Assessment of new scab control strategies in greenhouse trials

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Abstract

*Control of apple scab (*Venturia inaequalis*) in organic apple production is mainly based on copper and sulphur sprays. A reduction of the total amount of metallic copper is intended. New products or strategies to replace copper sprays at least partially are needed. In a research project, funded in BÖLN, biocontrol agents were assessed for their efficacy against apple scab in greenhouse trials by artificial inoculation of potted apple trees. Applications were done protectively or curatively and the rain fastness of products and mixtures was tested after protective applications. The formulated yeast 2H13 raised the efficacy of low copper doses to the significance level of the tenfold copper dose after protective application of the mixtures, but did not increase the curative activity of Vitisan. Neu 1143F significantly reduced scab severity after curative applications. Addition of wettable sulphur to Neu 1143F increased its curative activity. Pre-emptive and repeated application of homoeopatic plant strengtheners to the irrigation water increased the curative efficacy of the mixture of Vitisan + wettable sulphur on potted apple trees. Herewith, new tools for scab control strategies were identified and strategies using these tools should be further tested in field trials.*

Keywords: Apple scab, *Venturia inaequalis*, 2H13, copper, Biplantol, Vitisan, Neu 1143F

Introduction

Apple scab, caused by *Venturia inaequalis* 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.*, 2016). 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 (van Hemelrijck *et al.*, 2012; Kunz & Hinze, 2010; Kunz & Hinze, 2014). The addition of wettable sulphur to carbonates increased the curative efficiency (Hinze & Kunz, 2010). On the other hand, the addition of copper to carbonates reduced its curative effect significantly (Kunz & Hinze, 2014). Furthermore the protective activity and rain fastness of copper products were reduced after adding carbonates in the tank mixture (Kunz & Hinze, 2016). These examples demonstrate, that tank mixtures of fungicides or additives to fungicides could have positive effects (additive or even synergistic) in increasing the efficacy of the mixture compared to stand alone treatments. But also negative effects, decreasing the efficacy in the mixture compared to the stand alone treatments are possible. Therefore, new fungicides, additives and mixtures were tested in greenhouse trials under controlled conditions to select promising strategies to be tested in field trials.

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Material and Methods

Potted apple trees (‘Jonagold’ or ‘Gala’), were kept in a greenhouse. Daylight was prolonged to 14 h by artificial illumination. 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 16°C to 25°C and 100% relative humidity for 20 h in the dark. 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).

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

Preparation	**rate [%]**	**Supplier**
Spray Applications		
Blossom Protect™ ; *Aureobasidium pullulans*	0.15	bio-ferm GmbH
Buffer Protect; citric acid buffer	1.0	bio-ferm GmbH
Cuprozin® progress (Cup); copper hydroxide	variable	Spiess-Urania GmbH
Netzschwefel Stulln (NSS); wettable sulfur	0.25	Biofa AG
Neu 1143 F;	2.0	Neudorff GmbH KG
Squall®; polyethylene glycol mixture	0.25	KoGa Klein-Altendorf
Vitisan®; potassium bicarbonate	0.5	Biofa AG
2H13; formulated yeast strain	0.02	Bio-Protect GmbH
Watering		
Biplantol® agrar (Bip a); homoeopathic agent	*0.2	Bioplant Naturverfahren GmbH
Biplantol® mykos V forte (Bip m); homoeopathic agent	*0.2	Bioplant Naturverfahren GmbH

*Dose rate added to the irrigation water

Test products were 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 h before inoculation (protective or for rain fastness testing) or 24 hours 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 h after application with 30 L/m^2 (30mm) of water, and were inoculated consecutively (Kunz et al., 2008).

16 to 21 days after inoculation, the disease severity 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 severity of 10 shoots per treatment was calculated.

Effect of homoeopathic plant strengthening agents were tested by adding 0.2% of Bip a or Bip m to water, which was poured into pots (200 mL/pot) of apple trees (‘Gala’) every 2 weeks (Figure 3). 2 Shoots per tree were allowed to develop and were inoculated with *V. inaequalis* twice as described above. 24 h after each inoculation parts of the trees were sprayed curatively with a mixture of 0.5 % Vitisan + 0.25% NSS. Infections caused by *P. leucotricha* occurred naturally in the greenhouse. Two months after the first application of Bip’s, the shoots were removed and disease severity for *V. inaequalis* and *P. leucotricha* for each shoot was calculated as the average of the proportion of the diseased leaf area of all leaves. Biweekly pour treatments were continued using a mixture of 0.2% Bip a + 0.2% Bip m in the water for 8 weeks and new shoots emerged. Inoculation with *V. inaequalis* was done 5 weeks after the first treatment and the curative application of 0.5% Vitisan + 0.25% NSS 24 h after inoculation. All shoots were removed and evaluated as described above 7 weeks after the inoculation.

