

**Tests with Milsana<sup>®</sup> and *Brevibacillus brevis* for side-effects against *Typhlodromus pyri* (Acari, Phytoseiidae) and *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)**

Versuche mit Milsana<sup>®</sup> und *B. brevis* auf Nebeneffekte gegen *Typhlodromus pyri* (Acari, Phytoseiidae) und *Aphidius rhopalosiphi* (Hymenoptera, Braconidae)

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### Abstract

Data from laboratory range-finder and field test indicate that both biological agents, Milsana (VP 2000) and *B. brevis* WT, can be judged well-suitable for use in organic or integrated control systems. There were no negative effects recorded against *Typhlodromus pyri* (laboratory and field) and *Aphidius rhopalosiphi*. (laboratory). For further confirmation, limit tests under GLP (with the highest tested concentration) will be performed in the near future. When Myco-Sin was applied in combination with Milsana in the field, population levels of *T. pyri* were raised compared to the control.

### Keywords

*Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Reynoutria sachalinensis*, *Brevibacillus brevis*

### Introduction

Milsana<sup>®</sup>, a plant strengthener from *Reynoutria sachalinensis*, and *Brevibacillus brevis* (formerly *Bacillus brevis*), a biological plant protection agent, are targeted to control powdery mildew and /or grey mould (*Botrytis cinerea*) in cucumber, tomato and grape. For use in practice, the compatibility of the control agents with beneficial organisms is of great importance. Therefore, the objective of this study was to investigate possible side-effects on beneficial organisms which are sensitive to adverse effects of plant protection agents and are standard species for side-effect tests in EU registration. First results on the possible effects of Milsana<sup>®</sup> (VP 2000) and *Brevibacillus brevis* Wild-Type (WT) on *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera, Braconidae) and *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory under worst-case exposure conditions and from a field trial are presented.

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## Material and Methods

### Laboratory studies

Adults of *A. rhopalosiphii* and protonymphs of *T. pyri* were exposed to Milsana<sup>®</sup> (VP 2000) or *B. brevis* WT treated surfaces. The test substances were diluted in water and applied with a spray application volume of 200 l water/ha to glass plates or glass cover slides at rates up to 30 % for Milsana<sup>®</sup> and up to 9600 g/ha for *B. brevis*.

***A. rhopalosiphii*:** When dry, the glass plates were used to form the floor and the ceiling (treated surfaces inwards) of shallow arenas with an aluminium frame into which 10 adult wasps of equal sex ratio (age 48 h) were introduced (n = 2 per treatment). Water was applied as control (200 l/ha) and Perfekthion (active ingredient dimethoate) was applied at 0.30 ml product/200 l water/ha as toxic standard. During exposure the wasps were fed *ad libitum* with a honey water solution and a sugar water solution. Assessments of direct treatment effects were made after 30 min, 2, 24 and 48 h. Therefore the percentage of mortality was calculated for each replicate from the number of dead and moribund parasitoids in correlation to the number of released parasitoids. ***T. pyri*:** Glass cover slides were treated with the test substances. Deionised water was applied as control and Perfekthion was applied at 45 ml product/200 l water/ha as toxic standard. Twenty protonymphs were placed into each replicate unit (n = 3 per treatment). Assessments of direct treatment effects (mortality) were assessed after 3 and 7 days. During exposure the mites were provided with pollen of bean (*Vicia faba*) and birch (*Betula pendula*) *ad libitum*. Seven days after application the number of dead and missing mites was summed up for each replicate and calculated as mortality percentages.

The corrected mortality was obtained for both test species by comparing the values observed in the treated series with those in the control series, according to Abbotts formula, modified by Schneider-Orelli (1947). The mortality data of *A. rhopalosiphii* and *T. pyri* were analysed for significance using Fisher's Exact test (ZAR, 1984).

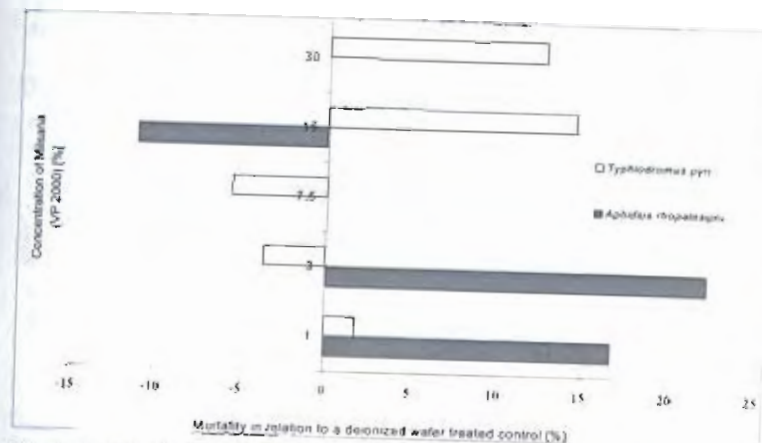
### Field trial

In 2000 in a vineyard at SLVA Trier an efficacy trial against *Plasmopara viticola* in grape plants of the cultivar 'Riesling' was conducted. (Completely randomised blocks with 4 replicates.) of Milsana<sup>®</sup> (VP 2000) was sprayed at a rate of 1.8 % together with Myco-Sin<sup>®</sup> (1 %). Application were conducted 8 times every 10 days (between 16.5. and 27.7.2000) with a back-pack motor sprayer (Geizhals M23). Besides disease incidence, the development of populations of *T. pyri* was recorded on 16.5., 14.6., 4.7. and 15.8.2000.

