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Control of apple scab by curative applications of biocontrol agents

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Abstract

In organic apple growing protective applications with copper, sulphur or lime sulphur are used for apple scab control. Protective applications have to be repeated when new leaves unfold. The timing of protective sprays depends on the weather forecast. If forecasted infection conditions fail to appear, treatments were for nothing. With curative control agents available, the number of treatments could be reduced. In greenhouse trials we tested control agents for their protective and curative efficiency against apple scab after artificial inoculation of potted apple trees. Applications were done 2 hours before inoculation, 5 hours after inoculation on wet leaves, 5 hours after inoculation during simulated rainfall or 24 hours after inoculation on wet or dry leaves. The optimal time of application differed between the preparations tested. Vitisan and OmniProtect had their highest activity when sprayed curative 24 hours after inoculation. Combinations were found, which revealed a high efficiency against apple scab from 2h before to 24 hours after inoculation. In a field trial apple scab was effectively controlled by curative applications of OmniProtect.

Keywords: apple scab, *Venturia inaequalis*, curative treatment, Vitisan, OmniProtect

Introduction:

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is the most important apple disease, causing economic losses in many apple production areas. Its significance is indicated by the fact that up to 20 fungicide treatments are performed per season to control apple scab disease. With modern, integrated disease control strategies the number of treatments could be reduced to 4 to 7 applications, using curative fungicides after conditions for scab infections are fulfilled (Bühler and Gessler, 1994; Triloff, 1994; Triloff, 1999). Simulation models based on weather data reliably predict infection periods but the success of these control strategies strongly depends on the availability of efficient curative fungicides. In the integrated production several chemical fungicides have been used until *V. inaequalis* developed resistances against these active ingredients (Jones, 1981; Kunz et al., 1997; Michel and Ließ, 2006).

In organic apple growing protective sprays with copper, sulphur or lime sulphur are used for apple scab control. But protective sprays have to be repeated when new leaves unfold and the timing of protective sprays depends on the weather forecast. If forecasted infection conditions fail to appear, treatments were for nothing. To improve the timing of treatments, organic growers started to apply lime sulphur during the period the fungus needs to germinate on the leave surface. The duration of the germination period depends mainly on the temperature, lasts from 5 to 20 hours and applications have to be done during rainfall on wet leaves. With curative control agents, which could be applied after the fungus has established the primary stroma beneath the cuticle, protective treatments could be avoided and the time span for the application after the start of germination could be prolonged. Holb et al. demonstrated the usefulness of curative applications of lime sulphur (Holb et al., 2003), but showed also phytotoxic effects and it is of great interest to find other curative preparations for apple scab control. In greenhouse trials we tested control agents for their curative efficiency against apple scab after artificial inoculation of potted apple trees and confirmed the curative activity of OmniProtect in a field trial.

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Material and Methods
Greenhouse trials:

Test plants. Potted apple trees of the cultivar Jonagold, grafted on M 9 rootstocks, were kept in a greenhouse at minimum 16/21°C (night/day) and a 14 h light period. The trees were fertilized weekly.

Inoculum. In June 2005, naturally infected leaves with sporulating scab lesions were collected in an orchard in Konstanz (cultivar Jonagold), which had been never treated with chemical fungicides. Conidial suspensions were obtained by shaking the thawed leaves in tap water and used to inoculate test plants like described beneath. After 14 to 21 days of incubation in the greenhouse, leaves with sporulating scab lesions were collected and stored in plastic bags at -70°C.

Test preparations were provided by Biofa AG. 15g/l Lime sulphur, 2.5 g/l Netzschwefel Stulln (active ingredient: sulphur), 10 g/l Biofa Cocana (a.i.: coco soap), 5 g/l Vitisan (a.i.: potassium bicarbonate) or 5g/l OmniProtect (a.i.: potassium carbonate) were suspended in tap water and sprayed onto the test plants until runoff.