Data analyses: The efficacy of the treatment was calculated by comparing the disease severity with the untreated control according to Abbott (Abbott, 1925).
The data set of each experiment was tested for equal variances using Bartlett's test. If variances were inhomogeneous, data were square root transformed. Data sets with homogeneous variances were subjected to a parametric analyses of variance followed by means separation by Tukey's Multiple Comparison Test ($p<0.05$). Otherwise, a non parametric analyses of variance (Kruskal Wallis-procedure) was computed, followed by means separation by Dunn`s Multiple Comparison Test ($p<0.05$).

Results

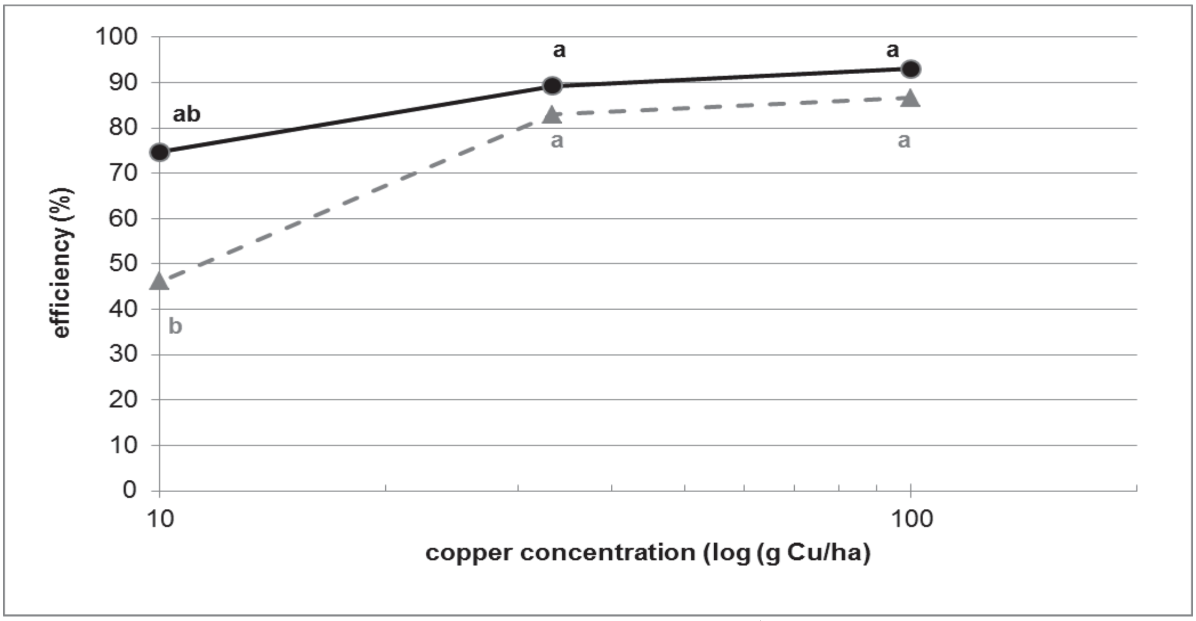


Figure 1: Dose-response relationships of Cuprozin progress (▲) without and with addition of 2H13 (●) if applied to apple shoots protectively just before inoculation with *V. inaequalis* conidia. Different letters indicate significant differences between all treatments in Dunn's Multiple Comparison test ($p<0.05$).

The formulated yeast 2H13 tended to increase the efficiency of Cuprozin progress (Cup) in different doses after application of tank mixtures to apple shoots 1 h before inoculation with *V. inaequalis* conidia. While 0.004 % Cup (corresponds to 10 g metallic copper/ha) was significantly less effective as 0.013 % or 0.04 % Cup, the mixture of 0.004 % Cup + 0.02 % 2H13 was on the same significance level as the higher copper doses (Figure 1). Applying Cup or the tank mixtures 20 h before inoculation with a subsequent spray irrigation of 30 mm 1h before inoculation, revealed no significant effects from the additives 2H13 or Squall (Figure 2).

