Influence of infection parameters on pear scab dynamics in organic orchards in The Netherlands, 2010-2013
B.G.H. Timmermans¹ and P.J. Jansonius¹

Abstract
Pear scab remains an important pest for organic pear production in The Netherlands. The disease incidence over the years shows a quite irregular pattern: for farmers in some years infection incidence seems to increase suddenly, whereas in other years incidence is rather low. In this project we tried to understand disease dynamics and quantify and influence various infections parameters.

In order to do this, we measured a number of infection related parameters in 8 organic orchards throughout the Netherlands from 2010 to 2013: amounts of leaf litter, ascospore production per unit of leaf litter, twig scab incidence (only 2012) and scab incidence. The results show large, perennial and robust differences in leaf litter amounts between orchards. Furthermore, the amount of ascospores per unit of leaf litter show a nonlinear relation with scab incidence the previous year, suggesting a threshold value above which spore density increases. Twig lesion numbers support this relation. We use this nonlinear relation, combined with various infection parameters, as a basis to understand (part of the) disease dynamics.

Keywords: conidia, leaf decomposition, leaf litter, Venturia pirina.

Introduction
For organic pear production in The Netherlands, pear scab caused by Venturia pirina Aderh. remains one of the major problems. Without a systematic strategy to control scab, in a few years’ time infection incidence can increase to dramatic figures. Even with a systematic control strategy, the infection incidence of pear scab shows a highly irregular pattern over years and orchards. This has been a trigger to start various projects to investigate infection biology and dynamics of pear scab and simultaneously to develop tools to control the epidemic.

In contrast to apple scab, pear scab can survive the winter both on scabbed leaves fallen on the orchard floor, and as lesions on twigs. Therefore in spring both ascospores formed on leaf litter and conidia formed on the twig lesions are present, and ascospores are not automatically the (only) starters of infection in spring. Although many factors are yet unknown, we argued that one of the main reasons for the unexpected and irregular infection incidence could be that there is no quantitative knowledge on the amounts of inoculum present for orchards in a specific year.

In the last few years, we developed a means of measuring leaf litter and number of ascospores per unit of leaf litter area at the time of the first major ascopore infections in spring (Timmermans & Jansonius, 2012). In the current contribution, we present the insights of an ongoing project in disease dynamics revealed by our perennial measurements.

¹ Department of Agriculture, Louis Bolk Institute, NL-3972 LA Driebergen, The Netherlands.
b.timmermans@louisbolk.nl
Material and Methods
In the winter of 2009/10, 2010/11, 2011/12 and 2012/13 fallen leaves have been collected in 8 organic Conference orchards, located throughout The Netherlands, in which standard commercial fungicide management has been applied. Sampling was done by collecting around 2-3 kg of fallen leaves from the soil. The leaves have been transported to the Louis Bolk Institute and placed in a field in wire-mesh cages with a small mesh at the bottom that prevented earthworms from entering.

In spring, during a heavy rain shower a plastic foil was placed at 50 cm height above the mesh cages, to prevent pre-measurement ascospore discharge. In spring (second half of April) the remaining leaf litter present in the 8 orchards was quantified. This was done by using a wire mesh (mesh size 4 mm) and placing it on the soil every 10-20 meters (depending on the length the orchards) in several rows throughout the orchards. The mesh was placed from tree-base to mid-row. On each placing location the amount of wire-squares that were filled with more than half with leaf litter, in one line of the mesh, was counted.

After a period of warm and dry weather leaves in each mesh cage were sampled (most years in the second half of April): the loose and slightly moist leaves at the top were mixed and a 25 g sample was taken from each mesh cage. Leaves were then incubated at room temperature in trays lined with wet tissue and covered with a porous plastic sheet for two weeks. Measurement of the ascospore number was done in a method according to Kollar (2000): the 25 g leaf material from each mesh cage was put into 1 l glass jars and submerged in 500 ml demineralised water, in which the leaves were shaken for 1 hour. Then, the material was poured out of the pots and sieved with a 0.25 mm sieve to remove the course material and subsequently with a 53 μm sieve to remove finer detritus. The filtrate was placed in a centrifuge for 5’ at 3300 rpm. The supernatant was pipetted into 1 ml of water and kept at -20° C until counting, that was done on subsamples in a Bürker counting chamber under a microscope at 400x magnification. A handful of the incubated leaves was spread out and a digital image was taken, from which the area per g leave was measured using image-J.

