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Tolerance increase to '*Candidatus* phytoplasma prunorum' in mycorrhizal plums fruit trees

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Abstract

The tolerance increase to the disease caused by '*Candidatus* Phytoplasma prunorum' in Japanese plum (*Prunus salicina*) after mycorrhizal inoculation has been evaluated. Results showed that after one-year growth, non-mycorrhizal European stone fruit yellows-inoculated plants, were negatively affected by the disease infection. In non-mycorrhizal and phytoplasma inoculated plants the percentage of premature death was 30%, whereas all the mycorrhizal plants inoculated with '*Ca*. P. prunorum' survived. The shoot length of mycorrhizal European stone fruit yellows-inoculated plants did not differ from the values recorded for healthy non mycorrizal plants, indicating that early mycorrhizal inoculation increases tolerance to European stone fruit yellows disease in one-year old *Prunus salicina* cv Angeleno grafted on GF-677 rootstock.

Key words: phytoplasma control, mycorrhiza, tolerance, ESFY.

Introduction

The cultivated Japanese plum (*Prunus salicina* L.) is severely affected by the '*Candidatus* Phytoplasma prunorum' associated with European stone fruit yellows disease (ESFY). This phytoplasma belongs to the apple proliferation group (Seemüller *at al*., 1998) or 16SrX-B subgroup (Lee *et al*., 1998), is transmited by *Cacopsylla pruni* (Carraro *et al*., 1998; Sabaté *et al*., 2007). Because phytoplasmas can be persistently spread by phloem feeding insects, great efforts have been done to control the vector. The spread of the disease cannot be prevented only with the application of insecticides and other control strategies have been focused on breeding for resistant or tolerant varieties and rootstocks. Additional alternatives modifying plant physiology can increase the tolerance to the disease. In this work, the effect of mycorrhizal inoculation on European stone fruit yellows disease on *P. salicina* has been evaluated.

Materials and methods

Plant Material

Forty plants of one year-old *Prunus salicina cv Angeleno* grafted on GF-677, obtained from *in vitro* culture were used in the experiment. Twenty plants were inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices*, Schenck and Smith (BEG 72). In March 2010, when plants were transplanted to 2 liter-volume individual containers, 10 g of *G. intraradices* soil inoculum were applied under the root system. Twenty plants were used as non-mycorrhizal control. Buds from 10-year-old infected *P. salicina* trees, previously determined as ESFY-positive by the PCR technique, were selected and grafted by chip budding on ten mycorrhizal and ten non-mycorrhizal plants in September 2010. The rest of the plants were used as healthy control plants (10 healthy mycorrhizal plants and ten healthy non-mycorrhizal plants). All trees were maintained in an insect-proof greenhouse to avoid possible insect phytoplasma transmission.

Six months after '*Ca*. P. prunorum' inoculation, once mycorrhizal colonization was confirmed in the roots systems of *G. intraradices* inoculated plants, trees were tested to corroborate the disease transmission. Symptoms expression, shoot lengths and stem diameter were also recorded one year later in order to evaluate the effect of mycorrhizal colonization.

DNA extraction and phytoplasma detection

DNA was isolated from approximately 1.0 g of fresh plant material, using the phytoplasma-enrichment procedure of Ahrens and Seemüller (1992). The phytoplasma detection in the ESFY-inoculated trees was carried out using the nested-PCR technique following the protocol of Garcia-Chapa *et al*., 2003.

Results

Results showed that after one-year growth, non-mycorrhizal ESFY-inoculated plants, were negatively affected by the disease infection.

In non-mycorrhyzal plants inoculated with the phytoplasma there was a high percentage of premature death (30%), whereas all mycorrhizal plants infected with '*Ca*. P. prunorum' survived (table 1).

Vegetative growth of non mycorrhizal plants is lower than growth of mycorrizal plants when they are infected by the phytoplasma, being the total shoot length of the outbreaks of 59 cm in the first ones opposite to 80 cm in the second ones (table 1). The shoot length of mycorrhizal ESFY-inoculated plants did not differ from the values recorded for healthy non mycorrizal plants (table 1).

Table 1. Survival after inoculation with '*Ca*. P. prunorum' and growth response of mycorrhizal (M+) and non mycorrhizal (M-) plants of 40 *Prunus salicina* plants cv Angeleno grafted on GF 677 rootstocks. Ten plants per treatment.

	M-		M +	
	Healthy	ESFY	Healthy	ESFY
% died after inoculation	0%	30%	0%	0%
Total shoot length (cm)	80	59	85	80

Discussion

The ESFY disease can exhibit different degrees of severity in infected trees. Some factors that could influence in the severity are the used variety, and also the graft/rootstock combination, as has been reported by Carraro and Kison (2001).

The initial and basic injury involved in graft-union pathology is the necrosis of rootstock sieve tubes. This necrosis blocks translocation, and causes the death of the fibrous roots of affected trees due to lack of carbohydrates (Lepka *et al*., 1999).

Previous studies, have already demonstrated that the inoculation with the AM fungus *G. intraradices* increases the tolerance versus different phytoplasmas such as '*Candidatus* Phytoplasma pyri' in the *Pyrus communis* OHF 333 rootstock (Garcia-Chapa *et al*., 2004; Lingua *et al*., 2002).

Since AM fungi colonize roots and use the host photosynthates, the failing of carbohydrate transport along the blocked phloem, and the decrease of starch in the roots due to phytoplasma infection should cause a decrease of the AM fungi growth, and the loss of the positive effects of the mycorrhizal symbiosis. However the results obtained in this work again support the hypothesis that AM fungi increase phytoplasma tolerance (Lingua *et al*. 2002). After one-year growth, ESFY-infected mycorrhizal plants did not differ from or even outgrew healthy control plants despite the presence of the disease, indicating that early mycorrhizal inoculation increases tolerance to ESFY disease in one-year old *P. salicina* cv Angeleno grafted on GF-677 rootstock.

Results obtained are encouraging. Further investigations are necessary in order to elucidate the mycorrhizal effect in '*Ca*. P. prunorum' infections.

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