Efficacy test. Five to ten leaves with conidia of *V. inaequalis* were thawed and shaken in tap water to obtain conidial suspensions. The three youngest completely unfolded leaves of the apple shoots were spray inoculated with $1x10^5$ conidia/ml until runoff and subsequently incubated at 18°C and 100% relative humidity for 20 h in the dark. The plants were subsequently kept under greenhouse conditions as described above. Test preparations were applied with the appropriate concentration either protective (2 hours before inoculation), during germination (5 hours after inoculation on wet leaves or 5 hours after inoculation during simulated rainfall) or curative (24 hours after inoculation on wet or dry leaves). Rainfall was simulated in the greenhouse (fig. 1) with a spray nozzle placed 2 m above the plants and the sprayed water quantity was measured with a pluviometer. 5 to 10 l water /m^2 were applied in 15 min.

Figure 1: Efficiency test in the greenhouse. Left: Application of test preparations to apple shoots during the germination period under simulated rainfall. Right: Untreated apple leaf 20 days after inoculation. Approximately 60% of the leaf surface is covered with sporulating scab lesions.

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16 to 21 days after inoculation, the disease incidence (fig. 1) for each shoot was calculated as average of the proportion of diseased leaf area of the three youngest inoculated leaves (Kunz et al., 1997). The average of the diseased leave area of 4 to 6 shoots per treatment was calculated.

The efficiency of the test preparation was calculated for each experiment by comparing the disease incidence with the untreated control according to Abbott (Abbott, 1925). The experiments were done at least twice.

Field trial. In an untreated orchard in Konstanz, planted in 1996, a field trial with five replications was conducted on the varieties Jonagold and Rewena in 2007 with single tree plots. OmniProtect (5g/l) was applied together with the surfactant TegoBetain CKD (1g/l) (Goldschmidt GmbH, Essen) 24h to 48 h after start of rainfall (approx. 1,000l/ha). Ten applications were done (April-04; May-06 (petal fall), 08, 15, 18, 30; June-02, 16, 22, 28) between green tip and the end of the shoot expansion. Treatments were applied to wet leaves except April-04, June 16 and June 28). On July 13th all the shoots were counted and evaluated for leave scab. On July 25th all fruits were counted and evaluated for fruit scab and were classified into 4 classes according to the russeted area. For each tree the russet index was calculated (Haug and Kunz, 2005).

Results

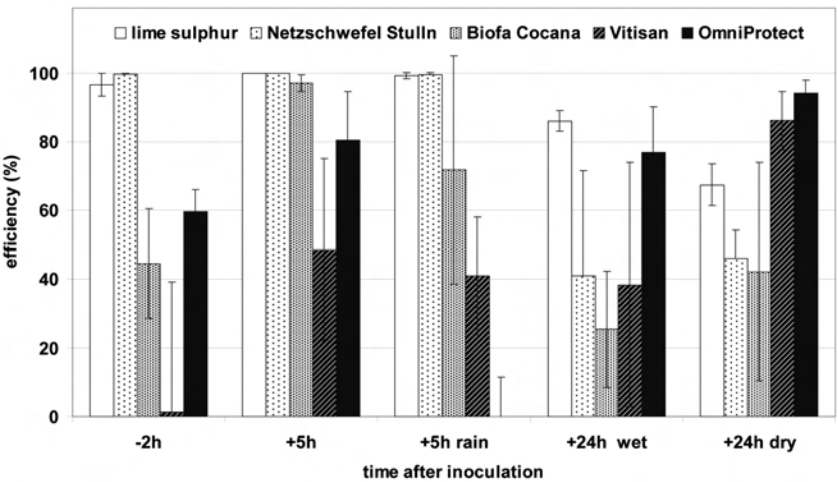

Figure 2: Efficiency of test preparations when applied to apple shoots protective 2 hours before inoculation with *V. inaequalis* conidia (-2h), during the germination period 5 hours after the inoculation either on wet leaves (+5h) or during simulated rainfall (+5h rain) or applied curative 24 hours after inoculation either on wet leaves (+24h wet) or on dry leaves (+24h dry).

