom blight disease symptoms (flower death) of fire blight are typically initiated in the spring on flowers as overwintering inoculum from cankers is disseminated to the surface of stigmas by rain and insects (Norelli *et al.*, 2003). The surface of the tips of stigmas particularly favors exponential growth of *E. amylovora* in the intercellular spaces

between papillae cells. Under conducive weather conditions, populations can double rapidly, and populations of 10^6 to 10^7 *E. amylovora* cells per flower are common (Taylor *et al.*, 2003). The availability of free moisture through rain or heavy dew is required to enable *E. amylovora* cells to migrate down the style where these cells infect flowers through natural openings present in the nectaries. This rapid buildup of *E. amylovora* populations on stigmas occurs over the course of bloom; however, the length of time required for populations to reach infective doses is less well known. The other factor to consider is the age of the flowers at time of initial contact of the *E. amylovora* cells. Apple flowers do not bloom uniformly, which means there are different aged flowers in a single cluster and throughout trees. This difference in age might be another impactful factor in the ability of *E. amylovora* population build up and consequent spread. If *E. amylovora* populations rise over the course of the bloom period, timing of when antibiotics are applied may aid in control of fire blight. A third factor to consider is the environmental conditions occurring during bloom, which impact the ability of the pathogen to divide. In this study, we examined if timing of antibiotic treatment, flower age, and weather conditions influence *E. amylovora* populations over the course of bloom.

Material and methods

Experiments were conducted in apple orchards located at the Michigan State University Plant Pathology Farm, East Lansing, MI, USA and at the Northwest Horticultural Research Center, Traverse City, MI, USA. Individual apple flowers were tagged the day prior to opening, and, depending on the experiment, flowers were inoculated after they were open for either 1, 3, or 5 days. The stigmas of the flowers were inoculated with $1 \times 10^{3-4}$ CFU *E. amylovora* 'Ea110' either in the morning or evening and sampled every 24 hours for 96-120 hours after inoculation. Several replicate experiments were started and run on different days to maximize the different weather conditions present. This experiment was repeated in 2016 and 2017 on various apple cultivars, including 'Golden Delicious', 'Gala', 'Jonathan', and 'Fuji.' In 2017, 20 additional flowers were inoculated at the same time as the flowers used to gather populations to rate disease incidence.

To explore the antibiotic application component, in 2016 and 2017 'Gala' apple trees were treated with either streptomycin, kasugamycin, oxytetracycline, or an untreated water control. Applications were made with an air blast sprayer. Stigmas of flowers open for one day were inoculated with $1 \times 10^{3-4}$ CFU *E. amylovora* 'Ea110' which was either preceded or followed by application of each registered antibiotic or a water check. Inoculation occurred either 2 hours (hrs) before (prior) or 2 hours after (post) the spray application. Populations were sampled every 24 hours for up to 96-120 hours after inoculation. Hourly weather data was collected using the MSU Enviroweather stations located within 1 km of the test orchards.

Results

Erwinia amylovora population dynamics on flowers open for 1, 3, or 5 days

In 2016, weather conditions at bloom were favorable for infection. The trend on 1 day open 'Jonathan' flowers showed that populations could reach carrying capacity in 72 hours, even if exposed to temperatures thought to be suboptimal for growth (Figure 1). The flowers open for 1 day supported 10 total generations of *E. amylovora* growth, with a generation time of 4.6 hours (Table 1). Population dynamics on three day open flowers followed a similar trend, however, these populations could grow 3 logs in 48 hours and maintained that population

throughout the sampling period (Figure 1). These 3 day open flowers had more generations faster than the 1 day open flowers; 12.6 generations and an average generation time of 3.8 hours (Table 1). The flowers inoculated after 5 days of being opened however, never reached the carrying capacity of 10^7 logs and crashed to the initial inoculated amount after four days (Figure 1). Table 1 also indicates that there were half as many generations of *E. amylovora* growth in the 5 day old flowers (6.6) and the generation time was much slower compared to growth on 1 or 3 day open flowers.

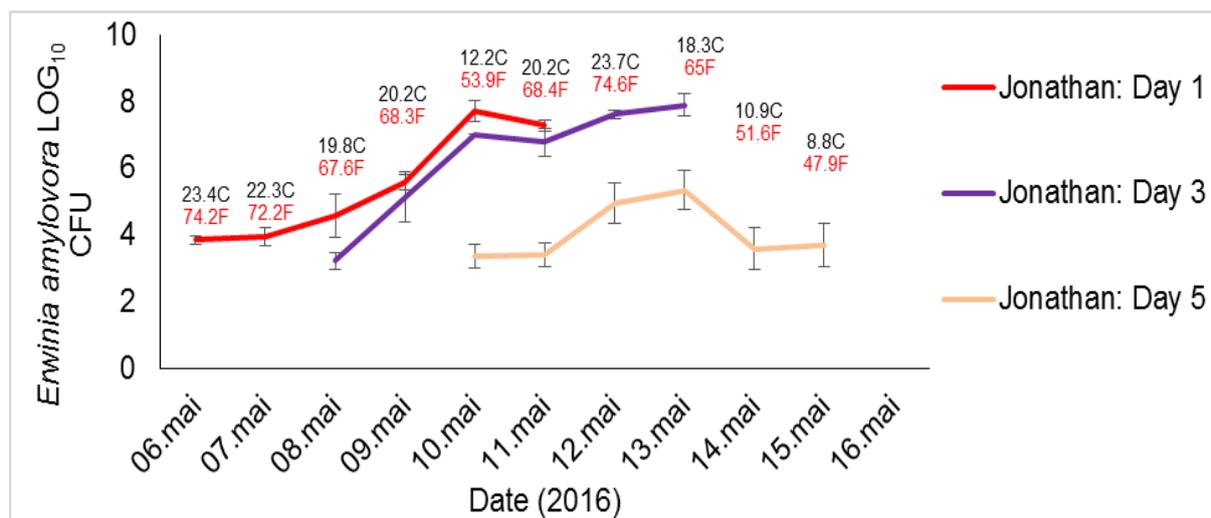


Figure 1. Graph depicting *Erwinia amylovora* LOG₁₀ CFU population trends over the course of apple bloom in 2016 on cv. 'Jonathan.' Flowers open for either 1, 3, or 5 days were inoculated at 10^{3-4} CFU on the first day of each trendline. Temperatures are the daily highs for the sample dates. Error bars represent standard error.

Table 1. The number of generations and average generation time for *Erwinia amylovora* Ea110 growing on 'Jonathan' flowers inoculated after 1, 3, or 5 days open in 2016. The number of generations are from $[n = (\log_{10}N_2 - \log_{10}N_1) / .301]$; generation time = $[time/n]$.

Flower	Number of generations	Generation time (hours)
2016 Jonathan one-day old	10.4	4.6
2016 Jonathan three-days old	12.6	3.8
2016 Jonathan five-days old	6.6	10.8

In 2017, temperatures were considered suboptimal for fire blight infection. These low temperatures kept *E. amylovora* populations low; it took the population approximately 120 hours to reach carrying capacity on 1 day open flowers (Figure 2). There were still 9.6 generations of growth with an average generation time of 12.5 hours for the 1 day open flowers (Table 2). In 2017 there were ample flowers inoculated to allow for disease rating. Though the populations could reach capacity, only 64% of flowers became blighted (Table 2). The carrying capacity was not reached on 3 or 5 day open flowers (Figure 2), as there were only 2.5 generations of growth on 3 day open flowers, and no growth was observed on 5 day

open flowers (Table 2). Even with low *E. amylovora* populations, 25% of the 3 day open flowers had symptoms of fire blight (Table 2).

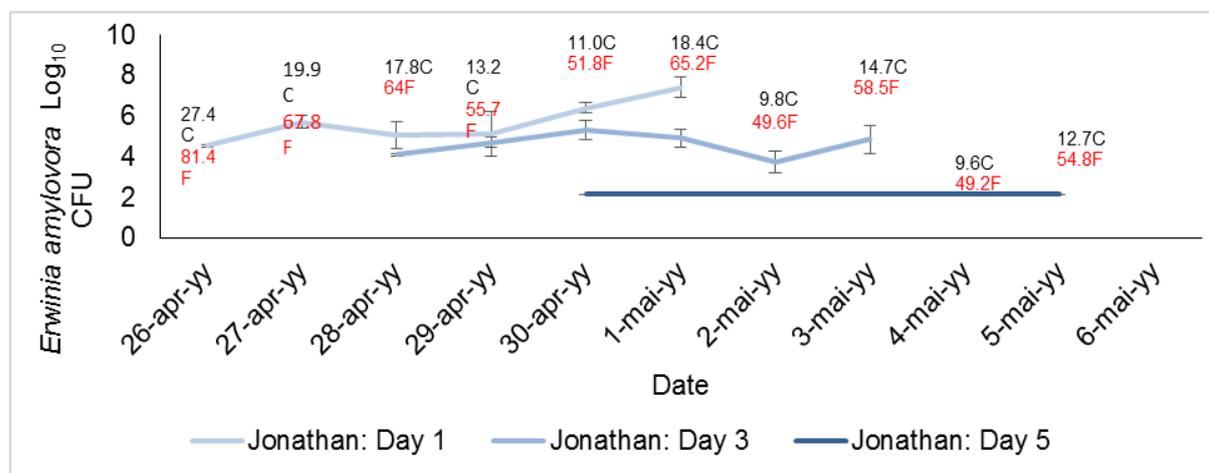


Figure 2. Graph depicting *Erwinia amylovora* LOG₁₀ CFU population trends over the course of apple bloom in 2017 on cv. 'Jonathan.' Flowers open for either 1, 3, or 5 days were inoculated at 10³⁻⁴ CFU on the first day of each trend line. Temperatures are the daily highs for the sample dates in both Celsius and Fahrenheit. Error bars represent standard error.

Table 2. The number of generations and average generation time for 'Jonathan' flowers inoculated after 1, 3, or 5 days open in 2017. The number of generations are from $[n = (\log_{10}N_2 - \log_{10}N_1) / 0.301]$; generation time = $[time/n]$. Disease incidence is the percentage of twenty inoculated flowers left to develop symptoms after the sampling period.

Flower	Number of Generations	Time of generation (hours)	Disease Incidence
2017 Jonathan one-day old	9.6	12.5	64.2%
2017 Jonathan three-days old	2.5	47.9	25.7%
2017 Jonathan five-days old	0	0	0%

Effect of antibiotic treatment on Erwinia amylovora population dynamics

In an experiment conducted in 2016, after a 48 hour lag, rapid growth of the *E. amylovora* on unsprayed flowers occurred, with the population reaching 10⁷ cells by the last day (Figure 3). Application of either kasugamycin or streptomycin resulted in populations remaining below 10³ for the entire experiment (Figure 3). Application of the bacteriostatic antibiotic oxytetracycline resulted in the population remaining below 10⁵ per flower until 96 hours post treatment (Figure 3).

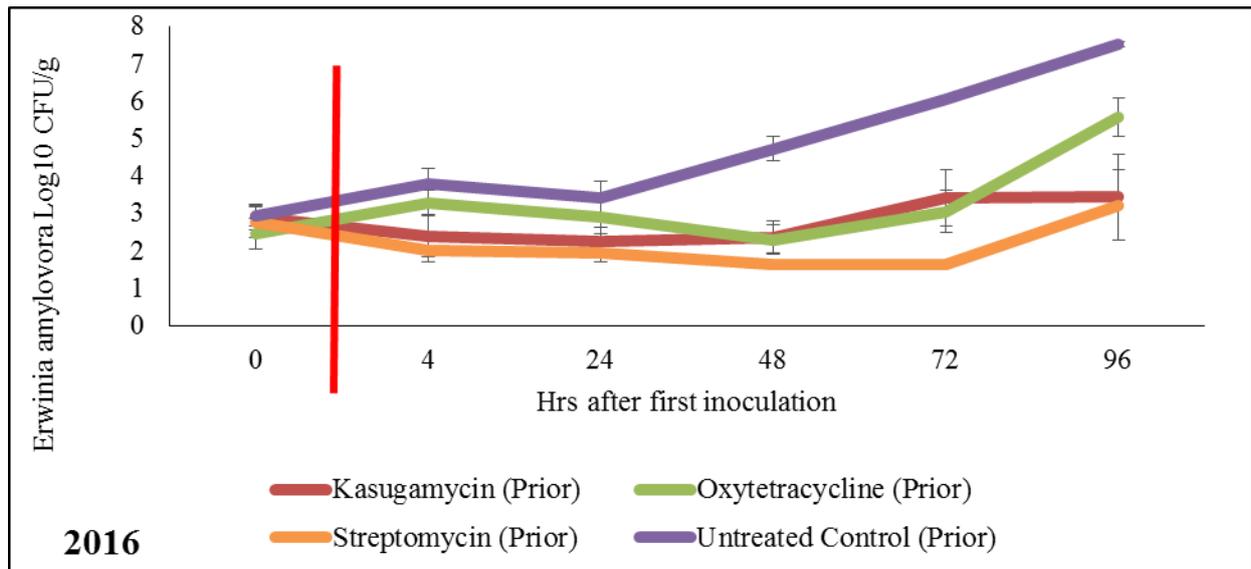


Figure 3. *Erwinia amylovora* population response inoculated prior to antibiotic application via air-blast on Gala flowers in 2016. The red line represents when the treatments were applied. Error bars represent standard error.

Discussion

Flower age is an important factor in the ability of *E. amylovora* to reach the carrying capacity of stigmas on apple as younger flowers enabled the bacteria to reach high population numbers quicker than on older flowers. We observed several log increases in growth of *E. amylovora* on flowers and rapid growth even on days when daytime maximum temperatures were as many as 8 °C less than the optimal growth temperature, reported to be 28 °C (van der Zwet *et al.*, 2012). Since we inoculated *E. amylovora* cells onto the flowers we sampled, our data suggest that if populations are established on stigmas, growth to 10^{6-7} cfu/flower can occur, even at lower than optimal temperatures.

Two antibiotics, kasugamycin and streptomycin, were both effective in holding *E. amylovora* populations at about 10^3 cfu/flower for up to 4 days after application. Both of these antibiotics are excellent bactericides for blossom blight control (McManus *et al.*, 2002; McGhee & Sundin, 2011), although streptomycin resistance in *E. amylovora* is problematic for disease control in several growing regions (McManus *et al.*, 2002; McGhee *et al.*, 2011). Oxytetracycline, which is bacteriostatic, was able to prevent the growth of *E. amylovora* cells for up to 3 days after application, following which the trajectory of population growth was similar to water-treated flowers.

In previous studies conducted under field conditions, others have shown that temperature, the presence of rain (which enables bacteria to migrate from stigma surfaces to the nectaries), and flower age are the determining factors in infection events (Thomson, 1986; Shwartz *et al.*, 2003; Taylor *et al.*, 2003). In addition, a minimum temperature of 18 °C was required for blossom blight epidemics (Thomson, 2000; van der Zwet *et al.*, 2012). Our data suggest that infection events can occur at even lower temperatures if cells are present on flowers. This study has helped us better understand the microbial ecology of *E. amylovora* on flowers, the potential for growth and infection on flowers differing in time open prior to colonization, and the effect of antibiotics on *E. amylovora* populations.

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Breeding for durable disease resistance in apple

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Abstract: In apple production there is a rising demand for high quality and regularly bearing apple varieties with durable disease resistance, allowing reduced pesticide input and therefore preventing residues on harvested fruits. Apple breeding has achieved considerable progress in developing disease resistant and tolerant varieties. However, relying only on single gene resistances such as *Rvi6* (*Vf*) against apple scab proved not to be a sustainable strategy as resistance breakdown is increasingly observed in orchards throughout Europe. Approaches are presented to develop commercially successful varieties with durable resistance against a range of important diseases.

Key words: apple breeding, fire blight, genetic resources, molecular markers, powdery mildew, scab

Introduction

Breeding can contribute to sustainable apple cultivation systems (Kellerhals, 2017). Disease resistance is an important breeding objective in many breeding programs worldwide. The range of diseases is about to increase as climate change, and international trade foster the emergence of new diseases. Classically, the focus in breeding was put on resistance to the fungal diseases scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). The next emerging important disease was fire blight (*Erwinia amylovora*). Currently, leaf blotch (*Marssonina coronaria*) as well as a range of storage diseases (e.g. *Neofabrea* spp., *Botrytis cinerea*) and canker (*Neonectria ditissima*), are becoming increasingly important. However, the share of disease resistant cultivars in practical fruit-growing is still very low (Switzerland 5%), even in organic fruit-growing. The majority of commercially grown scab resistant apple varieties still carries *Rvi6* resistance (Crosby *et al.*, 1992; Laurens, 1999, Kellerhals, 2017).

