Vitis 34 (3), 189–190 (1995)

Research Note

Effect of grapevine cultivar, strain of *Xylophilus ampelinus* and culture medium on *in vitro* development of bacterial necrosis

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Summary: Factorial experiments were designed to study the response of *in vitro* cultivated grapevine after inoculation with *Xylophilus ampelinus*, the causal agent of bacterial necrosis. Significant effects of culture medium, cultivar and strain were demonstrated. The possible uses for the *in vitro* test are discussed.

Key words: grapevine, bacterial necrosis, in vitro.

Introduction: The bacterial necrosis due to *Xylophilus ampelinus* (Willems *et al.* 1987), formally Xanthomonas ampelina (Panagopoulos 1969) is present in several grape-growing European regions where it can cause significant yield losses by reducing the yield and the longevity of affected vineyards (Panagopoulos 1987). Bacteria infected the vines through wounds and colonized the vascular tissues. The disease is transmitted by latently infected planting and grafting material. The extension of the disease sources is favored by pruning and mechanized cultural practices, rain, lack of copper sulfate treatment, and varietal susceptibility (Panagopoulos 1987; Ride *et al.* 1987). Sanitation and the planting of resistant cultivars can reduce the incidence of the disease (Ride *et al.* 1987; Lopez *et al.* 1987).

The symptoms have been obtained after *in vitro* inoculation of plantlets and it has been suggested that the cultivar susceptibility could be rated *in vitro* (Arregui *et al.* 1988; Ride and Bouquet, unpublished). The aim of this study was to evaluate in factorial experiments the performances of the *in vitro* method to assess the cultivar susceptibility and the pathogenic variation in the bacterium.

Materials and methods: Plantlets of Grenache (clone 224), Syrah (clone 73), and two intraspecific hybrids of *Vitis vinifera* Gramon (Grenache x Aramon) and Chasan (Listan x Chardonnay), were obtained from bud culture. Grenache and Gramon were rated very susceptible to bacterial necrosis in the vineyard and the greenhouse, whereas Syrah and Chasan were considered as tolerant. Cultures were initiated on a Galzy's medium coded G64 (Galzy 1964). Onto this medium, senescence and lignification limited the growth after 3 to 4 months and then micropropagation was seriously impaired. Another medium, performed by Galzy *et al.* (1990) and coded G90, was tested and was shown to increase the time between subcultures up to 6 months. The main difference is that potassium is brought with KNO_3 in G90 instead of KCl in G64. Therefore, G90 contains no Cl^- anions and more nitrate-N than G64. The plantlets were grown in non-sealed tubes at 25 °C with a 16h/8h photoperiod at 100 $\mu E\ m^{-2}\ s^{-1}$.

Strains XA2292 and XA2289, isolated from Ugni blanc in Charente-Maritime, were obtained from the « Collection Française de Bactéries Phytopathogènes », INRA, Angers, France. Preliminary observations indicated that XA2292 has a higher pathogenicity than XA2289. Strains were grown at 25 °C on agar slants containing per liter 5 g of yeast extract, 5 g of Bactopeptone, 10 g of glucose and 15 g of agar, pH 7.2. Bacterial suspensions of about 10^8-10^9 colony forming units per ml were prepared by suspending a 5 d-old culture from a test tube into 100 ml of sterile distilled water. Scissors were dipped in the suspension and plantlets having 8 to 12 nodes (2 months old) were decapitated between the second and third nodes from the top. Controls decapitated with scissors dipped in sterile distilled water were included. Plantlets were inspected for the presence of symptoms and the number of internodes with symptoms, i.e. canker, brown stripe or complete necrosis, was recorded for each plantlet, 4, 8 and 12 weeks after inoculation.

In Experiment 1, plantlets of Gramon and Chasan, micropropagated on G90 medium, were subcultured either on G64 or G90 and then inoculated with XA2292. For this experiment, the number of nodes and the height of the stem were recorded before inoculation. In Experiment 2, Grenache, Gramon, Chasan and Syrah grown on G90 medium were inoculated with XA2292 and XA2289. Experiments were replicated twice and were arranged in a complete randomized block design with 6 plantlets per plot and 4 replicates.

