Efficacy of biocontrol agents against apple scab in greenhouse trials

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Abstract

*Control of apple scab (*Venturia inaequalis*) in organic apple production is mainly based on copper and sulfur sprays. A reduction of the total amount of metallic copper is intended. New products to replace copper sprays at least partially are needed. In a research project, funded by the "Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft" biocontrol agents have been assessed for their efficacy against apple scab in greenhouse trials by artificial inoculation of potted apple trees. Applications were done protectively, as stop applications or curatively. In addition, the efficacy of protective applications followed by simulated rain was tested to proof the rain fastness of the products. New products and mixtures were tested during the last two years. In addition Curatio (lime sulphur) was tested for its protective efficacy and rain fastness if applied as stop application during rain.*

The optimal timing for the application differed between the preparations tested. Copper products had the highest efficiency when tested protectively. Rain fastness of the copper products depended on the formulation and the dose rate used. Adding carbonates to copper reduced the rain fastness of copper products. Some other products were identified with a good protective effect, but the rain fastness of the products was unsatisfying. Efforts should be invested to find suitable formulations or stickers. Stop applications during the germination of the fungus were effective using sulphur products or new products based on lime (Calcium hydroxide). Rain events during application of Curatio or after the application before the spray cover was dried, reduced the protective activity of Curatio. Therefore, applications of Curatio during germination of the scab fungus cannot be regarded as a spray cover for following infection periods. Besides the Carbonates, no other substances were identified with curative activity.

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

Introduction:

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is the most important apple disease, causing economic losses in all apple production areas with humid climate. Its significance in middle Europe is indicated by the fact that 50 % of the pesticide use in apple production is related with control of apple scab (van Hemelrijck *et al*., 2012). Conditions for infections by *V. inaequalis* are well known and simulation models predict ascospore release and the infection process. In organic apple growing scab control is focussed on the protective use of sulphur and copper products as well as additional sprays of lime sulphur during the germination period of the scab fungus (Zimmer *et al*., 2012). After the germination period, *V. inaequalis* infects the leave by penetrating the cuticle and establishing a primary stroma. Once an infection is established, curative compounds are needed to stop it. Carbonates were identified to have curative activity (Hinze & Kunz, 2012; Kunz & Hinze, 2011; Hinze & Kunz, 2010; van Hemelrijck *et al*., 2012). The addition of wettable sulphur to carbonates increased the curative efficiency (Hinze & Kunz, 2010). On the other hand, reduced the addition of copper to Vitisan or Omni Protect the efficacy of the carbonates significantly (Kunz & Hinze, 2014). Furthermore the protective activity and rain fastness of copper products were reduced after adding Vitisan in the tank mixture

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(Kunz & Hinze, 2014). This problem was addressed with further experiments using Armicarb and Copper in this study.

A screening for alternative products in the green house confirmed that sulfur and copper components had the highest protective efficiency and some additives like increased the rain fastness of wettable sulphur (Hinze & Kunz, 2010). New products derived from plant extracts were identified to have a high protective efficacy against apple scab. However, these products had a reduced rain fastness and are not ready for practical use (Kunz & Hinze, 2014). With the so far identified alternatives a replacement of copper in apple scab control in organic production is not possible (Zimmer *et al.*, 2012). Further products or product mixtures with high, reliable efficacy are needed. Therefore the assessment of potential biocontrol agents against apple scab was continued during the last two years and further experiments to assess the behaviour of the standard products under different conditions were done.

Material and Methods

Table 1: Used preparations (abbreviations), active ingredients, dose rate and supplier.

