Use of *Reynoutria sachalinensis* plant extracts, clay preparations and *Brevibacillus brevis* against fungal diseases of grape berries

Verwendung von Pflanzenextrakten aus *Reynoutria sachalinensis*, Gesteinsmehlen und *Brevibacillus brevis* gegen pilzliche Krankheiten an Weintrauben

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Abstract

R. sachalinensis plant extracts (Milsana®), clay preparations and *B. brevis* were tested for control of powdery and downy mildew and grey mould in organically grown grapes over three years. Milsana® was in all trials as effective against *U. necator* as sulphur, even in years with very high disease pressure. The plant extract showed also positive effects in *Botrytis* control. Myco-Sin® was in all trials as effective in controlling *P. viticola* as the copper containing agent FW 450. The combination of Milsana®, Myco-Sin® and *B. brevis* revealed the potential of *B. brevis* for control of *B. cinerea* in grape berries, and indicated additional positive effects towards the control of *U. necator*. The combined use of these biological control agents shows strong potential for replacement of copper and sulphur in organic viticulture.

Keywords

Grape vine, *Uncinula necator*, *Plasmopara viticola*, *Botrytis cinerea*, plant extracts, *Brevibacillus brevis*, *Reynoutria sachalinensis*

Introduction

Downy and powdery mildew, as well as grey mould are the major fungal diseases that cause yield and quality loss in grape berries. Plant strengthening agents as *R. sachalinensis* extracts (Milsana®) and the clay preparation Myco-Sin® and Ulmasud® have been shown to control powdery mildew and downy mildew, respectively, in different crops (Herger and Klingauf, 1990; Schmitt et al., 2001; Patzwahl and Kopf, 1998). The bacterial antagonist *B. brevis* interferes with fungal spore germination of *Botrytis cinerea* (Edwards and Seddon, 1992) and *Sphaerotheca fuliginea* (Schmitt et al., 1999) and showed strong reduction of grey mould infections in field trials in Chinese cabbage (Edwards and Seddon, 1992). However, it has not been tested to date in the field against grey mould on grape berries. The aim of the current study was therefore to test the effectiveness of these biological control agents, singly or in combination, against the different fungal pathogens of grapes in order to progress towards a combined control of all three diseases in organic viticulture.

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Material and methods

The field trials were performed in the years 1999–2001 by BÖW (Federal Registered Society of Ecological Viticulture) in organically cultivated vineyards at SLVA in Bad Kreuznach, SLVA Trier and at Schmalzried in Korb. Trials were arranged in completely randomised block design with 4 replicates per treatment. As standard treatments, wettable sulphur (Stulln, 0,6–0,2 %, 8 times) and the copper containing agent FW 450 (Dow Agro, 2510 g Cu/ha/a) were used. *R. sachalinensis* plant extract was applied preventively in different formulations: in 1999 Milsana® VP99 with a concentration of 0,5 %, in 2000 Milsana® VP2000 with a concentration of 1,8 % and in 2001 Milsana® VP2001 at a concentration of 1,2 %. Myco-Sin® and Ulmasud® were applied preventively at a concentration of 1%. *B. brevis* was fermented for 24 hours in tryptic soya broth and was applied after flowering at a 1 + 1 dilution. In 1999 spraying was performed on a weekly base, beginning in early May, ending in the middle of August. In 2000 and 2001, treatments were applied in 10 day intervals, starting in 2000 in middle of May, ending end of August. In 2001 treatment were applied from last third of May until last third of August.

Results and discussion

Figure 1 shows the disease severity on grape berries for *U. necator* (scale from 1 to 4) in trials performed with Milsana® in comparison to wettable sulphur in 1999 and 2001. For suppression of infection with *P. viticola,* Milsana was combined with either the copper containing agent FW 450 or with Myco-Sin®. In all trials, Milsana®, either in combination with FW 450 or with Myco-Sin®, controlled powdery mildew infection to the same degree or better than sulphur treatment (Figure 1). The same was recorded for disease incidence, which reached in 1999 100 and 98,5 % in the trials in Korb and Bad Kreuznach, respectively, and 74,1 and 62,6 %, respectively in 2001.

