Reduction in the use of fungicides in apple and sour cherry production by preventative methods and warning systems

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Content

Preface

The research project presented in this report was carried out at Aarhus University, Department of Food Science and University of Copenhagen, Department of Plant Biology and Biotechnology as a part of the research project "Reduction in the use of fungicides in apples and sour cherry production by preventive methods and warning systems" and financed by the Danish Ministry for Environment, Environmental Protection Agency.
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Sammenfatning og konklusioner

Formålet med dette projekt var at belyse hvorledes forbruget af fungicider i den danske æble- og surkirsebærproduktion kan reduceres ved brug af præventive metoder og ved at udvikle beslutningsstøttesystemer (DSS) til æbleskurv (***Venturia inaequalis***) og kirsebærbladplet (***Blumeriella jaapii***). Danske æbler dyrkes hovedsagelig til frisk konsum og havde i 2009 et total udbytte på 24.000 tons. Surkirsebær dyrkes hovedsageligt til industri, og i 2009 blev der produceret 14.820 tons.

Æbler og surkirsebær er højværdiafgrøder, hvor det er vigtigt at sikre produktkvaliteten ved at undgå angreb af skadevoldere Æbledyrkningen havde det højeste forbrug af pesticider inden for frugt- og bærsektoren med et behandlingsindex på 24,7 - 27,1 i gennemsnit over de tre år 1998 til 2000. Fungicidforbruget bidrog med et behandlingsindex på 20. Surkirsebær havde et behandlingsindex på 11 som gennemsnit af 1998-2000 og fungicider bidrog med et index på 8. En reduktion af fungicidforbruget i æbler og surkirsebær vil derfor bidrage væsentligt til en total pesticidreduktion i disse afgrøder i Danmark.
Projektet havde som delmål at undersøge
Æbleskurv (***Venturia inaequalis***):

1. Udslyngning af ascosporer i relation til lokale klimadata.
2. Virkningen af kvælstoftilførsel på udviklingen af æbleskurv på frøplanter i klimakammerforsøg og på frugtbærende træer i en æbleplantage.
3. Virkningen af kvælstoftilførsel på vækst, udbytte og frugtkvalitet for æblesorten 'Elshof'.
4. Optimering af DSS RIMpro til æbleskurvbekæmpelse under danske klimaforhold. Specifikt for at finde det bedste årlige startpunkt for simulering af infektions risici for æbleskurv.

Kirsebærbladplet (***Blumeriella jaapii***):

1. Kvantificering og udslyngning af sporer til etablering af primære infektioner.
2. Identificering af vinter konidier som primært inoculum.
3. Udvikling af DSS RIMpro til kirsebærbladplet. Specifikt for at finde det bedste årlige startpunkt for simulering af infektions risici for kirsebærbladplet.

Undersøgelserne viste, at stor tilførsel af kvælstof øgede risikoen for infektioner af æbleskurv, fordi der blev produceret flere sporer og fordi planterne var mere modtagelige for angreb. Men hvis det årlige forbrug af kvælstof blev holdt på 100 kg kvælstof per ha eller mindre i år med få primære æbleskurv infektioner var risikoen for skurvinfektioner meget lille. Tilførsel af kvælstof reducerede frugtfarven og en tilførsel på over 50 kg kvælstof per ha øgede ikke udbyttet eller frugtstørrelsen.

DSS RIMpro blev optimeret til danske forhold ved at Biofixpunktet 'første modne ascosporer' og ikke 'grønspids' blev brugt til simulering af infektions risici. Brugen af 'første modne sporer' som Biofix kræver en årlig bedømmelse af hvornår sporer er modne i det nedfaldne løv. I to ud af 85 regnperioder (2,4 %) underestimerede RIMpro mængden af ascosporer ved en potentiel

kritisk infektionsepisode. For at kunne bruge RIMpro optimalt er det nødvendigt at have mulighed for at benytte kurative fungicider. I de primære infektions perioder i 2007, 2008 and 2009 blev der totalt udført 19 forebyggende behandlinger mod æbleskurv. Hvis det havde været muligt at bruge kurative fungicider og DSS RIMpro med 'første modne ascosporer' som Biofix ville kun 3-4 behandlinger være blevet varslet. Brugen af dette system ville have betydet en reduktion på 79 % i fungicidforbruget til bekæmpelse af æbleskurv i de primære æbleskurvinfektionsperioder. Et perfekt DSS ville kun have varslet én nødvendig behandling.
For at forbedre det nuværende DSS system vil det være nødvendigt at se på andre metoder til at monitere ascosporeudslyngningen af ***Venturia inaequalis*** for at optimere modellen, der beskriver modning og udslyngning. De anvendte ascosporefælder ændrer sandsynligvis klimaet for bladene, hvorfra ascosporerne moniteres. Desuden bør udtagningsproceduren for blade, som udvælges til sporefælderne optimeres, således at de udvalgte blade bliver repræsentative for den aktuelle svampepopulation.
Projektet har bidraget med ny viden om ***Blumeriella jaapiis*** biologi. Vinterkonidier fra ***Blumeriella jaapii*** blev i projektperioden udslynget med samme mængde og under samme klimatiske forhold som ascosporer. Nogle vinterkonidier var større end tidligere beskrevet, men molekylære undersøgelser bekræftede, at de var ***B. jaapii*** sporer. Betydningen af vinterkonidier i infektionsprocesserne for kirsebærbladplet er stadig ukendt og bør belyses nærmere. For til fulde at forstå den primære infektionsproces for kirsebærbladplet er det nødvendigt med yderligere undersøgelser af svampens biologi.

Bedste frugtavlerpraksis til at bekæmpe kirsebærbladplet er ikke tilfredsstillende. En første version af et DSS for kirsebærbladplet er blevet udviklet i projektet. Der bruges 0,2 mm regn til at starte en udslyngning af ascosporer og 'første modne ascospore' bruges som det bedste nuværende Biofix. For at kunne bruge dette system er det nødvendigt at have årlige undersøgelser af hvornår ascosporerne er modne. Før modellen bør bruges i praksis, mangler der stadig basisviden om svampens biologi, samt en validering af systemet.
For at kunne bruge DSS RIMpro er det nødvendigt at have opdateret software, en computer og rådighed over lokale klimadata.

Summary and conclusions

The overall objective of the project was to reduce the use of fungicides in apple and sour cherry by optimizing preventive methods and by developing decision support system (DSS) for apple scab (***Venturia inaequalis***) and cherry leaf spot (***Blumeriella jaapii***).
Apple production in Denmark is mainly grown for fresh consumption on 1,681 ha, with a production of 24,000 tonne in 2009. Sour cherries were grown on 1,875 ha with a total production of 14,820 tonne mainly for juice (2009).

Apples and sour cherries are high value crops; where it is very important to secure the production by avoiding disease infections. Apple production had the highest use of pesticides in the fruit and berry sector with a treatment index of 24.7-27.1 as an average over three years 1998-2000. Fungicides made the major contribution with a treatment index of 20. Sour cherry production had a total pesticide treatment index of 11 as an average of 1998-2000, with fungicide treatments contributed with an index of eight. Reducing fungicide application in apple and cherry orchards will therefore substantially contribute to pesticide reduction in these crops in Denmark.

Research came out in this project includes:
For apple scab (***Venturia inaequalis***):

1. The ascospore discharge in relation to local climatically data.
2. The effect of nitrogen supply on the development of apple scab on seedling in the growth chamber and on mature apple trees in an apple orchard.
3. The effect of nitrogen supply on growth, yield and fruit quality of apple cv 'Elshof'.
4. Optimising the decision support system (DSS), RIMpro for apple scab control under Danish conditions. Especially on finding the best starting point (Biofix) for the seasonal simulations of the apple scab infection risk.

For cherry leaf spot (***Blumeriella jaapii***):

1. Quantifications and discharge of spores causing primary infections.
2. Characterization of winter conidia as primary inoculum.
3. Development of a DSS for cherry leaf spot. Especially on finding the best starting point (Biofix) for the seasonal simulations of cherry leaf spot infection risk.

The project documented that a high supply of nitrogen increased the risk of apple scab infection as more spores were produced and the trees were more susceptible. However, if the annual supply of nitrogen was kept at 100 kg nitrogen per ha or lower, the risk for increased apple scab infection risk was low in years with few primary infections of apple scab. Nitrogen supply reduced the amount of red skin surface on apples and a nitrogen supply higher than 50 kg per ha, did not increase fruit yield or fruit size.
The DSS RIMpro was improved for Danish conditions, as the use of 'first mature ascospores' and not 'greentip' as Biofix optimised the infection simulations. The use of 'first mature ascospores' as Biofix needs a yearly

evaluation of the start of the ascospore discharge. In 2 out of 85 rain events (2.4%), the RIMpro DSS underestimated the ascospore discharge in potentially critical situations. The access to curative fungicides is important to optimize the use of the RIMpro DSS. In the primary apple scab season of 2007, 2008, and 2009 in total 19 apple scab treatment were carried out by spraying preventive treatments. If curative fungicides and the RIMpro DDS with 'first mature ascospore' as Biofix had been available, only three to four treatments would have been applied. Therefore, use of the developed system would have lead to a 79% reduction in the fungicides used against apple scab in the primary season. A fully optimised DSS would only have shown one infection event that needed fungicide treatment.

To optimise the simulated ascospore discharge used for DSS RIMpro, it is necessary to improve the methods to monitor the ascospore discharge of ***Venturia inaequalis*** aiming at improvement of the models that describe the maturation and discharge of ascospores. The currant use of spore trap is likely to contribute to inaccurateness, as there are climatic differences between ascospore discharge in the spore traps and nature. The sampling method for leaves used for spore trapping should also be further investigated.
For cherry leaf spot (***Blumeriella jaapii***), winter conidia were caught at the same amount and circumstances as ascospores. Some winter conidia were larger than earlier described in the literature, but a molecular characterization verified that they were ***B. jaapii*** spores. The role of winter conidia in the infection process for cherry leaf spot is still unknown and should be investigated further. To understand the primary infection processes in cherry leaf spot more basic research in the disease biology is needed.
Best practise to control infection and disease development of cherry leaf spot is in sufficient. A first version of a DSS for cherry leaf spot has been developed using 0.2 mm rain to trigger the start of ascospore discharge and 'first mature ascospores' as the at present best known Biofix. Annual investigations of the start of spore discharge are needed to determine the best Biofix for the simulations of cherry leaf spot infections. This DSS prototype needs additional basic knowledge of the biology of ***Blumeriella jaapii*** and practical validation before it can be used in practise.
To use RIMpro, updated DSS software, a PC, and access to local weather data is necessary.

1. Introduction

1.1. Aim

To reduce the use of fungicides in apples and sour cherries by:

1. Developing IT-based decision support systems for apple scab and cherry leaf spot for Danish conditions.
2. Investigating the importance of winter conidia as a primary infection source for cherry leaf spot.
3. Investigating the timing and level of ascospore discharge in relation to meteorological data.
4. Starting research in monitoring of the spread of winter conidia for cherry leaf spot.
5. Studying the importance of nitrogen level on susceptibility of apple scab.

1.2 Hypotheses

1. The biology of ***Blumeriella jaapii*** and ***Venturia inaequalis*** is similar with respect to epidemiological characteristics. Therefore, RIMpro, a decision support system developed to manage apple scab in apple production will be adjusted for controlling cherry leaf spot on sour cherries. A similar warning program for cherry leaf spot, as for apple scab, with the use of the same meteorological data will be developed. However, it is necessary to monitoring the primary infection source for the two diseases.
2. Use of reliable warnings systems will contribute to a reduced and optimised use of fungicides in apple and cherry orchards.
3. Using less nitrogen fertiliser will reduce the level of apple scab infections in an apple orchard.
4. Winter conidia from ***Blumeriella jaapii*** causing cherry leaf spot are important as a primary infections source.
5. The outcome of this project will result in 20 to 25% reduction in the fungicide use in intensive apple and sour cherry production.

1.3. Fungicide use in Denmark

In 1995 to 1999, pesticides contributed to 10.7% of the total product value in the fruit and berry sector. This is the highest pesticide use in the horticultural sector in Denmark (Ørum and Christensen, 2001). Apple production had the highest use of pesticides with a treatment index of 24.7-27.1 as an average over the three years 1998-2000. Fungicides contributed with treatment index of 20 (Lindhard et al. 2003; Henriksen et al. 2003). Sour cherry production had a total pesticide treatment index of 11 as an average of 1998-2000 and fungicide treatments contributed with an index of 8 (Lindhard et al. 2003; Henriksen et al. 2003). Reducing fungicide application in apple and cherry orchards will therefore contribute substantially to pesticide reduction in these crops in Denmark.

To secure the production and the product quality it is very important to avoid diseases, as one initial infection in spring may initiate an epidemic which may destroy the whole crop. In such high value crops, it is difficult to reduce pesticide use. However, consumers are demanding a reduction in the risk of pesticide residues on food products. In apple production systems without the use of pesticides, the production may be reduced by 40-100% depending on the cultivar (Lindhard et al. 2003). The production of unsprayed or organic sour cherries is unattractive to the grower as the risk of disease is very high during the production season and resistant cultivars do not exist (Korsgaard and Lindhard Pedersen, 2007).

1.4. Apple scab caused by *Venturia inaequalis*

1.4.1. Introduction

Apple scab, caused by the ascomycete ***Venturia inaequalis (***conidial stage: ***Spilocaea pomi***), is worldwide the most important disease in the production of apples as measured by the potential economic loss, the cost and the amount of fungicides necessary to control the disease each year, the constant consideration of the growers, and the environmental impact of the control measures (MacHardy,1996). Apple scab occurs wherever apples are grown. It is usually not possible to produce apples commercially without an effective fungicide programme to control apple scab. If not controlled, the disease can cause extensive losses when humid and cool weather occurs during the spring. Losses result directly from fruit infections, or indirectly from reduced assimilation or defoliation, which reduces tree growth and yield.

Apple scab affects leaves, petioles, blossoms, sepals, fruit, pedicels, and rarely twigs (Jones and Aldwinckle, 1990). The spots are most noticeable on leaves (both upper and lower side) and fruit. The first symptoms of the disease are tupically found on emerging cluster leaves. Lesions first appear as olive-green spots with indefinite borders. With age, these spots become greenish-black with a velvety appearance, collared by the numerous emerging conidia. The number of lesions can vary from very few to several hundred per leaf. Severely affected leaves shrivel and fall to the ground. Defoliation may result in a reduced flower bud formation reducing bloom or fruit yield the following year.

Spots on young fruit result in deformation and cracking. Severely infected fruit may drop off prematurely. Infections on fruit later in the season are often not detectable until after harvest when the fruit are in storage. This is referred to as "pin-point scab" or "storage scab".

1.4.2. Disease cycle and parameters for disease development.

Although research in both the USA and Europe has shown that the conidia of the scab fungus can overwinter in trees on bud scales, the pathogen generally overwinters in leaves on the orchard floor (Figure 1.1). Ascospores are the major source of primary inoculum and are produced within pseudothecia that develop in fallen leaves during the winter months. First mature ascospores are ready to be released at about the time of bud break. Ascospores continue to mature and they are discharged until after flowering.

Ascospores are liberated during rain, and in the first hours after rain. When rainfall begins at night, discharge is strongly reduced and delayed until daybreak. Ascospores germinate in a film of water on the surface of leaves and fruit. The time required for the spores to germinate and infect the host tissue depends on the number of hours of continuous wetness and the temperature during the wet period. Lesions appear about 9 to 17 days following the infection. Optimal temperatures for lesion development are 16-24°C (Jones and Aldwinckle 1990).

Once the fungus is established in the leaf or fruit, conidia are formed on the surface of the lesion and become the source of secondary inoculum for the remainder of the season. Conidia are dispersed by rain splashing onto developing leaves and fruit. Several secondary cycles of conidial infection may occur during the growing season.
Apple varieties differ in their susceptibility to apple scab. The genetic variability within the fungal population enables the population to adapt to the host population.

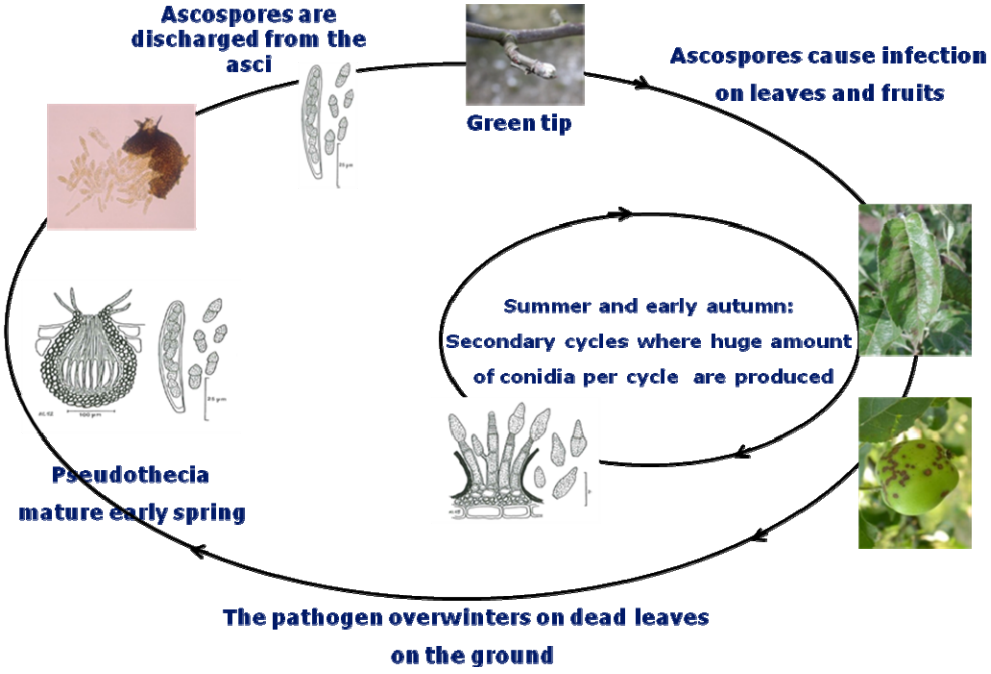

Figure 1.1. Disease cycle of apple scab caused by *Venturia inaequalis*.

1.4.3. Reduction and control

1.4.3.1. Resistant varieties

Scab resistant apple cultivars carrying resistance genes originating from several ***Malus*** species are commercially available. Most of these varieties carry the Vf-resistance gene from ***Malus floribunda*** (Machardy, 1996). In the integrated apple production, these varieties are hardly used. In the organic apple production in Europe their contribution can be as high as 30% of the yearly organic apple production (Korsgaard and Lindhard Pedersen, 2007). Most scab resistant apple varieties lack qualities compared to the standard commercial varieties in their production level, storability, taste and/or other aspects of consumer appearance. Some of these scab resistant varieties are relatively susceptible for other diseases such as powdery mildew, fire blight, ***Nectria*** or ***Gloeosporium*** fruit rot (Korsgaard and Lindhard Pedersen, 2007).

The apple scab fungus has overcome the Vf-resistance in most European countries. This means that this monogene scab resistance has become inefficient in most of the apple scab resistant cultivars available (Kühn and Pedersen, 2009). Newly released scab resistance cultivars are bred with polygene resistance. The general advice is to delay the loss of resistance by spraying with fungicides during spring in the primary apple scab infection periods.

1.4.3.2. Sanitation

The level of overwintering inoculum is an important factor in the success of the control of apple scab. High inoculum levels resulting from inadequate control in the previous year make it difficult to prevent infections. As the pathogen overwinters in leaves on the orchard floor, any measure that reduces the number of overwintering leaves on the orchard floor will reduce the inoculum and eases the control (MacHardy, 1996).

1.4.3.3. Fungicides

In most situations, repeated fungicide treatments are required during spring and summer every year to control the disease. Protective fungicides prevent the spores from germinating or penetrating plant tissue. To be effective, they must be applied to the surface of susceptible tissue shortly before infection occurs. Curative fungicides are capable of stopping the fungus when applied within a limited time after an infection has occurred.

In 2010, the Danish fruit growers had five fungicides available to control apple scab (Candit (Kresoxim-methyl), Delan WG (Dithianon), Kumulus S (sprøjesvovl), ND Mastana SC (Mancozeb), and Scala (Pyrumethanil) (http://planteapp.dlbr.dk/Middeldatabasen). Candit, Delan, and Scala have some curative effects. However, in practise Scala is mostly used against powdery mildew before 1 July and only three treatments can be applied per season as there is a high risk of as resistant (http://planteapp.dlbr.dk/Middeldatabasen).

1.4.4. Importance of nitrogen level for susceptibility for apple scab

In unsprayed apple production systems it has been shown that a reduction in the amount of nitrogen in the leaves reduced the susceptibility of the apples to apple scab (Kühn and Lindhard Pedersen 2009). The reason for this might be an increased content of phenols in leaves and fruit (Buchter-Weisbrodt, 1996; Leser and Treutter, 2005), or a reduced growth which causes a more open growth and thereby an increased evaporation from the leaves and a less favourable environment for the pathogen. In production systems with very low nitrogen availability, there is a risk that flower bud formation and fruit set and thereby the yield will be reduced (Lindhard and Bertelsen, 2002). The effect of sulphur to control apple scab increased in systems with low nitrogen supply compared to high nitrogen supply (Lindhard et al. 2005), and reduced nitrogen made the control of apple scab more effective.

1.5. Cherry leaf spot caused by *Blumeriella jaapii*

1.5.1. Introduction

Cherry leaf spot is caused by the fungus ***Blumeriella jaapii*** (conidial stage: ***Phloeosporella padi)***. Cherry leaf spot is, next to ***Monilia laxa***, the most important disease of both sweet, and sour cherries. Cherry leaf spot is

indigenous in the USA and Canada, but was first in reported in cherries in Europe around 1940 (Blumer, 1958) and in Denmark in 1948 (Neergaard, 1949). This disease has spread to all areas in Europe where cherries are grown (Jakobsen and Jørgensen, 1986).
The disease is particularly severe on sour cherries, and all commercial cherry varieties are susceptible to this disease (Wharton, 2003).

The disease mainly affects the leaves, but lesions may also appear on fruit, petioles, and fruit stems (Ogawa et al. 1995). Severely affected trees are often defoliated by midsummer, and produce dwarfed, unevenly ripened fruit with an inferior taste. Defoliation results in trees susceptible to winter frost, weak fruit buds, reduced shoot growth, and the death of fruit spurs or complete trees (Dutton and Wells, 1925).

One to three weeks after petal fall, the disease first appears as small, reddish-purplish spots on the upper surface of the leaves. These lesions rapidly enlarge, become brown or purple, and die from the centre out. Spots are irregular or round and occur over the entire leaf surface. Individual spots merge together to kill larger areas of the leaf. After six to eight weeks, the necrotic parts separate from healthy tissue and drop out, leaving a "shot-holed" appearance. During wet periods, cream-colored sticky spore masses appear in the centre of the spots on the lower side of the leaves. Infected leaves turn yellow and fall off. Entire trees can be defoliated before harvest. Symptoms similar to those on the leaves may also appear on leaf petioles and fruit pedicels, causing fruit to ripen unevenly. However, spots usually do not form on the fruit (Blumer, 1958).

1.5.2. Disease cycle and parameters for disease development

The fungus overwinters in dead leaves on the orchard floor (Figure 1.2). The ascospores are discharged during rainy periods for approx. six to eight weeks, starting at petal fall. The optimal temperature for ascospore discharge is 16°C and higher, with very few ascospores are discharged at temperatures below 8°C. These ascospores are spread by wind or splashing rain drops to the leaves. The ascospores stick to the leaf surface, germinate in a film of water, and penetrate the leaf through the stomata on the lower side of the leaves. Lesions appear on the upper leaf surfaces in about 10 to 14 days after the infection. The incubation period varies with temperature but can occur in as little as five days. Temperatures of 16-20°C are most favourable for disease development (Keitt et al. 1937). Sometimes the first sign of infection is on root suckers near the ground. The infection incidence in the primary cycle is limited and can easily be overlooked because the leaves are still small, the stomata on these leaves are still immature, and the temperatures are often low for efficient infection.

Once lesions have developed, during moist and rainy periods, many of summer conidia are formed from the acervuli on the lower side of the leaves. These conidia easily spread to other leaves by rain splash, causing severe secondary infections. Frequent rainfall during summer causes repeated secondary infection cycles leading to a rapid and progressive increase in the disease incidence, where eventually all leaves are infected, turn yellow, and drop.

Winter conidia are also produced in the overwintering leaves (Jakobsen and Jørgensen 1986; Pedersen and Hockenhull 1996; Bengtsson 2001; Bengtsson et al. 2006).

1.5.3. Importance of winter conidia for primary infections of cherry leaf spot

The life cycles of ***Blumeriella jaapii*** and ***Venturia inaequalis*** are very similar, but differ with respect to production of conidia during winter. In addition to 'summer' conidia the cherry leaf spot fungus also produces winter conidia in the overwintering leaves (Jakobsen and Jørgensen 1986; Pedersen and Hockenhull 1996; Bengtsson 2001; Bengtsson et al. 2006). The role of these winter conidia in the epidemiology of cherry leaf spot is unknown (Jones et al.1993; Bengtsson et al. 2006).

The ascospore discharge, as for ***Venturia inaequalis,*** is dependent on rain, temperature, and leaf wetness. The winter conidia are most likely spread by splashing raindrops as for the summer conidia, while the climatic conditions for discharge are very likely the same as for ascospores. To be able to develop a robust warning system for cherry leaf spot is it necessary to know the importance of the winter conidia as the primary infection source.

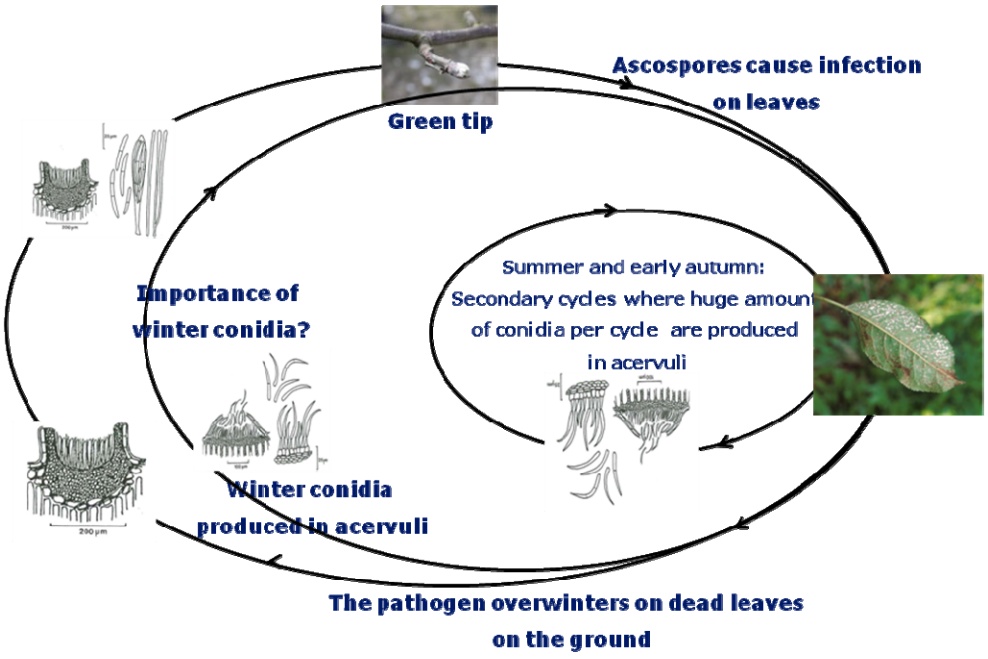

Figure 1.2. Disease cycle of cherry leaf spot (*Blumeriella jaapii*). Courtesy A.L. Jones and T.B. Sutton. http://www.google.com.

1.5.4. Reduction and control

1.5.4.1. Less susceptible varieties

Varieties differ in their susceptibility to cherry leaf spot, and ongoing research is focussed on identifying sources of resistance against this disease (Schulster, 2004). However, all commercial varieties of sweet and sour cherry are susceptible, and the application of fungicides is inevitable for an economically integrated or organic cherry production (Sjulin et al. 1989).

1.5.4.2. Sanitation

The fungus, as for the apple scab fungus, overwinters in dead leaves on the orchard floor. Improving leaf decay, and removing or destroying infected leaves before the new leaves emerge in spring are an important part of an integrated control strategy. The application of urea on the fallen leaves may help to reduce the inoculum (Bengtsson et al. 2006).

1.5.4.3. Fungicides

Fungicides are the primary means for managing cherry leaf spot. It is not difficult to control in sweet cherries, whereas control in sour cherries may be more challenging as these are more susceptible. Fungicide applications are mostly scheduled according to tree phenology, without considering the infection biology of the fungus. With minor variation, the following timing for fungicide applications has been recommended since 1919:

1. Petal fall or when the husks or flower remnants begin to split (shuck-split);
2. Ten to twelve days after shuck-split;
3. Immediately after the fruit is picked;
4. Ten to fourteen days later.

The first sprays are timed to prevent infection by ascospores. The next three sprays are aimed at preventing infection from both ascospores and conidia. The postharvest sprays protect the leaves from dropping prematurely.
In 2010, The Danish fruit growers had two fungicides to control cherry leaf spot (Delan WG (Dithianon) and ND Mastana SC (Mancozeb)). Delan may also have some curative effect (Anon, 2010).

1.6. Disease simulation models

1.6.1. Disease simulations models for apple scab

Mills Table (Mills, 1944) was the first initiative achieves rational use of fungicides against apple scab. Mills Table determined the minimum conditions of temperature and leaf weatness required for apple scab infection, and related ascospore discharge to weather conditions (Gadoury and MacHardy, 1982; Schwabe et al. 1989).

The first generation of simulations model were developed in the 1970s, they were not supported by a validation process and were written in obsolete computer language including EPIVEN by Kranz et al. (1973), Analytis (1973), VISIN by Jones (1978), APPLESCAB by Arneson et al. (1979) and the Minogue (1978) simulator.

The second generation of models were run on a PC or were associated to meteorological stations. Often information on model structure and algorithms was lacking and only outputs were provided. Examples are: Seem simulator by Seem et al. (1989), Biomat by Hofmaier (1994) and Mety by Boshuizena and Verheyden (1994).

Two systems are now widely used in Europe; 1: VENTEM as described by Xu and Butt (1993), which is also included in ADEM (Berrie and Xu, 2003) together with other apple diseases, and then included in MORPH, Methods of Research Practice in Horticulture, which is a support system for English growers (Rossi et al. 2007). 2: RIMpro elaborated by Trapman (1993), RIMpro is written in Visual Basic and has several improvements and updates. The program is described in technical literature but its algorithms are not known (Rossi et al. 2007). RIMpro has been widely validated in Europe (Trapman and Polfliet, 1997; Mattedi and Varner, 2000; Spanna et al. 2002), and RIMpro is now used by many fruit growers and advisory systems in Europe and Chile.

Rossi et al. (2007) developed an open apple scab simulation model called A-scab. The model combines information from the literature and actual biological data, it is completely open as the structure and algorithms are published and accessible. The model is not written in a special computer language but uses simple electronic sheets and the model has been validated under different epidemiological conditions (Rossi et al. 2007).

1.6.1.1. The decisions support system RIMpro

A decision support system (DSS) is supposed to collect, organize and integrate all types of information related to the production of a crop, to subsequently analyze and to interpret the information, and eventually recommend the most appropriate action or action choices (Agrios, 2005). RIMpro is developed by Marc Trapman (Trapman, 1993; Trapman, 1994; Trapman and Polfliet, 1997) and RIMpro is based on simulation models for main pests and diseases in apple and wine production systems. The models are driven by weather data gathered by on-farm weather stations, and weather forecast data. In addition to the Apple Scab module, RIMpro contains models for Sooty Bloch, Codling moth, Fire blight, and a first version of a model for Downy Mildew. In all cases, the models were developed in close collaboration with a project or expert group on the pest or disease in question, and with the potential end-users of the DSS. RIMpro is updated annually following progress in our knowledge and experience, as well as technical developments and possibilities.

RIMpro was originally developed as a single stand alone software. Following the needs of advisory services using RIMpro, the advisers-version of RIMpro was developed that allowed automatically weather data and weather forecast to be downloaded, and results to be uploaded to a website (as an example see: http://www.fruitweb.info/p_denmark.php).
RIMpro is widely used by advisory services and individual fruit growers (http://www.fruitweb.info/prog_scab.php). In Denmark, the advisory system has worked with RIMpro several years to obtain a better apple scab control. Both traditional and organic growers in Denmark and many other European countries (Italy in south to Norway in the north) use this system. RIMpro is used by around 500 individual fruit growers, and around 30 private and governmental advisory services in Europe to improve their decision making in crop protection.

To run the RIMpro program a Biofix (starting point) and local weather data is needed to initiate the calculations. Biofix is the date where the first ascospores in nature are observed to be mature and ready for discharge at the next rain event. This moment can be established accurataely by making regular observations of the development of pseudothecia under a microscope, or by wetting leaves and monitoring the ability of the pseudothecia to eject spores. The Biofix date can also be defined from the first ascospore projection as monitored in the field. In this case Biofix should be set a few days before the date where the first spores were observed. If none of this information is available, Biofix is set to the date of green tip of the main apple variety, presuming a similarity between development of the fungus and the host due to co evolution. In most apple production regions the Biofix date is found to be a date within a few days of green tip of apple. Recent research by Philion et al. (in press) suggested that mild, wet conditions in the last months before green tip led to the start of ascospore discharge before green tip, whereas dry condition pre budbreak led to the start of discharge after the beginning of vegetation of the host.

Danish trials have shown that the RIMpro was not completely adapted to climatic conditions in the spring (Lindhard Pedersen et al. 2005; Lindhard et al. 2006), which is an important period for apple scab control. If the control of the primary infections in the spring is efficient, the control is much simpler in the rest of the season, as the infection sources for the secondary infections are controlled. The pattern of the ascospore discharge appears to be different in the long cool Danish spring compared to other countries (Lindhard Pedersen et al. 2005; Lindhard et al. 2006). Therefore, it is necessary to adjust RIMpro to the Danish conditions, especially for the ascospore discharge pattern and the setting of the Biofix.

1.6.2. Disease simulations models for cherry leaf spot

A decision support system for cherry leaf spot was developed by Eisensmith and Jones (1981a; 1981b) in Michigan. They developed an empirical model for the prediction of infection efficiency based on temperature and humidity. The model is used by the extension services in Michigan and in several other states in the USA for the timing of fungicide applications, but has not been adopted by advisory services in Europe.

A warning system for cherry leaf spot has not been used in practise in Denmark. However in 1992-1995, Lindhard Pedersen and Løshenkohl (1997) conducted preliminary experiments, where they evaluated curative sprays at two warning levels; at low and high disease infection risk. The low level, sprays were applied on average three to nine times each year over a four-year period. Sprays at a high level reduced the number of sprays from one to four times yearly in the same period. In three out of the four years, the high spray level had sufficient control. In the year without sufficient control with high level sprays, the reduced disease control was due to a warning too close to harvest, where spraying is not allowed due to spray limits. This spray was not carried out, however caused infections. If a DSS should be used in practice, a special strategy is needed close to harvest (Lindhard Pedersen and Løschenkohl, 1997). This warning system has never been used due to a lack of user-friendly software and a lack of knowledge about the primary infection sources.
The existing warning system against apple scab combined with increased restrictions on pesticides use has increased the interest and the possibilities to develop a warning system against cherry leaf spot.

2. Materials and methods

2.1. Primary inoculum

2.1.1. Quantification of conidia and ascospores of *Blumeriella jaapii* on overwintering leaves

Quantification of ascospores and winter conidia and estimation of spore ratios were carried out in a pre-study in 2007 and continued in 2008 and 2009. In 2007, leaves infected with the cherry leaf spot fungus were collected from sour cherry trees (***Prunus cerasus*** cv. 'Stevnsbær') from an orchard at AU-Aarslev in the middle of October 2006. The leaves were placed on a nylon net (net size 0.65 mm^2) mounted on a wooden frame (50 x 50 cm), maintained with chicken wire and flower sticks (see Figure 2.1) and overwintered in a cherry orchard until spring 2007. In the middle of May 2007 a few lightly dried leaves were sampled and stored at 4°C until use. Primary inoculum of ***B. jaapii*** including washing-off of spores and quantifications was carried out according to a protocol modified after Bengtsson ***et al.*** (2006). Before washing-off spores, the leaf stems were removed and the leaves were divided by hand into four portions of 0.5 g (approx. seven leaves) and placed in 100 ml Erlenmeyer flasks. Five ml distilled water and one drop of tween-80 were added to each flask. The effect of soaking time was examined by allowing half of the leaf samples to incubate for 24 hours at 4°C and thereafter 1½ hour at room temperature, and these were compared with the remaining half of the leaf samples, which were only incubated at room temperature for 1½ hour. Thereafter, for each sample, 15 ml-distilled water was added, and the samples were shaken for one hour in a shaker (50 rpm/min). The samples were filtrated through three layers of gauze into clean 100 ml Erlenmeyer flasks, the leaf remains from each filtrate were further washed for 15 min in 10 ml distilled water, and again filtrated through gauze, and added to the first solutions. The samples were centrifuged for 15 min at 10,000 rpm (Beckman JA centrifuge). The pellet was dissolved in 2 ml of water and the number of ascospores and winter conidia were quantified under a microscope using a Fuchs-Rosenthal hemocytometer (quantified from five samples of 2 x 2 large squares). The number of ascospores and winter conidia are presented per gram leaf material and the ratio of ascospores and winter conidia were estimated.

Based on the results of the preliminary 2007 study, conidia and ascospores in spring 2008 and 2009 were quantified as described above. Samples of approx. 400 leaves infected with the cherry leaf spot fungus were picked from sour cherry trees (***Prunus cerasus*** cv. 'Stevnsbær') in an orchard at AU-Aarslev in the middle of October 2007 and 2008. In both years, the leaves were stored in mesh bags, hung in the tree crowns of sour cherry trees until the beginning of November, and thereafter placed in overwintering stores as described above. The overwintering took place until spring, where leaves were sampled following rain events.

Figure 2.1. Sour cherry leaves with cherry leaf spot kept in storage (left) and placed over winter under natural conditions at KU-LIFE (right).

2.1.2. Inoculum discharge

2.1.2.1. Ascospore discharge of Blumeriella jaapii and Venturia inaequalis

Unsprayed leaves of cv. 'Stevnsbær' infected by ***Blumeriella jaapii*** and leaves from apples cv. 'Jonagold' infected by ***Venturia inaequalis*** were picked in autumn just before leaf drop. The leaves were placed on vatex matting in wooden frames under apple or sour cherry trees, and held in place with chicken netting (Figure 2.1). Before bud break a few leaves were placed in a Wiesmann ascospore trap (Figure 2.2). The trap consisted of an aluminium slide holder that held standard microscope slides 5 mm above a bed of leaves. During rain, the leaves were wetted and the pseudothecia project their ascospores on the lower side of the glass slides. The spore trap was put directly on the soil, under the cherry or apple trees. After each rain period in the primary infection period the slides were exchanged. At each date the number of ascospores was counted on four slides randomly selected out of eight, using 200 time's magnification. Each slide was divided into 5 tracks, which were then divided into 4 sections, and spores were counted in all 20 microscope compartments. The counting was carried out until no more spores were discharge after rain events. If the rain period was more than one day, the number of ascospores was counted as discharged on the last day in the rain event with at least 0.2 mm of rain.

