Enhancement of degradation of fallen apple leaves

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***Abstract* – Leaves from organic apple trees were dipped with different organic materials and leaves were placed on the orchard floor in autumn. Leaf area and the amount of ascospores of *Venturia inaequalis* were measured in spring. The objective of this research was to find alternatives for urea that simulate the decomposition of apple leaves and reduces the ascospore production. In both years urea gave an increase of the leaf degradation and a significant reduction of the number of ascospores. The antagonist *Coniothyrium minitans* had no significant effect on the ascospore production in both years but decreased the leaf degradation. Beet pulp showed a significant reduction of the number of spores but reduced the leaf degradation rate. Applying extra earthworms increased the degradation.** [1]

INTRODUCTION

Scab (*Venturia inaequalis*) is the most important disease on fruit in the Netherlands. The fungus infects both fruit and leaves resulting in a lower production of quality apples. The life cycle of *V. inaequalis* consist of a sexual and an asexual phase. During the sexual phase the fungus lives on infected necrotic leaves. On these leaves ascospores are formed in pseudothecia during early spring. These ascospores are the primary inoculum in spring and will infect the newly formed leaves and fruit. It has been demonstrated that a lower number of fungicide applications were needed to control scab in low inoculum orchards (MacHardy, 1996). There are several methods to reduce *V. inaequalis* inoculum in orchards. One of the most effective methods is an application of 5 % urea at leaf fall. Urea stimulates the decomposition of leaves and reduces the amount of ascospores (Carisse and Dewdney, 2002). However, urea is produced synthetically and is not approved in organic culture guidelines.

The aim of the REPCO-project is to replace copper by other methods. One of the methods is to find alternatives for urea that simulate the decomposition of apple leaves and reduces the ascospore production.

MATERIALS AND METHODS

Orchard and equipment

A field experiment was carried out in 2003 and 2004 in the organic orchard of PPO in Randwijk, The Netherlands. The experiment was done on Jonagold (*Malus* x *domestica* Borkh.) on M 27 rootstock and pruned as slender spindles. The trees were planted in a single row. The trees were planted in 1999 at a planting distance of 3 x 1.1 m.

Treatments

Leaves from untreated trees were picked and placed between iron nettings and dipped in different solutions in December 2003 and 2004. The treatments of the dipping experiment in 2003 were: 1) untreated, 2) urea (5%), 3) amino acids (Aminosol, 1%), 4) *Coniothyrium minitans*, (Contans 0.4%) applied in autumn, 5) (*C. minitans*, Contans 0.4%) + amino acids, (Aminosol 1%) applied in autumn, 6) (*C.minitans*, Contans 0.4%) + compost tea, (Compara 5%) applied in autumn and 7) *C minitans*, (Contans 0.4%) applied in spring. The treatments of the dipping experiment in 2004 were: 1) untreated, 2) urea (5%), 3) beet pulp, (Vinasse 600 l/ha), 4) (*C. minitans*, Contans 0.4%), 5) *C. minitans*, Contans 0.8%), 6) untreated leaves with extra earthworms. The nettings (0.5 x 0.5 m) containing the treated leaves were placed on the grass in the orchard at 4 December 2003 and at 2 December 2004. Extra earthworms were applied to the soil the day before the leaves were placed.

Measurements

The leaf area was measured at the beginning of the decomposition experiment. A grid from transparent plexiglass was placed over the iron netting to estimate the total leaf area in cm^2. This grid was divided in compartments of 1 cm^2 and the number of compartments covering leaf surfaces was counted. Leaf degradation was measured twice at 17 March and 13 April 2004 and at 21 March and 18 April 2005.

Ascospore extraction in water by bubbling air was used to quantify the potential ascospore production. Leaf remains of 2 x 50 leaves per replicate were collected, air-dried (20°C, 70% RH for 24 h) and weight was determined. Subsequently, a sub sample of maximum 7-17 g air-dried leaf remains of each sample were spread in a plastic tray on moist filter paper. Trays were covered by a plastic bag and leaf remains were incubated in these moist chambers for 21 days (20°C, 12 h light per day) to allow maturation (of a substantial fraction) of asci. After incubation, leaf remains were transferred into 1000 ml-plastic bottles containing 150-350 ml water depending on the amount of leaf remains. Air (250 l per h) was bubbled through the water resulting in heavy turbulence during 2 h. Thereafter, the resulting

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suspension was passed through a sieve (1 mm mesh) to remove leaf debris. Two sub-samples (8 ml) of the suspension were stored at −20°C. Ascospore concentration in the suspensions was determined microscopically using a haemocytometer. Ascospore production was expressed as production per 100 leaves (originally fixed on the orchard floor in autumn).