Results: A few days after inoculation, tissues reacted by callusing and then necrosis. The necrosis was sometimes stopped at the first node but generally progressed along the stem. The internode firstly had brown stripe and then necrosed with cracks and cankers similar to those observed in the vineyard. When the necrosis appeared to have stopped at a node, the corresponding axillary bud developed to form a new stem which might thereafter have the symptoms and died. When the plantlet appeared less damaged, root formation was observed at callusing nodes.

In Expt. 1, G90 was more favorable with respect to growth than G64; the growth of Chasan was higher than Gramon for G90 but the reverse was observed for G64 (significant interaction, data not shown). The number of necrosed internodes was higher for plantlets grown onto G90 than for those grown onto G64 (Table). The cultivar effect was also significant 4 and 8 weeks after inoculation but not significant 12 weeks after inoculation.

In Expt. 2, the cultivar had a highly significant effect (Table). The tolerant cultivars (Syrah and Chasan) were clearly separated from the susceptible cultivars (Gramon and Grenache) 12 weeks after the inoculation. Strain XA2292 induced more quickly the symptoms than strain XA2289 but the difference between the two strains was not significant at the last observation (Table). The interac-

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tion observed 8 weeks after inoculation was due to the fact that symptoms were more acute for Grenache than for Gramon with XA2292 whereas no difference was noticed with strain XA2289. Strain XA2292 generally induced complete necrosis of internodes whereas only cracks were observed near the nodes with XA2289. The appearance of roots at nodes under the necrosis appeared more frequently with XA2289 than with XA2292 (7 *vs.* 29 % of the plantlets inoculated).

Table

Effect of cultivar, medium and strain of *Xylophilus ampelinus* on the number of necrosed internodes after inoculation of *in vitro* cultivated grapevine

	Weeks after inoculation		
	4	8	12
Experiment 1			
Cultivar			
Gramon	1.0	1.4	1.6
Chasan	0.5	0.9	1.4
Significance	***	**	ns
Medium			
G64	0.5	0.8	1.2
G90	0.9	1.5	1.8
Significance	***	***	***
Interaction	ns	ns	ns
Experiment 2			
Cultivar			
Gramon	1.1^{a}	1.8^{a}	2.5^{a}
Grenache	0.9^{ab}	1.7^{a}	2.8^{a}
Syrah	0.8^{b}	1.5^{b}	1.9^{b}
Chasan	0.3^{c}	1.0^{c}	1.8^{b}
Significance	***	***	***
Strain			
XA2292	1.0	1.7	2.4
XA2289	0.6	1.3	2.2
Significance	***	***	ns
Interaction	ns	**	ns

ns, **,*** non-significant at $P \leq 0.05$ or significant at $P \leq 0.01$ or ≤ 0.001, respectively (F test of ANOVA after the pooling of the data of both replicates of each experiment).
The means followed by the same letter are not significantly different at $P \leq 0.05$ according to Newman-Keuls test.

Discussion: The symptoms of bacterial necrosis can be reproduced on plantlets. This offers the possibility to study in controlled conditions the disease without the risk to disseminate the bacterium as already proposed (ARREGUI *et al.* 1988). The stem necrosis was often stopped at the node level where physical or chemical barriers probably exist. To estimate the progression of infection we propose to determine the number of internodes showing symptoms.

The effect of the medium indicated that the development of symptoms was dependent on the physiology of plantlets. Medium G90 with a higher nitrogen content appeared more favorable to growth and consequently to infection. The *in vitro* conditions seemed favourable to study the effect of nutrition on the disease. In particular, the hypothesis that high level of calcium in soil favors the tolerance of grapevine could be tested.

The ranking of cultivars was correlated with vineyard observations. Cultivars with large differences have been statistically separated but we suppose that it would be more difficult to distinguish cultivars with closer response and with intermediate level of susceptibility. However, the test appeared suitable to screen the most susceptible cultivars and new varieties in a breeding program.

It was also possible to demonstrate pathogenic differences between strains *of X. ampelinus.* The variation for pathogenicity among isolates of the bacterium from different geographic regions could be analyzed in order to characterize the populations of the pathogen and their evolution.

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