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Transmission of '*Candidatus* Phytoplasma mali' by root bridges under natural and experimental conditions

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Abstract

Transmission of '*Candidatus* Phytoplasma mali' could be demonstrated under experimental as well as under natural conditions in apple orchards in Trentino (Northern Italy). Experimental transmission by root bridges was obtained in a trial with 30 groups of two apple seedlings potted together. One seedling of each group was inoculated by grafting with '*Ca.* P. mali' in 2003 and the plants were kept under insect proof conditions. In 2006, three years after inoculation, 4 out of remaining 25 healthy test plants showed typical apple proliferation symptoms and tested positive for '*Ca.* P. mali' by specific PCR and immunofluorescence analysis. In all four cases the formation of root bridges between inoculum plant and receptor plant could be detected. Natural transmission by root bridges was studied in an 11-year's old apple orchard by treating cut apple proliferation-infected trees with the herbicide glyphosate in autumn 2005. In spring 2006, neighbouring trees showed not only herbicide symptoms suggesting root bridges but also typical apple proliferation symptoms indicating phytoplasma transmission. '*Ca.* P. mali' was detected by specific PCR in 57% of trees with herbicide symptoms.

Key words: apple proliferation, epidemiology, immunofluorescence, anastomosis, root nodule.

Introduction

The natural spread of '*Candidatus* Phytoplasma mali' was studied in Trentino (Northern Italy) since 1999 when a serious epidemic of apple proliferation (AP) disease started in this important apple growing region. It could be demonstrated that the disease is spread by aerial vectors: *Cacopsylla picta* (Foerster) and *Cacopsylla melanoneura* (Foerster) (Frisinghelli *et al.*, 2000; Mattedi *et al.*, 2007). However, it was observed, especially in medium-aged and old orchards, that infected trees were located adjacent in the same row or formed typical patches (Vindimian *et al.*, 2002). Natural root grafts between neighbouring trees were found during uprooting and/or excavations in these orchards. Furthermore, root contacts seem to be a common phenomenon in nurseries and orchards in this region (Adige Valley, Non Valley).

Therefore, the potential transmission of '*Ca.* P. mali' by root bridges was studied in more detail. First, the formation of root bridges and the subsequent transmission of the phytoplasma was studied under experimental conditions in an insect-proof screenhouse. Second, trials applying a systemic, only phloem-transported herbicide in slanting cuts in the bark of infected trees were performed in a medium-aged apple trees orchard in order to proof root contacts. Trees showing herbicide symptoms caused by compound translocation through a root bridge were further analysed by PCR for the transmission of '*Ca.* P. mali'.

Materials and methods

In February 2003, 60 healthy one-year-old Golden Delicious seedlings and 30 one-year-old AP infected micropropagated Golden Delicious plants were used. The roots of two different seedlings were inserted in a plastic hose (1 cm diameter and 5 cm length) to create a permanent contact. Then the seedlings, connected in this way, were put two by two in 30 pots (12 litres).

In August 2003, one of the seedlings of each pot was then graft-inoculated with the infected Golden Delicious plant using the approach grafting procedure. The pots were maintained in an insect-proof screenhouse for symptom observation.

Both the donor and the receptor plants were visually examined each year in autumn to check symptoms and PCR analysis were performed on samples from the branches of the seedlings to confirm the visual diagnosis.

PCR amplification was carried out with '*Ca.* P. mali' specific primer pairs AP5/AP4 and AP3/AP4 according to Jarausch *et al.* (1994) and fAT/rAS according to Smart *et al.* (1996).

The roots of the plants showing phytoplasma transmission were examined to locate where they grew together. Microscopic observations were performed to verify the connection point and '*Ca.* P. mali' was detected by specific immunofluorescence analysis as described by Loi *et al.* (2002).

