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Research Note

Transmission of grapevine yellows by *Oncopsis alni* (Schrank) (Auchenorrhyncha: Macropsinae)

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S u m m a r y : *Oncopsis alni* leafhoppers were captured on field-grown *Alnus glutinosa* that were infected by alder yellows phytoplasma. This pathogen is indistinguishable by RFLP of ribosomal and non-ribosomal DNA fragments from a phytoplasma associated with grapevine yellows in the same region. PCR tests revealed that approximately 11 % of the insects carried the pathogen. Their ability to inoculate grapevine was studied by transmission experiments. Three of 88 test plants developed typical symptoms of grapevine yellows, and the respective phytoplasma could be detected in symptomatic plants by PCR. Thus, *O. alni* was identified as a vector of a grapevine yellows of the elm yellows group.

K e y w o r d s : grapevine yellows, phytoplasma, vector, leafhopper, transmission, epidemiology, host plant.

Introduction: Two types of grapevine yellows (GY) affect *Vitis vinifera* L. in Germany. A stolbur group phytoplasma is associated with the widespread "Vergilbungskrankheit" that is present in almost all viticultural areas. A second type of GY has been reported from the Palatinate region of Germany (Palatinate Grapevine Yellows, PGY) and a phytoplasma of the elm yellows group was found to be associated with this disease (Maixner *et al*. 1995 b). Serological and molecular data revealed a close relationship of this pathogen to the phytoplasma associated with Flavescence dorée (FD) (Maixner *et al.* 1995 b), but Daire *et al.* (1997) were able to differentiate both diseases by RFLP analysis of non-ribosomal DNA fragments. Their results were supported by transmission experiments with *Scaphoideus titanus* Ball, the vector of FD, that failed to inoculate grapes with PGY (Boudon-Padieu and Maixner, unpubl. data). While PGY could be distinguished from some other elm yellows group phytoplasmas by RFLP analysis of ribosomal (Reinert and Maixner 1997) and non-ribosomal DNA fragments (Daire *et al*. 1997), no difference to the alder yellows phytoplasma (AGY) was found that is widespread in black alder (*Alnus glutinosa* L.) in Germany.

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Only two vectors of grapevine yellows have been identified so far. FD is transmitted by the leafhopper *S. titanus*, 'Vergilbungskrankheit' and 'Bois noir' by the planthopper *Hyalesthes obsoletus* Sign. (Maixner 1994; Sforza *et al.* 1998). While *S. titanus* is not yet present in Germany, another leafhopper, *Oncopsis alni* (Schrank), was identified as a vector of alder yellows (Maixner and Reinert 1999). We investigated the ability of this leafhopper to inoculate *V. vinifera* with AGY and the capability of the phytoplasma from alder to provoke symptoms of PGY in the inoculated grapes.

Material and Methods: Alder trees were tested for infection with AGY by polymerase chain reaction (PCR). Adult *O. alni* were collected from infected trees from June to August 1998 in both the Palatinate and Moselle areas of Germany. Transmission experiments were carried out in a growth chamber at 25 °C and a photoperiod of 16 h. The leafhoppers were fed in groups of 2-6 individuals in cages that contained a potted grapevine seedling each. They were removed from the plants after an inoculation period of one week and stored at -20 °C while dead insects were removed immediately. Grapes were grown in an insect-proof greenhouse at 20 °C and a photoperiod of 16 h for about three months. They were then hibernated at 10 °C under natural light conditions. Eighty-eight grapevine seedlings were inoculated by a total number of 381 leafhoppers.

Total DNA was extracted from leaf midribs of the inoculated grapevine seedlings as well as from individual insects as described by Maixner *et al.* (1995 a). DNA was also extracted from a periwinkle (*Catharanthus roseus* (L.) G. Don) infected by an isolate of PGY, and from field-grown *A. glutinosa* and grapevine with symptoms of PGY. PCR and RFLP analyses were carried out as described by Maixner and Reinert (1999). The primers fAY/rEY were used for the group-specific detection of elm yellows phytoplasmas, while an unspecific amplification with primers P1/P7 and subsequent digestion of the amplification products by *Alu*I allowed to compare the phytoplasmas detected in plants and insects.

