Vitis **44** (2), 99–100 (2005)

Research Note

Efficacy of *Metarhizium anisopliae* against *Hyalesthes obsoletus* (Auchenorryhncha: Cixiidae)

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K e y w o r d s : Bioassay, biocontrol, bois noir, entomopathogen, Grapevine yellows, Hyphomycetes, phytoplasmas, vector.

A b b r e v i a t i o n s : BCA = biocontrol agent, SEM = scanning electron microscope, VK = Vergilbungskrankheit.

Introduction: Vergilbungskrankheit (VK) or Bois noir of grapevine (*Vitis* sp. L.) is a widespread grapevine yellows disease in Europe. Infected grapevines decline showing specific symptoms like discoloration of leaves, incomplete lignification of shoots, abortion of inflorescences or shrivelling of berries. Phytoplasmas of the stolbur group were found to be associated with this disorder (Daire *et al.* 1994, Maixner *et al.* 1994), and the Cixiid planthopper *Hyalesthes obsoletus* Signoret was identified as a vector (Maixner *et al.* 1995; Sforza *et al.* 1998). Larval instars of this insect acquire the pathogens by feeding on the roots of herbaceous host plants such as *Convolvulus arvensis* L. and *Urtica dioica* L. while adults transmit them to grapevines during occasional feeding on this erroneous host. *H. obsoletus* as vector and herbaceous weeds as hosts for both, the phytoplasmas and the vector, play an important role in the epidemiology of VK. Therefore, management strategies have to focus on the control of host plants of phytoplasmas as well as on the decrease of vector population density. However, direct control of vectors by insecticides is impeded by the hidden life of *H. obsoletus* larval instars in the soil and the occurrence of planthoppers on non-cultivated plants. Parasitation by common parasitoids of Auchenorrhyncha such as Mymaridae, Dryinidae, and Pipunculidae (Maixner *et al.* 1998) is low and occasional parasitation by prostigmatid mites is not sufficient for efficient biological control. The subterranean life, on the other hand, could make *H. obsoletus* susceptible to entomopathogenic fungi such as *M. anisopliae*. *M. anisopliae* has a wide host range and is already in discussion as efficient BCA against other vine pests like phylloxera, *Daktulosphaira vitifoliae* Fitch, (Kirchmair *et al.* 2003, Huber *et al.* 2004).

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Material and Methods: At the beginning of the flight period in 2003 adult *H. obsoletus* were caught with a sweep net from *C. arvensis* and put into glass vials for transport. Plastic petri-dishes (diameter 8.5 cm) were filled with a mixture of plaster of Paris and charcoal (10:1) to a height of 5 mm, this was moistened with distilled water according to Green (1964). Twelve insects were put into each petri-dish. Shoots of *C. arvensis* seedlings served as food supply. Four female and 6 male samples (total: 120 individuals) were treated; the same amount was used as control.

Planthoppers were shaken to the surface of an opened petri-dish and sprayed with 150 µl of a *M. anisopliae* spore suspension (density: 5×10^4 spores·ml^{-1}). After treatment petri-dishes were covered immediately. The control samples were treated with sterilised tap water.

The strain of *Metarhizium anisopliae* var. *anisopliae* was Ma 500; it was provided by S. Keller, FAL-Reckenholz, Switzerland. The strain was grown on barley kernels according to Aregger (1992) and the inoculum stored at 4 °C until required.

All petri-dishes were controlled daily for 8 d and the number of dead and living planthoppers was recorded. Dead individuals were left in the petri-dishes until hyphae became visible. After spore formation the planthoppers were gathered and fixed in ethanol (75 % v/v). For SEM preparation the samples were dehydrated in an ascending ethanol series. After critical point drying the samples were mounted on aluminium specimen mounts and sputtered with gold. Micrographs were taken using a Philips ESEM scanning electron microscope.

Data were analysed with Statistica (Release 5.5; StatSoft Inc., 1984-1999) using the Shapiro-Wilk W-test and the nonparametric Mann-Whitney U-test.

Results and Discussion: The bioassay showed that *H. obsoletus* is susceptible to *Metarhizium anisopliae* Ma 500 applications: With the exception of day 2, mortality was significantly higher in the *Metarhizium*-treated samples (Fig. 1). Thus the mean duration of survival was significantly lower (4.41 d; $p < 0.001$) in the *Metarhizium* samples in comparison to the untreated control (5.53 d). The greatest effect of *Metarhizium* application was observed within the first 5 d of the experiment. Conidiophores and conidia of *M. anisopliae* were found on dead insects 5 d after treatment (Fig. 2).

