Evaluation of the efficacy of plant protection products against *Marssonina* blotch

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Abstract

In South Tyrol the first symptoms of Marssonina *blotch on apple leaves caused by* Marssonina coronaria *were observed in the year 2011. The disease with origin in Asia leads to a premature defoliation of infected trees only a few weeks after its outbreak.*

In order to find a reasonable way to control the pathogen in organic farming and to know more about the vulnerability of Marssonina coronaria *in future plant protection strategies, the efficacy of different plant protection products was evaluated in laboratory and in a field trial.*

The efficacy of different plant protection products allowed in organic farming against the described pathogen was analysed in laboratory with spore germination tests.

In addition to a calculated dosage of the respective product, dilutions of 30 %, 10 %, 3 %, 1 %, 0.3 % and 0.1 % of the applied dosage were tested. With generated dose-effect curves the fungicide potential of the tested products was calculated.

The evaluation of the efficacy of eight organic plant protection products was conducted in a field trial in 2013.

In both studies the best results were obtained with the acid clay-based product Ulmasud. But also lime sulfur and copper in different formulations provided an interesting effect on the pathogenicity of Marssonina coronaria*.*

Keywords: *Marssonina*, apple, spore germination test, field trial

Introduction

Marssonina blotch is a plant disease of different crops with the origin in Asia caused by *Marssonina coronaria*, the anamorph of *Diplocarpon mali*. In Italy, *Marssonina* blotch on apple trees was described first in 2001 in the region of Piemonte. In 2010, *Marssonina* blotch was detected in Switzerland and Germany, in 2011 in some regions of Austria (Steiermark). In 2011, the first symptoms of *Marssonina* blotch on apple leaves occured in South Tyrol. The infection with the fungal pathogen results in a quick shedding of leaves and the reduction of apple quality and quantity (Lindner, 2012). *M.coronaria* forms acervuli on leaves in June and July, containing asexual spores. After the break up of the fungal epidermis, numerous two-celled conidia are released and infect new leaves. In the later season microconidia (=spermatia) are formed in the acervuli, which lead after infection of leaves to the formation of the teleomorph *Diplocarpon mali*. The ascomycet forms ascospores at the end of april, leading to new infection of apple trees and the development of new acervuli (Lindner, 2012).

Material and Methods

In-vitro spore germination testing of different fungicides

In the spore germination tests, two-celled conidial spores of ten different strains of *Marssonina coronaria* were analysed. The fungal strains were taken from the strain bank

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of the Research Centre for Agriculture and Forestry Laimburg coming from different apple varieties in different locations in South Tyrol.

Spores of ten *Marssonina*-strains were incubated on Potato Dextrose Agar (PDA) for two weeks at 25°C. The visible Acervuli of *Marssonina coronaria* were cut into pieces and transferred to 1 mL aqua deion with an inoculation loop.

The solution containing the spores was shaked at 2000 rpm/sec on a laboratory shaker for 20 seconds. Out of the solution of the 10 different strains a spore mixture in equal parts with a cell density of 10^5- 10^6 cfu mL^{-1} was prepared.

The spore mixture was mixed 1:2 with a certain concentration of the respecitive fungicide and so a fungicide-spore mixture was prepared, to test its influence on germination rate of *Marssonina coronaria.*

30 µL of the fungicide-spore mixture were covered with a cover-glass on a polystyrole Petri dish in triple repetition.

The Petri dish was sealed with parafilm and put in a plastic box with wet cellulose paper to save the fungicide-spore mixture from drying out. The box was incubated for 48 hours at 25 °C. During the evaluation, 50 spores per repetition were counted and the germinated spores were quantified. As control, 30 µL of the diluted spore-mixture with a. deion was evaluated in triple repetition.

The four products with copper as active ingredient were all adjusted to 20 g of pure copper, in order to compare the four different formulations: copper oxychloride, copper hydroxide, copper sulphate and a liquid formulation of a product containing copper (Table 1).

Table 1: Overview of the plant protection products tested in the spore germination tests. In addition to the applied dose, dilution steps of 30 %, 10 %, 3 %, 1 %, 0.3 % and 0.1 % of applied dose were analysed.

