Yeast extract applications to reduce the primary ascospore inoculum of *Venturia inaequalis*

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Abstract

Ascospores of Venturia inaequalis*, released from pseudothecia on overwintered infected apple leaves, are the primary inoculum of the pathogen. In this study, a new sanitation strategy to reduce the ascospore inoculum was tested under orchard conditions during four overwintering periods (2011-2015). Apple scab infected leaves were collected at the beginning of leaf fall. Leaves were exposed in plastic trays (JKI Dossenheim) or on the soil (LVWO Weinsberg) and protected by a wire mesh. The leaf litter depots were treated up to four times in late autumn and winter with yeast extract preparations. In all four years the potential ascospore inoculum was reduced. Fourfold 30 % or 60 % yeast extract treatments were the most effective and reduced the ascospore discharge to 99-100 %. Twofold application of a 30 % yeast extract revealed a similar efficacy. Analyses of biological oxygen demand in the leaf litter depots indicated an increased microbiological activity. Additionally, the treatments enhanced the attraction of leaf litter for earthworms, which led to an accelerated ingestion rate. Leaves treated three times with a 6 % yeast extract or two times with a 10 % or 20 % yeast extract were removed completely until the beginning of ascospore maturity in 2013-2015. By comparison, up to 28 % of the untreated leaves were not ingested. The results demonstrated that the performed leaf litter treatments with yeast extracts reduced the primary ascosporic inoculum almost completely, and thus might contribute to a better management of the disease. Obviously, apple scab control was achieved by enhanced microbiological and earthworm activity.*

Keywords: apple scab, sanitation, biological control, sustainable fruit production, copper replacement

Introduction

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, is the economically most important apple disease worldwide (MacHardy, 1996). Scab management in organic fruit growing may require 30 treatments per season depending on weather conditions and disease pressure. In spring, infection is initiated mainly by ascospores released from pseudothecia on overwintered infected apple leaves (Hirst & Stedman, 1962a, b). In integrated fruit production the application of urea 5 % at the beginning of leaf fall is the standard method to reduce the overwintering inoculum of *V. inaequalis*. The application of urea accelerated leaf decomposition significantly (Jones & Aldwinckle, 1990), apparently due to quantitative shifts in microbial populations (Crosse *et al*., 1968; Burchill *et al*., 1965). Furthermore, the softening and degradation of leaf litter resulted in increased earthworm activity (Helling & Larink, 1998; Wright, 1972). However, in European fruit growing areas, urea is not registered for organic production. The aim of this study was to develop an alternative sanitation treatment with yeast extract preparations to suppress pseudothecial development and/or limit the discharge of acsospores of *V. inaequalis* by promoting leaf inhabiting microorganisms. The treatments should also stimulate earthworm activity to accelerate the leaf decay.

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Material and Methods

Preparation of nutrient solutions.

Urea (AppliChem GmbH, Darmstadt) and yeast extract (Bouillon N, LS, LEIBER GmbH, Bramsche), were dissolved in ultrapure water in the denoted concentrations (Table 1+ 2). Yeast extract 60 % was prepared with 600 g yeast extract (Fermentation S, LEIBER GmbH, Bramsche) dissolved in 1 l ultrapure water. The suspension was centrifuged for 30 min at 15 300 x g (Beckman JA-14, Beckman Coulter) at 21 °C. The supernatant was removed, the pellet resuspended in 1 l ultrapure water and centrifuged again. The combined supernatants were used as yeast extract 60 %. Yeast extract 20 % and 30 % were prepared following the same procedure with the use of 200 g or 300 g yeast extract.

Table 1: Application schedule for apple scab infected leaf litter treatments in Dossenheim.