From 120 insects, only 97 *Metarhizium*-treated could be evaluated, the other individuals were stuck to drops of condensed water. In the untreated control 87 out of 120 insects were left.

In the last 4 d of the experiment the high mortality in the control group was most likely caused by insufficient food supply due to the wilting shoots of *C. arvensis*.

Based on this study, future work will be required to verify the efficacy of *M. anisopliae* against *H. obsoletus* in laboratory and field experiments. Moreover it should be tested if larval instars can be infected as well. The improvement of application strategies, e.g. in grape nurseries and vineyards, and the development of second generation formulations of *M. anisopliae* for planthopper control are other important objectives to be tested. Standardized bioassay methods for

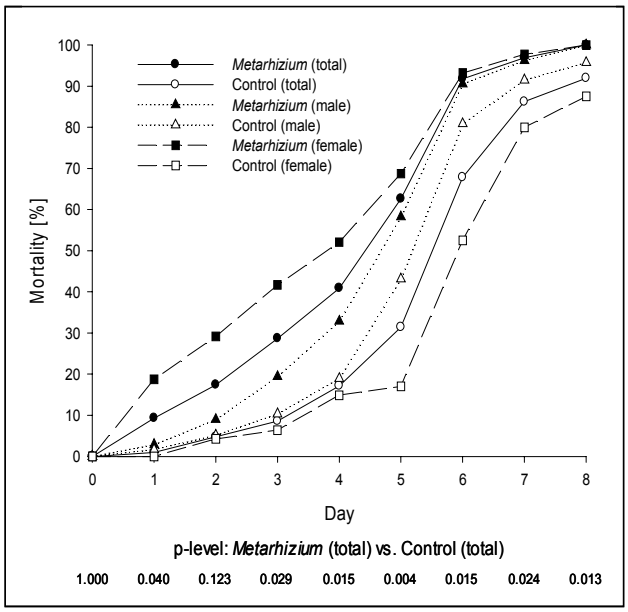

Fig. 1: Efficacy of *Metarhizium anisopliae* var. *anisopliae* (Ma 500) for control of *H. obsoletus* in bioassays. Percentage of mortality in line plots based on individual numbers; p-levels based on sample numbers (n = 10).

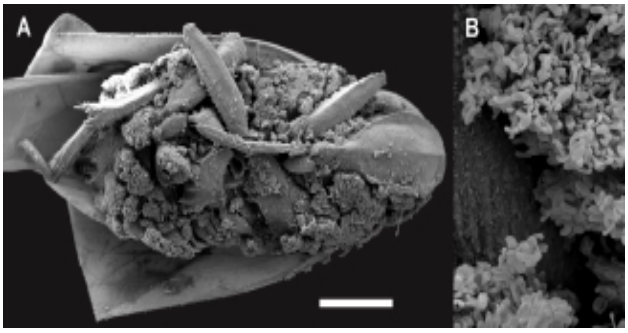

Fig. 2: Conidia of *Metarhizium anisopliae* on *H. obsoletus* (Bar: 500 µm in Fig. 2 A, 25 µm in Fig. 2 B).

high throughput screening of *Metarhizium* isolates and products are already available (BUTT and GOETTEL 2000; Standard protocols from EU RTD project FAIR6-CT98-4105). Additionally, standard protocols will be developed to organize field experiments efficiently (*e.g.* sampling, sample preparation, methods and tools to detect fungal residues in the environment/food chain), to demonstrate the improved efficacy of pathogens developed for *H. obsoletus* control, and to evaluate a distinct, more narrow host range of the specific isolates. An important point therefore is to determine potential risks to non-target organisms from the increased field exposure to specific *M. anisopliae* isolates. Previous field trials in vineyard soils showed no significant changes in abundance or diversity of soil invertebrates and soil fungi so far. Also no negative effects of *Metarhizium*-application on vine growth, yield, berry weight, total soluble solids (°Brix) or total titratable acidity of the must were found (HUBER *et al.* 2004). Further long-term investigations are in progress.

Acknowledgements: We are indebted to R. PÖDER and H. STRASSER, Institute of Microbiology, University of Innsbruck, Austria, for helpful discussions.

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Received July 18, 2004