Screening of biocontrol agents for their efficacy against apple scab

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Abstract

*Protective sprays with copper are included in spray schedules for apple scab control in organic fruit growing although the use of copper is under discussion. In a research project, funded by the "Bundesprogramm Ökologischer Landbau" biocontrol agents have been screened for their efficacy against apple scab (*Venturia inaequalis*) to find alternatives to copper. In greenhouse trials we tested biocontrol agents for their protective and curative efficiency against apple scab by artificial inoculation of potted apple trees. Applications were done 18 hrs before inoculation, 5 hrs after inoculation during simulated rain fall, 24 hrs or 48 hrs after inoculation. In addition, the efficacy of protective applications followed by simulated rain was tested. 23 biocontrol agents and 12 combinations of at least two biocontrol agents were involved in the tests.*

The optimal moment of application differed between the preparations tested. Sulphur and copper products had the highest efficiency when tested protectively. Some additives (e.g. NuFilm P, T/S forte) enhanced the rain fastness of protective sulphur fungicides. Five preparations reached their highest activity when sprayed curatively 24 hours after inoculation. The products with the highest curative efficiency are based on carbonates. Several combinations of preparations were found, which revealed a high efficiency against apple scab between 18 hrs before and 24 hrs after inoculation. The biocontrol agents and combinations with the highest efficacy will be tested in field trials within this BÖL-project.

Keywords: apple scab, *Venturia inaequalis*, curative treatment, rain fastness, carbonates, sulphur, copper

Introduction

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is the most important apple disease, causing economic losses in almost all apple production areas. Its significance is indicated by the fact that up to 20 fungicide treatments are applied per season to control the disease (Jamar et al., 2007; Kollar, 1997). Conditions for infections by *V. inaequalis* are well known and simulation models predict ascospore release and the infection process based on weather data and a weather forecast. In organic apple growing scab control is focussed on the protective use of sulphur, lime sulphur and copper. Additional sprays of lime sulphur during the germination period of the scab fungus are applied during severe infection periods. These specific applications are difficult to schedule as the time period for the application is often short and in most cases these sprays have to be done during rainfall and at night.

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 leaf. Once an infection is established, curative compounds are needed to stop it. With Vitisan and OmniProtect two compounds based on potassium carbonate were identified, which showed a curative activity (Kunz et al., 2008). The highest efficiency of Vitisan and OmniProtect was found when applied on dry leaves 24 hrs after inoculation. The mixture of Netzschwefel Stulln and either Vitisan or OmniProtect showed a high efficiency during the germination period and a curative action until 24 hrs after inoculation (Kunz et al., 2008).

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Different compounds based on carbonates have been tested for apple scab control during the last years, resulting in variable efficacies (Jamar et al., 2007; Kelderer et al., 2006; Pfeiffer, 2008; Tamm et al., 2006; Trapman, 2008). This can be explained by the influence of different formulations used, different weather conditions or by the moment of the application which has a considerable impact on the efficacy.

Therefore in this study, biocontrol agents were applied to artificially inoculated apple trees at different times during the infection process in greenhouse trials to evaluate the optimal timing for each product. The results will be considered in the setup of field trials done by KoGa Ahrweiler, KÖN Jork, KOB Bavendorf, LfL Dresden, who are cooperation partners in the described research project.

Material and Methods

Potted apple trees of the cultivar Jonagold, grafted on M 9 rootstocks, were kept in a greenhouse. The three youngest completely unfolded leaves of the apple shoots were spray inoculated with 10^5 conidia per ml until runoff and subsequently incubated at 18°C to 23°C and 100% relative humidity for 20 hrs in the dark. High humidity was ensured by using a humidifier. The plants were subsequently kept under greenhouse conditions. To prepare the inoculum suspension, leaves with conidia of *V. inaequalis*, stored at -80°C, were thawed and shaken in tap water (Kunz et al., 2008).

Table 1: Supplier, dose rate (rate), active ingredient and abbreviations (abbr.) of the preparations used.

