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Phytoplasma titer in diseased lavender is not correlated to lavender tolerance to stolbur phytoplasma

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Abstract

Yellow decline of lavender is associated with stolbur phytoplasma. It is an economically important disease in south-eastern France, and there is no curative control method. In the past few years, susceptible and tolerant lavenders (*Lavandula spp*.) or lavandins (*Lavandula latifolia* x *lavandula angustifolia*) were proposed as disease management strategy to growers. However, the bases of tolerance were unknown. In order to establish these possible bases, we compared the titers of stolbur phytoplasma in the leaves and shoots of different lavenders and lavandins, either sensitive or tolerant. Results showed that symptom severity was not correlated to the tolerance status of all cultivars tested, and that symptom severity was not correlated to the phytoplasma titer.

Key words: yellow decline of lavender, Q-PCR, stolbur phytoplasma, lavandin.

Introduction

In south-eastern France, lavender essential oil production is endangered by lavender decline. Lavander (*Lavandula angustifolia*.) and lavandin (*L. latifolia* x *L. angustifolia*) cultures are affected by yellow decline ("deperissement jaune" in French). The disease is associated with the presence of stolbur phytoplasma, which is transmitted by *Hyalesthes obsoletus* (Cousin *et al*., 1970, 1971, Moreau *et al*., 1974, E. Boudon-Padieu, personal communication). Stolbur phytoplasma is also spread by vegetative propagation through lavender and lavandin nurseries.

Up to now, no curative methods exist againsts stolbur phytoplasma. The only way is to eliminate diseased plants, apply insecticide treatments against insect vectors and plant phytosanitary certified material. Control of phytoplasma infection with resistant plant has always been very limited. However, as alternatives to susceptible plants, tolerant lavenders(ins) were proposed to growers to reduce the damage caused by lavender decline. Up to now, little is known about the mechanism of this tolerance, which could involve interactions between the plant and the phytoplasma or interactions between the plant and the insect vector. In order to identify the bases of tolerance to the decline, we intended to quantify the stolbur phytoplasma in lavenders(ins).

Materials and methods

Lavenders and lavandins were sampled in fields in South-East France. Lavenders were classified as susceptible (Lavande Bleue, lavande fine, Carla, B7, Maillettes, Matherone) or tolerant (Rapido, Diva). Lavandins were classified as susceptible (Abrial) or tolerant (Grosso, Sumian).

DNA extraction was done from 0.3-0.5 g of symptomatic leafy shoots with CTAB method and the final DNA pellet was resuspended in 50µl TE 1X (Murray and Thomson, 1980).

For setting an internal reference used in real-time PCR quantification, amplification of a 976 bp fragment of MAP gene was achieved using primers adkF2 (5'-GTTGGTCGCAGAATTTGTCC-3') and if1R2 (5'-CCAGAAACATAAGCGGTAATCGT-3') for 35 cycles (94°C 40 sec, 55°C 40 sec, 72°C 40 sec).

MAP PCR product was cloned in pGEM-T easy plasmid (Promega) following the manufacturer instruction. Q-PCR was carried out using primers MapStol-F and MapStol-R with a method modified from Pelletier and colleagues (Pelletier *et al*., 2009). The primers and probe for 'flavescence dorée' phytoplasma were removed, as well as the endogenous control targeting a grapevine tRNA which was replaced by a polyvalent endogenous control amplifying the COX gene (Weller *et al*., 2000). Results were analyzed using MxPro QPCR software (Agilent). The number of phytoplasmas was calculated for all plants, and the difference between mean values were evaluated by Chi-square tests.

Results

Obtainment of the quantification standard. A fragment of the stolbur phytoplasma MAP gene used for the detection (Pelletier *et al*., 2009) was amplified from DNA extracted from infected lavenders(ins) using primers adkF2 and if1R2. The obtained product was cloned in pGEM-T Easy and sequenced. The plasmid was then quantified and diluted in order to obtain a range standard from 10^1 to 10^8 plasmids/µl. This standard was tested in duplicate in each Q-PCR assay.

Absence of correlation between phytoplasma titer and symptom severity (severity index). A total of 2,500 lavenders(ins), susceptible or tolerant, were collected in 2008, 2009 and 2010. Each plant was assigned a severity index (from 0: no symptoms, to 7: dead plant) and

tested in Q-PCR assay 692 were positives for the presence of stolbur phytoplasma. Results showed that phytoplasma titer was not significantly correlated to the severity index.

The phytoplasma titer was measured in susceptible and tolerant lavenders(ins). Figure 1 shows the mean titer of phytoplasma in susceptible and tolerant lavandins in spring and autumn. There was a 2.4 fold and 1.2 fold differences in phytoplasma mean titer between susceptible and tolerant lavandins in spring and autumn respectively. However, these differences were not statistically significant (student impaired t-test). The same differences were observed between susceptible and tolerant lavenders in spring and autumn. The only significant difference in phytoplasma titer was observed for susceptible lavender between spring and autumn.

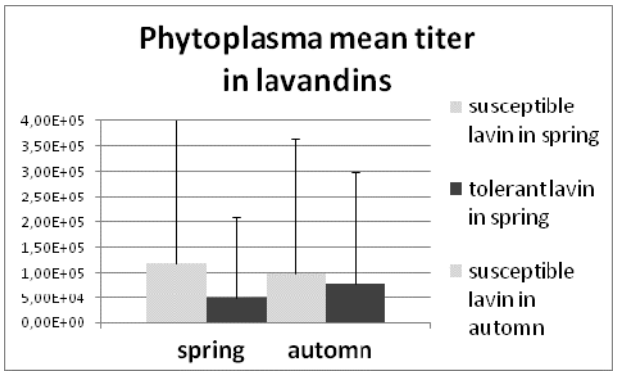

Figure 1. Histogram representing mean titer of phytoplasma (phytoplasma/µl) in susceptible (grey) or tolerant (black) lavandins (lavin), in spring and in autumn. Sampling was done in 2008-2009 and 2010.

Time course evolution of phytoplasma titer in lavenders(ins) of different susceptibility was studied. In one year, samples were collected every month on the same lavenders(ins) (from May to November 2010 except in August). Six different cultivars were analyzed: lavandins Abrial (susceptible, clonal), Grosso (tolerant, clonal), Blue-lavander (susceptible, populations), Rapido lavender (tolerant, controled population) and Diva (tolerant, clonal, in 2 different geographical locations). Quantification was achieved monthly.

Results showed that the phytoplasma titer evolution was not more rapid in susceptible plants as compared to tolerant plants. Most importantly, detection could become negative even when the plant sample had been previously tested positive, indicating the chronicity of the infection. Results concerning Diva lavender showed that disease evolution is dependant on the geographical location of the fields.

Discussion

Results showed that symptom severity was not correlated to lavender and lavandin susceptibility. Little differences in phytoplasma titer were observed between susceptible and tolerant lavenders(ins) in spring and autumn but they were not significant, only for susceptible lavenders. This might be due to temperature effect on the phytoplasma multiplication-rate.

The geographical location of the plants affected the disease development in Diva lavender, probably due to the influence of the climate on the stolbur phytoplasma multiplication, but also on the insect vector activity. Interactions between the plant and the insect vector could then be an important part of the tolerance mechanism of lavenders(ins) to stolbur phytoplasma.

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