Product name	applied dose (g-ml/hl)	Active ingredient	Distributor
Coprantol WG	62.5 (20 g pure Cu)	Copper oxychloride	Syngenta
Kocide 3000	43,4 (20 g pure Cu)	Copper hydroxide	Dupont
Poltiglia disperss	100 (20 g pure Cu)	Copper sulfate	Cerexagri
Heliocuivre	50 (20 g pure Cu)	Copper hydroxide – liquid formulation	Intrachem Biogard
Polisolfuro di calcio	800	Lime sulfur	Polisenio
Ulmasud	1000	Acid clay	Biofa
Fosfidor	400	Potassium phosphite	Agrimport
Vitisan	1000	Potassium bicarbonate	Biofa
Armicarb	500	Potassium bicarbonate	Helena Chimical
Mehltauschreck	1000	Sodium bicarbonate	Biofa
Wasserglas	1000	Sodium silicate	Thaler

In addition to a calculated dosage of the fungicide, dilution steps of 30 %, 10 %, 3 %, 1 %, 0.3 % and 0.1 % of the applied dosage were analysed in relation to the control reference.

After the fall down of effect under 50 % compared to the control reference, the evaluation was stopped, because the fungicide had no more relevant effect on the pathogen. Only Conidia, whose germ tube exceeded the half of the overall conidial length under the microscope at a magnification of 400, were counted as germinated.

Efficacy of different fungicides against *Marssonina* blotch in a commercial orchard

In 2012, a pre-screening test was performed in order to determine the appropriate products for the field trial in 2013. The application of products in 2012 was performed with a backpack sprayer in a commercial orchard in Gargazon (South Tyrol, Italy) with the apple cultivar Jazz.

Building on the results of the pre-screening test in 2012, an experimental trial in a commercial orchard with the apple cultivar Gold Rush was conducted in 2013 to study the efficacy of eight different plant protection products (Table 2). In addition to the eight products, four plant protection products provided by different companies, which are part of the project CO-FREE were carried out.

The orchard is situated in Ora (South Tyrol, Italy). The experimental products were applied once per week, starting on June 4th until October 7th. In total 19 treatments were carried out. A commercial motorized sprayer for experimental trials and a spray volume of 500 l water per meter canopy height were used. The application was preventive on dry leafs.

Table 2: Overview of the plant protection products tested in an experimental field trial in 2013 in 19 treatments from June 4th to October 7th.

Product name	Active ingredient	applied dose (g-ml/hl)	Distributor	application
Poltiglia disperss	Copper	50 (10 g pure Copper)	Cerexagri	1 x week
Polisolfuro di calcio	Lime sulfur	1000	Polisenio	1 x week
Ulmasud	Acid clay	1000	Biofa	1 x week
Vacciplant	Laminarin	75	Goimar	1 x week
Manzic	Mn, Zn	400	Protema Agri	1 x week
Vitisan	Potassium bicarbonate	1000	Biofa	1 x week
Armicarb	Potassium bicarbonate	300	Helena Chimical	1 x week
Amylo-X	Bacillus amyloliquefaciens D747	170	Intrachem	1 x week
Untreated Control	-	-	-	-

A randomised block design with two replicates of seven trees and two boarder trees each experimental unit was determined. Additional boarder rows prevented the individual experimental units from biasing.

The overall leaf fall (scale from 0-10; 0 = no leaf fall, 1 = 10 % of the leafs were fallen etc.) was visually estimated in the experimental units of the orchard on October the 14th.

The recorded data were analysed using a one-way ANOVA, followed by a Tukey HSD post hoc test.

Results

In-vitro spore germination testing of different fungicides

All products tested in spore germination tests (except Fosfidor) had a direct inhibitory effect on the germination of two-celled conidiospores of *M. coronaria*. Spore germination was inhibited by using the applied dosage of the products.

The products with the highest calculated fungicide potential in the laboratory test were Ulmasud and Polisolfuro di calcio (Table 3).

Table 3: Calculated fungicide potential of the products used in the spore germination tests

Product name	Applied dose (g/hl)	Minimal dose for 100% inhibiton of spore germination (g/hl)	fungicidal potential of applied dose
Coprantol WG	62.5 (20 pure Cu)	18,9 (6 pure Cu)	x3.3
Kocide 3000	43,4 (20 pure Cu)	13,2 (6 pure Cu)	x3.3
Poltiglia disperss	100 (20 pure Cu)	30,3 (6 pure Cu)	x3.3
Heliocuivre	50 (20 pure Cu)	15,2 (6 pure Cu)	x3.3
Polisolfuro di calcio	800	8	x100
Ulmasud	1000	3	x333
Fosfidor	400	-	no effect
Vitisan	1000	300	x3.3
Armicarb	500	150	x3.3
Mehltauschreck	1000	300	x3.3
Wasserglas	1000	1000	0

With the applied dose of 800 g/hl of the product Polisolfuro di calcio, the efficiency rate was 100 %. With 8 g/hl of the product the efficiency was still 100 %, which leads to a calculated fungicide potential of 100 (Figure 1).