In November 2005, AP-infected trees in an 11-year's old orchard were cut and the systemic, phloem-transported herbicide glyphosate was introduced in slanting cuts in the bark. In the following spring, herbicide symptoms were monitored on adjacent trees. In spring and in autumn 2006, AP-specific symptoms on these trees were recorded and PCR detection was performed as described above.

Results and discussion

'Ca. P. mali' transmission by root bridges could be demonstrated under experimental conditions. Three years after inoculation of the donor plants 4 out of 25

remaining healthy receptor plants showed clear symptoms of AP disease. Specific PCR and immunofluorescence diagnosis confirmed the presence of '*Ca.* P. mali' in these plants. Thus, a transmission rate of 16% could be obtained under these conditions. Examining the roots, spontaneous anastomosis was found in two cases outside the plastic hoses and in two cases in the induced contact area. Histological observations confirmed a tissue connection of sieve tube elements and phytoplasmas could be detected in these sieve tubes by immunofluorescence studies.

In 2005–2006, putative '*Ca.* P. mali'-transmission by root bridges could also be shown under field conditions in an 11-year's old orchard. In total, 134 AP-diseased trees were cut and treated with the herbicide glyphosate in November 2005. In spring 2006, herbicide symptoms were observed in 84 neighbouring trees. As it is well-known that glyphosate moves through the plants only within the phloem tubes (Casely and Coupland, 1985), this result indicates that the translocation of the herbicide from one plant to another happened through a phloem connection between the two plants due to a root bridge. In autumn 2006, 12% of the trees with herbicide symptoms showed typical AP symptoms for the first time. Specific PCR detection in all trees revealed the presence of '*Ca.* P. mali' in 57% of the trees with herbicide symptoms.

As *C. picta*, the main vector of '*Ca.* P. mali' in Trentino (Mattedi *et al*., 2007), was virtually absent from the region where the studied orchard is located, the obtained data indicate that '*Ca.* P. mali' transmission by natural root bridges might contribute in an important manner to the spread of AP disease in older apple orchards.

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References

CASELY J. C., COUPLAND D., 1985.- Environmental and plant factors affecting glyphosate uptake, movement and activity, pp. 93-123. In: *The Herbicide Glyphosate* (GROSSBARD E., ATKINSON D., Eds).- Butterworths, London, UK.

FRISINGHELLI C., DELAITI L., GRANDO M. S., FORTI D., VINDIMIAN M. E., 2000.- *Cacopsyilla costalis* (Flor,1981) as a vector of Apple Proliferation in Trentino.- *Journal of Phytopathology,* 148: 425-431.

JARAUSCH W., SAILLARD C., DOSBA F., BOVÉ J. M., 1994.- Differentiation of Mycoplasmalike Organisms (MLOs) in European fruit trees by PCR using specific primers derived from the sequence of a chromosomal fragment of the Apple Proliferation MLO.- *Applied and Environmental Microbiology*, 60: 2916-2923.

LOI N., ERMACORA P., CARRARO L., OSLER R., CHEN T. A., 2002.- Production of monoclonal antibodies against apple proliferation phytoplasma and their use in serological detection.- *European Journal of Plant Pathology,* 108: 81-86.

MATTEDI L., FORNO F., CAINELLI C., GRANDO M. S., JARAUSCH W. 2007.- Research on '*Candidatus* Phytoplasma mali' transmission by insect vectors in Trentino. *Acta Horticulturae*, in press.

SMART C. D., SCHNEIDER B., BLOMQUIST C. L., GUERRA L. J., HARRISON N. A., AHRENS U., LORENZ K. H., SEEMÜLLER E., KIRKPATRICK B. C., 1996.- Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region.- *Applied and Environmental Microbiology*, 62: 2988-2993.

VINDIMIAN M. E., CICCOTTI A., FILIPPI M., SPRINGHETTI M., DEROMEDI M., 2002.- Trasmissione di "apple proliferation" (AP) tramite anastomosi radicali.- *Petria*, 12: 375.

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