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Evaluation of vectoring ability of phytoplasmas by *Metcalfa pruinosa* Say (Homoptera: Flatidae) recently introduced in Europe

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Abstract: *Metcalfa pruinosa,* a neartic flatid introduced to Europe, is extremely polyphagous and might be a potential vector of phloem-inhabiting pathogens. The aim of the study was to evaluate the vectoring ability of the species for two different phytoplasmas. Phytoplasmas acquired on infected plants, did not appear to persist in the body of infected insects and no transmission was recorded.

Keywords: *Metcalfa pruinosa*, phytoplasma, flavescence dorée, clover phyllody, transmission.

Introduction

Since its first report in Italy in 1980 (Zangheri & Donadini, 1980), *Metcalfa pruinosa* has progressed to South France. Due to its multiple hosts and a phloem-feeder, it is considered as a potential vector of phloem pathogen agents such as phytoplasmas. Among grapevine yellows (GY) (Boudon-Padieu & Maixner, 1998), Flavescence dorée (FD) (EY-group phytoplasma) is epidemic because its vector, *Scaphoideus titanus*, is a vine-feeding leafhopper. Bois noir and Vergilbungskrankheit (stolbur phytoplasma) are GYs of variable importance as their vector, *Hyalesthes obsoletus*, does not live on grapevine. Known vectors of other European GYs feed on grapevine erratically (Maixner *et al*., 2000). *M. pruinosa* might be an alternative vector of FD, an efficient vector of any other GY or a vector of a new GY. Phytoplasma have been detected in wild specimen (Danielli *et al*., 1996). However, because phytoplasma vection is a specific process with circulation and multiplication in the insect body (Lefol *et al*., 1994), direct detection is not sufficient to demonstrate vectoring ability. Controlled trials for acquisition and transmission of phytoplasmas by *M. pruinosa* were conducted in 1999 and 2000 .

Material and methods

Phytoplasmas

FD and PHY (clover phyllody) phytoplasmas were maintained in broadbean (*Vicia faba*) in the insectarium by serial transmission with colonies of reared *Euscelidius variegatus* (Caudwell *et al*., 1972).

Phytoplasma acquisition, transmission and detection in insects

First instars of wild *M. pruinosa* were taken to the insectarium, caged on FD or PHY-infected broadbean or healthy broadbean control for 1-2 week periods. The insects were then transported to healthy broadbeans until they died, daily collected and stored at -20°C. Individual insects were tested with nested-PCR and RFLP of 16S rDNA of phytoplasma (Sforza *et al*., 1998).

Broadbeans were taken to the greenhouse and watched for symptom expression.

Results and discussion

Data of the experiments are summarised in Table 1. Insects obviously suffered from handling and survival was 5 to 26 days on broadbean in 1999 and 7 to 21 days in 2000.

FD detection and transmission
Sixteen insects out of 19 in 1999 and 18 out of 76 in 2000, tested FD positive. The PCR signal was high for insects dead on infected plants, even after only 5 days of acquisition. On the contrary, the signal was very low or negative for insects transferred to healthy test plants for more than 7 days. None of 7 test plants developed FD symptoms.

PHY detection and transmission
In 1999, 11 insects out of 16 tested positive for PHY. The signal was low or negative for insects transferred to healthy plants for 4 days or more. No symptoms occurred on test plants.

Control
Insects fed on healthy broadbean, tested negative in PCR for any phytoplasma.

Table 1. Duration of acquisition and transmission and phytoplasma detection in insects.

Phytoplasma (date)	acquisition (days)	transmission (days)	detection in insects	transmission
FD (1999)	5	0	8/8	/
	11	0	4/4	/
	11	4	3/3	0/1
	11	10	1/2	0/1
	11	15	0/2	0/1
Total			**16/19**	**0/3**
FD (2000)	7	0	3/8	/
	7	1	3/14	/
	7	2	2/6	0/2
	7	5	1/11	
	7	7	5/16	
	7	9	3/6 (weak)	0/2
	7	12	1/15 (weak)	
Total			**18/76**	**0/4**
PHY (1999)	11	7	4/5 (weak)	0/2
	11	8	3/5 (weak)	0/2
	11	9	3/3 (weak)	0/1
	11	10	1/3 (weak)	0/1
Total			**11/16**	**0/6**

Similar results were obtained with FD and PHY. The characterisation of one or the other phytoplasma in the body of insects fed on infected broadbeans and the absence of detection in insects fed on healthy plants, show that the phytoplasma detected were acquired by feeding. However, the weaker or no signals obtained from insects transferred to healthy broadbean, suggest that phytoplasmas were merely ingested in the guts and did not persist or multiply in

the insect body. This hypothesis is supported by the fact that no test plant was infected.

These experiments provide no indication that *M. pruinosa* could be a vector of FD or PHY. New trials will use other phytoplasmas and plants and better conditions for insects survival.

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