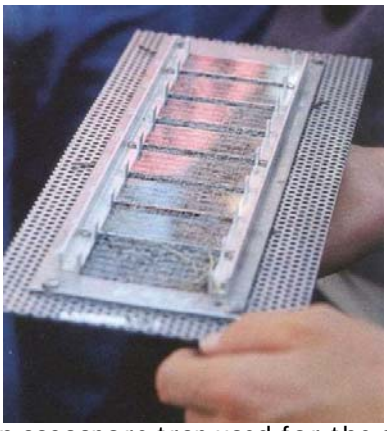

Figure 2.2. The Wiesmann ascospore trap used for the observations.

2.1.2.2. Discharge of potential winter conidia from Blumeriella jaapii

During the counting of ascospores from ***B. jaapii,*** large numbers of potential winter conidia were observed. The unknown spores had certain similarities to winter conidia from ***B. jaapii.*** These spores were also counted in 2008, 2009 and 2010. From each rain event the number of potential winter conidia spores was counted on four randomly selected slides out of eight using 200 times magnification. Each slide was divided into 5 tracks, which was then divided into 4 sections, and spores were counted in all 20 microscope compartments. The counting was carried out until no more spores were discharged after rain events.

Discharge of winter conidia by rain splash

In order to study the possible dispersal pattern of winter conidia by water splashes, a simple experimental setup was developed as outlined in Figure 2.3. Cherry leaves with clear cherry leaf spot symptoms were soaked overnight in water. Leaf material with acervuli was placed under a drop device. Conidia dispersed as a result of drops landing on the leaf, conidia attached themselves to a vertically placed slide coated with a thin layer of Vaseline. Conidia on each of four slides were counted under the microscope and the height of where they attached on to the slide was noted. The average number of dispersed conidia and the average dispersal distance were calculated.

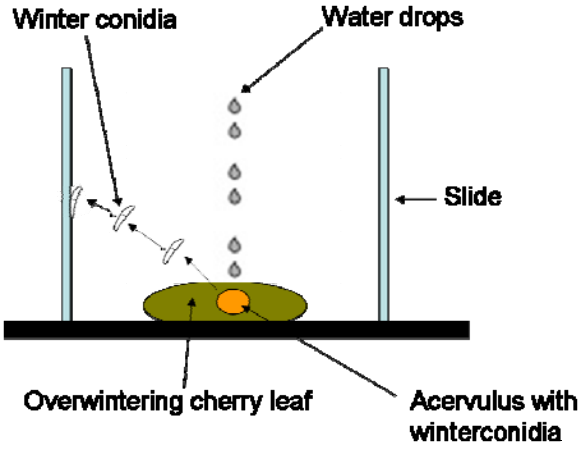

Figure 2.3. Experimental setup to examine dispersal of winter conidia by water splash.

2.1.2.3. Characterization of winter conidia from Blumerielle jaapii

Atypically long spores were regularly trapped on exposed glass slides in the sour cherry orchard. In order to determine whether these spores were winter conidia of ***B. jaapii***, molecular and morphological characterizations were carried out.

Molecular characterization

Specific detection of ***B. jaapii*** was based on amplification, sequencing, and alignment of the ITS1-ITS2 sequence. The primers shown in Table 2.1 were used to amplify the ITS1, 5.8S, and ITS2 sequence of two ***B. jaapii*** isolates and spores collected from the spore traps at AU-Aarslev.

Two single spore isolates of ***B. jaapii*** were obtained from acervuli on cherry leaves. MB364 was isolated from sour cherries ***Prunus cerasus*** cv. 'Stevnsbær' collected at AU-Aarslev and MB371 was isolated from cv. 'Stevnsbær' collected at the Pometum at University of Copenhagen, Høje Tåstrup, DK. Since ***B. jaapii*** is a very slow growing fungus and contamination can be problematic, different growth substrates were tested to optimise mycelium

production of the fungus. Six solid media were tested: potato dextrose agar (PDA), PDA+Chloramphenicol, PDA+ Streptomycin, PDA with a cellophane overlay, modified Melin-Norkrans (MMN) at pH4 (Chalot et al., 1994), and cherry leaf decoction agar (CheA) after Bengtsson (2002). Furthermore, two liquid media were tested: potato dextrose broth (PDB) and CheB (CheA without bacto agar). Based on the growth tests two media; PDA+cellophane and CheA were used for production of mycelium for DNA extraction simply by scraping off the mycelium. DNA was extracted using the Illustra™, and DNA extraction Kit phytopure™. PCR was performed with primers listed Table 2.1. The total reaction volume was 20 µl and contained 10 pmol of each primer, 1 x standard PCR buffer (Amplicon), 200 µM dNTP, and 2.5 U Taq polymerase (Amplicon). To each reaction 1 µl genomic DNA (10-20 ng) was added. The PCR reaction was performed using a MyCycler thermal cycler (Biorad, Hercules, CA, USA). An initial denaturation at 94˚C for 5 min was followed by incubation of 40 cycles of: denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec, and extension at 72°C for 30 sec. The PCR was completed with a final extension step at 72˚C for 5 min. The PCR products were visualised on ethidium bromide-stained 1.5% agarose gels run in TBE buffer. Bands of appropriate size were ligated in the pGem T-easy vector (Promega, Madison, USA) and transformed into competent ***E. coli*** GC5 cells (Amplicon, Copenhagen, Denmark). Inserts were checked by PCR reactions using the Sp6 and T7 vector primers on single colonies lysed by boiling in TE-buffer for 5 min. PCR products of appropriate band size were sequenced using the sequencing service from MWG. The resulting sequences were blasted in NCBI and aligned to obtain the ITS1, 5.8 S, and ITS2 sequence of ***B. jaapii***. Published ITS1-5.8S-ITS2 sequences from Gen Bank were aligned with the Clustal W software. Species from the three families ***Dermateaceae, Sclerotiniaceae and Hemiphacidiaceae*** all belonging to the order ***Helotiales*** were selected. Sequences were trimmed to align comparable sequences. The phylogenetic analysis was performed in the program MrBayes 3.1.2. (Ronquist and Huelsenbeck, 2003) using the Bayesian Inference method (Holder and Lewis, 2007; Heulsenbeck and Ronquist, 2001). The data set was examined assuming the complex evolution modet (GTR+I+) and using Markov Chain Monte Carlo (Yang and Rannala, 1997; Larget and Simon, 1999) as the tree sampling procedure. The analysis was run for 2,500,000 generations and every 500th tree value stored. Clade credabilities (The Baysian Posterior Probability), which gives the probability for a certain branch point to appear, were estimated to support tree nodes. The distance measured shows the expected DNA base change per site. The tree was drawn in TreeView with the species of ***Hemiphacidiaceae*** as outgroup.

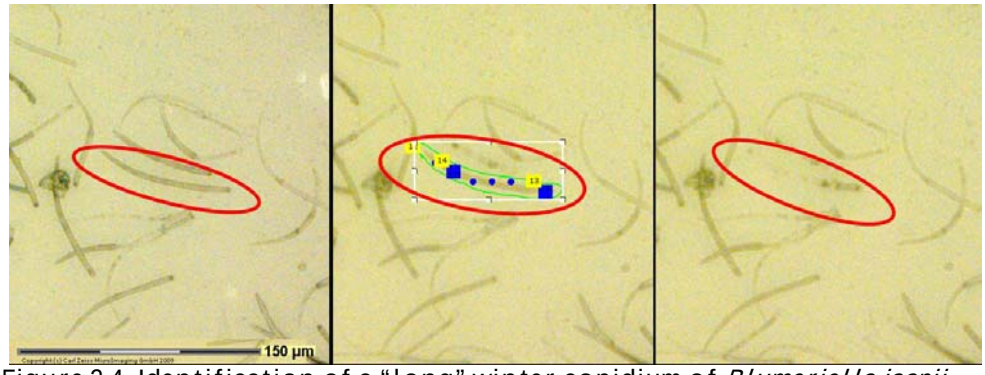

Figure 2.4. Identification of a "long" winter conidium of *Blumeriella jaapii* (left), marking of conidium (middle) and conidial material has been removed from the slide (right).

Spores (7-14 per sample) resembling the winter conidia type (2-3 µm x 40-80 µm or atypical 2-3 µm x 80-120 µm), were collected using a Zeiss PALM laser micro dissection system (Carl Zeiss Microimaging GmbH, Germany). Laser pulses lifted well-separated conidia that were marked (see Figure 2.4) from the slide and into a sterile 500 µl Micro Tube. Distinct conidia were marked and captured using the autoLPC of the PALM Robo software. In addition, DNA was extracted from winter conidia of ***B. jaapii*** by adding TE-buffer and boiling for 5 min. PCR, sequencing, and blasting was carried out as described above.

Table 2.1. Primer used to amplify the ITS1, 5.8S, and ITS2 sequence of *Blumeriella jaapii.*

Primer name	Sequence 5´→ 3´	Reference
ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
ITS2	GCTGCGTTCTTCATCGATGC	White et al. 1990
ITS3	GCATCGATGAAGAACGCAGC	White et al. 1990
ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
ITS1F	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns, 1993
FITSF1a	GCCTGTTCGAGCGTCATT	Ole Søgaard Lund, pers.com
FITSF2	ACCAGCGGAGGGATCAT	Ole Søgaard Lund, pers.com
FITSR1	TCCTACCTGATCCGAGGTCAA	Ole Søgaard Lund, pers.com
FITSR2	CTTGGTCATTTAGAGGA	Ole Søgaard Lund, pers.com

Morphological characterization

In order to assess the size of winter conidia, the length and width of conidia from acervuli developed on overwintering leaves were measured. Leaf pieces with the abaxial surface up were incubated on moist filter paper in moist chamber at 4˚C for 24 hours. Acervuli were identified using a dissection microscope and conidia were transferred from a separate acervulus to a glass slide using a sterile needle. Subsequently the conidia were mounted in aniline blue in water. Twenty conidia from each of three acervuli per leaf sample were measured (length x width) using a light microscope at x400 magnification. Conidia matching the morphological description of winter conidia (length 50-80 µm) and conidia(>80 µm), were measured on glass slides from the AU-Aarslev spore traps in 2009 and 2010.

2.2.1. Growth chamber experiments with *Venturia inaequalis*

Detailed studies of the effect of different nitrogen (N)-levels on apple scab development were conducted under controlled climatic conditions in growth chamber. The experimental setup was based on an apple seedling assay with plants being artificially inoculated with ***V. inaequalis*** conidia as described by Bengtsson et al. (2005 and 2006) and Hockenhull et al. **(**2005**).**

2.2.1.1. Production of inoculum

Conidia of a single spore isolate of ***Venturia inaequalis*** (MB363, isolated in 1998 from cv. 'Jonagold', AU-Aarslev were produced by the 'agar cellophane method' modified after Parker et al. (1995). Sterile cellophane membranes (diam. 8 cm) were placed on Potato Dextrose Agar (Scharlau Chemie, S.A.) in petri dishes and inoculated with a starter suspension of ***V. inaequalis.*** Cultures were subsequently incubated 10-14 days in darkness at 18°C. The spores produced were scraped into sterile water, filtered through polyester mesh, and centrifuged at 10,000 rpm (Beckman JA centrifuge) at 4°C for 30 min. The supernatant was discarded and the pellet consisting of spores was suspended in sterile water. The concentration was adjusted to 1.5 x 10^5

conidia per ml. The inoculum was stored in centrifuge tubes at –18°C until use.

2.2.1.2. Production of seed plants and optimization of growth medium and N application

Seeds of Malus ***domestica*** cv. 'Golden Delicious' and Malus ´Bittenfelder Sämlingr´ (Eichenberg Gehölzsamen GmbH, Miltenberg, Germany) were stored at 2°C. For stratification, seeds were primed under running tap water for 24 h, then surface sterilised for 1 min in 70% ethanol, 5 min in 2-3% sodium hypochlorite and finally rinsed three times in tap water. The seeds were placed in moist sand and incubated at 4°C for 3-8 weeks. Germinating seeds were sown in pots (5.5 cm diam.) containing the growth media described below.

Various compositions of growth media were tested in order to grow healthy plants at low N and high N levels. The following compositions were evaluated: sphagnum peat and sand with the ratios 4:1, 2:1, 1:1, 1:2 and 1:3, vermiculite and sand mixed 1:1 as well as all three media unmixed. Plants were placed on filter mats in trays, covered with plastic bags, and grown at 15-16°C (12 h light/12 h dark, Figure 2.5).

Nutrient solutions were applied either 14 or 21 days after the germinated seeds had been planted. For each tray holding 12 plants, 300 ml water was applied. The composition of the nutrient solution was modified after Jensen and Munk (1995). Each plant was supplied with 25 ml solution containing 0.02 ml of the following solutions: KNO_3 (202 g/l), $Ca(NO_3)_2$ (475 g/l), $MgSO_4$ (184 g/l) and NaH_2PO_4 (184 g/l). Thus, the low N-level corresponded to 2 mg N/pot. Medium N-level and high N-level was additionally supplied with NH_4NO_3 (32 g/l) resulting in 12 mg N/pot (medium N level) and 24 mg N/pot (high N-level). When needed, plants were watered with tap water. Plant growth at the different growth media and N-levels was continuously evaluated.

Figure 2.5. Apple seedlings grown in different growth media in climate chamber. Sand (left), sphagnum + sand 1:1 (middle) and sphagnum + sand 4:1 (right).

2.2.1.3. Inoculation of apple plants and disease severity assessment

Two weeks after application of the three nutrient solutions, twelve uniform plants with minimum 4 leaves were selected for each combination of growth media and N-level. Plants were placed on filter mats in trays and spray inoculated until run-off with a conidial suspension of ***V. inaequalis*** using a plastic handheld sprayer. Thereafter plants were immediately returned to the polyethylene cover for 48 h without light and under the same conditions as

described above. Light was again provided and holes were made in the cover to allow air exchange. Disease severity was assessed 14 days after innocultions (dai) on all individual leaves using the 0-7 scale derived from Croxall et al. (1952) and Parisi et al. (1993), see Table 2.2.

Table 2.2. Disease severity scale with symptom description.

Disease severity score	Symptom description
0	no visible symptoms
1	≤ 1% of scabbed leaf surface (sls);
2	1 <sls≤ 5%
3	5 <sls≤ 10%
4	10 <sls≤ 25%
5	25 <sls≤ 50%
6	50 <sls ≤ 75%
7	sls> 75%

The mean disease severity index was determined from the average score obtained from all inoculated leaves of each of the twelve plants per treatment.

2.2.1.4. Quantification of spore production on apple leaves

The spore production of diseased leaves was determined in two experiments 14 dai. Leaves (6 or 7 per plant) from each of twelve plants per treatment were sampled. The 6 or 7 leaves per plant were pooled, and cut into small pieces in 50 ml falcon tubes. After washing in 20 ml water with 0.01% tween and glass beads for 30 sec, the number of conidia in the washing fluid was counted with a Fuchs Rosenthal haemocytometer (three replications) and the spore production per plant for each treatment calculated. The diseases severity of the leaves were assessment before the spore production was measured.

2.2.1.5. Quantification of plant dry weight and plant N content

Plants, twelve per treatment, were harvested after14 days incubation at the different N-levels. Each replicate consisted of three plants. For dry weight determination, plants were cut above soil-level, packed in aluminium foil, and dried at 80°C for 24 h whereafter the percentage dry weight was calculated based on weighing before and after drying. Plants for N and carbon (C) quantification were harvested in a similar way, immediately frozen in liquid N, and subsequently stored at 20°C until analysis. The analysis was performed with Isotope ratio mass spectrometry (IRMS).

2.2.1.6. Quantification of disease severity using qPCR

Development of a qPCR method for quantification of ***V. inaequalis*** in leaves was carried out by TaqMan qPCR based on a method developed by Plant Research International, Holland (Jürgen Köhl, pers. com.). PCR was performed using the real-time PCR system Mx3000P (Stratagene, La Jolla, CA, USA).The primers TM-Vin FW/ TM-Vin-Rew and the probe TM-Vin-PR were kindly provided by J. Köhl). Each reaction of 20µµ µl contained 5 ng genomic DNA, 10 pmol of each primer, 5 pmol probe, and 10 µµµµl FastMix (Quanta).The PCR reaction was set up in duplicate for each sample. A standard series made with serial diluted pure ***V. ineapualis*** DNA of known concentration was included on each plate. Two PCR programs, namely 2-step and 3-step PCR, were tested to optimize the reaction. Two-step PCR was carried out with an initial denaturation step at 95°C for 10 min prior to amplification followed by 40 cycles of 15 sec at 95°C, 30 sec at 60°C.

Three-step PCR was carried out with an initial denaturation step at 95˚C for 10 min prior to amplification, followed by 40 cycles of 15 sec at 95°C, 30 sec at 58°C and 30 sec at 72°C. Fluorescence was detected after each cycle. Various combinations with 15 pmol of each primer, and 10 pmol probe were also tested.

As the TaqMan qPCR was unsuccessful, real time PCR based on SyBR was attempted. The primers TM-Vin FW/ TM-Vin-Rew were kindly provided by J. Köhl (Plant Research International, NL) were used to amplify DNA from ***V. inaequalis*** infected leaves. PCR was performed using the real-time PCR system Mx3000P (Stratagene, La Jolla, CA, USA). Each reaction of 25 µµµl contained 5 ng genomic DNA, 10 pmol of each primer, 12.5 µµµµl 2 x SYBR Green master mix (Stratagene) and 0.4µ µl of a 1000 x diluted reference dye (Stratagene).The PCR reaction was set up in duplicate for each sample. A standard series made with serial diluted pure ***V. ineapualis*** DNA of known concentration was included on each plate. The PCR was conducted as described for 3-step PCR above. Prior to amplification, an initial denaturation step was performed (95°C for 10 min.) This was followed by 40 cycles of 15 sec at 95°C, 30 sec at 58°C and 30 sec at 72°C. Fluorescence was detected after each cycle. After amplification, the melting curve was analysed to ensure amplification of only one PCR product. The PCR was performed on plant material sampled five days after inoculation with ***V. inaequalis***.

2.2.1.7. Data analysis

Data on disease severity, spore production, dry weight and N and C content were analysed by analysis of variance assuming a normal distribution. All analyses were run as one way anova with N-level as class variable and twelve replicats for each of the three N-levels. Variances were stabilised by appropriate transformation of data if necessary and means separated by LSD–values. Hypothesis were rejected at $P<0.05$. All data were analysed by PC-SAS (release 9.2; SAS Institute, Cary, NC).

2.2.2. Orchard

2.2.2.1. Experimental design

In spring 2004, two-year-old trees of the apple variety 'Elshof' on the rootstock M9 were established at AU-Aarslev (10^0 27´ E, $55^0$18´N), on a sandy loam soil, with a clay content of 11-15% at a planting distance 3.5 x 1.0 m. Every 14th tree was a pollinator. The pollinators were different cultivars of crap apple. Grass alleyways were established and herbicide treatment and drip irrigation (2 l water per tree per day) in the tree row was carried out.
In spring 2007, a split-plot design of apple control and nitrogen supply was established. Two similar sections of a ten-block design with totally randomised treatments of six levels of nitrogen were established. Each plot consisted of three trees separated by guard trees. Nitrogen application were divided evenly and supplied five times during the growing season: 1 April, 1 May, 1 June, 1 July and 1 August.
Treatments:

1. 0 kg Nitrogen
2. 25 kg Nitrogen
3. 50 kg Nitrogen
4. 75 kg Nitrogen
5. 100 kg Nitrogen
6. 200 kg Nitrogen

In one section apple scab was controlled by using the latest version of RIMpro decision support system. In the second section, apple scab was controlled using RIMpro decision support system but only in the primary ascospore season.

2.2.2.2. Disease assessment

Apple scab infections were analysed on all leaves on two annual shoots distributed on the west and east side of the tree in September 2007, 2008 and 2009. Leaves were graded in six classes: Leaf missing, no infections, 1, 2-4, 5-9 or >10 spots per leaves.

Infection severity (P) is calculated after Townsend and Heuberger (1943).

$$P = \frac{(\Sigma n*(v-1))*100}{(Vmax-1)*N}$$

Where:
n = number of leaves in the class
v = number of the class
Vmax = the first infection class
N = number of examined leaves

To estimate effects of the spraying and N supply on the decomposing of the leaves, the amount of overwintering leaves on the orchard floor was assessed early spring 2008. Each plot was scored using a scale from 1 to 9, where 1=no leaves left and 9=maximum leaves left.

2.2.2.3. Nitrogen and growth assessment

The content of inorganic N available for plants was determined in the 0-100 cm top soil layer in mid March 2007 and April, July and September 2007 and 2008. Eight sticks per plot were merged into one sample per plot. Nitrate-N and ammonium-N were determined calorimetrically according to slightly modified methods of Best (1976) and Crooke and Simpson (1971), respectively.

In each plot, leaf samples were collected four times during the 2007 and 2008 growing seasons in June, July, August and September 2007 and 2008 and one time during the 2009 season in August. At each time 50 leaves where collected from the centre part of the annual shoot from the three trees in each plot. The content of N was analysed according to standard protocols.
The diameter of the stem was determined 20 cm above the graft during winter in all years on all trees starting March 2007, before establishment of the treatments and ending December 2009. The trees were pruned as slender spindle.

2.2.2.4. Fruit yield and quality

Bloom, and thereby yield potential, was assessed during flowering in the spring of 2007, by giving a score per tree from 1-9, where 1=no flowers. In 2008 and 2009, yield potential was assessed by counting the total number of flower clusters per tree.

In 2007, 2008 and 2009 the actual fruit production was determined as number of fruit per tree, kg per tree and fruit weight. Using a colour grader fruit were graded into three classes due to their skin colour: less than 50% red skin, 50-75% red skin and more than 75% red skin. The internal fruit quality

was examined in cold stored fruit at three months after harvest, on five fruit from the centre tree in the plot. Fruit of the graded size 70-80 mm and a skin colour of 50-75% were selected for the quality evaluations. Fruit firmness was determined using a penetrometer (model FT 327) fitted with 11 mm tip and mounted in a drill press stand. Soluble solids were determined with a digital refractometer (RFM 330, Bellingsham + Stanley Ltd., Struers, DK).

2.2.2.5. Statistical analyses

The data on apple scab infections on leaves were analysed using a mixed model where the effects of treatments (main effects and interaction effects) were included as systematic effects. Blocks inside spraying treatment and individual are included as random effects. Statistical significance was defined at $p < 0.05$. Means were separated with LSD at the 5% level of significance. Based on the design, the tests for comparing spraying are nessesary but not suffficient for considereing the effect to be significant.
The data on scores on level of decomposed apple leaves on the orchard floor were analysed with GLM-procedure. Means separated with Duncans test and statistical significance defined at $p \leq 0.05$
The statistical analyses of data were performed using the procedure mixed of SAS (Version 9.2 www.sas.com). SAS Institute Inc. 2010. SAS/STAT®9.22 User's Guide. Cary, NC: SAS Institute Inc. Available Online at www.sas.com].

2.3. Development of RIMpro for apple scab and cherry leaf spot

2.3.1. Experimental design

The RIMpro DSS's are based on weather data driven simulation models for pests and diseases that are able to forecast phenological developments. To be able to build a simulation model there should be reliable information available on the biology and epidemiology of the organism in question. RIMpro is a dynamic population model made up of sub-processes that mimick the underlying biological developmental processes. Deterministic modelling and Continuous Simulation Modelling Programme (CSMP) is used.
Population models are, when all relevant information is known and incorporated, expected to be applicable for populations of the same organism under various geographical and climatic conditions. If missing information occurs, especially in the weather datasets, they are replaced with best possible guesstimate.

The limiting factor for the modelling is often due to information that is missing on sub-processes.

2.3.2. Onset of disease

2.3.2.1. Apple scab

To investigate whether the DSS for apple scab RIMpro is applicable to Danish conditions a trial in a 3-year-old planting of the apple cultivar 'Elshof' was established at AU-Aarslev. In a randomised block, design with four replicates, with plots of 10 trees, treatments against apple scab were carried out. All sprays were carried out using Delan WG (dithianon). The decision to spray against apple scab was made using RIMpro decision support system and carried out as a preventive spray just before expected infection of apple scab.

At every spraying event, one treatment with four replicates of 10 trees were left unsprayed to examine if this event caused apple scab infections. For controls, both total unsprayed trees and trees sprayed at every event were used.

In 2007, 2008 and 2009 when apple scab infections occured, apple scab (***Venturia inaequalis***) infections were analysed on all leaves on two annual shoot distributed on the west and east side of the tree. Leaves were graded in six classes: Leaf missing, no infections, 1, 2-4, 5-9 or >10 spots per leaf.

2.3.2.2. Cherry leaf spot

To develop a DSS for cherry leaf spot similar to the DSS for apple scab, it is important to know when infections occur. In spring 2007 a planting of cherry cv. 'Stevnsbær' on the rootstock colt, planting distance of 3 x 5 m, in a randomised block design with 4 replicates and plots of 5 trees was established at AU-Aarslev. Treatments against cherry leaf spot were carried out using Delan WG (dithianon). The decision to spray against cherry leaf spot was made by a cherry grower and carried out as preventive spray before expected infections. At every spray event one treatment with four replicates of five trees were left unsprayed to see if this event caused cherry leaf spot infections. For controls, both total unsprayed trees and trees sprayed at every event were used.

In 2007, 2008 and 2009 when cherry leaf spot infections occurred, cherry leaf spot (***Blumeriella jaapii***) infections were analysed on all leaves on two annual shoot distributed on the west and each side of the tree. Leaves were graded into six classes: Leaf missing, no infections, 1, 2-4, 5-9 or >10 spots per leaf.

2.3.3. Simulation of disease

Infection risk of ***Venturia inaequalis*** and ***Blumeriella jaapii*** were simulated using the DSS RIMpro, weather data, different starting point settings (Biofix), and actual data for ascospore and winter conidia discharge.
From 2007 to 2010, weather data was collected using a Campbell data logger at AU-Aarslev (10^0 27´ E, $55^0$18´N). The weather data included was temperature, relative humidity, precipitation, and leaf wetness. All data was measured in the canopy. RIMpro is a phenological model and needs a starting point (Biofix).

For modelling ***Venturia inaequalis,*** Biofix was set to first mature ascospores or greentip of the main apple cultivar 'Jonagold'.
For modelling ***Blumeriellea jaapii,*** three starting points were tested as possible Biofix:

1. First ascospore discharge observed in the field
2. Greentip for the crop
3. 1 January

Dispersed spores are necessary to initiate infections. For modelling both the actual number of ascospores and the number of ascospores plus winter conidia discharge are used to validate the simulation.
Complete leaf degradation was set in the model to 15 June to compensate for the situation that the leaf degradation in the spore trap was slow.

3. Results

3.1. Apple scab *Venturia inaequalis*

3.1.1. Ascospore discharge of *Venturia inaequalis*

Discharged ascospores of ***Venturia inaequalis*** were counted, Figure 3.1 shows the ascospore discharge of ***V. inaequalis*** from 2007 to 2010. The ascospore discharge started at the end of March and continued to mid June, occuring over 2-3 month. The average number of ascospores caught was approximately 32,000 per year with variation from 11,000 in 2009 to 58,000 in 2008 (Table 3.1). The number of rain events per year varied between 13 (2010) and 24 (2009).

Table 3.1. Ascospore discharge of *Venturia inaequalis* 2007- 2010.

Year	First spores caught (Date)	Last spores Caught (Date)	Total Number	Discharge period (Days)	Rain events with spores (Number)
2007	19 March	31 May	23,001	74	15
2008	13 March	16 June	57,863	96	21
2009	23 March	15 June	10,938	85	24
2010	29 March	31 May	13,036	64	13

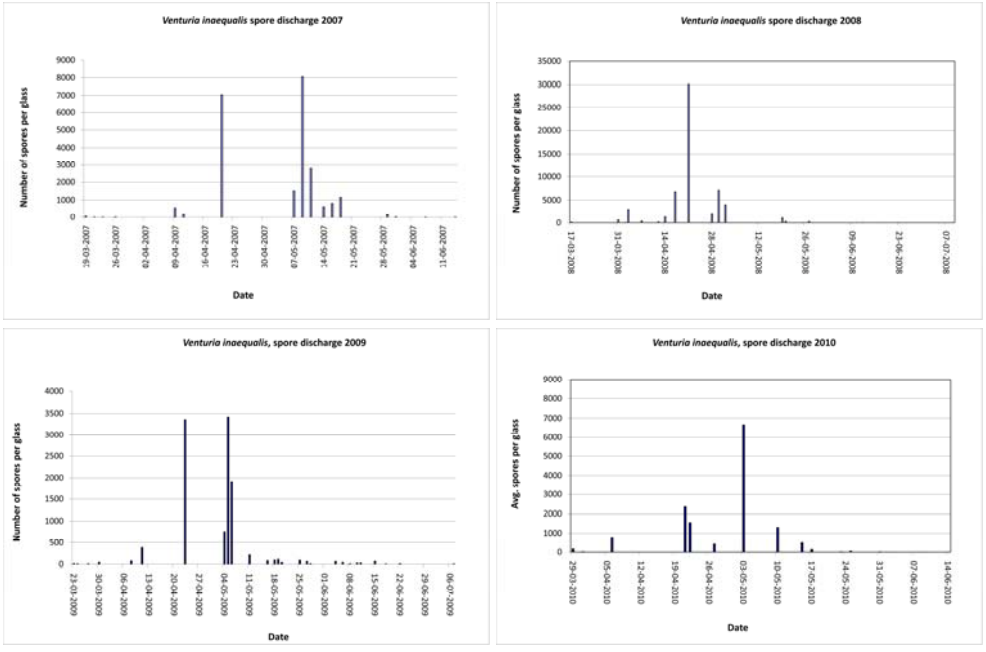

Figure 3.1. Discharge of ascospores from *Venturia inaequalis,* 2007 – 2010.

3.1.2. Effect of nitrogen on apple scab development

3.1.2.1 Growth chamber experiments with Venturia inaequalis

Various combinations of growth media (sand, vermiculite, and sphagnum peat based substrate) were tested in order to grow apparently healthy plants at low N as well as at high N levels. A mixture of sand and vermiculite resulted in very small and weak plants at low N-level (Figure 3.2) while plants grown in soil and sand mixtures (1:1, 2:1 and 4:1) grew well at all N-levels, although plants at high N were more dark green than those grown at the other N-levels (Figure 3.3).

3.1.2.2 Disease assessment

Inoculation of apple plants with ***V. inaequalis*** showed that the most reproducible effect of increasing N on scab severity in cv. 'Golden Promise' was obtained with a 1:1 mixture of sphagnum peat based growth substrate and sand (Figure 3.4). Therefore, this substrate mixture was used for all following plant assay with N application. However, due to a lack of high quality seed of cv. 'Golden Promise', seed from the cv. 'Bittenfelder Sämling' was used in the subsequent assays with N supply.

Figure 3.2. Apple plants of cv. 'Golden Promise' grown in a mixture of sand and vermiculite (1:1) at low nitrogen (N) (left), medium N (middle) and high N (right). Note apple scab development on the leaves (Photo: Marianne Bengtsson).

Figure 3.3. Apple plants of cv. 'Golden Promise' grown in a mixture of sphagnum peat soil substrate and sand (1:1) at low nitrogen (N) (left), medium N (middle) and high N (right). Note apple scab development on the leaves. Note the severe apple scab infection on medium and high N apple leaves (Photo: Marianne Bengtsson).

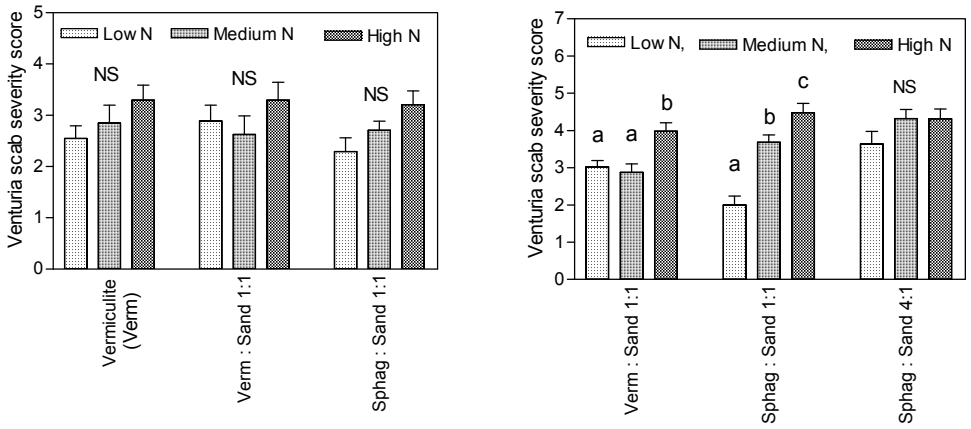

Figure 3.4. The effect of growth media and nitrogen (N) fertilization (low, medium and high N-level) on the apple scab severity score in seed plants of cv. 'Golden Promise'. Left figure represent experiment 1 and the right figure experiment 2. Mean values with different letters are significantly different at $P<0.05$, NS=no significant difference between N-levels. Mean values are presented, with standard error bars.

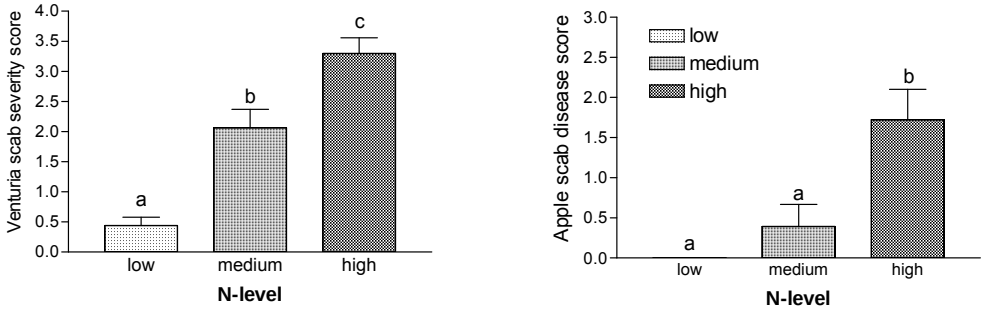

Figure 3.5. The effect of nitrogen(N) supply on the scab severity score on apple plants of cv. 'Bittenfelder Sämling' in two separate assays. Mean values with different letters are significantly different at $P<0.05$. Mean values are presented with standard error bars.

A significant effect of N-supply on apple scab severity score was also demonstrated in the more resistant cv. 'Bittenfelder Sämling'. Although there were some variation between the four essay where scab severity was scored (Figures 3.5 and 3.6), in general the plants supplied with the highest amount of N always had significantly higher disease scores than those plants at low N-level.

In two of the scab severity assays, the effect of N-level on ***V. inaequalis*** spore production was quantified (Figure 3.6). The two highest N-levels produced significantly more spores than the plants at the low N-level. Furthermore, the plots of conidia produced per plant against the apple scab score indicated that the lesions at high N-levels produced more spores per lesion unit than the lesions produced at low N-level.

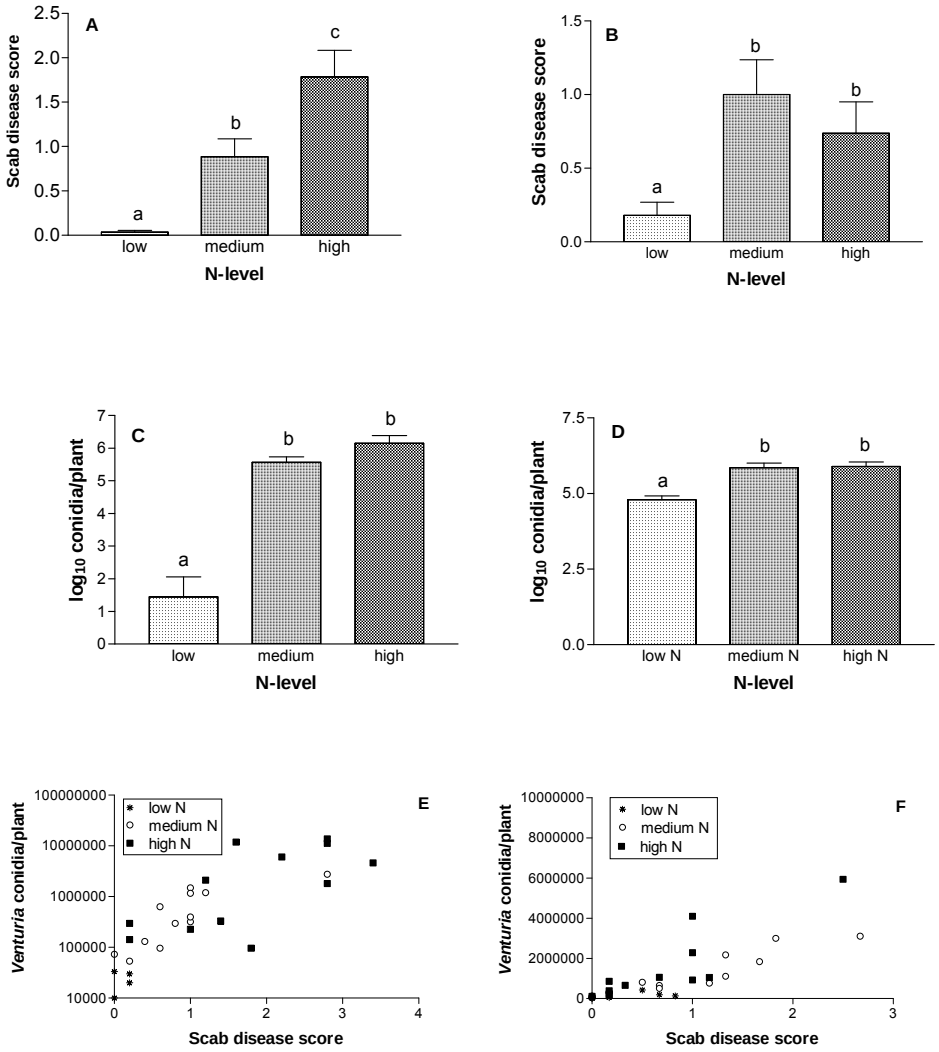

Figure 3.6. The effect of Nitrogen (N) application on apple scab disease severity on apple plants of cv. 'Bittenfelder Sämling' (A, exp 1 and B, exp 2). Effect of N application on spore production per plant (C, exp 1 and D, exp 2) and the relationship between spore production per plant and the disease score (E, exp1 and F, exp 2). Mean values with different letters are significantly different at $P<0.05$, with bars representing standard errors.