Year	Treatment	Date
	untreated control	
2011/12	yeast extract 6 %	21.11.11/ 19.12.11/ 16.01.12/ 16.02.12
2012/13	yeast extract 60 %	21.11.12/ 19.12.12/ 15.01.13/ 13.02.13
2013/14	urea 5 %	09.12.13/ 07.01.14/ 28.01.14/ 24.02.14
	yeast extract 30 %	
	yeast extract 30 % au	09.12.13/ 07.01.14
	yeast extract 30 % wi	28.01.14/ 24.02.14
2014/15	urea 5 %	03.12.14/ 13.01.15
	yeast extract 30 %	
	yeast extract 20 %a	
	yeast extract 20 % b	03.12.14/ 23.12.14
	yeast extract 20 % c	13.01.15/ 11.02.15

Table 2: Application schedule for apple scab infected leaf litter treatments in Heuchlingen.

Year	Treatment	Date
	untreated control	
2011/12	yeast extract 6 %	25.11.11/ 16.01.12/ 05.03.12/ 22.03.12
2012/13	yeast extract 6 %	23.11.12/ 08.01.13/ 13.02.13
2013/14	yeast extract 6 %	04.12.13/ 09.01.14
	yeast extract 30 %	
2014/15	yeast extract 6 %	15.12.14/ 23.12.14
	yeast extract 10 %	
	yeast extract 20 %	

Leaf litter treatments

At the beginning of leaf fall in 2011 (October 27), 2012 (November 5), 2013 (November 12), and 2014 (October 30), infected apple leaves from different cultivars were collected in commercial orchards in Germany (Würzburg, Heilbronn). The experimental studies were conducted in Heuchlingen at the State Research Institute for Viticulture & Pomiculture (LVWO) in Weinsberg and the Julius Kühn-Institute (JKI) in Dossenheim (Germany). In the orchards, leaves were air-dried and sheltered from rain under natural conditions until they were exposed in plastic trays (55 x 30 x 7 cm, 80 g dry weight (DW) leaves) covered with a wire mesh in Dossenheim. The trays were perforated with 3 mm holes, so that the rainwater could drain to the ground. Set up for earthworm experiments at the LVWO consisted of evenly distributed leaves on the soil protected by a wooden frame with a wire mesh (85 cm x 59 cm, 200-250 g DW leaves). Each assay was repeated at least three times on different locations in the orchard. The soil has been prepared before experimental setup with a Ladurner machine (mechanical weed control) at the same time as in the organic orchard of LVWO.

The leaf litter depots were treated up to four times in late autumn and winter with yeast extract preparations listed in Table 1+2. Urea 5 % treatments were used as a standard to evaluate the effectiveness of the yeast extract preparations. In Dossenheim all solutions were sprayed on top of the leaves until beginning of runoff (~ 100 ml). The first treatments were performed, after approximately 90 % of the annual leaf fall was completed. At LVWO 150 ml / wire mesh were sprayed, which corresponded to 300 l water/ha.

Potential ascospore release and earthworm activity

During the period of ascospore release, the potential ascospore discharge was determined weekly according to the water bath method (Kollar, 1998). The efficacy of leaf litter treatments on the cumulative ascospore potential of *V. inaequalis* compared to untreated control was calculated. The percentage of leaf decay due to earthworm activity was assessed visually two or three times a month.

