# Assessment of biocontrol agents for their efficacy against apple scab

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## Abstract

*Control of apple scab (*Venturia inaequalis*) in organic apple production is based on copper and sulphur 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 18 hrs before inoculation, during the germination 5 hrs after inoculation during simulated rain fall or curatively 24 hrs after inoculation. In addition, the efficacy of protective applications followed by simulated rain was tested to proof the rain fastness of the products. 36 different products were involved in the tests during the last four years.*

*The optimal timing of application differed between the preparations tested. Copper products had the highest efficiency when tested protectively. 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 to improve the rain fastness of these products.*

*Applications during the germination of the fungus were effective using sulphur products. Only one of the alternative products tested reduced scab symptoms under these conditions. Carbonates reached their highest activity when sprayed curatively 24 hours after inoculation. Combinations with sulphur and stickers improved the curative activity of carbonates. No other substances were identified with high curative activity. Mixtures of copper and carbonates reduced the protective effect and rain fastness of copper products as well as the curative effects of carbonates.*

**Keywords:** apple scab, *Venturia inaequalis*, 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 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 (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 (Hemelrijck *et al.*, 2012; Hinze and Kunz, 2010; Hinze and Kunz, 2011; Hinze and Kunz, 2012). The highest efficiency of Vitisan (potassium bicarbonate) and Omni Protect (potassium carbonate) was found when applied on dry leaves 24 hrs after inoculation. The addition of wettable sulphur to carbonates increased the curative efficiency (Hinze and Kunz, 2010).

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A screening for alternative products in the green house confirmed that sulphur and copper components had the highest protective efficiency and some additives like Algo Vital, Saponin, Nu-Film P or Goemar fruton increased the rain fastness of wettable sulphur (Hinze and Kunz, 2010).

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 for protective or curative efficacy against apple scab was continued during the last four years.

## Material and Methods

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

| Preparation | rate [%] | Supplier |
|---|---|---|
| Algicin, seaweed extracts | 0.2 | LfULG Dresden-Pillnitz |
| Algo Vital Plus (AlgoVital); seaweed extracts | 0.5 | Biofa AG |
| Antica; Alpha-Hydroxysäuren | 0.5 | ChiPro GmbH |
| Armicarb®; formulated Potassium bicarbonate | 0.5 | Spiess-Urania GmbH |
| Cera-Schwefal® 700 flüssig (Cera-Schw.); sulphur | 0.338 | KoGa Klein-Altendorf |
| Chitoplant; Chitosan | 0.1 | ChiPro GmbH |
| Cuprozin® flüssig; copper hydroxide | | Biofa AG |
| Cuprozin® progress (Cup.prog); copper hydroxide | 0.12* | Spiess-Urania GmbH |
| Cuprum; copper | 0.1 | KoGa Klein-Altendorf |
| Funguran®; copper oxychloride | | Biofa AG |
| Funguran® progress (Fung.prog); copper hydroxide | 0.086* | Spiess-Urania GmbH |
| HF-Pilzvorsorge (HF-Pilzv.); fennel oil | 0.4 | Biofa AG |
| Hydrocal super (Hydrocal); hydrated lime | Dusted | Verblasetech. Schneider |
| Kaliwasserglas (KaliWG); silicic acid and $K_2O$ | 0.5 | Biofa AG |
| Kumulus® WG (Kumulus); wettable sulphur | 0.25 | BASF AG |
| Lime sulphur | 1.5 | Biofa AG |
| LMA; potassium-aluminium-sulphate | 2 | Chevita GmbH |
| Netzschwefel Stulln (NSStulln); wettable sulphur | 0.25 | Biofa AG |
| Nu-Film P (NuFP); pinolene | 0.03 | Intrachem Bio, Germany |
| Omni Protect (Omni); potassium carbonate | 0.5 | Bio-Protect GmbH |
| P1-Extrakt; extract from *Glycyrrhiza glabra* | 5 | Trifolio-M GmbH |
| Phyto Vital; Lignin derivates | 2 | Ligmeda Consult |
| Phytocare; extract from *Macleaya cordata* | 0.5 | Proagro GmbH |
| Plantacare; surfaces active agents | 0.52 | Bio-Protect GmbH |
| Potassium hypo chlorite (KCLO); | 8 | KoGa Klein-Altendorf |
| SAP-Ex; extract from *Sapindus saponaria* | 5 | KoGa Klein-Altendorf |
| Saponin; extract from *Yucca schidigera* | 1 | KoGa Klein-Altendorf |
| Sergomil L60 ; copper fertilizer | 0.15 | Proagro GmbH |
| Steinhauers Mehltauschreck (NaHCO3); sodium bicarbonate | 0.5 | Biofa AG |
| Temauxin AS; acetylsalicylic-acid | | Temmen GmbH |
| Temauxin S; salicylic-acid | 2 | Temmen GmbH |
| Trifolio TS-forte (TS-forte) | 0.25 | Trifolio-M GmbH |
| Ulmasud®; acidic rock powder | 1 | Biofa AG |
| Vacciplant®; laminarin | 0.075 | Belchim crop protection |
| Ventex; formulated potassium carbonate | 0.6 | Bio-Protect GmbH |
| Vitisan®; potassium bicarbonate | 0.5 | Biofa AG |