3.1.2.3. Nitrogen and dry weight determination

The effect of N-supply on the N and carbon (C) content and on dry weight of apple plants was determined in two separate assays. Plant material was sampled following 14 days growth at the three various N-levels (after the time-point of inoculation with the pathogen) and subsequently analysed. Increasing the N-level in nutrient solutions resulted in a significantly increased in plant N-content in both assays, with the two highest N-levels resulting in almost equal N-contents (Figure 3.7). On the other hand, the C-content did not differ significantly between treatments (Figure 3.8). Similarly the dry weight of plants was also unaffected by N-supply, except in one of the assays where the high N-level plants resulted in a significantly higher dry weight than for plants in the two other treatments (Figure 3.9).

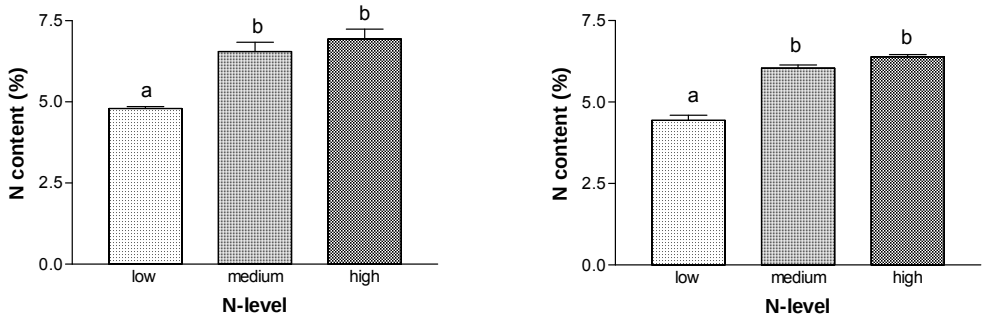

Figure 3.7. The effect of nitrogen (N) application on the N-content of apple plants of cv. 'Bittenfelder Sämling' in two assays. Mean values (n=7) with different letters are significantly different at $P<0.05$, where bars represent standard errors.

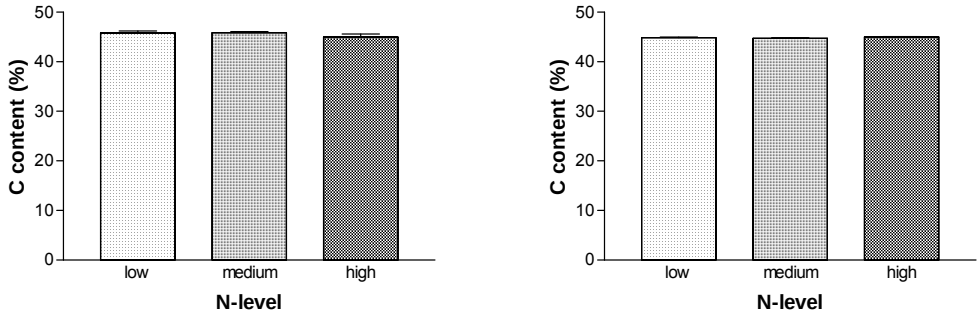

Figure 3.8. The effect of nitrogen (N) application on the carbon (C)-content of apple plants of cv. 'Bittenfelder Sämling' in two assays. Mean values with different letters are significantly different at $P<0.05$, where bars represent standard errors.

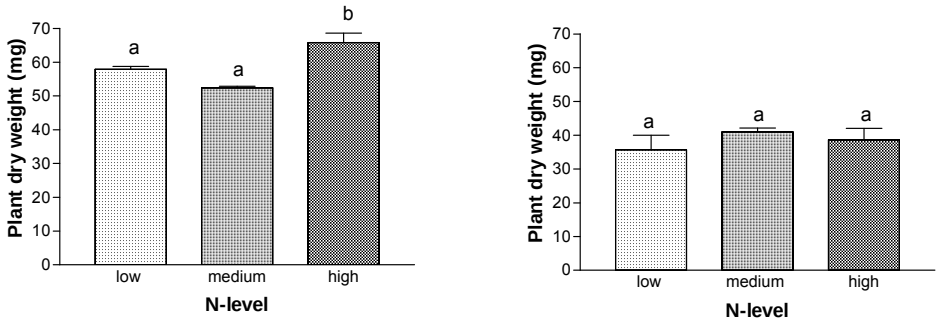

Figure 3.9. The effect of nitrogen (N) application on the dry weight of apple plants in two assays. Mean values with different letters are significantly different at $P<0.05$, where bars represent standard errors.

3.1.2.4. Quantification of Venturia inaequalis using QPCR

Optimization of a TaqMan qPCR method for quantification of ***V. inaequalis*** in leaves was based on primers and a probe develop at Plant Research International, Holland. However, we were unable to obtain useful qPCR results using our PCR machine and FastMix from Quanta. The PCR efficiency of the reactions was below 50% even with pure ***Venturia*** DNA, and optimization of primer and probe concentrations as well as PCR program (2-step or 3-step PCR) did not improve the PCR efficiency.

The attempt to use the TaqMan primers for real time PCR with SYBR green showed that the primers amplified two products and therefore they were unsuitable for quantification ***V. inaequalis*** in leaves.

3.1.3 Orchard

3.1.3.1 Disease assessment

In 2007, six primary scab treatments were carried out and in total 12 treatments against apple scab. Treatments were carried out as preventative treatments before expected infection according to RIMpro DSS-system.

In spring 2007, no primary infections were observed. By the middle of September, a small infection from secondary infections was assessed (Table 3.2). There was a slightly larger infection of apple scab on leaves with reduced sprays. No infections on fruit were found (data not shown). There was a slightly larger infection of ***V. inaequalis*** on leaves of tress that were supplied with 200 kg nitrogen during the growing season (Table 3.2).

Table 3.2. Apple leaves (cv. 'Elshof') with and without apple scab and infection severity at different nitrogen treatments, middle September 2007. LSD=least significant difference and NS=not significant.

Treatment	% leaves Dropped	% leaves with no scab	% leaves with 1 spot	% leaves with 2-4 spots	% leaves with 5-9 spots	% leaves with 10 or more spots	Infection Severity
1. 0 kg N	4.1	95.5	0	0.1	0.2	0	0.26
2. 25 kg N	4.3	94.9	0.3	0.1	0.1	0.3	0.47
3. 50 kg N	4.7	95.2	0.1	0	0	0.1	0.08
4. 75 kg N	2.8	96.9	0.2	0	0.1	0.1	0.10
5. 100 kg N	4.3	95.0	0.3	0.2	0.2	0.1	0.33
6. 200 kg N	3.8	93.3	0.7	0.6	0.6	1.2	2.02
LSD 5%	NS	NS	0.44	0.44	0.33	0.36	0.62
Spraying							
RIMpro Whole season	4.4	95.6	0.0	0.0	0.0	0.0	0.00
RIMpro Primary season	3.4	94.4	0.5	0.3	0.4	0.6	1.08
LSD 5%	NS	NS	0.28	0.28	0.33	0.27	0.40

In 2008, apple scab was controlled eight times in the primary apple scab season and in total 12 sprays were carried out.

No infection on either leaves or fruit were found in 2008. In April 2008, we scored the level of decomposed leaves on the soil surface (Table 3.3). In 2007 a reduced apples scab control (RIMpro primary season) left less leaves on the soil surface compared to RIMpro whole season control (Table 3.3). A very high level of nitrogen supply had a tendency to reduce the decomposing of leaves (Table 3.3).

Table 3.3. Score of level of decomposed apple leaves (cv. 'Elshof') on the soil surface, where 1=no leaves left and 9=all leaves left for two spraying strategies.

Treatment	RIMpro primary season	RIMpro Whole season	Average of spraying strategies
1. 0 kg N	2.4 a	3.3 ab	2.9 ab
2. 25 kg N	2.4 a	3.0 b	2.7 b
3. 50 kg N	2.6 a	3.1 ab	2.9 ab
4. 75 kg N	2.8 a	3.5 ab	3.2 ab
5. 100 kg N	2.8 a	2.9 b	2.9 ab
6. 200 kg N	2.9 a	3.7 a	3.3 a
Sprays	2.6 b	3.3 a	

Values followed by the same letter within a columns do not differ significantly at p≤0.05.

Table 3.4. Apple leaves (cv 'Elshof') with and without apple scab and infection severity at different nitrogen treatments, middle September 2009. LSD=least significant difference and NS=not significant.

Treatment	% leaves dropped	% leaves with no scab	% leaves with 1 spot	% leaves with 2-4 spots	% leaves with 5-9 spots	% leaves with 10 or more spots	Infections severity
1. 0 kg N	5.3	93.5	1.0	0.1	0.0	0.1	0.43
2. 25 kg N	4.6	94.2	0.9	0.2	0.0	0.1	0.37
3. 50 kg N	4.6	93.4	1.2	0.4	0.2	0.2	0.78
4. 75 kg N	4.6	92.9	1.2	0.7	0.5	0.3	1.26
5. 100 kg N	4.4	94.1	0.7	0.4	0.2	0.2	0.72
6. 200 kg N	4.1	93.5	1.2	0.7	0.1	0.3	1.06
LSD 5%	0.71	0.28	NS	NS	NS	NS	NS
Spraying							
RIMpro Whole season	5.1	93.0	1.0	0.5	0.2	0.3 a	0.91
RIMpro Primary season	4.1	94.2	1.1	0.3	0.1	0.1 a	0.64
LSD 5%	0.84	0.90	NS	NS	NS	NS	NS

In 2009, six primary scab treatments were carried out and in total 11 treatments against apple scab. A small apple scab infection occurred and no differences were found on the infection level on leaves either due to spray or to supply of nitrogen (Table 3.4). No important infections of apple scab on the fruit occurred. Scab on fruit only occurred on five fruit on one single tree.

3.1.3.2 Growth and nitrogen assessment

No differences occurred in vegetative growth and nitrogen (N) content of soil and leaves due to spraying strategies.

The vegetative growth measured as total stem diameter and growth of stem diameter from 2007 to 2009, increased significantly with a N supply of 50 kg or higher. There were no additive effect on growth with a supply higher than 50 kg N (Table 3.5). Increasing N supply significantly of 50 kg N per ha or more increased the level of nitrogen in the leaves each year. In August 2009 the general level of N in the leaves was high (Table 3.5).

The level of free N in the soil in 2007 and 2008 are shown in Figure 3.11. High supply of N was reflected by the amount of free N in the soil both years. However, there were big differences between the level of free N in the soil for 2007 and 2008. The differences in free N in the soil for the two years was only refected in the level of nitrogen in the leaves in September 2008. The N content in the leaves was more stable even when the N supply fluctuated (Figure 3.10).

Table 3.5. Stem diameter 2009, growth of stem diameter 2007 - 2009, and Nitrogen(N) in percent of dry matter for cv 'Elshof' in august 2007, 2008 and 2009. LSD=least significant difference and NS=not significant.

Treatment	Steem dia mm Autumn 2009	Growth mm 2007-2009	Total nitrogen in leaves % 2007	Total nitrogen in leaves % 2008	Total nitrogen in leaves 2009 %
1. 0 kg N	54.3	15.2	2.08	2.20	2.31
2. 25 kg N	54.7	15.3	2.18	2.22	2.57
3. 50 kg N	57.3	18.6	2.23	2.30	2.68
4. 75 kg N	56.1	17.0	2.39	2.35	2.77
5. 100 kg N	56.7	16.8	2.46	2.33	2.76
6. 200 kg N	56.8	17.5	2.53	2.44	2.82
LDS 5%	2.78	2.77	0.22	0.19	0.17

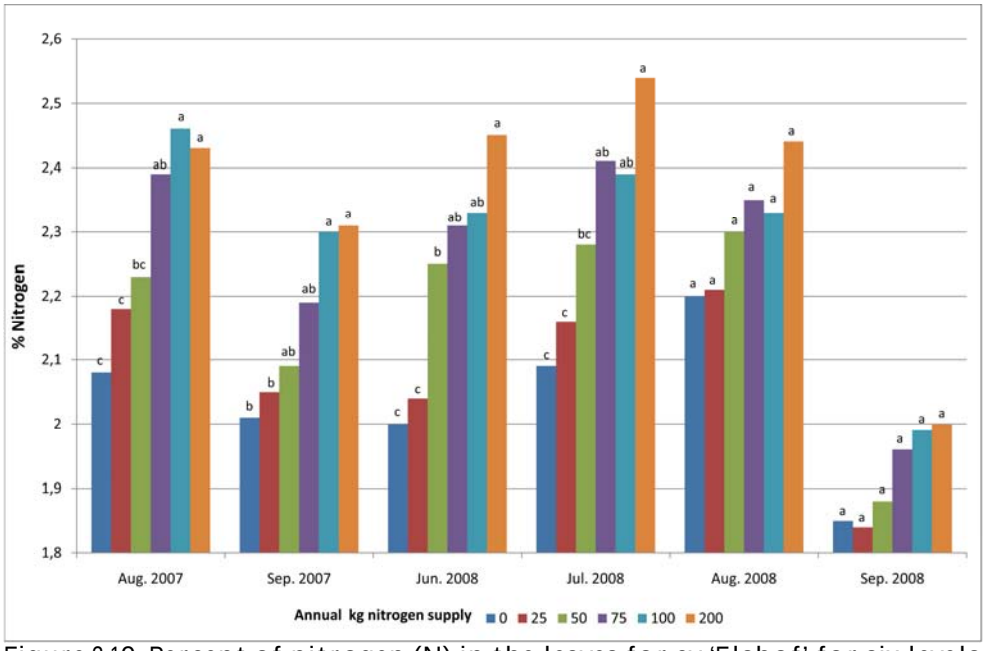

Figure 3.10. Percent of nitrogen (N) in the leaves for cv 'Elshof' for six levels of nitrogen supply in 2007 and 2008 at AU- Aarslev. Values with the same letter for the same date do not differ significantly at p≤0.05.

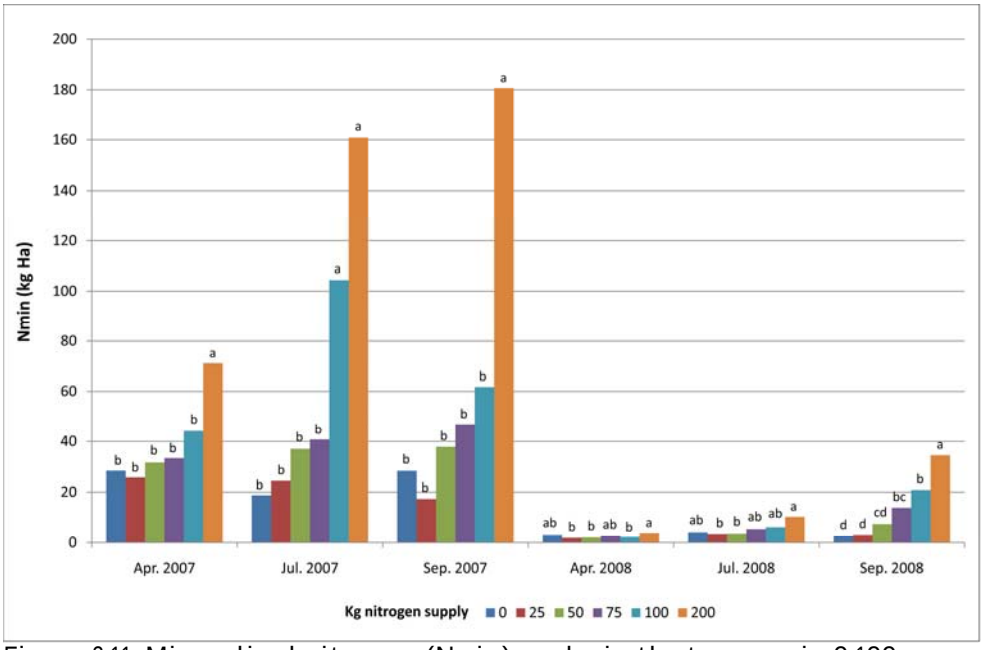

Figure 3.11. Mineralised nitrogen (Nmin) per ha in the tree row in 0-100 cm depth for cv 'Elshof' for six levels of nitrogen supply in 2007 and 2008 at AU-Aarslev. Values with the same letter for the same date do not differ significantly at p≤0.05.

3.1.3.3 Fruit yield and quality

No differences occurred in yield and fruit quality due to the spray strategies.

Increased N supply increased fruit size, reduced fruit firmness and reduced the level of surface colour on the fruit (Tables 3.6 and 3.7). In 2007, there were no differences in fruit yield and sugar content of the fruit (Tables 3.6 and 3.7).

Table 3.6. Flowering, yield, and fruit size for six nitrogen (N) levels on cv 'Elshof' in 2007. LSD=least significant difference and NS=not significant.

Treatment	Flowering Score 1-9, 9=max	Yield kg/tree	Number fruit/tree	Fruit size G
1. 0 kg N	5.4	11.6	69	170
2. 25 kg N	5.6	12.5	72	175
3. 50 kg N	5.4	11.2	63	177
4. 75 kg N	5.5	11.6	66	177
5. 100 kg N	5.8	10.8	60	181
6. 200 kg N	5.3	11.2	62	182
LSD 5%	NS	NS	0.56	1.6

Table 3.7. Percent apple fruit in three grades for red colour on the surface, fruit firmness, and sugar content for six nitrogen (N) levels on cv 'Elshof' in 2007. LSD=least significant difference and NS=not significant.

Treatment	% fruit <50 % red skin	% fruit 50%< red skin<75%	% fruit > 75 % red skin	Firmness N	Sugar %
1. 0 kg N	4.6 d	14.1 d	81.3 a	54.9 a	15.9 a
2. 25 kg N	10.5 c	20.3 c	69.1 a	51.3 a	15.6 a
3. 50 kg N	16.9 b	22.7 bc	60.4 c	52.3 a	16.0 a
4. 75 kg N	15.5 b	24.4 ab	60.1 c	52.2 a	15.7 a
5. 100 kg N	18.6 ab	23.8 b	57.6 c	50.9 a	15.8 a
6. 200 kg N	22.0 a	27.3 a	50.6 d	51.2 a	16.1 a
LSD 5%	5.37	4.34	3.15	0.71	NS

No N supply reduced the fruit size and increased the level of surface colour on the fruit (Tables 3.8 and 3.9) as seen in 2007. No significant differences in apple fruit yield and internal quality measured as firmness and sugar content occurred in 2008.

Table 3.8. Flowering, yield and fruit size for six nitrogen (N) levels on cv 'Elshof' in 2008. LSD=least significant difference and NS=not significant.

Treatment	Flowering no. clusters/tree	Yield kg/tree	Number fruit/tree	Fruit size g
1. 0 kg N	140	10.3	59	176
2. 25 kg N	114	11.7	64	185
3. 50 kg N	161	11.4	61	191
4. 75 kg N	129	10.3	54	192
5. 100 kg N	111	9.2	47	194
6. 200 kg N	176	11.6	64	187
LSD 5%	0.53	NS	8.8	12.8

Table 3.9. percent apple fruit in three grades for red colour on the surface, fruit firmness and sugar content for six nitrogen (N) levels on cv 'Elshof' in 2008. LSD=least significant difference and NS=not significant.

Treatment	% fruit <50% red skin	% fruit 50%< red skin<75%	% fruit > 75% red skin	Firmness N	Sugar %
1. 0 kg N	18.2	31.4	50.3	49.8	14.9
2. 25 kg N	28.9	34.7	36.4	51.3	15.5
3. 50 kg N	30.0	34.5	35.5	51.1	15.5
4. 75 kg N	36.1	38.8	25.2	51.3	15.5
5. 100 kg N	33.0	34.5	32.6	51.9	16.2
6. 200 kg N	37.7	34.7	27.5	49.7	15.3
LSD 5%	12.12	NS	5.12	NS	NS

In 2009 a supply of 100 kg N gave the highest yield and the largest fruit (Table 3.10). The lowest number of flower clusters, fruit and yield were seen with the highest and lowest supply of N (Table 3.10). The lowest number of fruitlets were removed at no N supply (Table 3.10). Even the differences between fruit colouring were smaller between the N treatments in 2009 than in 2007 and 2008. Low N supply gave a higher colouring of the fruit skin. In 2009 all fruit very heavily coloured (Table 3.11). In 2009 no differences were observed in internal fruit quality occurred (Table 3.11).

Table 3.10. Flowering, yield, and fruit size for six nitrogen (N) levels for cv 'Elshof' in 2009. LSD=least significant difference and NS=not significant.

Treatment	Flowering no. clusters/tree May 2009	Number fruitlets removed	Yield kg/ tree	Number fruit/tree	Fruit size g	Flowering no. clusters/tree May 2010
1. 0 kg N	131	38	10.6	85	127	90.4
2. 25 kg N	164	65	12.6	107	120	85.6
3. 50 kg N	131	64	13.9	102	137	88.0
4. 75 kg N	143	62	12.3	92	135	80.2
5. 100 kg N	194	72	14.1	103	139	66.9
6. 200 kg N	124	73	11.6	89	134	95.6
LSD 5%	52.3	3.4	1.21	2.1	15.5	1.17

Table 3.11. Percent fruit in three grades for red colour on the surface, fruit firmness and sugar content for six nitrogen (N) levels for cv 'Elshof' in 2009. LSD=least significant difference and NS=not significant.

Treatment	% fruit <50 % red skin	% fruit 50%< red skin<75%	% fruit > 75 % red skin	Firmness N	Sugar %
1. 0 kg N	0.6	2.1	97.4	41.4	15.2
2. 25 kg N	0.2	0.5	99.3	39.5	14.8
3. 50 kg N	0.6	2.9	96.5	38.1	14.7
4. 75 kg N	0.6	4.5	94.9	39.5	14.9
5. 100 kg N	1.4	3.9	94.8	39.2	14.9
6. 200 kg N	1.1	5.0	93.9	40.8	15.3
LSD5%	NS	1.1	2.7	NS	NS

3.1.4. Development of RIMpro for apple scab

3.1.4.1. Onset of disease

Apple scab caused by Venturia inaequalis

Decisions for when to spray the experimental apple orchard were bases on the RIMpro decision system and the weather forecast. In 2007, the spring was early, warm, and dry. In agreement with RIMpro predictions, spraying against primary infections was carried out prior to expected infection events: 16 April, 5 May, 11 May, 21 May, 31 May, 7 June, and 18 June, leaving one treatment with four replicates unsprayed at the actual date and all other treatments sprayed. Using this schedule, it was possible to investigate if the actual warning event caused an infection. Apple scab was assessed in the different treatments and compared to apple scab in unsprayed plots and plots with full spray programmes.

Spray treatmetns were Delan WG (dithianon), Delan is a preventive fungicide that has an effect for approximately one week after spraying, depending on temperature and precipitation.

No important infections of apple scab occurred in 2007 and there were no significant differences in the incidence of apple scab between the timing of spraying, sprayed and unsprayed were found (Table 3.12).

Table 3.12. Symptoms of apple scab on leaves of apple cultivar 'Elshof' on annual shoots mid September 2007. For treatment 3 to 9, spraying was omitted as compared to the full spraying, at the specified date.

Treatment Unsprayed at the specified date	Number of leaves	% leaves without scab	% leaves with one spot	% leaves with 2-4 spots	% Leaves with 5-9 spots	% leaves with 10 or more spots	Infection Severity
1. Unsprayed	1357	96.6 ab	0.3 a	0.3 a	0.0 b	0.1 a	0.36 a
2. Full spraying plan	1402	98.0 a	0.3 a	0.2 a	0.1 a	0.0 a	0.26 a
3. 16 April	1354	96.8 ab	0.1 a	0.2 a	0.0 b	0.1 a	0.29 a
4. 5 May	1360	98.0 a	0.2 a	0.1 a	0.0 b	0.0 a	0.08 a
5. 11 May	1006	97.6 ab	0.0 a	0.1 a	0.0 b	0.0 a	0.06 a
6. 21 May	1333	97.5 ab	0.5 a	0.1 a	0.0 b	0.1 a	0.24 a
7. 31 May	1587	95.9 b	0.0 a	0.0 a	0.0 b	0.0 a	0.00 a
8. 7 June	1397	97.6 ab	0.2 a	0.1 a	0.0 b	0.0 a	0.08 a
9. 18 June	1433	96.6 ab	0.1 a	0.1 a	0.0 b	0.0 a	0.04 a

Values followed by the same letter in columns do not differ significantly at p≤0.05

In 2008, the spring was early, warm and dry. Spraying against primary apple scab infections was carried out 31 March, 12 April, 25 April, 7 May, 16 May, and 27 May. No apple scab infections on leaves or fruit occurred in 2008. Data is not shown.

In 2009, preventive spraying to control primary infections of apple scab were carried out 24 April, 3 May, 16 May, 27 May, 4 June and 10 June. Unsprayed trees had a small infection that mainly developed at the apple scab infection event around 3 May (Table 3.13).

Table 3.13. Symptoms of apple scab on leaves of apple cultivar 'Elshof' on annual shoots mid July 2009. For the treatment 3 to 8, spraying was omitted as compared to the full spraying, at the specified date.

Treatment Unsprayed at the specified date	% leaf drop	% leaves without scab	% leaves with one spot	% leaves with 2-4 spots	% Leaves with 5-9 spots	% leaves with 10 or more spots	Infections Severity
1. Unsprayed	0.1 a	97.7 bc	1.3 a	0 b	0	0	0.33 a
2. Full plan	1.3 a	98.7 ab	0 b	0 b	0	0	0.00 b
3. 24 April	0.9 ab	99.0 ab	0.2 b	0 b	0	0	0.04 b
4. 3 May	1.5 a	97.3 c	1.1 a	0.1 a	0	0	0.33 a
5. 16 May	1.2 ab	98.8 ab	0 b	0 b	0	0	0.00 b
6. 27 May	0.5 ab	99.5 a	0 b	0 b	0	0	0.00 b
7. 4 June	0.2 b	99.5 a	0.3 b	0 b	0	0	0.08 b
8. 10 June	0.7 ab	99.3 a	0 b	0 b	0	0	0.00 b

Values followed by the same letter in columns do not differ significantly at p≤0.05

3.1.4.2 Development of RIMpro for apple scab

The RIMpro simulations needs a Biofix date to start the seasonal calculations (Table 3.14). Under Danish conditions, the first ascosporic discharge in each year was recorded a considerable time before the start of vegetation of the apple trees. The Biofix date of greentip in 'Jonagold' and a simulated date for first mature ascospores was used and set a few days before first ascospore discharge.

Table 3.14. Biofix settings for RIMpro simulations for apple scab 2007-2010.

Year	First ascospore discharge observed in the field	Date of greentip 'Jonagold'	Difference in days between first ascospore discharge and greentip	RIMpro Biofix first mature ascospores
2007	18 March	7 April	-20	15 March
2008	13 March	29 March	-16	10 March
2009	23 March	3 April	-11	20 March
2010	29 March	8 April	-10	25 March

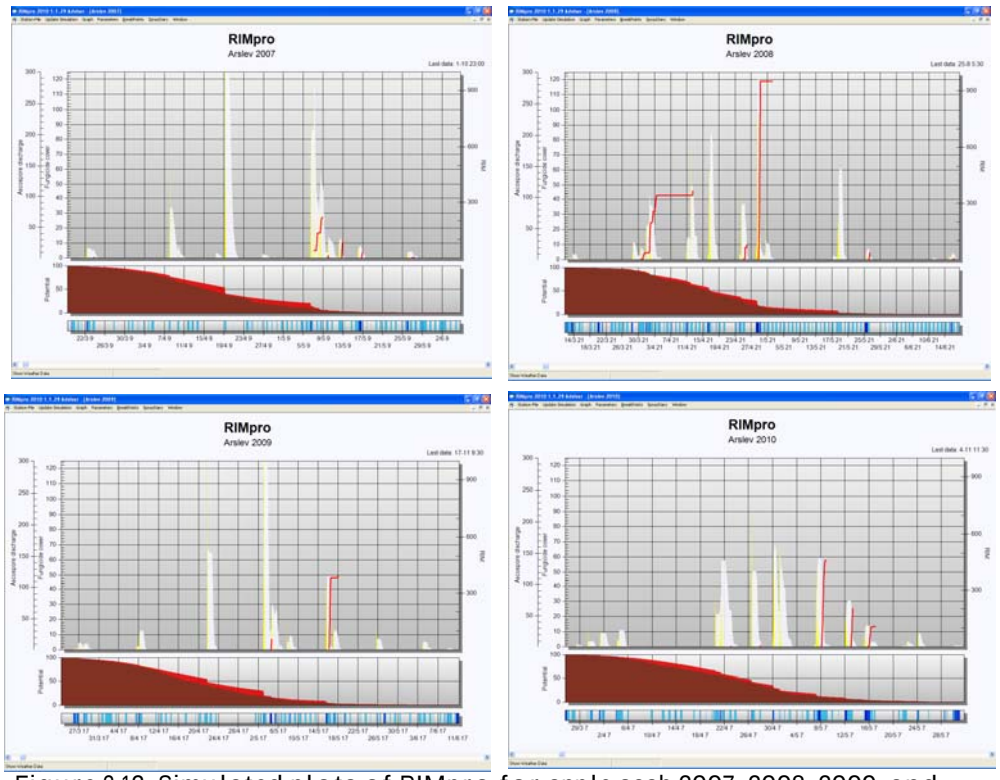

Figure 3.12. Simulated plots of RIMpro for apple scab 2007, 2008, 2009, and 2010 with Biofix set as the date for the first mature ascospores.
From top: Yellow: simulated ascospore discharge; white: simulated ascospore survival on leaves; red: simulated quantity of spores entering the host plant=relative infection measure (RIMs);
Middle: Brown: simulated ascospore potential which is diminishing during spring.; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.
In an orchard with low level of apple scab inoculum and a robust cultivar a RIM value below 250 is considered a low risk.

Table 3.15, shows that the observed ascospore discharge during each rain event fits to the ascospore discharge as calculated by the RIMpro-model. The Biofix for the model was set a few days before the first ascospores were observed in the trap, which is regarded as best practice. Table 3.16 summarize the validation results.

In all four years (2007-2010) the general distribution of the simulated ascospore discharge was close to the observed discharge pattern. During the rain periods in which most spores were found, the model also produced the highest number of spores. However, in some cases the peak discharge in the trap was found at the beginning of the rain period, and in the simulation model in the second part of the wetness period, or vice-versa. In 81.2% of the rain events the simulated ascospore discharge was at the same magnitude as the observations. In 9.4% of the rain events the model overestimated the amount of ascospores that were discharged in nature. However overestimations are not critical for the effectiveness of the apple scab management. An overestimation could result in an additional fungicide treatment that is unnecessary, but would not result in a failed scab control.

However, in 9.4% of the rain events the model underestimated the ascospore discharge. In most cases the day before or after the underestimation a high number of ascospores was determined. The underestimation would in these cases not have caused a serious problem in scab management, as the day before or after the DSS calculations there would has been an indicated necessity for a fungicide treatment. In two episodes (18-20 April 2007, and 5-6 May 2009) the underestimation could have been critical for the effectiveness of the scab control when the following wetness period would have been long enough to cause a serious infection.

Table 3.15. Rain events, precipitation, discharged ascospores of *Venturia inaequalis* as observed in the spore trap, and the ascospore projection as calculated by the RIMpro simulation model. The Biofix for the model was set at first mature ascospores Evaluation period 2007-2010.

	Observed					RIMpro simulations Biofix=first mature spores	
Rain period Days	Rain (mm)	Date the rain ended	Date end slide changed	Counted ascospore (No.)	Counted ascospores in the season (% of total)	Estimated ascospores in the season (% of total)	Validation
2007							**Biofix 15/3**
16/3-19/3	14.5	19/3	19/3	51	0.2	< 0.1	Acceptable
20-21/3	3.5	21/3	21/3	5	< 0.1	< 0.1	Acceptable
22-23/3	2.7	23/3	23/3	4	< 0.1	< 0.1	Acceptable
24/4	1.7	24/3	26/3	8	< 0.1	< 0.1	Acceptable
8-9/4	0.6	9/4	9/4	545	2.4	3.5	Acceptable
10/4	0.4	10/4	11/4	185	0.8	0.3	Acceptable
18-20/4	1.9	20/4	20/4	7045	30.8	3.5	Too low
27/4+7/5	7.7	7/5	7/5	1533	6.7	14.9	Too high
8-9/5	6.4	9/5	9/5	8058	35.2	26.8	Acceptable
10-11/5	2.4	11/5	11/5	2795	12.2	5.6	Too low
12-14/5	4.0	13/5	14/5	612	2.7	8.6	Too high
15-16/5	1.3	16/5	16/5	808	3.5	< 0.1	Acceptable
17/5	33.3	17/5	18/5	1159	5.1	< 0.1	Acceptable
26-29/5	8.2	29/5	29/5	147	0.6	3.7	Acceptable
30/5	30.7	30/5	31/5	13	0.1	0.6	Acceptable
6/6	4.7	6/6	7/6	1	< 0.1	< 0.1	Acceptable
13-14/6	0.9	14/6	14/6	1	< 0.1	< 0.1	Acceptable
2008							**Biofix 10/3**
10-13/3	19.4	13/3	13/3	66	0.1	< 0.1	Acceptable
14/4-15/3	6.2	15/3	17/5	136	0.2	< 0.1	Acceptable
21/3-25/3	14.7	25/3	25/3	10	< 0.1	< 0.1	Acceptable
26/3-27/3	0.6	27/3	27/3	12	< 0.1	< 0.1	Acceptable
29/3-31/3	3.9	31/3	31/3	681	1.2	< 0.8	Acceptable
¼-2/4	4.0	2/4	2/4	46	0.1	3.0	Acceptable
¾	2.8	¾	¾	2993	5.2	5.1	Acceptable
5/4-6/4	0.8	6/4	7/4	406	0.7	0.1	Acceptable
12/4	6.8	12/4	12/4	194	0.3	2.7	Acceptable
13/4	0.1	13/4	14/4	1341	2.3	7.4	Too high
17/4	9.0	17/4	17/4	6850	11.8	0.1	Too low
18/4	0.4	18/4	21/4	30129	52.0	17.4	Peak. but still too low
25/4	0.8	25/4	25/4	5	< 0.1	0.7	Acceptable
26/4	1.7	26/4	28/4	2081	3.6	7.9	Acceptable
29-30/4	19.0	30/4	30/4	7170	12.4	33.0	Peak but

							too high
1-2/5	1.2	2/5	2/5	4000	6.9	2.9	Acceptable
18-19/5	2.3	19/5	19/5	1116	1.9	0.5	Acceptable
20/5	3.0	20/5	20/5	305	0.5	15.0	Too high
26-27/5	26.1	27/5	27/5	270	0.5	2.1	Acceptable
11-12/6	0.3	12/6	12/5	41	0.1	0.3	Acceptable
14-16/6	4.7	16/6	16/6	11	< 0.1	0.4	Acceptable
17/6	7.3	17/6	17/6	17	< 0.1	0.6	Acceptable
20-21/6 +23/6	5.7	23/6	23/6	22	< 0.1	0.1	Acceptable
24/6+ 27-30/6	11.1	30/6	30/6	7	< 0.1	< 0.1	Acceptable
6/7-9/7	6.9	9/7	9/7	5	< 0.1	< 0.1	Acceptable
2009							**Biofix 20/3**
23/3	2.0	23/3	23/3	13	0.1	< 0.1	Acceptable
24/3	6.9	24/3	24/3	7	0.1	< 0.1	Acceptable
25/3+27/3	5.9	27/3	27/3	8	0.1	< 0.1	Acceptable
28-29/3	8.7	29/3	30/3	47	0.4	< 0.1	Acceptable
8/4	2.8	8/4	8/4	77	0.7	3.2	Acceptable
9/4	2.5	9/4	11/4	384	3.5	< 0.1	Acceptable but too low as only 0.2 mm rain in database
22-23/4	2.7	23/4	23/4	3357	30.9	13.2	Peak but too Low
3-4/5	10	4/5	4/5	756	6.9	43.8	In nature most spores on second day
5/5	4	5/5	5/5	3418	31.4	< 0.1	Too low
6/5	7.2	6/5	6/5	1910	17.6	5.3	Too low
7-8/5+9/5	7.6	9/5	11/5	218	2.0	7.5	Acceptable
16/5	3.0	16/5	16/5	83	0.8	16.2	Too high
17-18/5	7.0	18/5	18/5	97	0.9	4.5	Acceptable
19/5	2.2	19/5	19/5	112	0.4	0.1	Acceptable
20/5	3.2	20/5	20/5	41	0.9	< 0.1	Acceptable
23/5+25/5	0.5	25/5	25/5	94	0.6	4.6	Acceptable
26-27/5	8.2	27/5	27/5	64	0.1	0.6	Acceptable
28/5	8.4	28/5	28/5	12	0.6	0.7	Acceptable
4/6	1.0	4/4	4/6	65	0.4	0.1	Acceptable
5-6/6	2.7	6/6	6/6	43	0.2	0.5	Acceptable
8/6	0.3	8/6	8/6	8	0.3	< 0.1	Acceptable
10/6	8.6	10/6	10/6	27	0.2	0.7	Acceptable
11/6	0.4	11/6	11/6	29	0.3	< 0.1	Acceptable
12-13/6	39.0	13/6	15/6	68	0.6	< 0.1	Acceptable
18/6	1.0	18/6	18/6	4	< 0.1	< 0.1	Acceptable
20/6+22/6	4.5	22/6	22/6	13	< 0.1	< 0.1	Acceptable
2010							**Biofix 25/3**
27/3	20	27/3	29/3	173	1.2	0.4	Acceptable
31/3	1.5	31/3	31/3	22	0.2	1.0	Acceptable
¾	10	¾	6/4	786	5.6	5.7	Acceptable
21/4	6.3	21/4	21/4	2363	16.8	5.7	Too low
22/4	3.4	22/4	22/4	1551	11.1	9.9	Acceptable
23/4+27/4	1.0	27/4	27/4	429	3.1	13.8	Too high
30/4-2/5	7.7	2/5	3/5	6664	47.5	30.6	Acceptable
7/5-9/5	17.1	9/5	10/5	1304	9.3	16.0	Too high
12-13/5	11.1	13/5	15/5	496	3.5	8.4	Acceptable
16-17/5	21.9	17/5	17/5	144	1.0	4.6	Acceptable
22/5	2.2	22/5	23/5	18	0.1	1.0	Acceptable

24-25/5	3.8	25/5	25/5	55	0.4	2.5	Acceptable
26/5+28/5 +29-31/5	22.6	31/5	31/5	18	0.1	0.2	Acceptable
2/6	0.9	2/6	2/6	6	< 0.1	< 0.1	Acceptable
7-8/6	45.2	8/6	8/6	2	< 0.1	< 0.1	Acceptable
9/6	11.3	9/6	9/6	2	< 0.1	< 0.1	Acceptable
10-11/6	10.5	11/6	11/6	0	< 0.1	< 0.1	Acceptable
12-13/6	14.9	13/6	14/6	3	< 0.1	< 0.1	Acceptable

Table 3.16. Validaton of simulation with Biofix set at first mature ascospores in 2007-2010 for apple scab.

