Phytopathol. Mediterr. (2009) 48, 285–290

Susceptibility to *Armillaria mellea* root rot in grapevine rootstocks commonly grafted onto Teroldego Rotaliano

Daniele PRODORUTTI[1], Federica DE LUCA[2], Lorenza MICHELON[2] and Ilaria PERTOT[1]

[1]Fondazione Edmund Mach, via E. Mach 1, 38010
and
[2]SafeCrop Centre–Istituto Agrario di San Michele all'Adige, via E. Mach 1,
38010 S. Michele all'Adige (TN), Italy

Summary. Armillaria root rot is an increasing problem in some grapevine-growing areas in north-eastern Italy (Trentino Province). The susceptibility of seven grapevine rootstocks (Schwarzmann, 3309 C, 101-14, Teleki 5C, SO4, Kober 5BB and 41 B; all grafted with Teroldego Rotaliano) to *Armillaria mellea* was evaluated in a five-year investigation. Two inoculation methods were also compared: young grapevine plants were either transplanted to a substrate that had been inoculated with *A. mellea* (Method A), or *A. mellea* rhizomorphs were inserted under the root bark after the root bark had been lifted up with a scalpel (Method B). Plants inoculated with Method A had higher infection and mortality rates than plants that were inoculated with Method B, demonstrating that root wounding does not lead to higher *A. mellea* infection. The significantly higher mortality and infection rates of 3309 C as compared with Teleki 5C in the final year of the study suggest that a Teroldego Rotaliano vineyard established on 3309 C will suffer greater losses than would a similar vineyard established on Teleki 5C. Rootstocks that were intermediate in their response to infection (Schwarzmann, Kober 5BB, and 41B) may offer moderate levels of resistance since with these rootstocks the mortality and infection rates were not significantly different from those of Teleki 5C. Since all rootstocks became infected, however, no rootstock is completely immune.

Key words: autochthonous grape variety, Armillaria disease, Armillaria resistance.

Introduction

Root rot caused by *Armillaria* spp. is an increasing problem in some grapevine-growing areas of northern Italy (Trentino Province), especially in the Piana Rotaliana region, where the grape (*Vitis vinifera* L.) variety Teroldego Rotaliano is the most popular (comprising about 99% of the Registered Designation of Origin area; Fellin, personal communication, 2007). *Armillaria mellea* (Vahl.: Fr.) P. Kumm. reduces plant vigor and fruit quality and frequently kills the plant in the last stage of infection (Aguin-Casal *et al*., 2004). Rhizomorphs in and around the roots and white fans of mycelia under the bark are typical signs of the disease (Raabe, 1988; Fox, 2000). *A. mellea* can survive for several years in the soil as a saprophyte on root residues. This long-lasting inoculum is particularly problematic for vines in new vineyards planted on previously infected sites (Pertot *et al*., 2008).

Immunity to *Armillaria* spp. has not so far been reported in the genus *Vitis*. Raabe (1979) tested several grape rootstocks for resistance to *Armillaria* spp. and found that, two years after inoculation, rootstocks differed significantly in the incidence of Armillaria infection. Baumgartner and

Corresponding author: D. Prodorutti
Fax: +39 0461 615500
E-mail: daniele.prodorutti@iasma.it

Rizzo (2006) studied the relative resistance of eight grapevine rootstocks in California. Two years after inoculation they also found significant differences in infection incidence, but not in the vine mortality rates. They concluded that rootstock 3309 C was the most susceptible to the disease. Similar results were reported by Mansilla *et al*. (2001), who found that 3309 C was the first to show the aerial symptoms of Armillaria infection, before the other rootstocks tested.

Teroldego Rotaliano is commonly grafted onto a number of rootstocks: Schwarzmann (*V. riparia* × *V. rupestris*), 3309 C (*V. riparia* × *V. rupestris*), 101-14 (*V. riparia* × *V. rupestris*), Teleki 5C (*V. berlandieri* × *V. riparia*), SO4 (*V. berlandieri* × *V. riparia*), Kober 5BB (*V. berlandieri* × *V. riparia*) and 41 B (*V. vinifera* × *V. berlandieri*), which yield the highest quality wines (Malossini and Fellin, 2002). Empirical observations in vineyards suggest a correlation between rootstock vigor and the timing of Armillaria symptoms, with more vigorous rootstocks exhibiting later infection dates.