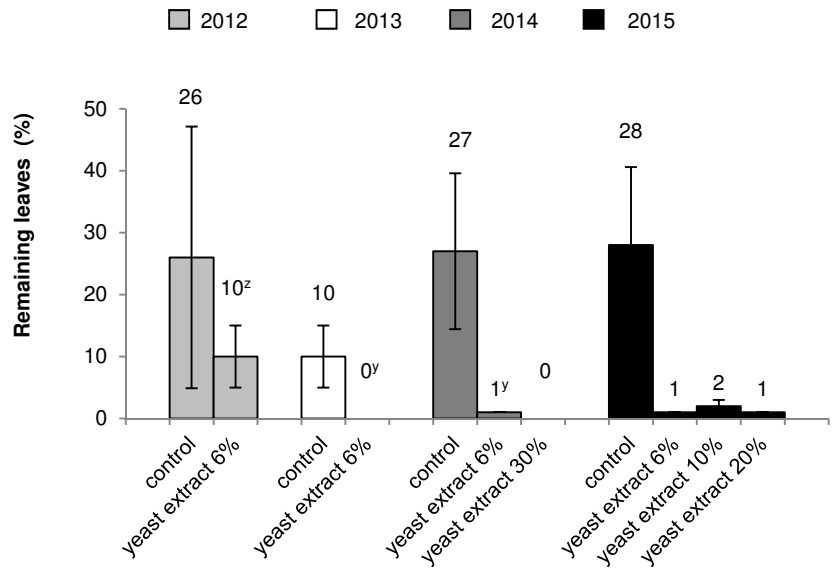
Detection of aerobic metabolism

In the season 2014/2015, leaf litter depots were sampled every four weeks, respectively. 1 g DW leaf samples were incubated with 10 ml ultrapure water in OXI-TOP devices (WTW, Weilheim) to measure the biological oxygen demand (BOD). OXI-TOP devices consist of a glass bottle (510 ml) with an insert underneath the top carrying two NaOH pellets to bind the released carbon dioxide. The glass bottles were closed with measuring heads including piezo-resistive electronic pressure sensors. Oxygen uptake was detected as reduced pressure every 24 h at a constant temperature of 20 °C in course of 5 days.

Results

Leaf litter decay due to earthworm activity (LVWO Weinsberg)

In the seasons 2012-2015, leaf litter treatments with yeast extracts enhanced the leaf decay due to earthworm activity (Figure 1, 2). Threefold application of a 6 % yeast extract or twofold application of a 10 % or 20 % yeast extract resulted in an almost complete reduction of leaf litter.



[Y]threefold application, [Z]fourfold application

Figure 1: Remaining leaves in leaf litter depots at the beginning of ascospore maturation in 2012-2015 in Weinsberg. Leaves were treated two times with yeast extract preparations between November and February. Error bars = ± standard error of the mean.

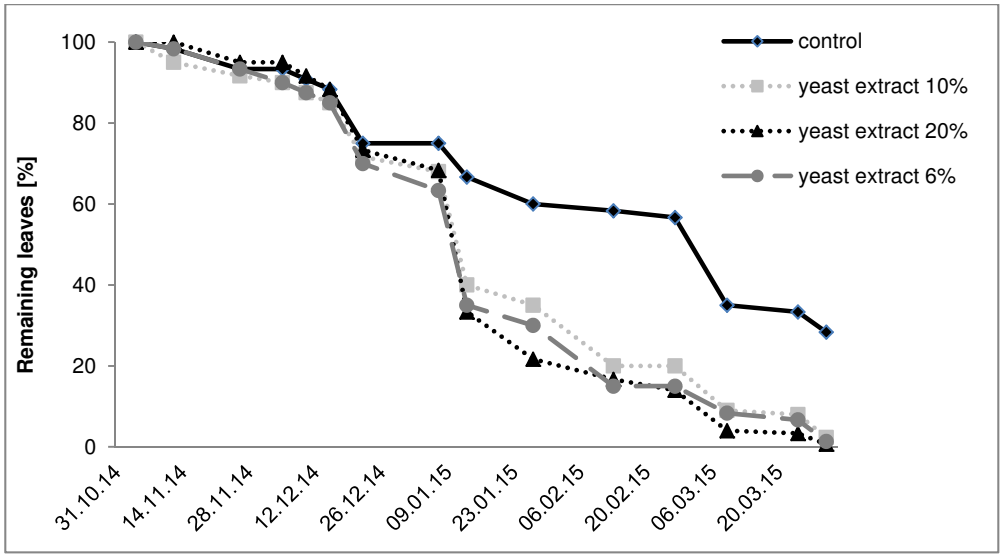


Figure 2: Decomposition rate of apple scab infected leaf litter depots due to earthworm activity. Leaf litter treatments were performed on 15 December 2014 and 23 December 2014, in Weinsberg.

Ascospore potential in leaf litter depots (JKI Dossenheim)

In the years 2012-2014, a fourfold treatment with urea 5 % reduced the ascospore discharge nearly completely (Figure 3). The effect of yeast extract preparations was correlated to the applied concentration. A 6 % yeast extract reduced the ascospore inoculum to 78 %. Higher yeast extract concentrations of 30 and 60 % reduced the ascospore amount nearly completely.