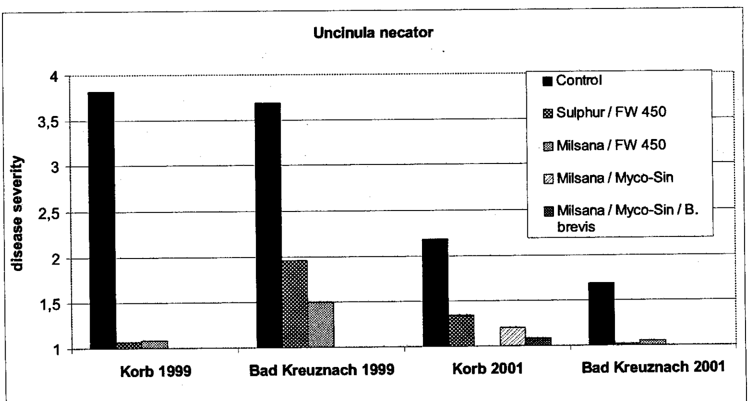

Figure 1: Disease severity of *U. necator* on grape berries. Dates of evaluation: Korb 1999 on 22.08.; Bad Kreuznach 1999 on 04.08.; Korb 2001 on 30.08.; Bad Kreuznach on 21.08.

The combination of Milsana® and Myco-Sin® with *B. brevis* resulted in even better control of *U. necator* with respect to disease severity and incidence. Comparable results were obtained in small scale trials against powdery mildew of cucumber, where additive effects were found when *B. brevis* was applied in combination with *R. sachalinensis* (Seddon and Schmitt, 1999).

In the trials against *P. viticola*, the clay preparations Myco-Sin® and Ulmasud® were tested in comparison to the copper containing agent FW 450. In all three years, the effectiveness of Myco-Sin® and Ulmasud® - applied together with sulphur for control of powdery mildew - was comparable to that of the copper containing agent FW 450 (Figure 2). In Trier 2000, the combination of Myco-Sin® with Milsana® resulted in significant reduction of infection with *P. viticola* (severity and incidence) compared to the control, but the effectiveness was significantly lower than for the other treatments. Disease incidence in the control plots reached 100 %, while the treatments showed levels of 74, 75 and 71 % respectively. Since the disease level was extremely high in this year and the combination of Myco-Sin® and Milsana® was not tested in the other trials, further results, including tests with the new formulated plant extract of *R. sachalinensis* , are needed before drawing final conclusions.

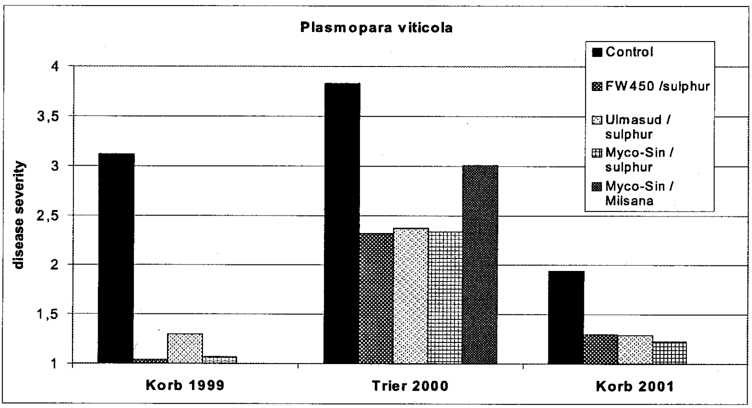

Figure 2: Disease severity of *P. viticola* on grape berries. Dates of evaluation: Korb 1999 on 22.08.; Trier 200018.09.; Korb 2001 on 30.08.

Disease incidence in the control plots in Korb 1999 reached 93,7 % and was reduced most by Myco-Sin® or FW 450 resulting in 5,3 and 3,5 %, respectively. In Korb 2001, disease incidence in the control plots reached 68,6 %, while treatment with Myco-Sin®, Ulmasud® and FW 450 showed levels of 15, 18,9 and 19,8 %, respectively. The high potential of Myco-Sin® for control of downy mildew infection on grape berries was also observed by Kast and Heller (1999).

In all trials, disease severity of *B. cinerea* was strongly reduced by all treatments under investigation (Figure 3). In the trial in Korb 2000, there was no difference between the effectiveness of Milsana® applied together with FW 450, and Milsana® applied together with Myco-Sin with respect to disease severity (Figure 3). Also, disease incidence was reduced from 100 % in the control plots to 87,4 and 87,9 %, respectively.

