

# Effects of several beetroot Vinasse products on foliage decomposition and ascospore formation of *Venturia inaequalis* on remaining leaf material

S. Buchleither, A. Bohr, S. Späth, U. Mayr

## Abstract

*In a three year research project, funded by the "Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft" the effect of the beetroot Vinasse products „Biorga“, „Biofa“ and „Provita“ were tested with regard to decomposition and ascospore formation on Jonagold cultivars. All products were sprayed shortly before natural leaf fall in 2011-2013, each diluted to 33 % (2011, 2012) respectively 25 % (2013). The treated orchard had a uniformly high incidence of apple scab (*Venturia inaequalis*). After spraying, an accurately defined amount of leaves with uniformly infestation of apple scab was picked from the trees and put outdoor into wire mesh cages during the winter. Each treatment was replicated threefold, including an untreated control. Additionally the Vinasse product "Biofa" (25 %) was tested separately only with regard to ascospore formation in comparison to an untreated control in a second trial.*

*Leaf degradation in the mesh cages was annually observed visually between November and April. To evaluate the amount of ripe ascospores on remaining leaves in spring, spores were twice discharged using the water bath method according to Kollar (2000). In every year we observed faster leaf decomposition in nearly all Vinasse treatments which lead to a considerably reduced amount of leaves left at the beginning of the ascospore release season. Except in 2011 the treatment with "Biofa"- Vinasse and in 2013 "Biorga"- Vinasse was not different from the untreated control. However, in the majority of cases effects was not significant because of high standard deviation. Concerning the amount of ripe ascospores on remaining leaf material, all Vinasse products showed good effects in every year. Reduction rates between 14 – 89 % compared to the untreated control was determined. Also in trial 2, treatment with "Biofa"-Vinasse lead to a reduction of ascospores on remaining leaves between 27 – 84 % compared to the untreated control.*

**Keywords:** beetroot Vinasse, apple scab, *Venturia inaequalis*, decomposition

## Introduction

Vinasse treatment sprayed on the leaves shortly before leaf fall is traditionally playing part in 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 influence the maturation process of ascospores, thus leading to a altered amount of ascospores on the remaining leaves. 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 l/ha and 400 l/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 l/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 %.

Regarding these experiences, 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 results of a three year trial at the Kompetenzzentrum Obstbau-Bodensee with three different Vinasse products "Biorga", "Provita" and "Biofa". The products were compared with an untreated control relating to acceleration of decomposition and ascospore formation on the remaining leaves.

## **Material and Methods**

The trial was placed in an organic *Jonagold* orchard on rootstock M9 with a uniformly high level of scab infections throughout the site. The treatment was carried out each year in November with beginning of leaf fall. The Vinasse products "Biorga", "Provita" and "Biofa" were applied in a dilution of 33 % (2011, 2012) respectively 25 % (2013). In a second trial only the Vinasse product "Biofa" was applied with a 25 % concentration in a different orchard but also on the variety *Jonagold* (M9). Treatments were applied with a motorized sprayer using a spray volume of 500 l per hectare and meter foliage height. After treatment, leaves with obvious and uniform scab infections were picked in the several treatments. After picking, the leaves were air dried for 24 hours before respectively 150 g were weighed and put into wire mesh cages to overwinter in the field. Each treatment was replicated threefold. The depots prevented the leaves to fly away but allowed natural exposure to the weather. To investigate the effect of the Vinasse products on ascospore formation, one set of wire cages was put on a synthetic canvas cover in order to prevent earthworm activity, but together with some soil on top of the canvas to simulate natural conditions. In order to prevent ascospore discharge prior to the scheduled date of counting, the cages were roofed some days before the expected rain. Ascospores were yearly counted in April and a second time in May, during the peak period of ascospore release. In 2011, spores in trial 2 were only counted once. To determine the number of ripe spores, the water bath method of Kollar (2000) was used. Leaves were dried at room temperature overnight and torn into pieces of approx. 1 cm<sup>2</sup>. Strong leaf 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 plankton cytometer (Hydro-Bios, Germany). Each sample was shaken again directly before it was put into the plankton cytometer 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. Each solution was counted out twice.

To observe the effect of the Vinasse products on foliage decomposition, another set of mesh cages was put on the bare soil. This set was examined visually between November and the beginning of ascospore season in the next year in regard to differences in rate and timing of decomposition. At the end of the trial the weight of the remaining leaf material was assessed.

