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Research Note

Identification of *Xiphinema index* in an Austrian vineyard

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K e y w o r d s : nematode, longidorids, *Xiphinema vuittenezi*, species-specific primers, virus vector.

The ectoparasitic Longidorid nematode *Xiphinema index* has a worldwide economic importance because of its ubiquitous presence and ability to transmit *Grapevine fanleaf virus* (GFLV). Infection with GFLV leads to low yield, reduced fruit quality and a shorter lifespan of vines (Andret-Link *et al*. 2004). Although GFLV was detected in various Austrian viticultural regions (Gangl *et al*. 2000, 2001, 2003), and despite the fact that the soil-borne nature of fanleaf disease had been observed in Austria well over a century ago (Rathay 1882, 1883), its nematode vector *Xiphinema index* has never been encountered, contrary to other species of the *Longidoridae* family, predominantly *Xiphinema vuittenezi*, *X. pachtaicum* and other species. In this study soil samples from a vineyard in Burgenland, where vines displayed typical symptoms of GFLV infection, were analyzed for the presence of *X. index* by morphological and molecular analyses, using species specific PCR primers.

Material and Methods: For the isolation of nematodes in a vineyard in Burgenland soil samples including fine roots were collected in the rhizosphere of two *Vitis vinifera* cvs 'Neuburger' (2 and 25 years old) and 'Zweigelt' (5 years old), showing symptoms of GFLV infection. Roots were visually analyzed for nematode feeding symptoms. Leaf samples of the corresponding host plants were collected for GFLV testing by ELISA, using a commercially available kit (Bioreba, Switzerland). Nematodes were extracted from 1.5 kg of soil by a modification of Cobb´s method (Shurtleff and Averre 2000) using a sieve with 200 µm meshes. The genus of nematodes was determined initially by morphological characteristics (Thorne and Allen 1950). An authentic population of *X. index* was used as a positive control. Species identification within the genus *Xiphinema* was performed by multiplex PCR analysis of pooled samples. DNA isolation was carried out by placing 15 nematodes in 25 µl of lysis buffer (1x Taq DNA polymerase buffer and 60 µg of proteinase K ml^{-1}) between two glass slides and crushed gently. The homogenate was taken up carefully with a pipette, transferred to a 0.5 ml Eppendorf tube and frozen at -80 °C for 15 min followed by subsequent incubation at 60 °C for 1 h and at 95 °C for 15 min. PCR was carried out with the following cycles: 95 °C for 3 min, followed by 39 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, ending with 1 cycle at 72 °C for 5 min. The primers A-ITS1, I27, D24, V18, ITA26 were used (Wang *et al*. 2003). Amplification products were separated on a 1.5 % agarose gel in 1 x TAE buffer.

Results and Discussion: Although GFLV is present in various Austrian viticultural regions (Gangl *et al*. 2000, 2001, 2003), surveys for nematodes of the *Longidoridae* family detected predominantly *X. vuittenezi* and *X. pachtaicum* as well as other species, which are not vectors of GFLV. This left unanswered the question of how GFLV spreads in Austrian vineyards. In fact, *X. index* was never identified, probably due to the morphological similarity with *X. vuittenezi* and perhaps due to a lower abundance.

Grapevines with characteristic fanleaf symptoms tested positive for GFLV in ELISA. Examination of fine roots showed signs typical for nematode feeding. Analyses of soil samples resulted in 10-14 individual nematodes of different developmental stages per kg soil, with a head-region and other characteristics typical for the genus *Xiphinema.* Species definition, however, was not possible, because morphological and morphometric diagnostic characteristics of female adults, as currently used for *Xiphinema* ssp. identification, exhibit extensive plasticity. The availability of molecular markers specific for the most important *Xiphinema* species is of a major advantage. Molecular differentiation according to Wang *et al*. (2003) should result in a 349 bp band for *X. index*, a 813 bp band for *X. diversicaudatum*, a 591 bp band for *X. vuittenezi*, and a 414 bp band

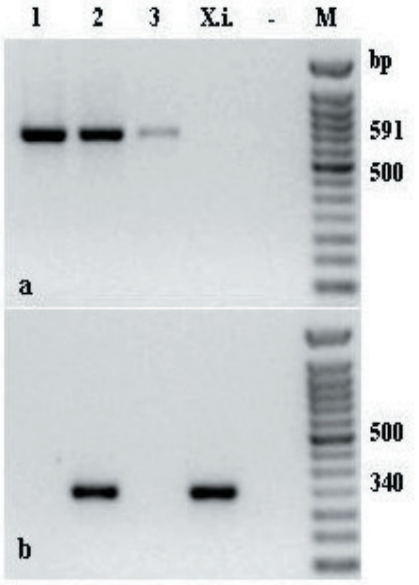

Figure: Molecular analysis of nematode populations (1-3) from the rhizosphere of three GFLV-infected grapevines using species specific primers for **a**) *X. vuittenezi* and **b**) *X. index*. *X. index* was identified in a mixture with *X. vuittenezi* in sample No. 2. X.i. = *X. index*, - = negative control, M = molecular size marker, bp = basepairs.

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for *X. italiae*. Indeed, the species specificity, sensitivity and reliability of the primers were confirmed by Hübschen *et al*. (2004).

All nematode populations analysed by PCR consisted of *X. vuittenezi*, except for one which also contained *X. index*. Bands specific for *X. diversicaudatum* and *X. italiae* could not be detected. These results prove, for the first time, that *X. index,* the recognized vector for the detrimental grapevine fanleaf disease, is present in the vineyards of a specific Austrian region. It can be expected that the use of species specific primers, as described here for samples from other viticultural regions in Austria, will reveal a more widespread occurrence of *X. index*. As nematicides are banned by the Common Agricultural Policy (CAP) of the EU, these results emphasize the importance of alternative defence strategies in modern viticulture, such as genetically improved rootstocks exhibiting virus resistance (Maghuly *et al*. 2005).

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