# Application of Beetroot Vinasse – Influence on Foliage Decomposition and Ascospore Potential on Remaining Leaf Material

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# Abstract

The beetroot vinasse products "Biorga", "Biofa" and "Provita" were tested on Jonagold cultivars. All three products were sprayed shortly before natural leaf fall in November 2010, each diluted to 33 % (400 I vinasse per 800 I water per hectare). The chosen orchard had a uniformly high incidence of apple scab (Venturia inaequalis). After the spraying a predefined amount of leaves (150 g) with strong symptoms of apple scab was picked from the trees and put outdoor into wire cage depots to overwinter. Each treatment was replicated threefold, including an untreated control and a unit sprayed with a 10 % sugar solution.

The two aspects of the trial required two different settings. Firstly, a set of cage depots was put directly on the ground in order to monitor the decomposition process of the leaves. Secondly, another set of cages was put on a synthetic canvas cover in order to stop decomposition and thus keep the leaf material as intact as possible to examine its ascospore potential. Leaf degradation was observed regularly between November 2010 and March 2011. To evaluate potential inoculum, ascospores were discharged using the water bath method according to Kollar (2000). This was first done in early April 2011 (beginning of the ascospore release season) and repeated in mid April and early May 2011 (peak of the season).

The vinasse products "Biorga" and "Provita" reduced the amount of leaves left in March 2011 by around a third. For "Biofa" this accelerating effect was not found. Concerning the amount of ripe ascospores, all three vinasse treatments showed no effect at the first sampling date early in the spore release season. However, at the two latter sampling dates during the main phase of the release period, a reduction of at least 55 resp. 75 % compared to the untreated control was found.

The described findings are only based on one-year results and should be interpreted with appropriate cautiousness. The trial will be repeated with the same setting in 2011/12.

Keywords: beetroot vinasse, apple scab, Venturia inaequalis, inoculum, decomposition

## Introduction

Some organic apple growers in the Bodensee region in Southern Germany use vinasse, sprayed on the leaves shortly before leaf fall, as part of their strategy towards apple scab (*Venturia inaequalis*). The idea of a vinasse application is to accelerate the decomposition of the leaves and therefore reduce the potential scab inoculum for the following year.

The accelerating effect of vinasse is largely agreed upon. However, the application of vinasse might also stimulate the maturation process of ascospores, thus leading to a higher percentage of ascospores on the remaining leaves.

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The research results concerning this question vary extremely. Köhl (2007) found an ascospore reduction of 95 % when dipping leaves in vinasse solutions which would equate to doses of 200 I/ha and 400 I/ha respectively when recalculated for a commercial orchard application. Contrary to this result, Timmermans et al. (2010) tested vinasse in Conference pears and found an increase in ascospore potential (concerning *Venturia pirina*) by 45 % after an application of 500 I/ha vinasse diluted 1:1 with water. Pfeiffer et al. (2004) observed a moderate increase of ascospore potential after a vinasse treatment with a concentration of 3 %.

All in all, the subject is not finally clarified yet. The results may also differ due to the various vinasse products and different concentrations used. This article summarises the implications of a one-year trial at the Kompetenzzentrum Obstbau-Bodensee with the three different vinasse products "Biorga", "Provita" and "Biofa". The products were compared in regard to acceleration of decomposition and ascospore potential on the remaining leaves. Also part of the trial was an untreated control and a treatment with sugar solution.

#### **Material and Methods**

The trial was placed in a *Jonagold* orchard with a uniformly high level of scab-infections throughout the site. The treatment was carried out on the 3<sup>rd</sup> of November 2010 when the most severely infected leaves at the end of the long shoots started to fall. The vinasse products "Biorga", "Provita" and "Biofa" were applied in a dilution of 33 % (400 I vinasse and 800 I water per hectare). After the spraying, the trees were dripping wet on both upper and lower side of the leaves. Additionally a treatment with a 10 % sugar solution and an untreated control were added. Directly after the spraying was completed, leaves with obvious and uniform scab infections were picked off the trees and put into wire cages to overwinter in the open field. Each cage was filled with 150 g fresh leaf material and each treatment was replicated threefold. The depots prevented the leaves to fly away but allowed natural exposure to the weather. One set of wire cages was put on a synthetic canvas cover in order to prevent earthworm activity (together with some soil on top of the canvas to re-enact natural conditions) and another set was put on the bare soil (see Fig.1).



Figure 1: Wire mesh cages with treated leaves in the fall of 2010 put on synthetic canvas cover to prevent earthworm activity (left) and on the bare soil to monitor natural decomposition (right).