## **Results**

### Effect on foliage decomposition

Expect treatments with "Biofa" in 2011 and "Biorga" in 2013, all Vinasse products lead to a faster decomposition compared to the untreated control. Due to accelerate decomposition in the Vinasse treatments, a considerably reduced amount of leaves left at the beginning of the next ascospore release season was measured. Particularly in years with a generally

poor and slow decomposition like 2011, the influence of the Vinasse products was obvious. At the beginning of March 2011, in the untreated control still 37 % of the leaves were left in the cage. For “Biorga” and “Provita” this percentage was 12 % respectively 5 %. Thus the leaf matter was reduced to around one third compared to the untreated control. Only in 2011, the treatment sprayed with the Vinasse “Biofa” showed no reduction. But also in years with good conditions for decomposition like 2012, when leaf reduction was commonly high between November and January, Vinasse treatments led to a lower amount of remaining leaves left in April. This is also valid for the year 2013, when decomposition of leaf material was generally high. However, considering standard deviation, the effects of the Vinasse treatments were not significant except for “Provita” and “Biorga” in 2011. As figures 1b – 3b represents, standard deviation was generally high in the several treatments.

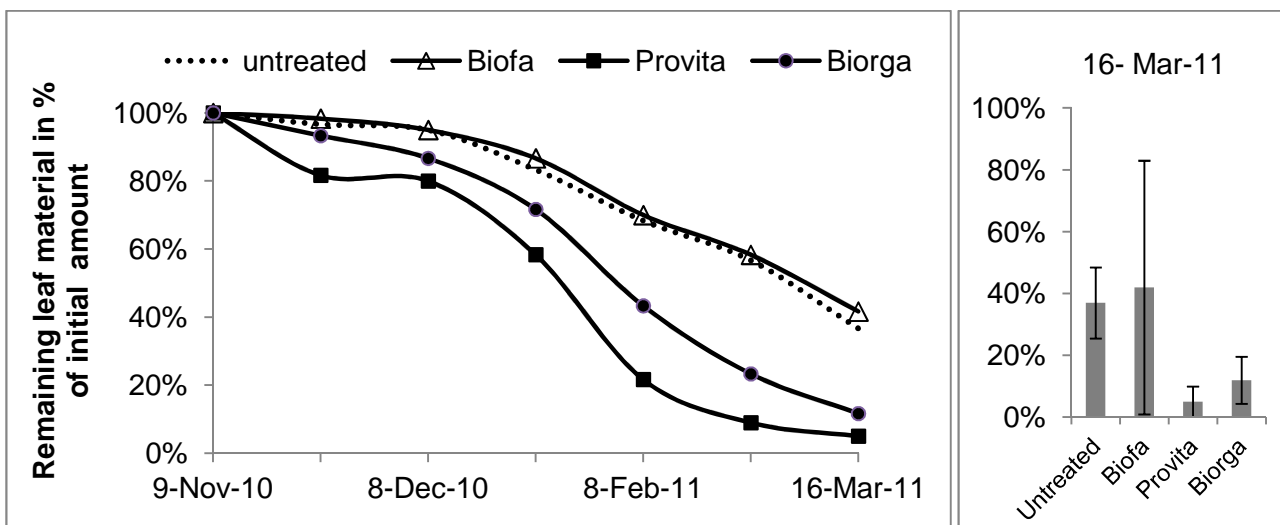


Fig. 1: a) Progression of leaf degradation after autumn treatment with three different beetroot Vinasse products compared with an untreated control in 2010/11. b) Final amount of leaf material (%) and standard deviation in the several treatments