*This Copper compounds were used with 300 g cu/ha.

Potted apple trees of the cultivar Jonagold, were kept in a greenhouse. Daylight was prolonged by artificial illumination so that growing shoots were available during the whole year. 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 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).

Test products were usually supplied by the companies or by the project leader (Jürgen Zimmer, KoGa Klein-Altendorf) (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 hrs after inoculation during simulated rainfall (stop application) or 24hrs after inoculation on 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 17hrs 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. 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 (1925). Experiments were done at least twice and the average efficiency is given in the figures. The data reported here were acquired from 2010 to 2013.

## Results

Twenty-one products were tested for their protective activity against apple scab in comparison to Netzschwefel Stulln (NS Stulln), which reduced apple scab symptoms by 98 % in average (Hinze and Kunz, 2010). Nine products showed comparable or higher efficiency than NS Stulln. Three new copper products (Funguran progress, Cuprocin progress, Sergomil), two sulphur products (Cera-Schwefal and lime sulphur), three plant extracts (P1-Extract, Phytocare, SAP-Ex) and a formulated potassium carbonate (Ventex) (fig. 1). Artificial rain (30 mm) between application and inoculation reduced the efficiency of NS Stulln from 98 % to 89 % (Hinze and Kunz, 2010), indicating that rain fastness of the protective products is crucial for a good performance in the field. The efficiencies of lime sulphur, Fung. prog. and Cup. prog. were not reduced by 30 mm rain between application and inoculation, whereas the efficiencies of the other products was reduced to 25 % in worst case (fig. 1). SAP-Ex, Cera-Schw. and P1-Extract were so far not tested for rain fastness. This should be done before the products can be recommended for the field.

We tested also additives to NS Stulln to enhance rain fastness identifying Goemar fruton, Nu-Film P, Saponin and Algo-Vital as suitable additives in the 2010 study (Hinze and Kunz, 2010). In the recent experiments TS forte enhanced the rain fastness and the efficacy of NS Stulln best. In contrast mixtures of copper products with Vitisan showed reduced efficiency with or without rain compared to the application of copper products alone (fig. 1).

In 2013 the new copper formulations Fung. prog. and Cup. prog. were introduced. Dose response relationships of these new products compared to Funguran and Cuprocin flüssig showed a higher efficacy of the new products after protective applications if the amount of metallic copper was reduced to 100g/ha or even to 30g/ha (fig. 2). The copper fertilizer Sergomil showed also a high efficiency with low amount of copper in this experiment. However, its rain fastness is not sufficient for a good performance in the field (fig. 1).

