Efficacy of alternative substances to control apple scab by leaf litter treatment

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Abstract

Currently, a frequently used and effective strategy to control apple scab in organic fruit growing is the application of copper in combination with sulphur. With regard to the intended EU-wide prohibition of copper as a plant protectant, it is thus of the utmost importance to find comparable alternatives. This study therefore focuses on the decrease of the infection threat during spring by the reduction of sporulation from the leaf litter. This reduction is to be achieved by treatments of the leaf litter with substances, that potentially have a direct fungicidal effect on the apple scab pathogen itself or enhance microbial competition, and, secondly, show an indirect effect by increasing the attractiveness of the treated apple leaves for earthworms and consequently their decomposition.

In addition to several plant extracts, two yeast extracts, a soya-containing medium and different individual components were tested. First results show, that casein-peptone, TSB (Tryptic Soy Broth) and casamino acids can reduce the ascospore potential by more than 90 % compared to the untreated control. Furthermore, the first experiments indicate, that one of the yeast extracts leads to an accelerated decomposition of the leaves by earthworms.

Keywords: *Venturia inaequalis*, apple scab, leaf litter, ascospore potential, organic fruit production

Introduction

Apple scab (*Venturia inaequalis*), which is a serious problem in apple growing regions worldwide mainly overwinters on fallen leaves. During rain events in spring, pseudothecia release ascospores, which constitute the principal source of primary inoculum (MacHardy *et al.* 2001; MacHardy 1996). Aim of this study is a reduction of the ascospore potential in spring without removal of the organic matter from the orchard. This especially offers a solution for smaller organic fruit growers to reduce ascospore dose of fallen leaves without removing the leaf litter from the orchard as it is done with vacuum foliage collectors. Several media and plant extracts were tested considering a direct fungicidal effect on the apple scab pathogen and an acceleration of the leaf decomposition by attraction of earthworms. Germination tests of conidia were made prior to the selection of plant extracts and media to estimate their potential fungicidal effect. The experimental design of the presented work is based on the previous study of Kollar and Pfeiffer (2003) and several results of former years (Pfeiffer, 2010; Pfeiffer *et al.*, 2004; LVWO Weinsberg, 2006; LVWO Weinsberg, 2009). These investigations revealed that yeast extract seems to have a beneficial effect on the palatability of apple leaves for earthworms.

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Material and Methods

In November 2010 dried leaves that were heavily infected with apple scab were exposed in the open field in two different ways.

In the orchard of Weinsberg, the individual leaf depots (200g/0,5m²) were placed directly onto the ground and covered with wire meshes. From January until March 2011 (January 17th 2011, February 1st 2011, February 23th 2011, March 11th 2011 and April 25th 2011) different plant extracts, nutrition media and yeast extracts were applied to the individual leaf litter depots (each 150 ml, corresponds to 300l/ha) (table 1). Each treatment was replicated three times.

To determine a potential fungicidal effect of the applicated media, ascospore dose was measured with the water bath method published by Kollar (2000). Therefore, samples were taken between March 3rd 2011 and June 6th 2011 in regular intervals of 4-12 days.

Leaf litter was in direct contact to the soil to determine the palatability of the treated apple leaves for earthworms. Between January 11th 2011 and June 21st 2011 a visual rating to record degradation of the leaf litter and activity of earthworms was done every 6-26 days, according to weather and soil conditions (% of amount at the beginning of the experiment).

Table 5: Extracts, media, concentrations and number of treatments on the trials of Dossenheim and Weinsberg 2010/2011.

agent	Weinsberg	Dossenheim
Water control		4x
Untreated control	x	
Tryptic soy broth 9%	5x	4x
Tryptic soy broth 9%,		4x
Tryptic soy broth ethanol extract 80 %		4x
Tryptic soy broth ethanol precipitate 80 %		4x
Tryptic soy broth 9%, heat treatment		4x
Yeast extract *Foodgreen* 1,1 %	3x, 5x	
Yeast extract *Leiber* 1,1 %	3x, 5x	4x
Yeast extract *Leiber* ethanol extract 80 %		4x
Yeast extract *Leiber* ethanol precipitate 80 %		4x
Yeast extract *Leiber* heat treatment		4x
Yucca saponin, 1,5 % (vol %)	5x	4x
Primrose root extract 6%*	5x	4x
Saponaria officinalis 10% (6% vol)*		4x
Quillaja officinalis (bark)10% (6% vol)*		4x
TSForte 1 %		4x
Palmarosa 0,1% + TSForte 1%		4x
Peptone casein 6 %		4x
Peptone Soy 6 %		4x