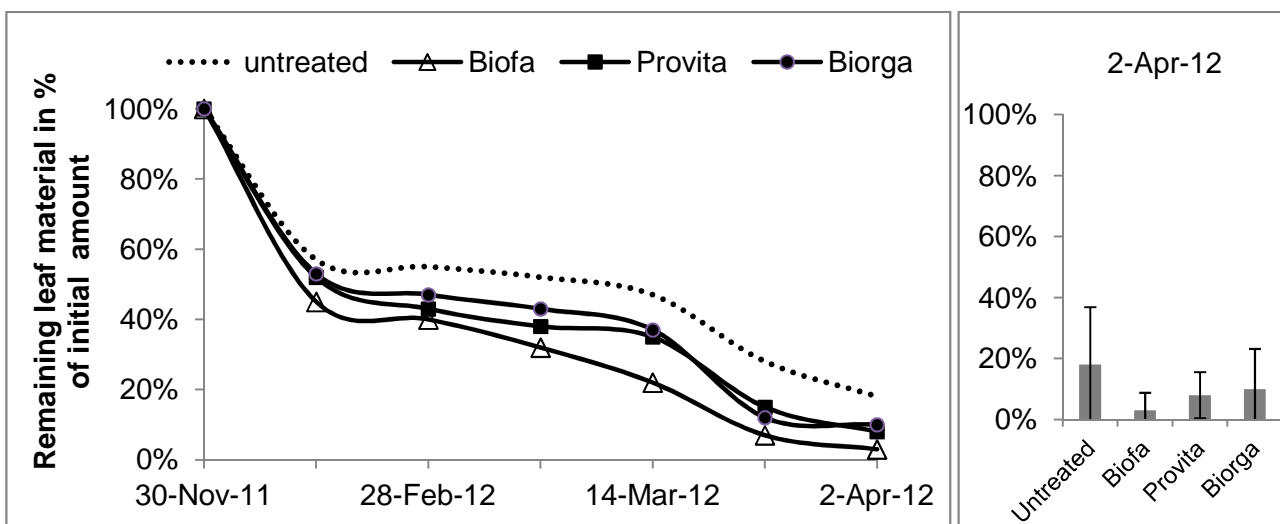


Fig. 2: a) Progression of leaf degradation after autumn treatment with three different beetroot Vinasse products compared with an untreated control in 2011/12. b) Final amount of leaf material (%) and standard deviation in the several treatments

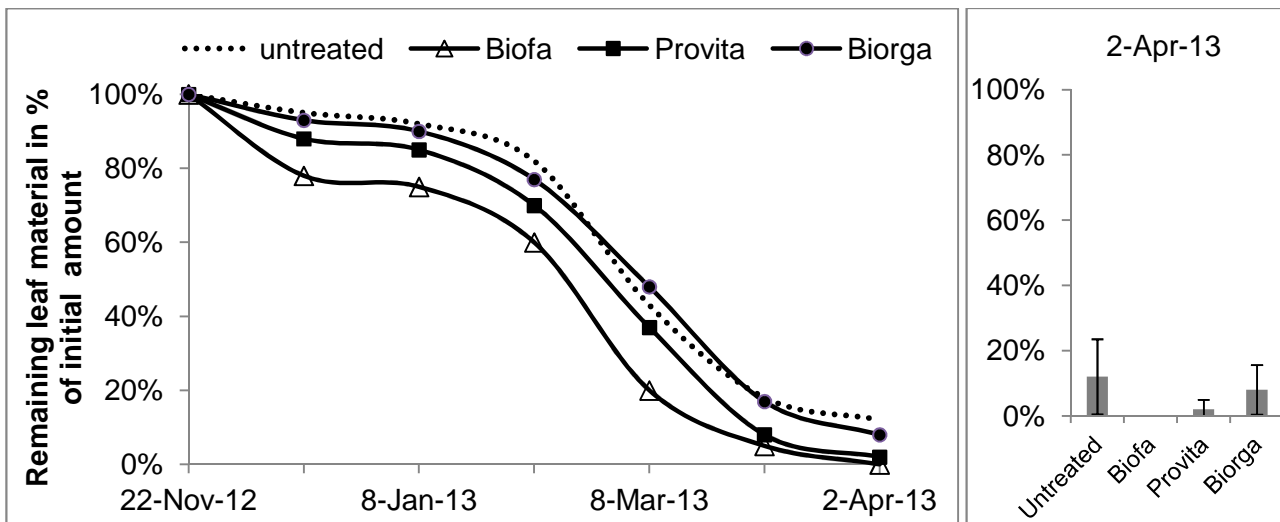


Fig. 3: a) Progression of leaf degradation after autumn treatment with three different beetroot Vinasse products compared with an untreated control in 2012/13. b) Final amount of leaf material (%) and standard deviation in the several treatments

### Effect on ascospore formation

Except first sampling date in 2012 (April 8) the amount of ascospores in all Vinasse treatments was considerably lower than in the untreated control at every sampling date. In 2011 the leaves of the untreated control discharged around 640.000 ascospores per gram leaf matter at the first sampling date in April. The corresponding number for the Vinasse-treated leaves was 283.000 (Biofa), 260.000 (Provita) and 265.000 (Biorga), i.e. the spore potential was reduced 56 to 60 %. At the second date in May 2011, the Vinasse-treated leaves discharged 72 % (Biofa), 52 % (Provita) respectively 52 % (Biorga) less of ascospores than the untreated leaves (see fig. 4).