Disease resistance based on a single major gene can be overcome by new evolving strains of the pathogens. This will occur mainly due to mutation or by selection of the virulent strains, which are already present at low frequency in the population while increasing the surface planted with the resistant cultivar. Modern molecular selection tools allow for resistance pyramiding and therefore more durable resistance. Since the end of the 1980s there have been tremendous developments in the area of molecular methods and tools, and they are now more frequently implemented in classical breeding programs. Several EU projects (Table 1) as well as research activities mainly in USA (such as RosBREED, www.rosbreed.org) and New Zealand have considerable impact on this development.

More than 18 race-specific apple scab resistance genes have been identified and mostly mapped on different chromosome regions of the apple genome (Bus *et al.*, 2011, Jha *et al.*, 2009). Approaches followed by Agroscope in Wädenswil to pyramid several scab resistances and to combine them with powdery mildew resistance and fire blight resistance or tolerance are promising (Baumgartner *et al.*, 2015).

Table 1. EU projects to foster the development and application of modern molecular apple breeding

Project	Acronym	Coordinator	Project period
European Apple Genome Mapping Project	EAGMAP	Graham King (UK)	1994-1996
Durable Apple Resistance in Europe	DARE	Yves Lespinasse (F)	1998-2001
High Quality Disease Resistant Apples for a Sustainable agriculture	HIDRAS	Luca Gianfranceschi (IT)	2003-2007
Bridging the gap between genomics and fruit breeding	FRUITBREEDOMICS	François Laurens (F)	2011-2015

Baumgartner *et al.* (2015; 2016) described an efficient application and proof of concept of the pyramiding strategy using SNP molecular markers. The validation of the pyramids included the inoculation of progeny plants displaying different resistance gene setups with an apple scab strain overcoming the *Rvi6* resistance. The effect of the other resistance gene, *Rvi2*, if present, could be clearly observed. A complementary approach to pyramided scab resistances is exploiting the broad genetic basis in disease tolerance which is present in selected traditional local varieties (Kellerhals *et al.*, 2012). In order to introgress resistances originating from small-fruited wild apples, the ‘fast track’ breeding is a promising approach to speed up the generation cycle in apple breeding without using a genetic modification (Volz *et al.*, 2009; Baumgartner *et al.*, 2011; 2014). The ‘fast track’ breeding approaches establish optimal growing conditions to young apple seedlings in growth chambers or glasshouses, allowing them to switch as early as possible from the juvenile to the adult phase, where flower and fruit formation is possible.

Material and methods

Pyramiding scab resistances

Phenotypic screening. Phenotyping for scab resistance responses of the seedlings was performed under glasshouse conditions. The seedlings were inoculated at the four-leaf stage using a hand-sprayer containing a conidial suspension of *V. inaequalis* (4.3×10^5 conidia per ml). The inoculum originated from infected leaves of former seedling screenings in the glasshouse supplemented with conidia of leaves from susceptible orchard trees not treated with fungicides. Following inoculation, the temperature was kept at 18 to 21 °C and high humidity established using several humidifiers. During the first 48 h after inoculation, relative

humidity (RH) was kept close to 100% to prevent evaporation of the leaf droplets containing the *V. inaequalis* conidia. For the following 5 days, humidity was reduced to approx. 80% RH, subsequently raised again close to 100% RH for 2 days to promote fungal sporulation, and then again reduced to ~80% RH. The phenotypes were evaluated 14 days after inoculation (DAI) using the scale of Chevalier *et al.* (1991) (Table 2). Plants scored in classes 0 to 3b were considered resistant. Stellate chlorosis and necrosis symptoms, if present, were also recorded.

Table 2. Phenotypic scoring according to Chevalier *et al.* (1991)

Class	Phenotype
0	no visible symptoms
1	pinpoint pits
2	spots or necrotic lesions and no sporulation
3a	necrotic lesions, some with sparse sporulation
3b	restricted sporulation
4	unrestricted sporulation

Marker assisted selection. Leaf samples for molecular analysis were taken from the seedlings at the four leaf stage for DNA extraction prior to *V. inaequalis* inoculation. Marker screening was accomplished following the protocol of Frey *et al.* (2004) using multiplex PCRs with fluorescently labelled primers. Data analysis was performed with GeneMapper™ Software (Applied Biosystems). The following loci related to scab (*Rvi*), powdery mildew (*Pl*) and fire blight (FB) resistance with their corresponding marker alleles were used for selection of the desired combinations of resistances (Table 3).

Table 3. Marker set applied for marker assisted selection and scab resistance pyramiding

Resistance	Used marker – Amplicon (Type)	Reference
<i>Rvi2</i>	CH05E03 - 172 (SSR), OPL19 - 438 (SCAR)	Jansch <i>et al.</i> (2015)
<i>Rvi4</i>	CH02C02a - 182 (SSR), Hi22d06 - 132 (SSR)	Jansch <i>et al.</i> (2015)
<i>Rvi6</i>	CHVf1 - 164 (SSR)	Vinatzer <i>et al.</i> (2004)
<i>Pl1</i>	AT20 - 458 (SCAR)	Markussen <i>et al.</i> (1995)
<i>FB_F7</i>	AE10 - 380 (SCAR), GE-8019 - 403 (SCAR)	Khan <i>et al.</i> (2007)

Fire blight inoculation tests

Genotypes were grafted on rootstock M9 T337 (12 replicate trees per genotype). ‘Gala Galaxy’ was included as a susceptible reference, ‘Enterprise’ as a resistant reference. Grafted plants in tubes with substrate were allowed to develop for five to six weeks in a glasshouse (temperature: 16-24 °C, RH: approx. 65%). Afterwards, the plants were transferred to the quarantine glasshouse. Plants with a minimum shoot length of 10 cm were inoculated as described by Momol *et al.* (1998). *E. amylovora* (Swiss strains ACW 610 *Rif.*, specified concentration = 10⁹ cfu per ml) inoculum was injected into the shoot tip using a syringe of

0.6 × 25 mm (Gr. 16, 23 gauge) to pierce the shoot just above the youngest unfolded leaf. The length of the visually fire blight-free shoot part as well as the length of the necrotic lesion (cm) was measured 7, 14 and 21 days after inoculation (DAI). To estimate susceptibility, percent lesion length (lesion length divided by total shoot length) was calculated for each time point and compared to percent lesion length of ‘Gala Galaxy’ (set at 100%).

Fast Track Breeding

Introgression of *Fb_E* and *FB_MR5* at Agroscope apple breeding started in 2008 (Figure 1) as described by Kellerhals *et al.* (2017). Pseudo-backcross generations (pBC) were performed using high quality and productive apple varieties and selections as displayed in Figure 1.

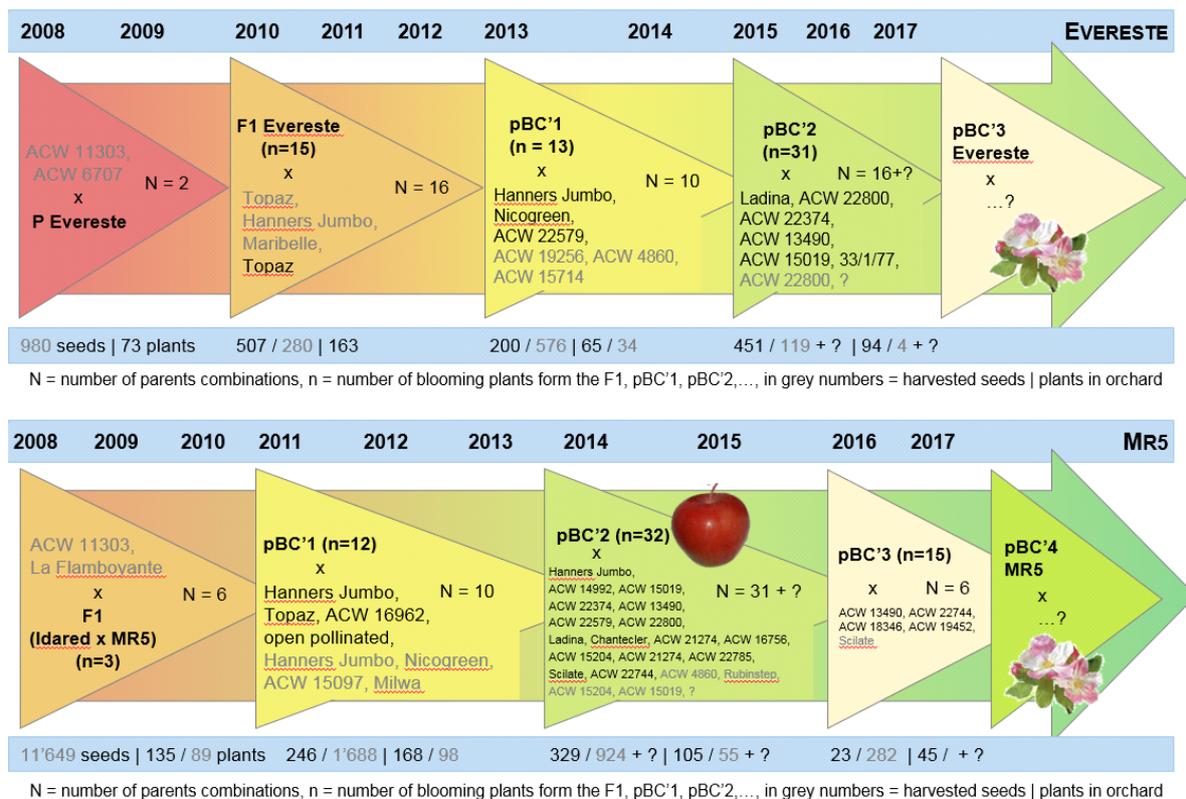


Figure 1. Fast Track breeding timelines with the fire blight resistances from ‘Evereste’ (*Fb_E*) and *Malus × robusta* 5 (*FB_MR5*) (updated from Kellerhals *et al.*, 2017).

Progeny plants were selected using markers linked to the *Fb_E* or *FB_MR5* fire blight resistance (Table 4) and other resistance gene markers such as *Rvi6* according to Kellerhals *et al.* (2017). Individuals carrying the desired resistances are useful parents for the next introgression cycles. Currently, 80 plants of the pBC'3 generation of *Fb_E* (‘Evereste’) and 49 plants of the pBC'4 generation of *FB_MR5* (*Malus × robusta* 5) are grown in the glasshouse. During the next two years, flowers of both resistance sources are expected in pBC'4 plants. To develop commercially suitable cultivars with durable fire blight resistance, both resistance loci will be pyramided in the following backcross generation. Cultivars additionally including scab and powdery mildew resistance are envisaged.

Table 4. Marker set used for marker assisted selection in fast track fire blight resistance breeding

Resistance	Used marker – Amplicon (Typ)	Reference
<i>Fb_E</i>	ChFbE06 - 260 (SSR)	Parravicini <i>et al.</i> (2011)
<i>FB_MR5</i>	FEM47 - 218 (SSR), FEM19 - 157 (SSR)	Fahrentrapp <i>et al.</i> (2013)

Results and discussion

Pyramiding scab resistances

Figure 2 shows scoring results from the artificial screening for phenotyping scab resistance responses (cross: ‘ACW 16102’ (*Rvi2*, *Rvi4*, *Rvi6*, *Pl1*) × ‘ACW 22750’ (*Rvi6*, *FB_F7*), $n = 384$) segregating for a range of scab resistance markers (*Rvi2*, *Rvi4*, *Rvi6*). The percentage of plants scored in classes 1 to 3 b according to Chevalier *et al.* (1991) and considered resistant and in class 4 showing unlimited sporulation and considered susceptible, were 85 and 15, respectively. According to the cross combination, 93% of the progeny plants were expected to be resistant. The genotypic segregation based on the molecular marker analysis is shown in Figure 3. The alignment of phenotypic and genotypic data indicates in general a good correspondence of phenotypic and genotypic results. A total of 34 seedlings (8.9%) showed an inconsistency between the resistance prediction by markers compared to the phenotypically observed symptoms. Thirtyone genotypes predicted by marker analysis to be resistant were phenotypically susceptible, i. e., *Rvi2* (2 seedlings), *Rvi4* (1 seedling), *Rvi6* (23 seedlings), *Rvi6Rvi6* (*Rvi6* homozygous, 3 seedlings), *Rvi2Rvi4* (1 seedling) and *Rvi2Rvi6* (1 seedling).

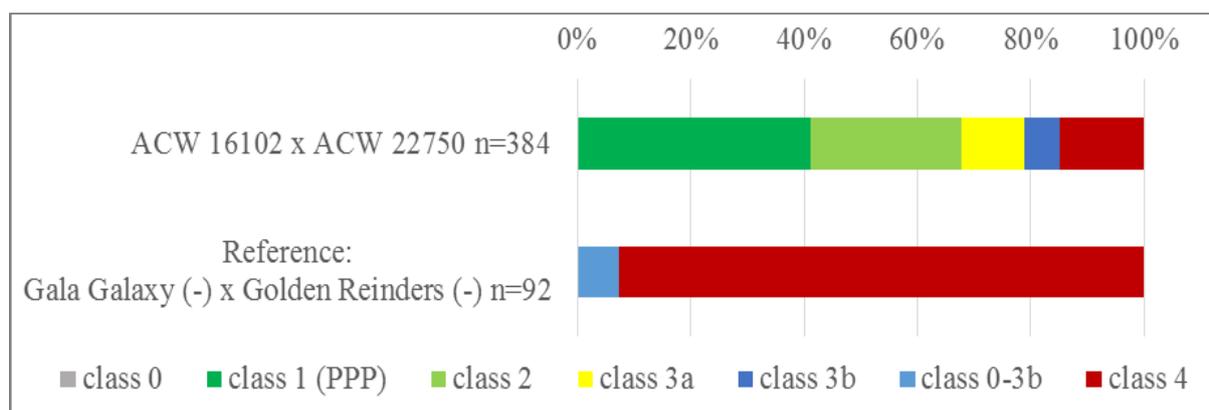


Figure 2. Segregation of progeny (cross: ‘ACW 16102’ (*Rvi2*, *Rvi4*, *Rvi6*, *Pl1*) × ‘ACW 22750’ (*Rvi6*, *FB_F7*), $n = 384$) for phenotypic scab resistance responses, classes according to Chevalier *et al.* (1991), compared to susceptible reference (cross: ‘Gala Galaxy’ × ‘Golden Reinders’, $n = 92$)

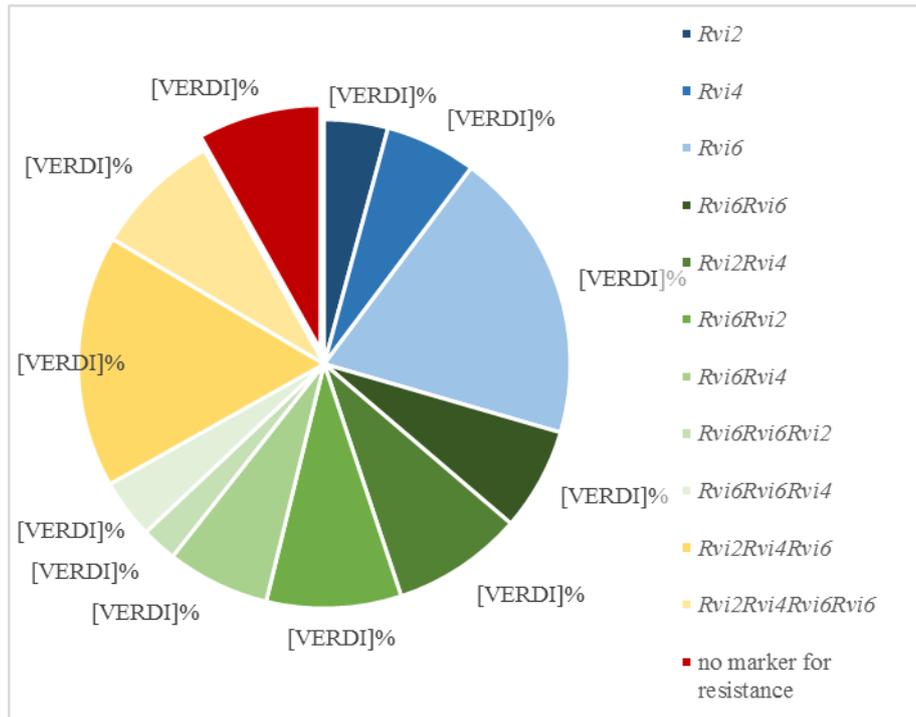


Figure 3. Segregation of progeny plants (cross: ‘ACW 16102’ (*Rvi2*, *Rvi4*, *Rvi6*, *PI1*) × ‘ACW 22750’ (*Rvi6*, *FB_F7*), $n = 384$) for scab resistance markers (*Rvi2*, *Rvi4*, *Rvi6*). Blue = single resistance hetero- or homozygous, green = double-pyramid resistance hetero- or homozygous, yellow = triple-pyramid resistance hetero- or homozygous.

