Differences in leaf litter, ascospore production and infection of pear scab (*Venturia pirina)* in Dutch organic orchards

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Abstract

The last two years we measured the amounts of leaf litter and ascospore production per unit of leaf litter area in 7 organic pear orchards throughout the Netherlands. In one of the orchards, adapted managements strategies were implemented two years ago, being grass/clover that is grown as ground cover on the tree-strip, and organic cattle-manure that replaces chicken manure pellets, in order to stimulate the earthworm population and change the palatability of the leaf litter.

First results indicate large differences between orchards in percentage of ground covered by dead leaves at the time of major ascospore infections, but also in number of ascospores per cm^2 leaf litter and in resulting potential ascospore dose. We used these data, together with weather data (temperature, rainfall), in a simple multivariate analysis to gain insight in the dynamics of the system. In 2010, 85 % of the variation in pear scab was explained with a model with rainfall during summer and the amount of ascospores per unit of leaf area. In 2011, 81 % of the variation was explained by a model with the amounts of ascospores per unit leaf area and the potential ascospore dose. In the adapted management experiment we measured no changes in leaf litter in the treatments yet.

We discuss that our first results show that, to a limited degree, leaf litter was indeed important for the scab epidemic in 2011, whereas in 2010 the high amount of rainfall in the second part of the growing season must have led to a high conidial infection pressure. Surprisingly, in both years ascospore number per unit leaf area was of more importance than leaf litter area or potential ascospore dose. This raises questions on for example the correlation with branch-lesions that we did not measure, and whether the number of ascospores per unit of leaf litter is a direct or an indirect factor that steers the scab incidence in the orchards.

Keywords: Conference, farmyard manure, leaf decomposition, disease cycle, rainfall

Introduction

In The Netherlands, pear scab (*Venturia pirina* Aderh.) is a major fungal problem in organic pear orchards, and a constant worry for the farmers. During the last few years, a number of organic pear farmers, who formerly had only small percentage of scab infected fruits in their orchards, have seen increases in the scab incidence. This irrespective of their intensive management that formerly seemed enough to control the fungus.

To control scab, one way to handle the problem can be to search for new and more effective additives that can be used. These can be sprayed directly on trees and fruits, or used as indirect system-controls to steer towards a more scab-suppressing orchard condition. Two years ago, we reported about an experiment in which we used beetroot Vinasse on leaf litter to decrease potential ascospore dose in Conference (Timmermans *et al.*, 2010). Preliminary experiments in more controlled conditions (Köhl, 2007) reported promising results but after translating these to practical measures on field scale, we

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measured the opposite, which could be due to variation in Vinasse quality or the application method we used.

In contrast with apple scab (caused by *Venturia inaequalis* Cooke) pear scab can overwinter not only on the leaf litter covering the soil, but also on the braches of the pear trees as lesions. Therefore, ascospores formed on leaf litter are not the only starters of infection in spring.

In order to gain insight in the disease cycle of pear scab and the role of ascospores, 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. First visual impressions were that there were quite big differences between orchards, and we devised adapted managements strategies to see whether we could steer differences, or whether they are (partly) intrinsic to local soil and (micro)climate.

Material and Methods

The orchards: collecting leaves

This project has been performed with leaves from 8 commercial orchards, throughout The Netherlands, in which standard commercial fungicide management has been applied. In the winter of 2009-2010 and 2010-2011 fallen leaves have been collected in the 8 orchards, by walking and collecting around 2-3 kg of fallen leaves from the soil throughout each of the orchard-fields. Many small samples were taken to obtain a reliable sample. The leaves have been transported to the Louis Bolk Institute and after mixing 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 ascospore discharge.

Measurement of leaf litter

In spring of 2010 and 2011, at the time we presumed the major ascospore discharges would take place (21, 22, 26 April, 15, 17 May 2010; 14, 19, 20 and 21 April 2011) 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 adapted in length to be half the row distance of each orchard, and placed from tree-base to mid-row. On each placing location the amount of wire-squares that were filled with leaf litter, in one line of the mesh, was counted (totals of 54 – 163 counts per orchard).

Measurement of the potential ascospore number per unit of leaf area

After a period of warm and dry weather (around 20°C during the day) leaves in each mesh cage were sampled (April, 2010 and 2011): the loose and slightly moist leaves at the top were mixed and a 25 g sample was taken from each mesh cage. The wet and compacted lowest level of leaf litter was not sampled. 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 were spread out and a digital image was taken, from which the area per g leave was measured using image-J.

Measurement of scab infection

In each orchard, also 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, 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 (Biofruit advies b.v.) and the authors.