At the first sampling date in 2012 there was no difference between the amount of ascospores in the three Vinasse treatments and the untreated control. However, the discharged amount of ascospores in the Vinasse treatments was reduced between 32 – 44 % compared with the untreated control at the second sampling date in May.

In 2013 altogether the highest reduction rates were measured in the Vinasse treatments. In April 2013 quantified number of ascospores in the Vinasse treatments was 75 % (Biofa), 56 % (Provita) respectively 85 % (Biorga) lower than in the untreated control. In May 2013 still reduction rates between 46 – 56 % were measured in the Vinasse treatments. Instead of 526.000 spores per gram leaf matter in the untreated control only 229.000 (Biofa), 258.000 (Provita) and 284.000 (Biorga) spores were counted in the several Vinasse treatments.

In other publications the level of ascospores is presented in ascospores/ml. Measurement unit “ascospores per gram leaf litter” must be divided by 50 to receive this value.

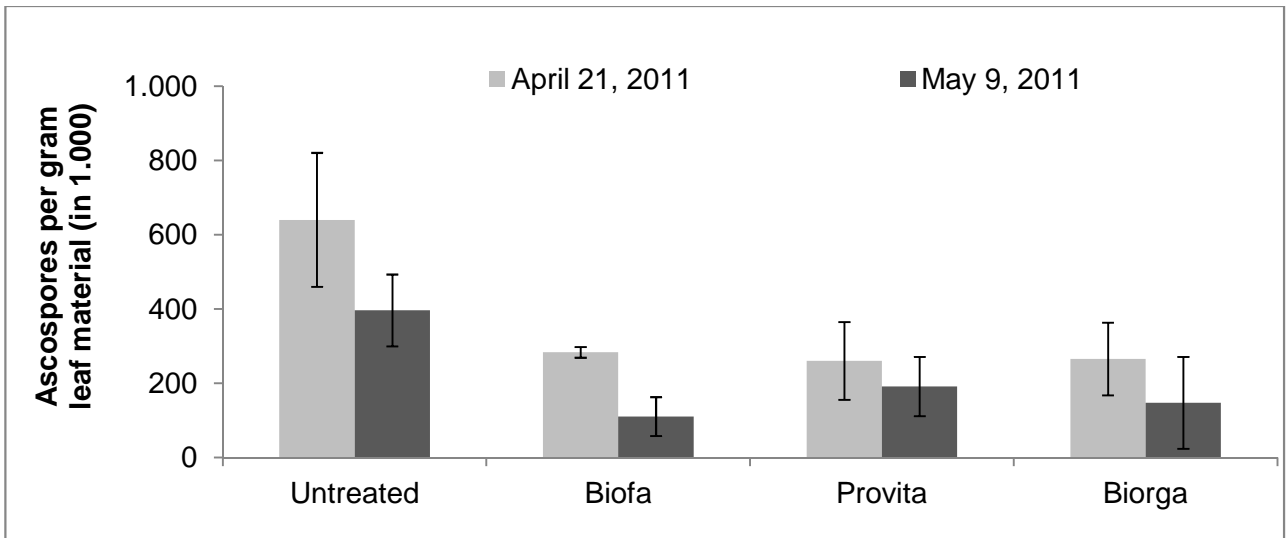


Fig. 4: Mean amount of discharged ascospores in water bath analysis at two sampling dates in 2011 (n=3 replications per treatment and date). Error bars represent standard deviation.