Year	Total Events of rain periods	Validation of the simulation result		
		Too Low	Acceptable	Too High
2007	17	2	13	2
2008	24	2	20	2
2009	26	3	21	2
2010	18	1	15	2
Total	85	8 (2 potential critical)	69	8
Percent		9.4 (2.4 potential critical)	81.2	9.4

3.2. Cherry leaf spot *Blumeriella jaapii*

3.2.1. Primary inocullum

3.2.1.1 Quantification of conidia and ascospores of Blumeriella jaapii on overwintering leaves

Quantification of spores on overwintering cherry leaves and estimation of spore ratios were carried out in spring and early summer 2008 and 2009 using two methods namely soaking the leaves either for 1.5 h at 20°C or for 24 h at 4°C (Tables 3.17 and 3.18). In 2008, the mean numbers of ascospores and winter conidia were almost identical irrespectively of washing method, and ascospores were not identified until 21 April with both methods. In 2009, the method with 1.5 h soaking resulted in approximately 20% higher production of both ascospores and winter conidia as compared to soaking for 24 h at 4°C. Since the difference between the two methods for estimation of primary inoculum potential on overwintering leaves was minimal, data for the two methods were pooled accross 2008 and 2009 for comparison with the data on discharged conidia and spores (see Tables 3.19 and 3.20).

Table 3.17. Production of *Blumeriella jaapii* ascospores and winter conidia on overwintering cherry leaves soaked either 1.5 h at 20°C or 24 h at 4°C. Leaves sampled in 2008.

Date	1.5 h leaf soaking at 20°C			24 h leaf soaking at 4°C		
	Asco-spores	Winter conidia	% asco-spores	Asco-spores	Winter conidia	% asco-spores
14 April	0	2000	0	0	8000	0
21 April	3000	9375	24	1500	20500	7
28 April	22000	58500	27	24500	73000	25
19 May	40000	342000	10	59500	415500	13
27 May	76500	234500	25	77000	200500	28
12 June	46500	81500	36	27000	57000	32
Mean values	31333	121313	21	31583	129083	20

Table 3.18. Production of *Blumeriella jaapii* ascospores and winter conidia on overwintering cherry leaves soaked either 1.5 h at 20°C or 24 h at 4°C. Leaves sampled in 2009. Leaves were sampled after rain events.

Date	1.5 h soaking at 20°C			24 h soaking at 4°C		
	Asco-spores	Winter conidia	% asco-spores	Asco-spores	Winter conidia	% asco-spores
08 April	12000	12000	50	4000	5000	44
11 April	13875	9375	60	21000	2000	91
23 April	64500	4000	94	29000	2000	94
04 May	37500	34000	52	91500	15500	86
05 May	35500	18500	66	73000	7000	91
06 May	130000	68000	66	121000	50500	71
11 May	112500	40500	74	72000	46500	61
16 May	176000	43500	80	190000	85000	69
18 May	97000	102000	49	64500	102000	39
19 May	133000	390000	25	100000	258000	28
20 May	142000	240500	37	59500	76500	44
25 May	129500	156500	45	106500	173000	38
27 May	40000	69500	37	43500	55500	44
28 May	35000	63500	36	50000	149500	25
04 June	38500	89500	30	29000	51500	36
06 June	118000	130000	48	75000	65000	54
08 June	110000	155000	42	45500	64500	41
Mean values	83816	95669	48	69118	71118	49

3.2.1.2 Inoculum discharge

Discharge of ascospore and spores suspected to winter conidia of ***Blumeriella jaapii*** are shown in Figure 3.13. Ascospore discharge of ***Blumeriella jaapii*** started from 2 April until 9 May depending of the year. The potential winter conidia followed the same pattern, starting and ending at approximately the same dates as the discharge of ascospores. The duration of the discharge period varied from 22 days in 2007 to 83 days in 2008. The average number of ascospores caught was approximately 5000 per year with variation from 3400 in 2009 up to 9600 in 2008. The average number of winter conidia was approximately 4700 with variation from 300 in 2010 to 10300 in 2008. The numbers of winter conidia were low in 2010 (Table 3.19).

Table 3.19. Discharge of ascospores and potential winter conidia of *Blumeriella jaapii* 2007- 2010.

Year	Rain events with spores (No.)		First spores caught (Date)		Last spores caught (Date)		Total Number		Discharge period (Days)	
	Asco-Spores	Winter Conidia	Asco-Spores	Winter Conidia	Asco-spores	Winter conidia	Asco-spores	Winter conidia	Asco-spores	Winter conidia
2007	7	11	9 May	-	31 May	-	4198	-	22	-
2008	20	20	2 April	2 April	23 June	23 June	9666	10281	83	83
2009	22	22	11 April	11 April	26 June	26 June	3359	3646	77	77
2010	10	10	21 April	22 April	31 May	25 May	3485	294	41	34

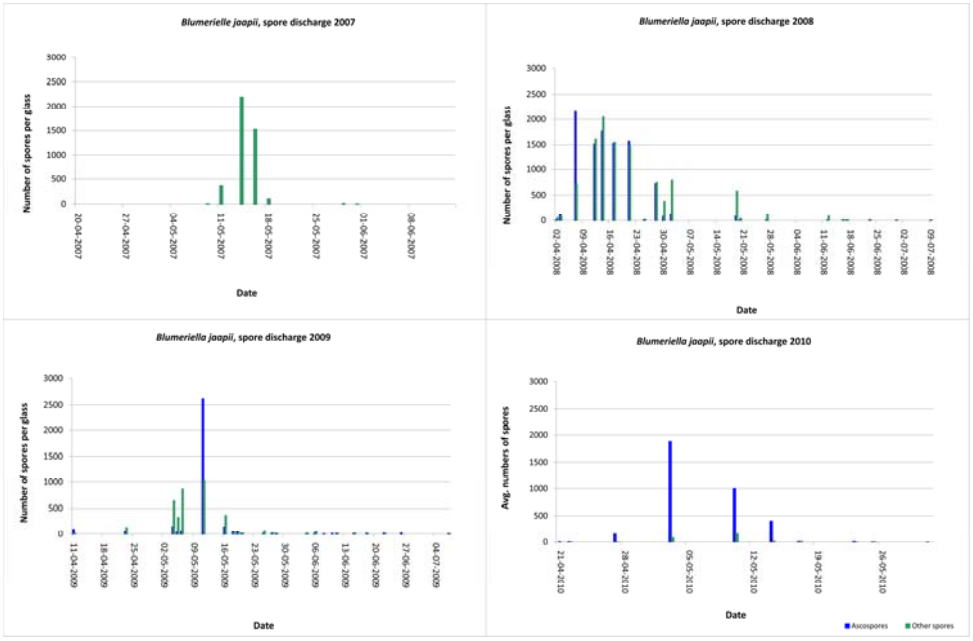

Figure 3.13. Discharge of ascospores (Blue) and winter conidia (Green) for *Blumeriella jaapii* 2007-2010.

Discharge of potential winter conidia

In Tables 3.20 and 3.21 the discharge of ascospores and winter conidia are compared to the inoculum potential on overwintering leaves after rain events at the same dates. In 2008 a high rate of ascospore and winter conidia discharge was associated with a relatively low inoculum potential, while in periods with low spore discharge the inoculum potential of both spore forms was high (Table 3.20). There was a tendency for a similar relationship in 2009, as the high ascospore discharge rates at 4 and 11 May were associated with relatively low values for inoculum potential of the two spore forms. In both years, the inoculum potential of both ascospores and winter conidia was high until mid-June, although the major discharge of ascospores had occurred by the end of April/mid-May.

Table 3.20. Discharge of ascospores and winter conidia of *Blumeriella jaapii* ascospores compared to the potential production of ascospores and winter conidia on overwintering leaves sampled after rain events, in 2008.

Date	Discharged spores (Spores/glass)			Inoculum potential on overwintering leaves (Spores/ g leaf)		
	Asco-spores	Winter conidia	% asco-spores	Asco-spores	Winter conidia	% asco-spores
14 April	1763	2044	46	0	5000	0
21 April	1566	1480	51	3200	23150	12
28 April	711	741	49	19833	65833	23
19 May	83	577	13	58667	396000	13
27 May	8	111	7	76750	217500	26
12 June	3	86	3	36750	69250	35

Table 3.21. Discharge of ascospores and winter conidia of *Blumeriella jaapii* ascospores compared to the potential production of ascospores and winter conidia on overwintering leaves after a rain events, in 2009.

Date	Discharged spores (Spores/glass)			Inoculum potential on overwintering leaves (Spores/ g leaf)		
	Asco-spores	Winter conidia	% asco-spores	Asco-spores	Winter conidia	% asco-spores
08 April	-	-	-	8000	8500	48
11 April	89	19	82	17438	5688	75
23 April	52	123	30	46750	3000	94
04 May	143	640	18	64500	24750	72
05 May	32	324	9	48000	14667	77
06 May	60	865	6	125000	59250	68
11 May	1760	1025	72	92250	43500	68
16 May	137	362	27	183000	64250	74
18 May	40	29	58	80750	116500	41
19 May	40	33	55	116500	324000	26
20 May	11	17	39	100750	158500	39
25 May	8	65	11	118000	116500	50
27 May	14	23	38	41750	62500	40
28 May	9	7	56	42500	106500	29
04 June	5	19	21	33750	70500	32
06 June	20	47	30	96500	97500	50
08 June	4	3	57	77750	109750	41

Discharge of winter conidia by water splash

The water splash experiment showed that both small and big water drops could discharge winter conidia from mature acervuli. The largest dispersal distance from the acervulus was approximately 3 cm (Table 3.22).

Table 3.22. Average number of winter conidia discharged and the average dispersal distance in relation to drop size and fall height of the drops.

Drop size	Fall height of drops (cm)	Mean dispersal distance (mm)	Mean number of dispersed winter conidia
Small	15	3.8	0.2
Medium	15	11.3	1.3
Large	15	30.1	4.3
Small	25	25.1	12.5
Medium	25	30.4	1.9
Large	25	12.6	0.3

3.2.1.3. Characterization of winter conidia of Blumeriella jaapii

Morphological characterization

The length of winter conidia on overwintering cherry leaves was measured microscopically following incubation in moist chamber at 4°C for 24 h. The variation in mean length of conidia between acervuli at each sampling date varied slightly (Figure 3.14). However, the length distribution was highly variable between sampling dates. Less than 8% of the conidia were >80 µm on 16 May and 6 June while on 5 April, 6 May and 8 June, approximately 20% of the measured conidia had a length > 80 µm (Figure 3.14). This possibly reflects different levels of acervulus maturation. In Figure 3.15, the average length and distribution of conidia measured on overwintering leaves in 2009 is given. Approximately 10% of the conidia were either <50 µm or >80 µm. Of measured conidia derived from acervuli on overwintering leaves had a maximum length of 105 µm and the minimum length of 40 µm.

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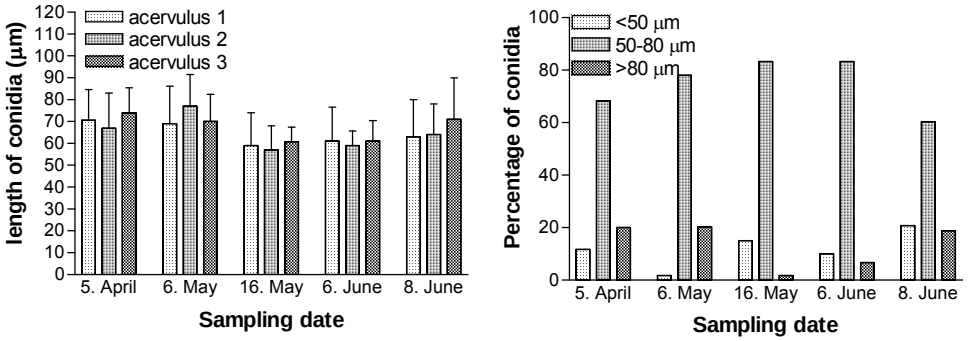


Figure 3.14. Length of winter conidia produced on overwintering leaves 2009 after moist incubation at 4°C for 24 hours of the leaves. Twenty conidia from each of three acervuli per sampling date were measured, bars represents standard deviation of the means (left). Size distribution of conidia at each sampling date (right). Data is presented as means, With standard error bars.

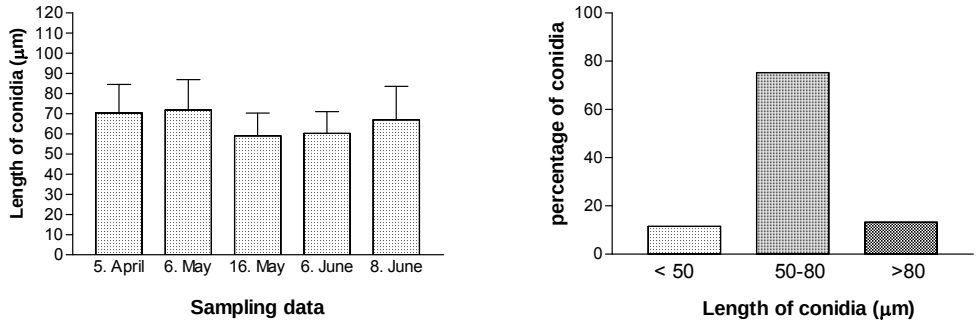


Figure 3.15. Average length of winter conidia produced on overwintering leaves 2009 after moist incubation at 4°C for 24 hours of the leaves, bars represents standard deviation of the means (left). Percent of winter conidia in three size catagories washed from overwintering leaves in 2009 (right). Data is presented as means, with standard error bars.

In 2009 and 2010, conidia of ***B. jaapii*** were measured on the slides from the spore traps. The average length of discharged conidia in 2009 was about 75 µm at both sampling dates (Figure 3.16.). The length distribution showed that approximately 25-30% of the conidia were >80 µm while no conidia <50 µm were observed on the slides. Of all measured conidia on the spore trap slides in 2009, the maximum length was 112.5 µm and the minimum length 52.5 µm.

In 2010, the discharged potential winter conidia were larger than in 2009 as the average length was between 80 to 90 µm (Figure 3.17). This was also reflected in the length distribution as more than 60% of the conidia were >80 µm and no conidia were <50 µm. Of all measured conidia on the spore trap slides in 2010, the maximum length was 127.5 µm and the minimum length 52.5 µm.

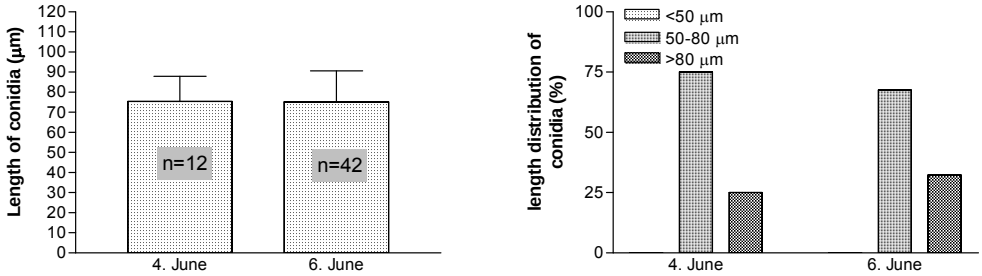


Figure 3.16. Average length of winter conidia discharged at AU-Aarslev in 2009. Bars represents standard deviation of the means (left). Length distribution of the discharged winter conidia (right).

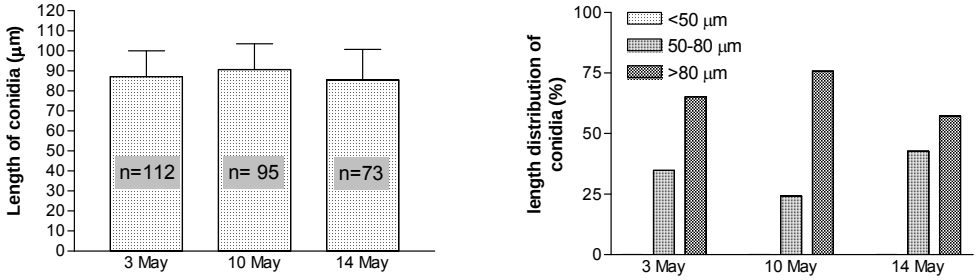


Figure 3.17. Average length of winter conidia discharged at AU-Aarslev in 2010. Bars represents standard deviation of the means (left). Length distribution of the discharged winter conidia (right).

Molecular characterization

In order to obtain the full length ITS1, 5.8S, ITS2 sequence of ***Blumeriella jaapii*** the primers denoted in Figure 3.18 were used. It should be noted that the ***ITS1*** primer was unable to amplify ***B. jaapii*** DNA, which initially was challenging since this primer normally amplifies fungal DNA without problems.

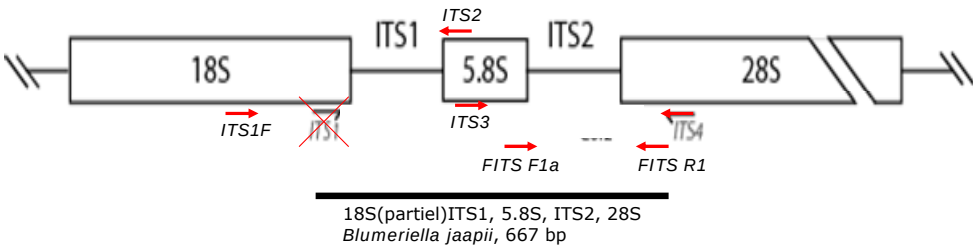


Figure 3.18. Mapping of primers used to amplify the ITS1, 5.8S, ITS2 sequence of *Blumeriella jaapii*.

From isolate MB371 a sequence of 532 bp was obtained covering 18S(partial), ITS1, 5.8S and ITS2. From isolate MB364 only 362 bp covering 5.8S, ITS2 and 28S(partial) was obtained. The two ***B. jaapii*** sequences were 100% identical in 5.8S and ITS2 (Figure 3.19). There are no ITS1 and ITS2 sequences of ***B. jaapii*** available in the GenBank. Blasting of

the ***B. jaapii*** ITS sequence revealed that the sequence had an identity of 86-87% with three genera (***Marssonina***, ***Thedgonia***, ***Chadophora***) of the Helotiales. According to morphology, both ***Marssonina*** and ***Blumeriella*** belong to the Dermaetaceae family of Helotiales. A neighbour-joining tree derived from ITS1, 5.8S, ITS2 sequences from the GenBank and the obtained ***B. jaapii*** sequence is shown in Figure 3.18. Sequences from species belonging to three families of Helotiales were included in the alignment from which the tree was created using ***Rhabdocline*** species of Hemiphacidiaceae as outgroup. The analysis revealed two main clades being Sclerotiaceae with 11 species and Dermateaceae with 11 species. ***B. jaapii*** grouped within the species all belonging to the Dermateaceae family (Figure 3.20).

An ITS sequence of 311 bp was obtained from a sample of long spores (>80 µm), that was detected on slide from the spore trap at AU-Aarslev using a laser micro dissection microscope. Alignment of the sequence showed a 100% identity with the ITS sequence of the ***B. jaapii*** isolate MB371 (Figure 3.19). On the other hand, blasting of the large spore sequence showed only 80% identity to a ***Thedgonia*** species. Therefore, it was concluded that the large spores belonged to ***B. jaapii.***

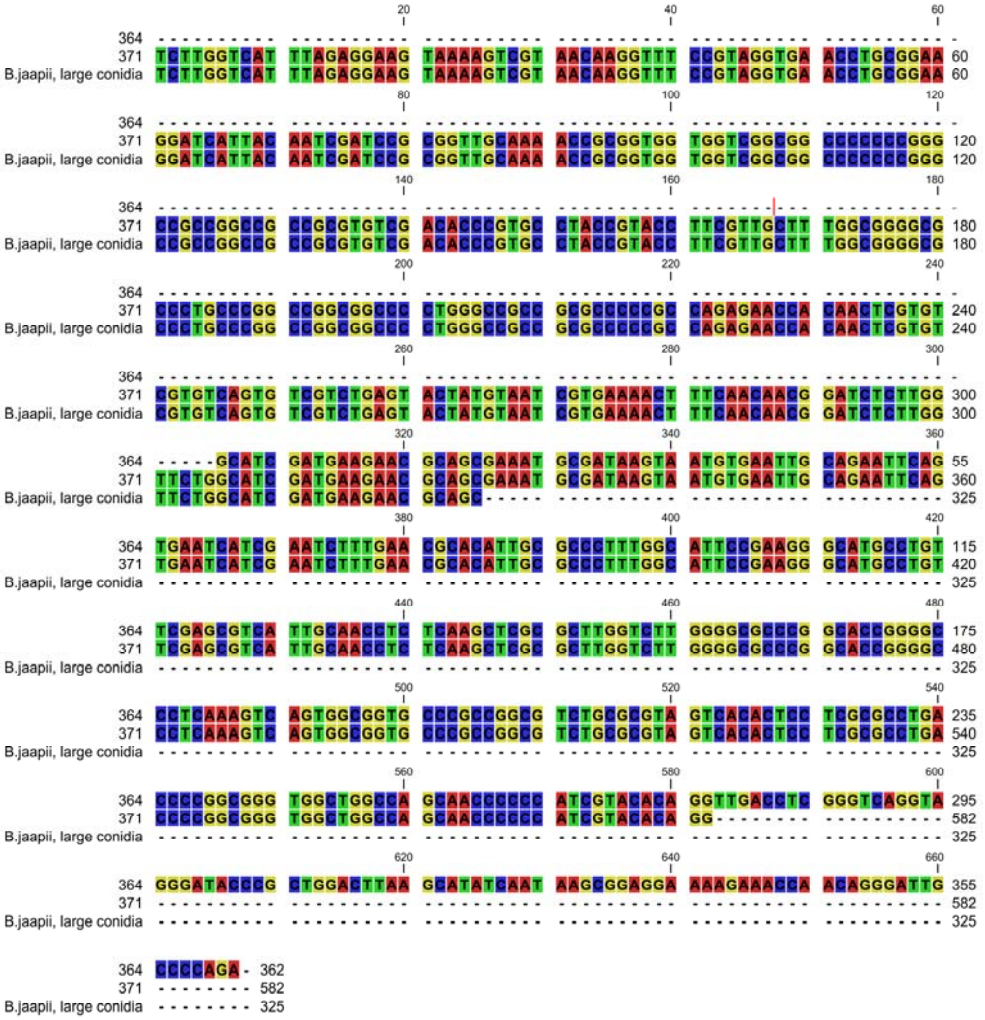


Figure 3.19. Alignment of the ITS sequences of the two *Blumeriella jaapii* isolates MB371 and MB364 and the ITS1 sequence from large winter conidia discharged from overwintering cherry leaves from cv 'Stevnsbær' in 2010.

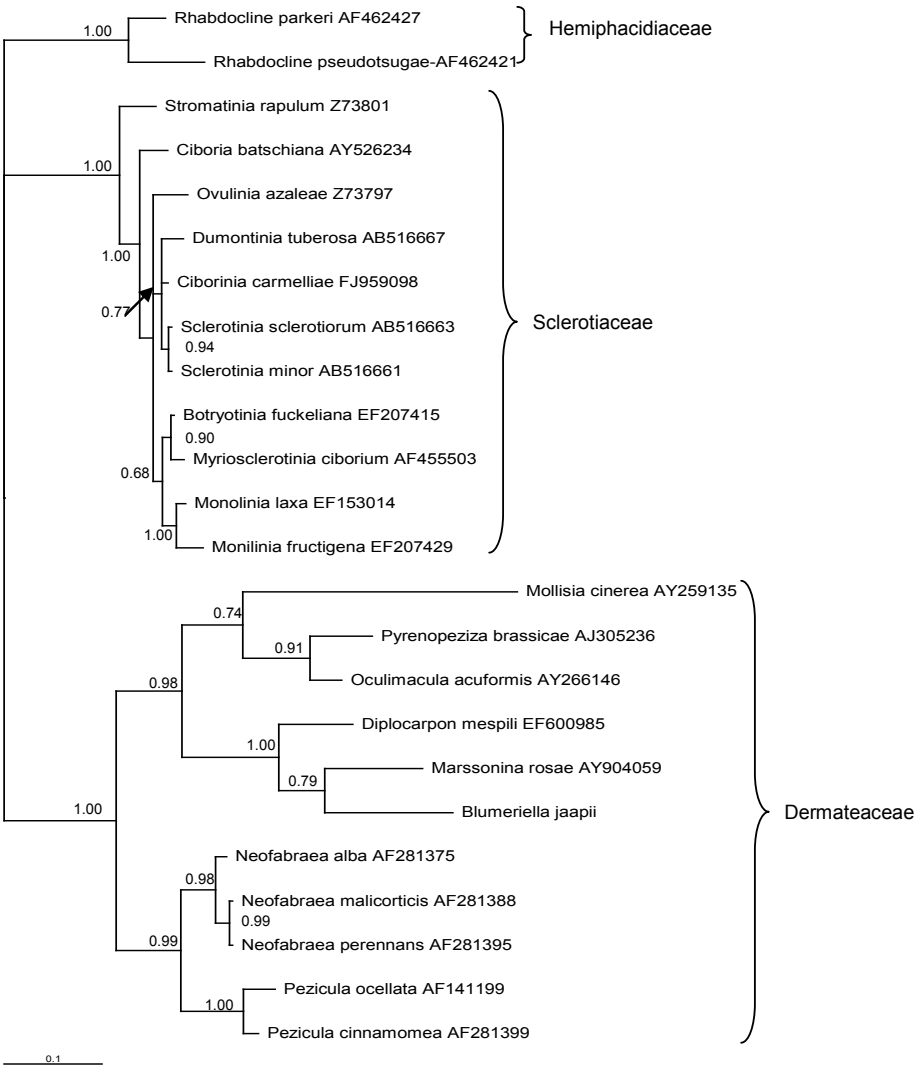


Figure 3.20. Bayesian phylogenetic tree derived from ITS1,5.8S,ITS2 sequences from GenBank of species within Helotiales. *Rhabdoline* species were used as outgroup. The obtained sequence from *Blumeriella jaapii* (B.jaapiiITS1, ITS2) was also included. Clade credibility values are shown at branch points.

3.2.2. Development of RIMpro for cherry leaf spot

3.2.2.1. Onset of disease

Cherry leaf spot caused by Blumeriella jaapii

Decisions on when to spray were carried out using best practise in combination with the weather forecast. In 2007, the spring was early, warm, and dry. Spraying was carried out prior to expected infection events on the following dates: 7 May, 21 May, 31 May, 7 June and 18 June, leaving one treatment with four replicates unsprayed at the actual date and all other treatments sprayed. Spray treatments were Delan WG (dithianon), Delan is a preventive fungicide that has an effect for approximately one week after spraying, depending on temperature and precipitation. No primary infections of cherry leaf spot were found.

In 2008, sprays were carried out 2 May, 21 May, 13 June, 9 July, and 22 July. The spring was early, warm, and dry. No primary infections were found.

In 2009, sprays were carried out 18 May, 15 June, 1 July, 16 July, and 21 September. The full spray plan kept a reasonable control of cherry leaf spot until 15 July (Table 3.23) but late in the season severe infections occurred (Table 3.24). The control, was not sufficient to control cherry leafspot in 2009. The infection controlled by the spray carried out 18 May and 1 July resulted in a significantly lower percentage of leaves without leaf spot as compared to the other treatments. There was also a tendency that the infections were partially controlled by the sprays on 15 June and 16 July. The most important infection events may have occurred around these dates (Table 3.24). The spray treatment carried out on 21 September had no effect on cherry leaf spot (Table 3.24). During August and September ***Blumeriella jaapii*** developed and the assessment in October revealed that the unsprayed trees were heavily infected and 90% of the leaves dropped premature (Table 3.24). The sprays carried out were not sufficient to control cherry leaf spot. The calculations of the infection incidence did not include leaf drop, and therefore the infection severity for unsprayed trees is in correct (Table 3.24).

Table 3.23. Symptoms of cherry leaf spot on annual shoots 10 October 2009. For the treatment 3 to 5, spraying was omitted as compared to the full spraying, at the specified date.

Treatment Unsprayed at the specified date	% leaf drop	% leaves without leaf spot	% leaves with one spot	% leaves with 2-4 spots	% leaves with 5-9 spots	% leaves with 10 or more spots	Infections severity
1. Unsprayed	5.9 a	57.0 c	9.3 a	13.5 a	7.1 a	7.2 a	21.6 a
2. Full spraying plan	3.7 a	90.6 a	3.7 d	1.3 c	0.4 c	0.3 c	2.2 c
3. 18 May	4.5 ab	69.7 b	8.2 ab	9.6 b	4.4 b	3.6 b	13.7 b
4. 15 June	4.6 ab	85.4 a	6.3 bc	2.6 c	0.6 c	0.5 c	3.8 c
5 . 1 July	3.5 b	91.1 a	4.0 cd	1.1 c	0.1 c	0.2 c	1.9 c

Values followed by the same letter in columns do not differ significantly at p≤0.05

Table 3.24. Symptoms of cherry leaf spot on annual shoots 10 October 2009. For the treatment 3 to 7, spraying was omitted as compared to the full spraying, at the specified date.

Treatment Unsprayed at the specified date	% leaf drop	% leaves without leaf spot	% leaves with one spot	% leaves with 2-4 spots	% leaves with 5-9 spots	% leaves with 10 or more spots	Infections severity
1. Unsprayed	91.7 a	0.0 c	0.0 b	0.1 c	0.1 c	8.0 d	8.2 c
2. Full spraying plan	11.1 b	42.1 b	9.3 a	14.2 a	7.5 ab	15.9 bc	30.9 a
3. 18 May	12.3 b	40.2 b	9.2 a	14.3 a	8.3 ab	15.8 bc	31.5 a
4. 15 June	13.7 b	33.2 b	9.0 a	14.2 a	10.4 a	19.5 ab	36.6 a
5 . 1 July	14.9 b	29.9 b	9.4 a	12.6 ab	9.3 a	23.9 a	39.5 a
6. 16 July	13.6 b	39.1 b	8.2 a	10.5 ab	9.7 a	18.8 ab	33.5 a
7. 21 September	10.6 b	55.2 a	9.8 a	9.0 b	5.3 b	10.1 cd	21.0 b

Values followed by the same letter in columns do not differ significantly at p≤0.05

3.2.2.2. Simulation of disease

Cherry leaf spot

A preliminary version of a simulation model for primary infections of ***Blumeriella jaapii*** was constructed based on published information on maturation, discharge, and germination of this disease. The program was run with input of weather data collected in 2007, 2008, 2009, and 2010 at AU-Aarslev using greentip as the Biofix date.

Observed spore release events were related to the dates of the rain events as found in the recorded weather data. In 2007, only ascospores were counted. In 2008, 2009 and 2010 both ascospores and conidia spores were counted on each observation date were accumulated day degrees over 4°C was calculated from the 30 min recordings of climate data. The cumulative percentage of trapped spores was plotted against accumulated day degrees starting at:

1. First of January, presuming development of ascospores start as soon as temperatures reach 4°C.
2. Greentip of main variety of sour cherry 'Stevnsbær', presuming to co-evolution of ***Blumeriella jaapii*** and its host that there is a synchronisation between pathogen and host.
3. First observed discharge of spores in the spore trap, presuming that from this event onwards further maturation and discharge would be driven by temperature.

First spores of ***B. jaapii*** were found in the spore trap 10 to 24 days after greentip (Table 3.25). In each year >90% of the spores were captured before the middle of May (Figure 3.21A).

Table 3.25. Biofix settings for RIMpro simulations for cherry leaf spot 2007-2010.

Year	First spores trapped following rain	Start of vegetation sour cherry cv. 'Stevnsbær'	Difference in date	Biofix RIMpro Calculation
2007	7 May	1 April	+ 24	5/5
2008	1 April	22 Marts	+ 10	29/3
2009	8 April	24 Marts	+15	6/4
2010	20 April	29 Marts	+ 21	18/4

Plotting the cumulative spore release against the accumulated day degrees >4°C staring January 1. An almost linear relationship occurred between the main spore production period (10-80%) and the accumulated day degrees over 4°C, but there was a great variation in the starting point of the spore discharge each year (Figure 3.21B). A different Biofix point was needed as the starting point for the calculation of maturation, discharge, and infection by model.

In order to find a Biofix as accommon starting point for the four data sets, the cumulative spore release was plotted against the moment of greentip (Figure 3.21C) and the date that first spore release was recorded (Figure 3.21D). Using green tip of sour cherry as the Biofix point did not reduce the variation between years.

Using the date that the first spores were released as the starting point reduced the variation in the cumulative discharge pattern between 2007, 2008, and 2010. In the 2009 dataset however there was a much slower increase in the first 15% of the spore release than in the other years (Figure 3.21D). In the monitoring method and the weather, there were no obvious differences in conditions between 2009 and the other years. However with the current knowledge, using "first trapped spores" is the best possible Biofix for the model.

Figure 3.21 A

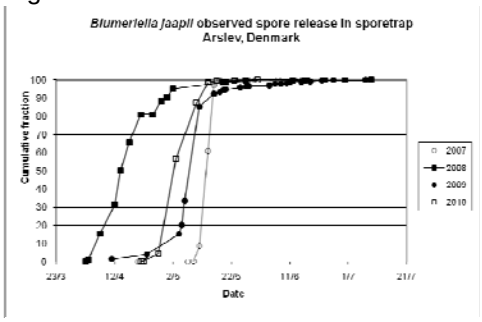


Figure 3.21 B

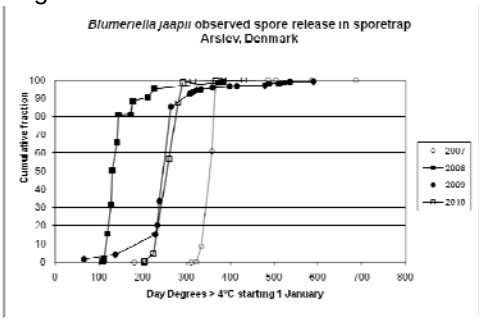


Figure 3.21 C

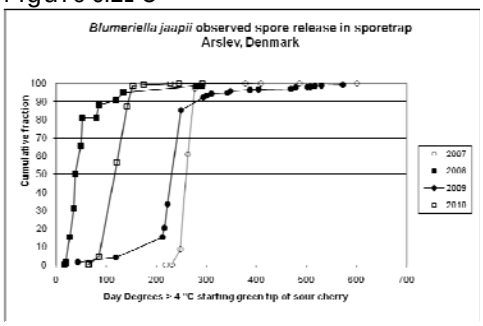


Figure 3.21 D

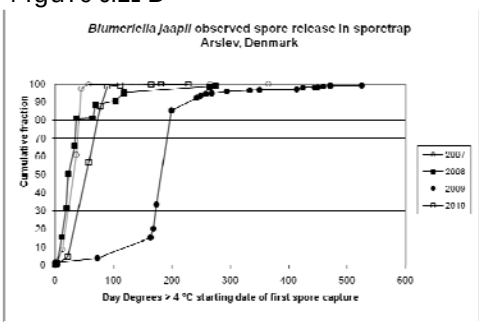


Figure 3.21A-D. Cumulative spore release of *Blumeriella jaapii* (ascospores and winter conidia) for 2007, 2008, 2009, and 2010 plotted against A: Data of spore discharge. B: Day Degrees >4°C starting 1 January. C: Day Degrees >4°C starting greentip of sour cherry, cv 'Stevnsbær' and D: Day Degrees >4°C starting at date for first discharged spore.

3.2.2.3 RIMpro-Blumeriella simulation

Figure 3.22 shows plots of RIMpro for cherry leaf spot run for 2007-2010 with the Biofix set at estimated first mature ascospores. According to the simulations, the rain events which led to spore discharge only very rarely led to infection. During the annual last discharge events, only a small proportion of the spores found the ability to germinate and infect the plant (Table 3.26 and Figure 3.22). Following the processing of the 2010 weather data by the model, no primary infections of ***Blumeriella*** should have taken place.

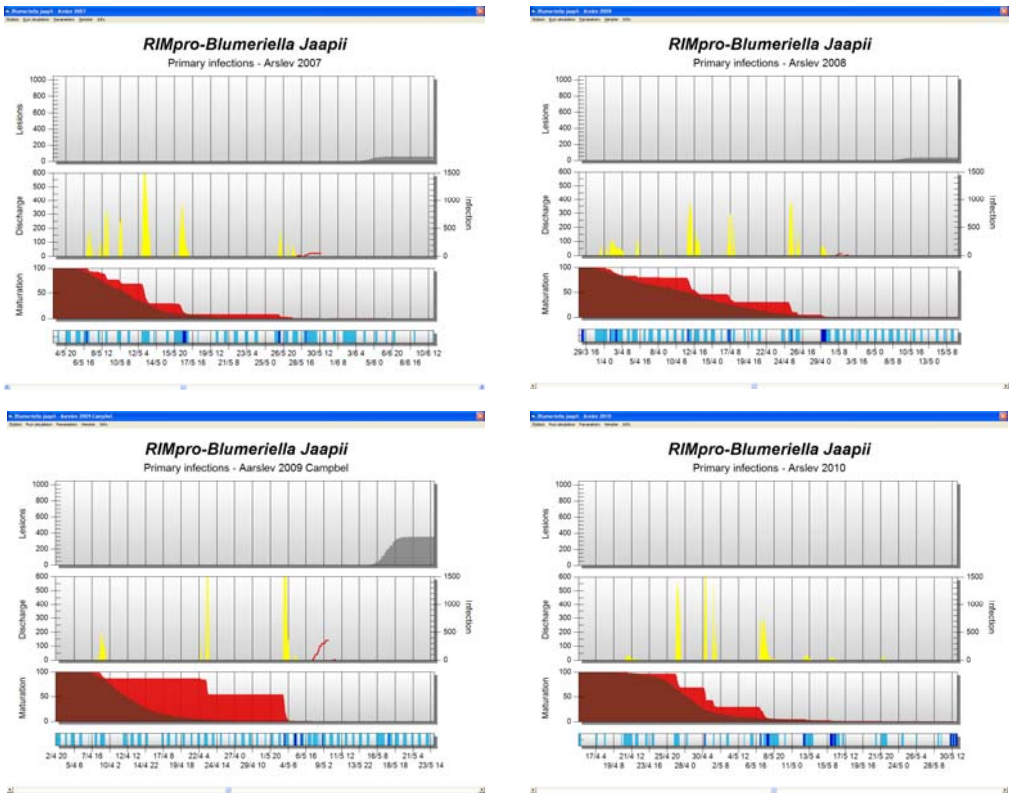


Figure 3.22. Plots of RIMpro for cherry leaf spot 2007, 2008, 2009, and 2010 using first observed spore release as Biofix and primary infections source as ascospores+winter conidia.
From top: Grey: simulated number of lesions; Yellow: simulated ascospore discharge; red: simulated quantity of spores entering the host plant=infection.
Middle part: Brown: Simulated ascospore potential which is diminishing during spring; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.

Table 3.26. Basic data for use in the RIMpro simulation model.
Rain events, date for changing slides, number of discharged ascospores and winter conidia,
Probit (cumulative mature spores (Y) in probit from the accumulated day degress (X) as : Y = 2.51 + 0.01X, with 90% confidence bands at Y+ 0.814 and Y- 0.814), degree days >4°C starting at 1 January, greentip 'Stevnsbær' and date for first spore discharge 2007-2010.