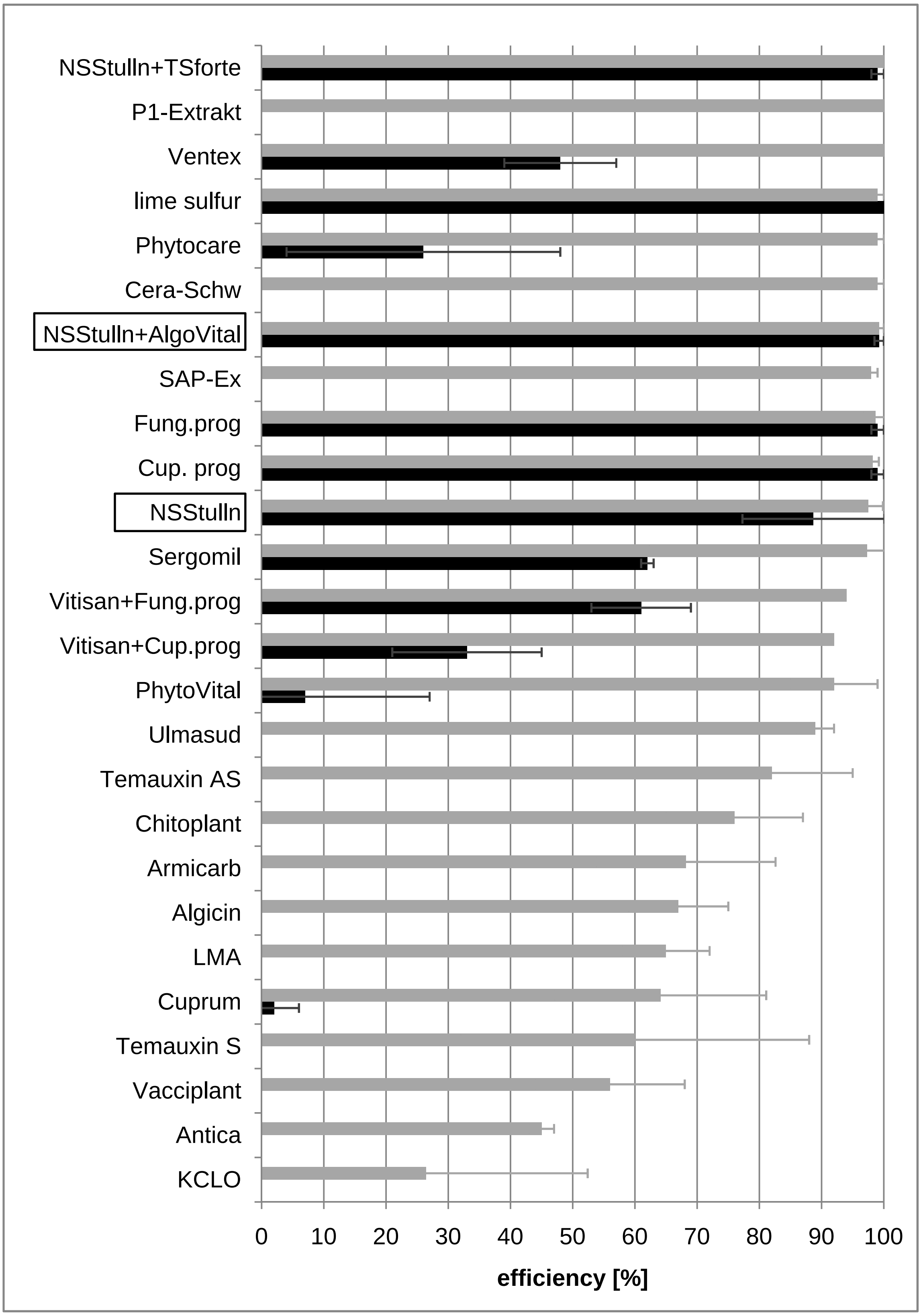


Figure 1: Efficiency of preparations and mixtures applied to apple shoots protectively, 18 hrs before inoculation with *V. inaequalis* conidia without (grey columns), or with 30 mm overhead irrigation (black columns) before the inoculation. If black columns are missing, the compound was not tested with irrigation. Average and standard deviation from at least two trials are given. Standard preparations (Hinze and Kunz, 2010) are framed.