Three seedlings predicted by markers to not carry *Rvi2*, *Rvi4* or *Rvi6* were found phenotypically resistant (Figure 4). Hypotheses explaining the genotype-phenotype incongruences have been presented by Gyga *et al.* (2004).

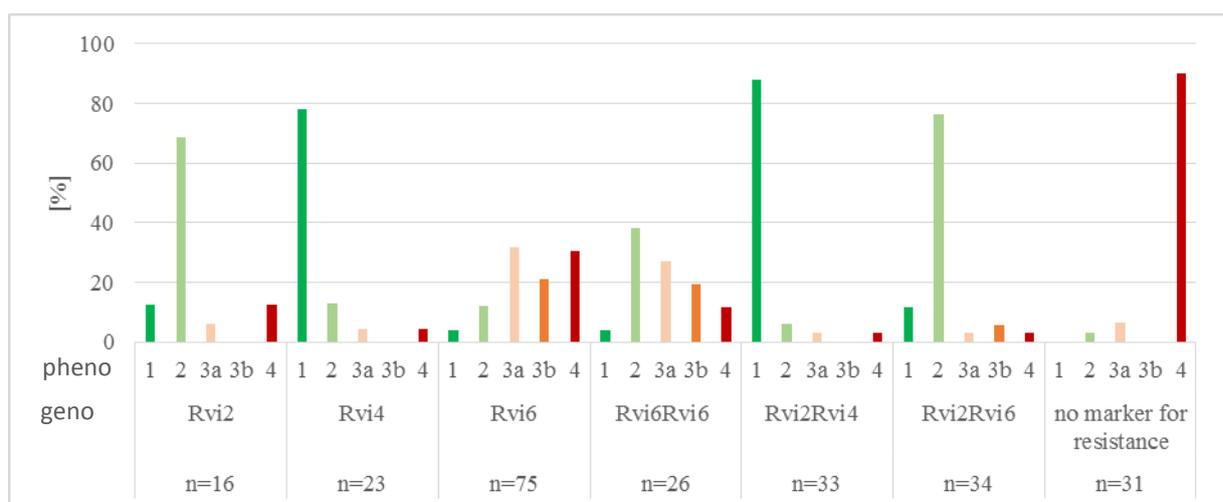


Figure 4. Alignment of phenotypic and genotypic data (cross: ‘ACW 16102’ (*Rvi2*, *Rvi4*, *Rvi6*, *PI1*) × ‘ACW 22750’ (*Rvi6*, *FB_F7*), every resistance group is set to 100%, pheno = phenotype, geno = genotype.

Fire blight inoculation tests and Fast Track breeding for fire blight resistance

The results of fire blight shoot inoculation of 38 traditional heirloom apple accessions performed in 2017 in the glasshouse revealed a large variation in the severity level among the accessions tested (Figure 5). However, compared to former tests (Gassmann *et al.*, 2014), in this set of genotypes there were no accessions more susceptible than the susceptible reference cultivar ‘Gala Galaxy’. For a conclusive susceptibility ranking at least two glasshouse tests and one artificial flower inoculation test should be considered. From the 38 apple accessions displayed in Figure 5, ‘Süsser Zila’ and ‘Brunnerapfel’ are considered in crosses of the breeding program so far.

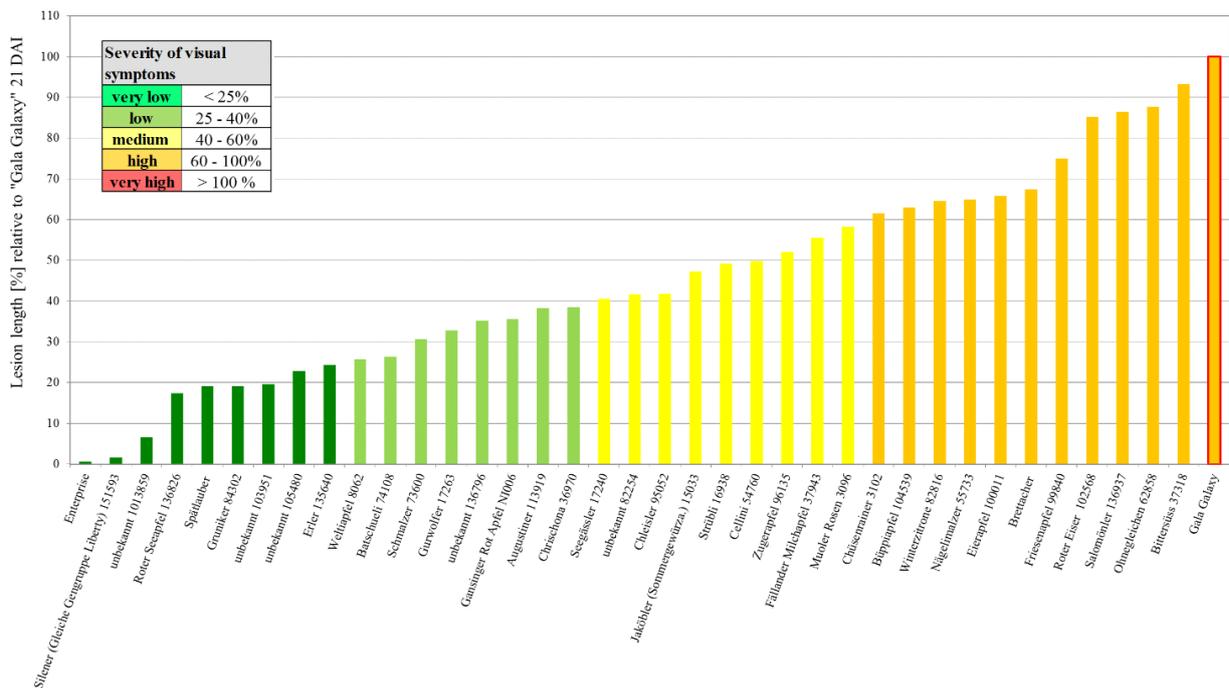


Figure 5. Severity of visual fire blight symptoms of 38 traditional Swiss apple accessions. The severity level is expressed as mean value of percent lesion length (lesion length 21 DAI divided by total shoot length) relative to ‘Gala Galaxy’ (set at 100%). ‘Enterprise’ and ‘Gala Galaxy’ are the tolerant and susceptible controls, respectively.

The phenotypic resistance level of selected pBC’2 and pBC’3 genotypes found by marker analyses to carry *FB_MR5* (6 genotypes) was tested (Figure 6). All progeny plants were less susceptible than ‘Gala Galaxy’. Five genotypes showed a severity level of visual fire blight symptoms classified “very low”, not statistically different from the resistant control ‘Enterprise’. For one genotype carrying *FB_MR5*, the severity of the visual symptoms was classified “medium”. This plant showed a statistically significant higher severity level compared to the tolerant control ‘Enterprise’. This genotype is most probably an outlier and needs further evaluation of molecular data and phenotyping.

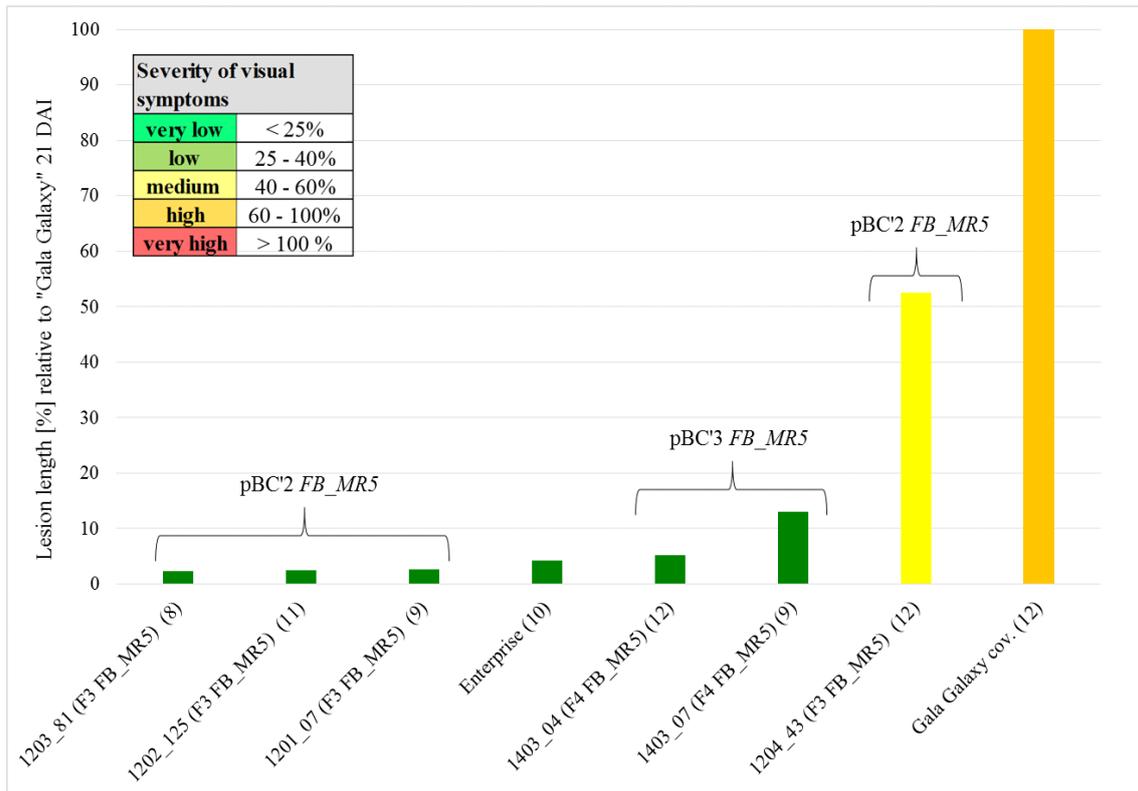


Figure 6. Severity of visual fire blight symptoms of four pBC'2 and two pBC'3 offsprings from *Malus* × *robusta* 5. The severity level is expressed as mean value of percent lesion length (lesion length 21 DAI divided by total shoot length) relative to 'Gala Galaxy' (set at 100%). 'Enterprise' and 'Gala Galaxy' are the tolerant and susceptible controls, respectively. Number in brackets equals number of replicates per genotype.

Apple varieties with durable disease resistance are useful for low input production systems. As resistances based on single genes are vulnerable to breakdown, alternatives have to be considered. A promising approach is pyramiding several resistances against the same disease in one genotype. For apple scab, advanced selections with up to three resistance genes (i.e., *Rvi2*, *Rvi4* and *Rvi6*) are available. Apple selections with a high level of fire blight resistance combined with commercial fruit quality are under development. Where sources for disease resistance are still small-fruited wild apples, 'fast track' breeding helps to successfully speed up the generation cycle and to introgress wild apple resistance in a high quality apple genetic background. High-speed breeding to introgress resistances originating from wild apples can also be achieved using a transgenic method called early flowering (Flachowski *et al.*, 2011; Le Roux *et al.*, 2012; van Nocker & Gardiner, 2014). Another possibility considering genetic modification is the development of cisgenic apples as reported by Kost *et al.*, 2015. According to Schouten, *et al.* (2006) a cisgenic plant does not contain any transgenes and has been genetically modified with one or more genes isolated from a crossable donor plant. Currently, in Europe cisgenic crops are still under GMO regulation.

Apple genetic resources are a valuable source for identifying a range of traits potentially useful in breeding. Accessions carrying polygenic resistances are suitable parents in breeding programs, provided they combine an acceptable level of other important features such as fruit quality, storability and regular productivity. The phenotypic screening of accessions to identify genotypes with a good level of resistance to fire blight, scab and powdery mildew

allows for incorporation of such genotypes in breeding approaches and potential use in fruit-growing. A range of apple varieties with low scab, mildew and fire blight susceptibility and good fruit quality was released or is in the pipeline. However, disease-resistant apples have to fulfill the high-quality expectation of the market and the consumers. Commercial impact of disease-resistant apple varieties is still low and efforts are needed to improve this situation.

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Evaluation and optimization of the use of plant resistance inducers in apple orchard (PEPS project)

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Abstract: In the frame of the PEPS project supported by the French Ministry of Agriculture, five plant resistance inducers (PRIs) were tested both in orchard and in controlled conditions. The first work package of the project was to screen in laboratory about thirty commercialized products in order to select the five most efficient apple defence activators. The products are then tested throughout the PEPS network of experimental orchards for their ability to protect against apple scab and storage diseases. In parallel to orchard trials, several studies are performed in controlled conditions in order to identify factors that can influence PRI efficiency and to study additional properties of the products. This paper is a mid-term review of the project.

Key words: apple scab, elicitors, storage diseases, *Venturia inaequalis*

Introduction

Plant resistance inducers (PRIs), or elicitors, could be useful to reduce the use of pesticides, particularly in the frame of the French Ecophyto Plan, which plans to reduce the use of pesticides by 50% in 2025. This type of product is now completely understood to be unable to replace chemicals, but could be helpful when integrated in a low input phytosanitary schedule. However, their integration into pest management programs, if existing, remains too rare due to the lack of efficiency and/or reproducibility of the products in protecting crops under production conditions. The mechanisms of elicitation are complex, and could be influenced by several factors, abiotic or biotic (Walters *et al.*, 2013). The project PEPS supported by the French Ministry of Agriculture (Casdar 2014-2018), aims to understand how to integrate these new agricultural inputs into apple orchard pest management, focused on apple scab and storage diseases. The project gathers 10 French partners acting in the fruit sector: the National Institute for Agricultural Research (INRA), the Technical Institute for Fruits & Vegetables (Ctifl), the French Institute for Cider Productions (IFPC), 6 Fruit Experimental Stations belonging to 5 structures (La Morinière, Invenio/2 sites (Limousin and

Ste. Livrade), Cefel, Cehm, La Pugère), the Research Group on Organic Farming (GRAB), and the Horticultural School of Niort.

The project is organized into 5 work packages:

- WP1 concerns the screening of commercial products through the qPFD patented tool (Brisset & Dugé de Bernonville, 2011; Brisset, 2013; Marolleau *et al.*, 2013) to select the best ones as elicitors to be tested in orchards and in controlled conditions.
- In WP2, the majority of the technical partners test the selected PRIs in orchards against apple scab (8 locations) and against storage diseases (4 locations).
- WP3 is developed by 4 partners, segmented in several subtasks aimed to study some conditions influencing the efficiency of PRIs in controlled conditions (greenhouses), such as apple varieties, abiotic stresses, and other inputs.
- WP4 concerns transfer of knowledge, dissemination of information and results of the trials to the growers, fruit technicians, researchers, etc., and WP5 is devoted to the management of the project.

Material and methods

Screening of commercial elicitors (WP1)

The “qPFD” tool (qRT-PCR monitoring the expression of 28 genes involved in plant defence mechanisms in apple) was applied on a set of 28 commercial products, marketed in France as “biostimulant”, “plant strengtheners” and “stimulators of natural defence”. Trials were carried out by a service provider working for INRA (licensor of the patent), by applying each compound on apple seedlings cultivated in greenhouse, sampling leaf discs and performing molecular analysis. Following this analysis, 5 PRIs showing the best activity in the elicitation of defence genes were selected to be used in further trials: ASM (analog of salicylic acid, coded A), a potassium phosphite (coded B), a foliar fertilizer containing a phosphite (coded C), potassium bicarbonate (coded D) and another foliar fertilizer based on a mix of organic and mineral ingredients (coded E).

Trials in orchard against apple scab (WP2)

Trials are performed in the 8 locations of the network, with at least 2 PRIs within the 5 ones previously selected. All partners use the same protocol, but not always the same variety: in the network, there are cvs. Gala, Golden Delicious, Pink Lady[®], Braeburn for eating apple, and cv. Fréquin Rouge for cider apple.