Preparation	**abbr.**	**active incredient**	**rate [%]**	**supplier**
Algo Vital Plus	Algo Vital	*seaweed extracts*	0.5	Biofa AG
Armicarb	Armicarb	potassium bicarbonate	0.5	KoGa Ahrweiler
Biofa Cocana	Cocana	Coco soap	1	Biofa AG
BlossomProtect	BP	*Aureobasidium pullulans*	1.2	Bio-Protect GmbH
Cuprozin	Cuprozin	copper	0.1*	Spiess-Urania GmbH
Goemar fruton sp.	Goemar	nitrogen, magnesium sulphate, water-soluble boron	0.25	Spiess-Urania GmbH
HF-Pilzvorsorge	HF-Pilz.	fennel oil	0.4	Biofa AG
Hydrocal super	Hydrocal	hydrated lime	dusted	Verblasetechnik Schneider
Kaliwasserglas	KaliWG	silic acid, potassium oxid	0.5	Biofa AG
Kumulus WG	Kumulus	sulphur	0.25	BASF AG
Lime sulphur	Lime s.	sulphur	1.5	Biofa AG
Mycosin	Mycosin	acid rock powder	1	Biofa AG
Netzschwefel Stulln	NS Stulln	sulphur	0.25	Biofa AG
Nu-Film P	NuFP	pinolene	0.03	Intrachem Bio, Germany
OmniProtect	Omni	potassium carbonate	0.5	Bio-Protect GmbH
Saponin	Saponin	extract from *Yucca schidigera*	1	KoGa Ahrweiler
SPU-01010-F	SPU1010	copper	0.476*	Spiess-Urania GmbH
SPU-02610-Z	SPU2610	(surfactant)	0.05	Spiess-Urania GmbH
SPU-02720	SPU2720	copper	0.086*	Spiess-Urania GmbH
SPU-02980	SPU2980	copper	0.24*	Spiess-Urania GmbH
Steinhauer's Mehltauschreck	Steinhauers	sodium bicarbonate	0.5	Biofa AG
TGS	TGS	*Trichoderma*	1	Gerlach GmbH & Co. KG
VitiSan	Vitisan	potassium bicarbonate	0.5	Biofa AG

*Copper compounds are used with 300 g cu/ha.

Test products were usually supplied by the companies (tab.1). The compounds were suspended in tap water and sprayed onto the dry test plants until run off 18 hrs before inoculation (protective or for rain fastness testing), 5 hours after inoculation during simulated rainfall or 24 or 48 hrs after inoculation on wet or dry leaves (curative). Rainfall was simulated with a spray nozzle placed 2 m above the plants, and its amount was measured with a pluviometer. To test the rain fastness of a product, plants were irrigated 18 hrs after application with 30 l/m^2 of water, and were inoculated consecutively (Kunz et al., 2008). 16 to 21 days after inoculation, the disease incidence for each shoot was calculated as the average of the proportion of the diseased leaf area of the three youngest inoculated leaves (Kunz et al., 1997). The average of the diseased leaf area of 5 shoots per treatment was calculated. The efficiency of the tested compound 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.

Results

In the greenhouse trials, an average of 35% of leaf area was covered with sporulating scab lesions on untreated shoots due to the artificial inoculation of the shoots with conidia of *V. inaequalis*, indicating a high disease pressure in the trials.