Adapted management experiment

In one of the orchards with a history of high scab infection and a visibly bad decomposition of overwintering leaves, two adapted management strategies were applied starting in winter 2009-2010. The orchard is located in the province of Flevoland on clay soil and has older V-shaped Conference trees and little light on the soil. Row distance is 3.5 m and the trees are planted 1.2 m apart from each other and have 4 branches each. Each management strategy was performed in two repetitions of 54 m length and 3 rows width. Treatments were:

- Control: autumn 750 kg/ha organic chicken pellets (3% N), spring 500 kg/ha organic chicking pellets enriched with beetroot vinasse (8% N), tillage 2 times a year (autumn, spring)
- Adapted manure treatment: march 20 ton/ha cattle manure (in 2010 mixed with compost) instead of the chicken pellets
- Adapted tillage: no tillage
- Adapted undergrowth: grass/clover (*T. repens* cultivar Alice, sown in spring 2010 and resown in spring 2011)

Statistical analysis was done using R (R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org).

Results

Leaf litter on the soil in the 8 orchards varied a lot (Figure 1A.). Orchards 1,2,4 and 8 had very small amounts of leaf litter in both years, whereas others (6,7,5) had quite some leaf litter in both years. Strikingly, two orchards in Zeeland seemed to have much more leaf litter in 2011 than in 2010.

Also, there were large differences in the potential amount of ascospores on the leaf litter as was measured under conducting conditions in the lab (Figure 1B.). Multiplying these measurements gives the potential amount of ascospores per ha (potential ascospore dose) in each of the orchards, as a measure of potential infection pressure by ascospores (Figure 1C.). Finally there was also large variation in scab infection in the two years (Figure 1D.).