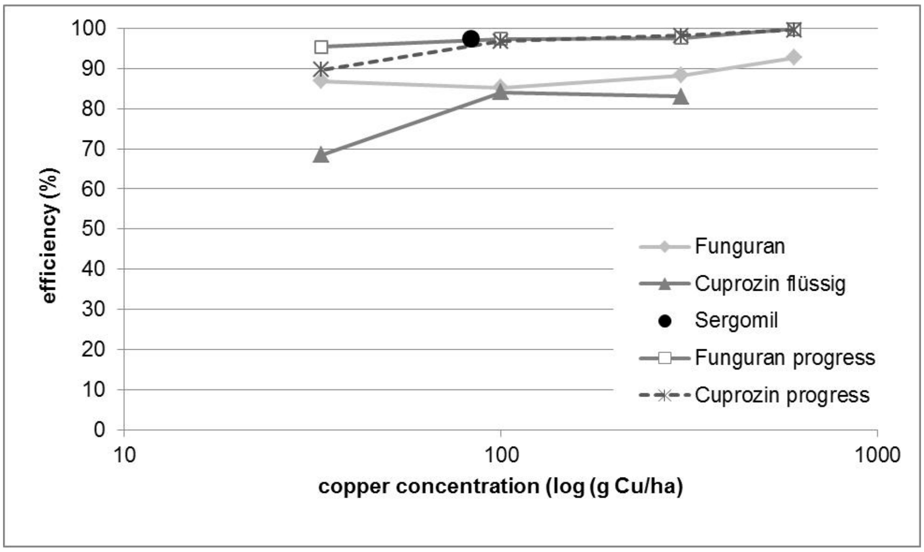


Figure 2: Dose-response relationship of copper products if applied to apple shoots protectively, 18 hrs before inoculation with *V. inaequalis* conidia. Averages from at least two trials performed in 2012 and 2013 are given.