The aim of the study was to evaluate the susceptibility to Armillaria root rot of several rootstocks that are commonly grafted with Teroldego Rotaliano. Two methods for inoculating grapevine with *A. mellea* were tried, one simulating natural infections under field conditions, and the other based on one of the methods reported by Baumgartner and Rizzo (2006) with modifications.

Materials and methods

The susceptibility of seven grapevine rootstocks (Schwarzmann, 3309 C, 101-14, Teleki 5C, SO4, Kober 5BB and 41 B, all grafted with Teroldego Rotaliano) to *A. mellea* root rot was evaluated. Vines were potted in 12-L pots containing a sterile mixture of peat and pumice (3:1) and were grown under natural conditions in S. Michele all'Adige (Italy). Pots were separated from the soil with a layer of pebbles. During this period all vines had similar growth with no sign of Armillaria symptoms. One year after planting, vines were inoculated using two different methods. With Method A, vines were transplanted to a substrate previously inoculated with *A. mellea*-infected roots. With Method B, *A. mellea* rhizomorphs grown in culture were inserted under the root bark of the plants. Method A was selected because it simulates the infections under natural conditions and also takes into account the effect of the vigor and rooting habit of rootstocks on infection. Method B is a modification of the wound plus inoculation method reported by Baumgartner and Rizzo (2006), using a less persistent inoculum (rhizomorphs rather than of infected pear wood).

The inoculum of Method A consisted of grapevine roots naturally infected with *A. mellea* and collected from a vineyard in Piana Rotaliana (vineyard no. 18; Pertot *et al*., 2008). Five stunted and/or dead plants were selected and the roots colonized with typical white Armillaria mycelium, and rhizomorphs were collected. *A. mellea* was isolated and identified as described by Pertot *et al*. (2008). *A. mellea* infected roots were cut into small pieces (5 cm) and these pieces were added (approximately 1:50, v:v) to the sterile peat and pumice mixture. The inoculated potting mix was incubated for six months at 22°C and high humidity to promote *A. mellea* growth, and then used as the soil for Method A plants.

In Method B, an isolate of *A. mellea* (18A; Pertot *et al*., 2008) obtained from an infected grapevine from the same vineyard was used as inoculum. This isolate was cultured on 1% malt extract agar (MEA; Oxoid, Hampshire, UK) for 30 days at 22°C. To inoculate the plants, the bark of the main roots (older than one year) was lifted (wounds 3×0.5 cm) with a scalpel, forming a gap between the bark and the root below the bark. *A. mellea* rhizomorphs (0.5 cm long) were then inserted into this gap, underneath the bark. Five roots per plant were inoculated. Each of these roots was inoculated three times, twice in the first year of the trial (in April and August) and once in the second year (in April). A sterile mixture of peat and pumice (3:1) was used to transplant the vines in Method B, and the untreated controls.

The study used a randomized complete block design, with four blocks of five inoculated plants for each combination of inoculation method and rootstock per block. Twenty plants of each rootstock were not inoculated (half of these were wounded as in Method B, but not inoculated). Uninoculated vines served as the untreated controls and were randomized in the blocks like the other vines (five vines per rootstock per block). All the potted plants were kept outdoors under natural conditions. Plants were fertilized twice each growing season with Nitrophoska Gold® (NPK 15-9-15, a slow-release fertilizer, Compo Agricoltura S.p.a., Cesano Maderno, Italy). This experiment was rep-

licated once: it started in 2000 and was repeated in 2001.

Vines were evaluated for *A. mellea* in September, each year for five years, by gently removing the potting mixture and examining the roots and crowns of all vines. Dead vines were also counted at this time. To verify *A. mellea* root infection, we checked for rhizomorphs in direct contact with or penetrating into the roots, and for the typical *A. mellea* fan-leaf mycelia under the bark of the rotted roots and crowns. Dead plants with no visible sign of *A. mellea* mycelia or rhizomorphs were put into plastic bags which were then sealed and kept in the dark under humid conditions for 30 days to promote the growth of the fungus. After this period the roots were again examined for *A. mellea*.