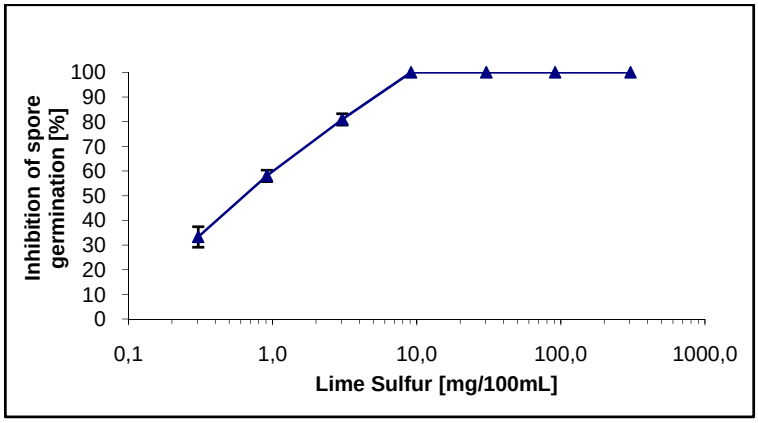

Figure 1: Dose-effect curve with std. deviation of the fungicide Polisolfuro di calcio (800 g/hl) regarding the inhibition of spore germination of two-celled conidia of *M. coronaria*.

The fungicide with the highest fungicide potential in spore germination tests against *M. coronaria* was the acid clay based product Ulmasud. Up to a delution of 0.3 % the product showed an inhibition of spore germination of 100 % (Figure 2). This equals to 3 g/hl of product, so that the fungicide potential is 333 in relation to the applied dose of the product (1000 g/hl)

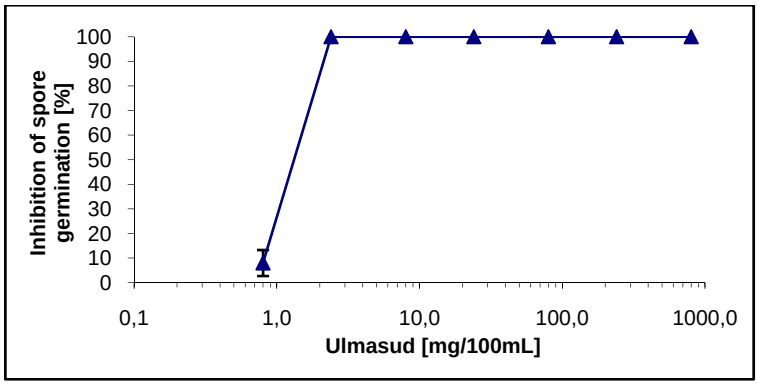

Figure 2: Dose-effect curve with std. deviation of the fungicide Ulmasud (1000 g/hl) regarding the inhibition of spore germination of two-celled conidia of *M. coronaria*.

The four products with copper as an active ingredient were all adjusted to 20 g of pure copper in the spore germination tests, in order to compare the different formulations copper oxychloride, copper hydroxide, copper sulphate and a liquid formulation of a copper containing product. Regarding the fungicide potential of the different formulations, there were no differences between the tested products. The 100 % inhibition of spore germination of copper against *M. coronaria* was determined to at least 6 g/hl. Starting from an applied dose of 20 g/hl, a fungicide potential of 3.3 can be calculated for all four tested copper formulations (Figure 3).