The latter set was examined visually between November 2010 and March 2011 in regard to differences in speed and timing of decomposition. At the end of the trial the weight of the remaining leaf material was assessed.

The set on the canvas cover was used to count the amount of ripe ascospores on the leaves at three different points in time. In order to prevent ascospore discharge prior to the scheduled date of counting, the cages were roofed some days before the anticipated rain. Ascospores were counted at the beginning of the spore ripening in early April and again in

the middle of April and in early May during the peak period of ascospore release. To determine the number of ripe spores, the water bath method of Kollar (2000) was used. Leafs were dried at room temperature overnight and torn into pieces of approx. 1 cm<sup>2</sup>. Strong veins were taken out. 1 g of the remaining leaf material was suspended in 50 ml of distilled water and shaken at 100 rpm for one hour. Immediately after the shaking the fluid was mechanically decanted into another flask to stop the discharge process. In the next step the spores were quantified with a 0.5 ml Kolkwitz planktoncytometer (Hydro-Bios, Germany). Each sample was stirred up again directly before it was put into the planktoncytometer in order to prevent spores from sinking to the bottom or clinging to the flask. Then the ascospores were allowed to settle for at least 10 minutes and 30 squares were counted under a microscope.

## Results

#### Decomposition process

Leaves treated with "Biorga" resp. "Provita" were decomposed faster which lead to a considerably reduced amount of leaves left at the beginning of the ascospore release season. At the beginning of March the untreated control still had 37 % of the leaves left in the cage. For "Biorga" and "Provita" this percentage was 12 % resp. 5 %. Thus the leaf matter was reduced to around one third compared to the control. The treatment sprayed with the vinasse "Biofa" showed no reduction and the sugar solution rather slowed than fastened the decomposition process in this trial: In the middle of March 50 % of the initial leaf matter was still in the wire cages (see Fig. 2).

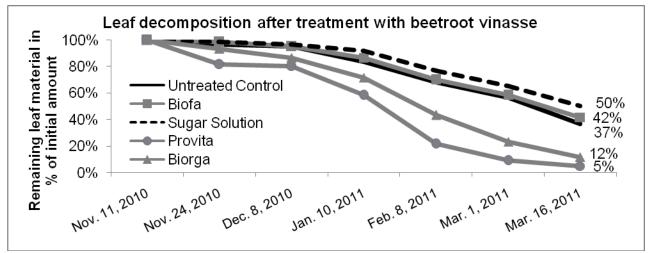


Figure 2: Process of leaf degradation after autumn treatment with three different beetroot vinasse products and sugar solution.

## Ascospore ripening process

At the first date of analysis there was no significant difference between the amount of ascospores in the three vinasse treatments and the untreated control. At the second date of analysis (middle of April), the leaves of the untreated control discharged around 640,000 ascospores per gram leaf matter. The corresponding number on the vinasse-treated leaves was between 260,000 and 283,000, i.e. the spore potential was reduced by 55 to 60 %. At the third and last sampling date, the vinasse-treated leaves contained at least 75 % less ascospores than the untreated leaves – the strongest reduction measured in this trial but with an unexplained high spread in the untreated control (see Fig. 3).

The sugar solution showed no clear effects. At the first and second sampling date, the leaves treated with sugar solution again discharged more spores than the control-leaves. However, at the last date of analysis a lower spore potential was found.

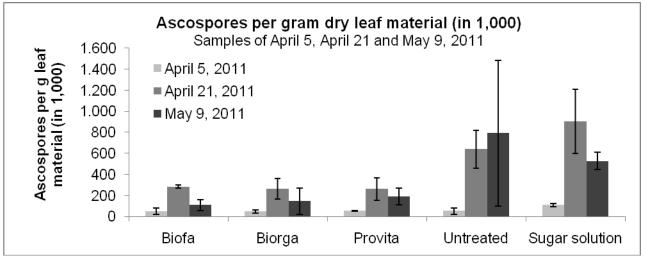


Figure 3: Mean amount of discharged ascospores at the three dates of water bath analysis (n=3 replications per treatment and date). Error bars represent standard deviation.

## Discussion

The vinasse products "Biorga" and "Provita" accelerated the leaf decomposition process considerably. This effect could so far not be shown for "Biofa". This might result from the difference in composition of the vinasse products which will be analyzed in the next year of the trial. The hypothesis that the application of vinasse has a negative influence on the ascospore potential of the remaining leaves could not be affirmed – for two of the three dates of analysis the opposite was found. Nevertheless, these conclusions are based on a one-year field trial only. Due to a high standard deviation in the result of the untreated control at the sample taken in May, the results are only significant for the sample of April 21, 2011. The research will be repeated in 2012, possibly with more replications, in order to verify the results.

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