The modalities to be compared are:

- Reference IPM (REF): Classical treatments against scab during the primary season (time of season for ascospore release). Mainly based on preventive fungicide applications before rain. Additional applications are recommended after infection (curative spray) when the peak of ascospore ejection shows a RIM value above 300 (when using the RIMpro model), or if the preventive product had been washed off, or when unprotected leaves had merged. Scab sprays are stopped at the end of the primary season except if scabbed leaves are present in the orchard.
- A modality called “low input” (LI): The same treatments and strategy as REF, but with risk-taking decided in the project committee: The first year, in 2015, we decided to not apply a curative chemical after a peak of ascospores when the RIM value was

below 800. The following years, we decided to “forget” a spray against a scab contamination in April or May, at least two weeks after the stage “tight cluster”.

- Several modalities consisting in the LI modality completed by each one of the selected PRIs sprayed in an average of a 7-day frequency from the stage “tight cluster” until the end of the primary season. The used rate is calculated on the basis of rate/ha registered or recommended by the supplier, in a volume of 400-500 l/ha.

The design is a Fisher’s block design with 3 to 4 replicates. Untreated control, to evaluate the local scab pressure, is not included in the design.

The level of infection is noted for leaves and fruit in June after the end of the primary season, and only on fruit at harvest. Fifty shoots and 100 fruits per elementary plot are observed, and the number of shoots representing at least one scabbed leaf and scabbed fruit are counted.

Trials in controlled conditions (WP3)

Experiments are conducted with the five selected PRIs by four partners and aim at understanding how to influence induced resistance under field conditions:

- is there a genotype effect: 11 commercial cultivars are compared either in protection assays against apple scab or by means of defence analysis after treatment.
- does environment modulate plant response to PRIs: High stress temperature during the day and/or low stress temperature during the night, applied before and/or after PRI treatment are compared to standard assay conditions.
- do other orchard inputs interfere (positively or negatively) with PRI: PRIs are applied in mixture with 15 inputs (fungicides, insecticides, thinning agents, fertilizers) and compared to PRIs applied alone.
- what is the persistence of action of PRIs: The delay between PRI treatments and inoculation or tissues sampling for defence analysis is increased from 3 days to 14 days.

The work package also aims at quantifying the major apple allergens in fruits collected by the various partners involved in WP2 on trees treated by PRIs either in spring or before harvest. These allergens are PR proteins whose synthesis is supposed to be induced by PRI application.

Finally, the five PRIs are tested for their effectiveness against three other major pests and pathogens of apple: *Erwinia amylovora* (fire blight), *Dysaphis plantaginea* (rosy apple aphid) and *Podosphaera leucotricha* (apple powdery mildew).

Results and discussion

In 2015, the overall level of scab was too low in all the treated plots, meaning that the risk-taking was insufficient to get significant differences between the modalities; in many places, all of the ascospore peaks were credited by a RIM value above 800 so that the rules determining the decision could not be correctly applied.

In 2016, the gap in fungicide schedule in the majority of sites gave more scab on shoots than in the reference plots, allowing significant differences according to the PRIs. On fruit, the overall incidence was relatively low, and the percentage of scabbed fruit was not significantly different.

According to the experimental sites, the total number of PRI applications, integrated in the fungicidal program, varied from 7 to 12 per year. The best efficacy was obtained with phosphites (PRI B and C), giving sometimes better results on shoots than the chemical reference, and on fruits a lower, but not significant tendency. PRI A provided good results mostly everywhere, except in Pink Lady[®]. Potassium bicarbonate did not result in sufficient effectiveness when applied each week: this compound is better known to have a post-infection effect (application is usually recommended in a slot of 200-300 degree-hours after the beginning of scab infection) (Philion & Joubert, 2015). In these trials it was positioned in fact before infection (as inducer), and did not show efficiency in these conditions. The foliar fertilizer coded PRI E provided good results in one site, and no effect in another site; its composition is based on several plant extracts of which some could promote defence mechanisms.

Trials in orchard against storage diseases (WP2)

Only four experimental sites are involved in this action, with cultivar Pink Lady[®], Goldrush, and Opal. Pre-harvest sprayings are mainly targeted on *Neofabraea vagabunda*, the major causal agent of Bull eye's rot (usually called "*Gloeosporium*" rot) in France. At least three items are compared:

- Untreated control (only in pre-harvest).
- Chemical reference: Spraying of conventional chemicals applied preferably before a rain (Giraud & Coureau, 2014), from 5-6 weeks before harvest (captan or dithianon), another one at 2-4 weeks before harvest (boscalid + pyrachlostrobine) according to rain events, and one 3-7 days before harvest (fludioxonil).
- PRIs sprayed alone, with a 7-day frequency, at the same rate as in the apple scab trials.

At harvest, 100 fruits per elementary plot are picked and stored 4 to 6 months in regular atmosphere cold rooms. The number of fruits infected by *N. vagabunda* is counted after opening the cold rooms and if necessary after a 15 days shelf-life.

No significant effect of PRIs A, C, D and E has been noted up to now. Only phosphite B is sometimes efficient, but not always. In the future, presence of phosphite residues should be analyzed.

Trials in controlled conditions (WP3)

Experiments are underway and results should help to give recommendations for an optimal use of PRIs in the field.

Conclusions

The presented work is a mid-term report of the project. The first conclusion is that it is possible to reduce the number of fungicide treatments against scab and replace a part of them by plant resistance inducers. Phosphites seem to be the most promising products. However, phosphites are known to be phytotoxic, so that the total number of treatments could not exceed 6-8 per year. Phosphites may produce residues in pre-harvest applications. The phosphite B was recently registered in the Biocontrol list in France, allowing it in reducing the index of frequency of treatments requested in Ecophyto Plan 2025.

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Polyploidy influences *Malus × domestica* – *Venturia inaequalis* interactions

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Abstract: In this study, performed at KU Leuven in 2016-2017, we aimed at unravelling the effect of polyploidy and defense priming with fosetyl-aluminium in apple (*Malus × domestica* Borkh.) on resistance to apple scab (*Venturia inaequalis*). At first, macroscopic disease symptoms were analyzed in the apple scab susceptible (primed and unprimed) diploid and tetraploid genotypes. Next, the evolution of *V. inaequalis* DNA at the molecular level was quantified via qPCR analysis. Finally, we performed RNA-sequencing to analyze differences in gene expression upon the various treatments. Macroscopic scab symptoms in the leaves indicated different degrees of susceptibility with the highest degree of symptoms in unprimed diploid plants, and the lowest in primed tetraploid plants. Over all the treatments, we could conclude that polyploidy in ‘Gala’ reduced sporulation by 38.3% in comparison to the diploid genotype. Results were confirmed with qPCR. The amount of *V. inaequalis* DNA was decreased by 63.5% in unprimed and 61.5% in primed tetraploid plants in comparison to diploids. Moreover, significant differences between diploids and tetraploids were identified in all transcriptomic comparisons. We can conclude that polyploidy substantially improves the resistance of apple plants to *V. inaequalis*. The effect can even be increased by priming the defense response. The observed beneficial phenomenon is supported by differences in gene expression profiles.

Key words: apple scab, non-model crop, biotic stress, transcriptomics



New threats of postharvest diseases in pome fruit

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Abstract: Postharvest diseases caused by fungi can give huge economical losses for growers. The climate is changing and new conditions lead to new opportunities for different plant pathogenic fungi. Fungal diseases normally more common in southern countries are now occurring more in the north. Furthermore, retail is requesting fruit with lower residues than the maximum residue limits set by the authorities and with as few active substances as possible. This causes changes in pre-harvest treatments and can contribute to a shift in the population of fungi present in orchards. Every year different fruit samples are delivered to pcfruit npo (Belgium) for identification of fruit rot pathogens. Over the past seasons several (new) fungal diseases have been detected. Infections of pears with *Phialophora malorum* caused great losses in 2015 and 2016. In those years *Tilletiopsis* spp., a pathogen that was rarely observed, gave problems on apple. Last years, again more pear orchards were infected with *Stemphylium vesicarium*, causing problems in the orchard as well as after storage. To get an idea concerning the different diseases and their contribution to the total amount of postharvest fruit rot on apple and pear in Belgium, a survey was done in different auctions and grading companies from December 2015 till April 2016. The survey indicated that on apple *Botrytis cinerea* remained the most important pathogen, with 41% of the decayed fruit infested with the fungus. *Neofabraea* spp. was the second most found pathogen on apple during storage, but also *Monilinia* spp., *Fusarium* spp. and *Penicillium* spp. continued to be important pathogens on apple. On pear, *Botrytis cinerea* accounted for more than 66% of the infested fruit, indicating that it is still the main pathogen on this fruit. However, also *Monilinia* spp., *Fusarium* spp. and *Alternaria* spp. were found on diseased pear fruit, and the incidence of *Stemphylium vesicarium* was higher compared to the preceding years.

Furthermore, out of diseased pear fruits from several orchards during summer 2016, one specific pathogen was isolated by cutting a block of 0.5 × 0.5 mm out of the pear fruit at the margin of diseased-healthy tissue and placing it on PDA medium. The pathogen was identified as *Botryosphaeria dothidea*. Until now in Europe, this pathogen was only found on pears in Italy. As no research was yet performed to define the efficacy of different fungicides against this pathogen on pear, *in vitro* fungicide trials on agar in Petri dishes were set up in Belgium in 2016-2017. These tests indicated that the products Switch (cyprodinil + fludioxonil), Geoxe (fludioxonil) and Bellis (pyraclostrobin + fludioxonil) obtained the best efficacy for inhibition of mycelial growth of this pathogen. For Luna Experience (fluopyram + tebuconazole) and Penbotec (pyrimethanil) a moderate to good efficacy was observed in this *in vitro* trial. For Merpan (captan) and Hermosan (thiram) only a moderate efficacy was observed. To get a clear indication of the efficacy of the fungicides in the orchards, additional *in vivo* tests and field trials were performed in pear. This test confirmed the results from the *in vitro* trials, and after treatments with Switch, Geoxe and Bellis hardly any symptoms appeared on the pear fruit after artificial inoculation with *B. dothidea*. After treatments with Luna Experience and Penbotec some infections occurred, but the growth of the lesions was reduced

compared to treatments with Merpan or Hermosan. However, larger lesions appeared as compared to treatments with Switch or Geoxe.

These trials indicate that all tested fungicides have an efficacy against *B. dothidea*, but that Bellis, Switch and Geoxe obtained the best efficacy. The efficacy of trifloxystrobin towards *B. dothidea* was earlier demonstrated by Brown-Rytlewski and McManus (2000), however, the trials were performed only on cankers in apple trees and not on fruits. Although the economic importance of this pathogen is still limited, further research is needed to unravel important times of infection and, in concordance, the best times for treatments. These results then need to be interpreted to adjust the actual treatment schedules against fruit rot pathogens to obtain the most optimal schedule for the fruit grower based on the 'fruit rot history' of his orchard.

Key words: apple, *Botryosphaeria*, fungal disease, pear

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Emerging and threatening postharvest diseases in pome fruit in the Netherlands

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Abstract: Pome fruit may remain for up to 12 months in storage, during which time fruit rot diseases may develop. Despite the use of fungicides and improved storage technologies, postharvest diseases still remain an important limiting factor for the long-term storage of apples and pears. Postharvest diseases of pome fruit are caused by a range of fungal pathogens. Typically, symptoms of disease occur after several months in cold storage with controlled atmosphere. In this study, packinghouse surveys of postharvest diseases on stored apple and pear fruit were conducted from 2012 to 2016. Decayed apple and pear fruits were sampled from commercial packinghouses, representing orchards of various apple and pear producing areas and cultivars in the Netherlands. Approximately 300 samples were analyzed during the storage seasons from 2012 to 2016. A sample consisted of 10-15 representative decaying fruits from each grower lot. The survey revealed that the most important postharvest pathogens were *Cadophora luteo-olivacea* causing side rot on pears, and *Fibulorhizoctonia psychrophila* as the causal agent of lenticel spot on apple and pear. Also, new problems were noticed caused by pathogens not earlier described in the Netherlands on apple or pear, such as *F. avenaceum* on pear and apple, *Neonectria candida* and *Neofabraea kienholzii* on pear, and *Colletotrichum godetiae* and *Truncatella angustata* on apple.

Key words: fruit rot, fungal pathogens, storage

Introduction

Apple (*Malus domestica*) and pear (*Pyrus communis*) are important fruit crops in the Netherlands. The main apple and pear cultivars are 'Elstar' and 'Conference', comprising 40 and 75% of the respective production areas. Fruits are stored under specific controlled atmosphere (CA) conditions for up to 11 months, depending on the cultivar and volume to be marketed. As fruits may be stored for an extended period, postharvest diseases caused by various fungal pathogens can be a limiting factor to long-term storage. The aim of the study was to determine the main causal agents of postharvest diseases of apple and pear fruits in the Netherlands.

Material and methods

In this study, packinghouse surveys of postharvest diseases on stored apple and pear fruits were conducted from 2012 to 2016. Decayed fruit were sampled from commercial packinghouses, representing orchards of various apple and pear producing areas and cultivars

in the Netherlands. Approximately 10-15 representative decaying fruits from each grower lot were analysed. In total, approximately 300 samples were analyzed during the storage seasons from 2012 to 2016.

Results and discussion

In this survey, postharvest rots caused by *Botrytis cinerea* and *Penicillium* spp. was generally not regarded as problematic, because incidences were generally very low (1-5%). This type of decay is in general easily controlled by fungicide applications prior to harvest. Also, avoidance of wounds during harvest (special instructions have been made for pickers) reduces problems with *Botrytis* and *Penicillium* during storage. Other pathogens, such as *Neofabraea* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp., were isolated at low frequencies and were considered of minor importance. Also, new diseases were noticed, and the different pathogens and symptoms are described below.

***Cadophora luteo-olivacea* causing fish eye or side rot on ‘Conference’ pears**

Symptoms of side rot disease of pear fruit were mainly found on ‘Conference’, showing typical round to oval, dark-brown and slightly sunken spots (size 0.5 to 1.0 cm in diameter) appearing after six or more months of cold storage in controlled atmosphere. The symptoms were present in most years, however, disease incidences varied strongly between years. Disease incidences were sometimes up to 90%. Remarkably, very firm and green pear fruit were heavily affected.

***Fibulorhizoctonia psychrophila* causing lenticel spot on ‘Elstar’ apples and ‘Conference’ pears**

Disease incidences ranged from very low to >75%. The symptoms started as small brown to black spots (1-5 mm²) that originated from lenticels. The spots enlarged in a circular fashion and became sunken as the disorder progressed. The centre of the lesion was depressed, often with cracks and mycelium in the centre. One fruit could have several to many lesions. In prolonged cold storage, the disease developed further, and gradually decay of the whole fruit occurred.

***Fusarium avenaceum* causing postharvest decay of ‘Conference’ pears and wet core rot on ‘Elstar’ apples**

In general, low incidences of 1% to 5% were recorded on pears of ‘Conference’. Lesions showed brown and watery circular necrosis, were slightly sunken, often with visible whitish, yellowish or pink mycelia covering the lesions. Symptoms of apple wet core rot were observed on cv. Elstar after 4 to 6 months’ in controlled atmosphere storage. Apples exhibited light brown wet rots, initially developing in the core and subsequently spreading into the surrounding cortex, often with a white to rose-reddish mycelium.

***Neonectria candida* causing postharvest rot on ‘Conference’ pears**

Occasionally, storage rots were observed when storage crates were contaminated with orchard soil. The lesions showed brown and watery circular necrosis, were slightly sunken, and displayed whitish to yellowish mycelia covering the lesions.

***Neofabraea kienholzii* causing bull's eye rot on 'Conference' pears**

Symptoms of bull's eye rot caused by *N. kienholzii* were observed in 2015 on 'Conference' pears in storage in the Netherlands for the first time. Bull's eye lesions on apple and pear fruits are generally caused by four *Neofabraea* species: *N. alba*, *N. malicorticis*, *N. perennans* and *N. kienholzii*. Independent of the species, the symptoms appear as flat or slightly sunken circular lesions, which are brown, often lighter brown in the centre.

***Colletotrichum godetiae* causing bitter rot on 'Golden Delicious' apples**

This is the first report of bitter rot caused by *C. godetiae* on apple fruit in the Netherlands. Currently, bitter rot is not an important disease in apples in the Netherlands. However, it is spread worldwide and considered one of the most important diseases causing considerable crop losses, and may become an emerging problem in the Netherlands in the near future.

***Truncatella angustata* causing postharvest rot on 'Topaz' apples**

In January 2016, light peel damage caused by hot water treatment was observed on 'Topaz' apples from an organic orchard. Also, up to 15% of the 'Topaz' apples showed typical rot lesions of an unknown causal agent. The lesions showed brown, irregular necrosis and were slightly sunken.