		Trapped spores			Spores number	Spores percent	Probit	DD= degree days >4C starting		
Rain events Dates	Date changing slide	Asco	Conidia	Total Sum of asco and conidia	Cumu-lative	Cumu-lative		1 Jan.	Green tip	First spore dis-charge
2007						**2007**				
	20/4	0		0	0	0.0		180		
6/5	7/5	0		0	0	0.0		311	219	
7. 8/5	9/5	5		5	5	0.1	1.96	322	231	0
10.11/5	11/5	359		359	364	8.7	3.64	334	248	12
12. 13. 14/5	14/5	2194		2194	2558	60.9	5.28	358	263	36
15. 16/5	16/5	1523		1523	4081	97.2	6.91	366	277	44
17. 18/5	18/5	106		106	4187	99.7	7.79	379	293	57
19. 26. 27.28/5	29/5	10		10	4197	100.0	8.49	486	378	164
29.30/5	31/5	1		1	4198	100.0		504	409	182
6/6	7/6	0		0	4198	100.0		587	486	265
13. 14/6	14/6	0		0	4198	100.0		687	601	365
2008										
¼-2/4	2/4	7	49	56	56	0.3	2.23	108	16	0
¾	3/4	105	82	187	243	1.2	2.75	111	19	3
5/4	7/4	2155	704	2859	3102	15.6	3.99	119	27	11
12/4	12/4	1510	1609	3119	6221	31.2	4.51	127	35	19
13/4	14/4	1763	2044	3807	10028	50.3	5.01	130	38	22
17/4	17/4	1527	1545	3072	13100	65.7	5.40	141	49	33
18/4	21/4	1566	1480	3046	16146	80.9	5.88	144	52	36
25/4	25/4	12	14	26	16172	81.1	5.88	172	80	64
26/4	28/4	711	741	1452	17624	88.4	6.19	177	85	69
29-30/4	30/4	79	377	456	18080	90.6	6.32	211	119	103
1/5	2/5	120	785	905	18985	95.2	6.66	226	134	118
18-19/5	19/5	83	577	660	19645	98.5	7.17	372	280	264
20/5	20/5	4	39	43	19688	98.7	7.23	383	291	275
26-27/6	27/5	8	111	119	19807	99.3	7.46			
11/6	12/6	3	86	89	19896	99.7	7.80			
14. 15. 16/6	16/6	2	12	14	19910	99.8	7.90			
17/6	17/6	3	11	14	19924	99.9	8.05			
20.21.23/6	23/6	5	9	14	19938	100.0	8.32			
2009										
8-9/4	11/4	89	19	108	108	1.5	2.84	65	43	0
22-23/4	23/4	52	123	175	283	4.0	3.25	137	119	72
3-4/5	4/5	143	640	783	1066	15.2	3.97	228	212	163
5/5	5/5	32	324	356	1422	20.3	4.17	233	216	168
6/5	6/5	60	865	925	2347	33.5	4.57	238	222	173
7.9/5	11/5	2603	1025	3628	5975	85.3	6.05	264	249	199
16/5	16/5	137	362	499	6474	92.4	6.43	307	294	242
17-18/5	18/5	40	29	69	6543	93.4	6.51	314	301	249
19/5	19/5	40	33	73	6616	94.4	6.59	323	310	258
20/5	20/5	11	17	28	6644	94.8	6.63	333	342	268
23.25/5	25/5	8	65	73	6717	95.9	6.74	359	348	294

26-27/5	27/5	14	23	37	6754	96.4	6.80	398	387	333
28/5	28/5	9	7	16	6770	96.6	6.83	415	404	350
4/6	4/6	5	19	24	6794	97.0	6.88	479	469	414
5.6/6	6/6	20	47	67	6861	97.9	7.04	489	479	424
8/6	8/6	4	3	7	6868	98.0	7.06	509	502	444
10/6	10/6	11	0	11	6879	98.2	7.10	515	508	450
11/6	11/6	13	11	24	6903	98.5	7.18	524	517	459
12-13/6	15/6	10	18	28	6931	98.9	7.31	536	530	471
18/6	18/6	14	1	15	6946	99.2	7.39	590	573	525
20/6	22/6	18	12	30	6976	99.6	7.64			
25.26/6	26/6	19	0	19	6995	99.9	7.98			
.	7/7	7	3	10	7005	100.0				
2010										
21/4	21/4	6	0	6	6	0.2	2.05	203	64	0
22/4	22/4	8	2	10	16	0.4	2.37	204	65	1
27/4	27/4	158	1	159	175	4.6	3.32	224	85	21
30/4.1.2/5	3/5	1881	87	1968	2143	56.7	5.17	260	121	57
7/5. 8/5	10/5	1002	160	1162	3305	87.5	6.15	280	141	77
3/5	14/5	401	20	421	3726	98.6	7.20	292	153	89
16.17/5	17/5	12	14	26	3752	99.3	7.45	314	175	111
22/5	23/5	12	3	15	3767	99.7	7.73	367	228	164
24.25/5	25/5	3	7	10	3777	99.9	8.27	384	245	181
28.30.31/5	31/5	2	0	2	3779	100.0		431	292	228

4. Discussion

4.1. Apple scab (Venturia inaequalis)

4.1.1. Importance of primary inoculum for *Venturia inaequalis*

Venturia inaequalis ascospores are an important primary infection source for onset of apple scab epidemics. Also conidia from wood are known to be a possible source of primary infection, but only under extremely favourable conditions for the disease. Primary infections by overwintering conidia occassionally occur in orchards with very susceptible apple varieties, which were severely infected by apple scab in the previous year and had finished their growth late that season. In commercial apple orchards, ascospores are considered the only source for primary infection.

In the present study the ascospores were caught in a 'Wiesmann' ascospore trap. It is assumed that the conditions in the spore trap resembled that of the surroundings measured air temperature used for the DSS calculations resembled the temperature experienced by the leaves in the spore trap, the rain and humidity measured corresponded to the humidity conditions in the spore trap, and the leaf sample in the spore trap were representative of the total population of apple leaves in an orchard. These assumptions may not have been met.

Leaves in the trap were covered with glass slides. If the spore trap was placed in direct sunlight the temperature under the glass slides could be 10 to 20°C higher than the air temperature measured at 2.0 m height (Peter Triloff, not published). The covering of the leaves by glass slides may also may change the humidity on the leaves in the trap compared to nature. During rain events with only little precipitation the leaves in the trap may not become completely wetted, and ascospore discharge may be incomplete. In additions the spore trap only provides room for a maximum of 15 apple leaves, whereas ascospore maturation is known to be variable from leaf to leaf. The limited sample of the population could be a source of error. In future studies it is suggested to investigate methods to monitor the ascospore discharge of ***Venturia inaequalis*** with the aim of evaluating or improving models that describe the maturation and discharge of ascospores. The sampling method for leaves used for spore trapping should also be improved.

Another source of error might be the fact that the ascospores were not counted every day, but after a rain event. To identify the day the ascospores were released, the number of ascospores counted was assigned to the last day with at least 0.2 mm rain, meaning that the spores counted on a certain day might have been discharged over several previous days.

4.1.2. Nitrogen and apple scab development

Apple seedlings of cv. 'Golden Promise' and cv. 'Bittenfelder Sämlings' grown in different substrates in climate chambers at three nitrogen (N) levels clearly showed that increasing the N-level increased the severity of the apple scab infection as well as the production of spores that could enter the next disease cycle. The N-induced increase in scab severity and sporulation was correlated with an increase in leaf N-content. A similar relationship was found in barley-powdery mildew system (Jensen & Munk, 1995). In addition, scab lesions at high N tended to produce more spores per lesion unit than lesions developed at low N-level, which is in agreement with Leser and Treutter(2005) who reported that a higher percentage of ***Venturia inaequalis*** infected leaves occurred on trees at high N-level than at low N-level. However, very little differences occurred in carbon content and dry weight of the plants. This showrd that the plant growth in these rather small seedlings was not significantly affected by the N content.

Investigation of the effect on N supply on the infections of apple scab in an orchard was set up. As only very few and small scab symptoms developed during the 3 years it was difficult to evaluate the effect on apple scab infection risk in an orchard. However a very high N supply of 200 kg per ha per year showed higher infection of apple scab. The N content in the seedlings varied from 5-7% N in the dry matter and the highest level of nitrogen in the leaves in the orchard was 2.82%. The recommended N content in Denmark is between 2.2 and 2.5% nitrogen in dry matter (Vang-Pedersen et al. 1977). These results showed that high N supply and content in the plant gave a higher risk for development of infection and an increased spore production. However, if the supply of N was kept at a maximum of 100 kg N per ha per year the risk for increased apple scab infection was low in years with low disease infections.

4.1.3. Nitrogen and apple production

Increased N supply increased the N level in the soil especially if the annual supply was 100 kg N per ha or above. The N uptake and level in the leaves increased significantly with a supply of 50 kg/ha and above. The level of N in the soil was very different between years 2007 versus 2008, however this was not clearly reflected in the leaf samples. The differences between available N in the soil may be due to less precipitation in the winter 2007-2008 and thereby less N leaching or a higher soil temperature in 2007, which increased the mineralisation of N. To supply the trees with a certain amount of N it is important to know the level of available N in the soil in the spring. Soil samples to determine available mineralised N would then be necessary.

A supply of N at 50 kg/ha or more increased the tree growth, measured as stem diameter. The flowering and the yield was not influenced by the N supply in 2007 and 2008, but there was a reduction in yield if no N was supplied. In 2007 and 2008, no N supplied reduced the fruit development, shown as fruit size in gram. The N supply had no clear effect on the internal quality of the fruit measured as fruit firmness and sugar content. However, a high N supply very clearly reduced the amount of red skin surface, this was also found by Kühn and Lindhard Pedersen (2009).

4.1.3 Development of a decision support system (DSS) for apple scab (*enturia inaequalis*)

The DSS RIMpro was tested under Danish conditions throughout the project period. The optimal way of using a decision support system (DSS) is to carry out the fungicide treatment based on when the DSS estimated a high infection risk. By then the rain event has taken place and an infections is more likely. For this use, it is necessary to have access to fungicides which have a curative effect on the fungi, however, this was not the case every year. In the first year of the project period only the preventive fungicide dithianon was available. The decision on timing of the apple scab control treatments in the trial therefore had to be made prior to an expected infection event. However, still using RIMpro and the weather forecast for the best timing of the treatments. In 2007, 2008 and 2009 only very few infections of apple scab developed in the well managed research orchard of the cultivar 'Elshof', both in the systems where treatments were carried out only in the primary apple scab season and where treatment continued throughout the whole growing season.

On average, 6-7 fungicide treatments were applied annually to control apple scab in the primary apples scab season, preventive treatments prior to an expected rain event based on RIMpro with 'green tip' as Biofix (Table 4.1).

However, very few apple scab infection in practice took place at the time of green tip (Tables 3.12 and 3.13). As the actual sprays were carried out prior to an expected infection and not all expected infection periods occurred therefore more treatments events were carried out than infection events (Table 4.1).

The DSS RIMpro using 'green tip' as Biofix predicted too many infection periods in the three rather dry springs of 2007-2009. RIMpro predicted 1-3 severe apple scab infection each year (Table 4.1).

Table 4.1. Actual treatments to control apple scab carried out and simulated infection events using DDS RIMpro with two Biofix sets for 2007 to 2009.

	Biofix: greentip	Biofix: green tip		Biofix: first mature spores	
Year	Actual spraying carried out in the primary infection period	Major infection events RIM value > 250	Minor infection events RIM value 100-250	Major infection events RIM value > 250	Minor infection events RIM value 100-250
2007	7	3	1	0	1
2008	6	2	0	2	0
2009	6	1	2	1	0

In most apple producing regions in Europe first ascospore discharge is recorded a few days before or after bud break (Annex A). However, in Denmark the time between green tip and the actual start of ascospore releases varied considerable between years. The difference was 10-20 days between first ascospore discharge and green tip in 2007 to 2010 (Table 3.14). The discharge of ascospores started 10-20 days before start of vegetation of the apple trees. The correlation coefficients between observed ascospore discharge and expected discharge of the 'New Hampshire model', 'RIMpro with Biofix green tip' and 'RIMpro first ascospore release' showed that the best fit occurred when Biofix was set to 'first ascospore release' (Annex A). This striking difference and variation in the relative penology of apple tree and apple scab for Denmark, was most probably caused by the mild and wet winter maritime conditions in Denmark. In other apple production regions

"Green tip of apple" is a useful alternative to the more complex observations on maturation of pseudethecia or management of spore traps to establish the Biofix date for RIMpro. In Denmark these observations were necessary to find the right date to initiate the RIMpro simulations each year.

When the simulation for the risk of apple scab infections were run with Biofix set as 'first mature spores' (Tables 3.15 and 3.16) 81% of the rain events in 2007-2010 had an acceptable estimation in the RIMpro model, 9.4% were underestimated and 9.4% of the rain events were overestimated. In total two rain events, which were under estimated could have been critical for the effectiveness of the scab control if the following wetness period would have been long enough to cause a serious infection.

With a perfect DSS system and curative fungicides available only one treatment against apple scab would have been necessary in the primary seasons of 2007, 2008 and 2009, 3 May 2009 event that actually lead to an infection (Table 3.13). In practise, 19 treatments were carried out using preventive fungicides before expected infections. If the combination of curative fungicides and RIMpro with Biofix set as 'first matures spores' would have been used perfectly, only 3-4 treatments to control apples scab would been needed (Table 4.1).

For an apple grower this means that based on best practise available in 2007 to 2009, 19 treatments would have been carried out to control apple scab in the primary apple scab season. However only one treatment was necessary. If curative fungicides and the new RIMpro version had been available only 3-4 treatments would have been applied.

To improve the simulations of RIMpro it would probably be worth working on a better method to monitor and thereby predict the ascospore discharge, as the climatic conditions in the spore traps probably do not fit to nature and the amount of leaves used for evaluating discharge should be selected to represent a reasonable part of the apple scab population.

4.2. Cherry leaf spot (*Blumeriella jaapii*)

4.2.1. Importance of primary inoculum for *Blumeriella jaapii*

Ascospores of ***B. jaapii*** are considered as the most important source of primary inoculum (Garcia and Jones, 1993; Pedersen and Løschenkohl, 1997). However, winter conidia of ***B. jaapii*** are also produced in overwintering leaves (Pedersen and Hockenhull 1996; Bengtsson et al. 2006). The role of the winter conidia in the epidemiology of cherry leaf spot is not known (Jones 1995, Bengtsson et al. 2006). When counting discharged ascospores of ***B. jaapii*** using a 'Wiesmann' spore trap, we identified large numbers of spores that morphologically resembled winter conidia, which are described to be 2-3 x 50-80 µm (Blumer, 1958). However, a considerable ratio of the observed conidia was longer than the description. A morphological characterisation of ***B. jaapii*** winter conidia from acervuli on overwintering cherry leaves in 2008 and 2009, showed that approximately 10% of the conidia were >80 µm with a maximum length of 105 µm, which proved that winter conidia are often considerably longer than previously described. A molecular method based on species specificity of the ITS1 and ITS2 regions of ***B. jaapii*** was developed in the project. Using this method we were able to show 100% identity between an ITS1 sequence from a sample of discharged

potential winter conidia >80 µm and a single spore isolate of ***B. jaapii*** (Figure. 3.19). We therefore concluded that ***B. jaapii*** winter conidia were discharged and caught in 'Wiesmann' ascospore traps. Measuring discharged winter conidia from the traps in 2009 and 2010 showed a maximum length of 113 and 128 µm, respectively and furthermore 25% and 60% of the discharged conidia were >80 µm in 2009 and 2010, respectively.

In 2008, 2009 and 2010 these potential winter conidia (including spores>80 µm) were counted as well as the ascospores. In 2008 and 2009, their discharge period was similar to the ascospores and the amount of winter conidia accounted for approximately 50% of the total discharged ***B. jaapii*** spores. In 2010 the winter conidia only accounted for approximately 10% of the discharged ***B. jaapii*** spores and the discharge period was 1 week shorter than that for the ascospores. Investigation of the inoculum potential of ***B. jaapii*** showed that the potential number of spores in the overwintering leaves remained at high levels following each rain events, which resulted in discharge of both ascospores and winter conidia In 2008 the number of potential winter conidia was five times higher than the amount of ascospores and in 2009 the inoculum potential was similar for the two spore types. However, no obvious relationship between actual discharge of both spore types and the estimated inoculum potential could be established The actual discharge period for ***B. jaapii*** varied more than for ***V. inaequalis*** in two out of four years the discharge started late and ended early One mechanism of winter conidia discharge is potentially rain splash, a model study showed that winter conidia could be discharged by water drops hitting a mature acervulus leasing to dispersion up to 3 cm. However, this discharge mechanism is probably limited for the conidia caught in the spore trap, as the slides of the trap covered the overwintering leaves and thereby prevented, rain drops from directly hitting the acervuli.

In conclusion we have shown that ***B. jaapii*** winter conidia are considerably longer than earlier described, that they occur in high numbers on overwintering leaves and that they discharge in high numbers mostly coincidently with ascospores. Therefore it is likely that ***B. jaapii*** winter conidia play an important role in the primary infections of cherry leaves. However, their infection capacity, incubation rate, and development on the host still need to be investigated.

4.2.2 Development of a decision support system (DSS) for cherry leaf spot (*Blumeriella jaapii*)

For cherry leaf spot, no primary infection was observed in 2007 and 2008, but later in these seasons smaller secondary infections occurred. Earlier in other trials secondary infections developed where no primary lesions where found (Keitt, 1937; Niederleitner and Zinkernagel, 1999).

In July 2009 primary infections were observed. Sprays were carried out according to normal practice. This method was not sufficient as trees sprayed with the full spray plan had severe infections in September 2009. The unsprayed trees had extremely severe infections, in September 92% of the leaves dropped premature due to infections. The infection controlled by a spray carried out 18 May had a relatively good effect on disease control in the middle of July, whereas the treatments carried out 15 June and 1 July seemed to have no effect on cherry leaf spot infection middle of July. However, when we looked at the infections established middle September all treatments had

severe infections. Primary infections established in May and June developed severe secondary infections. Cherry leaf spot seemed to be rather slow in establishing primary infections. No or very few infections were observed despite the large discharge of both ascospores and winter conidia observed in 2007 and 2008, perhaps the primary infections were difficult to detect. In 2009, a primary infection developed due to a large discharge of both ascospores and winter conidia. Later in the 2009 season severe secondary infection occurred.

To develop a DSS for cherry leaf spot, the effect of rain on the amount of ascospores released and the effect of temperature on discharge were investigated (Annex B). As little as 0.2 mm rain were sufficient to trigger strong ascospore discharge, but the amount of rain was not correlated to ascospore discharge. Ascospore discharge occurred at both low and high temperatures, and there was no distinct lower threshold or optimum temerature, but the rate of discharge increased with temperature (Annex B).

To determine the best Biofix for cherry leaf spot, different starting points for the development of ascospores were tested. Garcia and Jones (1993) found 4°C to be the lower, developmental threshold for maturation of ascospores of ***Blumeriella jaapii.*** The dates of first ascospore discharge were expressed as day degrees (DD) over 4°C. Different staring points for this maturation were tested. The best but not perfect starting point was found to be 'first mature ascospores'. After this evaluation, 'first mature ascospores' were used as Biofix for the DSS cherry leaf spot simulations.

A first version of a DSS for cherry leaf for primary infections of ***Blumeriella jaapii*** was constructed based on the above knowledge and published information on maturation, discharge, and germination. The program was run with input of weather data collected in 2007, 2008, 2009, and 2010 at AU-Aarslev. According to the infection simulations, only very small infections were observed in 2007 and 2008, which fits to the actual observations that no primary infections occurred in these years. In 2009, a small primary infection was simulated and a small primary infection occurred, which later developed into a severe secondary infection.

The first version of the DSS for cherry leaf spot showed a good correlation between the prediction of infection and to the actual spore discharge. However, setting the right Biofix for this model was critical as most spores were produced in a minor interval following the Biofix and the perfect Biofix has not yet been identified. In this research Biofix setting for 'first mature spores' fitted in 3 out of 4 years. More knowledge about the establishment of the primary infection and a better model for Biofix setting is necessary to develop a better DSS for cherry leaf spot.
A DSS for cherry leaf spot is necessary to develop an effective control of cherry leaf spot with the right timing of spray treatments to control the disease. Normal practise of spraying 5 times yearly is not sufficient. In years with no primary infections these sprays are un necessary and in 2009 where a severe infection occurred these five spays was insufficient or wrongly timed to effectively control the disease.

5. Conclusions

5.1. Apple scab (*Venturia inaequalis*)

The influence of nitrogen (N) management on apple scab

- High supply of N increased the risk of apple scab infection as more spores were produced and the trees were more susceptible.
- If annual supplies of N were kept at 100 kg N per ha or lower, the risk for increased apple scab infection risk was low in years with few primary infections of apple scab.
- N supply reduced the amount of red skin surface on apples.
- N supply higher than 50 kg per ha, did not increase yield or fruit size in these trials.

Development of decision support system (DSS) for apple scab

- The actual Biofix setting of RIMpro is very important in Denmark.
- Biofix for apple scab in Denmark has to be 'first mature ascospores' and not 'green tip'.
- The use of 'first mature ascospores' as Biofix needs a yearly evaluation of the start of the ascospore discharge.
- In 2 out of 85 rain events (2.4%) the RIMpro DSS underestimated the ascospore discharge in a potentially critical situation.
- Access to curative fungicides is important to optimize the use of DSS.
- In the primary apple scab season 19 apple scab preventive treatment were carried out over a three-year period. If curative fungicides were available and the RIMpro DDS was set with 'first mature ascospore' as Biofix, only three to four treatments would have been necessary.
- Using the DSS a 79% reduction in the level of sprayed fungicide against apple scab in the primary season would have resulted.
- An optimized DSS would only have identified one infection event that needed treatment.

5.2. Cherry leaf spot (*Blumeriella jaapii*)

Primary inoculum of cherry leaf spot

- Winter conidia of cherry leaf spot were caught at the same frequency and under the same conditions as ascospores.
- Some winter conidia are larger than earlier described.
- The role of winter conidia in the infection process for cherry leaf spot still needs to be investigated.
- To understand the primary infection processes in cherry leaf spot more basic research in the disease biology is needed.

Development of DSS for cherry leaf spot

- Best practise for control is currently insufficient for cherry leaf spot control.
- 0.2 mm rain was sufficient to trigger strong ascospore discharge.
- The amount of rain was not correlated to ascospore discharge.
- Currently, ‘first mature ascospores’ is the best known Biofix.
- A first version of DSS for cherry leaf spot has been developed.

6. Perspectives

6.1 Apple scab (*Venturia inaequalis*)

The Decision support system (DSS) RIMpro has been used and validated in a number of European countries. The present study has demonstrated the importance of adjusting a system to the 'local' conditions. For using RIMpro in Denmark to control apple scab the setting of the 'Biofix' (starting point of the DSS) is very important. Whilst green tip is used om other countries as the Biofix date this is not suited to Danish conditions. In the Danish climate, the Biofix must be set as 'first mature ascospores', this should be re-evaluated each year.

To optimise the simulated ascospore discharge used for DSS RIMpro it is necessary to look at the methods to monitor the ascospore discharge of ***Venturia inaequalis*** with the aim of evaluating or improving models that describe the maturation and discharge of ascospores. The use of 'Wiesmann' spore traps were not optimal, therefore other methods should be developed. The sampling method for leaves used in the spore traps should also be improved.

Access to curative fungicides is essential as the RIMpro gives a warning for disease when a rain event has taken place and induced an infection risk. At this time, the spores of ***Venturia inaequalis*** are likely to have started to grow on the leaves. To control these infections curative fungicides are needed. To use RIMpro; updated DSS software, a PC, and access to local weather data is necessary.

If curative fungicides were available and applied based on RIMpro, 79% of the fungicide treatments against apple scab could have been saved over the three years 2007, 2008, and 2009. The outcome would have been optimal apple scab control and reduced expenses for salary, fungicides, and petrol. The number of fungicide treatment per year is timed by RIMpro; this timing depends on the actual climate in an individual year. In some years no fungicide treatments can be saved, but the optimal timing of the fungicides needed.

To optimise nitrogen (N) supply it is important to know the level of available nitrogen in the soil in the spring. Soil samples to determine available mineralised nitrogen is firstly necessary

The optimum use of nitrogen in a drip irrigated intensive 'Elshof' orchard was 50 kg N per ha supplied from April to August. The fruit yield was high and the fruit were more red that for higher N supply. This amount of N supplied (50 kg N per ha) is not expected to induce a high apple scab infection risk even in years with severe infections.

6.2 Cherry leaf spot (*Blumeriella jaapii*)

The present study has demonstrated the importance of knowing the biology of the target pathogen before developing a DDS system. We have shown that winter conidia may play an important role in primary infection of cherry leaf spot, as they are present at a similar frequency as ascospores. However it is not known if they cause primary infections of cherry leaf spot. Some years primary infections of cherry leaf spot occur, but they are not detected. Despit a lack of primary infections, secondary infections may occur later in the season. Basic research to understand the infection processes in cherry leaf spot is needed.

The first version of a DSS for cherry leaf spot has been developed. The first version of the DSS simulated the actual primary infections in 2007 to 2010 quite well. However, testing the program is needed in practise to see how well it predicts infections in years with severe infections. Basic knowledge is needed to effectively model and run the system. Validation of the DSS is needed.

One of the most important outcome of the study are that annual measurements of the start of spore discharge are needed to determine the correct Biofix, as the starting point for the simulations of cherry leaf spot infections.

The recommendations of RIMpro would be improved if curative fungicides were available as the DSS gives a warning for disease when a rain event has taken place and induced an infection risk. To use RIMpro, updated DSS software, a PC, and access to local weather data is necessary.

7. References

Agrios, G.N. 2005. Plant Pathology, fifth ed. Academic Press. Pp 922.

Analytis, S. 1973. Methods of analysing epidemics as exemplifeid by apple scab, ***Venturia inaequalits*** (Cooke) Hader. Acta Phytomedica 1. Verlag Paul Parey, berlin (DE).

Anonym, 2010. Håndbog for Frugt og Bæravlere. 2010. Udgivet af Frugt og Grønt Rådgivningen. Pp 128.

Arneson, P.A., Oren, T.R., Loria, R., Jenkins, J.J., Goodman, E.D. and Cooper, W.E. 1979 APPLESCAB: a pest management game. NACTAJ 23: 61-62.

Bengtsson, M. 2001. Biological control of the overwintering stages of cherry leaf spot (***Blumerialla jaapii***) and apple scab (***Venturia inaequalis***) Ph.D. thesis, Department of Plant Biology, KVL.

Bengtsson, M., Green, H., Leroul, N., Pedersen, H.L. and Hockenhull, J. 2006. Effect of autumn application of urea on saprotrophic fungi in off-season leaf litter of sour cherry and evaluation of fungal isolates to reduce primary inoculum of ***Blumeriella jaapii.*** Journal of Plant Disease and Protection 113: 107-112.

Bengtsson, M., Hockenhull, J. and Neergaard, E. de. 2006. Histopathological study of the effect of post leaf fall application of urea on saprotrophic development of the cherry leaf spot pathogen, ***Blumeriella jaapii.*** Journal of Plant Diseases and Protection 113: 214-218.

Bengtsson, M., Jørgensen, H.J.L., Pham, A., Wulff, E. and Hockenhull, J. 2006. Screening of organically based fungicides for apple scab (***Venturia inaequalis***) control and a histopathological study of the mode of action of a resistance inducer. Proceeding from 7th International IOBC/wprs Workshop on Orchard Diseases, 31 August – 4 September 2005, Piacenza, Italy. IOBC /wprs Bulletin 29(1): 123-127.

Bengtsson, M., Jørgensen, H.J.L., Wulff, E. and Hockenhull, J. 2006. Prospecting for organic fungicides and resistance inducers to control scab (***Venturia inaequalis***) in organic apple production. Proceeding from European Joint Organic Congress, 30-31 May, Odense, Denmark, pp 318-319. Online: http://orgprints.org/7395/01/BENGTSSON_etal_Prospecting.pdf

Berrie, A.M., and Xu, X.M. 2003. Managing apple scab (***Venturia inaequalis***) and powdery mildew (***Podosphaera leucotricha***) using Adem™. International Journal of Pest Management. 49: 243-224.

Best, E.K. 1976. An automated method for determining nitrate-nitrogen in soil extracts. Queensland J. Agr. and Animal Sci. 32: 161-166.

Blumer, S. 1958. Beitrage zur Kenntnis von ***Cylindrosporium padi.*** Phytopathol. Zeitschr. 33: 263-290.

Boshuizen, A.J., and Verheyden, C. M. 1994. An agrometeorological network for warnings against scab in Belgium. Norwegian Journal of Agricultural Sciences. Supplement 17: 429-441.

Buchter-Weisbrodt, H., 1996. Phenole gegen schorf. Obstbau 2: 62-64.

Crooke, W.M. and Simpson W.E. 1971. Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J. Sci. Food. Agr. 22: 9-10.

Croxall, H.E., Gwynne, D.C. and Jenkins, E.E. 1952. The rapid assessment of apple scab on leaves. Plant Pathology 1: 39-41.

Duttonm W.C. and Wells H.M, 1925. Cherry leaf spot. Special bulletin. Michigan State College, Agricultural experiment station, Section of forestry 147. pp16.

Eisensmith, S.P. and Jones, A.L. 1981a. A model for detecting infection periods of ***Coccomyces Hiemalis*** on sour cherry. Phytopathology 71: 728-732.

Eisensmith, S.P. and Jones, A.L. 1981b. Infection model for timing fungicide applications to control cherry leaf spot. Plant Disease: 955-958.

Garcia S.M and Jones A.L. 1993. Influence of temperature on apothecial development and ascospore discharge by ***Blumeriella jaapii***. Plant Disease. 77: 776-779.

Gadoury, D.M and MacHardy, W.E. 1982. A model to estimate the maturity of ascospores of ***Venturia inaequalis***. Phytopathology. 72: 901-904.

Henriksen, K., Hansen, C.W., Pedersen H.L., Paaske K. and Andersen L. 2003. Muligheder for forebyggelse og alternativ bekæmpelse inden for Gartneri og frugtavl. Bekæmpelsesmiddelforskning fra Miljøstyrelsen, nr 71. pp 86. www.mst.dk

Hockenhull, J., Bengtsson, M., Wulff, E., Jørgensen, H.L.J., Collinge, D., Pedersen, H.L., Paaske, K. and Nørbæk, R. 2005. Control of scab in organic apple growing (StopScab). Final report. Research in organic farming (2000-2005) (DARCOF II). http://www.okoforsk.dk/projekt/foejoII/i14/slut.pdf

Hofmaier, C. 1994. BIOMAT – a smart tool for apple scab control. Norwegian Journal of Agricultural Sciences. Supplement 17: 253-256.

Holder, M. and Lewis, P.O. 2003. Phylogeny estimation: traditional and Bayesian approach. Nature Rev. Gen. 4: 274-284.

Jakobsen, H. and Jørgensen, K. 1986. Cherry Leaf Spot. Danish investigations of perennation state and primary infection in sour cherries. Tidsskrift for Planteavl 90: 161-175.

Jensen, B. and Munk, L. 1995. Nitrogen-induced changes in colony density and spore production of ***Erysiphe graminis*** f.sp. ***hordei*** on seedlings of six spring barley cultivars. Plant Pathology 46: 191-202.

Jones, A.L. 1978. Analyses of apple scab epidemics and attempts at improved disease prediction. Proceedings of the Apple and Pear Scab Workshop. Kanas City. Michigan. 19-22.

Jones, A.L., Ehret, G.R., Garcia, S.M., Kesner, C.D. and Klein, W.M. 1993. Control of cherry leaf spot and powdery mildew on sour cherry with alternate-side applications of fenarimol, myclobutanil and tebuconazol. Plant Disease 77: 703-706.

Jones A.L. and Aldwinckle, H.S. 1990. Compendium of Apple and Pear Diseases. APS Press, pp 125.

Keitt, G.W., Blodgett, E.C., Wilson, E.E and Magie, R.O. 1937. The epidemiology and control of cherry leaf spot. Wis. Agric. Exp. Stn. Res Bull. 132. pp.117.

Korsgaard M. and Lindhard Pedersen H. 2007. Frugt og bær, landbrugsforlaget pp. 304.

Kühn, B.F. and Pedersen, H.L. 2009. Cover crops and mulching effects on yield and fruit quality in unsprayed organic apple production. European Journal of Horticultural Sciences. 74: 247-253.

Kranz, J., Mogk, M. and Stumpf, A. 1973. EPIVEN- a simulator for apple scab. Zeitschrift für pflanzenkrankheiten 80: 181-187.

Larget, B. and Simon, D.L. 1999. Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16: 750-759.

Leser, C. and Treutter, D. 2005. Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. Physiologia Plantarum 1123: 49-56.

Lindhard H. 2003. Vurdering af muligheder for forebyggelse og alternativ bekæmpelse i Frugt og Bær. Arbejdsrapport fra Miljøstyrelsen Nr. 38 pp 131. www.mst.dk .

Lindhard Pedersen, H. and Bertelsen, M. 2002. Alleyway groundcover management and scab resistant apple varieties. ECO-FRU-VIT. 10th International Conference on Cultivation technique and Phytopathological problems in Organic Fruit-Growing and Viticulture. p. 16-21.

Lindhard Pedersen, H., Christensen L.P., Bengtsson M.V., Paaske K. and Hockenhull J. 2005. Organic field-testing of compounds to control apple scab (***Venturia inaequalis***) in combination with alleyway cover crops. Poster til 7th International IOBC/WPRS Workshop on Orchard Diseases. Italy Aug-Sep. 2005.

Lindhard Pedersen, H., Linddal Pedersen, K. and Paaske, K. 2005. Evaluating the use of RIMpro and Metos weather stations for control of apple

scab (***Venturia inaequalis***) in Denmark 2002-2005. Poster til 7th International IOBC/WPRS Workshop on Orchard Diseases. Italy Aug-Sep. 2005.

Lindhard Pedersen, H. and Løschenkohl B. 1997. Testing of a warning system against cherry leaf spot (***Blumeriella jaapii***). Gartenbauwissenschaft 62: 197-201.

Lindhard H., Pedersen, K.L and Paaske K. 2006. RIMpro kan reducere antallet af sprøjtninger. Frugt og Grønt, 2: 72-74.

MacHardy, W.E. 1996 Apple Scab: Biology, Epidemiology, and Management. APS Press, 545 pages.

Mattedi, L. and Varner, M. 2000. Produzione integrate attraverso la conoscenza delle pricipali malattie fungine del melo e della vite. Arti Grafiche La Commerciale-Borgogno, Bolzano.

Mills, W.D. 1944. Efficient use of sulphur dusts and sprays during rain to control apple scab. Cornell Ext. Bull. 630. 4 pp.

Minogue, K.P. 1978. A mathematical model for epidemics of apple scab. PhD Dissertation, MacGill University.

Neergaard, P. 1949. Kirsebærbladpletsyge (***Higginsia hiemalis***) – en for Danmark ny, alvorlig kirsebærsygdom. Gartnertidende 46: 487-489.

Niederleitner, S. and Zinkernagel, V. 1999. Primary infection and epidemic development of the cherry leaf spot disease Blumeriella jaapii (Rehm) von Arx. J. Plant Dis. Prot. 106: 88–97.

Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K. and Uyemoto, J.K. 1995. Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN, USA, 128 pages.

Parisi, L., Lespinasse, Y., Guillaumés, J. and Krüger, J. 1993. A new race of ***Venturia inaequalis*** virulent to apples with resistance due to the Vf gene. Phytopathology 93: 533-537.

Parker, D.M., Hilber, U.W., Bodmer, M., Smith, F.D., Yao, C. and Koller, W. 1995. Production and transformation of conidia of ***Venturia inaequalis***. Phytopathology 85: 87-91.

Pedersen, H.L. and Hockenhull, J. 1996. Effects of urea on the formation of apothecia and winter conidia of ***Blumeriella jaapii*** (Cherry Leaf Spot) and associated microfungi on overwintered cherry leaves. Gartenbauwissenschaft 61: 257-261.

Ronquist, F. and Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Rossi, V., Giosue, S., and Bugiani, R. 2007. A-scab (Apple-scab), a simulation model for estimating risk of ***Venturia inaequalis*** primary infections. Journal compilation. Bulletin OEPP/EPPO Bulletin 37: 300-308.

Schluster M., 2004. Investigation on resistance to leaf spot disease (***Blumeriella jaapii***) in cherries. J. of Fruit and Ornamental plant research, Special ed. 12: 275-279.

Schwabe, W.F.S., Jones, A.L. and van Blerk, E. 1989. Relation of degree-day accumulation to maturatin of ascospores of ***Venturia inaequalis***. South Africa. Phytophylatica 21: 13-16.

Seem, R.C., Shoemaker, C.A., Reinolds, K.L. and Eschenbach E.A. 1989. Simulation anf optimization of apple scab management. IOBC/WPRS Bulletin 2: 66-87.

Sjulin, T.M., Jones A.L., and Andersen R.L. 1989. Expression of partial resistance on cherry leaf spot in cultivars of sweet, sour, duke an European ground cherry. Plant Disease 73: 56-61.

Spanna, F., Cotroneo A., Galliano, A. and Vittone, F. 2002. Validation of the model RIMpro for the spread of apple scab in the fruit growing area of Piemonte. Notiziario sulla protezione delle piante 15 NS: 315-320.

Townsend, G.R. and Heuberger, J.W. 1943. Plant Disease Reporter. 27: 340-343.

Trapman, M.C. 1993. Control of scab on the basis of RIM. De Fruitteelt 50, 31-34.

Trapman, M.C. 1994. Development and evaluation of a simulation model for ascospore infections of ***Venturia inaequalis***. Norwegian Journal of Agricultural Sciences 17: 5-67.

Trapman, M.C. and Polfliet, M. 1997. Management of primary infections of apple scab with the simulation program RIMpro: review of four years field trials. IOBC Bulletin 20: 241-250.

Vang-Petersen O., Kaack. K and Rasmussen P.M. 1977. Kvælstof til frugttræer. iii effect på frugters farve og indhold af syre, sukker og aromastoffer. Tidsskrift for Planteavl. 81: 159-164.

Wharton, P.S. 2003. Screening cherry ferm plasm for resistance to leaf spot. Plant disease: 471-477.

Xu, X.M. and Butt, D.J. 1993. PC-based disease warning system for use by apple growers. Bulletin OEPP/EPPO Bulletin 2: 595-600.

Yang, Z. and Rannala, B. 1997. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo method. Mol. Biol. Evol. 14: 717-724.

Ørum. J.E. and Christensen J. 2001. Produktionsøkonomiske analyser af mulighederne for en reduceret pesticidanvendelse i dansk gartneri. Rapport fra Fødevareøkonomisk Institut.