Samples of any mycelium growing on dead or symptomatic plants were cultured (Worrall and Harrington, 1993; Pertot *et al.*, 2008) to confirm the presence of *A. mellea* according to Pertot *et al.* (2008) and the absence of other pathogens. Five years after the initial inoculation, all the vines were uprooted and once again examined, as they had been in previous years.

The incidence (%) of *A. mellea* on the vines and vine mortality due to the disease was evaluated annually for each inoculation method and for each rootstock. For each treatment, the area under the disease progress curve (AUDPC) was calculated using the formula

$$\sum_{i=1}^{n-1} [(t_{i+1} - t_i)(y_i + y_{i+1})/2],$$

where y=mortality or frequency of infected plants, t=years after inoculation and n=total No, of assessments.

Parametric and non-parametric statistical tests were conducted using the Statistica 7.1 software package (StatSoft, Tulsa, OK, USA). The Kolmogorov-Smirnov test for two independent samples was used to detect statistical differences between the inoculation methods. Each year, Kruskal-Wallis analysis was used to compare the frequencies of infection and mortality between the rootstock treatments. ANOVA and Tukey's test were used to compare the AUDPC values of the different rootstock treatments and to compare the results of the repeated experiments. Each year's AUDPC data was arcsin$\sqrt{}$/1000 - transformed before ANOVA was conducted.

Results

In the second year after inoculation vines started to show symptoms of *A. mellea* infection (reduced plant vigor and premature reddening of leaves) and to die. This is similar to the progress of natural infections in new vineyards, in which initial symptoms never occur until two or three years after planting (Perazzolli *et al.*, 2007).

The data of the two experiments were pooled because no effect of the variable "experiment" was observed ($P>0.05$). In the years of the study, even though the differences between rootstocks remained much the same, vines inoculated with Method A had higher infection rates and mortality rates ($P<0.05$) than plants inoculated with Method B. During the five years of the experiment, infections developed in 59.3% of vines inoculated with Method A, and 38.5% of the plants inoculated with this method died. Of the vines inoculated with Method B, 8.6% became infected in the course of the experiment, and 4.3% died. From the second year (when vines started to die) to the fifth year after inoculation, the rates of cumulative mortality from *A. mellea* infection showed a linear trend with a constant increase of 7% of dead vines per year with Method A and 1% with Method B (Fig. 1).

None of the non-inoculated vines died or developed *Armillaria* infection in the test period. It is concluded that Method A, based on infected substrate, is more effective than Method B. In our assessment of the susceptibility of the different rootstocks to *A. mellea*, we therefore examined only vines inoculated with Method A.

In vines inoculated with Method A, the incidence of *A. mellea* infection and the rates of mortality due to *A. mellea* in each year did not differ significantly between individual rootstocks ($P>0.05$, Kruskal-Wallis test; data not shown). In the fifth year after inoculation, the percentage of dead vines in individual rootstocks (mean of the two experiments) varied from 40% in Teleki 5C to 73% in 3309 C and the frequency of infection ranged from 60% in Teleki 5C to 100% in 3309 C. However, in the AUDPC values from year 5 of the experiment, 3309 C had significantly higher mortality and infection rates than Teleki 5C, Schwarzmann, Kober 5BB and 41 B. The infection rate of 3309C was also significantly higher than that of 101-14 C (Table 1).