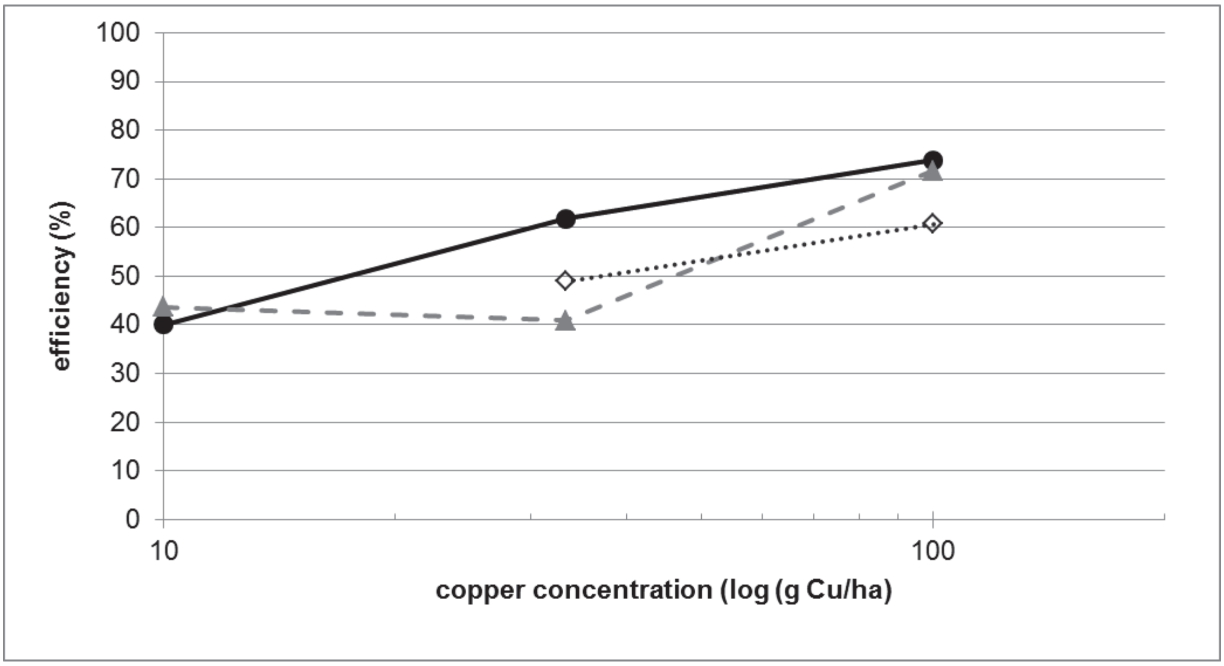


Figure 2: Dose-response relationships of Cuprozin progress without (▲) and with addition of 2H13 (●) or Squall (◇), if applied to apple shoots protectively 18 h before inoculation with *V. inaequalis* conidia. Shoots were affected by 30 mm rain 1 h before inoculation. No significant differences between the treatments as indicated by Dunn's Multiple Comparison test (p<0.05).

Table 2: pH-value in the spray suspension, scab severity (% ± standard deviation) on apple leaves and efficiency of the treatment after curative application.

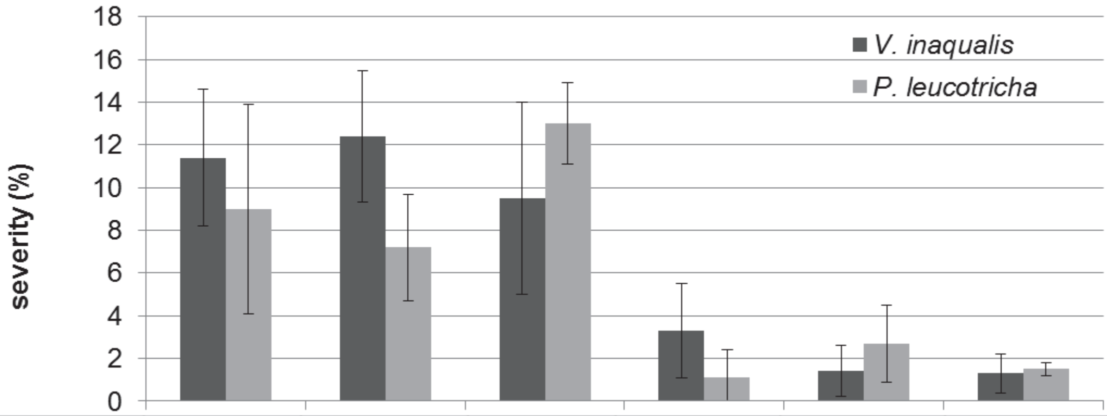
Treatment	pH	Rate (%)	Severity (%)	Efficiency (%)	*Tukey's
untreated		-	29.3 ± 11.5		a
Vitisan	8.7	0.5	3.1 ± 3.8	**89**	b
Neu 1143F	6.5	2	6.6 ± 6.0	**78**	b
Neu 1143F + Vitisan	8.1	2 + 0.5	3.3 ± 4.7	**89**	b
Neu 1143F + Buffer Protect	3.7	2 + 1	10.4 ± 10.3	**64**	b
Neu 1143F + Squall	6.2	2 + 0.25	11.7 ± 11.8	**60**	b
Neu 1143F + NSS	6.1	2 + 0.25	3.2 ± 7.3	**89**	b
Vitisan + 2H13	8.8	0.5 + 0.02	4.0 ± 3.6	**87**	b
Vitisan + Blossom Protect	nd	0.5 + 0.15	6.1 ± 6.7	**79**	b
Vitisan + Squall	8.8	0.5 + 0.25	9.3 ± 8.1	**68**	b