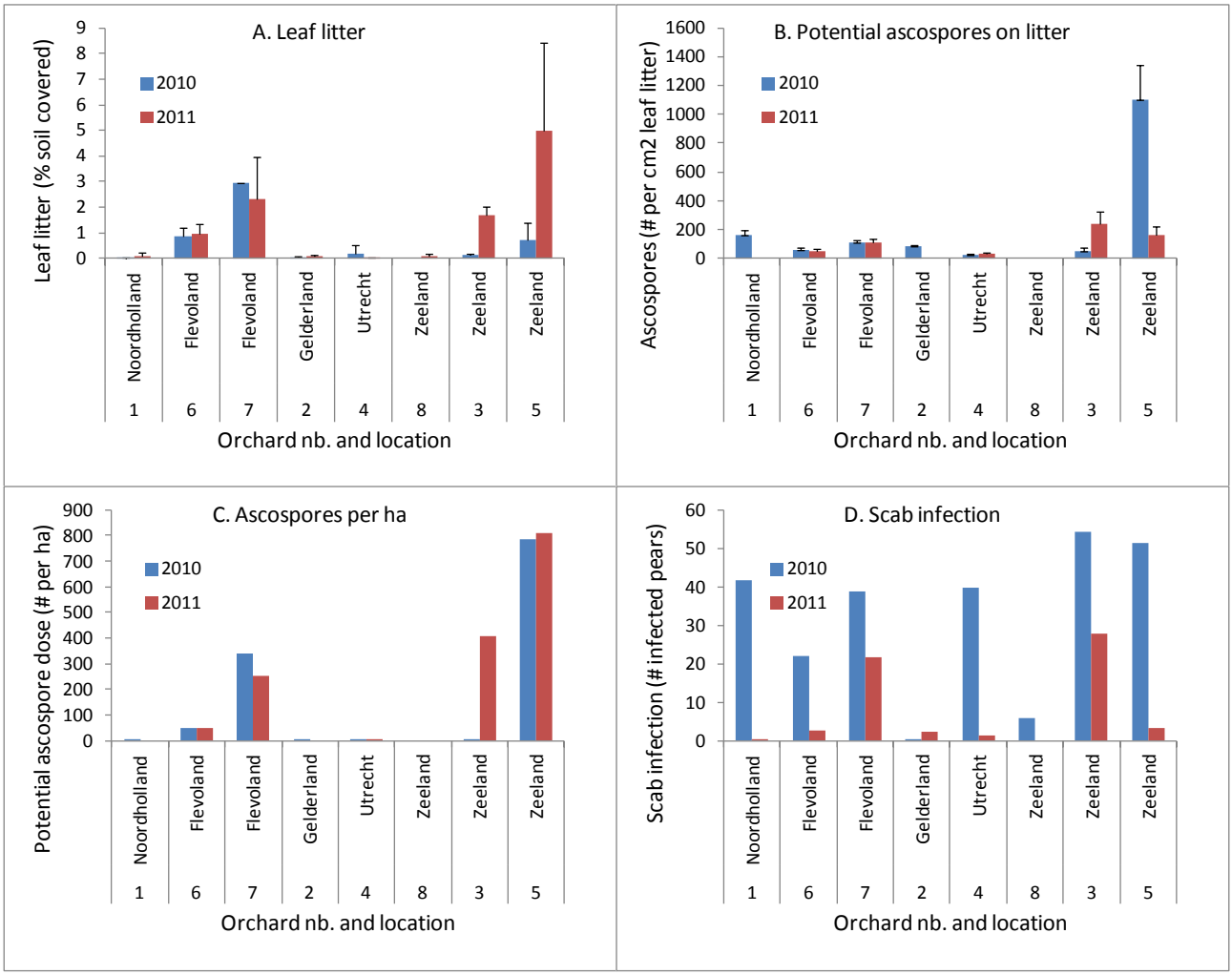

Figure 1. Leaf litter (A) (error bars indicate standard deviation), potential ascospore density on leaf litter (B) (error bars indicate standard error), potential number of ascospores per ha (C) and scab infection (D). Scab infection between the two years cannot be compared as it was measured somewhat differently (see materials and methods).

To show the intricacy of the system, scab infection was plotted against potential ascospore dose (Figure 2): there was no clear relation in both years. In 2010, there seems a sort of triangular shape in the data, indicating a potential multivariate relationship. In 2011, the situation is different: in one of the orchards (number 5) a high infection pressure by ascospores seems to coincide with a low number of infected fruit.

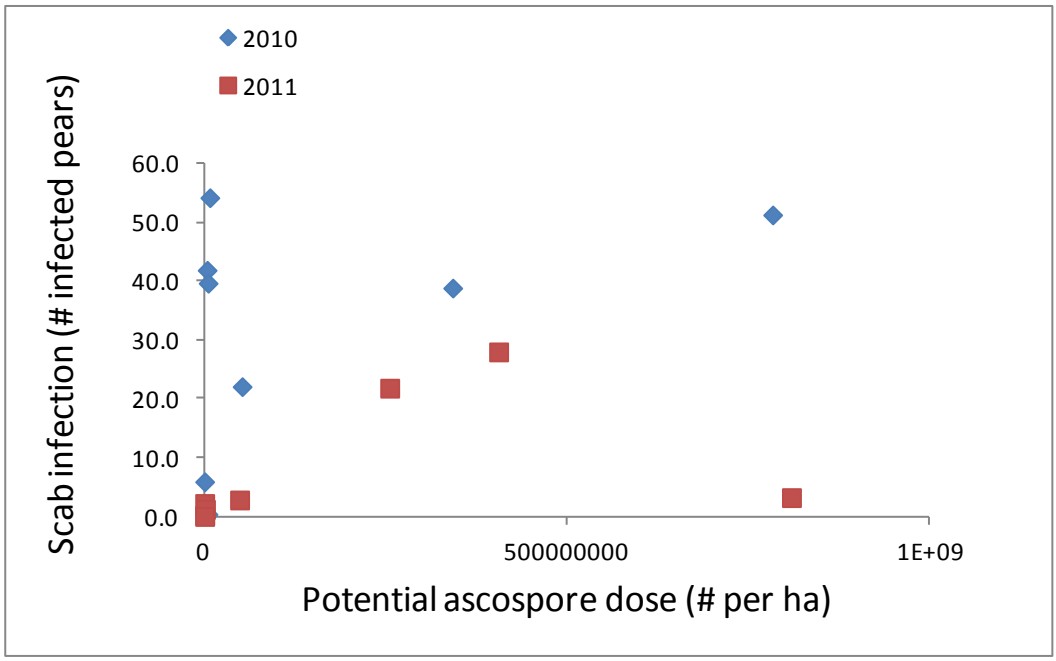

Figure 2. Scab infection plotted against potential ascospore dose in 2010 and 2011. The general level of scab infection between the two years cannot be compared as it was measured somewhat differently (see materials and methods).

That data presented above were used to do some multivariate statistics (Figure 3). In 2010, we find a statistical model that can explains 86% of the variation in the scab infection in the orchards, that contained rainfall from July till September (p=0.01) and the potential number of ascospores on the leaf litter in the orchards (p=0.046). Third important factor was the amount of leaf litter but this was not significant (and is not shown). In 2011 we find a statistical model that explains 82% of the variation of the scab containing again the potential number of ascospores on the leaf litter (p<0.01) and the potential ascospore dose (p=0.07). The two points dominating the relation are orchards 7 and 3, having both a high potential ascospore dose in 2011. In our analysis of scab incidence in the year 2011, we also tested the amount of variation explained by the scab incidence measured in 2010, but it had no significant impact.

