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INTRODUCTION

Despite the numerous diseases caused by phytoplasmas on cultivated and wild plants worldwide, few resistant species or varieties have been identified. Some studies performed on woody plants, like apple or coconut trees are the exception (for review Seemuller and Harries 2009). Thus, inoculations tests of different *Malus* species by the apple proliferation phytoplasma have shown that *Malus sieboldii* presents little symptoms and that phytoplasmas multiply less in *M. sieboldii* than in traditional *Malus domestica* cultivars or rootstocks (Bisognin *et al.* 2008). In the case of grapevine, intraspecific (*Vitis vinifera* cultivars) and interspecific (rootstocks) variability in plant sensitivity to the Flavescence dorée (FD) disease is well known; some inoculated rootstocks even show no symptoms of the disease (Schvester *et al.* 1967, Moutous *et al.* 1977). But this variability has not been studied in controlled conditions of inoculation and has not been characterized in term of phytoplasma titre in the plant. Our study consists in evaluating the sensitivity of major cultivars and rootstocks by recording the symptoms, the % of infected plants, and by measuring the phytoplasma titre in the plants after inoculation by the vector *Scaphoideus titanus* in high confinement greenhouse. Greenhouse experiments are completed by symptom observations and phytoplasma quantification in vineyards.

MATERIALS AND METHODS

Young plantlets (20-30 cm high) of Cabernet Sauvignon (CS), Merlot, Chardonnay and Pinot Noir cultivars, Selection Oppenheim 4 (SO4), Kober 5BB (5BB), 3309 Couderc (3309), Millardet et de Grasset 41B (41B), Nemadex Alain Bouquet, Riparia Gloire de Montpellier (RGM) rootstocks issued from in vitro multiplication were grown in high confinement greenhouse (25°C, L16:D8 photoperiod). Infectious *S. titanus* were obtained by acquisition on broad beans infected with FD phytoplasma (FDp), strain FD-PEY05 (Papura *et al.* 2009). Insects were transferred by groups of 7 onto grapevine plants for 1 week. Each experiment was performed by inoculating 14 to 16 plants per accession tested, including the sensitive CS as a positive control. After the transmission period, insect survival rates were recorded and insects were collected for further phytoplasma detection. Three, 5 and 10 weeks post-inoculation (wpi), 4 to 6 plants were collected. Stems, petioles and midribs were dissected, weighed and total DNA was extracted. Quantification of FDp cells in each plant was performed by quantitative real-time PCR on the *tuf* gene. For each time of sampling and for each grapevine accession, the symptoms, the % of infected plants and the mean phytoplasma titre were recorded.

RESULTS AND DISCUSSION

Survival of *S. titanus* on 5BB, 3309, S04, 41B and RGM was higher than or equivalent to the survival on CS which ranged between 69 and 86 %. It is not surprising as these *Vitis* species are native hosts of the vector in North America. Insects survived less on Pinot Noir, Merlot and Nemadex. In comparison with CS, the number of infected plants was higher for Chardonnay, 3309 and 41B, slightly lower for RGM Pinot N and 5BB, lower for SO4 and Merlot. No Nemadex plants were found infected. The first symptoms (coloration of leaf blades and veins, rolling of the leaves) appeared at 6 weeks post inoculation (wpi) for CS and 1 week later for Chardonnay and Pinot N. No specific symptoms could be observed at 10 wpi for the other accessions. Generally, the mean phytoplasma titre increased between 3, 5 and 10 wpi. However, for some accessions such as Chardonnay, SO4, 41B, 5BB and Merlot, it stabilized or even decreased at 10 wpi. Whatever the time, the ratio between CS and Chardonnay phytoplasma titre never exceeded 4. For 3309, RGM and Pinot N, the ratio which was high (17 to 115) at 3 wpi, decreased over the time to reach at 10 wpi ratio values similar to that of Chardonnay. For SO4, the high ratio measured at 3 wpi was slightly reduced at 10 wpi. The ratio was stable for 41B (28 to 30) but drastically increased for Merlot and 5BB (27 to 100 and 74 to 625 respectively).