* water extract of 10 g plant material/100 ml water, diluted to 6 % aqueous solution

In Dossenheim contact of the leaves to the ground was avoided by putting them into sowing-boxes (each filled with 70 g) and covering the soil with saran tissue. Treatment of the leaves was done on January 11th 2011, February, 16th 2011, February 27th 2011 and March 7th 2011. In total 18 different treatments were tested with one replication each. Samples to record ascospore potential were taken between March and June 2011 every

week. To determine the amount of microorganisms via CFU, a sample (each 1g) of every leaf depot was taken at the beginning of march. Leaf samples were crushed in liquid nitrogen and shaken in 99 ml distilled water for 15 min. A series of dilution of this solution was plated on TSA agar enriched with cyclohexemid (0,4 µg ml-1) and incubated for three days at 20 °C. The number of CFUs was calculated according to the formula *CFU/ml= number of colonies*solution*10*.

Intention of this work was to test a broad range of substances, which potentially reduce infection pressure during spring. According to this the experimental design constitutes a temptative test with up to 18 variants and n ≤ 3 and statistical assessment is not provided.

Results

Weinsberg: A considerable reduction of the leaf litter by earthworms could be detected in February. At this date, more than 50 % of the leaf litter treated with *Leiber* yeast extract was already degraded, while all other variants showed a slower reduction. This tendency, a promoting effect of *Leiber* yeast extract, could be observed over the period of the whole experiment. With the end of March, an accelerated leaf decomposition was also visible for the TSB variant. In the mid of April more than 95 % of the leaf litter treated with *Leiber* yeast extract or TSB were decomposed. The value of all other variants was between 15 and 20 %. In general, the ascospore potential was very low during the experimental period of spring 2011. Compared to the control, all of the media tested in Weinsberg showed a reduction of the ascospore potential. Due to the fast leaf decomposition of *Leiber* yeast extract and TSB, the ascospore potential of these variants could only be determined until mid of April. The most effective media were *Foodgreen* (3x) and Yucca saponin with a reduction rate of about 50 %. *Foodgreen* (5x) and Primroot showed a lower efficacy with a ascospore potential of 70 and 85 % compared to control, respectively.