Preparation	rate [%]	Supplier
Armicarb®; formulated Potassium bicarbonate	0.5	Spiess-Urania GmbH
CuCabs, copper	0.3*	Agrolytix GmbH
Cuprozin® progress (Cup.prog); copper hydroxide	0.04*	Spiess-Urania GmbH
Curatio; lime sulfur	1.5	Biofa AG
Cuprum; copper	0.1	KoGa Klein-Altendorf
Funguran® progress (Fung.prog); copper hydroxide	0.0286*	Spiess-Urania GmbH
Hydrocal super (Hydrocal); hydrated lime	Dusted	Verblasetech. Schneider
Kalkmilchlösung (lime suspension in water)	10	KoGa Klein-Altendorf
Kalkwasser (disolved lime)	100	KoGa Klein-Altendorf
Kumulus® WG (Kumulus); wettable sulfur	0.25	BASF AG
Netzschwefel Stulln (NSStulln); wettable sulfur	0.25	Biofa AG
Nu-Film P; pinolene	0.03	Intrachem Bio, Germany
P1EC-159; extract from *Glycyrrhiza glabra*	2.06	Trifolio-M GmbH
Saccalia; extract from *Reynoutria sachalinensis*	0.1	Syngenta
Vitisan®; potassium bicarbonate	0.5	Biofa AG

*For this Copper compounds the rate corresponding to 100 g Cu/ha is given.

Potted apple trees of the cultivar Jonagold, were kept in a greenhouse. Daylight was prolonged by artificial illumination so that growing shoots were available year-round. The three youngest completely unfolded leaves of the apple shoots were spray inoculated with 105 conidia per ml until runoff and subsequently incubated at 16 °C to 23 °C and 100 % relative humidity for 20 hrs in the dark. High humidity and by this leave wetness 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 -20 °C, were thawed and shaken in tap water (Kunz *et al.*, 2008).

Test products were usually supplied by the companies or by the project leader (Jürgen Zimmer, KoGa Klein-Altendorf) (Table 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), 5hrs after inoculation during simulated rainfall (stop application) or 24 hrs after inoculation on dry leaves (curative). For simulating rainfall a spray nozzle was placed 2 m above the plants, and the amount of water was measured with pluviometers placed in the pots. To test the rain fastness of a product, plants were irrigated 17 hrs after application with 30 l/m^2 (30 mm) of water, and were inoculated consecutively (Kunz *et al.*, 2008). In one experiment testing the built up of an effective cover of Curatio, plants were irrigated with different amounts of water immediately after application or at different time points after application.

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. The average of the diseased leaf area of 5 shoots per treatment was calculated. The efficacy of the tested compound was calculated for each experiment by comparing the disease incidence with the untreated control according to Abbott (Abbott, 1925). Experiments were done at least twice and the average efficacy is given in the figures and tables.