Leaf decomposition data were subjected to analysis of regression. T-probabilities were calculated for pair wise comparison of treatment means. The number of ascospores was log10 transformed before analysis. After transformation the data was subjected to analysis of variance (ANOVA). Significant F-tests ($P<0.05$) were followed by al Least Significance Difference (LSD)-test for pair wise comparison of treatment means using $LSD_{0.05}$ values.

Results and Discussion

Year 2003-2004

Leaves dipped in urea gave significantly higher leaf degradation measured on 17 March 2004 (Table 1). On 13 April the treatment with urea was no longer significant different from the untreated control. The treatments *C. minitans* applied in spring and *C. minitans* + amino acids applied in autumn had significant lower leaf degradation compared with the untreated on 13 April. In the case of the dipping experiment significant differences were found in the ascospore production (Table 1). Leaves treated with urea had a significantly lower ascospore production per 100 leaves. The antagonist *C. minitans* had no effect on the ascospore production.

Table 1. Effect of the different treatments on the leaf degradation and the number of ascospores per 100 leaves in 2003-2004.

Treatment	Leaf decomposition (%) 17 March		13 April		Number of ascospores	
Untreated 1	24.5	b	57.7	ab	3301400	b
Untreated 2	23.6	b	49.8	bc	3721900	b
Urea	40.6	a	61.7	a	363200	a
Amino acids	23.4	b	47.9	bcd	5444500	b
C. minitans autumn	20.0	b	44.9	bcd	6939200	b
C. minitans + Amino acids	22.2	b	36.9	cd	9665200	b
C. minitans + Compost tea	26.1	b	51.0	bc	5162000	b
C. minitans spring	18.0	b	33.0	d	2851300	b
F prob.	<.001		0.002		0.009	

Year 2004-2005

Leaves dipped in urea showed a significantly enhanced leaf decomposition compared to the control (assessment at 21st of March, Table 2). Adding extra earthworms increased the leaf degradation even more than urea. Dipping the leaves in *C. minitans* (0.8%) decreased the leaf degradation. On 18 April no significant effect was found between the control, urea and the treatment with extra earthworms. *C. minitans* showed a significantly lower leaf degradation both the concentrations and beet pulp (600 l/ha) gave the lowest leaf degradation. Significant differences were found in the ascospore production for different treatments leaves (Table 2). Urea caused a significantly lower ascospore production per 100 leaves. Also the treatment with beet pulp (600 l/ha) reduced the ascospore production. The antagonist *C. minitans* had no significant effect on the ascospore production at both concentrations.

Table 2. Effect of the different treatments on the leaf decomposition and the number of ascospores per 100 leaves in 2004-2005

Treatment	Leaf decomposition (%) 21 March		18 April		Number of ascospores	
Untreated	24.5	c	89.9	a	7873170	b
Urea	41.5	b	91.2	a	87291	a
Beet pulp	20.2	cd	46.2	c	223091	a
C. minitans 0.4%	18.4	cd	81.2	b	2821694	ab
C. minitans 0.8%	12.9	d	80.7	b	6587825	b
Extra earth-worms	68.8	a	92.0	a	7873170	b
F prob.	<.001		<.001		<.001	

The treatments '*C. minitans* + amino acids' and '*Coniothyrium minitans*' applied in spring caused a significantly lower decomposition compared with the untreated control. *C. minitans* might have a negative effect on the decomposition. On the other hand the treatment '*C. minitans* + compost tea' did not show an effect on the decomposition compared to the control. A lower decomposition rate of the leaves treated with *C. minitans* was also found in 2005.

Applying extra earthworms increased the decomposition with 44 % compared to the control. This shows the importance of the earthworms in reducing the amount of inoculum sources during winter and spring.

Conclusions

Applying extra earthworms increased the decomposition compared with the control. In 2005 beet pulp showed a significant reduction of the number of spores but also reduced the leaf degradation rate.

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