Annex A

Evaluation of two models for the prediction of the discharge of ascospores by *Venturia inaequalis* under Danish conditions (2002-2009)

Introduction

In the period 2002-2005 differences were noticed between the seasonal pattern of discharge of ascospores by ***Venturia inaequalis*** as monitored in an ascospore trap at AU-Aarslev, and the discharge as estimated by the apple scab simulation model RIMpro that is used by advisors in Denmark as a decision support tool for apple scab management (Lindhard Pedersen et al. 2005). The question was raised: Is it necessary to adapt the RIMpro model for Danish climatic conditions?

The New Hampshire ascospore maturation model (Gadoury and MacHardy. 1982) is a widely accepted model to estimate the maturation of ascospores of ***Venturia inaequalis*** based on a heat sum over 0°C starting at the day the first mature ascospores are found. This model has been validated in several states in the USA, in Canada, in Italy, and in Norwegian conditions. The maturation process is driven by temperature, however minimum humidity is required at the leaf surface that contain the pseudothecia. Several authors reported a better fit of the model to their field data when they interrupted the heat sum calculations during prolonged dry periods (3, 4, 5, 6).
RIMpro is a dynamic simulation model that estimates the relative risk of infections by apple scab and it is widely used by advisers and apple growers as a decision support tool. The program is based on the current state of knowledge on the infection biology of the apple scab fungus. Boxcar routines are used to mimic dispersion in development processes. The model is driven by data gathered from on-farm weather stations. The discharge of ascospores is an intermediate outcome of the first three processes in the RIMpro model:

1. The maturation of the ascospore population is calculated by an algorithm comparable with the New Hampshire model, however the RIMpro model is driven by a non linear relation to temperature instead of a linear relationship. Maturation is interrupted during prolonged dry periods (Stensvand et al. 2005).
2. The diurnal behaviour of the fungus is taken into account. Under equal temperature and wetness conditions the discharge rate during the night is 95% lower than under daytime conditions.
3. The discharge process is triggered by rain. At temperatures below 8°C the discharge rate decreases with temperature until zero at 0°C (Stensvand et al. 1997).

The data on ascospore discharge, as determined by AU-Aarslev in the years 2002 to 2009, was compared to the ascospore discharge pattern as estimated by both models.

Material and methods

Scab infected leaves of the apple variety ‘Jonagold’ were overwintered outside. Before bud break a few leaves were plased on the bottom of a ’Wiesmann’

ascospore trap (Figure 1). The trap consisted of an aluminium slide holder that held standard microscope slides about 5 mm above a leaf bed. During rain the leaves were wetted and the pseudothecia projected their ascospores onto the lower side of the glass slides. The spore trap was placed directly on the soil, in open air. After one or several days with rain the slides were exchanged, and the number of ascospores was counted on four slides. The number of ascospores counted was assigned to the last day with at least 0.2 mm of rain.

From 2002 to 2006 a Metos Compact weather station was used to collect the weather data. In 2007 to 2009 a Campbell data logger was used to collect the weather data. Temperature, relative humidity, precipitation and leaf wetness measurements were collected at canopy height.

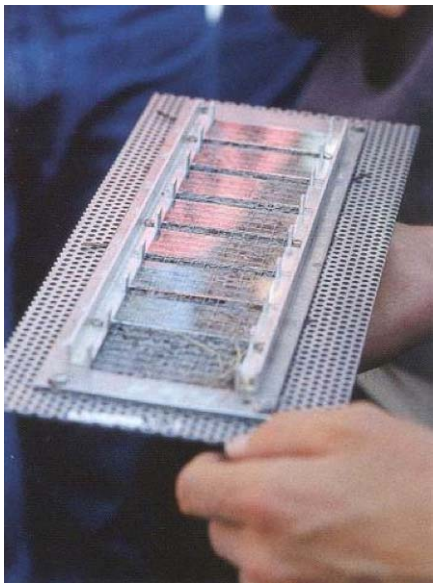


Figure 1. The 'Wiesmann' ascospore trap used for the observations.

For the New Hampshire model the temperaure sum over 0°C was calculated in day-degrees using the weather data at 30 min resolution. The model used first mature spores as Biofix. However for this evaluation for each year, the date that first ascospores were discharged into the spore trap was used as Biofix. From this Biofix date the model calculated cumulative mature spores (Y) in probit from the accumulated heat sum (X) as : $Y = 2.51 + 0.01X$, with 90% confidence bands at Y+ 0.814 and Y- 0.814.

The RIMpro model version 2010 was used with standard parameter settings. The model also used a Biofix. Calculations were run with both first observed discharge in the ascospore trap, and greentip of 'Jonagold' as Biofix. Complete leaf degradation was set in the model to 15 June to compensate for the situation in the spore trap where leaf degradation was slow.

For comparison and regression analysis all results were processed to cumulative percentages of discharged spores. The probit values resulting from the calculations with the New Hampshire model were back-transformed to cumulative percentage mature spores.

The observed pattern of discharge was plotted against the estimations from both models over time. Observed cumulative percentage discharge in the trap

was regressed against the cumulative percentage discharged spores as estimated by both models.

Results

Biofix

Both models require the date of first mature ascospores to initialize the calculations. In general, this stage is reached close to the phenological stage of 'greentip' of apples; the emergence of the first susceptible plant tissue on the host. For this reason the instruction for RIMpro is to use 'the date of greentip of the main apple variety' as Biofix if no other information is available. If the date of first physiological mature ascospores, or the date of first ascospore discharge is known, Biofix is set to this date, or some days earlier so the date of first simulated ascospore discharge meets the first observed discharge.

In the AU-Aarslev data, the actual first ascospore discharge was from 20 days before greentip, to 10 days after greentip (Table 1) On average the first ascospores at AU-Aarslev were found in the traps 10 days before greentip.

Table 1. The difference in days between greentip of 'Jonagold' and the first observed discharge of ascospores in Denmark. AU-Aarslev 2002-2007.

Year	Greentip 'Jonagold'	First ascospores	Difference in days
2002	28 March	15 Apr.*)	(18)
2003	10 Apr.	3 Apr.	-7
2004	3 Apr	19 March	-15
2005	1 Apr.	11 Apr.	10
2006	19 Apr.	10 Apr.	-9
2007	7 Apr.	18 March	-20
2008	29 March	13 March	-16
2009	3 Apr.	23 March	-11

*) not accurate to be used as Biofix as there was no rain recorded between 13/3 and 11/4.

General observations

The average period over which ascospores were captured each spring was 83 days. In the pronounced maritime climate in Denmark precipitation was recorded on 48% of these days. The average rainfall during the ascospore discharge period was 135 mm (Table 2).

Table 2. General information on the ascospore discharge period of ***Venturia inaequalis*** in Denmark. AU-Aarslev 2002-2007.

Year	Duration of ascospore discharge (days)	Number of days with rain	Total amount of rain (mm)	Number of times counted	Total number of ascospores per slide
2002	75	42	106.9	20	4356
2003	57	37	132.3	21	12042
2004	97	50	147.4	25	270761
2005	78	32	111.5	19	28122
2006	58	39	131.8	17	18768
2007	89	36	149.7	17	22902
2008	118	43	159.0	23	57885
2009	91	37	143,6	26	10880
Average	83	40	135		

Results for the individual years

Table 3. Correlation coefficients (r^2) of the fit between observed and expected ascospore discharge according to two Models. Aarslev 2002-2007.

Year	New Hampshire Maturation model	RIMpro	RIMpro
	Biofix First discharge	Biofix Greentip	Biofix First discharge
2002	0.93	0.97	0.95
2003*	0.75	0.52	0.59
2004	0.96	0.89	0.95
2005	0.76	0.87	0.76
2006	0.87	0.85	0.93
2007	0.89	0.99	0.92
2008	0.80	0.92	0.99
2009	0.93	0.89	0.94

*2003 data excluded in conclusion, as they were atypical.

2002

In 2002, the relative humidity (RH) data mere missing from the weather data file. For processing with RIMpro, missing RH values were substituted with a constant 72% RH. Greentip was noted on 28 March but no rain was recorded between 19 March and 11 April. During the first two rain events on 12 April and 14 April. 24% of the seasonal total of ascospores were captured indicating many spores had become mature in the trap during the weeks around greentip when no rain was recorded. In this situation the Biofix on first mature spores could not accurately be established.
Observed ascospore discharge correlated well with the ascospore discharge and maturity estimated by both models, however with an offset of about 20% mature spores (Figure 1).

The angle of the regression curve indicated that the maturation of the spore population in the leaf bed in the trap was slower than in the models (Figure 2 and Table 3).

2003

In 2003 there was a poor fit between the observed and estimated ascospore discharge pattern (Figure 3). The maturation of the spores in the trap was much faster than estimated by the models, resulting in a poor correlation for both models (Figure 4 and Table 3).

2004

Observed ascospore discharge correlated well to the estimated cumulative ascospore discharge with minimal offset (Figure 5). The maturation speed of the spores in the trap was about 10% higher than in the models. Using “first mature spores” as Biofix resulted in a better fit to the observed data than the use of greentip (Figure 6 and Table 3).

2005

As in 2003, maturation of spores in the trap was much faster than estimated by the models based on air temperature (Figure 7). Correlations were poor. The curves indicated that the maturation speed in the trap was around 30% higher than estimated (Figure 8 and Table 3).

2006
In the 2006 data there was a reasonable correlation between observed and simulated maturation (Figure 9). Again maturation speed as observed in the trap was higher than estimated by the models (Figure 10 and Table 3).

2007
On 19 April 2007 the first massive ascospore release was observed in the trap. During this event the weather station recorded 1.8 mm of precipitation, but the leaf wetness sensors seemed to malfunction and did not recording any wetness either during or after the full rain event. The data file was corrected by adding four hours of leaf wetness following the rain event.

In 2007, for the fist time the observed ascospore maturation was later than predicted by the New Hampshire model. This is probably the result of some longer intervals without rainfall in March and April (Figure 11).

The cumulative ascospore discharge as calculated by RIMpro using first ascospore release as Biofix correlated well to the observations. When Biofix was set to greentip the correlation was poorer, and ascospore maturation was predicted too late (Figure 12 and Table 3).

2008
In 2008 again maturation of the ascospore population in the trap proceeded more rapidly than predicted by the two models (Figure 13), resulting in a poor fit of the observed date to the results of the simulation (Figure 14 and Table 3).

2009
In 2009 the maturation in the spore traps was slightly slower than expected by the New Hampshire model, but slightly faster than expected by the RIMpro model (Figure 15). Using first ascospore release as Biofix for the RIMpro model resulted is better fit of the simulation results to the observations (Figure 16 and Table 3).

Discussion
Both ascospore models estimated the maturation and discharge of ascospores in an orchard situation using algorithms derived from previous field and laboratory research.
This evaluation of the models for Danish conditions was based on the assumption that:

1. The ascospore discharge pattern, as observed in the ascospore trap, resembled the ascospore discharge pattern in the orchard.
2. The temperature and humidity conditions experienced by the apple leaves in the spore traps were identical to the temperature and humidity conditions measured by the weather station which were used to drive the models.

In the experimental setup and available historical data these assumptions were not necessarily met.
The ‘Wiessman’ trap offers space for only a few apple leaves. It is not evident that the pseudothecia in these few leaves represent the maturation of ascospores in the total population of apple leaves in a orchard. This evaluation is based on the average number of ascospores per glass slide during each individual rain event, but considerable variation in the number of ascospores per glass slide were observed.

The apple leaves holding the potential ascospore inoculum in the trap were covered with glass slides. In the case the trap is in direct sunlight, the temperature under the glass slides could be 10 to 20°C higher than the air temperature measured at 2.0 m height (Peter Triloff, and Marc Trapman observations, unpublished data). These two finding indicate that maturation speed in the trap may have been increased.

1. Early Biofix: In the trap at AU-Aarslev the first ascospores were found on average 10 days before greentip. In past years in other fruit growing regions in Europe observations in the variation in number of days between first ascospore discharge and greentip have been found, but on average first discharge under laboratory or field conditions coincided with the greentip stage of the main variety (2009: average +0.75 day, see Annex 1).
2. Accelerated maturation: In five out of the eight years maturation speed, as observed from the ascopore discharge in the trap, was considerably faster than predicted by the New Hampshire model (a model that is only driven by temperature). In most evaluations ascopsores have been found to last longer than predicted by the New Hampshire model.

Covering the leaves by glass slides may result in leaves in the trap that are not completely wetted during rain events with little precipitation, therefore ascospore discharge may be incomplete. The ascospores were not counted after every rain event, but after a rain period. Therefore the number of ascospores counted was assigned to the last day with at least 0.2 mm of rain. Those may result in an error, as the spores counted on a certain day may have been discharged over several previous days. The assignment of the captured spores an the actual day of rain was not done when interpretating the data set (Lindhard Pedersen et al. 2005).

Conclusions

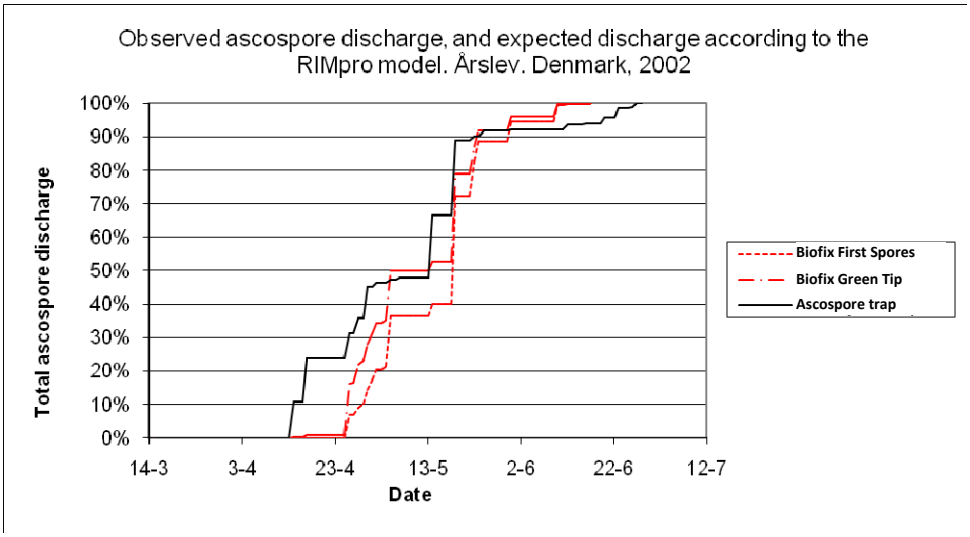
Biofix

As the time between greentip and the actual start of ascospore release in Denmark varies considerable between years, actual Biofix setting of RIMpro seems to be even more important than in other countries. Combination of the data in Tables 1 and 3 revealed that Biofix set to 'greentip' or 'first ascospore release', 'whatever comes first' yielded correlation coefficient of 0.97, 0.95, 0.87, 0.93, 0.97, 0.91 and 0.94 for 2002, 2004, 2005, 2006, 2007, 2008 and 2009 respectively (excluding 2003 due to an atypical dataset).This is better than the correlations recently published for the New Hamshire model, at r^2=0.82 or r^2=0.90 (Gadoury and Seem. 2004).
To be able to establish the Biofix for the maturation models even more accurately it has become practise to check for discharge in the laboratory. In the weeks approaching, greentip apple leaves should be brought in the laboratory and wetted and monitored to determine whether they are already prepared to eject ascopores at the next rain event.

Developmental rate

Although the correlation coefficients between observed and simulated cumulative ascospore discharge for the RIMpro model with Biofix as first spore discharge were over 0.9 in the six out of the eight years included in this evaluation, maturation speed, as observed from the ascopore discharge in the trap was considerably higher than that predicted by the models in the five out of eight years. It is questionable whether the temperature received by the apple leaves in the spore trap was identical to the measured air temperature that drives the models.

From this evaluation there was no clear evidence that the ascospore maturation process of ***Venturia ineaqualis*** populations in Denmark is different to that of ***Venturia ineaqualis*** populations in other fruit growing areas.



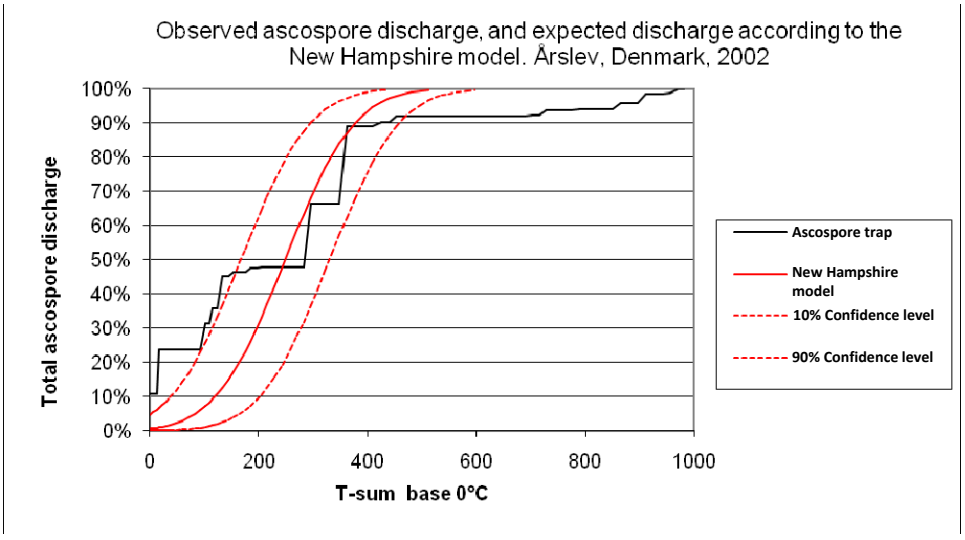
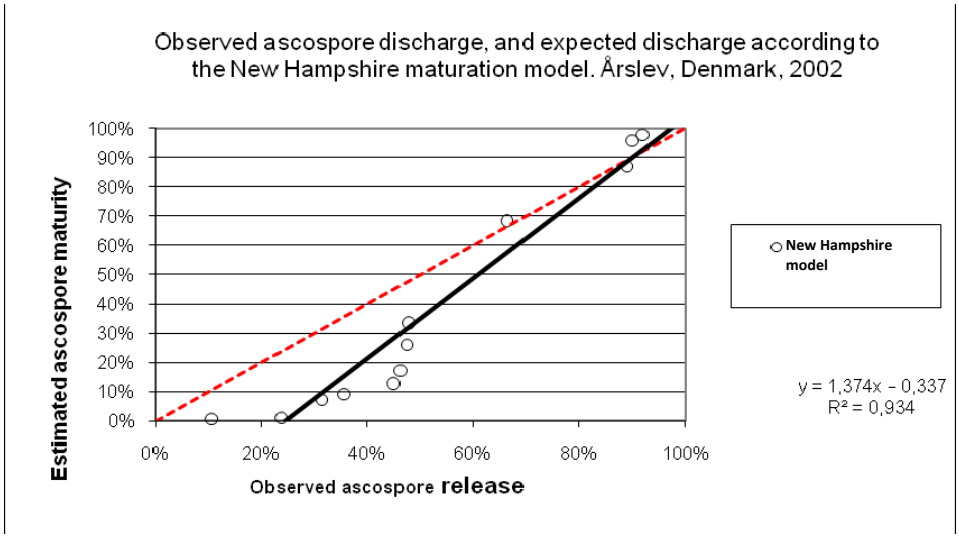
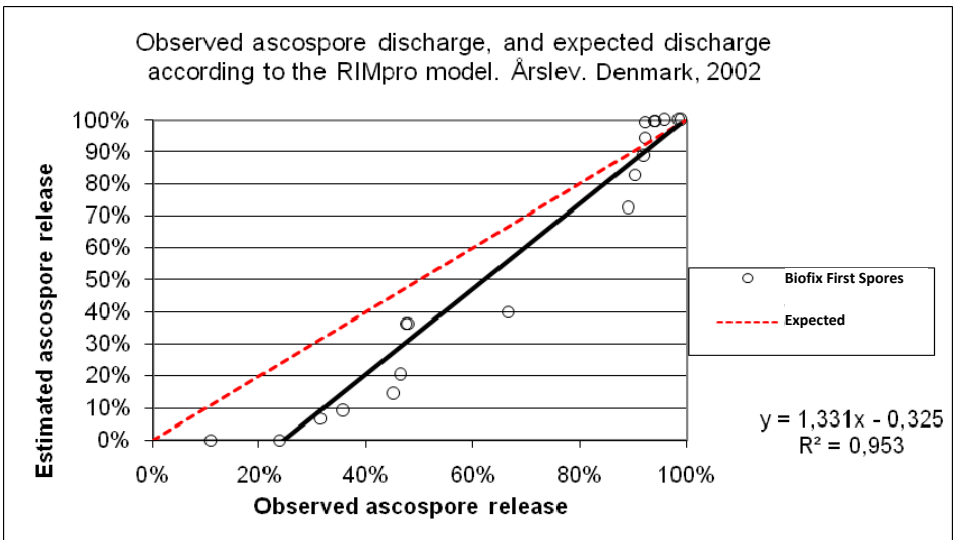


Figure 1. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro-model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2002. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





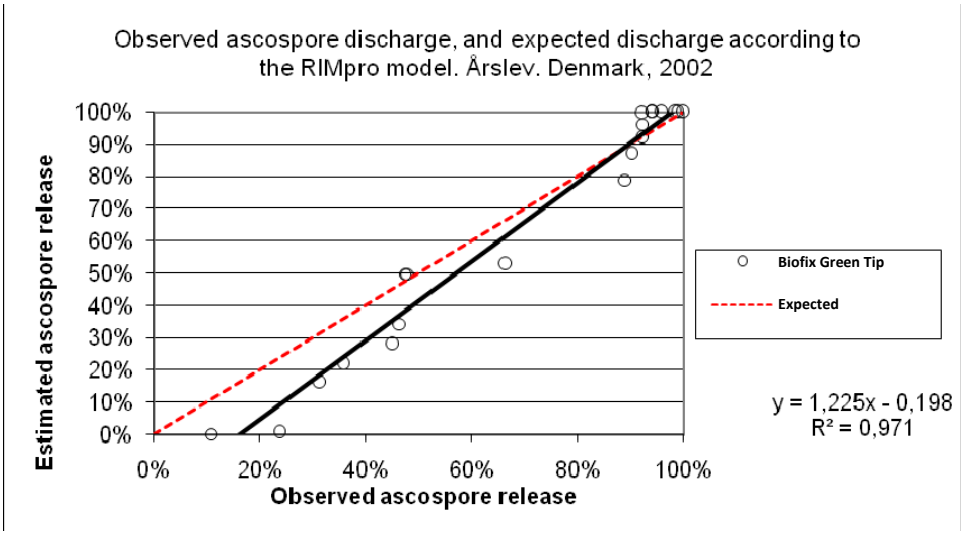
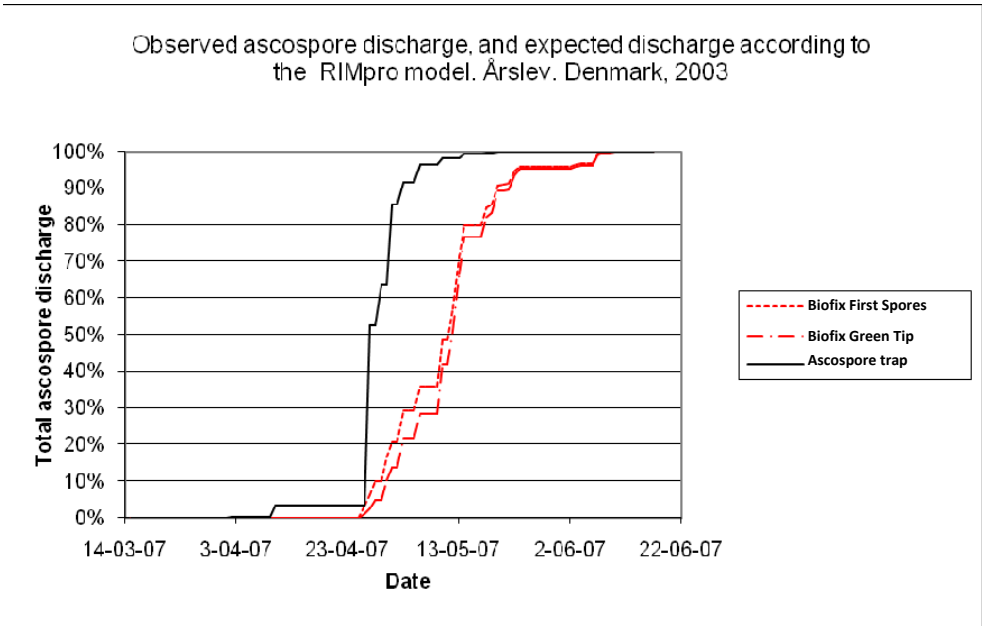


Figure 2. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle) and RIMpro-model with Biofix 'greentip' of 'Jonagold' apples' (Bottom) for AU-Aarslev, Denmark in 2002.



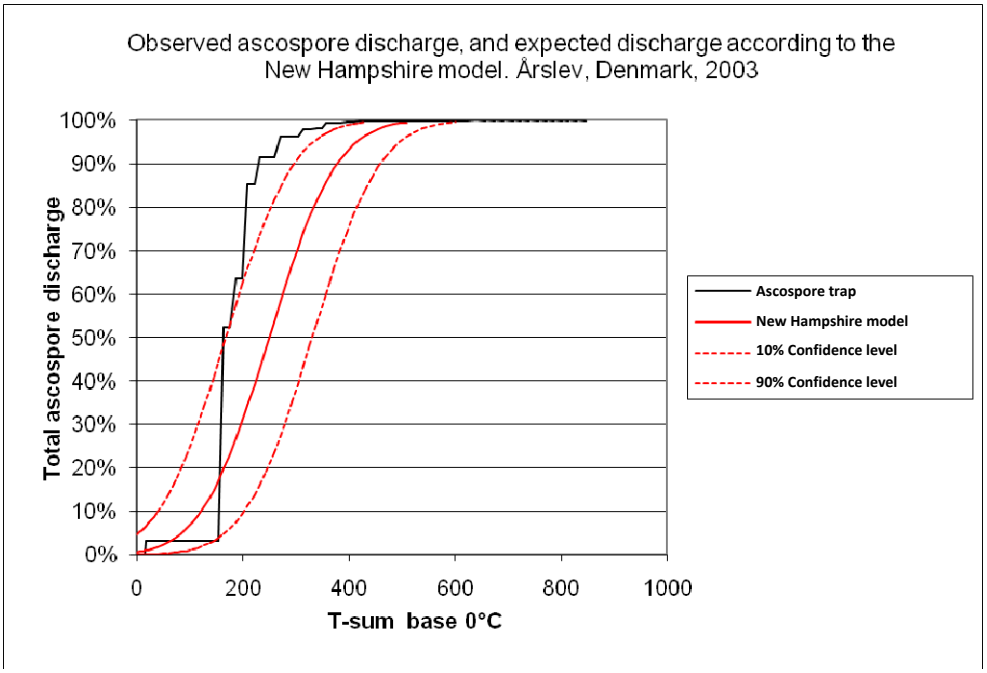
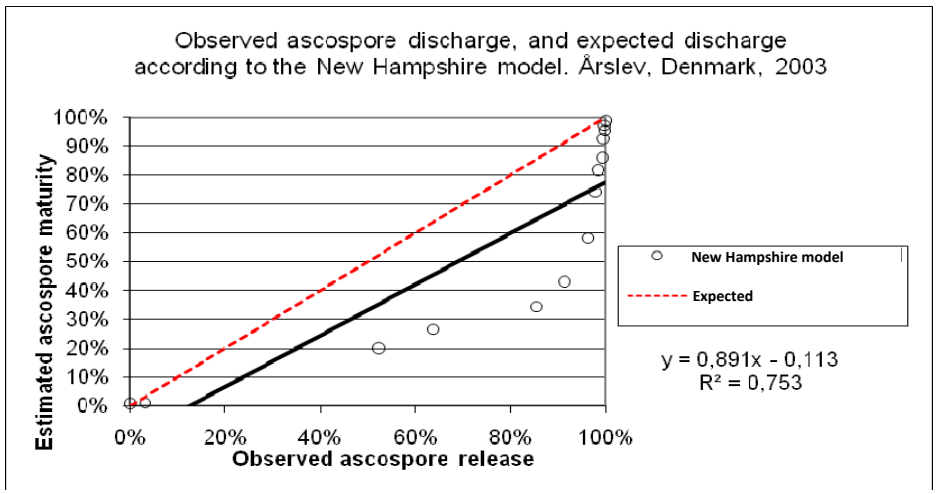
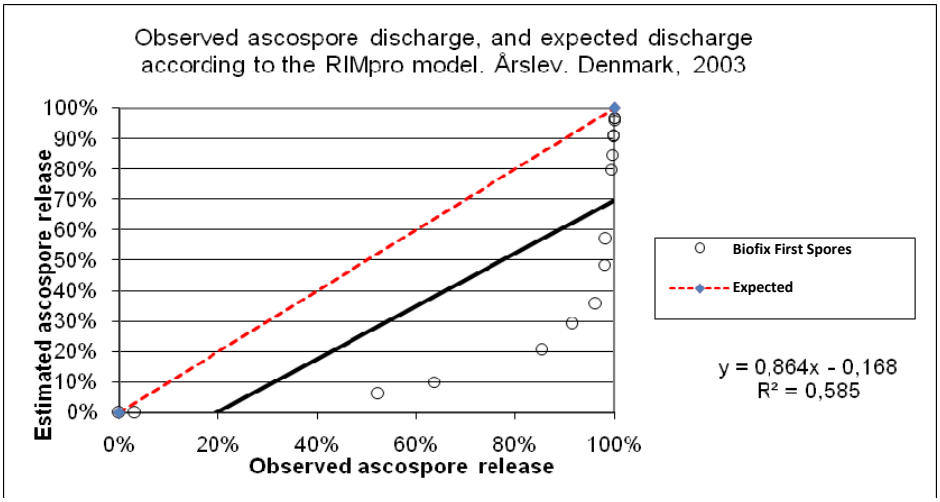


Figure 3. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2003. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





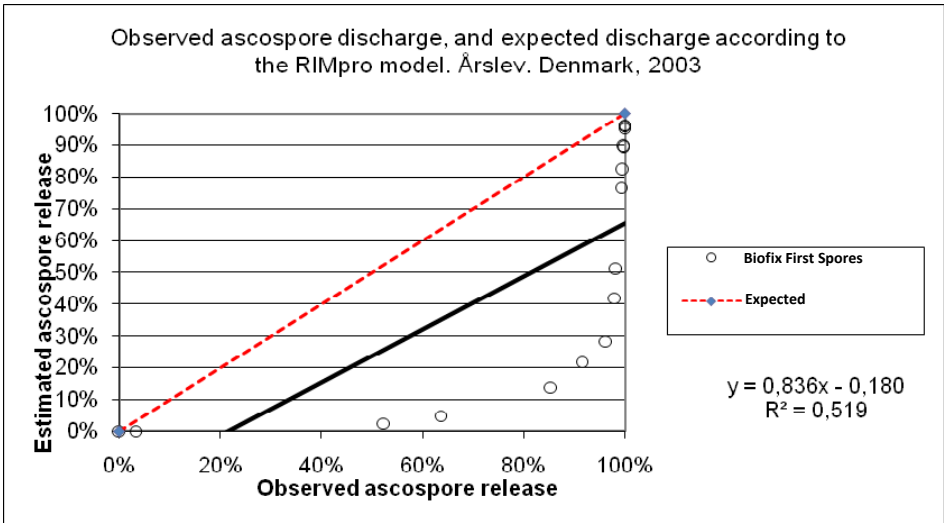


Figure 4. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples (Bottom) for AU-Aarslev, Denmark in 2003.

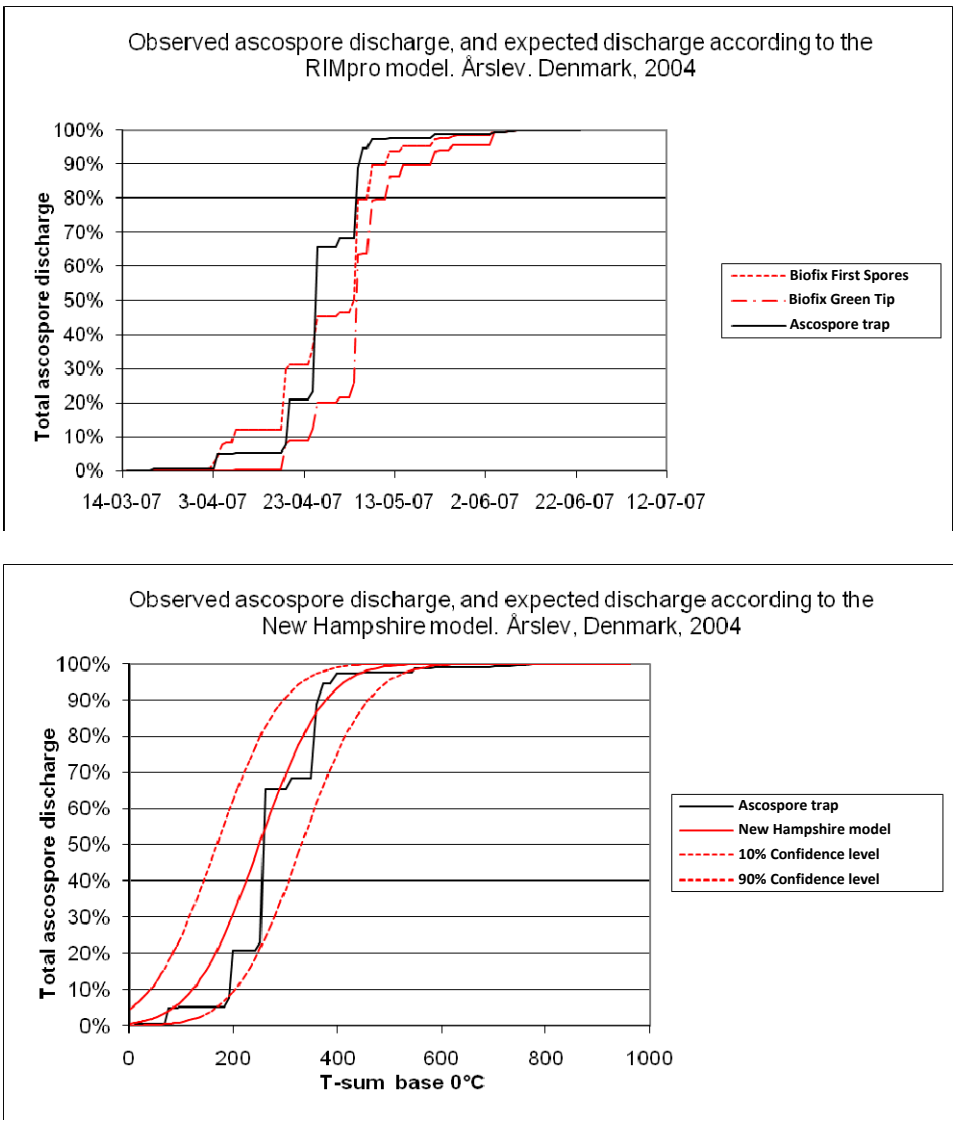
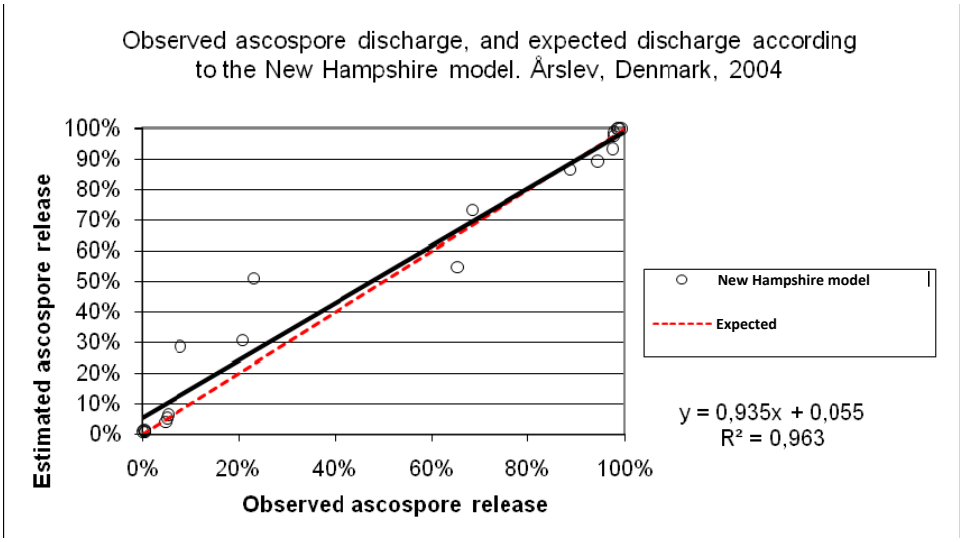
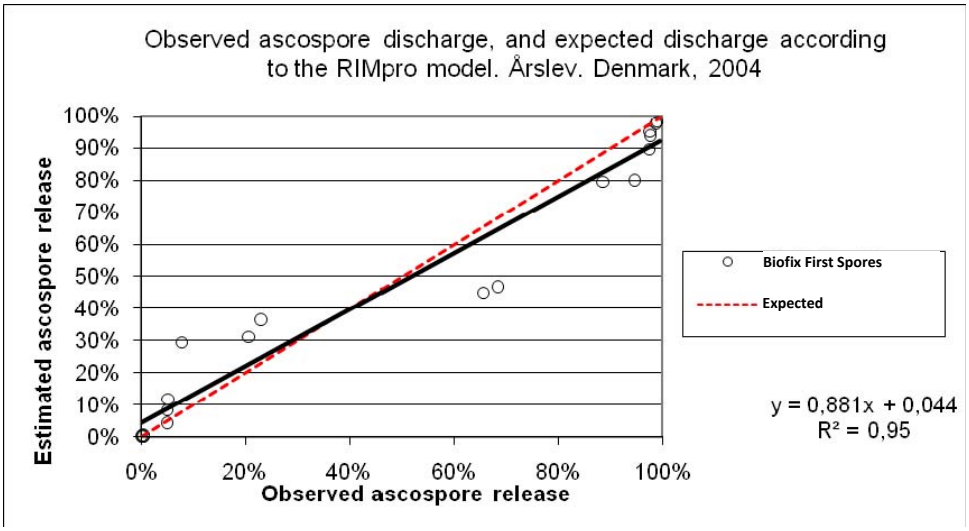


Figure 5. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2004. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





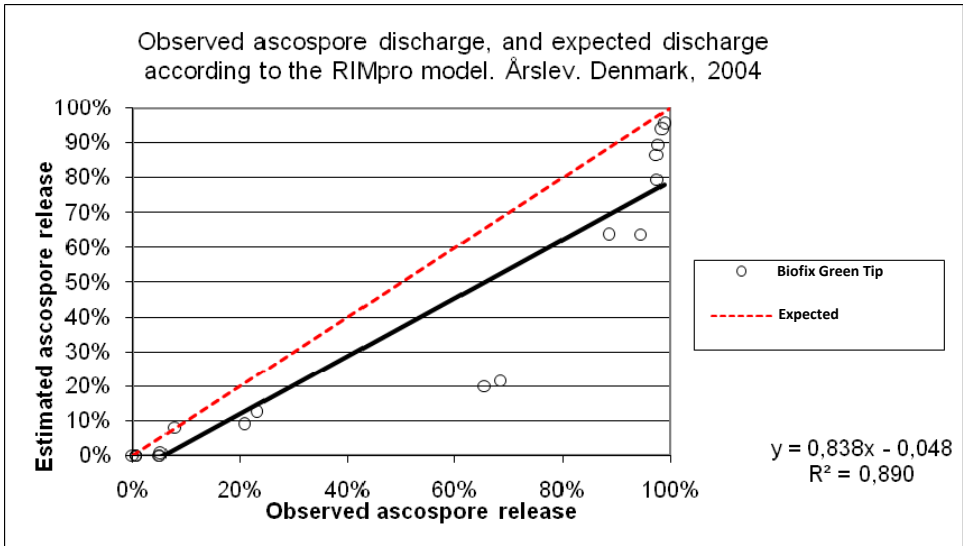


Figure 6. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples (Bottom) for AU-Aarslev, Denmark in 2004.