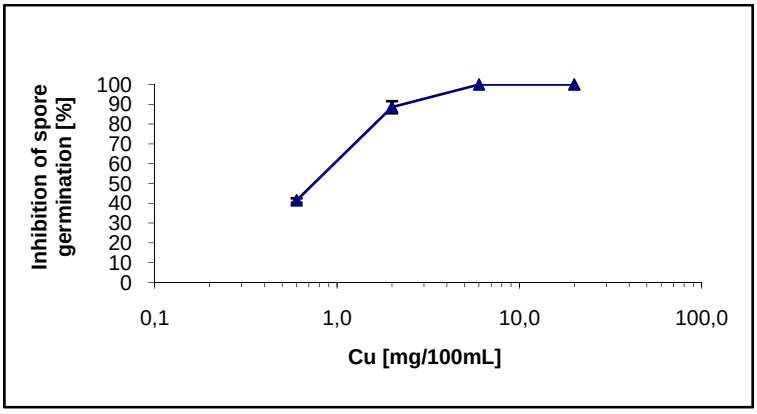

Figure 3: Dose-effect curve with std. deviation of the fungicide Poltiglia disperss (20 g/hl pure copper) regarding the inhibition of spore germination of two-celled conidia of *M. coronaria*.

Fosfidor had no direct effect on the germination of the two-celled conidiospores of *M. coronaria*, Wasserglas inhibited spore germination only at the maximal applied dose of 1000 ml/hl. The three bicarbonate products Vitisan, Armicarb (Potassium bicarbonate) and Mehltauschreck (Sodium bicarbonate) inhibited spore germination up to a delution of 30 % of applied dose, so a fungicide potential of 3.3 was calculated for the three products (Table 3).

Efficacy of different fungicides against *Marssonina* blotch in a commercial orchard

In the field trial in 2013, the infection pressure of *M. coronaria* in the whole orchard was very high, what led to 52.5 % premature leaf fall in the untreated control. This result did not differ statistically significant from Vacciplant (60.5 %), Amylo-X (50 %) and Manzic (52.5 %). The treatments with the products containing Potassium bicarbonate, Vitisan (35 %) and Armicarb (35 %) and Polisolfuro di calcio (22.5 % of leaf fall) with lime sulfur as an active ingredient, tended to have a positive influence on the reduction of leaf fall caused by *Marssonina* blotch. But the differences to the untreated control were negligible and not statistically significant. Poltiglia disperss (15 %) and Ulmasud (5 %) were the only products, which differed statistically significant from the untreated control (Table 4).

The four additional products of the companies in the CO-FREE project had no effect on *Marssonina* blotch.

Table 4: Efficacy of different fungicides against leaf fall caused by *Marssonina* blotch in a field trial in 2013 at Ora (South Tyrol, Italy)

Treatment	leaf fall [%]	N	Std. Deviation	Std. Error of Mean	Tukey HSD*
Poltiglia disperss	15.0	2	14.1	10.0	ab
Polisolfuro di calcio	22.5	2	3.5	2.5	abc
Ulmasud	5.0	2	0.0	0.0	a
Vacciplant	60.5	2	17.7	12.5	d
Manzic	52.5	2	10.6	7.5	cd
Vitisan	35.0	2	7.1	5.0	abcd
Armicarb	37.5	2	3.5	2.5	abcd
Amylo-X	50.0	2	7.1	5.0	bcd
Untreated Control	52.5	2	3.5	2.5	cd

*different letters indicate statistically significant differences (Tukey HSD test: P=0.05)

Discussion

Different strategies are used to control premature leaf fall caused by *Marssonina* blotch. A better orchard and canopy management, which contains the reduction of the inoculum level by removing overwintered fallen leaves can be carried out (Lindner, 2012). Potential plant treatment strategies can be taken into consideration to reduce defoliation of trees.

The evaluation of plant protection products most commonly used in organic farming, which are acid clay, lime sulfur and copper, showed considerable potential for *Marssonina* blotch control.

The best product in this study for disease control of *Marssonina* blotch in organic farming was Ulmasud: the highest fungicide potential in laboratory (333x) was calculated, and leaf fall in the organic orchard was reduced up to approximately 90 % according to Abbott (1925). But also a large number of burn spots were detected on leaves in the field trial in 2013.

As an alternative, the product Polisolfuro di calcio reduced leaf fall in the trial orchard by 57.1 % according to Abbott (1925). Also the calculated fungicide potential of 100 showed the possible potential of the fungicide for *Marssonina* blotch control.

Copper as an active substance reduced premature defoliation by 71.4 %. There were no differences of inhibition of spore germination between formulations of four tested products in laboratory.

In additional field trials, higher amounts of pure copper (applied dosage of 10 g/hl) are needed to be tested. In India, good results in *Marssonina* blotch control were obtained with copper oxychloride at a concentration of 0.3 % (Sharma, 2004).

Acknowledgements

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