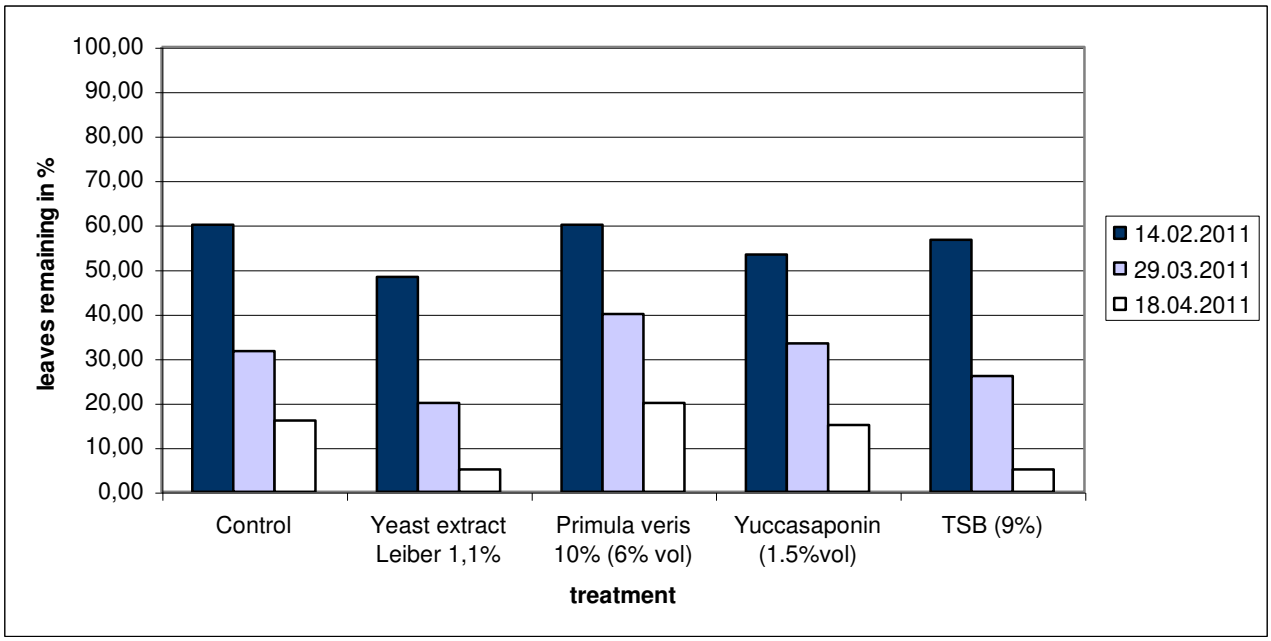

Figure 6: Leaf decomposition trial in Weinsberg 2010/2011. Untreated control in comparison with yeast extract *Leiber*, *Primula versis*, Yuccasaponine and TSB. Until 14.02: 2 treatments, 29.03 and 18.04: 5 treatments.

Dossenheim: Compared to Weinsberg the ascospore dose in Dossenheim was very high. The most effective reduction of discharged ascospores was observed for Peptone casein, TSB (9%) and Casamino acids with an ascospore potential of 2, 4 and 5 % compared to the control. By treatment with *Saponaria officinalis* and Yuccasaponin discharge of ascospores was lowered to 60-65 %. *Quillaja officinalis*, yeast extract *Leiber* and extract of *Primula veris* were the less effective variants, with an ascospore potential of more than 80 % compared to control.

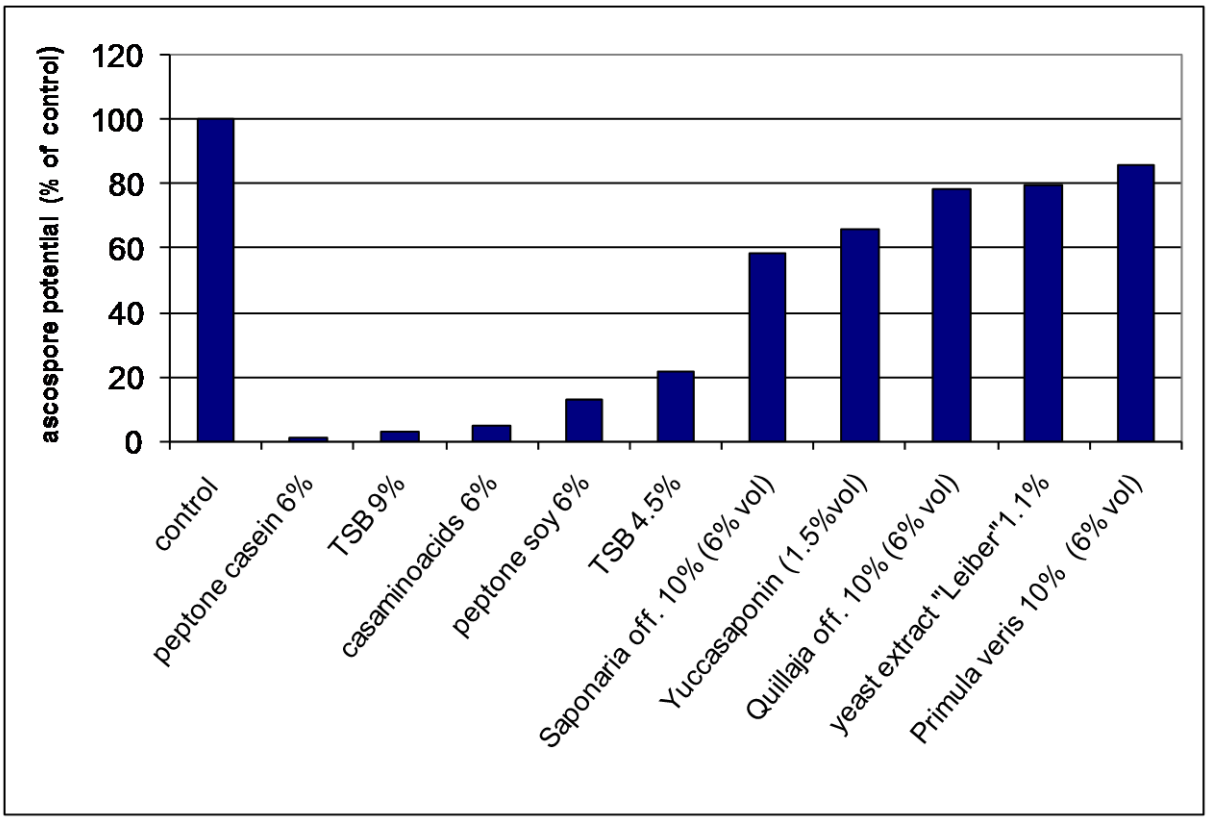

Figure 7: Cumulated ascospore potential (in % of the control) in Dossenheim up to June 2011.