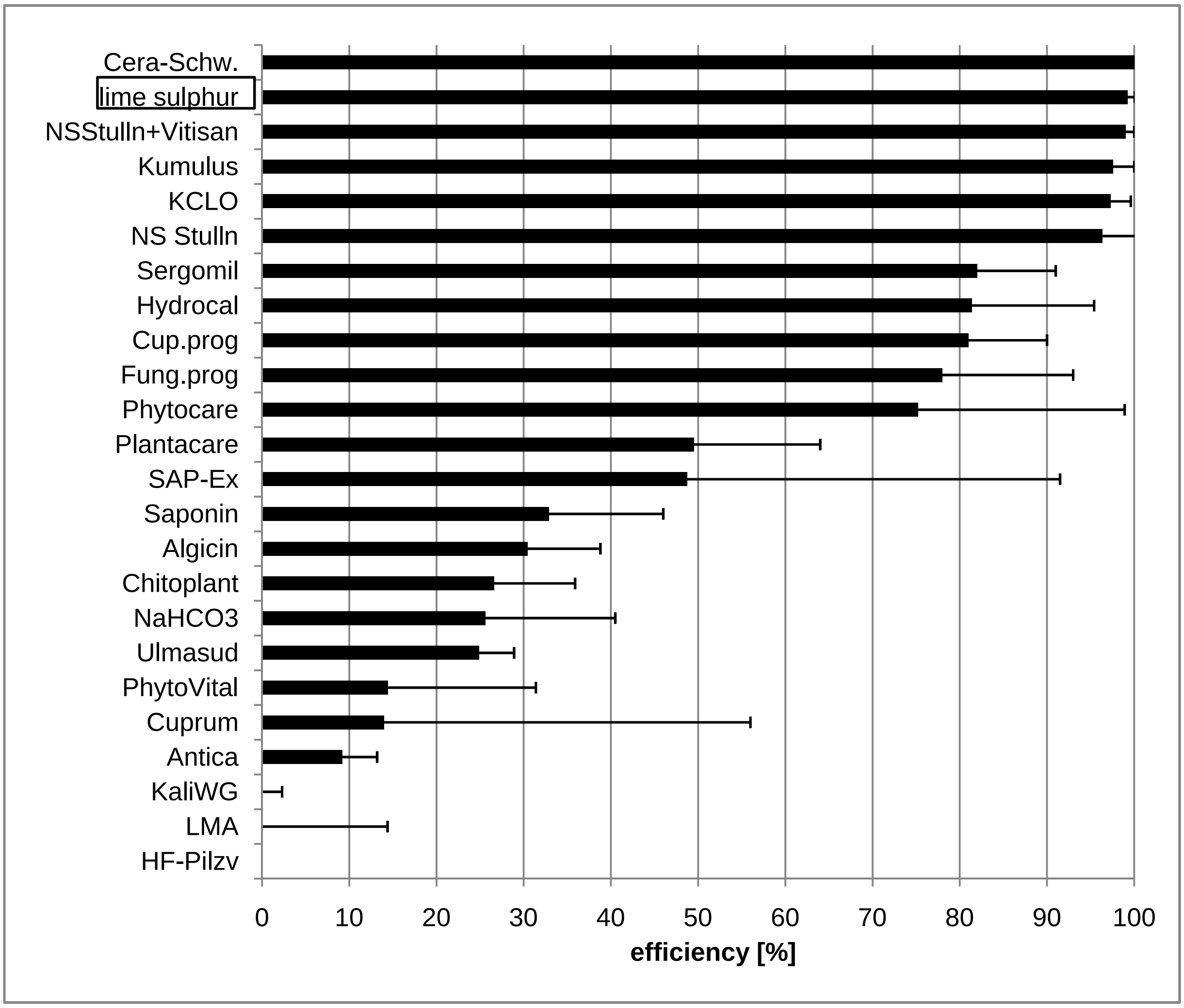


Figure 3: Efficiency of preparations and mixtures applied 5 hrs after inoculation to apple shoots during the germination of conidia of *V. inaequalis*. Application was done during overhead irrigation. The average and standard deviation from at least two trials are given. Standard preparations (Hinze and Kunz, 2010) are framed.

Twenty-three products or mixtures were tested during the germination period of the scab fungus under artificial rain in comparison to lime sulphur. Only the sulphur products (Cera-Schw., NSStulln+Vitisan, Kumulus, NS Stulln) and the Potassium hypo chlorite (KCLO) reduced scab symptoms under these conditions by more than 95 %. The copper products and all the others showed reduced effects under these conditions (fig. 3).

Thirteen products or mixtures were tested curatively 24 hrs after inoculation on dry leaves in comparison to carbonates and the combination Omni Protect + NS Stulln + NuFilm P, which reduced scab incidence best under these conditions (Hinze and Kunz, 2012). None of the new tested products reduced scab symptoms comparable to the carbonates. To get worse, the addition of a copper product to a carbonate reduced the curative effect of the carbonates (fig. 4).