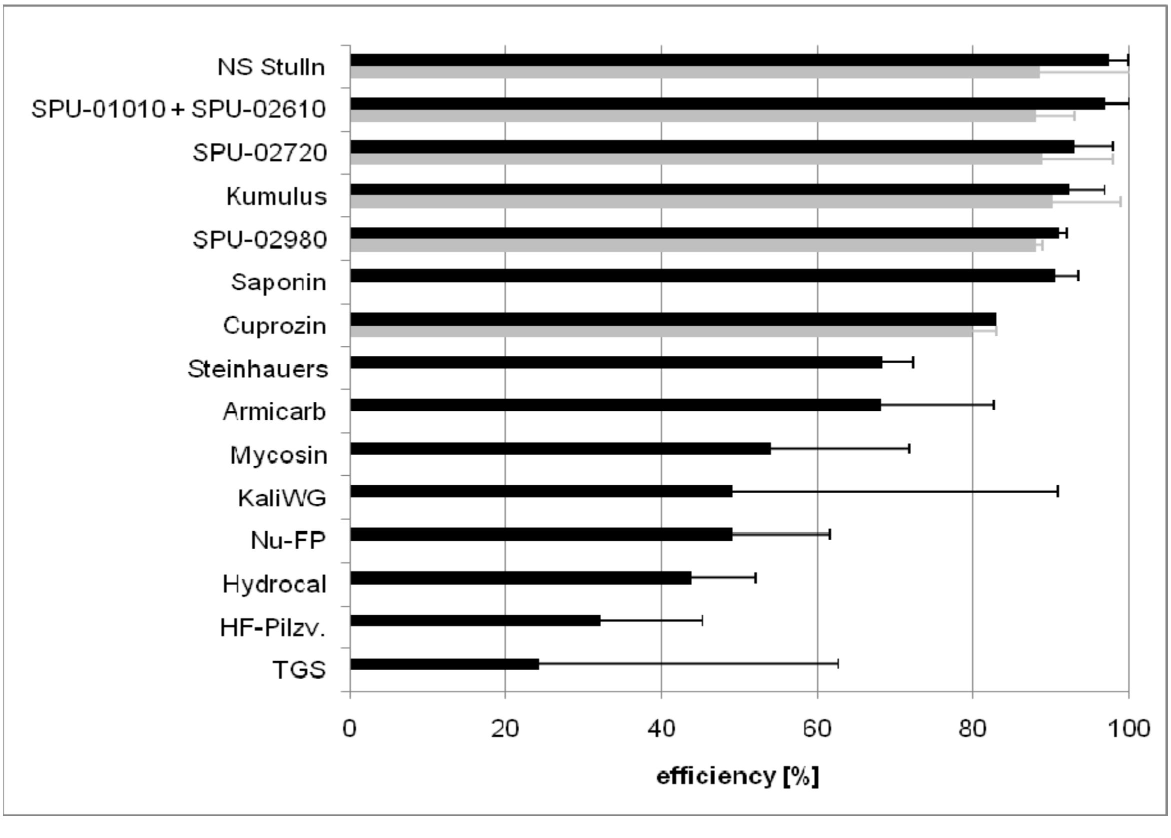

Figure 1: Efficiency of preparations applied to apple shoots protectively, 18 hrs before inoculation with *V. inaequalis* conidia without (black columns), or with 30 mm overhead irrigation (grey columns) before the inoculation. When grey columns are missing, the compound has not been tested with irrigation. Average and standard deviation from at least two trials are given.

Wettable sulphur, some copper formulations and Saponin reduced the number of scab lesions by more than 90%, when applied **protectively** (fig. 1). Artificial rain (30 mm) between application and inoculation slightly reduced the efficiency of all tested products.

Using Netzschwefel Stulln it was reduced from 98% to 89%. Therefore additives were tested in order to increase the rain fastness of Netzschwefel Stulln (sulphur). The additives had no influence on the efficiency of Netzschwefel Stulln without artificial rain. However, the addition of Saponin, AlgoVital Plus, Nu-Film P, Goemar fruton sp., Mycosin or Vitisan to Netzschwefel Stulln tended to increase its rain fastness (fig. 2). Netzschwefel Stulln, lime sulphur and the mixtures of Netzschwefel Stulln with Vitisan or OmniProtect reduced scab incidence nearly completely, when applied during germination under overhead irrigation **5 hrs after** inoculation (fig. 3). More preparations have to be tested under these conditions.