In conclusion, the most important pathogens in terms of incidence and severity were *Cadophora* spp., causing side rot on pear, and *Fibulorhizoctonia psychrophila*, causing lenticel spot on apple and pear.

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Characterisation of *Pseudomonas syringae* pathogenicity on various species of fruit trees

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Abstract: *Pseudomonas syringae* is an important pathogen on a wide range of hosts, including pome fruits. Bacterial canker of fruit trees is caused by two *P. syringae* pathovars with a different host range. *P. syringae* pv. *syringae* has a wide host range, including pome fruits, while cherries and plums are predominantly infected by *P. syringae* pv. *morsprunorum*. Twenty-four isolates of *P. syringae* originating from various diseased hosts, including apple and pear, were selected to characterise their pathogenicity on different fruit tree species and related hosts. These isolates were identified as *P. syringae* using biochemical and phenotypic characterisation by LOPAT and GATTa tests for determination of species and pathovars, respectively. Also, 16S sequencing was performed to confirm their identity. The pathogenicity of the isolates was tested by inoculation of bacteria on immature fruitlets of 14 hosts and potted plants of four hosts, including apples and pears. The tested isolates varied strongly in their pathogenicity depending on the inoculated host species. Several isolates showed strong aggressiveness to more than one host species.

Key words: bacterial diseases, hosts, symptoms

Introduction

Pseudomonas syringae is an important pathogen to a wide range of plant species and occurs in all fruit growing areas in the world. Bacterial diseases (canker, bacterial decline, gummosis, blossom blast, dieback, spur blight, twig blight, bud bacteriosis) of fruit trees are mainly caused by two *P. syringae* pathovars with different host range (Young, 1995). *P. syringae* pv. *syringae* can cause damage to many commercially grown fruit species, including pome fruits, while cherries and plums are predominantly infected by *P. syringae* pv. *morsprunorum* (Bultreys & Kaluzna, 2010).

During surveys in Latvia, symptoms resembling *P. syringae* infections were observed on traditionally grown fruit species (apple, pear, stone fruits), and also on "new" fruit crops – seabuckthorn, hazelnuts, rowan, and related wild hosts. Pathogenic bacteria (mainly *Pseudomonas* spp.) were obtained from diseased plants and isolates are maintained in the collection at Institute of Horticulture. The study was initiated to characterise their pathogenicity on various fruit tree species and related hosts.

Material and methods

Bacterial isolates originating from various diseased plants were selected to characterise their pathogenicity on different fruit tree species and related hosts. Most of the isolates were identified as *P. syringae* using LOPAT and GATTa tests (Lelliott *et al.*, 1966; Schaad *et al.*, 2001), and 16S sequencing (Scortichini *et al.*, 2005). The pathogenicity of the isolates was tested by inoculation of bacterial suspensions on immature fruitlets of 14 hosts in moist chambers according to Kałużna & Sobiczewski (2009) and potted plants of four hosts in growth chambers. The pathogenicity was evaluated in four separate bioassays: 1) Bioassay 1 – bacterial isolates were inoculated on woody and succulent tissues of potted lilacs, apple and seabuckthorn; 2) Bioassay 2 – bacteria were tested on immature fruitlets of 14 fruit hosts, including pome fruits; 3) Bioassay 3 – verification of results of bioassay 2; 4) Bioassay 4 – pathogenicity of bacterial isolates was tested on succulent and woody tissues of potted seabuckthorn.

Results and discussion

In total, the pathogenicity of 26 *P. syringae* isolates was tested on immature fruitlets of 14 fruit hosts and potted plants of three hosts. The tested isolates varied strongly in their pathogenicity depending on the inoculated host species and type of the tissues. Several isolates showed strong aggressiveness to more than one host, especially *P. syringae* pv. *morsprunorum* isolates were highly aggressive on immature fruitlets in both tests. In immature fruitlet tests, sweet cherries, Mahaleb cherries and sour cherries were the most severely damaged, but less severely damaged were apples and plums. The pathogenicity of isolates in several cases did not correlate with the host where from they have been isolated. More studies and tests on the plants are needed to compare and evaluate the pathogenicity of *P. syringae* isolates on different fruit crops and are currently in progress.

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Ongoing molecular study of *Stemphylium vesicarium* resistance to dicarboximides and phenylpyrroles by site-specific allele replacement into Histidine Kinase 1 gene

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Abstract: Four classes of sensitivity to dicarboximides were established by *in vitro* growth inhibition assays for field strains of *Stemphylium vesicarium*, the causal agent of pear brown spot: S (sensitive), S+ (low resistance), R1 (moderate resistance), R2 (high resistance). Cross-resistance to fludioxonil was only detected in the R2 phenotype. Previous studies correlated the dicarboximide resistance class with single aminoacid substitutions observed in a two-component histidine kinase (HK1), corresponding to single nucleotide polymorphism (SNPs) in the nucleotidic sequence of the *SvHK1* gene. The goal of this ongoing study is to define the role of SNPs in the *SvHK1* sequence on dicarboximide resistance by the replacement of the S allele with S+, R1 or R2 alleles. Protoplasts from a sensitive strain, whose genome has been *de novo* sequenced, were transformed to obtain knock-out *SvHK1* lines. PCR-based screening of mutants indicated in the Δ *SvHK1*-5 line and its monoconidial derived strains a single, complete, site-specific and stable insertion of the linear disruption vector in the *SvHK1* site. The selected mutant will be transformed with the complementation vectors carrying one of the S+/R1/R2 alleles, and complemented strains will be tested for the expected acquired resistance level. Assessment of the role of *SvHK1* SNP mutations in *S. vesicarium* resistant phenotypes will allow us to develop a Digital Droplet PCR assay to determine resistant allele-frequency in populations collected in monitored areas.

Key words: brown spot of pear, site-specific mutation, iprodione, fludioxonil, field resistance risk



Molecular identification of *Venturia asperata* from atypical scab-like symptoms on apples in Italy

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Abstract: Apple scab-like symptoms were observed at the end of July in 2012 in Northern Italy (Cesena) on fruits of apple cv. Modì carrying the *Rvi6* major resistance gene to *Venturia inaequalis*. The aim of this work was to identify the causal agent of the atypical scab-like symptoms by molecular techniques. Symptomatic fruits were collected during May in one orchard in 2015. Ten monoconidial isolates were obtained through recovering of the conidia from about 10 fruits. Conidial suspensions were then streaked on Petri dishes of water agar amended with streptomycin sulfate. After 24 h of incubation at 20 °C, single germinated spores were selected under stereomicroscope, then picked up and placed on PDA amended with three antibiotics. The isolates were cultivated at 20 °C until molecular characterization together with the reference strain of *Venturia asperata*. Amplification of ITS fragments was carried out to specifically amplify rDNA of *V. asperata*, *V. inaequalis* and *Venturia pirina*. Approximately 4-5 hyphae were removed from each isolate and transferred without DNA extraction to a PCR tube with the addition of BSA. DNA amplification was obtained for all isolates by primers specific for *V. asperata*, while no amplification was observed using primers specific for *V. inaequalis* and *V. pirina*. These results point out the presence of *V. asperata* from the atypical scab-like symptoms, and further studies are in progress to have a more precise identification of the pathogen also by morphological and pathogenic assays.

Key words: “Modì”, ITS fragments, *Venturia inaequalis*, *Venturia pirina*, *Rvi6* resistant gene



Quantitative comparison of apple scab severity in chemically treated apple trees by visual and molecular screening

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Abstract: *Malus × domestica* is severely threatened by *Venturia inaequalis*, the causal agent of apple scab. So far apple scab is controlled by extensive fungicide treatments, positioned according to infection models including spore release. Despite this support, the scab inoculum is still increasing yearly. In order to improve scab control, a reliable quantitative molecular approach to support visual observations of the disease in the orchards is needed. In a study performed at Pcfuit in 2015, we aimed at comparing macroscopic apple scab symptoms with molecular quantification of the *V. inaequalis* DNA together with a comparative analysis of pathogen colonization in three different types of apple leaves: rosette and basal and apical extension shoot leaves. Four different scab chemical control programs were applied to ensure differential expression of symptoms in the scab-susceptible 'Jonagold' cultivar. The macroscopic symptoms appearing after natural infections were visually assessed, and the quantification of fungal DNA in the leaves was performed using specific qPCR. During the phase of primary scab infections, as well as during the conidial phase later on, equivalent development of disease severity and differences between treatments were detected by macroscopic and molecular quantification. Molecular quantification well correlated to visual observation both on rosette leaves as on the extension shoot leaves.

Key words: apple scab, non-model crop, biotic stress, pathogen screening, chemical control



Pre-harvest infection of apple fruits cv. Cripps Pink by *Diplodia seriata*, *D. mutila*, *Phacidiopycnis washingtonensis* and *Phacidium lacerum* in the Maule Region, Chile

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Abstract: The Chilean apple industry is one of the major exporters of fresh apples in the world with an export volume of 760,000 tons. The Maule Region is the largest production area of apples in Chile, where more than 60% of Chilean commercial apples are planted. Apple rots are one of the major problems for the Chilean fresh apple industry. Recently, several species of fungi have been described causing pre- and post-harvest rots of apple in the USA, Germany and Chile. However, the infection timing of fruits during pre-harvest is unknown in the Maule Region, Chile. Therefore, the present study was conducted to assess the susceptibility of apple cv. Cripps Pink inoculated with *Diplodia seriata*, *D. mutila*, *Phacidiopycnis washingtonensis* and *Phacidium lacerum*. The fruits were wounded and inoculated at 15 and 7 days prior to harvest using conidial suspensions and mycelial plugs in two localities in the Maule Region. All fruits were placed in commercial boxes and stored at 0 °C. Over the 60 days of storage, lesions were observed in all apples inoculated at both 15 and 7 days before harvest. Apples inoculated with *P. washingtonensis* and *P. lacerum* developed greater lesions than the *Diplodia* species. This study demonstrated that the four fungal species are able to develop rots during cold storage when the fruits of cv. Cripps Pink were inoculated before harvest in two apple orchards in the Maule Region of Chile.

Key words: apple rots, *Diplodia*, *Phacidiopycnis*, *Phacidium*



Silverleaf disease in apple orchards: Understanding and preventing an increasing problem

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Abstract: Silverleaf, caused by the Basidiomycete *Chondrostereum purpureum*, is a disease of fruit crops that has been increasing in Chilean apple orchards. It produces wood necrosis and foliar silvering, induced by a fungal enzyme (endoPG). The objectives were to understand the disease epidemiology, its effects on fruit yield and quality and to develop a non-destructive method for detection of the pathogen. Studies were conducted from 2014 to 2016, in the south of Chile. Spore release was recorded in field and laboratory, and its correlation with meteorological conditions determined. Foliar symptoms were assessed in 57 Gala Brookfield orchards, and the effects on physiology, yield and fruit quality in 10 of these orchards. Antibodies were synthesized based on the endoPG's sequence and a DAS-ELISA protocol developed. Symptomatic leaves from naturally and artificially inoculated plants and from asymptomatic nursery plants were analyzed, and the presence of the fungus was confirmed through PCR and isolation on 25% PDA. The disease was present in 95% of the surveyed orchards, and chlorophyll content and water uptake were most affected. Yield was reduced in 60% and fruit calcium in 26% of the orchards. EndoPG levels and symptom intensity were positively correlated, and the fungus was detected in asymptomatic plants. Silverleaf showed a high incidence and economic importance for the apple industry in Chile. The disease is not yet under control, however, detection methods as presented above may become an efficient tool for its prevention.

Key words: silverleaf disease, *Chondrostereum purpureum*, endoPG detection



Distribution of the infection speed of ascospores of *Venturia inaequalis*

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Abstract: In many apple production areas where spring is wet, fungicides are applied in relation to rain events that trigger ejection of ascospores of *Venturia inaequalis*, which are responsible for primary infections of apple scab. Past studies have established the rate of spore ejection during rain in relation to light and temperature, and the minimum wetting time required for infection at different temperatures. Simulation software like RIMpro use this information to calculate the infection risk and help time spray applications. However, the distribution of the infection speed of spores that have landed on leaves was never studied and assumptions are used. To estimate the distribution of speed, we precisely inoculated ascospores of *Venturia inaequalis* on potted trees and placed them in infection chambers at different temperatures. Trees were gradually taken out, quickly dried and then incubated in standard conditions until lesions were enumerated. The number of lesions increased with infection time for the different temperatures up to a plateau corresponding to the infection time of the slowest spores. The infection curve was best modelled using the monomolecular function starting at the minimum infection duration, 100 degree-hours (DH) base 0 °C. Initial results between 4 °C to 24 °C suggest half the spores can succeed infection within 140 DH. Although our results match the mean values in RIMpro, it is likely the better understanding of the distribution will improve decisions under field conditions.



Management of pear black rot (*Stemphylium vesicarium*) in Conference orchards using a new infection model, a growth regulator, and inoculum reduction

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Abstract: Pear black rot (*Stemphylium vesicarium*) is an important disease in pear production in Europe. The disease is present in most pear orchards in Netherlands and Belgium. Despite frequent fungicide applications losses vary between years and orchards from less than 1%, to over 50% of the production. In published fungicide trials not more than 80-90% effectiveness could be reached. Current knowledge and understanding of the infection biology, host susceptibility, and expert experience was implemented in a simulation model for the infection biology of *S. vesicarium*. The model can be used for the timing of fungicide applications. In 2016, trials to compare control strategies were run in nine pear orchards with a history of black rot infestation. Two or three fungicide applications on moments indicated by the infection model reduced the number of infected fruits by 60%. The standard schedule of seven fungicide applications reduced the number of infected fruits by 71%. Two additional soil treatments with *Trichoderma* increased control efficacy to 83%.



Management of leaf scar infections by *Neonectria galligena* in Kanzi[®] orchards using a new infection model

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Abstract: The commercially valuable apple cultivar Kanzi[®] is highly susceptible to *Neonectria galligena*. The period of autumn leaf fall is considered an important period for new infections on leaf scars. A model for the infection biology of *N. galligena* was developed based on published data and expert experience. The model was used for the timing of fungicide applications in 16 Kanzi[®] orchards in the autumn of 2014, 2015 and 2016. Observations on the level of leaf scar infections were made in April the following year. The efficacy of fungicide applications according to the model was compared to the result of the standard fungicide program. Results will be presented.



Pathogenicity of fungi isolated from cankers of pome fruit trees on fruits in the storage

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Abstract: The emergence of fruit rot diseases and increasing damages in the orchards and storages, lack of data on their causes and control possibilities are among the main concerns of the pome fruit growers. Several pathogenic fungi are known to cause cankers on the trees in orchards and also fruit rots in orchards and storages (e. g. *Neofabraea* spp., *Monilinia* spp.). The knowledge on these diseases is still not sufficient, and their significance in many areas is not known. The aim of the present study was to elucidate ability of various fungi isolated from tree cankers of apple and pear to cause fruit rots in the storage. Four apple and four pear cultivars differing in tolerance to fruit rots were used for the studies. Pathogenicity on fruits was characterised for 20 fungal isolates belonging to *Neofabraea* spp., *Fusarium* spp., *Diaporthe* spp., and *Valsa* spp. in several storage experiments. The differences in ability to cause fruit rot were observed among isolates belonging to the same species and among the species. Eight of thirteen tested isolates originating from tree cankers were also able to cause fruit rot. *Neofabraea* strains and one *Fusarium* sp. isolate were most aggressive, and they caused significant damages on most of the tested cultivars. The more aggressive and virulent (degree of damages and ability to infect more cultivars) were *Neofabraea* spp. and isolates originating from tree cankers.

Key words: apple, pear, fruit rot, canker, *Neofabraea*

Introduction

Apples and pears occupy an important niche in the fruit growing industry in Latvia. The emergence of fruit rot diseases and increasing damages in the orchards and storages, lack of the data on their causes and control possibilities are among the main concerns of the pome fruit growers. Several pathogenic fungi are known to cause cankers on the trees in orchards and also fruit rots in orchards and storages. Among these fungi, *Neofabraea* species are well-known pathogens of pome fruits causing storage rots and cankers (Jong *et al.*, 2001; Gariepy *et al.*, 2005; Henriquez *et al.*, 2005).

In Latvia, damages by storage diseases for apples may vary between 4 and 59%, depending on cultivar and farm (storage) (Grantina-Ievina, 2015). Among detected pathogens *Neofabraea* spp. (*N. alba* and *N. malicorticis*) were identified as dominating (0.3-50.9%) (Grantina-Ievina, 2015). The knowledge about these diseases is still not sufficient, and their significance in many areas is not known (Pešicová *et al.*, 2017). The present study aimed to elucidate ability of various fungi isolated from tree cankers of apple and pear to cause fruit rots during the storage.