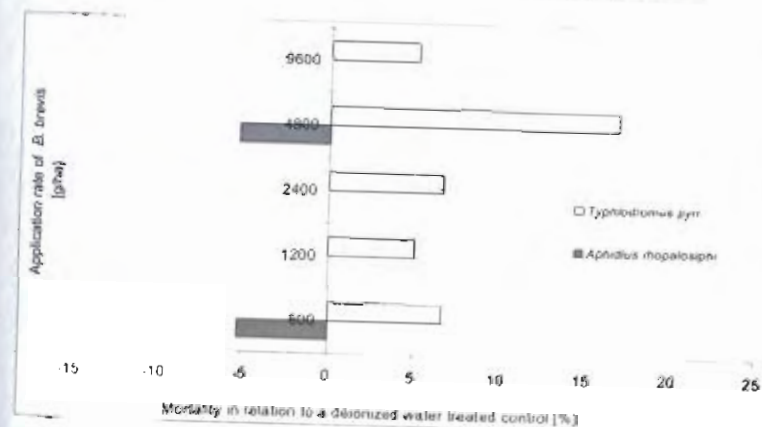
## Results and Discussion

The mortality rates of *A. rhopalosiphii* and *T. pyri* after exposure to Milsana<sup>®</sup> (VP 2000) treated glass plates compared to the control are shown in Figure 1. Even at the highest concentration of 30 % Milsana<sup>®</sup> (VP 2000), which covered the the-

oretical highest possible amount (accumulated residues due to successive applications of the plant extract), the corrected mortality rates for *A. rhopalosiphii* and *T. pyri* did not exceed the trigger value of 30 %. Significant difference to the control (P=0.05) were found for both test species with the toxic standards. The same was found for *T. pyri* with 15 % Milsana<sup>®</sup> (VP 2000), probably due to biological variability. Both studies with Milsana<sup>®</sup> (VP 2000) met the criteria for validity. (The control mortality was 10.0 % for *A. rhopalosiphii* and 8.3 % for *T. pyri* and the mortality in the toxic standard was 100 % for *A. rhopalosiphii* and 96.7 % for *T. pyri*).



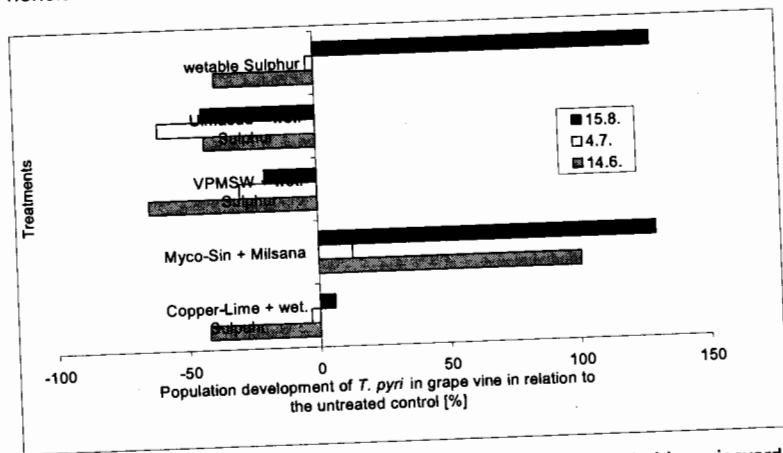
**Figure 1:** Corrected mortality of *T. pyri* and *A. rhopalosiphii* after exposure to Milsana (VP 2000) on glass plates (range finder test). The control mortality is set to 0 %.



**Figure 2:** Corrected mortality of *T. pyri* and *A. rhopalosiphii* after exposure to *B. brevis* on glass plates (range finder test). The control mortality is set to 0 %.

Figure 2 shows the mortality rates of *A. rhopalosiphi* and *T. pyri* after exposure to *B. brevis* WT treated glass plates compared to the control. Up to an application rate of 9600 g/ha no corrected mortality rates above the trigger value of 30 % were detected, neither with *A. rhopalosiphi* nor with *T. pyri*. Significant difference to the control ( $P=0.05$ ) were found for both test species with the toxic standards. *B. brevis* WT at 4800 g/ha showed the same, again possibly due to the biological variability. The studies with *B. brevis* WT met also the validity criteria. (Mortality rate being 5.0 % for *A. rhopalosiphi* and 1.7 % for *T. pyri* and the mortality in the toxic standard being 100 % (*A. rhopalosiphi*) and 96.7 % (*T. pyri*), respectively.) According to BBA criteria (Brasse and Rothert, 1993) Milsana® (VP 2000) as well as *B. brevis* WT can be classified as harmless.

The results of the test with Milsana (VP 2000) were corroborated by observations from a field trial in grape where as well as the efficacy against phytopathogens also the population development of *T. pyri* was monitored. Treatment with Milsana® (VP 2000) in combination with the plant strengthener Myco-Sin® resulted in an increase in the population level on all evaluation dates compared to the control, while in the other treatments, where sulphur was included, the population was decreased on most dates or throughout the whole experiment (Figure 3). In laboratory studies performed with *Trichogramma cacoeciae* Marchal (Hymenoptera, Trichogrammatidae) (Hafez *et al.*, 1999), application rates of *R. sachalinensis* extract as used in practice were also classified as harmless.



**Figure 3:** Changes in population levels of *T. pyri* relative to the control in a vineyard at SLVA Trier in 2000

## Acknowledgements

The trials were funded by the EU (FAIR CT98-4413). Information on the project is available under [www.biocombi.net](http://www.biocombi.net)

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