In conclusion, Chardonnay like CS, can be considered as highly sensitive to FDp, Pinot N can be considered as moderately sensitive and Merlot as the less sensitive to FDp. This is in agreement with former field observations (Boudon-Padieu 1996). Furthermore, field surveys confirmed that the level of symptoms and FDp titre were significantly lower in Merlot than in CS. Although 3309 and RGM rootstocks did not present any symptoms, they exhibited high multiplication of the phytoplasma and can therefore be considered as tolerant to FDp. It was also the case for non-symptomatic "wild" rootstocks regrowth surrounding FD outbreaks. On the contrary, the low FDp multiplication in 5BB could make this rootstock a potential source of resistance to FD disease. Insect survival rate on *Muscadinia rotundifolia*-derived intergenic hybrid Nemadex was quite low and Nemadex could not be infected by FDp. Resistance to insect might explain such results as muscadine hybrids also appeared to be a good source for resistance to the nematode *Xiphinema index* (Esmenjaud *et al.* 2010).

Experiment	Accession	% of insect survival	Nb of infected plants/total inoculated	Symptom appearance at wpi	Mean phytoplasma titre in nb of cells/ug nucleic acids ± SE **(CS*/accession ratio)**		
					3 wpi	**5 wpi**	**10 wpi**
1	CS	86	13/14	6	$5x10^3 \pm 3.6x10^3$	$7.4x10^4 \pm 4.1x10^4$	$7.1x10^3 \pm 5.8x10^3$
	Merlot	62	9/14	-	$1.8x10^2 \pm 1.7x10^2$ **(27)**	$4.2x10^2 \pm 4.7x10^2$ **(175)**	$7 x10^1$ **(100)**
2	CS	69	14/15	6	$2.5x10^4 \pm 1.6x10^4$	$6.2x10^4 \pm 1.3 x10^4$	$1.6x10^5 \pm 7.1x10^4$
	3309	96	15/15	-	$1.5x10^3 \pm 1x10^3$ **(17)**	$2 x10^4 \pm 1.5x10^4$ **(3)**	$4.5x10^4 \pm 3.1x10^4$ **(3)**
	RGM	76	12/14	-	$2.2x10^2 \pm 1.8x10^2$ **(115)**	$5.6x10^3 \pm 3.9x10^3$ **(11)**	$3.8x10^4 \pm 2.6x10^4$ **(4)**
	SO4	84	12/15	-	$9.1x10^2 \pm 7.4 x10^2$ **(27)**	$1.4x10^4 \pm 2.4x10^4$ **(4)**	$7.9x10^3 \pm 6.3x10^3$ **(20)**
3	CS	83	11/15	6	$2.9 x10^4 \pm 3.9x10^4$	$1.1x10^5 \pm 1.2x10^5$	$1.8 x10^5 \pm 4.7x10^4$
	Chardon.	83	14/15	7	$7.4x10^3 \pm 7.9x10^3$ **(4)**	$6.1 x10^4 \pm 9.1x10^4$ **(2)**	$6.2 x10^4 \pm 7x10^4$ **(3)**
	Pinot N	73	10/15	7	$6.9x10^2 \pm 3.7x10^2$ **(42)**	$1.1x10^4 \pm 1.9x10^4$ **(10)**	$3.6x10^4 \pm 4.6x10^4$ **(5)**
	41B	91	11/14	-	$1x10^3 \pm 7.9x10^2$ **(28)**	$6x10^3 \pm 6x10^3$ **(18)**	$6.1x10^3 \pm 8.2x10^3$ **(30)**
	5BB	81	11/16	-	$3.9x10^2 \pm 2.2x10^2$ **(74)**	$3.4x10^3 \pm 2.7x10^3$ **(32)**	$2.9x10^2 \pm 2x10^2$ **(625)**
	Nemadex	41	0/15	-	-	-	-

* CS values from the same experiment were taken as a reference; wpi : weeks post-inoculation; -: no symptoms or no ratio.

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