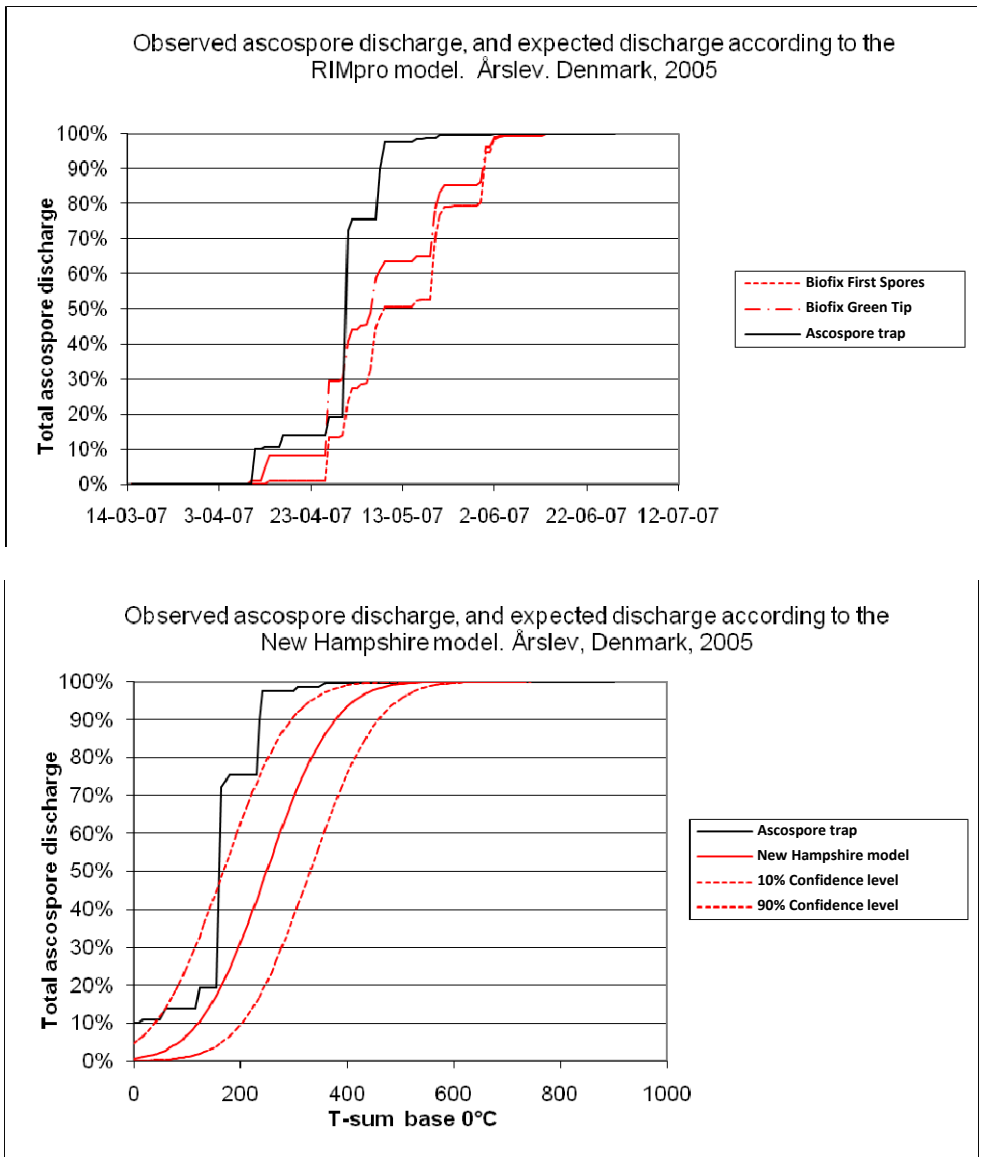
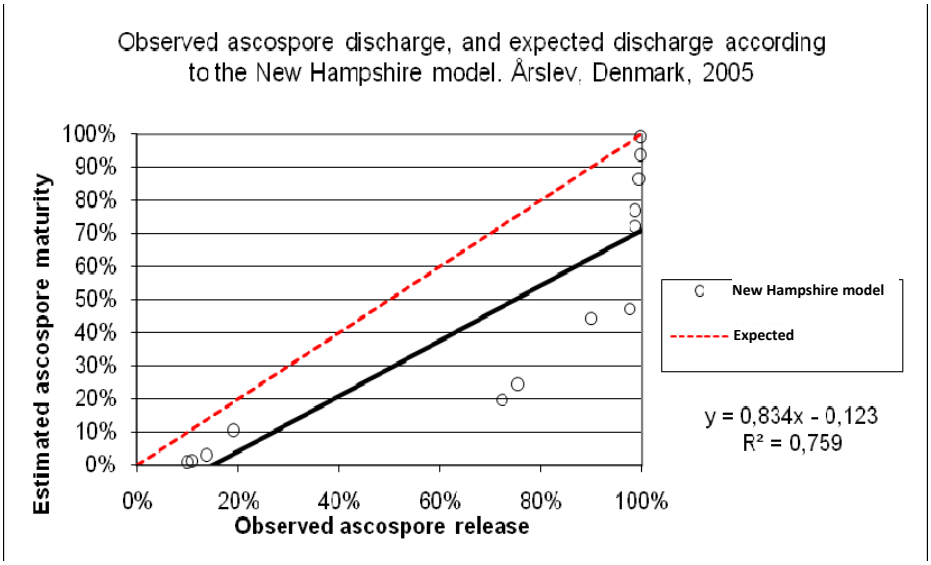
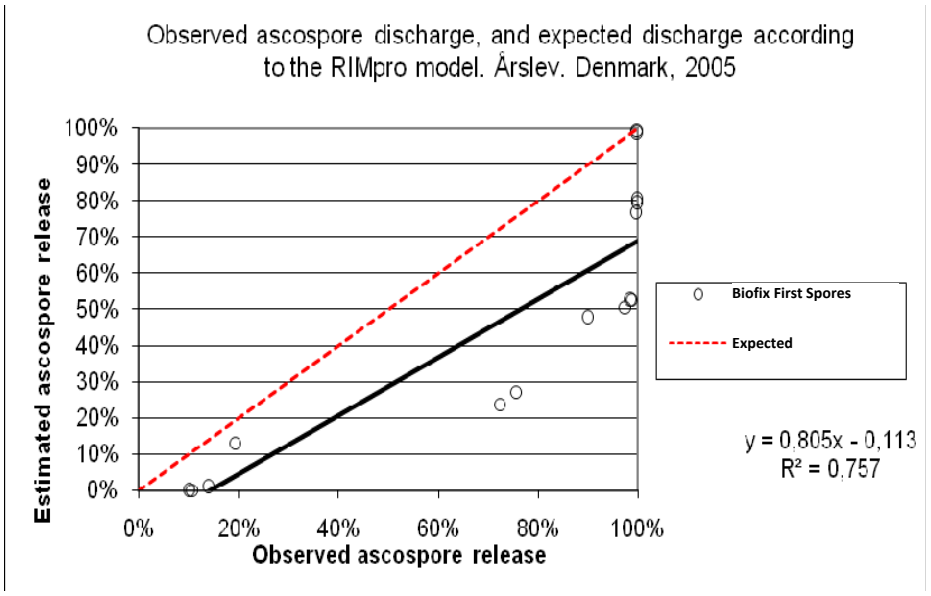


Figure 7. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2005. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





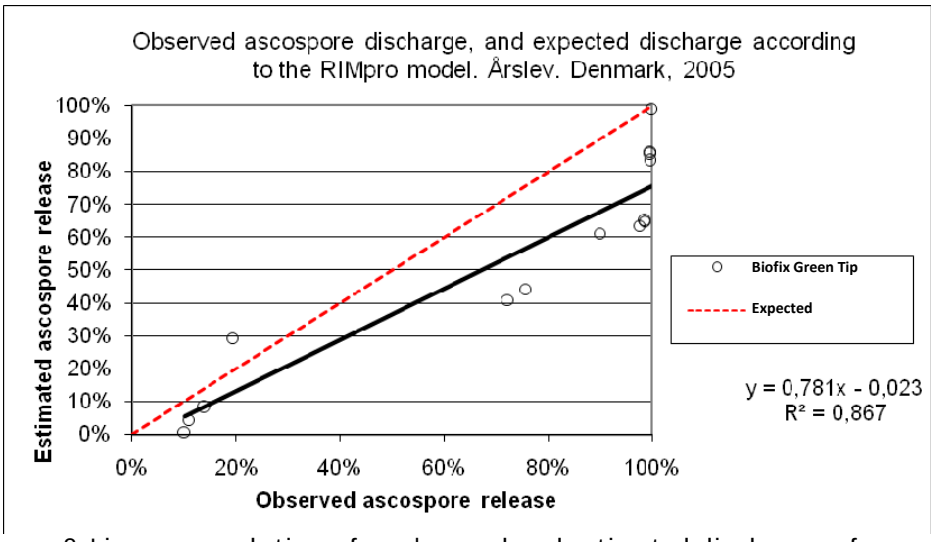


Figure 8. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples (Bottom) for AU-Aarslev, Denmark in 2005.

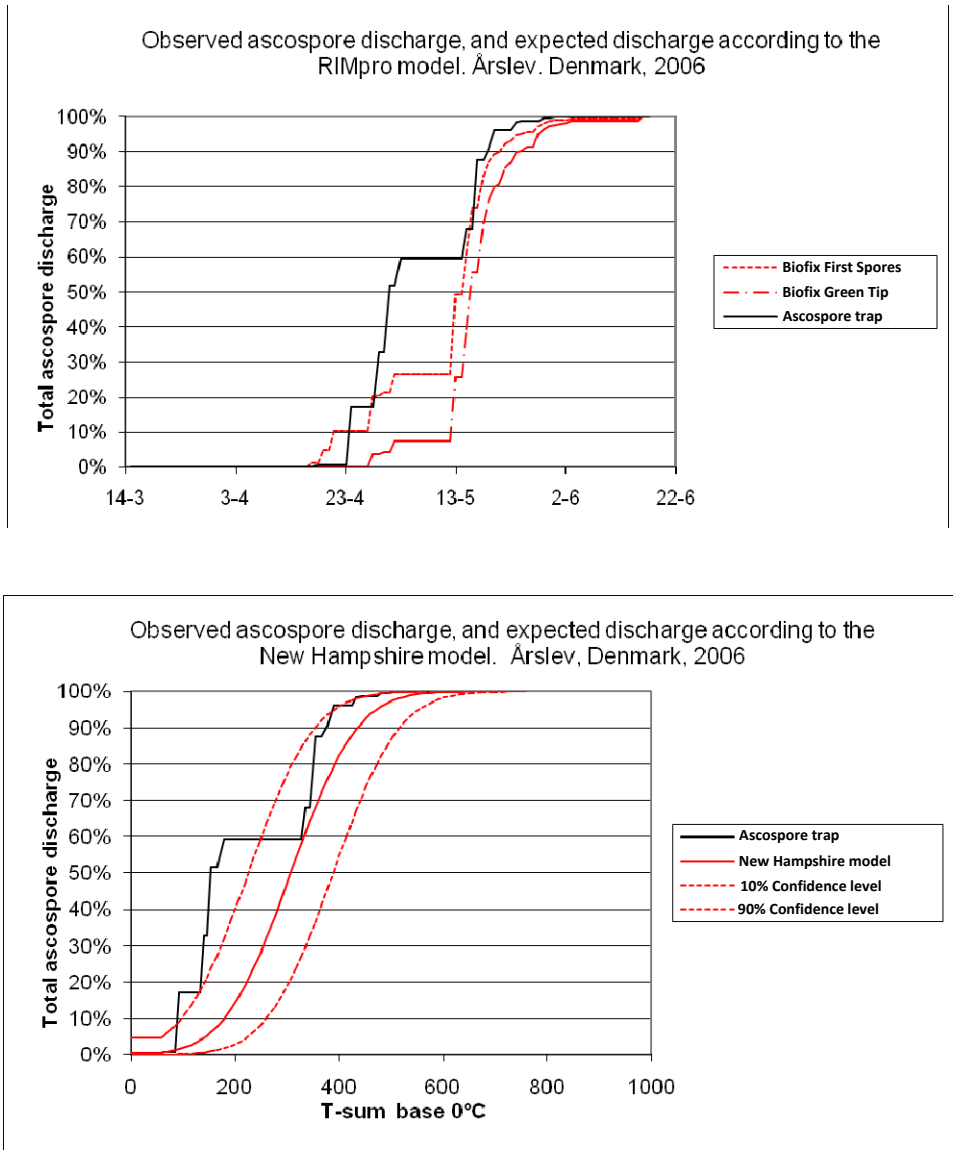
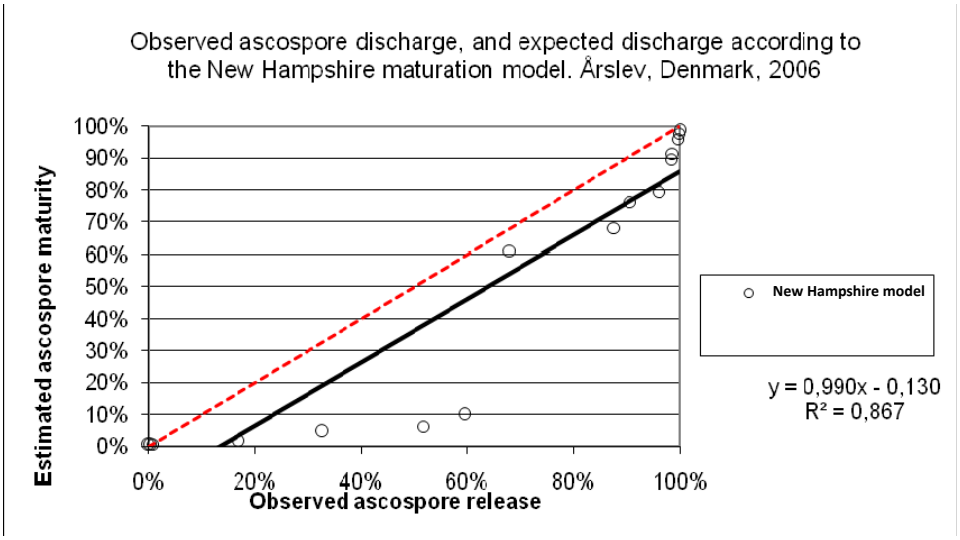
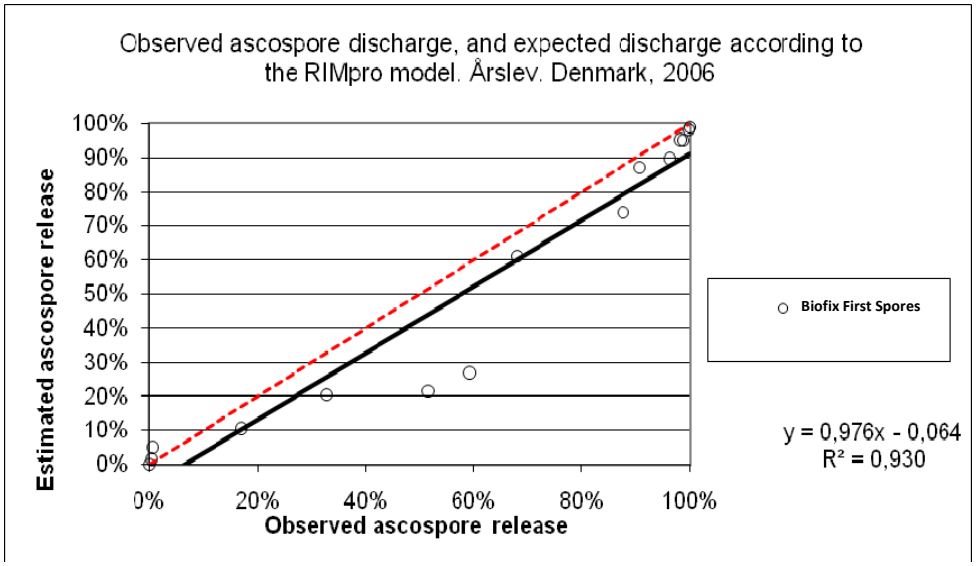


Figure 9. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2006. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





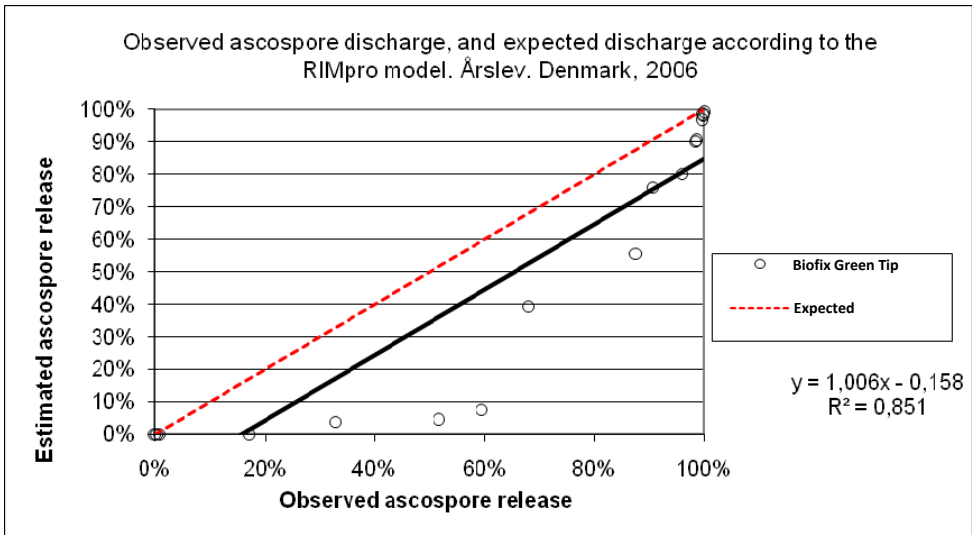


Figure 10. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples (Bottom) for AU-Aarslev, Denmark in 2006.

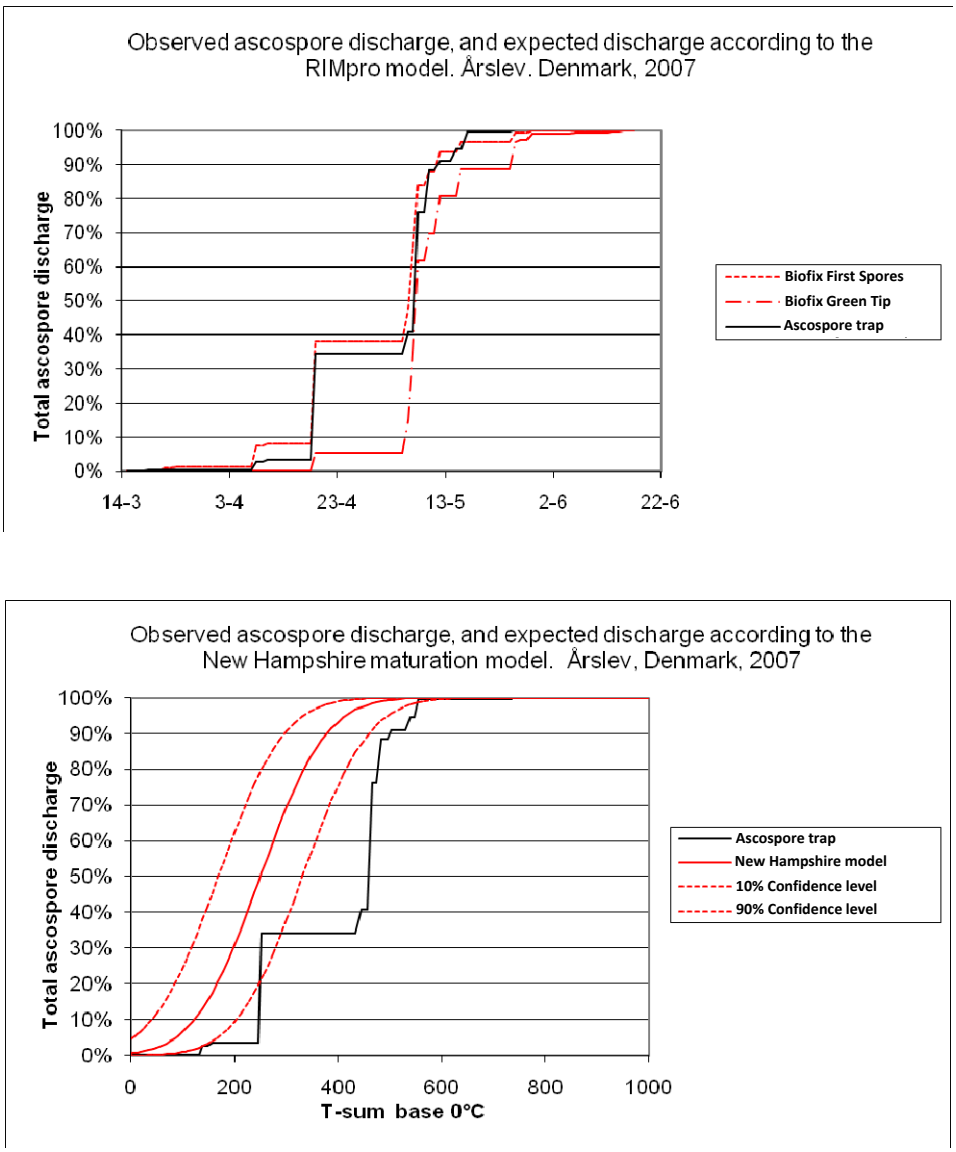
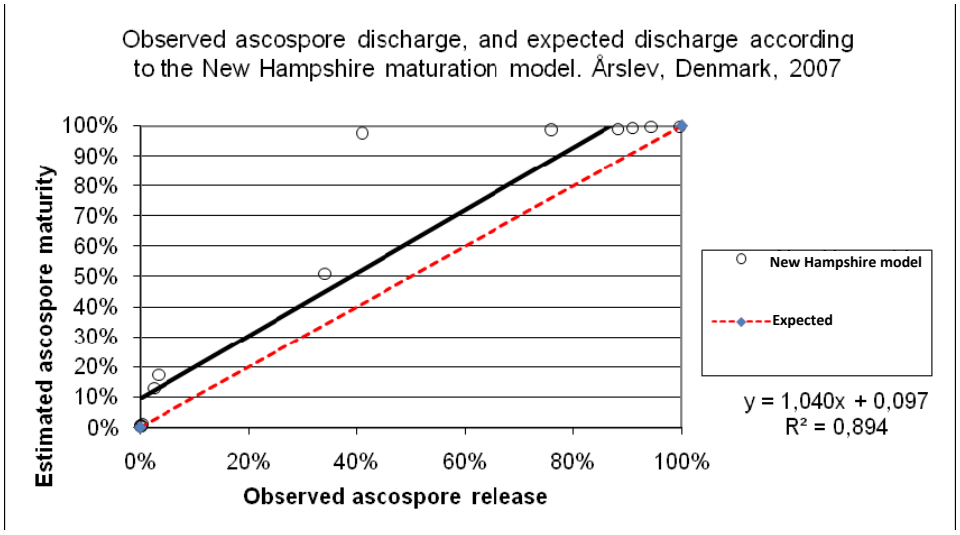
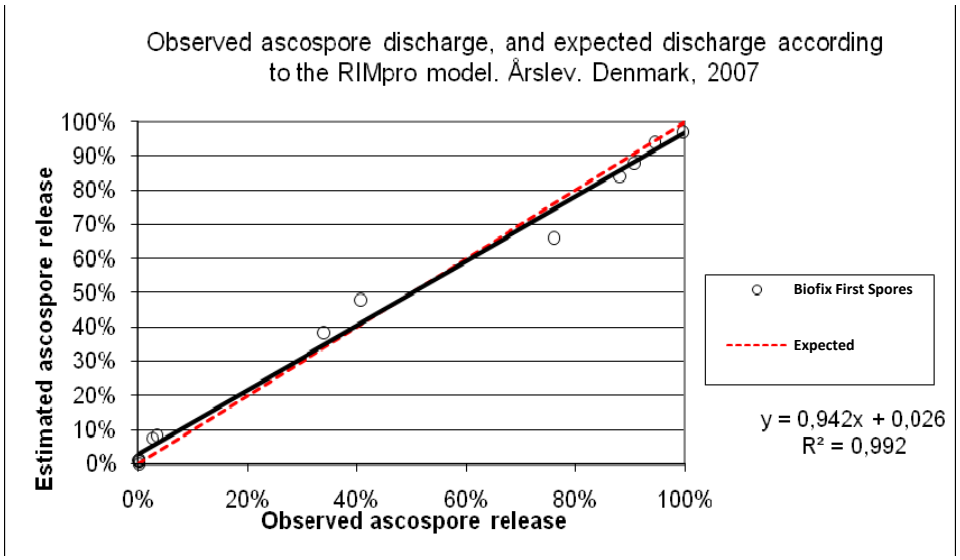


Figure 11. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2007. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





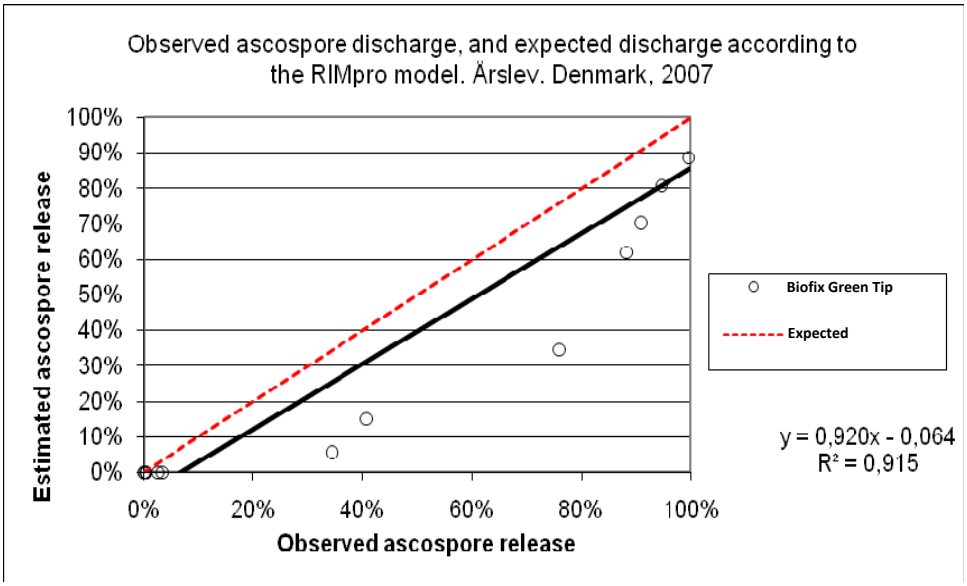


Figure 12. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples' (Bottom) for AU-Aarslev, Denmark in 2007.

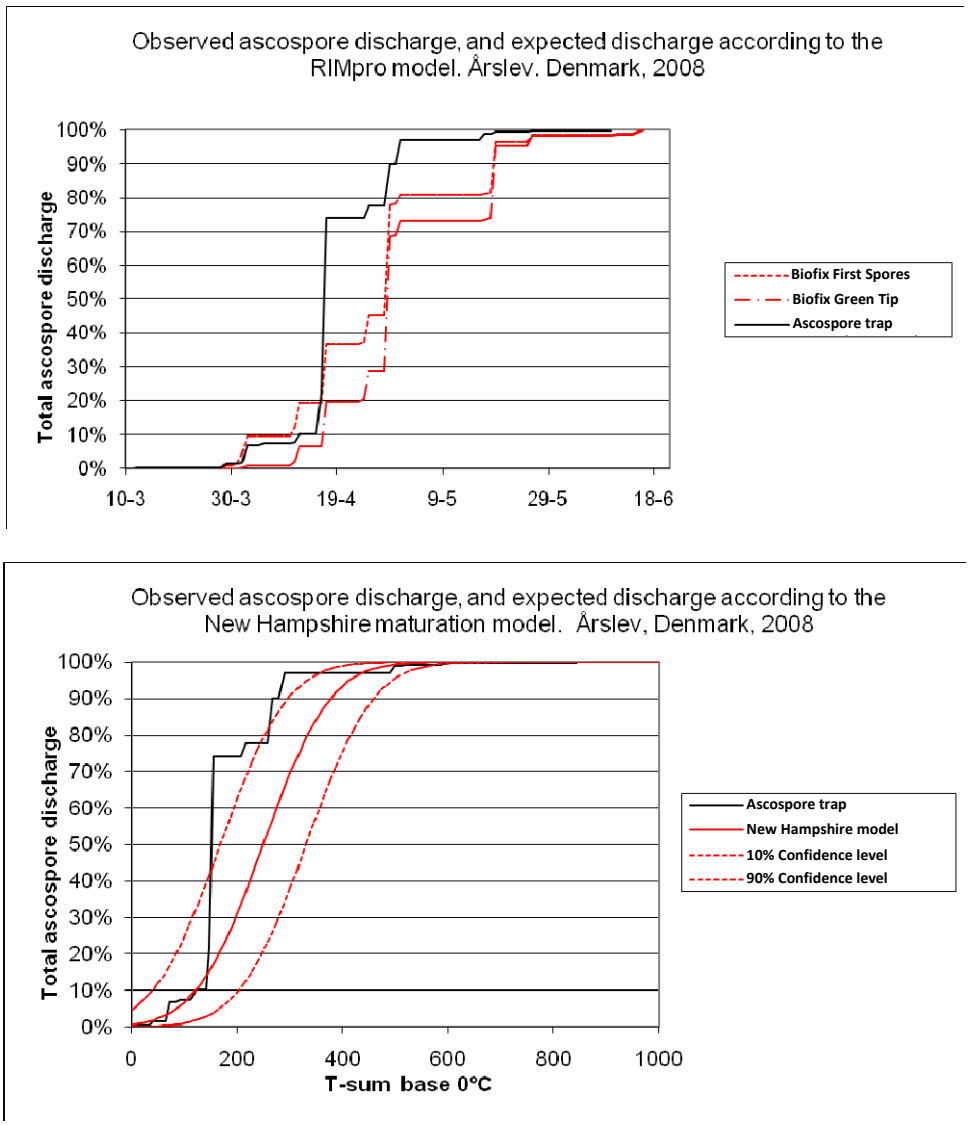
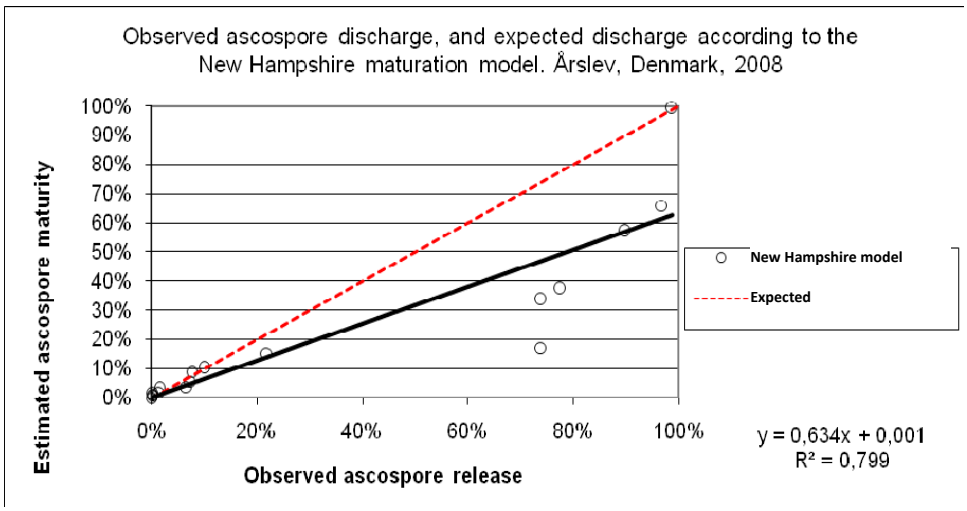
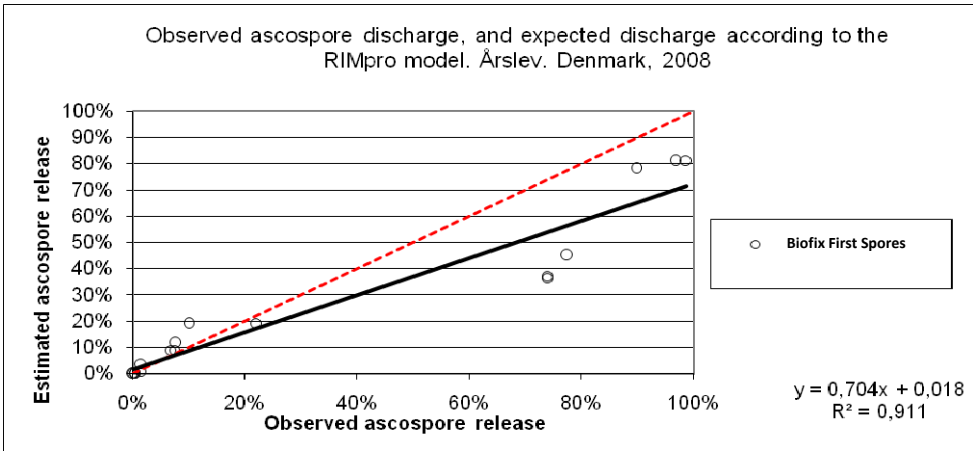


Figure 13. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2002. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





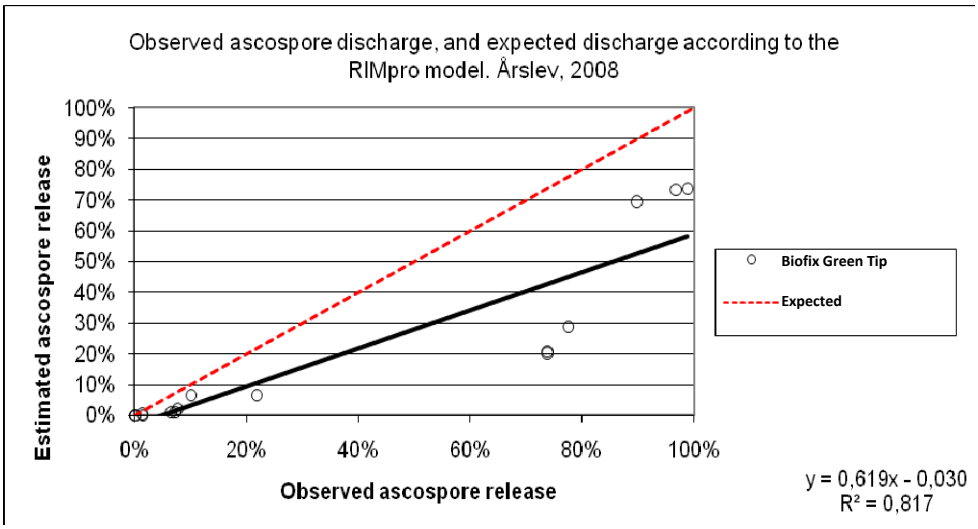


Figure 14. Linear correlations for observed and estimated discharge of ascospores of *Venturia inaequalis* according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle), and RIMpro model with Biofix 'greentip' of 'Jonagold' apples' (Bottom) for AU-Aarslev, Denmark in 2008.