Results

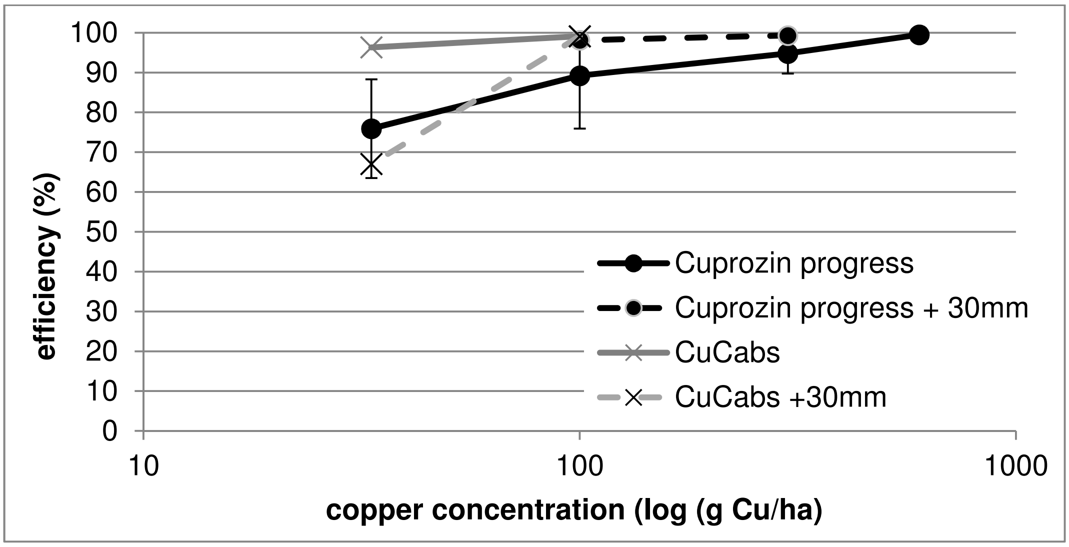

Figure 1: Dose-response relationship of copper products if applied to apple shoots protectively, 18 hrs before inoculation with V. inaequalis conidia. A 30 mm rain event was simulated 1 hr before inoculation, if indicated (+30 mm). Standard deviation is shown only for Cuprocin progress.

Cuprocin progress was used as standard in many trials with protective applications in different dose rates, from which a dose-response relationship was deduced (Figure 1). 0.12 % Cup. prog. (corresponding to 300 g Cu/ha) revealed an efficacy of 95 % in average (Number of trials (N)=6). Applications of 0.04 % Cup. prog. (100 g Cu/ha) gave variable results from 80 % to 100 % control (N=15). Lower doses were not satisfying. Rain events of 30 mm 17 hrs after application of Cup. prog. and just before inoculation with conidia, did not reduce the efficacy. Using CuCabs the efficacy of a low dose of copper (33 g Cu/ha) tended to be higher than with Cup. prog. However, this low dose lost efficacy after 30 mm of rain (Figure 1). From this results the reduction of the copper dose below 100g Cu/ha is not recommendable for both products.

In addition to the results already published (Kunz & Hinze, 2014; Hinze & Kunz, 2010) new products and mixtures were tested for their protective activity against apple scab and for rain fastness (Figure 2). Curatio as well as copper products (min. 100 g Cu/ha) reduced scab symptoms by more than 95 %, even after an artificial rain event of 30 mm. For NS Stulln the reduced rain fastness was confirmed. The copper preparation Cuprum and the plant extract P1EC159 showed an interesting high protective activity. For both products rain fastness was very low revealing a total loss of scab control after 30 mm of rain, although P1EC was mixed with the sticker NuFilmP.