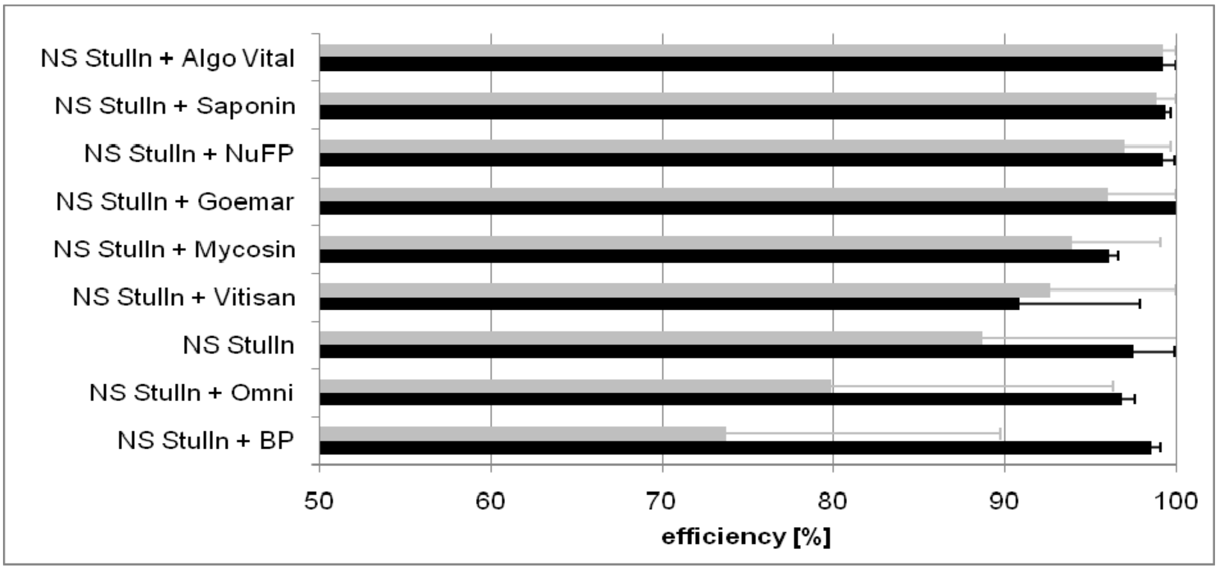

Figure 2: Efficiency of mixtures protectively applied to apple shoots, 18 hrs before inoculation with conidia of *V. inaequalis*, without (black columns), or with 30 mm overhead irrigation (grey columns) before inoculation. Average and standard deviation from at least two trials are given.

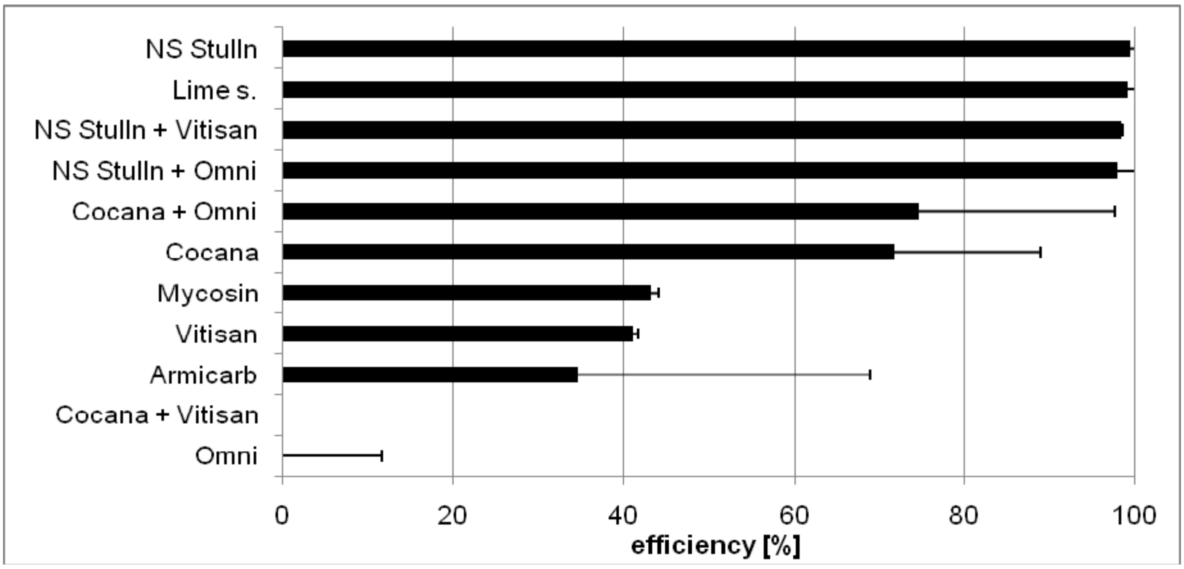

Figure 3: Efficiency of preparations applied to apple shoots during the germination of conidia of *V. inaequalis* 5 hrs after inoculation. Application was done during overhead irrigation. The average and standard deviation from at least two trials are given.

Curative applications **24 hrs after inoculation on wet leaves** represent the most critical condition to reach high efficacies. Under these conditions only the mixture of Netzschwefel Stulln with OmniProtect reduced disease incidence by more than 90%, followed by Armicarb, a mixture of Netzschwefel Stulln and Vitisan, Steinhauer's Mehltauschreck, lime sulphur and OmniProtect (fig. 4).

