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Research Note

A tripartite aseptic culture system for grape (*Vitis* spp.), phylloxera (*Daktulosphaera vitifoliae*) and mites (*Tarsanemus* sp.)

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Introduction: Grape phylloxera (Homoptera: Phylloxeridae) is one of the most important insect pests of grapes. The monophagous dwarf aphid parasitizes leaves and roots of susceptible hosts damaging the vines and eventually destroying them. The use of resistant rootstocks for phylloxera control was the first example of successful pest control by biological means in grape, but there is evidence that this resistance is being overcome by new strains of this pest. The evolution of new strains necessitates both the development of new rootstocks and research on integrated control methods including the search for parasites, parasitoids or other insect pathogens.

This paper presents a method for maintaining phylloxera and an insect antagonist under aseptic conditions and shows that under certain conditions *Tarsanemus* can eliminate phylloxera populations.

Material and methods: *Vitis labrusca* (from the collection of the Universität Hohenheim) was micropropagated in a dual culture system as described by Forneck *et al.* (1996) in growth chambers at 25 °C, with 30-40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (16 h) light. *V. labrusca* was used as phylloxera host because the leaf galls of this species are partially open allowing observations within the galls without disrupting the system. Phylloxera eggs were gathered from a greenhouse-based colony originating from Bingen (Germany), the *Tarsanemus* sp. mite eggs from plants in our greenhouse. Both kinds of eggs were surface-sterilised according to Grzegorczyk and Walker (1997) either separately or together; they were inoculated simultaneously using a sterile brush.

Results and Discussion: The pyhlloxera population established readily on roots and leaves of *V. labrusca* forming leaf galls and nodosities, respectively. This was not hampered by the mite populations which, due to the smaller inoculum, built up more slowly. After a lag period, mites were observed associated both, with dead and live phylloxera of all stages including eggs. It was clear, that the mites caused the phylloxera's death because continuous observation showed that none of the aphids survived the interaction. Within 2 d they died and started to decay. Under normal conditions phylloxera stages, even without nutrient supply, survive at least for 5 d under septic conditions. Mites were first observed in leaf galls and on the plant's apex together with phylloxera larvae. Their number increased rapidly and they migrated over the entire plant, their contact with phylloxera morphs being accidental rather than due to a purposeful search. Some mites were also found to feed on the plant leaves and roots laying eggs close to the root hairs. Dead morphs of phylloxera infested with mites decayed considerably faster than usual and mites could be observed laying up to 10 eggs near or on a single phylloxera body. The eggs hatched after 5-7 d and the larvae migrated away from the empty carcass. Both male and female mites could be observed (Figure), the latter laying 2-3 eggs over a 5-7 d period. All tripartite cultures were discontinued after approximately 80 d because the phylloxera populations had been eliminated. Although no microbes could be detected at that time, the mite's feces covering the nutrient agar might be a problem for longer experiments.

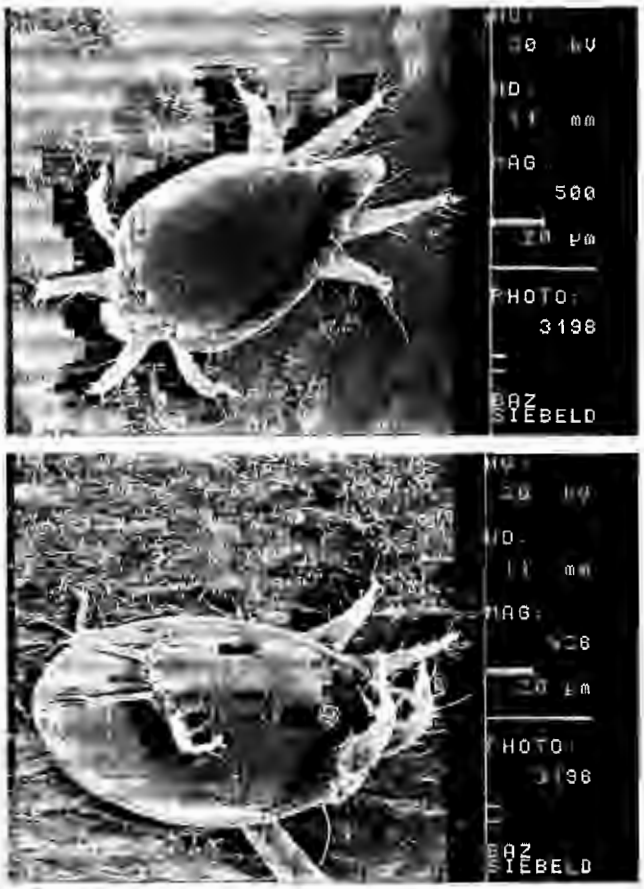

Figure: Male (above) and female *Tarsanemus* sp.

Tarsanemus may never be a suitable organism for phylloxera control in the field because at this time we know little about the mite's ecological habitat, feeding preferences and host specificity. Nevertheless, this method maintaining grape, phylloxera and the prospective antagonists under aseptic conditions provides the opportunity to study population dynamics, feeding behavior and other aspects of parasites, parasitoids or insect pathogens of phylloxera which could increase the ability to biologically control phylloxera.

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