Material and methods

Apple cvs. Iedzēnu, Spartan, Aroma, Monta and pear cvs. Conference, Condo, Mramornaya, Beloruskoye Pozdnaya, BP-8965 differing in tolerance to fruit rots were used for the studies. Pathogenicity on fruits was characterised for 20 different fungal isolates (*Neofabraea* spp., *Fusarium* spp., *Diaporthe* spp., *Valsa* spp., *Acremonium* spp.) known as causing agents of cankers, fruit rots or saprobes in two repetitive storage experiments. The tested fungi have been isolated from fruit tree cankers or damaged fruits during our previous studies or were obtained from the CBS culture collection.

Three non-wounded fruits were inoculated with two mycelial discs of sporulating cultures for each cultivar and isolate combination. Agar discs were used on non-inoculated control fruits. Inoculated fruits were kept in containers sealed with plastic film and stored in standard fruit storage for three months. Development of rots was monitored once a week, and final measurement of rots was done after three months of the storage.

Results and discussion

The aim of the present study was to elucidate ability of various fungi isolated from tree cankers of apple and pear to also cause fruit rots during storage. Among 20 of the tested fungal isolates, eleven caused fruit rot in the storage on one or more apple or pear cultivars. The differences in ability to cause fruit rot were observed among isolates belonging to the same species and among the species. Eight of thirteen tested isolates originating from tree cankers were also able to cause fruit rot. *Neofabraea* spp. strains and one *Fusarium* sp. isolate were most aggressive and caused significant damages on most of the tested apple and pear cultivars. The more aggressive and virulent (degree of damages and ability to infect more cultivars of both hosts) were *N. perennans* and *N. malicorticis* strains originated from cankers. These species are well known as aggressive pathogens on apple and pear, causing lenticel rot and cankers in several pome fruit growing regions (Pešicová *et al.*, 2017). Isolates of *Diaporthe*, *Valsa* and *Acremonium* did not cause fruit rot in the two experiments. Tests on plants are in progress to characterise further aggressiveness and virulence of these fungi on plants of the same apple and pear cultivars used in the storage experiments.

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European apple canker: developing novel control strategies

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Abstract: European apple canker is a devastating disease of apple, causing losses at each stage of tree and fruit production from the nursery stage, through tree establishment to postharvest. Our research programme on European apple canker at NIAB EMR spans the whole chain from apple breeding to post harvest control, the outputs from which will lead to a systems approach for disease control. The approaches and early results from three projects will be described; firstly, the development of detection techniques will be presented and how we are using them to better understand the localization of the pathogen during infection. Secondly, work evaluating control strategies for canker will be presented including the effect of biological soil amendments, rootstock genotype and application technologies. Finally, early work on developing our understanding of how microorganisms that live within plant tissues (endophytes) influence canker susceptibility will be presented.

Key words: canker, *Neonectria ditissima*, detection, control



Possible entry points of *Neonectria ditissima* during propagation of apple trees

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Abstract: *Neonectria ditissima*, the cause of European canker, may enter apple trees during propagation. However, when and where the fungus infects during propagation have been subjects for discussion. Both young transplants of rootstocks and rootstocks at a size ready for grafting or T-budding were infected by *N. ditissima* after artificial inoculation. Among the rootstocks tested, B9 (the second most used rootstock in Norway) was significantly more susceptible than M9, which is the most commonly used apple rootstock in Norway and elsewhere. If budwood was collected from trees with different numbers of lesions with European canker, higher lesion numbers on the mother-trees gave more infected trees following grafting or T-budding than if budwood was collected from trees with fewer or no lesions. Our experiments showed that rootstocks may become infected by *N. ditissima* through various wound sites during propagation and that contaminated scion wood may host the pathogen.

Key words: budwood production, inoculum, *Malus × domestica*, *Nectria galligena*, nursery

Introduction

On apple trees, latent infections of the ascomycete *Neonectria ditissima*, the cause of European canker, may be initiated during the nursery phase. An experiment in the UK documented the phenomena both with natural infections and artificial inoculation (McCracken *et al.*, 2003). However, at what time the natural infections occurred, how they occurred and from what sources the inoculum originated was not documented. We assume that the possible sources of inoculum when propagating trees are rootstocks, budwood or inoculum entering from the surrounding vegetation. Conidia of *N. ditissima* are available most of the year, while ascospores may be present in more defined periods, depending on climatic conditions (Weber, 2014).

The objective of the present experiments was to assess rootstocks and budwood as potential inoculum sources for apple trees.

Material and methods

Rootstocks

Details of the experiment are reported elsewhere (Børve *et al.*, 2018). It consisted of both transplants and fully sized rootstocks for propagation. They were inoculated with map pins (Talgø and Stensvand, 2013) or spore suspensions and were kept for eight to 12 weeks before final assessment.

Budwood assessment

Shoots collected as budwood at time of T-budding and grafting were cut in pieces and incubated after different pre-treatments (e. g. freezing). The buds on the pieces were assessed for mycelial growth and sporulation of *N. ditissima*.

Trees made of budwood from mothertrees with cankers

The experiment consisted of trees made either by T-budding or grafting, with budwood collected from mothertrees with either no, a few (2-3) or many (4-7) cankers. The budwood was collected during two growing seasons, and the T-budded/grafted trees started to grow in the corresponding two seasons (2014 and 2015). Trees were made with T-budding or grafting of cvs. Discovery or Summerred on rootstocks Antonovka, B9 or M9. Young trees were assessed for cankers up to 38 months after propagation.

Results and discussion

Rootstocks

All tested rootstocks were infected by *N. ditissima*. Symptoms started to develop ca. 4 weeks after inoculation. Generally, more infections developed on B9 than on M9, and disease development was faster on newly developed than on 1-year old wood (Figure 1). Symptoms developed rather quickly on the rootstocks, and it is likely that possible infections on commercial rootstocks may be discovered prior to delivery for grafting if properly inspected.

Budwood assessments

On the buds on budwood pieces, we found mycelial growth and conidia identified as *N. ditissima* on 3 of 4598 (0.065%) buds assessed after incubation. This shows that buds may contain the fungus, although in low numbers.

Trees made of budwood from mothertrees with cankers

Significantly more cankers developed on trees with budwood originating from mothertrees with the highest number of cankers (Figure 2). This clearly indicates that budwood collected from apple trees infected by *N. ditissima* may increase the risk of transferring the pathogen to the new trees.



Figure 1. Incidence (%) of symptomatic infections caused by *Neonectria ditissima* recorded in days after inoculation with map pins containing mycelia of the fungus; in defeathering wounds of either newly emerged growth or 1-year old wood. Mean of three experiments of M9 and B9.

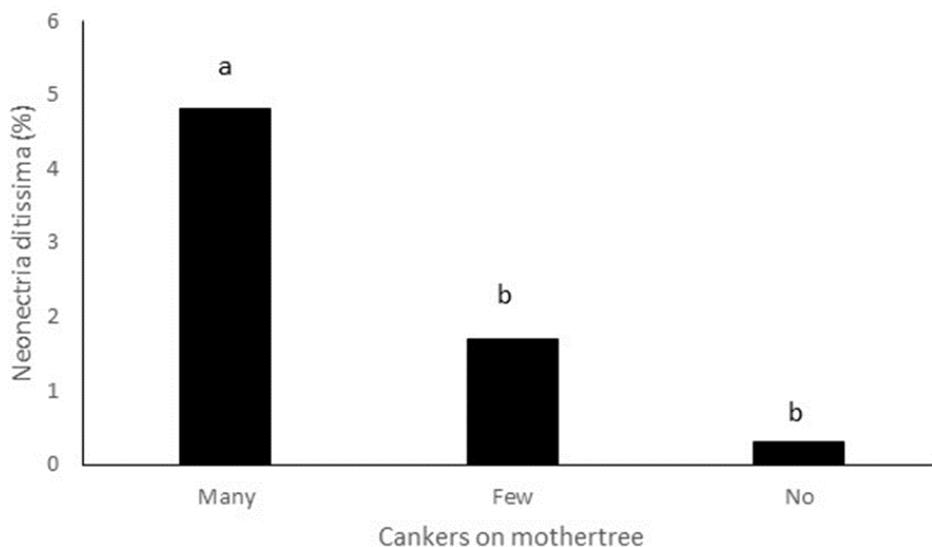


Figure 2. Incidence (%) of *Neonectria ditissima* on T-budded and grafted trees made of budwood from mothertrees with no, a few (2-3) or many (4-7) cankers assessed for up to 38 months after propagation. Mean of two cultivars, three rootstocks and two years and in total 372 trees made of budwood from each mothertree category.

Budwood is cut for T-budding in late summer, and such buds may be exposed to conidial inoculum of *N. ditissima* for a shorter period than for budwood collected during winter. Furthermore, if budwood grows in a climate where ascospores from new perithecia start to spread during autumn, the T-budding will have taken place before ascospores have started to release from newly developed perithecia. Generally, there may thus be less inoculum on the

budwood during T-budding than during grafting. If conidia or ascospores are present on the surface of the budwood, the spores may be transported by capillary into the scion or the rootstock immediately following grafting. On 12 of 13 grafted trees in our experiments where infection occurred, cankers developed in the area between the scion and the rootstock, indicating that the budwood itself may carry inoculum of *N. ditissima* at time of grafting.

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Genetic Research on European canker at SLU: tools developed and knowledge gained

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Abstract: European canker, caused by the fungus *Neonectria ditissima*, is the most serious disease in apple production in Sweden. Spores of the fungus infect trees and fruit through natural and man-made wounds, and damage branches, the main trunk and fruit in storage. The trees may become infected also during propagation. No effective control measures are available. There is an urgent need for cultivars with high levels of resistance. Canker research at SLU in Sweden has been conducted since 2010 in collaboration with national and international partners, with a focus on plant resistance tests, resistance mechanisms, genetic basis of plant resistance, genetic diversity in the fungus, early disease diagnostics. The main results are: a) Efficient and reliable spore inoculation-based plant resistance tests have been developed and successfully applied in germplasm screening and in other studies; b) Differentially expressed genes have been identified in an RNA-seq study on the partially resistant cultivar 'Jonathan' and the highly susceptible cultivar 'Prima' of which several co-localized with previously identified QTL intervals; c) A pathogenic isolate of the fungus has been successfully transformed with the fluorescent protein mCherry, enabling new approaches in plant: pathogen interaction studies; d) Putative QTL for infection percentage have been found; e) Genetic diversity of the pathogen has been investigated and mapping of virulence loci by GWAS (genome wide association study) is in progress.

Key words: fruit tree canker, *Neonectria ditissima*, apple, resistance



The sources of *Rosellinia necatrix* infections in northern Israel's deciduous orchards

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Abstract: *Rosellinia necatrix* causes wilting and death of apples and other deciduous trees in northern Israel. The objectives of the present study were to identify and locate sources of inoculum of the fungus. About 60% of the infested plots were located near a Mediterranean oak maquis forest, and the infection apparently spread from the edge of the orchard to its core. Isolates collected from different orchards appeared to be genetically different from each other, except for three plots that belonged to different farmers but were located near each other. In three plots, isolates collected from different trees in the same orchard were genetically identical, whereas in one plot, in which the soil was not native, different genetic groups were observed.

Key words: *Dematophora necatrix*, genetic diversity, Mediterranean oak maquis forest

Introduction

White root rot disease, caused by the fungus *Rosellinia necatrix* Hartig (anamorph *Dematophora necatrix*, phylum: Ascomycotina), is damaging a wide range of important crops, including ornamental plants and many fruit trees such as apple, sweet cherry, nectarine, peach and avocado. The objectives of the present study were to identify and locate the sources of inoculum of *R. necatrix* by using broad surveys of infested plots at various locations, and to assess the spatial relationship between infection probability by the fungus and the distance of the plot from potential sources.

Material and methods

Spatial analysis

To assess the probability of *R. necatrix* infecting orchards in relation to possible sources of inoculum, roots of recently dead trees from 95 orchards in northern Israel were examined for presence of the pathogen. Distances from the closest potential inoculum source were measured with the ArcMap platform (ESRI 2013), and logistic regression analysis was conducted to assess the infection probability. Forty-nine isolates of *R. necatrix* from apple roots, grapevines, cherry, terebinth (*Pistacia palaestina*), and oaks (*Quercus* spp.) were obtained during 2012-2015 from locations of the Golan and Galilee.

Compatibility assay (CA)

Mycelium incompatibility assays of all possible combinations were performed as described by Armengol *et al.* (2010).

Total DNA was extracted from pure cultures of 49 isolates. PCR-ISSR (ISSR1, 2, 4, 5) and UP-primer (As4 and GA8G) analyses were performed as described by Armengol *et al.* (2010) and Ikeda *et al.* (2005). Only clear and reproducible bands were considered. All assays were repeated three times.

Results and discussion

Of the 95 surveyed deciduous orchards, about 60% were found to be infested with *R. necatrix*. All the infested orchards belonged to 14 settlements, in nine of which the infested plots were located at the boundary of the Mediterranean maquis oak forest. The mean distance of infested orchards from the closest maquis was 1271 m, whereas that of healthy orchards was 2572 m. The average distance of infested orchards from nearby infested orchards was 335 m, whereas that of healthy orchards was 2257 m (Figure 1) Regression analysis yielded a significant relationship between distance and probability of infestation, with $\beta_0 = 0.88$ ($p = 0.001$), $\beta_1 = -0.001$ ($p = 0.016$), and Nagelkerke's $R^2 = 0.13$, suggesting that infection probability decreases with increasing distance.



Figure 1. Soil infection probability as a function of the distance from the closest potential source of infection. *T-Test, $P < 0.01$. ■ Healthy, □ infested

ISSR 1, 2, 4, and 5 yielded 10, 5, 14, and 14 polymorphic bands, respectively. The universal primers AS4 and CA8G yielded 12 and 5 clear, easy-to-score polymorphic bands, respectively. According to the genetic analysis and compatibility assay, the 49 isolates divided into 28 different genetics groups. Three isolates from the Netu'a apple orchard, 4 isolates from the Gish apple orchard, and 11 isolates from the Mas'ade experimental plots had the same genetic band pattern and did not form boundaries in the incompatibility assay. Nine isolates from the Metula experimental plot were divided into 6 genetic groups. Most of the isolates were genetically different from each other, except for one pair and one group of three isolates that were genetically and morphologically identical as also suggested by the mycelial CA. Isolates from different deciduous orchards appeared to be genetically different from each other, except for the three neighboring plots in Mas'ade, that belonged to different farmers but were located next to each other. These isolates shared the same band pattern in the genetic analysis, and did not form a boundary in the CA.

Results from the survey indicate four possible main sources of infection: (i) Mediterranean maquis forests located near agricultural lands; (ii) transfer of soil within orchards; (iii) movement of inoculum inadvertently carried out by farmers from nearby orchards or distant plots in the same farm; and (iv) contagion via roots of adjacent trees within the orchard.

The full results of this work were published by Dafny-Yelin *et al.* (2018).

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Molecular detection and quantification of apple scab (*Venturia inaequalis*) in fruit

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Extended Abstract: Apples are an important fruit crop in Belgium. In 2015, more than 280 000 tons of apples were produced, and the majority was exported (Vlam, 2017). To guarantee good prices, the fruit need to be of excellent quality and preferably free of blemishes. This may not be easy to achieve because of several diseases. One of these is apple scab, caused by *Venturia inaequalis*. The Belgian temperate climate is ideal for this pathogen (Bowen *et al.*, 2011). Even with extended use of fungicides, scab is still a threat to our orchards (MacHardy *et al.*, 2001). Often growers notice that after a few months of storage, previously symptomless fruit may develop typical scab symptoms, making them less desirable for the fresh market (Tomerlin & Jones, 1983). In this study, we aimed to detect and quantify latent scab infections in apple peel and use this technique as a predictive tool to evaluate the risk of scab development during the storage.