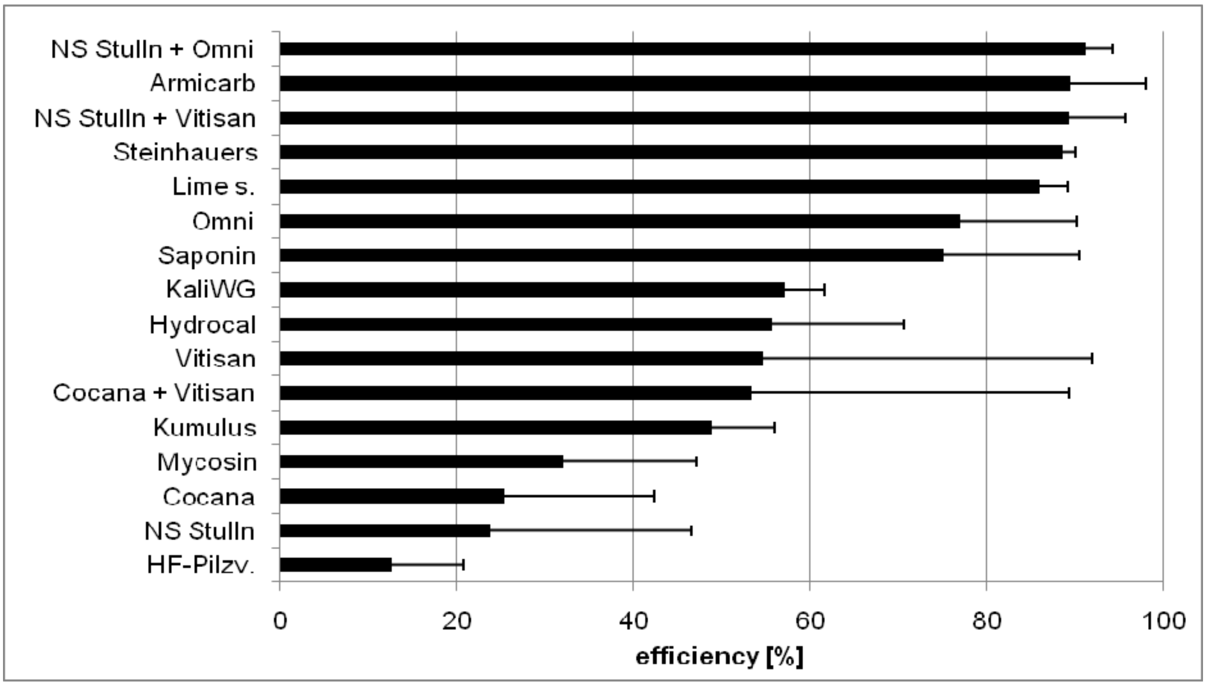

Figure 4: Efficiency of preparations or mixtures applied to apple shoots 24 hrs after the inoculation with conidia of *V. inaequalis* on wet leaves. The average and standard deviation from at least two trials are given.