In 2014 (Figure 4), a twofold treatment with a 30 % yeast extract was sufficient to reduce the ascospore inoculum of *V. inaequalis*. The efficacy of the treatments did not depend on the date of application. In 2015, the 30 % yeast extract showed a lower efficacy. The effect of a 20 % yeast extract depends on the timing of application. Treatments in winter (yeast extract 20 % c) (13.01.15; 11.02.15) were the most effective and reduced the ascospore

discharge to 81 %. An application early after leaf fall (03.12.14) and a second one in winter (13.01.15) reduced the ascospore inoculum to 48 % (yeast extract 20 % a). Applications in late autumn (yeast extract 20 % b) (03.12.15; 23.12.15) did not result in a reduction of ascospore potential.

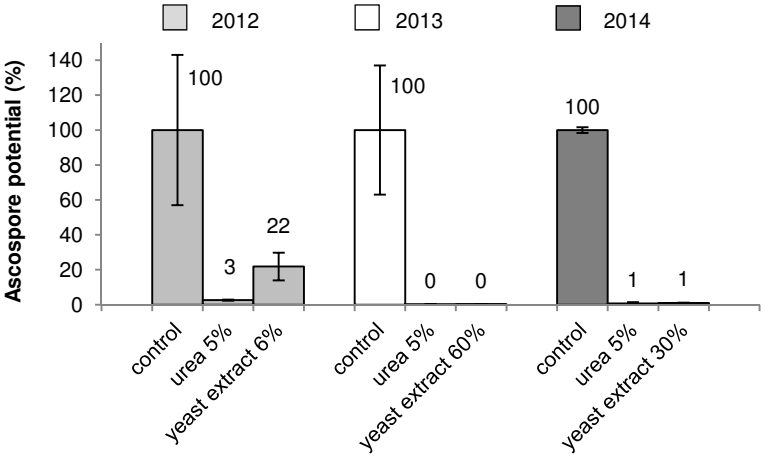


Figure 3: Cumulative ascospore potential in fourfold treated leaf litter depots in 2012-2014. Error bars = ± standard error of the mean.

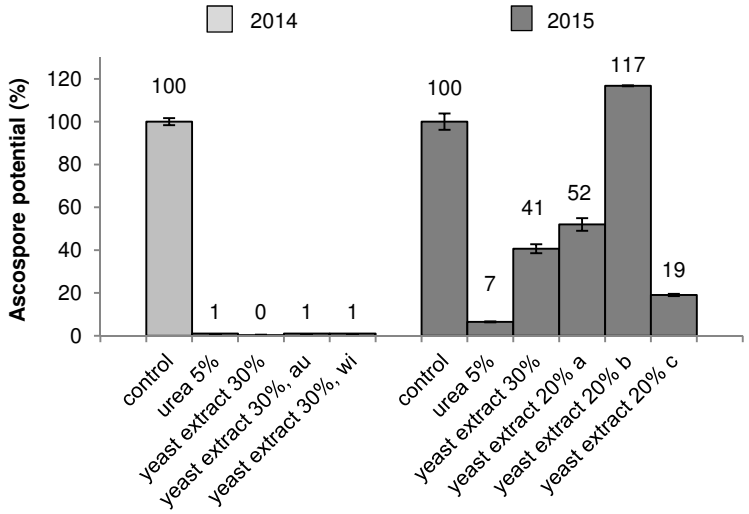


Figure 4: Cumulative ascospore potential in twofold treated leaf litter depots in 2014-2015. Error bars = ± standard error of the mean.

Biological oxygen demand (BOD)

Leaf litter treatments with yeast extracts promoted the microbial activity compared to untreated control (Figure 5). The highest BOD_5 values (50 mg/ml) were measured in the 30 % yeast extract depot, approximately one week after the second leaf litter treatment. In the 20 % yeast extract depot, highest BOD_5 (41 mg/ml) was observed one month after the second leaf litter treatment.