Greenhouse trials: In the greenhouse trials, on an average 35% of leaf area was covered with sporulating scab lesions on untreated shoots due to the artificial inoculation of apple shoots with conidia of *V. inaequalis*. Lime Sulphur or Netzschwefel Stulln reduced scab lesions to a high extend when applied protective or during the germination period (fig. 2).

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The efficiency of Lime Sulphur and Netzschwefel Stulln decreased when applied curative. Biofa Cocana or OmniProtect had only moderate efficiencies and Vitisan had no effect when applied protective. Biofa Cocana had a high efficiency (97%) when applied during the germination period on wet leaves, but under simulated rainfall the efficiency decreased to 72% and curative applications with Biofa Cocana had only slight effects. Vitisan and OmniProtect had only moderate or no efficiency when applied during the germination period or curative on wet leaves. But Vitisan and OmniProtect reduced scab incidence by 85% and 94% when applied curative on dry leaves (fig. 2).

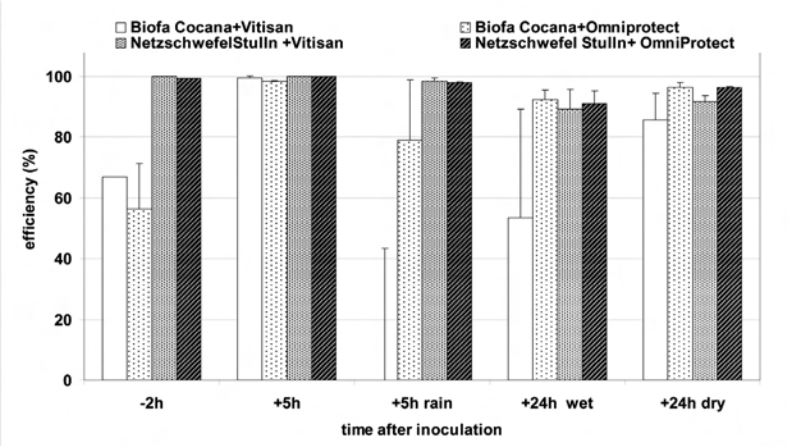

Figure 3: Efficiency of mixtures of test preparations when applied to apple shoots during the germination period 5 hours after the inoculation with conidia of *V. inaequalis* either on wet leaves (+5h) or during simulated rainfall (+5h rain) or applied curative 24 hours after inoculation either on wet leaves (+24h wet) or on dry leaves (+24h dry).

The efficiency of the test preparations strongly depended on the time of application during the infection process. We combined different preparations to prolong the time period of high efficiency. The mixture of Biofa Cocana with Vitisan prevented scab symptoms when applied during the germination period on wet leaves, but not under simulated rain. Protective or curative applications on wet leaves of this mixture had a moderate efficiency and curative applications on dry leaves had a high efficiency (86%). The mixture of Biofa Cocana and OmniProtect showed a high efficiency (80 to 98%) when applied after inoculation (fig. 3). Mixtures of Netzschwefel Stulln with either Vitisan or OmniProtect both reduced scab incidence by more than 90% regardless of the time of application.

Field trial: In 2007 the period between green tip and petal fall was rainless and therefore no infection conditions for apple scab occurred. After bloom heavy rain periods caused infection conditions for apple scab in May and June. With ten curative applications with OmniProtect scab incidence on leaves was significantly reduced by 87% on Jonagold and by 94 % on Rewena (tab. 1).

On treated trees no apples with scab symptoms were found the 25th of July. The russet index on treated fruits increased significantly on both varieties (tab. 1). A further assessment at harvest was not possible as many fruits were lost due to brown rot infections.

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Table1: Scab incidence on leaves (% shoots with scab symptoms) was evaluated on July the 13th. Total number of fruits, scab incidence on fruits and fruit russet was evaluated on July the 25th. Mean values of untreated and treated trees from each variety were compared with a unpaired T-Test. Different letters indicate a significant difference (p<0.05).