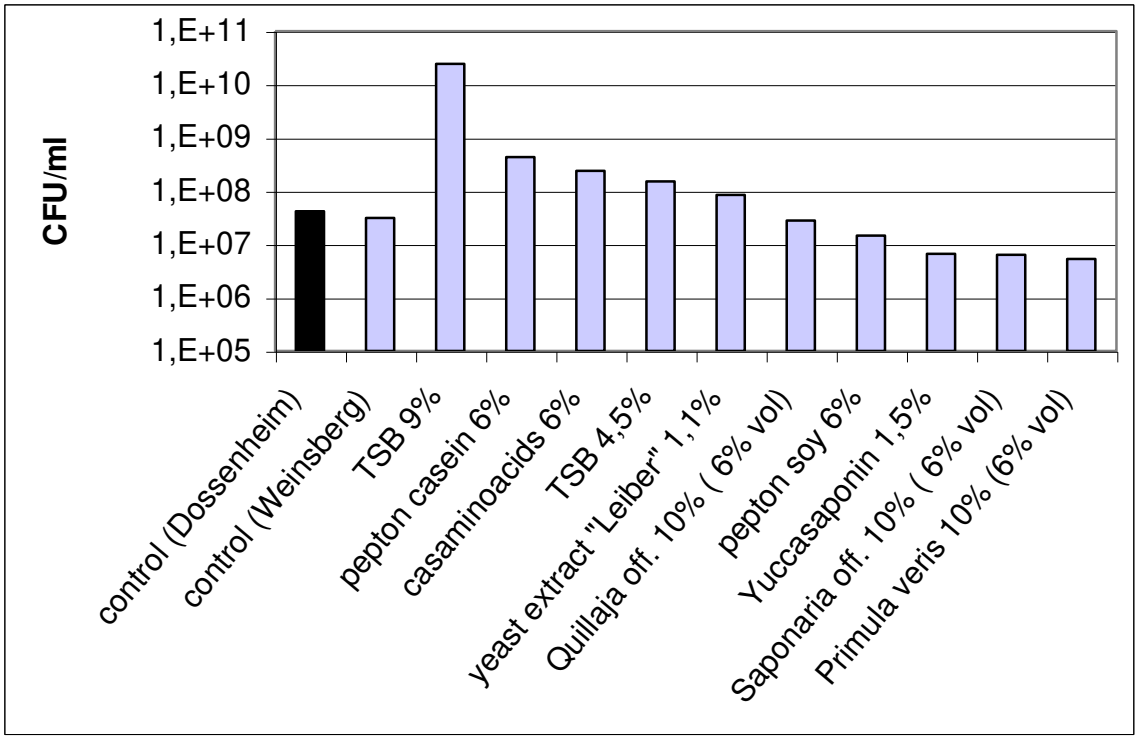

Figure 8: Microbial colonization of leaves depending on treatment. Results for the trial in Dossenheim supplemented by control value of Weinsberg.

Evaluation of the CFUs showed, that the number of bacterial colonies was significantly increased for TSB 9%, peptone casein, casamino acids and TSB (4,5 %) compared to control. The saponin containing plant extracts (Yucca, Saponaria, Primula) as well as the yeast extract did not have any effect on the colonisation of the apple leaves with bacteria.

Discussion

The initial results have shown, that peptone casein, casamino acids and TSB are promising substances to reduce the ascospore potential of fallen leafs. The trial in Weinsberg additionally showed an accelerated leaf decomposition by treatment of the leaves with TSB or *Leiber* yeast extract. Because of this, in the mid and end of April decomposition of the leaf depots with contact to the ground was proceeded so far, that no more leaves were present when ascospore potential increased clearly at the end of April. The results for *Leiber* yeast extract confirm with the research work of the former years, where activity of earthworms was enhanced as well. The results of ascospore discharge align with the microbiological examination, where the variants with the most effective reduction of the ascospore potential had the highest number of bacterial colonies. In the following period of the project, additional substances are to be tested for their direct or indirect effect on the apple scab pathogen. Furthermore the results of the most promising media of the research work 2010/2011 have to be confirmed. Also a combination of media with fungicide effects and those which promote leaf decomposition will be done.

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