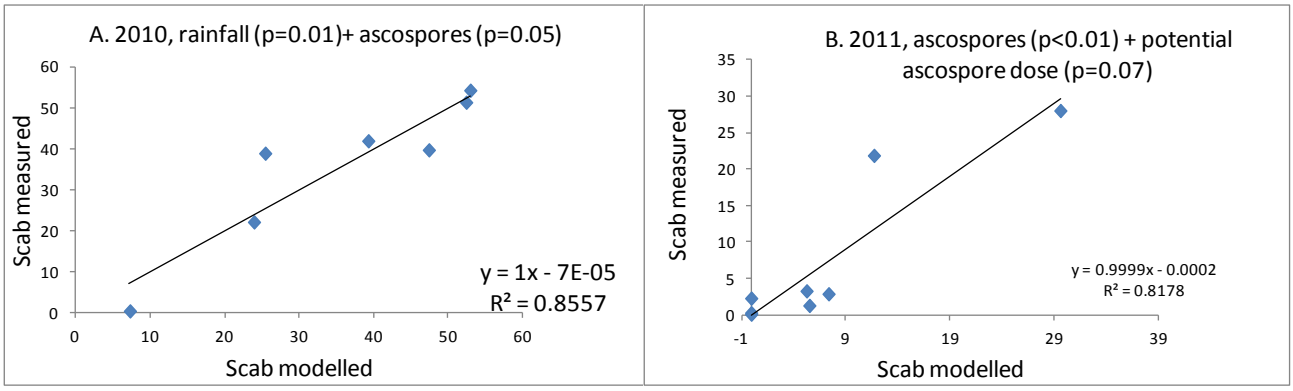

Figure 3. Variation in scab modelled by the best statistical models, containing rainfall from July till September and the potential ascospore density on leaf litter in 2010, and the potential ascospore density on leaf litter and the potential ascospore dose in 2011.

Finally, we measured the amounts of leaf litter separately in the various adapted management strategies in our experiment (located in orchard 7 in the former figures) (Figure 4). Within the orchard there are quite some differences in percentage of soil area covered. However, the were no clear changes by the management strategies yet.

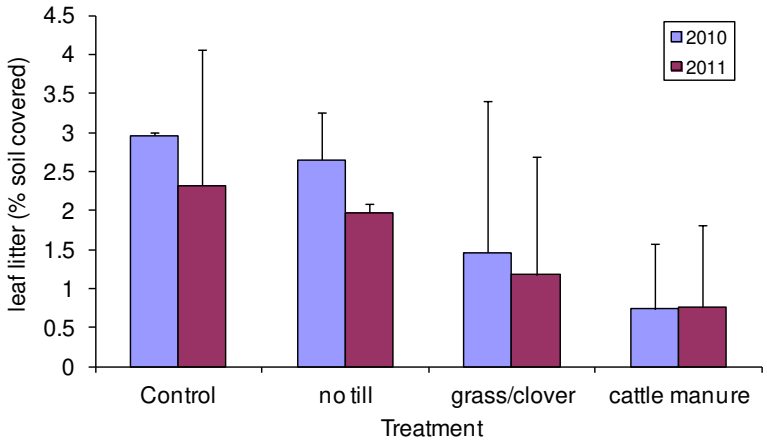

Figure 4. Leaf litter in de adapted management experiment in 2010 and 2011. Error bars indicate standard deviation.

Discussion

Back to our research aim: what insights in the disease cycle of pear scab do we get from these preliminary results? First of all, we can see that there are large variations, both in infection parameters and in scab incidence, within the range of orchards in our research. Both can have large annual variations: others reported the relations between autumn scab incidence and spring ascospore production (Horner & Horner, 2002). We find some correlations here: scab incidence in 2010 explained 45 % and 30 % of the variation in ascospore per unit leaf litter and potential ascospore dose, but no relation with scab incidence in 2011.

The presented data suggests that in 2010, a year with a very wet end of the summer in The Netherlands, rainfall in this period has been the main factor that explained variation in the scab incidence. Rainfall during the first part of the growing season explained only a small part of the variation. Furthermore, our results show that ascospores formed on leaf litter play a role but this is not the whole story. Strikingly, the ascospore number per unit of leaf litter area was of more importance in explaining variation in scab incidence than our calculated potential ascospore dose. There are at least two possible explanations. One could be that in the field, conditions for ascospore formation and/or infection success are not very favourable, and that the large differences in number of ascospores become a regulation factor. A second explanation can be that the number of ascospores on leaf litter is correlated with conidia from branch lesions in the same orchard. If we highlight for example orchard 1 in 2010 this becomes more clear: here, there were almost no leaves on the soil and therefore only a very low potential ascospore dose. There were still quite a number of ascospores per unit of leaf litter area, and scab incidence in 2010 was quite high. This would suggest an influence of branch lesions in this orchard. It would be very interesting to measure this additionally.

We have to mention that our analyses are based on correlations, and can never proof a causal relationship. Therefore, we included an experiment in one of the orchards, orchard

7, in our research. With adapted management, we try to increase leaf litter decomposition here, and hope to report some results in the future.

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