In Korb 2001, disease incidence in the control plots reached 89,7 % and disease severity was 2,66. Here, treatments with Milsana® in combination with Myco-Sin® resulted in disease incidence of 60,3 % and disease severity of 1,93. When *B. brevis* was applied in addition, disease was reduced further down to 1,5 disease severity and 29,8 % disease incidence and was thus equally effective as treatments with FW 450 and sulphur. This is the first time that the potential of *B. brevis* to reduce grey mould in grape berries in the field has been demonstrated.

Under less severe disease pressure, as in Bad Kreuznach 2001 (disease severity 1,71 and disease incidence 47,4 % in control plots), Milsana® in combination with FW 450 was more effective than sulphur in combination with FW 450, resulting in a disease severity of 1,14 and disease incidence of 11,8 % for the Milsana® / FW 450 treated plots compared to 1,31 and 22,6 % for the sulphur / FW treated plants. Effectiveness of *R. sachalinensis* plant extracts on *B. cinerea* infections has been reported earlier for other systems (Herger and Klingauf, 1990). The data also support earlier findings that copper can reduce grey mould infection.

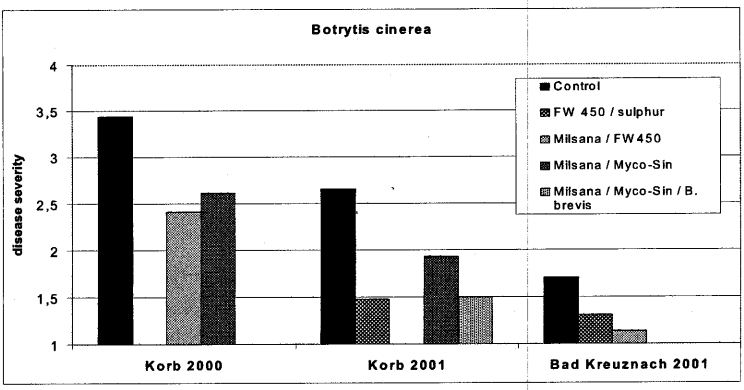

Figure 3: Disease severity of *B. cinerea* on grape berries. Dates of evaluation: Korb 1999 on 22.08.; Korb 2001 on 30.08.; Bad Kreuznach on 21.08.

In Korb 1999, complete yield loss occurred in control plots due to the high level of infection with *U. necator* and *P. viticola*. In contrast to this, in plots treated with sulphur / FW 450 and Milsana® / FW 450, 7 t/ha and 8 t/ha were harvested, respectively. Sugar content was recorded as 91 Oe, and 103 Oe, respectively.

In Trier 2000, only *P. viticola* occurred. Control plots yielded 3,42 t/ha, while treatments with FW 450, Ulmasud® or Myco-Sin® in combination with sulphur yielded 6,46, 6,49 and 6,69 t/ha, respectively. Myco-Sin® in combination with Milsana® resulted in greater than 10 % more harvested berries than in the control plots and reached 3,91 t/ha. Sugar content was 61 Oe in berries from control plots, and 74, 69, 59 and 68 Oe in berries treated with FW 450, Ulmasud® and Myco-

Sin® in combination with sulphur and Myco-Sin® in combination with Milsana®, respectively.

Final Conclusions: Milsana® showed consistent and effective disease control against *U. necator* suitable even for replacement of sulphur. Furthermore, effects were found against *B. cinerea* which should be followed-up. Myco-Sin® was repeatedly effective in *P. viticola* control at levels suitable for copper replacement. *B. brevis* for the first time has shown promising control of *B. cinerea* in grape berries. Furthermore, positve effects were also demonstrated against *U. necator.* More definitive trials must be carried out to clarify the extend and effectiveness of this biocontrol agent. For optimisation of disease control and cost-effective application, synergistic and additional effects of the control agents when applied in combination have to be further examined, and alternating applications with optimised formulations should be tested. Overall, results indicate that the combination of the 3 agents lead to higher levels of disease control and are a promising base for biological control of the three most damaging fungal diseases in organic viticulture.

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