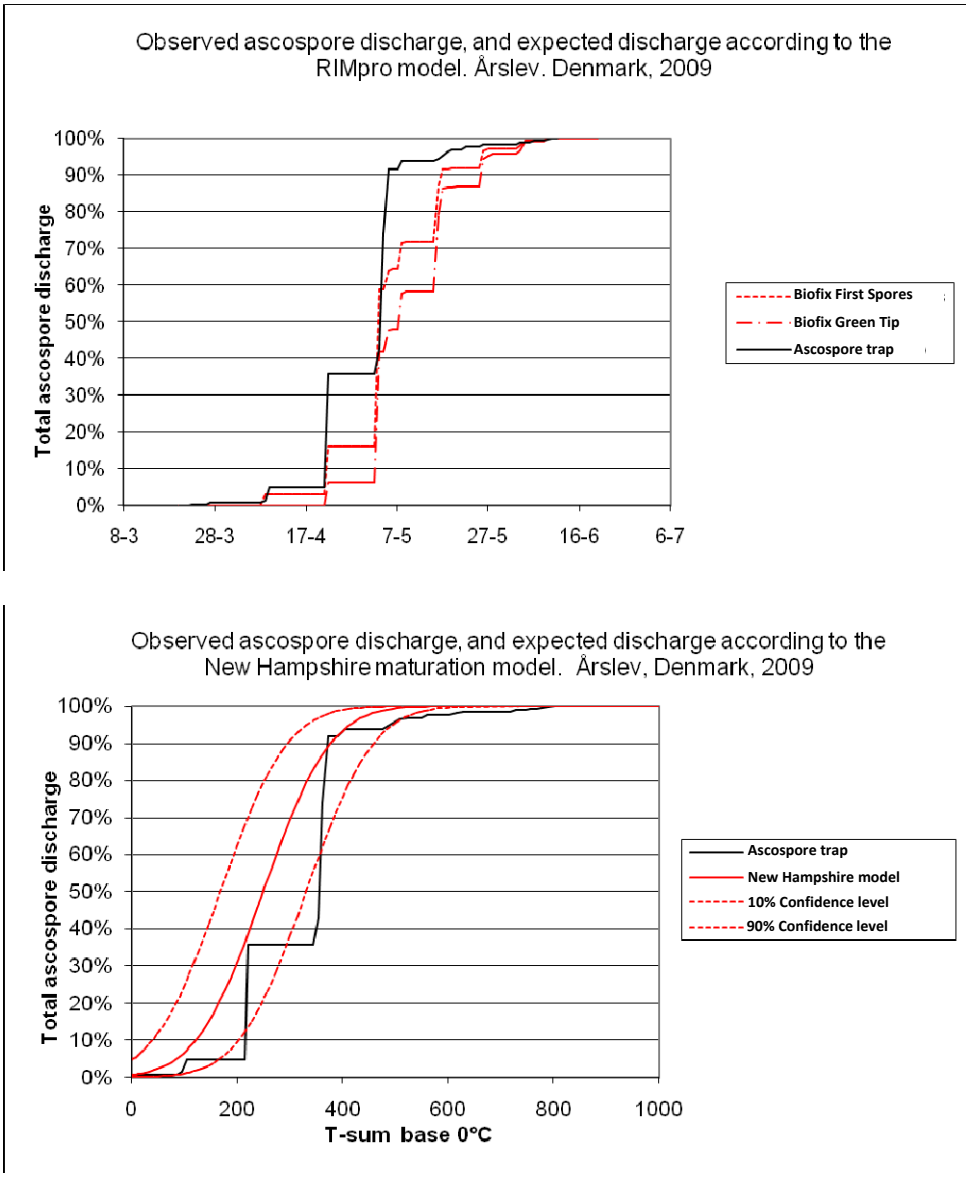
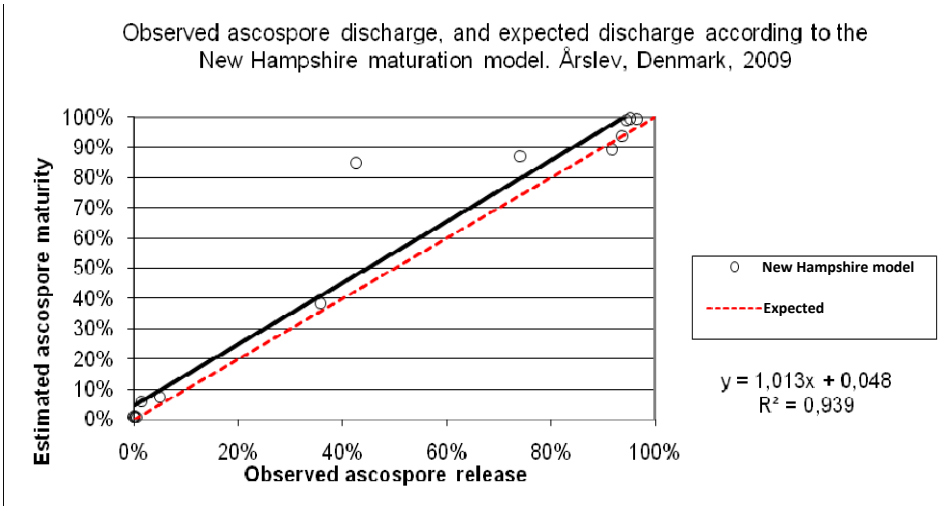
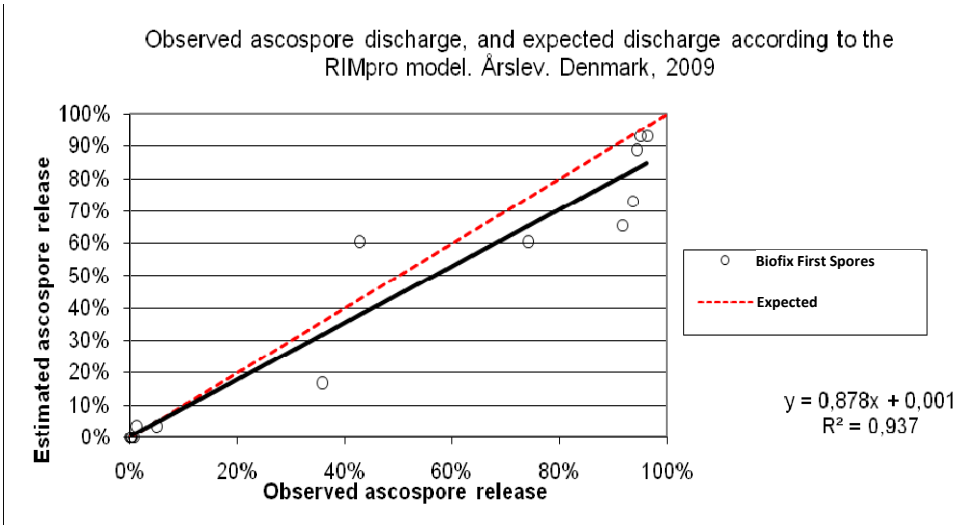


Figure 15. Observed and estimated discharge of ascospores of *Venturia inaequalis* according to RIMpro model (Top) and New Hampshire model (Bottom) for AU-Aarslev, Denmark in 2009. RIMpro estimation used two Biofix dates: 'First ascospore discharge' and 'greentip' of 'Jonagold' apples.





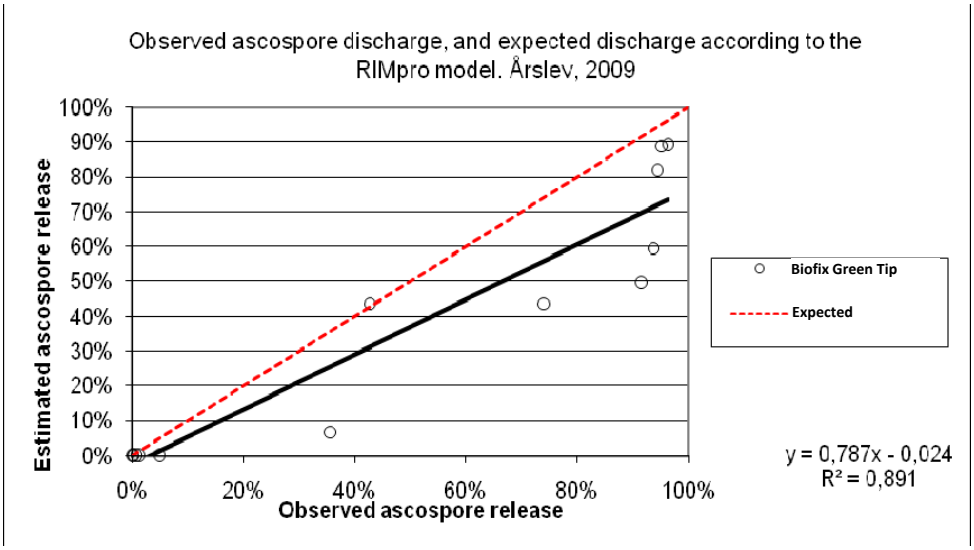


Figure 16. Linear correlations for observed and estimated discharge of ascospores of ***Venturia inaequalis*** according to New Hampshire model (Top), RIMpro model with Biofix 'first discharge of ascospores' (Middle) and RIMpro model with Biofix 'greentip' of 'Jonagold' apples (Bottom) for AU-Aarslev, Denmark in 2009.

Literature:

(1) Lindhard Pedersen, H., Linddal Pedersen, K. and Paaske, K. 2005. Evaluating the use of RIMpro and Metos weather stations for control of apple scab (***Venturia inaequalis***) in Denmark 2002-2005. IOBC/wprs Bulletin Vol. 29(1) 2006.

(2) Gadoury, D.M., and MacHardy, W.E. 1982. A model to estimate the maturity of ascospores of ***Venturia ineaqualis***. Phytopathology 72: 901-904.

(3) Gadoury, D.M., and Seem, R.C. 2004. A Comparison of methods used to estimate the maturity and release of ascospores of ***Venturia ineaqualis.*** Plant Disease 88: 869-874.

(4) St-Arnaud, M. 1990. Evaluation au Qubec dún modele de prediction de fa fin de la periode annuell d'ejection des ascospores du ***Venturia Ineaqualis.*** Phytoprotection 71: 17-23.

(5) Rossi,V 1999. Field evaluation of some models estimating the seasonal pattern of airborne ascospores of ***Venturia ineaqualis.*** Phytopathology 147: 567-575.

(6) Stensvand, A., Eikimo, H., Gadoury, D.M., and Seem, R.C. 2005. Use of a rainfall frequency threshold to adjust a degree-day model of ascospore maturity of ***Venturia ineaqualis.*** Plant Disease 89: 198-202.

(7) Stensvand, A., Gadoury, D.M., Amundsen, T., Semb, L. and Seem, R.C. 1997. Ascospore release and infection of apple leaves by conidia and ascospores of ***Venturia ineaqualis*** at low temperatures. Phytopathology 87: 1046-1053.

Annex 1: RIMpro Biofix data 2009

Region	Organisation	First visibly mature spores in squash mount	First dicharge L= Lab F= Field	First Greentip And cultivar	Proposed Biofix	Days from Greentip
Latvia	LPPRC, Riga	11-4-09	L=26-4-09	24-4-09	26-4-09	2
Norway	Bioforsk, Oslo Western Norway	4-4-09 -	F=27-4-09 -	Gravenstein 14-4-09 1-4-09 / 4-4-09	- -	13
Denmark	Hort. Res. Centre Arslev	-	F= 23-3-09	Jonagold 3-4-09 Elstar 4-4-09	23-3-09	-11
North Germany	Versuchsanstallt Jork	23-3-09	F= 9-4-09	Jonagold 20-3-09	23-3-09	20
Netherlands	PPO Randwijk	-	L=14-3-09 (F= 17-3-09)	Jonagold 18-3-09 Elstar 22-3-09	17-3-09	-4
Belgium	PCFruit, Sint-Truiden	16-2-09	L=6-3-09 (F= 17-3-09)	Jonagold 16-3-09	16-3-09	-10
	CRA-W Gembloux	-	L=13-3-09	Jonagold 16-3-09	13-3-09	-3
Mid Germany	DLR Rheinpfalz, Neusttadt	-	F= 27-3-09	Jonagold 31-3-09 Braeburn 31-3-09 Gala 16-3-09	26-3-09	-4
South Germany	MABO (Lake Constance)	19-3-09	L=26-3-09	Jonagold 26-3-09	26-3-09	0
Switzerland	Güttingen (Lake Constance)	-	F=30-3-09	Golden 6-4-09 Gala 6-4-09	30-3-09	-6
	Wädenswill (Lake of Zurich)	-	F=16-4-09	Golden 6-4-09 Gala 6-4-09	6-4-09	10
	Frick FIBL	-	F=28-3-09	Topaz 6-4-09 Gala 3-4-09	30-3-09	-6
Loire France	La Moreniere	4-3-09	L=19-3-09 F=25-3-09	Golden 12-3-09 Braeburn 2-3-09 Gala 5-3-09	19-3-09	7
Limosin France	Perlim	-	F= 4-3-09	none	4-3-09	-
South-West France	CTIFL (Bergerac)	20-2-09	L = 4-3-09 F= 4-3-09	Golden 16-3-09 Braeburn 10-3-09 Gala 10-3-09	4-3-09	12
	CEFEL (Montauban)	26-2-09	L = 2-3-09 F= 4-3-09	Braeburn 2-3-09 Gala 5-3-09	2-3-09	0
South-East France	(Avignon) SICOLY (Rhone)	10-3-09	F= 28-3-09	Golden 24-3-09 Braeburn 17-3-09 Gala 17-3-09	10-3-09	4
	INRA-UERI Gotheron (Drome)	30-3-09	F= 24-3-09	Golden 19-3-09	30-3-09	5
Trentino Italy	Res. Centre St Michele	-	L=11-3-09	Golden 14-3-09 Fuji 15-3-09	12-3-09	-3
Spain	IRTA, La Tallada d'Emporda (Girona)	24-2-09	L =5-3-09	Gala 15-3-09 Golden 27-3-09	5-3-09	-22

Annex B.

Development of an infection model for cherry leaf spot *(Blumeriella jaapii)*

Discharge of ascospores

No results correlated cumulated number of discharged ***Blumeriella jaapii*** ascospores trapped in the field with actual heat sums or moisture conditions. This work is necessary to develop an infection model for CLS.

The effect of rain on ascospore discharge

Mature ascospores of ***Blumeriella jaapii*** were discharged during rain events. Based on information for ***Venturia inaequalis*** it is known that as little as 0.2 mm of rain is sufficient to trigger ascospore discharge. Keitt et al. (1937) stated for ***Blumeriella jaapii*** that "the ascocarps must be thorougly wet before discharge can begin". In Keitt et al. (1937) records were kept on the epidemiology of cherry leaf spot (CLS) over the period 1916 to1935. During this time period there were many occassions of ascospore discharge with as little as 0.1 inch (2.54 mm) of rain.

Niederleiter and Zinkernagel (1999) stated that there was a correlation between the number of discharged ascospores and the amount of rainfall. However the data of Niederleiter and Zinkernagel (1999), did not support this conclusion as the graphically presented data showed that the number of discharged ascospores was not always correlated with the amount of rainfall. In addition the differences in the number of ascospores discharged could be explained by the population distribution during the maturation process, differences in temperature during the discharge event, or differences in duration on the rainfall period.
Niederleiter and Zinkernagel (1999) recorded ascospore discharge events at below 0.5 mm of rain. In the main period of ascospore maturation they also recorded ascospore discharge on days without rain, but these days were following immediately after days with several mm of rain. This fits to the observation of Keith (1937), where the strongest ascospore discharge occurred at the end of the wetness periods. In the first hours after the leaves have become dry the number of discharged ascospores per hour was up to 10 times higher than during the wetness period.

The effect of the amount of rain during the wetness period on the discharge of ascospores in the individual discharge periods for ascospores of ***Blumeriella jaapii*** at AU-Aarslev for 2008 are shown in Figure 1 and 2. As little as 0.2 and 0.4 mm of rain was sufficient to trigger a strong ascospore discharge. Higher quantities of rain did not lead to more ascospores to be discharged. Therefore, 0.2 mm of rain will be used as a trigger for the start of ascospore discharge in the model, no quantitative effect of the amount of rain on the ascosporic discharge will be calculated.

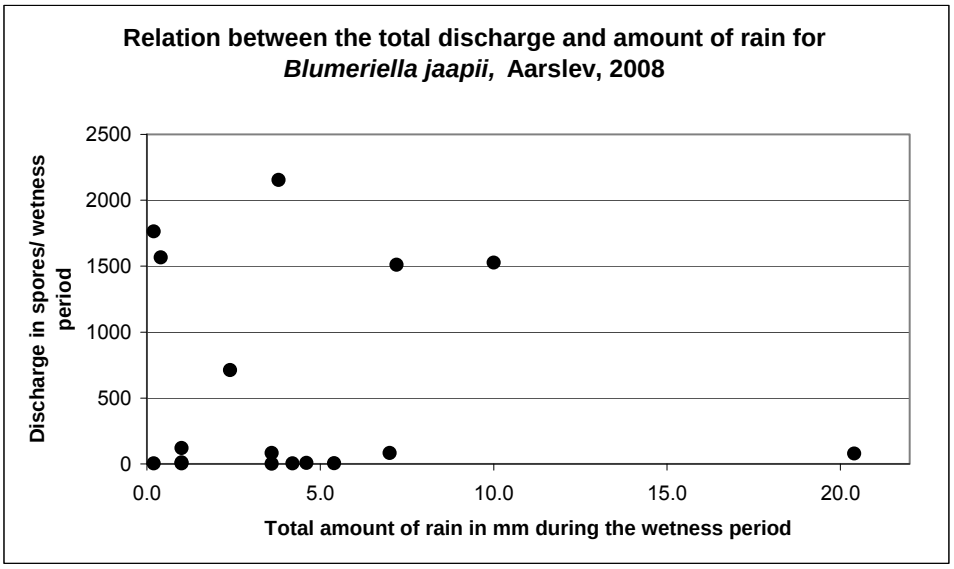


Figure 1. Plot between the discharge of *Blumeriella jaapii* ascospores and the amount of rain during the wetness events at AU- Aarslev, 2008.

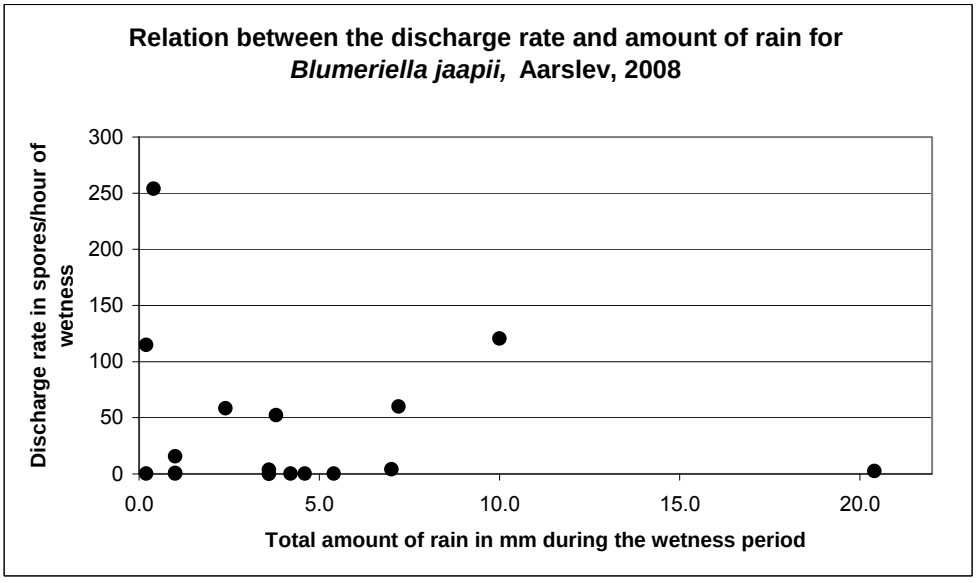


Figure 2. Plot between the discharge rate of *Blumeriella jaapii* ascospores and the amount of rain during the wetness events at AU-Aarslev, 2008.

The effect of temperature on ascospore discharge

Keitt et al. (1937) studied the effect of temperature on the discharge of ascospores. Ascospore discharge occurred at all temperatures tested in the range from 4 to 36°C. At 36°C ascospore discharge was completed in 24 h, at 4°C discharge continued on a consistently low level for 11 days. The data from Keitt et al. (1937) will be used for the model to calculate the discharge rate as a function of temperature.

Garcia and Jones (1993) studied the effect of temperature on the discharge rate in the range from 8 to 30°C in 2 h intervals. Although the experimental setup was totally different, the relationship between temperature and discharge rate was surprisingly comparable between the two studies (Keitt et al. 1937; Garcia and Jones, 1993).

The effect of the average temperature during the wetness period on the discharge of ascopores in the individual discharge periods for AU-Aarslev, 2008 are shown in Figure 3. During the coldest wetness period (3.3°C) a considerable number of spores (16% of the yearly total) was discharged. The strongest discharge rate was measured at an average temperature of 7.2°C. Higher temperatures did not lead to higher discharge rates (Figure 3). These practical findings are inconsistent with the laboratory results published by Keith (1937) and Garcia and Jones (1993), who both published very low release rates at low temperatures.

In the field data, maturation appeard to be the overruling factor in the ascospore discharge pattern. During a wetness event the mature spores were discharged, regardless of the amount of rain or the average air temperature. The same can be concluded from the data published by Niederleiter and Zinkernagel (1999).

Discharge appeared to be possible at both low and high temperatures. There was no distinct lower threshold, and no distinct optimum, but the discharge rate increases with temperature. According to these data the discharge rate for ***Blumeriella jaapii*** was much lower than for ***Venturia inaequalis*** were 80% of the mature spores were discharged within 2 h after the start of rain.

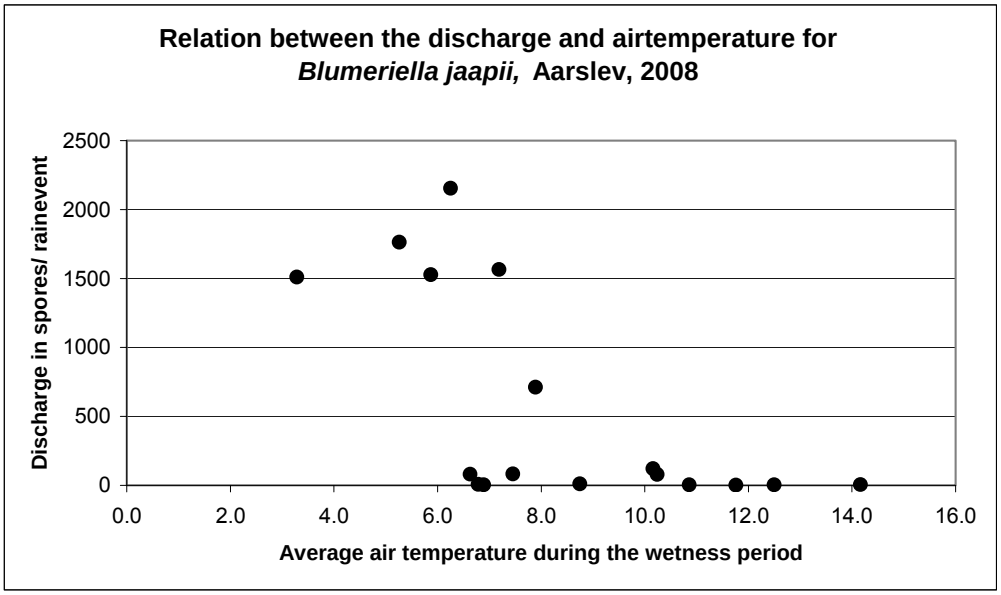


Figure 3. Plot to show the discharge rate of *Blumeriella jaapii* ascospores and average air temperature during the wetness period at AU-Aarslev, 2008.

Biofix of ***Blumeriella jaapii***

In the test run the Biofix for the model was defined as ”2 days before the first rainy day that caused ascospore ejection” (Table 1).

Table 1. Biofix settings for the model.

Year	First ascospores found in spore trap	Rain that caused this first discharge	Biofix used for model test runs
1994	20/5	17/5	15/5
1995	8/5	7/5	5/5
2007	9/5	7/5	5/5
2008	2/4	1/4	29/3

Simulation of infection model for cherry leaf spot

The four available datasets on ascospore discharge of ***Blumeriella jaapii*** at AU-Aarslev from 1994, 1995, 2007 and 2008 and local climate data were used for the simulations.

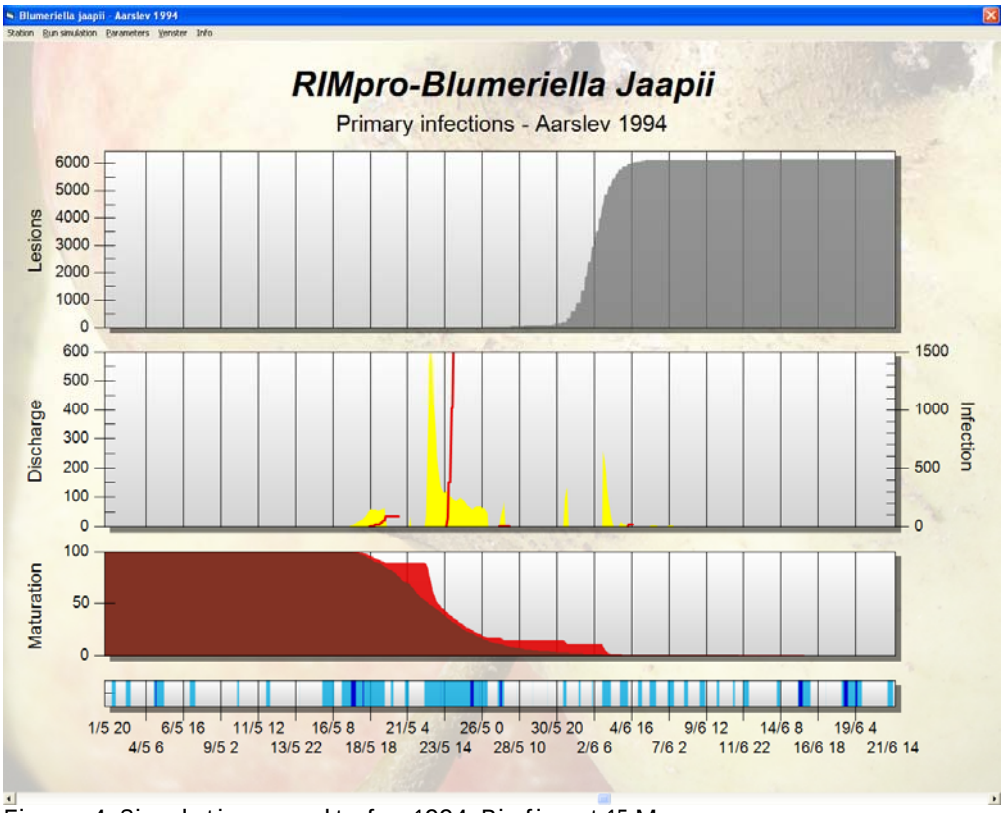


Figure 4. Simulation results for 1994. Biofix set 15 May.
From top: Grey: simulated number of lesions; Yellow: simulated ascospore discharge; red: simulated quantity of spores entering the host plant=infection.
Middle part: Brown: Simulated ascospore potential which is diminishing during spring; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.

Table 2. Results of the observations on ascospore discharge at AU-Aarslev in 1994. Date for rain event larger than 0.2 mm, date for rain event used in the model, actual number of discharge ascospores and percentage of discharged ascospores.

Rain events > 0.2 mm Date	Observations date	Ascospores no	Percentage ascospores
17, 18/5	20/5	1735	15
12, 22, 23, 24/5	24/5	3872	33
25, 26/5	27/5	3423	29
27/5	30/5	1819	16
31/5	1/6	1	0
2, 3, 4, 5, 6/6	6/6	371	3
9/6	9/6	37	0
10, 13/6	13/6	8	0
15, 17/6	17/6	48	0
18, 19, 20/6	20/6	92	1
21, 22/6	22/6	144	1
25, 26/6	27/6	89	1

In 1994 the model underestimated the ascospore discharge on 27 May, and overestimated the discharge at the beginning of June (Figure 4 and Table 2). The model calculated one minor infection, and one very severe primary infection (Figure 4). In in 1994, in the untreated plots in the field trials a strong ***Blumeriella jaapii*** epidemic developed (Lindhard Pedersen and Løschenkohl, 1997).

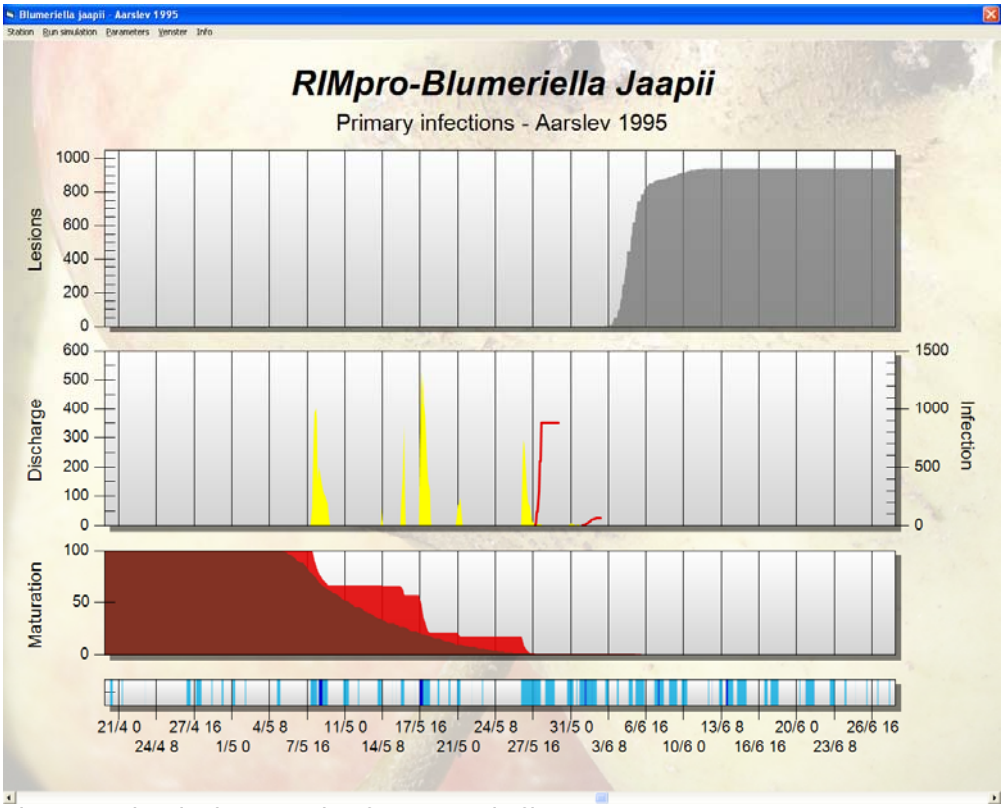


Figure 5. Simulation results for 1995. Biofix set 5 May.
From top: Grey: simulated number of lesions; Yellow: simulated ascospore discharge; red: simulated quantity of spores entering the host plant=infection.
Middle: Brown: Simulated ascospore potential which is diminishing during spring; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.

Table 3. Results of the observations on ascospore discharge at AU-Aarslev in 1995. Date for rain event larger than 0.2 mm, date for rain event used in the model, actual number of discharge ascospores and percentage of discharged ascospores.

Rain events > 0.2 mm Date	Observations date	Ascospores no	Percentage ascospores
7/5	8/5	9	0
8/5	9/5	25	1
9/5	11/5	102	4
14, 15, 16, 17, 18/5	18/5	219	8
20, 22/5	22/5	734	27
25, 26, 27/5	29/5	462	17
30, 31/5	31/5	595	22
1/6	2/6	200	7
5, 7/6	7/6	112	4
8, 9/6	9/6	86	3
10, 12/6	12/6	10	0
13, 14, 15/6	16/6	41	2
17, 18/6	19/6	41	2
20/6	21/6	37	1
21/6	23/6	12	0
24/6	26/6	2	0
27/6	5/7	32	1

In 1995 the model overestimated the first discharge period 8-9 May. On 22 and 31 May 1995 more spores were found in the trap than predicted by the model (Figure 5 and Table 3). The model calculated one moderate and one minor infection (Figure 5). In 1995 the untreated plots in the field trials an epidemic developed, but it was not as severe as 1994 (Lindhard Pedersen and Løschenkohl, 1997).

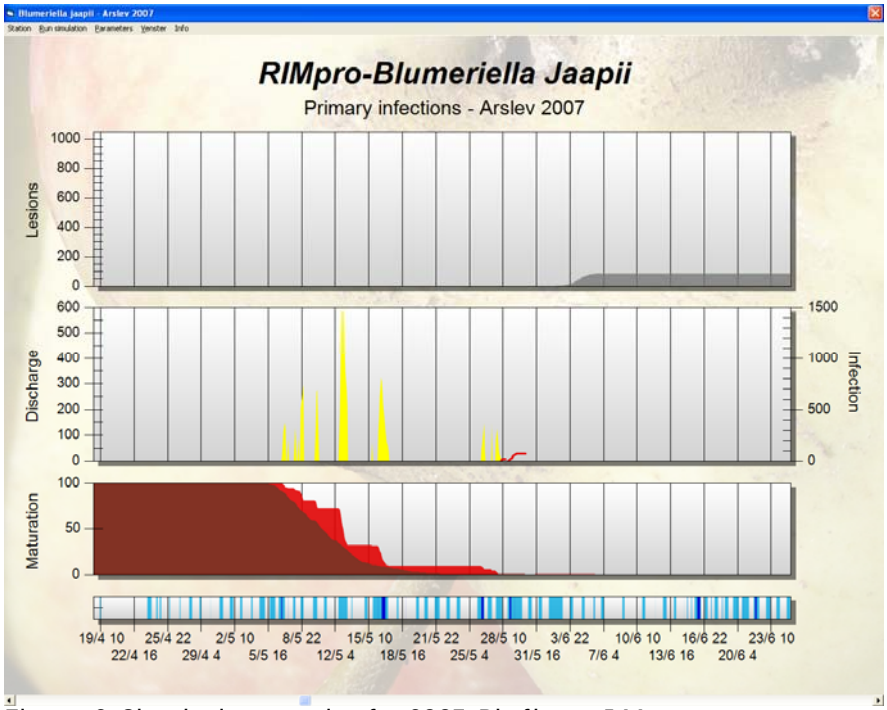


Figure 6. Simulation results for2007. Biofix set 5 May.
From top: Grey: simulated number of lesions; Yellow: simulated ascospore discharge; red: simulated quantity of spores entering the host plant=infection.

Middle: Brown: Simulated ascospore potential which diminished during spring; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.

Table 4. Results of the observations on ascospore discharge at AU-Aarslev in 2007. Date for rain event larger than 0.2 mm, date for rain event used in the model, actual number of discharge ascospores and percentage of dischargd ascospores.

Rain events > 0.2 mm Date	Observations date	Ascospores no	Percentage ascospores
17, 18/5	20/5	1735	15
12, 22, 23, 24/5	24/5	3872	33
25, 26/5	27/5	3423	29
27/5	30/5	1819	16
31/5	1/6	1	0
2, 3, 4, 5, 6/6	6/6	371	3
9/6	9/6	37	0
10, 13/6	13/6	8	0
15, 17/6	17/6	48	0
18, 19, 20/6	20/6	92	1
21, 22/6	22/6	144	1
25, 26/6	27/6	89	1

In 2007 the main period of ascospore discharge was accurately predicted by the model. During the last discharge periods at the end of May, less spores were found in the traps than simulated by the model (Figure 6 and Table 4). The model calculated only one very light infection resulting from the last ascospore discharge, and this was consistent with the 2007 field observations on disease development. In 2007 the trials at AU-Aarslev no infections by ***Blumeriella jaapii*** were seen. In commercial orchards only a very late infections (23 July) showing a few symptoms resulting from secondary infections were observed.

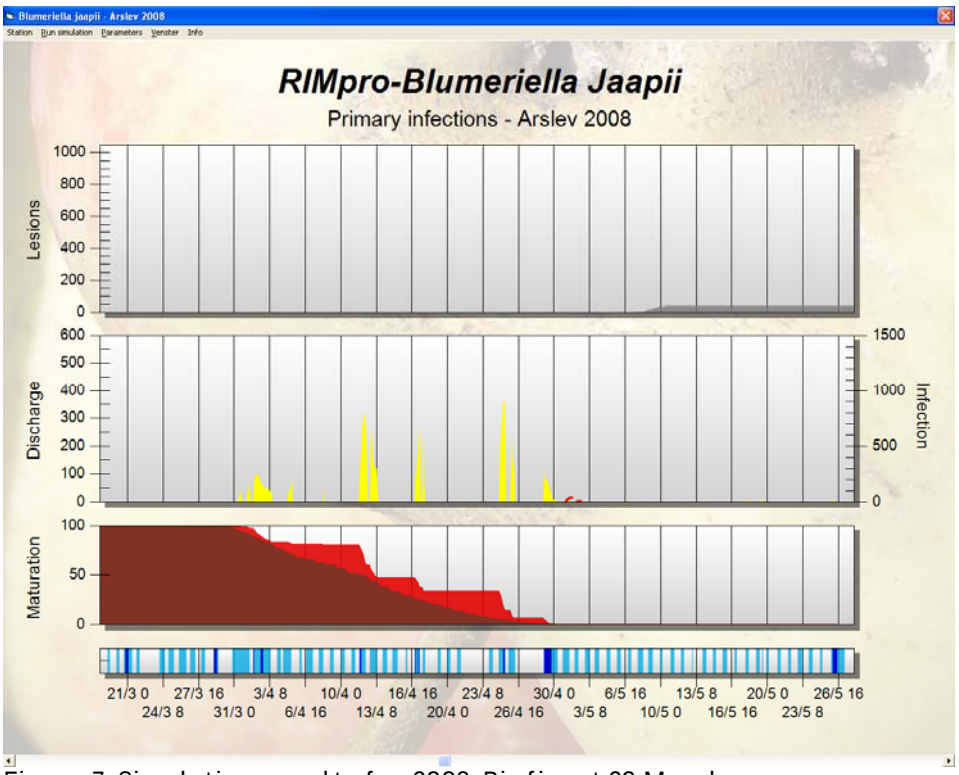


Figure 7. Simulation results for 2008. Biofix set 29 March.
From top: Grey: simulated number of lesions; Yellow: simulated ascospore discharge; red: simulated quantity of spores entering the host plant=infection.
Middle: Brown: Simulated ascospore potential which is diminishing during spring; red: simulated mature ascospores ready to be discharged at next rain event.
Bottom: Dark blue: Measured rain events; light blue: Measured leaf wetness.

Table 5. Results of the observations on ascospore discharge at AU-Aarslev in 2008.
Date for rain event larger than 0.2 mm, date for rain event used in the model, actual number of discharge ascospores and percentage of discharged ascospores.

Rain events > 0.2 mm Date	Observations Date	Ascospores no	Percentage ascospores
¼	2/4	7	0
2/4	3/4	105	1
5/4	7/4	2155	22
8, 11/4	12/4	1510	16
12/4	14/4	1763	18
16/4	17/4	1527	16
17/4	21/4	1566	16
24/4	25/4	12	0
25/4	28/4	711	7
29/4	30/4	79	1
30/4, 1/5	2/5	120	1
17/5	19/5	83	1
19/5	20/5	4	0
26/5	27/5	8	0
11/6	12/6	3	0
14, 15/6	16/6	2	0
16/6	17/6	3	0
19, 20, 21, 22/6	23/6	5	0
23/6	30/6	1	0

In 2008 the model underestimated the first ascospore discharge event, but cosely simulated the remainder of the discharge period (Figure 7 and Table 5).

The model calculated only one very light primary infection resulting from the last ascospore discharge (Figure 7). This was consistent with the 2008 field observations on disease development. In 2008, both in the trials at AU-Aarslev and in commercial orchards no primary infections were seen.

Improvement of the model and the user interface

In December 2007 the first version of the model was coded in Visual Basic 6.

In 2008 the model was embedded in the RIMpro decision support software to make use of the general in and output routines, and effort was made to stabilize the user interface. An interface was added to be able to read the weather data from the meteorological Campbell station placed at AU-Aarslev from the Internet.

Technically the model is now available for use by researchers, advisers and growers who have access to the relevant data from a weather station.

Literature

1. Garcia, S.M and Jones, A.L. 1993. Influence of Temperature on apothecial development and ascospore discharge by ***Blumeriella jaapii***. Plant Disease. 77: 776-779.
2. Keitt, G.W., Blodgett, E.C., Wilson, E.E and Magie, R.O. 1937. The epidemiology and control of cherry leaf spot. Wis. Agric. Exp. Stn. Res Bull. 132. pp. 117.
3. Lindhard Pedersen H., and Loschenkohl, B. 1997. Implementation of a warning system to control cherry leaf spot (***Blumeriella jaapii***) under European conditions. Gartenbauwissenschaft, 62 (5): 197-201.
4. Niederleitner, S. and Zinkernagel, V. 1999. Primary infection and epidemic development of the cherry leaf spot disease ***Blumeriella jaapii*** (Rehm) von Arx. J. Plant Dis. Prot. 106: 88–97.

Summary
The overall objective of the project was to reduce the use of fungicides in apple and sour cherry by optimizing the Dutch decision support system RIMpro for Danish conditions. For apple scab it was demonstrated that RIMpro could be improved by changing Biofix (model start) from 'green tip' to 'first ascospore discharge'. The project also documented that a high supply of nitrogen increased the risk of apple scab infection. For cherry leaf spot it was shown that major adjustments of the model and basic knowledge concerning the disease are necessary before further testing in sour cherry.

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