*Different letters indicate significant differences in Tukey's Multiple Comparison Test (<0.05) after square root transformation.
nd=not determined

Vitisan and Neu 1143F reduced scab incidence significantly after curative applications 24 h after inoculation. Tank mixing both products did not increase efficacy. The performance of Neu 1143F tended to be improved by the addition of wettable sulfur to the spray suspension, but not by addition of Squall or Buffer Protect. 2H13, Blossom Protect or Squall did not increase the efficacy of Vitisan after curative applications (Table **2**).

Evaluation of all leaves of untreated apple shoots ('Gala') after 8 weeks growth in the green house and two inoculations with V. inaequalis revealed a severity of 11% and 9% for *V.*

inaequalis and *P. leucotricha* (Figure 3), respectively. Whereas 4 pour treatments with Bip a or Bip m did not significantly reduce disease severity, curative applications of the mixture Vitisan + NSS significantly reduced severity of both pathogens as well as the combinations of pour treatments with Bip's and curative applications of Vitisan + NSS. Highest efficiencies against apple scab (88%) were found with the combinations of Bip's with curative applications of Vitisan + NSS (Figure 3).



efficiency (%)		0 ; 21	17 ; 0	71 ; 88	88 ; 71	88 ; 83
Tukey's; Dunn's	a ; ab	a ; ab	a ; a	b ; c	b ; bc	b ; bc
21.03.2017	-	Bip a	Bip m	-	Bip a	Bip m
03.04.2017	-	Bip a	Bip m	-	Bip a	Bip m
06.04.2017	Inoculation *V. inaequalis*					
07.04.2017	-	-	-	Vitisan+NSS	Vitisan+NSS	Vitisan+NSS
19.04.2017	-	Bip a	Bip m	-	Bip a	Bip m
19.04.2017	Inoculation *V. inaequalis*					
20.04.2017	-	-	-	Vitisan+NSS	Vitisan+NSS	Vitisan+NSS
03.05.2017	-	Bip a	Bip m	-	Bip a	Bip m
18.05.2017	Evaluation					

Figure 3: Severity (% ± standard deviation) of *V. inaequalis* or *P. leucotricha* (natural infections) on 6-8 apple shoots per treatment. Bip a or Bip m were applied by watering the plants. Vitisan + NSS were sprayed 24h after inoculation with *V. inaequalis*. Different letters indicate significant differences between treatments in Tukey's or Dunn's Multiple Comparison Test (<0.05), respectively.

In the second experiment, disease severity was 11% and 2% for *V. inaequalis* and *P. leucotricha,* respectively. No significant effects were seen against *P. leucotricha* from any treatment, due to the low infection pressure. Pouring the pots with the mixture of Bip a + Bip m again did not reduce apple scab symptoms. However, the curative application of Vitisan + NSS (efficiency 77%) as well as the combination of pouring with Bip a + Bip m in addition to curative application of Vitisan + NSS (efficiency 82%), significantly reduced apple scab severity, revealing in the highest efficiency in the combination.

Discussion

In organic apple growing, scab control is based on the protective use of copper and sulphur, complemented by the so called stop applications with Curatio (lime-sulphur) during the germination of ascospores (Kunz & Hinze, 2016; Zimmer *et al.*, 2016). As the use of copper is under discussion for ecological purposes, new copper formulations, and additives to copper are under investigation to reduce the input of metallic copper (Cu) in apple orchards.