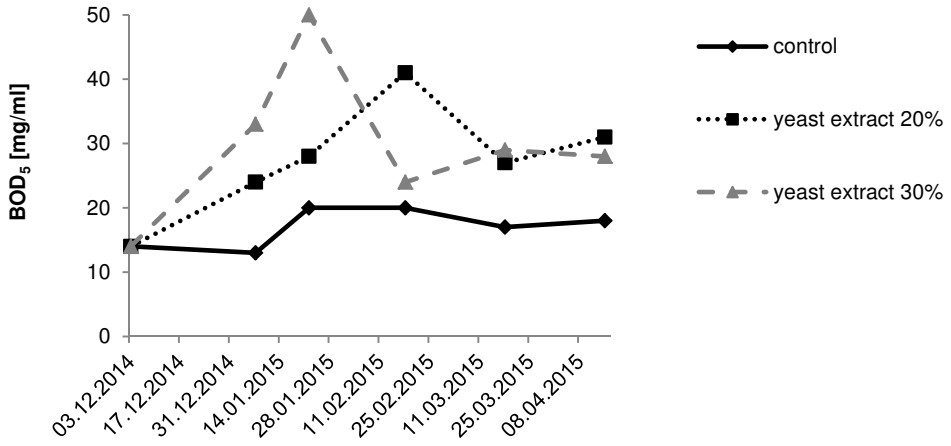


Figure 5: Biological oxygen demand (BOD_5) in leaf litter depots treated two times (3 December 2014; 13 January 2015) with a 20 % or 30 % yeast extract.

Discussion

Similarly to earlier findings (Rüdiger *et al.*, 2012), sanitation treatments with yeast extract preparations in late autumn and winter reduced the ascospore inoculum of *V. inaequalis* significantly. A 6 % yeast extract preparation revealed a low efficacy compared to the more concentrated 30 and 60 % yeast extracts. Twofold application of a 30 % yeast extract in 2013/14 showed the same effect as a fourfold application, respectively. Therefore, the twofold application seems to achieve sufficient impairment of pseudothecia. The efficacy of the treatments did not depend on the date of application. Applications in late autumn resulted in a similar reduction of ascospore inoculum as applications in winter. However in 2015, the effect of a 20 % yeast extract on the ascospore potential differed significantly depending on the date of application. Furthermore, the reduction of ascospore potential was much lower as compared to the effects in 2014. A reason for the lower efficacy might be the lower yeast extract concentration. In addition, the amount of precipitation after the second leaf litter treatment until ascospore maturation differed significantly in both years. In 2013/14 the amount of precipitation in the autumn treated depot was 79 mm and in the winter treated depot 55 mm. In 2014/15 the amount of precipitation was much higher, 181 mm in the autumn treated depot and 89 mm in the winter treated depot. Rainfalls may have washed off the yeast extracts followed by a reduced effect on the development of microorganisms.

Leaf litter treatments with yeast extracts led to an increase of microbiological activity. A 30 % yeast extract, which resulted in the highest BOD_5 values, was also the most effective in ascospore reduction. The increased microbiological activity in the leaf litter depots may have caused a stronger competition for space and nutrients between *V. inaequalis* and other microorganisms. In addition, bacteria and fungi may act as antagonists caused by mycoparasitism (Benyagoub *et al.*, 1998) or antibiosis (Heye & Andrews, 1983; Hossain *et al.*, 2009). The microbial activity in the yeast extract depots was increased already three weeks after the first leaf litter treatment. It can be assumed that the initiation of fruiting bodys which occurs two or three weeks after leaf fall (Mac Hardy, 1996) might be disturbed.

The application of yeast extracts affected the feeding activity of earthworms and caused accelerated leaf decay. In 2013-2015, the threefold application of a 6 % yeast extract was so efficient that all leaves were incorporated in the soil before ascospore maturation.

Ultimately, nearly the entire ascospore potential could be reduced. Earthworms are known to be selective in their choice of apple leaf litter. The preference is significantly correlated with palatability (Satchell, 1967) and the state of leaf decomposition. The increased microbial activity in the present study promoted leaf degradation and therefore stimulated the attraction of leaf litter for earthworms.

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