Apple fruits from ten orchards in the main fruit growing area of Belgium were sampled. Seven organic orchards, two IPM orchards and one minimally sprayed and heavily infected orchard at the research station of Sint-Truiden were selected. Symptomless fruits were picked at harvest in September and stored in controlled atmosphere at 1 °C. Three replicates of 10 apples each were sampled at every sampling date during the harvest. Samples were collected monthly from September until April. The fruits were scored individually using a scab index ranging from zero (no symptoms) to four (heavily infected). Because the fungus resides in the peel, only the peel (approx. 2 mm thick) was used for further examinations. The peel was macerated, and DNA was extracted with the Nucleospin plant II kit. A novel in-house developed sensitive qPCR method was used to detect and quantify *V. inaequalis*. The disease severity index (DSI) was calculated using the following calculation;

Tomerlin and Jones (1983) stated that only few (7% and 0%) symptomless harvested fruits developed lesions during storage. We observed lesions in fruit from all orchards during storage. The monthly evaluation showed an increase of scab incidence during storage. Fruit harvested from the heavily infected orchard showed the highest number of lesions, while the less infected orchards showed less symptoms after storage. Almost no symptoms were detected before December in fruit from any of the orchards. In general, fruits from organic orchards showed scab symptoms more rapidly than fruits from IPM orchards. The severity of symptoms depended strongly on the amount of disease incidence during the season. The newly developed molecular detection technique showed real promise. The technical replicates showed little or no variation, while the biological replicates showed some variation. In most of the symptomless fruit samples collected at harvest, the presence of *V. inaequalis* was detected. A clear difference was seen between the different orchards. A correlation was found

between the DSI in April and the amount of the fungus detected by qPCR in September. After further optimization, this technique may be used as a predictive tool to evaluate the risk of scab symptom development during storage.

Key words: latent apple scab, qPCR

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Characterization of the apple and pear bark microbiota

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Abstract: Bark is considered an important overwintering site for pathogenic, beneficial and saprophytic microorganisms and can act as a reservoir of potential plant pathogens and biocontrol agents. However, the majority of the studies regarding plant-associated microbial communities are focused on the phylloplane, fruits and the rhizosphere, while bark is poorly investigated. The aim of this study was to assess the composition of fungal and bacterial communities of pear and apple barks. Results showed that the amount of cultivable colony forming units (CFUs) and the composition of fungal and bacterial communities differed according to bark age and plant species.

Key words: apple and pear microbiota, bark-associated microorganisms, Illumina sequencing, metabarcoding

Introduction

Plants are hosts to endophytic and epiphytic microbial communities that establish beneficial, detrimental or neutral associations with their host (Lodewyckx *et al.*, 2002). Most of the studies about the composition and dynamics of plant-associated microbial communities have been focused on rhizosphere, phylloplane and fruits, while only few studies regarding bark and flower microbial communities have been carried out (Lambais *et al.*, 2014). Particularly, bark can host pathogenic, beneficial and saprophytic fungi (Buck *et al.*, 1998; Martins *et al.*, 2013), possibly acting as a reservoir of potential plant pathogens and biocontrol agents. Despite the theoretical relevance of bark-associated microbial communities in plant health, little is known about this subject. We aimed to investigate the composition of fungal and bacterial communities residing on bark of apple (*Malus domestica*) and pear (*Pyrus communis*) using a metabarcoding approach.

Material and methods

Barks of apple cvs. Golden Delicious and Gala and pear cvs. Abate and Williams were sampled in triplicate from one year-old shoots (new barks) and three-four year-old branches (old barks) at the dormancy stage. Bark samples were ground with sterile stainless steel jars in presence of 2.5 ml of a cold (4 °C) sterile isotonic solution (0.85% NaCl). The viability of

culturable fungi and bacteria was assessed using a plating method (Cappelletti *et al.*, 2016), and colony forming units (CFU) were determined for fungi and bacteria at seven and five days after incubation at room temperature, respectively. DNA was extracted from ground samples, and ITS2 and V5-V7 regions of 16S rDNA were amplified for the identification of fungi and bacteria, respectively. An Illumina high throughput sequencing, followed by bioinformatic analyses, was carried out to identify the bark-associated microorganisms.

Results and discussion

A higher number of fungal and bacterial CFUs was recovered in old as compared with new barks, while plant species only affected the number of fungal CFUs. Of all the fungal reads, 97% were attributed to one species. A total of 430 fungal operational taxonomic units (OTUs) was found. The richness of the fungal communities of bark was affected by plant variety, while the diversity was influenced by bark age and plant variety (Table 1). Of the bacterial reads, 95% were assigned to one genus. Totally, 824 bacterial OTUs were recovered. Richness of the bacterial communities was affected by bark age, plant species and plant variety, while the diversity was affected by bark age (Table 2).

Table 1. Fungal richness and diversity, estimated by the number of OTUs and by the Simpson's index, respectively. Double and triple asterisks indicate significance of $p < 0.05$ and $p < 0.01$, respectively, NS means not significant.

Variable	OTUs p -value	Simpson's index p -value
Bark age	0.083	0.000
Plant variety	0.005	0.000
Plant species	0.814	0.782

Table 2. Bacterial richness and diversity, estimated by the number of OTUs and by the Simpson's index, respectively. Double and triple asterisks indicate significance of $p < 0.05$ and $p < 0.01$, respectively, NS means not significant.

Variable	OTUs p -value	Simpson's index p -value
Bark age	0.013	0.000
Plant variety	0.001	0.897
Plant species	0.006	0.539

The composition of fungal and bacterial communities was influenced by bark age, plant species and plant variety. Particularly, abundances of dominant fungal and bacterial taxa differed according to the bark age. Among fungal and bacterial communities, the presence of genera that potentially comprised pathogenic and beneficial species will be investigated.

Our results contribute to the knowledge of bark, a poorly studied, even important, plant organ (Arrigoni *et al.*, 2018). Despite that bark represents a difficult environment (Buck *et al.*, 1998), roughness and cavities could provide an optimal overwintering site for many

microorganisms. This study demonstrates the presence of numerous fungal and bacterial communities residing on bark, partially shared with bulk soil (Martins *et al.*, 2013) and leaves (Lambais *et al.*, 2014).

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New insights into the microbiome of apple fruit surface cv. Pinova through metagenomics

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Abstract: Plant microbial communities (microbiota) living on the surface of fruit have been the source of the majority of biocontrol agents (BCAs), but their role as a community has been poorly studied so far. A pioneering assay using high-throughput sequencing (HTS) has been carried out to get insight into the microbiota of apple (cv. Pinova) surface through metagenome sequencing. Fruit of cv. Pinova was sampled in Belgium during autumn 2013, and then the microbiota was isolated. After DNA extraction, the HTS assay generated 14.5 Gbases, which were assembled in 133.888 contigs. These contigs provided useful information on taxonomic composition of the microbiota. A total of 1863 bacterial species and 1194 fungal species have been identified. Our results demonstrated a very diverse microbial community on conventionally treated apple cv. Pinova, and its role needs to be characterized.

Key words: microbiota, high-throughput sequencing, *Malus domestica*

Introduction

Carposphere microbiota can host plant pathogens and may thus play an important role in the development of plant diseases, including post-harvest fruit diseases causing significant worldwide losses in apple (Leff & Fierer, 2013). Currently, synthetic fungicides represent the main source to control postharvest decay of apple (Sivakumar & Bautista-Baños, 2014), but the use of chemicals is going to be restricted because of the development of resistance to many fungicides by major postharvest pathogens (Hahn, 2014).

Plant microbial communities (microbiota) living on the surface of fruit have been the source of the majority of biocontrol agents (BCAs), but identifying and determining the relative frequency of microorganisms cannot be readily achieved by culturing methods. More than 99 % of microorganisms on the earth are unculturable with known culturing techniques. The emergence of metagenomics with high-throughput sequencing technologies has enabled researchers to capture a comprehensive view of complex bacterial and fungal communities, which comprise unculturable species (Abdelfattah *et al.*, 2017).

Therefore, a better insight into the microorganism composition and diversity on apple trees is important in understanding the potential for biocontrol to succeed. The objective of the present study was to characterize the bacterial and fungal communities on the surface of apple cv. Pinova using a metagenomic approach.

Material and methods

The microbiota from the carposphere of conventionally grown apple fruit of cv. Pinova was evaluated through classical plating assays and using the innovative metagenomics approach. Sampling was performed in October 2013 in an orchard of cv. Pinova planted 18 months earlier. Four replicates composed of eight fruit each collected from four plants were sampled, and then fruits were washed with phosphate buffer (pH = 6.5) and shaken at 130 rpm for 20 min at room temperature.

The washing solution was immediately filtered with sterile filters, pellets were washed with 2 ml sterile water, and then the suspensions were centrifuged at 13.000 rpm for 10 min. After discarding the supernatants, the pellets were added into a lysing matrix tube, and the FAST DNA SPIN kit for soil (MP Biomedicals) was used to extract DNA according to instructions of the manufacturer.

The extracted DNA was subjected to whole genome amplification using the GenomePlex[®] Whole Genome Amplification Kit (Sigma-Aldrich, USA) kit according to manufacturer instructions.

The library preparation for sequencing was carried out using the kit DNA Truseq (Illumina), and the sequencing was done on Hiseq 2000 from Illumina at DNAVision for 2×100 nt length. Generated sequences were demultiplexed, and their quality controlled using standard Illumina pipeline. The sequences were further pooled and analysed using the standard pipeline of MG-RAST and the standard pipeline option for quantitative metagenomics analysis.

Taxonomical annotation of sequences was made using M5NR database (Minimal e-value $1e-5$, minimum 60% of identity and minimum alignment length of 15 bp).

Results and discussion

In the present study we characterize the bacterial and fungal communities on the surface of cv. Pinova apple fruit, a cultivar partially resistant to apple scab (*Venturia inaequalis*), conventionally treated during the growing season. Using a metagenomics approach based on high-throughput sequencing (HTS), we accurately estimated the natural microbial population size at harvest time and investigated on the presence of putative apple fruit pathogens or putative biocontrol species in the complex community of the carposphere.

The HTS assay generated 14.5 Gbases, which were assembled into 133.888 contigs. These contigs provided useful information on taxonomic composition of the microbiota.

Our results generally demonstrated high fungal and bacterial diversity across the fruit samples. The percentage of total sequence reads assigned to Eubacteria was 12.1% whilst to Eucaryotes was 87.1% among which 83.2% were fungi. The total of 1.863 bacterial species and 1.194 fungal species has been theoretically identified*.

The microbiota was dominated by Eucaryotes, predominantly fungi, but the number of bacterial species assigned within all the samples was higher than fungal species. Among them, several contigs were assigned to known apple pathogens and others to species or genus of known BCA strains.

These preliminary results are under deeper investigation and need to be confirmed, but they underline a very diverse microbial community whose role needs to be characterized.

* In next-generation sequencing (NGS) technologies the characterization and identification of known microorganisms at strain/species levels remain challenging, mainly due to the lack of high-resolution tools and the extremely diverse nature of microbial communities.

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Fungi associated with cankers and diebacks of fruit trees in Latvia and their pathogenicity on various fruit tree species

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Abstract: Cankers, diebacks and wood rots are widespread on fruit trees and are caused by a variety of pathogenic fungi. Among these, diseases caused by pathogenic species belonging to *Diaporthe*, *Valsa* and *Botryosphaeria* are considered as the most damaging to their hosts. The research was initiated when severe canker and dieback symptoms and tree death were observed in fruit tree orchards in Latvia. To identify the causes of observed tree diseases, orchard surveys and samplings were performed from May to September. The samples from branches and trunks with symptoms of cankers and dieback were collected. Fungi were isolated from surface sterilised plant tissues on potato dextrose agar, sub-cultured in pure cultures and preserved for further studies. The isolated fungi were characterised and identified by morphological characters and sequencing of the ITS region. Several fungal isolates were selected and tested for their pathogenicity on seedlings of *Malus domestica*, *Pyrus communis*, *Prunus mahaleb* and *Prunus cerasifera*. During the surveys, overall decline, severe canker and dieback symptoms often causing a death of the plants was observed. Fungal isolates belonging to the known pathogenic genera causing of tree cankers and dieback, such as *Diaporthe*, *Cytospora* and *Monilinia* have been identified. The pathogenicity of the fungal isolates varied depending on the inoculated plant species.

Key words: pathogenicity, apple, pear, stone fruits, *Diaporthe*, *Cytospora*

Introduction

Due to the global trade of plant material, governmental policies banning a range of fungicides, changes in climate and growing technologies new and previously considered as minor diseases are emerging. Several canker, dieback and wood rot diseases are widespread on fruit trees and are caused by a variety of pathogenic fungi. Among these, diseases caused by pathogenic species belonging to *Diaporthe*, *Valsa*, *Neofabraea*, *Neonectria*, and *Botryosphaeria* are considered as the most damaging to their hosts globally or depending on growing regions (Nakatani & Fujita, 1997; Sakuma, 1997; Xu *et al.*, 1998; Garipey *et al.*, 2005; Henriquez *et al.*, 2006; Wang *et al.*, 2011).

Severe canker and dieback symptoms often causing the death of the infected trees have recently been observed in fruit tree orchards in Latvia. The research was initiated to investigate on fungi involved and to identify the causal agents of observed diseases.

Material and methods

Orchard surveys and samplings were performed from May to September. The samples from branches and trunks with symptoms of cankers and dieback were collected. Fungi were isolated from surface sterilised plant tissues on water and potato dextrose agars, sub-cultured in pure cultures and preserved for further studies. Fungal isolates were preserved on oat-meal agar, and potato agar slants at +4 °C. The isolated fungi were characterised and identified by morphological characters, sequencing of the ITS/5.8S region with universal primers, and comparing of obtained sequences with data in GenBank.

The virulence of 126 isolates (identified as *Epicoccum* spp., *Phomopsis* spp., *Monilinia*, *Fusarium* spp., *Cytospora* spp.) isolated from apple, pear and stone fruits with cankers and dieback symptoms was tested in two pilot inoculation experiments on seedlings of *Malus domestica*, *Pyrus communis*, *Prunus mahaleb* and *P. cerasifera*. Plants were inoculated with mycelial plugs of the fungi using a T-cut method and monitored for symptom development during the two seasons in the greenhouse.

Results and discussion

More than 150 apple, pear, sweet and sour cherry, and plum orchards were surveyed. In total, 565 woody samples from branches and trunks with symptoms of cankers and dieback were collected. More than 3000 fungal isolates were obtained in pure cultures, and possible pathogens and representatives of each group were preserved in our fungal collection.

Fungi, belonging to the known pathogenic genera causing tree cankers and dieback, such as, *Diaporthe* (*Phomopsis*), *Valsa*, *Leucostoma* (*Cytospora*), *Neofabraea* (*Cryptosporiopsis*), and *Monilinia*, were identified. Among the isolates tested in pilot inoculation tests, *Phomopsis* spp., *Cytospora* spp., and *Fusarium* spp. isolates were most virulent and able to cause canker and dieback to more than one host. Several of the tested *Diaporthe* spp. and *Fusarium* spp. strains isolated from plums and pears were able to cause severe canker on all of the tested hosts, indicating their broad host range and aggressiveness.

The research is in progress for more accurate identification (multi-locus sequencing and phylogeny, morphological characterization) of fungal species involved and to evaluate their role in canker and dieback diseases of fruit trees in Latvia.

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Epidemiological factors and cultivar sensitivity affecting severity of apple canker in Finland

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Abstract: The economic importance of apple canker has increased in Finland in recent years. The causal organism of apple canker is *Neonectria ditissima*. The perfect (ascospore-forming) stage of the fungus disperses by wind or rain-splash and the imperfect (conidia-forming) stage by rain-splash. The temperature and moisture requirements for perithecia development, maturation, and finally ascospore ejection are not known in the Finnish climatic conditions. Also, very little is known about the canker susceptibility of eastern or continental types of cultivars grown in Finland. To better understand the geoclimatic potential of the disease development and the monthly temporal trends of disease conducive weather periods, the Finnish weather data was used to run a canker risk model. In the experimental part of the ongoing study, the densities of airborne ascospores are studied with volumetric air samplers placed in orchards in two different locations of southern Finland in 2017-2019. Trapped *Neonectria* spores will be counted under a microscope and species identity confirmed from DNA. The spore trapping results will be combined with weather data, and with visual observations of perithecia amounts and maturity in the orchards. Cultivar tolerance to artificial infection by *N. ditissima* is tested in a greenhouse in 2017-2018. In 2017, isolated ascospores were used as the pathogen inoculum and one-year old potted trees of 66 cultivars were wound-inoculated. The trial will be repeated in 2018. Later, sensitivity of frost damages in different cultivars will be evaluated by inoculating wounds induced by controlled freezing. Based on the early results, canker risk may extend beyond the current main apple growing area. Cultivar phenology and the timing of high-risk weather conditions require further observations. Various levels of disease susceptibility or tolerance were observed in both the modern and traditional cultivars, indicating potential for improvement of resistance by breeding.

