Effects of beetroot Vinasse on ascospore formation of *Venturia pirina* in a one-year field trial on an organic Conference orchard

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Abstract

In a one year experiment under orchard conditions in an organic Conference orchard, a single treatment with 500 l/ha beetroot Vinasse was tested as a means to reduce the formation of ascospores by Venturia pirina *on the leaf litter.*

Vinasse was applied with an orchard sprayer at the beginning of the leaf-fall period, diluted 1:1 with water.

Directly after the completion of the leaf-fall large amounts of leaves were gathered in the orchard and kept during the winter in wire mesh cages on bare soil. The fine wire mesh did not allow large earthworms to come in and take away the leaves.

In the next spring, after a period of warm, dry weather samples were taken from these cages for the detection of ascospores. This was done using a water bath method, both immediately and after an incubation period in the laboratory.

Vinasse applied in this way did not result in a reduction of the potential ascospore discharge as was expected based on previous (laboratory) results, but on the contrary to an increase in potential ascospore discharge of 45%. This result differs from results in earlier trials. A satisfying explanation could not be found thus far. For the time being the leaf-fall treatment with Vinasse against scab in practice should be carefully considered.

Keywords: beet residue, Pear, Scab, *Venturia inaequalis*

Introduction

Scab in pears (*Venturia pirina*) remains a large problem in organic orchards. Available means (sulphur, lime sulphur and potassium bicarbonate) are weak and may cause some leaf damage in cultivar Conference. In pear, the fungus can survive the winter on the fallen leaves on the ground as is also the case in apples, but also as lesions on the branches. To keep the infection pressure as low as possible, it is important to prevent ascospore formation on leaf litter as one of the primary infection sources. Firstly this can be realized by decreasing the amount of leaf litter (and hence the amount of lesions and ascospores), and secondly by decreasing the amount of ascospore per unit of leaf litter. In conventional orchards, the latter is done by fungicides or urea, which are not available for organic orchards. Here, the use of beetroot Vinasse, a rest product from the sugar industry that contains a low percentage of nitrogen and high concentrations of potassium and phosphorus, seems a promising alternative. In the EU project REPCO, leaves picked in fall from Jonagold apple trees, submerged in beetroot Vinasse and placed on the soil during winter had 95% reduction of ascospore number of *Venturia inaequalis* after lab incubation in spring (Köhl, J., 2007). A two-year experiment was performed by PPO, funded by LAMI, in which leafs in fall, at their latest stage at the trees, were wetted with Vinasse two to four times using an irrigation installation and then kept during winter in field conditions.

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Ascospore counting was done after lab incubation in spring and comparable decreases in ascospore production were measured (not published yet). In the current project we tried to translate and upscale the use of beetroot Vinasse into a measure to decrease potential ascospore discharge in Conference in practice.

Material and Methods

The experiment was done in an organic orchard with Conference pear trees, in 4 repetitions, each containing 8 measurement trees. Two treatments were tested: a control treatment and a treatment in which the leaves were sprayed with beetroot Vinasse.

Beetroot Vinasse was sprayed once, with a dose of 500 litre Vinasse mixed with 500 litre water per ha, on 28 October 2008. This was the beginning of the leaf-fall, and at the moment of spraying the pear trees had lost around 5% of their leaves. The spraying was not done earlier to prevent damage to leaves that were still green as much as possible, and not later because the fluid covers all leaves easier if they are still at the tree. The spraying was done during an afternoon, with almost no wind and dry weather conditions. The product was well visible on the leaves, and coverage seemed homogenously, in the form of droplets due to the viscosity of the mix. In the days after spraying, the weather remained dry. The fallen leaves of the eight measurement trees in each field were collected almost completely, resulting in 3 kg leaves per field, and placed in wire mesh cages with a small mesh at the bottom that prevented earthworms from entering. The mesh cages were kept in the field during the winter, and in spring transported to another location near the measurement laboratory, where they were kept in an identical way. In spring, during a heavy rain shower a plastic foil was placed at 50 cm height above the mesh cages, to prevent ascospore discharge.

After a period of warm and dry weather (around 20°C during the day) leaves in each mesh cage were sampled (sampling dates 15-4-2009 and 22-4-2009): first, the dried-out top layer of leaves was gently shifted to the side, then the loose and slightly moist leaves underneath 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 sampled on the second sampling date were incubated at room temperature in trays lined with wet tissue and covered with a plastic sheet for one week.

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.

For the leaves that were sampled on the first sampling date, 2 subsamples were counted for each concentrate, and for leaves that were sampled on the second sampling date it was decided to increase this number to 3 subsamples for each concentrate.

All statistics were done with Genstat (version 11.1.0.1575).

Results

After spraying pear trees with beetroot Vinasse the leaves coloured brown directly, skipping the normal stages of yellow and orange, whereas leaves at trees in de not sprayed treatments showed the normal coloration process as is seen in autumn. No effects were noticed on the leaf formation in the spring after spraying.