In the greenhouse trials, copper dose rates corresponding to 100 g Cu/ha were the lower limit for a satisfying disease control (Kunz & Hinze, 2016). For a further reduction alternative control agents or partners for mixtures are needed.

The yeast strain 2H13 increased the efficacy of low copper doses against *Phytopthora infestans* in a tomato leaf disc assay and against *Pseudoperonospora cubensis* in potted cucumber, if applied in tank mixtures (Kunz *et al.*, 2016). In this study, 2H13, formulated as wettable granules, was tested in combination with Cuprozin progress in different doses against apple scab, and the positive effect of the Yeast on low copper doses was confirmed. The efficacy of a copper dose corresponding to 10 g Cu/ha was raised to the same significance level as 100 g Cu/ha by adding 2H13 to the spray suspension for a protective application (Figure 1). This indicates that a copper reduction to 10% would be possible, after adding 2H13. However, irrigation of the apple shoots 20 h after application of the mixtures of 2H13 + Cup and 1 h before inoculation with *V. inaqualis*, revealed in reduced efficacies of the mixtures (Figure 2), compared to the trial without rain event (Figure 1). The effect of 2H13 on the efficacy of copper was only visible with the dose of 33.3 g Cu/ha in the trial with rain event. This indicates that in the field, were scab infections are always linked to rain events, higher doses of copper will be needed, to get the additive effect of 2H13. In field trials a copper reduction to 50% should be tested with 2H13.

After 30 mm rain, the sticker Squall had no positive effect on the efficacy of Cuprozin progress in both doses tested (Figure 3). Furthermore, Squall was added to Neu 1143 or Vitisan for curative applications against apple scab and again no positive effects were visible (Table 2).

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,). Mixtures of carbonates and sulphur 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. Neu 1143F reduced scab severity significantly after curative application and its efficiency was close to that of Vitisan (Table **2**). The mixture of Neu 1143F + Vitisan did not increase the efficacy compared to Vitisan as stand-alone treatment. The pH-value in the tank mixture Vitisan + Neu1143 F was slightly reduced compared to that of Vitisan alone. As the high pH-value is crucial for the efficacy of Vitisan, further investigations should be done before the tank mixture with Neu 1143F could be recommended. Although, the yeast preparations 2H13 or Blossom Protect did not decrease the pH of Vitisan in the tank mixture, they had no positive effect on its curative activity against apple scab.

Primary stroma of *V. inaequalis* develop under the cuticle of apple leaves (Kunz *et al.*, 1997). To stop the infection curatively, the active ingredient has to reach the primary stroma under the cuticle. Spray suspensions of Neu 1143 F had a nearly neutral pH of 6.5. As transport of active substances through the cuticle could be triggered by pH, the addition of Buffer Protect was tested, which decreased the pH-value in the spray suspension to 3.7. Buffer Protect had no positive effect on the efficacy of Neu 1143F. Addition of wettable sulphur increased the curative efficacy of Neu 1143 F (Table **2**). This positive effect of wettable sulphur on curative treatments is known also for Vitisan and other carbonates (Kunz & Hinze, 2014). However, the efficacy of curative treatments with carbonates + wettable sulfur in field trials were often not satisfying (Zimmer *et al.*, 2016). Therefore, strategies are needed to further increase the curative activity of the known products.

Pre-emptive and repeated applications of homoeopathic plant strengthening remedies represent an environmentally friendly tool to contribute to plant health (Würthle & Borlinghaus, 2017). The homoeopathic plant strengthening agents were solved in water and poured into the pots. In the first trial, Biplantol agrar and Biplantol mykos V forte were compared, and in the second trial a mixture of the two products were used. In both trials, the homoeopatic agents did not significantly reduce disease severity of *V. inaequalis* or *P. leucotricha* on shoots of potted apple trees. Curative applications of the mixture Vitisan + NSS significantly reduced severity of both pathogens as well as the combinations of pour treatments with homeopathic agents and curative applications of Vitisan + NSS. In both trials, the pre-emptive applications of Biplantol increased the curative efficacy of Vitisan + NSS.

With the yeast 2H13 as additive to copper, Neu 1143F as a curative fungicide and the homeopathic plant strengtheners, new tools for scab control strategies were identified in this study. Strategies should be further tested in field trials.

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