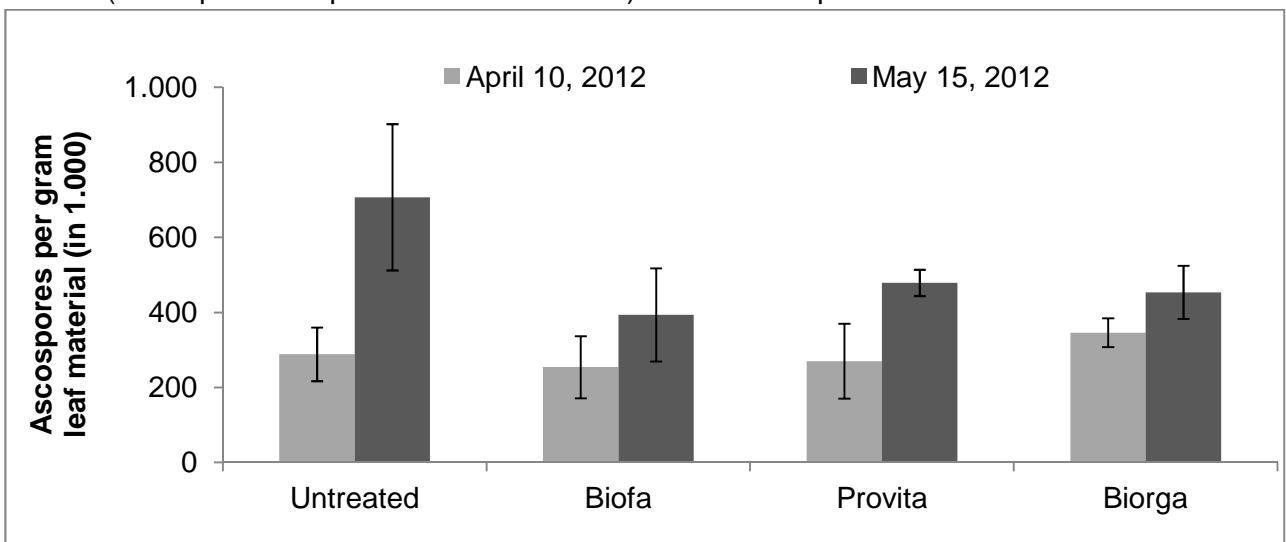


Fig. 5: Mean amount of discharged ascospores in water bath analysis at two sampling dates in 2012 (n=3 replications per treatment and date). Error bars represent standard deviation.

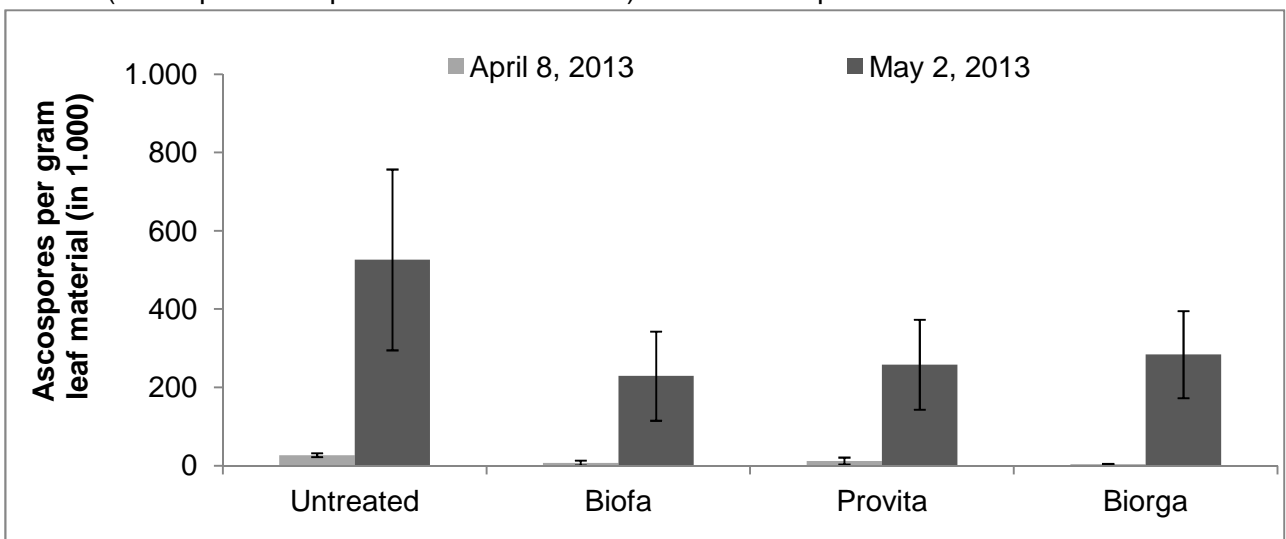


Fig. 6: Mean amount of discharged ascospores in water bath analysis at two sampling dates in 2013 (n=3 replications per treatment and date). Error bars represent standard deviation.