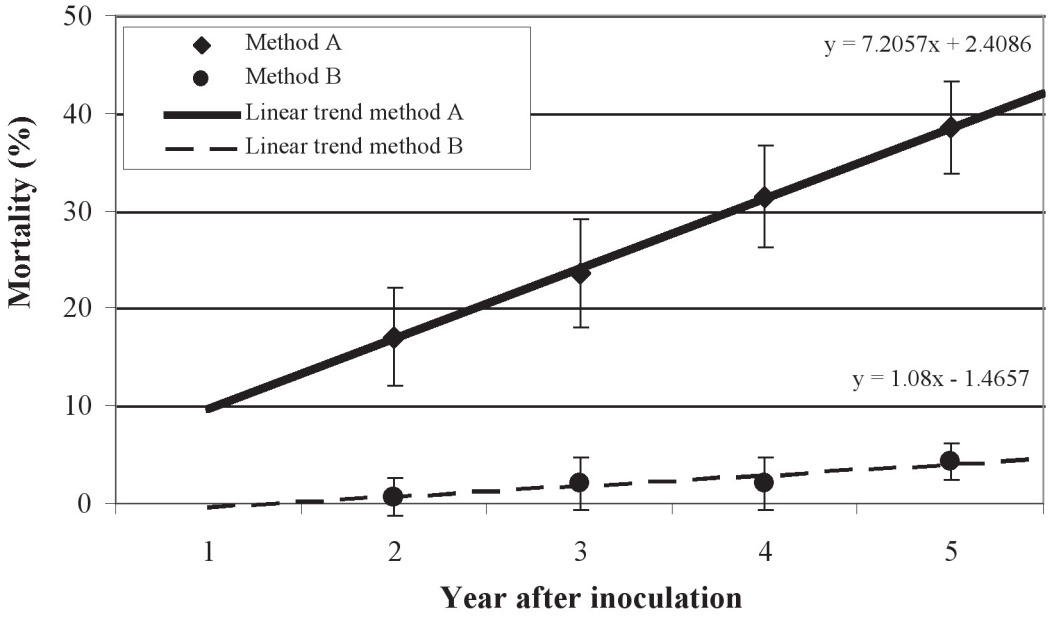

Fig. 1. Cumulative mortality (%) due to *Armillaria mellea* infection of grapevine rootstocks planted in *A. mellea*-infected substrate (Method A) or inoculated by inserting rhizomorphs under the root bark (Method B), in the course of a five-year study. Vertical bars represent standard errors. Each value represents the mean of two experiments.

Table 1. Mortality and infection rates from *Armillaria mellea*, expressed as the Area Under Disease Progress Curve at the fifth year after inoculation (AUDPC), for seven grapevine rootstocks planted in infected substrate (Method A).

Rootstock	Mortality (AUDPC)		Infection (AUDPC)	
Teleki 5C	56.7[a]	a[b]	70.0	a
Schwarzmann	73.3	ab	83.3	ab
Kober 5BB	90.0	ab	110.0	ab
41 B	96.7	ab	110.0	ab
101-14 C	110.0	abc	116.6	ab
SO4	166.7	bc	176.6	bc
3309 C	230.0	c	243.3	c

[a] Data of two experiments were pooled. For each rootstock, values represent the mean of all replicates of the two experiments (40 vines).
[b] Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Tukey's test.

Discussion

Our study examined new grapevine rootstocks and demonstrated that rootstock immunity to *A. mellea* is not likely to exist, but that there are different levels of resistance. Significantly higher mortality and infection rates (AUDPC values) in 3309 C than in Teleki 5C, in the final year of the study, suggest that a new vineyard of Teroldego Rotaliano grafted on 3309 C is likely to suffer he-

avier losses than a new vineyard grafted on Teleki 5C. The three rootstocks intermediate in their response to infection (Schwarzmann, Kober 5BB, and 41B) may offer moderate levels of resistance, since it was found that mortality and infection rates of these rootstocks were not significantly different from those of Teleki 5C. Moreover, the finding that 3309 C is most susceptible to the disease corresponds to what has already been reported in other studies (Mansilla *et al*., 2001; Baumgartner and Rizzo, 2006).

Teleki 5C had significantly lower mortality and infection rates (expressed as AUDPC) than SO4 and 3309 C. This is in contrast with Baumgartner and Rizzo (2006), who found that Teleki 5C was not different from 3309 C in infection or in mortality rates. This discrepancy could be related to the fact that Method A is probably more prone to reflect the effect of rootstock vigor (Teleki 5C is more vigorous than 3309 C) than was the method employed by Baumgartner and Rizzo (2006).

When calculated over a short period (two years), the differences in the mortality rates of the different rootstocks were too small to be significant (Baumgartner and Rizzo, 2006) but they became significant when calculated over a period of five years as in our study. Two years seem however to be sufficient to show differences in the frequency of infection, as reported by Raabe (1979) and Baumgartner and Rizzo (2006).