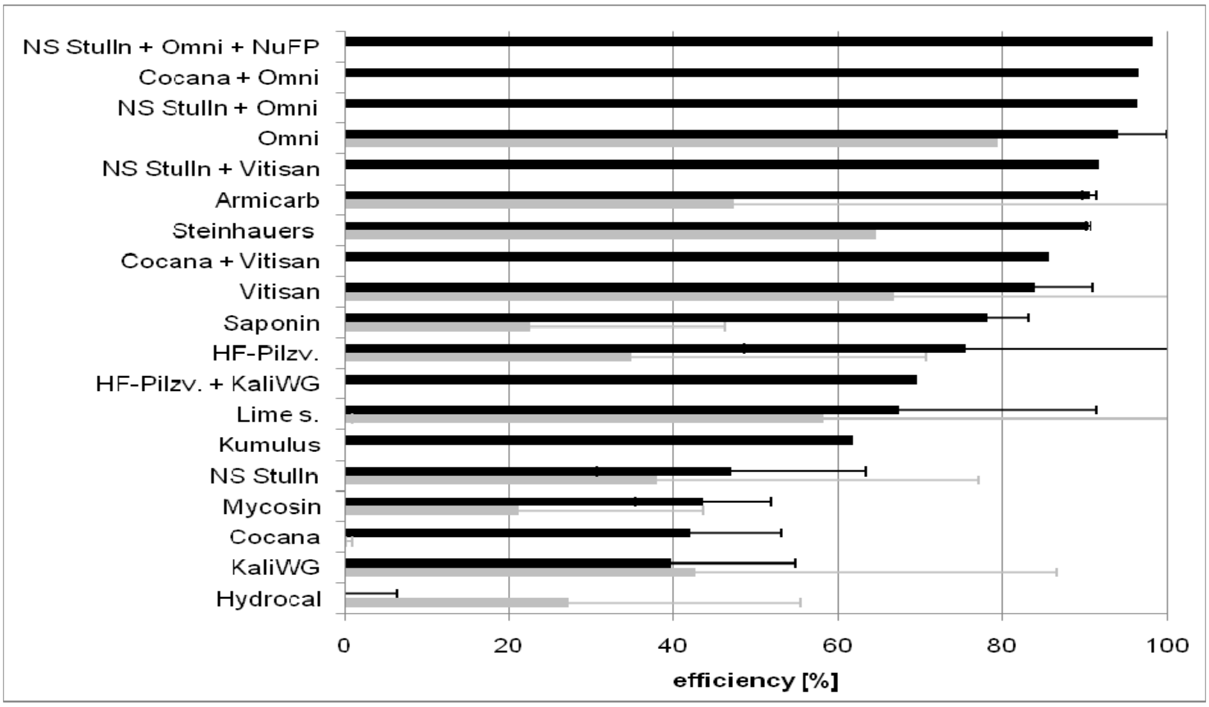

Figure 5: Efficiency of preparations and mixtures applied to apple shoots 24 hrs (black columns) or 48 hrs (grey columns) after the inoculation with *V. inaequalis* conidia on dry leaves. Where the grey column is missing, the preparation has not been tested 48 hrs after inoculation. The average and standard deviation are given.

Treatments containing potassium- or sodium carbonates exhibited the highest efficiencies when applied curatively **24 hrs after inoculation on dry leaves**. Under these conditions a combination of OmniProtect with Netzschwefel Stulln and NuFilm P reduced scab incidence by 98% (fig. 5), and should be further tested after a longer period post inoculation. The extension of the post inoculation period to **48 hrs** reduced the efficiency of the tested preparations.

Discussion

In organic apple growing scab control is based on the protective use of copper. However, the use of copper is under discussion for ecological purposes. Therefore alternative control agents are needed. Wettable sulphur and lime sulphur are known to have a high protective efficacy, but there is doubt about the rain fastness of wettable sulphur. In practice sprays of lime sulphur during the germination period of scab spores are applied after high ascospore releases. These specific applications are difficult to schedule as the time frame for the application is short and in most cases applications have to be done during rainfall and at night. Kunz et. al (Kunz et al., 2008) proposed curative applications of potassium- and sodium carbonates and other potential control agents, which gave variable control in field trials.

Conditions for infections by *V. inaequalis* are well known and simulation models predict ascospore release and calculate the infection process on the basis of weather data. In the greenhouse trials different situations during the infection process of the scab fungus were simulated. Protective applications were done 18 hrs before inoculation with conidia and rain fastness of the products was tested by overhead irrigation before inoculation. The artificial inoculation simulates the start of a rain event. With the rain conidia or ascospores are dispersed in the orchard, germinate and build appressoria on the leaf cuticle. At temperatures of 18°C this epicuticular germination period lasts 6 to 8 hrs. We applied test compounds 5 hrs after inoculation during overhead irrigation, as in the orchards in most cases rain falls during the infection process. After the germination period *V. inaequalis* infects the leaf by penetrating the cuticle and establishing a primary stroma between the cuticle and the epidermis of the leaf. Once an infection is established curative preparations are required to stop a further development of the mycelium.

The efficiency of the test products strongly depended on the moment of application during the infection process. Copper formulations had a protective activity with an acceptable rain fastness. The use of copper during germination or as curative application has not been tested so far in our test-system. However, the addition of copper increased the efficacy of sulphur in field trials, when applied during germination with overhead irrigation (Kelderer et al., 2000).

Wettable sulphur had a high efficacy when applied protectively. Its rain fastness was improved by several additives (Nu-Film P, AlgoVital, Goemar fruton) and it is also applicable during the germination period, but not as a curative. Vitisan and OmniProtect are not suitable as protective sprays (Kunz et al., 2008), but had a promising curative activity. The same was found for Steinhauers Mehltauschreck and Armicarb in this study. Saponin had a moderate efficiency when used protective or curative and it enhanced the rain stability of Netzschwefel Stulln. Further investigations on combinations with other control agents should be done.

None of the control agents had a high efficacy during the entire infection process. Therefore we combined different preparations to prolong the time period of high efficacy. The mixture of Netzschwefel Stulln with OmniProtect prevented scab symptoms under all conditions tested, except in the rain fastness test, where the combination was less effective than Netzschwefel Stulln on its own. The addition of Nu-Film, AlgoVital or Goemar fruton should be tested to increase rain fastness of the combination.

Because the use of fungicide combinations during the whole season would be too expensive, strategies for the alternate use of different products should be developed, where each products to be used at its best performance. Together with our cooperation partners these strategies will be developed and tested in field trials the next years.

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