Strikingly, the number of ascospores measured on the leaves after spraying with beetroot Vinasse was higher at both sampling dates, compared to the number of ascospores per gram dry leaf in the control treatment without spraying (Figure 1). The increase was not significant for the first sampling date, when total number of ascospores was lower, probably due to the fact that there had been no additional inoculation period. For the second sampling date, the number of ascospores was 45% higher on leaf litter that was sprayed with beetroot Vinasse, and the result was significant (p=0.029).

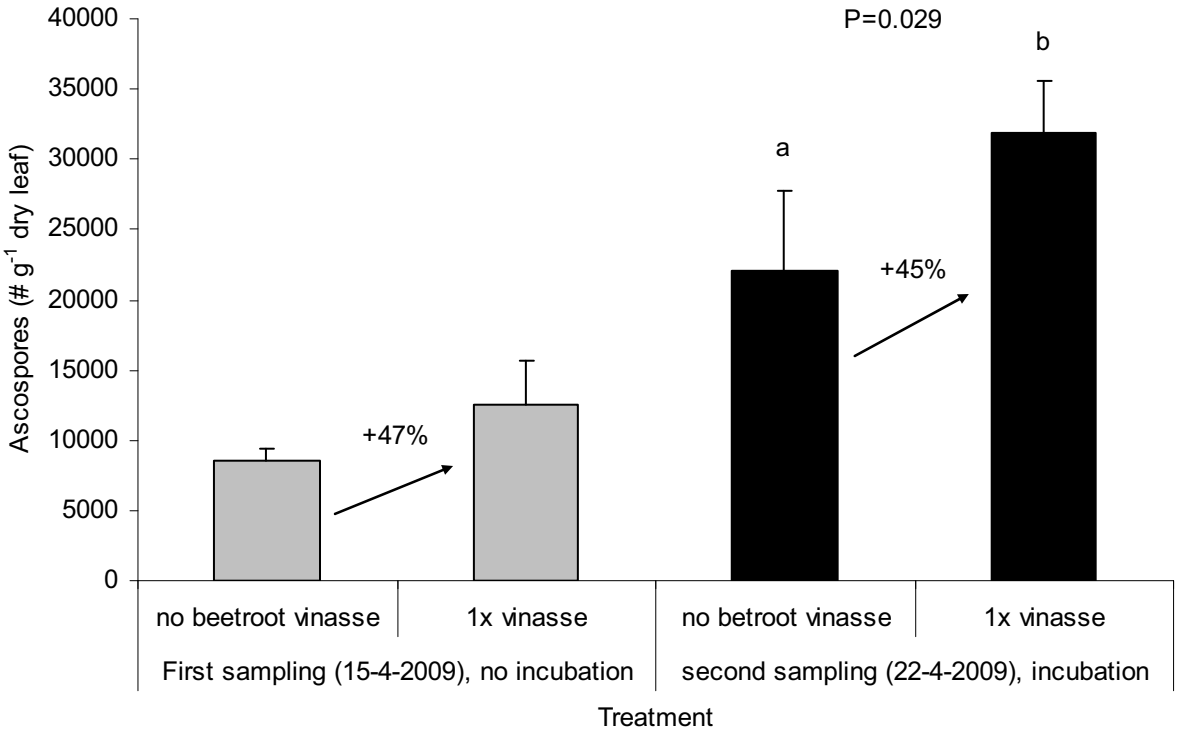

Figure 1: The number of ascospores measured at two sampling dates at the leaves in the mesh cages. Letters indicate a significant difference on the second sampling date (P=0.029), and the arrows with percentages indicate the relative increase in ascospore number. Error bars indicate standard error of the mean.

Discussion

Clearly, the results show that in our experiment the treatment with beetroot Vinasse did not result in a decrease of ascospore formation, but resulted inn an increase instead. This seems contradictory to findings in other projects for *Venturia inaequalis* on apple (Köhl, 2007), in which Vinasse decreased ascospore formation almost comparable to the use of urea. In an other experiment, Pfeiffer *et al*. (2004) found a large increase of ascospore potential in apple leaf samples treated with a 3% Vinasse solution for *Venturia inaequalis*. Of course, we realize that this is a much lower concentration that the 50% solution we used. The most probable explanation for the differences in the results of the REPCO experiment (Köhl, 2007) and the experiment in pear that we presented is the variability in the quality of beetroot Vinasse. For example it can be speculated that it is the high salinity

of Vinasse, known also to have undesirable effects in soil applications (Tejada & Gonzalez 2005; Madejón *et al*., 2001) has an inhibiting effect on fungal growth. Application at a low concentration (as done by Pfeiffer *et al*., 2004) would then at least not result in a decrease in ascospore formation. In our experiment we did not measure the quality of the Vinasse, and we can not be sure that it had its normal salinity.

The results presented here show that use of beetroot Vinasse is not ready for application in practice yet: stimulation of the potential ascospore production in an organic orchard could have quite undesirable effects as a result. And therefore we conclude that an in-depth investigation into the mechanism of the effect of Vinasse on ascospore formation should be done in order to be able to predict its effects.

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