### Effect on ascospore formation – trail 2:

Except second sampling date in 2012 (May), Vinasse treatments generally lead to considerable reduction of spores. In 2011, when spores were counted only in May, 168.000 spores on leaves of the untreated control but only 42.000 spores on the Vinasse treated leaves were counted. Thereby a 75 % reduction of spores was given in the Vinasse treatment. In April 2012 a spore reduction of 27 % compared to the untreated control was measured in the Vinasse treatment. In 2013 the number of counted spores was 81 % (April) respectively 67 % (May) lower than in the untreated control.

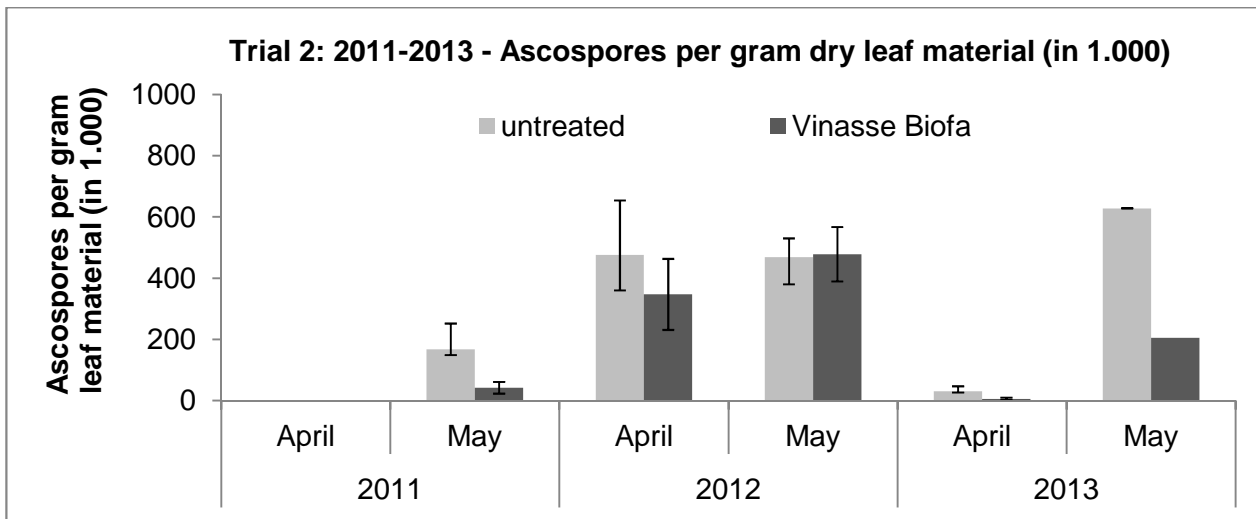


Fig. 6: Mean amount of discharged ascospores in water bath analysis at two sampling dates in 2011-2013 in trial 2 (n=3 replications per treatment and date). Error bars represent standard deviation.

### **Discussion**

Within three years the Vinasse product “Provita” accelerated the leaf decomposition process considerably compared to the untreated control. For the products “Biorga” and “Biofa” this could only be observed in two years. Based on the results of 2011, it can be supposed that the positive influence on leaf decomposition is obvious particularly in years with generally poor conditions for decomposition. With the reduction of leaf material in the orchard, the reduction of apple scab inoculum can already be achieved. However, in the majority of cases effects was not significant because of high standard deviation. Within the three years of the trial Vinasse products additionally had an influence also on ascospore maturation. The number of spores produced on the Vinasse treated leaves was generally lower than in the untreated leaves. A value of 250.000 ascospores per gram leaf material generally represents a very high infection risk. Indeed, the amount of ascospores on the remaining leaves was reduced considerably in the vinasse treatments compared to the untreated control, but still enough spores remained to cause a high respectively very high infection risk. However, this additional fact confirms that Vinasse products seem to have clear influence on apple scab inoculum. However further investigations are necessary to transfer this results into practice. It is possible, that the premature picking of the leaves changes conditions for spore formation. It is also possible that Vinasse is more durable on leaves in the depots than directly on the trees because of different rainwash effect. In this case, impact duration of the Vinasse products is longer in the mesh cages. At least, the hypothesis that the application of Vinasse has a negative influence on the ascospore potential of the remaining leaves could not be confirmed with this trial.

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