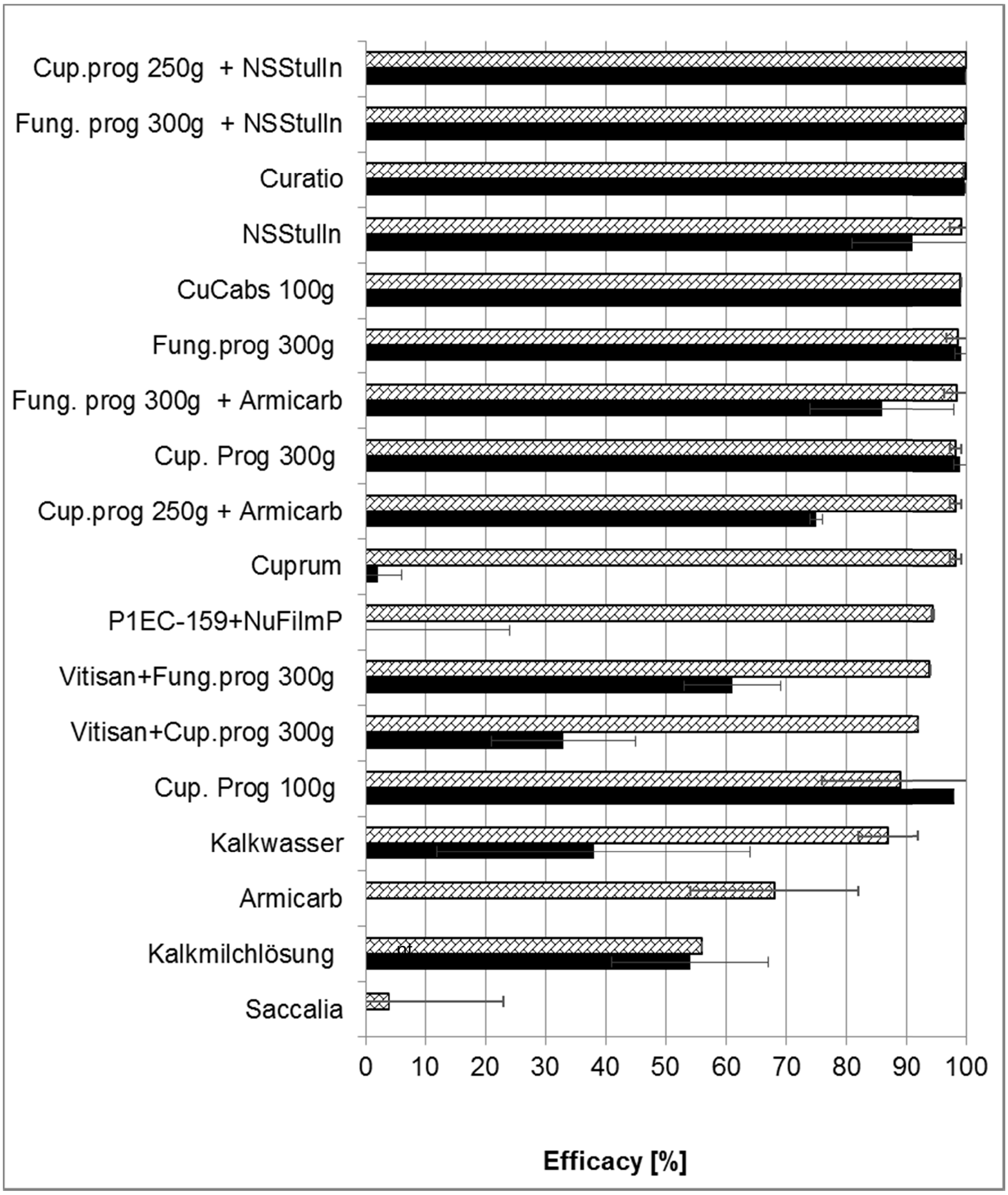

Figure 2: Efficacy of preparations and mixtures applied to apple shoots protectively, 18 hrs before inoculation with V. inaequalis conidia without (scattered columns), or with 30 mm overhead irrigation (black columns) before inoculation. Nt= not tested. Average and standard deviation from at least two trials are given.

Two preparations based on lime were tested. Kalkwasser had an efficacy of 87 % and had no rain fastness. Kalkmilchlösung had only low effects without and with rain. The plant extract Saccalia had no effect in this test system.

Mixtures of copper products with wettable sulfur controlled scab nearly completely, even after 30mm of rain, whereas the mixtures of Armicarb with copper products had a reduced rain fastness compared to the copper products applied as standalone treatments (Figure 2). This effect was already shown using Vitisan in mixtures with copper products (Kunz & Hinze, 2014).