Differences in infection and mortality rates may be due to differences in the rooting patterns of the rootstocks. If *A. mellea* inoculum occurs near the roots, a rootstock with a limited root system, such as 3309 C, is killed and shows aerial symptoms more quickly than a vigorous rootstock, such as Kober 5BB, which reacts to the infection by quickly producing new roots, thus postponing plant death (Fregoni, 2005).

The results also confirm the hypothesis that frequent root wounding, like that entailed by Method B, does not raise *A. mellea* infection (Baumgartner and Rizzo, 2006) to levels that are caused by the constant occurrence of a persistent inoculum. Other studies describe methods of inoculating grapevine rootstocks using infected wood pieces placed close to the roots or secured to the root collar of plants (Mansilla *et al*., 2001; Baumgartner and Rizzo, 2006). These methods also hastened infections and the onset of symptoms. Our Method A, though it required many months to become effective, better reflected infections under field conditions, while allowing a satisfactory comparison to be made between rootstocks.

In conclusion, a weak rootstock, such as 3309 C, may show more symptoms and higher mortality than others, but this higher susceptibility should not cause the rootstock to be automatically excluded from a new vineyard. In areas cultivated with Teroldego Rotaliano and that also have a high risk of *A. mellea*, reducing the amount of inoculum in the soil by carefully eliminating old infected roots and intercropping with a species not susceptible to *A. mellea* would probably do more to control the disease than relying on the inherent resistance of any of the rootstocks examined here. The choice of a rootstock for Teroldego Rotaliano should therefore be based mainly on other criteria: the fruit quality produced by the rootstock, the most practical local irrigation system and the soil characteristics of the vineyard.

Acknowledgements

The authors thank U. Malossini for help with the rootstock collection and for expert agronomic advice. This work was supported by AGRIBIO and SafeCrop Center, both funded by the Autonomous Province of Trento and the Cantina Rotaliana di Mezzolombardo.

Literature cited

Aguin-Casal O., M.J. Sainz-Oses and J.P. Mansilla-Vazquez, 2004. *Armillaria* species infesting vineyards in northwestern Spain. *European Journal of Plant Pathology* 110, 683–687.

Baumgartner K. and D.M. Rizzo, 2006. Relative resistance of grapevine rootstocks to Armillaria root disease. *American Journal of Enology and Viticulture* 57, 408–414.

Fox R.T.V. (ed.), 2000. *Armillaria Root Rot: Biology and Control of Honey Fungus*. Intercept, Andover, UK, 222 pp.

Fregoni F., 2005. *Viticoltura di Qualità*. Phytoline, Affi (VR), Italy, 404–417.

Malossini U. and F. Fellin, 2002. *Nel Segno del Teroldego*. Cantina Rotaliana di Mezzolombardo, Trento, Italy, 123 pp.

Mansilla J.P., O. Aguin and M.J. Sainz, 2001. A fast method for production of *Armillaria* inoculum. *Mycologia* 93, 612–615.

Perazzolli M., S. Faccin, F. Schwarz, P. Gatto, I. Pertot, C. Gessler and C. Moser, 2007. Functional characterization of grape defence genes to improve the biocontrol activity

of *Pseudomonas fluorescens* against *Armillaria mellea*. *Bulletin OILB/SROP* 30(6), 293–296.

Pertot I., D. Gobbin, F. De Luca and D. Prodorutti, 2008. Methods of assessing the incidence of Armillaria root rot across viticultural areas and the pathogen's genetic diversity and spatial–temporal pattern in northern Italy. *Crop Protection* 27, 1061–1070.

Raabe R.D., 1979. Testing grape rootstocks for resistance to the oak root fungus. *California Plant Pathology* 46, 3–4.

Raabe R.D., 1988. Armillaria root rot. In: *Compendium of Grape Diseases* (R.C. Pearson, A.C. Goheen, ed.), APS Press, St. Paul, MN, USA, 35–36.

Worrall J.J. and T.C. Harrington, 1993. Methods for research on soilborne phytopathogenic fungi. In: *Heterobasidion* (L.L. Singleton, J.D. Mihail, C.M. Rush, ed.), APS Press, St. Paul, MN, USA, 86–90.

Accepted for publication: July 12, 2009