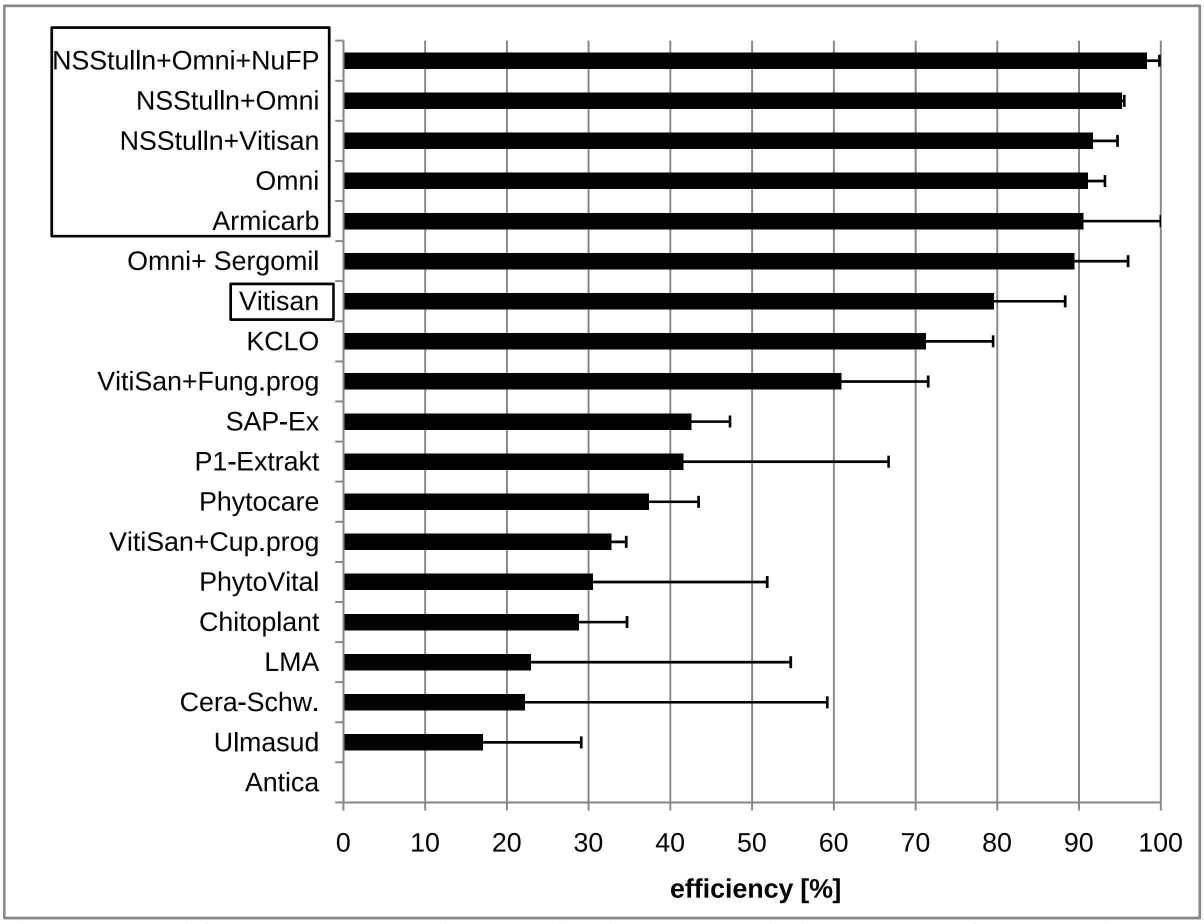


Figure 4: Efficiency of preparations and mixtures applied to apple shoots 24 hrs. after the inoculation with *V. inaequalis* conidia on dry leaves. The average and standard deviation are given. Standard preparations (Hinze and Kunz, 2010) are framed.

## 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. With new copper formulations recently introduced to the market, the input of metallic copper can be reduced (Zimmer *et al*., 2012). In addition, alternative control agents are needed. Wettable sulphur 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 (Hinze and Kunz, 2010), as scab infections are always accompanied with rain fall and by this removing of active ingredients from the leaves by washing. Some plant extracts (P1-

Extract, Phytocare, SAP-Ex, Saponin) showed promising effects after protective applications. However, the products are not ready for commercialization at the moment. Suitable formulations are needed which improve the rain fastness and stability of the products.

Lime sulphur showed good rain fastness if applied to dry leaves and it is highly efficient if applied protectively and during germination of the fungus. Applications during germination are done to wet leaves or during rain fall and under these conditions no lime sulphur coverage of the leaves to control subsequent infections will be reached (data not shown). Therefore during rain periods of several days, applications have to be done daily what is not further allowed with lime sulphur. Alternative products are needed for this purpose. Only wettable sulphur products and KCLO showed activity if applied during the germination period of the scab fungus in our study.

Carbonates proofed to have a curative activity against apple scab (Hemelrijck *et al*., 2012; Hinze and Kunz, 2010; Hinze and Kunz, 2012; Kunz *et al*., 2008). No other substances suitable for organic growing with curative activity were identified. Mixtures of carbonates and sulphur and addition of additives increased the curative effect of carbonates (Hemelrijck *et al*., 2012; Hinze and Kunz, 2010) 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 rain fastness and protective efficiencies compared to copper products and the mixtures had reduced efficacy compared to carbonates if applied curatively. We assume that this incompatibility is caused by different pH-values needed by the different products. Besides the search for new alternative products for scab control in organic apple production further effort is 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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