Key words: apple canker, *Neonectria ditissima*, ascospores, epidemiology, resistance breeding

Introduction

In Finland, apple canker has been recognized for a long time as a disease of local or moderate importance. For example, Gauffin *et al.* (1906) distributed an apple canker management advice for apple growers as early as in 1906. Recently the expenses of canker management have increased simultaneously, with changes in cultivation technology, choice of plant material types, and with import of plant material and use of more sensitive rootstocks and less winter hardy cultivars. Similar increase is reported e.g. from some areas in Norway (Børve *et*

al., 2015), and the importance of breeding new tolerant cultivars for the Nordic countries has been identified (Nybom *et al.*, 2015).

Apple canker, caused by the ascomycete *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman, is known to be very severe in latitudes above 52 ° (Beresford & Kim, 2011). In a disease conducive, cool and humid climate, infection pressure may stay high, and various types of wounds serve as infection routes throughout the year (Weber, 2014). Because leaf scars pose as the most numerous wound type per tree, leaf drop in autumn is usually regarded as the most important period for chemical canker management. Infections that have taken place during a late leaf-drop do not develop visible disease symptoms before spring or early summer, whereas new infections in the spring or early summer have a shorter latent period of 2-3 weeks (Crowdy, 1952; Weber, 2014).

In the Köppen climate classification, the southernmost parts of Finland fall into the type “humid continental mild summer, wet all year”. Apple production in those semi-maritime climatic regions in Finland (mainly latitudes between 60 and 62 °N) is characterized by a short growing season where few modern cultivars reliably reach vegetative maturity, and occasionally extremely cold winters (Kaukoranta *et al.*, 2010). Orchards or home gardens with highly prevalent and severe apple canker symptoms can be found within the main apple production areas. However, autumn and especially winter temperatures are mostly lower than the optimal temperatures (+11 to +16 °C) observed practically and experimentally for *Neonectria* infection and disease development (Dubin & English, 1975; Latorre *et al.*, 2002; Beresford & Kim, 2010). Understanding the epidemiological factors relevant for temporality of pathogen spread in the Finnish conditions, as well as the host cultivar susceptibilities, are required for efficient disease management.

The aims of this on-going study are to identify the weather conditions of high risk for ascospore dispersal, and to evaluate cultivar resistance in spring-like conditions.

Material and methods

The rule-based risk model of apple canker by Kim & Beresford (2011) was coded as a SAS (SAS Institute Inc., USA) model and run using gridded three-hourly weather data from Finland in 2003-2016, provided by the Finnish Meteorological Institute.

The densities of airborne ascospores were studied with volumetric air samplers (7-day Burkard traps, Burkard Manufacturing Co, UK) placed in orchards in two different locations of southern Finland (Figure 1). The first trapping period was from early April to late June in 2017. The trapped *Neonectria* ascospores were counted under a microscope using stratified random field sampling. Species identity will be confirmed later from DNA. The spore trapping results are combined with weather data, and with visual observations of perithecia amounts and maturity in the orchards.

Cultivar sensitivity to infection by *N. ditissima* is tested by artificial inoculation in 2017 and 2018. In the first trial in spring 2017, isolated ascospores were used as the pathogen inoculum. Six dormant one-year old potted plants per cultivar were introduced in a greenhouse with conditions mimicking spring (short day-length, cool night temperature). Plants were organized in a row-column design with inoculation date as a main blocking factor. The conidial wound inoculation method (Van de Weg, 1987; Garkava-Gustavsson *et al.*, 2013) was modified as follows: Three vegetative apical buds on the main shoot per plant were removed with a scalpel. Inoculum was prepared from excised natural perithecia that were incubated for 2 hours in sterile double-distilled water and crushed to release ascospores (Crane *et al.*, 2009). Ascospore number was determined with hemacytometer under a

microscope and adjusted when necessary. A droplet of 15 μl (approx. 200 ascospores) was pipetted on a fresh wound, and the wound was covered with vaseline for one week. Samples of spore suspensions were kept in the greenhouse overnight and the germination rates of spores were assessed next day using a microscope. Water was used to mock-treat non-inoculated control wounds (3 per cultivar). The symptoms were recorded and lesion size measured every second week for 14 weeks, after which the experiment was finished.

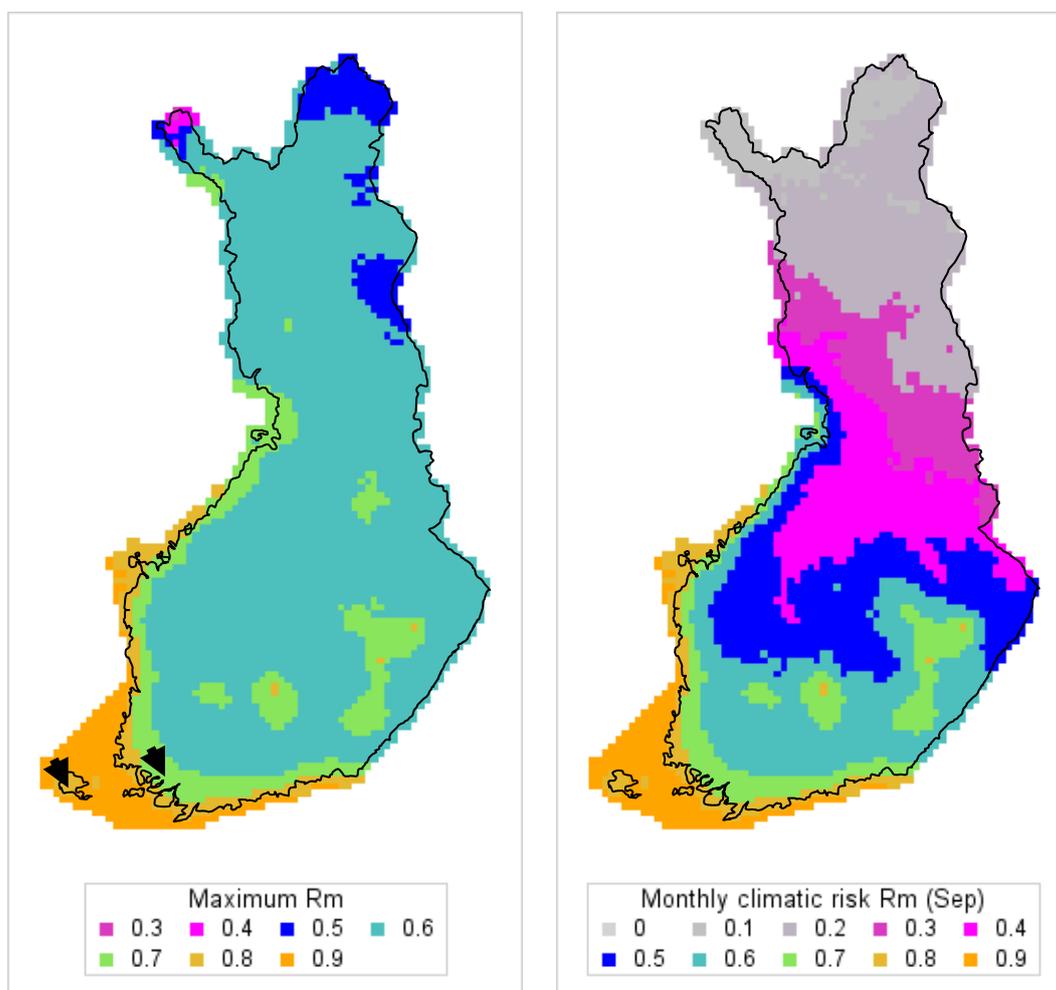


Figure 1. Maximum rule-based risk model values in Finland based on gridded weather data in 2003-2016. Left: average of annual maximum R_m values. Right: average monthly R_m value for September across the years. In the map on the left side, the approximate locations of the two spore traps in Åland Islands and in southwestern continental Finland are pointed with arrowheads.

Results and discussion

In the climatic risk model, the maximum R (R_m) values over 0.75 are associated with regions of severe disease levels (Kim & Beresford, 2012). The R_m values in Finland reached 0.8 in the whole coastal areas and archipelago of southern Finland and the Åland Islands (Figure 1). Furthermore, regions of relatively high risk values (0.7) were observed in the lake areas of

continental southern Finland. According to the model, the highest average monthly risk values were in September in most parts of Finland, which corresponds to the time of the main harvest season.

The second highest average monthly risk values were reached in June, coinciding with the detachment of petals or rosette leaves, which leave potential wounds for pathogen entry (Swinburne, 1975). The time of leaf drop in autumn and winter cultivars (October-November) was not, on average, associated with such high risk values, probably due to the rapidly decreasing autumn temperatures (results not shown).

Ascospore trapping in spring 2017 resulted in low numbers of ascospores. No clear peaks in spore abundance were evident. The detected ascospores were trapped during May or June, a period with maximum day temperatures ranging from +5 to +18 °C.

Ascospores used in the cultivar inoculation trial had germination rates of 30-70% an overnight after their use for inoculation. At 12 weeks post inoculation, 68% of the spore-inoculated wounds had developed lesions of various sizes. No lesions developed in the mock-inoculated wounds. Cultivar-specific differences were observed in infection rate and disease development. The test included several traditional or local cultivars, selected for their Finnish origin or a long cultivation history in the national apple germplasm collection in Finland. The trial will be repeated with the same set of cultivars to reduce any year × cultivar interaction, a factor that should be carefully controlled in *Neonectria* inoculation studies (Garkava-Gustavsson *et al.*, 2016).

Few studies have compared the canker tolerance in the traditional Nordic cultivars with the modern cultivars. For example, the locally important and winter hardy Nordic “cinnamon apple” cultivars (in Finnish, Kaneliomena, in Swedish, Kaneläpple) closely related to ‘Korichnoe Polosatoe’, a Russian cultivar from the 19th century, show low infection percentage in controlled inoculation experiments (Garkava-Gustavsson *et al.*, 2016), and are known for a relatively good field tolerance in Finland.

In conclusion, the results from the risk model will help to better focus the on-going experimental epidemiological studies. In some cultivars, not only the genetic canker tolerance but also phenology (e.g., timing of leaf drop in the autumn or rapid shoot extension in the spring) may influence their field tolerance. To develop an efficient integrated canker management program, more information of the infection routes in the Finnish climate is required.

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Epidemiological studies on *Diplocarpon mali* in Austria

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Abstract: In Austria, apple blotch (caused by *Diplocarpon mali*) is a relatively new threat, mainly to organic and extensive apple production. Severe leaf symptoms and premature leaf fall due to this pathogen were first discovered in Austrian orchards in 2011. Depending on climatic conditions, symptoms have appeared since then in different severity. Leaf spots usually develop on the upper surface of mature leaves. The fungus produces conidia in acervuli on infected tissue, which spread infectious conidia throughout the growing season. Diverging investigations about the overwintering stage of the fungus (sexual / asexual state) have been published. The aim of the experiments was to gather information about the disease development after leaf fall and during winter as well as the further development of the fungi after tree dormancy. The results should provide data on the source of inoculum for primary infections and the dispersal of the pathogen. Therefore, morphological studies on diseased apple leaves collected in Austrian orchards were carried out from September to May in 2015-2017. To determine the possible time of primary infections in the field, spore traps were used for detecting mature conidia released from overwintered leaves. The development of disease symptoms and conidia production was also assessed after inoculations of different apple cultivars under greenhouse conditions. In current experiments, there is no evidence for the development of apothecia during the dormant season. However the maturation of acervuli was monitored. Evaluation of further results is in progress and will be presented.

Key words: *Diplocarpon mali*, apple, epidemiology



Development and incidence of twig scab in pear

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Abstract: Pear scab is one of the main diseases of pear. Disease is caused by the fungus *Venturia pirina* which is highly similar to causal agent of apple scab *Venturia inaequalis*. The biology of both pathogens is comparable and applications of plant protection products usually are carried out in one time. However, according to annually observations at the same weather conditions pear fruits are heavier damaged by scab than apples. Observations showed that one of the reasons may be appearance of the twig scab on pears. The aim of this study was to evaluate the incidence level of scab on pear shoots and to follow development of the disease during the vegetation season. The incidence and severity of scab was assessed on widely grown cultivars in commercial orchards from 2012 to 2013. Observations regarding pear scab development were carried out in an organic orchard on cultivar 'Mramornaja' from 2012 to 2013. Scab was assessed on pear leaves, pear fruits and shoots to evaluate an incidence and severity level of the disease. Twig scab incidence and severity varied among cultivars and orchards. The most susceptible cultivars to twig scab were 'Mramornaja' and 'Mlejevskaja Rannaja'. The highest resistance under field conditions was observed for cultivar 'Conference'. The first symptoms of pear scab on the leaves, fruits and young shoots of cultivar 'Mramornaja' were found at the end of May and in early June in both years of observations.

Key words: *Venturia pirina*, severity, cultivars



***Venturia inaequalis* biofix estimation under the conditions of a dry spring in Bulgaria**

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Abstract: In 2017, observations on the conditions for development of the pseudothecia of *V. inaequalis* have been carried out. Winter was snowy and extremely cold with temperatures below 20 °C. After snow melted in the beginning of March, leaf samples from locations all around Bulgaria were monitored with the squash mount method. The data obtained were evaluated in comparison with records from previous seasons. Generally, the biofix (actual discharge of ascospores based on a moist chamber laboratory test) was late in 2017. Usually it takes place in the first half of March and at about green tip stage. In the present season, it was in most regions in the end of March or beginning of April, which phenologically corresponded to early or full bloom stage in the main apple varieties. Such a trend was also recorded in 2012 when the spring was similarly very dry just after the pseudothecia were formed. In 2017, first fruiting bodies appeared a little bit late due to the cold winter and then followed a 30 day period of very dry, warm weather. Analysis of climate features, pathogen and tree development was performed. During the period of asci maturation, it seems that moisture was the limitation factor. This was confirmed by the huge difference in development of fruiting bodies on single leaves, which presumably overwintered in more moist spots of the ground floor.

Key words: apple scab, forecast, biofix, IPM



Evaluation of decision support system RIMpro in forecasting of apple canker in Latvia

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Abstract: European canker, caused by *Neonectria ditissima* Tul. & C. Tul., is a serious apple disease, which affects orchards in different parts of Europe, including Latvia. The model "Apple canker" in the decision support system RIMpro, developed in Netherlands (Bio Fruit Advice), has been available in Latvia since autumn of 2014. The aim of the present study was to evaluate the suitability of the model for climatic conditions of Latvia. This study presents results of observations of the disease from October 2014 to December 2016 in 9 apple orchards in Latvia with on-site meteorological stations and supplementary orchards located nearby. Apple orchard "Pūre" was chosen as the main observation place, because it has convenient location for regular assessments and a meteorological station is in use there all year around. Assessments were made on medium old canker wounds. Samples were analysed by microscope to observe fungal structures or spores of *N. ditissima*. Field observations of development of the disease were compared with the forecast of the RIMpro model. In most cases, information about development of *N. ditissima* provided by the "Apple canker" model corresponded to field observations.

Key words: model, *Neonectria ditissima*, orchards, wounds



Apple scab monitoring and forecasting in Latvia

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Abstract: Protecting apple orchards against apple scab [*Venturia inaequalis* (Cooke) Wint.] is one of the most important issues in Latvian horticulture. Minimal use of chemicals in fruit-growing is important condition in integrated fruit production. IOBC guidelines for integrated fruit production prescribe use of forecasting systems in plant protection for a precise pesticide use. In Latvia, the Latvian Plant Protection Research Centre (LPPRC) started investigations for adaption of the decision support system RIMpro for apple scab control in 2003. In the first four years (2003-2006), the aim of the investigations was to verify conformity of the scab infection risk given by RIMpro to the on-site scab ascospore discharge intensity by use of microscope slide spore traps, as well as to determine the border values for signaling when fungicide application is necessary. In the next two years (2007-2008), the main objective was to reduce the risk of developing fungicide resistance in apple orchards. In 2012, observations were started to determine the precise “biofix” for the apple scab forecast in RIMpro. During the recent ten years (2007-2017), RIMpro has been available also for Latvian apple growers. For precise weather information, ten weather stations are placed in apple orchards across Latvia. During critical infection periods for apple scab, growers follow forecasts on the LPPRC web page and receive e-mails with fungicide coverage. Each of the farms using the RIMpro forecast are inspected twice per year during the growing season to evaluate the efficacy of the application program. In average, six yearly fungicide applications against apple scab have been necessary according to RIMpro, but whether or not to spray should always be a co-decision with the grower, the adviser and the decision support system.

Key words: DSS, RIMpro, relative infection measure program, fungicides, biofix