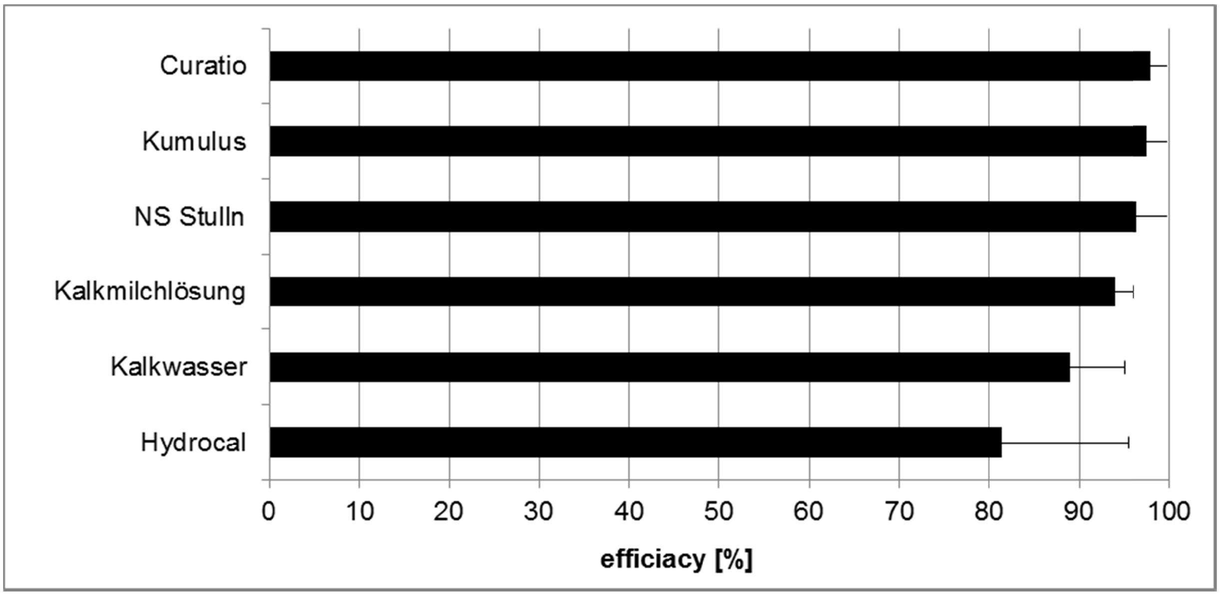

Figure 3: Efficacy of preparations applied 5 hrs after inoculation to apple shoots during the germination of conidia of *V. inaequalis* (stop application). The average and standard deviation from at least two trials are given.

Kalkmilchlösung and Kalkwasser were also tested in the so called stop application and reduced symptom development by 94 % and 89 %, respectively (Figure 3). These preparations based on lime showed higher efficacy than Hydrocal (hydrated lime) dusted on the leaves. Curatio or wettable sulfur (Kunz & Hinze, 2014) performed better than the lime preparations (Figure 3) under these conditions.

Curatio showed a high efficacy in stop applications (Figure 3) and also if applied protectively (Figure 2). Additional tests were done for its protective efficacy, if the rain event occurred during application or short afterwards to simulate the application of Curatio as a stop application. The question was if and under which condition this application during rain leads to a protective spray cover preventing infections of a subsequent infection period. The application of Curatio 18 hrs before inoculation reduced scab incidence by 99% as expected from former trials (Table 2). Fogging of low amounts of water (1-5 mm) during the application of Curatio and short after, reduced the efficacy against the subsequent infection to 87-91 % (Table 2). Heavy rain during the application (15 mm) reduced the efficacy to 77 %. 15 mm rain 1, 2 of 4 hrs after the application did also reduce the efficacy to around 90 %. Leaves were not dried completely 4 hrs after the application of Curatio in this experiment. After drying for 17 hrs Curatio proved to have a good rain fastness. Even 30 mm water applied 17hrs after the application of Curatio did not reduce the efficacy of the product in former trials (Table 2). The experiment demonstrated that Curatio sprays should be allowed to dry completely before a rain event to keep its full efficacy as protective cover against a consecutive infection period.

Table 2: Scab incidence (% leaf area covered with sporulating lesions), standard deviation (SD) and efficacy compared to the untreated control on apple leaves after application of 1.5% Curatio and subsequent overhead irrigation (mm) at different time points.

treatment	**Overhead irrigation (mm) at time point after treatment**					**Scab Incidence (%)**	**± SD**	**efficacy (%)**
	0 h	**1h**	**2h**	**4h**	**17h**			
Untreated						**32,7**	34,0	
Curatio						0,4	1,1	**98,7**
Curatio	1					4,2	8,9	**87,1**
Curatio	3					4,4	10,3	**86,6**
Curatio	5					3,1	5,7	**90,5**
Curatio	15					7,5	12,5	**77,1**
Curatio		15				4,7	5,6	**85,5**
Curatio			15			3,1	6,2	**90,4**
Curatio				15		5,4	7,8	**83,5**
Curatio average					30			**99,8**

Kalkmilchlösung and Kalkwasser were also tested curatively 24 hrs after inoculation on dry leaves and did not show any effect (data not shown).