Results and Discussion: *O. alni* is a strongly monophagous leafhopper on alder (Ossianilson 1981). We found it frequently on *A. glutinosa*. However, a few specimen were also caught on sticky traps or by sweep net in affected vineyards adjacent to alder trees. While *O. alni* survived well on alder seedlings during previous transmission trials, none of the 381 leafhoppers used in our experiments survived the feeding period on grapevine. Four of 80 (5 %) leafhoppers from Palatinate and 17 of 113 (15 %) leafhoppers from the Moselle area tested positive with primers fAY/rEY (Figure, A). On an average, 11 % of *O. alni* were found to carry a phytoplasma of the elm yellows group.

Only one of 88 inoculated grape seedlings developed symptoms of grapevine yellows within three months after inoculation. This plant ceased growing and died so fast that it could not be used for DNA extraction. Two more seedlings developed typical symptoms of GY after dormancy, such as rolling of leaves, lack of lignification and black

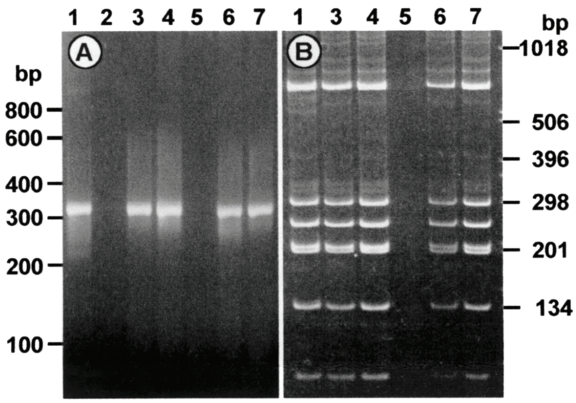

Figure: Specific detection of elm yellows group phytoplasmas by PCR with primers fAY/rEY (**A**); *Alu*I restriction profiles of a DNA fragment that had been amplified with primers P1/P7 (**B**).
1 = Periwinkle isolate of PGY; 2 = Healthy *Alnus glutinosa* seedling; 3 = Infected *A. glutinosa* on which *Oncopsis alni* was captured; 4 = *O. alni* used to inoculate grapevine; 5 = Healthy seedling of *V. vinifera*; 6 = Seedling of *V. vinifera* inoculated by *O. alni;* 7 = Infected *V. vinifera* cv. Scheurebe from a Palatinate vineyard.

pustules along the shoots. These plants were tested positive in PCR with elm-yellows specific primers, however, only a faint band could be achieved with one of the samples. No DNA was amplified from all other seedlings. In total, one of 33 seedlings (3 %) inoculated with *O. alni* from the Palatinate region and one of 55 seedlings (2 %) on which leafhoppers from the Moselle area were fed were found positive with primers fAY/rEY.

*Alu*I digestion of DNA fragments amplified with primers P1/P7 revealed identical restriction fragment patterns in samples obtained from alder, diseased grapevine from the field and a PGY isolate in periwinkle as well as from *O. alni* and the experimentally inoculated grapevine seedlings (Figure, B). This profile can be distinguished from fragment patterns obtained from other elm yellows group phytoplasmas such as rubus stunt or elm yellows (REINERT and MAIXNER 1997).

The results of the experimental inoculation of grapevine seedlings by *O. alni* collected from AGY infected *A. glutinosa* led to the conclusion that *O. alni* is not only the vector of alder yellows (MAIXNER and REINERT 1999) but is also able to transmit this phytoplasma to grapevine, thus inducing symptoms of grapevine yellows in the infected plants. In the field, PGY is normally restricted to old vines growing in vineyards along creeks or rivers where *A. glutinosa* is present. This behaviour can be explained by the strong adaptation of *O. alni* to alder that prevents a frequent inoculation of grapevine even if the infestation of the leafhopper population is fairly high. However, more detailed investigations are required to understand the epidemiology of PGY. It will be necessary to search for PGY in other viticultural areas where infected alders are growing. Furthermore, the ability of other vectors of elm yellows group phytoplasmas to transmit PGY has to be investigated. *Macropsis fuscula* (Zetterstedt), for example, the vector of rubus stunt disease, is also present in the areas affected by PGY.

As long as *O. alni* remains the only vector of PGY, the economic risk of this disease seems to be low. The situation would change, however, if a transmission cycle from grape to grape by another, ampelophagous vector could be established. Therefore, care has to be taken to avoid the introduction of potential vector species and known vectors of elm yellows group phytoplasmas into areas where PGY occurs.

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