In each orchard, the scab infection was measured. In 2010 this was done by randomly picking 1000 fruits (picking one side of a tree fully, every 10-20 m) in 2 of the rows were the leaves were collected and the leaf litter was measured in spring. The fruits were then scrutinized for scab infections. In 2011, 2012 and 2013 the scab infections were measured by walking through each orchards and randomly turning 500 pears per orchards, and scrutinize them for infections, by Marc Trapman (Biofruitadviesb.v.) and the authors.

In the first week of February 2013, in all orchards 40-50 twigs were collected randomly, in the same rows as the other measurements. In some orchards, pruning was just finished and we collected - cut but not yet dried - twigs from the soil surface. Twigs were packed in plastic bags, and kept at 5°C until measurement in April. Then, the length of the twigs was measured and the number of twig lesions was counted.

Results
Pear scab incidence shows an irregular pattern, with large fluctuations between years and orchards (Fig. 1a), in the four years of our measurements. In 2010, six of the eight orchards had over 20 percent of pear scab incidence. In the following years, the variation in scab incidence between orchards was larger.

A first step to understand differences in scab incidence is to look at differences between orchards: we find significant and robust differences in amounts of leaf litter coverage in April between the eight orchards (Fig 1b).
Figure 1: (a.) Pear scab incidence measured at the harvest, in 2010 – 2013, as total percentage of infected pears and (b.) litter coverage of the soil in April, averaged over 2010 – 2013. Error bars indicate standard error, different letters indicate significant differences. Zeel-2 (8.8 % litter coverage) was left out of the statistics because of structural changes that were (are being) made in the orchard.

Secondly, in our measurements, both ascospore densities per unit of surface of leaf litter, and twig lesion numbers tend to show an increase in the following year after more than 30 % of pear scab in the year before (Fig. 2).

Figure 2: The number of ascospores (a.) developing on the surface of the leaf litter, in relation to pear scab in the previous year (data from 2010, 2011), and the number of twig lesions (b.) in relation to pear scab in the previous year in 2012.

Finally, we like to present the relation that the frequency of scab incidence exceeding a certain level had with leaf litter coverage of the soil (Fig 3.) In 2010 to 2013 these frequencies of scab incidences show significant trends with leaf litter coverage: the more leaves, the more often scab-problems.

Discussion
Firstly our data show that there is a nonlinear relation between pear scab and spore densities in the following year that increases above around 30 % of pear scab (equals 8 % of pears selected out for industry). This shows that farmers that in the long term want to lower scab incidences should aim to not exceed the level. Furthermore, our data show a perennial relation: after a year with over 30 % scab incidence, spore density increases and the following year the control strategy should be adapted in strictness. For apple, such a relation is known between ascospore production and leaf scab (Horner & Horner, 2002).
Secondly, results show that in the year 2010 only two orchards did not have relatively high scab incidences. Those two orchards happen to be the ones with the lowest leaf litter coverage of the soil. Following 2010, (increased spore densities) four orchards did manage to keep scab incidences below 10% in 2011 to 2013, and again those were the four lowest in leaf litter coverage. Overall, frequencies of scab incidence exceeding a certain level are correlated to the large and robust differences in leaf litter, suggesting a promoting effect of leaf litter and spore densities, that influences the orchards scab ‘performance’ on long term. Important is to realize that these results can help to form a theory, but cannot proof that there is a causal relation between leaf litter and scab incidence in pears (as there is for apple, Holb 2006): we did not change leaf litter coverage and see a corresponding change is scab incidence. There could also be a third factor underlying the correlation, for example tree densities or growth rates.

Acknowledgements
We want to thank the members of the organic pear working group greatly for the help and support in doing this work. We thank Marc Trapman for his advice and help in measuring the scab infection in a number of orchards in 2011.

References