Discussion

In organic apple growing scab control is based on the protective use of copper and sulfur. However, the use of copper is under discussion for ecological purposes. With new copper formulations, the input of metallic copper can be reduced (Zimmer *et al.*, 2012). In the greenhouse trials described here, copper dose rates corresponding to 100 g Cu/ha were the lower limit for a satisfying disease control. For a further reduction alternative control agents or partners for mixtures are needed. Wettable sulfur products are known to have a high protective efficacy, but their rain fastness should be improved by addition of suitable additives like Nu-Film P or Trifolio TS-forte (Kunz & Hinze, 2014; Hinze & Kunz, 2010), as scab infections are always accompanied with rain fall and by this with washing off of active ingredients from the leaves. Some plant extracts (P1-Extract, Phytocare, SAP-Ex, Saponin) showed promising effects after protective applications, with a lack of rain fastness (Kunz & Hinze, 2014). In this study a new formulation of the P1-extract (P1EC-159) was tested with the sticker Nu-Film P and we found no improvement in rain fastness compared to the former trials. The products are still not ready for commercialization. Suitable formulations are needed which improve the rain fastness and stability of the products. Two new preparations based on lime were tested. Whereas the efficacy in the so called stop application was high, the protective effect was not satisfying. So the products could be used as alternatives to Curatio in the stop application rather than as alternatives to copper used protectively. Curatio (lime sulfur) showed good rain fastness if applied to dry leaves and it is highly efficient if applied protectively and during germination of the fungus (stop application). The stop applications are done to wet leaves or during rain fall. Under these conditions the built up of a stable spray cover from lime sulphur was not possible. Even 1 mm of rain applied during the application of Curatio reduced the control of apple scab of a subsequent inoculation (Table 2). Therefore during rain periods of several days, applications of Curatio have to be done daily after each ascospore release. Daily applications are not covered by the registration. Alternative products are needed for this purpose. Wettable sulphur products and KCLO showed activity if applied as stop application (Kunz & Hinze, 2014) and the new tested lime preparations. However, the Kalkwasser was applied undiluted and the Kalkmilchlösung in a dose rate of 10 % (100 L/ha). The high doses would be a challenge for the logistics with these products.

Carbonates proofed to have a curative activity against apple scab (Hinze & Kunz, 2012; Hinze & Kunz, 2010; Kunz *et al.*, 2008; van Hemelrijck *et al.*, 2012; Kelderer *et al.*, 2010). No other substances suitable for organic growing with curative activity were identified. Mixtures of carbonates and sulfur and addition of additives increased the curative effect of carbonates (Hinze & Kunz, 2010; van Hemelrijck *et al.*, 2012) and these mixtures if applied to dry leaves will also have a protective effect on subsequent infection periods. This was also expected for mixtures of copper products and carbonates. But the greenhouse trials showed that such mixtures had reduced efficacy compared to carbonates if applied curatively and had reduced rain fastness and protective efficacy compared to copper products (Kunz & Hinze, 2014). These findings for Vitisan (potassium bi-carbonate) were confirmed for the formulated product Armicarb in this study. Therefore, copper products should not be used in tank mixtures with the carbonates. In contrast to the mixtures of copper with carbonates, mixtures of sulfur and copper performed very well in our test systems and could be recommended. However, as both copper and sulfur are used protectively the practical benefit is questionable. This study demonstrated, that alternatives to copper are still not found and that further efforts are also needed to characterize existing products. Knowledge on the behaviour under different weather conditions and in combination with different products should be available for reliable recommendations to the growers.

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