	Jonagold		Rewena	
	untreated	**treated**	**untreated**	**treated**
% shoots with scab	16.8 (a)	2.2 (b)	6.3 (a)	0.4 (b)
% efficiency	-	**87**	-	**94**
Number of fruits	130	342	317	89
% fruits with scab	0 (a)	0 (a)	2.0 (a)	0 (b)
Russet index	1.06 (a)	3.86 (b)	1.37 (a)	3.90 (b)

Discussion

Conditions for infections by *V. inaequalis* are well known and simulation models predict ascospore release and calculate infection periods on the basis of weather data. Modern integrated scab control uses protective fungicides short before forecasted rain starts and curative fungicides after conditions for infections are fulfilled (Triloff, 1999). In organic apple growing scab control is mainly based on protective use of sulphur, lime sulphur and copper. Additional applications of lime sulphur during the germination period of the scab fungus are done during heavy infection periods. These specific applications are difficult to schedule as the time period for application is short and in most cases applications must be done during rainfall and often during night.
In our greenhouse trials different situations during the infection process of the scab fungus were simulated. Protective applications were done short before inoculation with conidia of the fungus. The artificial inoculation simulates the start of rain. With the rain conidia or ascospores are dispersed in the orchard, germinate and build appressoria on the cuticle of young leaves. At temperatures of 18°C this epicuticular germination period lasts 6 to 8 hours. We applied test preparations 5 hours after inoculation in a closed room, were fog led to wet leaves. This was sufficient to get high infection rates without measurable rainfall. Many test preparations were highly efficient under these conditions. In the orchards in most cases rainfall will occur during the infection process. Therefore we did additional tests under simulated rainfall. With the exception of lime sulphur and Netzschwefel Stulln the efficiencies of the test preparations decreased when used during simulated rain. After the germination period *V. inaequalis* infects the leave by penetrating the cuticle and establishing a primary stroma between the cuticle and the epidermis of the leave. Once an infection is established curative preparations are needed to stop it. With Vitisan and OmniProtect two preparations based on potassium carbonate were identified, which showed a curative activity. At 18°C both preparations could be used 24h after inoculation, which corresponds to 432 degree hours. At a mean temperature of 10°C we expect a curative effect until 40 hours after start of rain. The highest efficacy of Vitisan and OmniProtect was found when applied on dry leaves 24 hours after inoculation. If the leaves are still wet, the addition of Netzschwefel Stulln is useful.

The mixture of Netzschwefel Stulln and either Vitisan or OmniProtect showed a high efficiency during the germination period and a curative action until 24 hours after inoculation. When used combined with wettable sulphur, Armicarb (a.i. potassium bicarbonate) was most effective in trials in Switzerland (Tamm et al., 2006). Different preparations based on potassium bicarbonates have been tested for apple scab control during the last years (Jamar et al., 2007; Kelderer et al., 2006; Tamm et al., 2006).

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Variable efficacy of potassium bicarbonate was found in field trials (Kelderer et al., 2006). This could be explained by the influence of different formulations used, or by the fact that the timing of application has a considerable impact on the efficacy. In most field trials bicarbonates are applied protective. However, our data show that a curative use is more efficient. Vitisan as well as OmniProtect showed a higher curative activity than lime sulphur, which is the only fungicide used in organic growing for curative control of apple scab (Holb et al., 2003).

Ten curative applications of OmniProtect led to a significant reduction of leave and fruit scab in a field trial in Konstanz 2007. These confirmed the results from the greenhouse trials. But OmniProtect increased fruit russet clearly. Six of the ten applications were done during the four weeks following bloom. This is the most critical period for fruit russet. In another trial in Konstanz applications of OmniProtect in July did not increase fruit russet on Golden Delicious (data not shown). This indicates that OmniProtect and the similar preparation Vitisan should not be used during bloom and four weeks after bloom. Nevertheless both preparations are useful curative supplements for scab control before bloom and in summer.

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