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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

Ilaria Pertot[a,*], Davide Gobbin[a], Federica De Luca[a], Daniele Prodorutti[b]

[a]*SafeCrop Centre, Istituto Agrario di San Michele all'Adige, via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy*
[b]*Plant Protection Department, Istituto Agrario di San Michele all'Adige, via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy*

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Abstract

Armillaria mellea (Vahl:Fr.) P. Kumm. is the causal agent of root rots of numerous perennial woody plants, including grapevine. In grapevine, *A. mellea* infections cause reduced plant vigour, poor grape quality and, in the last stage of the disease, plant death. No post-infection control agents are available for use against this disease. Disease management programmes rely solely on prophylactic measures. Therefore, knowledge of the distribution pattern of the disease over time, disease incidence and actual losses due to the disease across geographical areas is crucial. We evaluated the use of aerial photographs, surveys of growers' experiences and field assessments to estimate the incidence of *Armillaria* root rot in a viticultural area (140 ha) situated in the Piana Rotaliana region (Italy). Results of the 4-year assessment were that 25% of the vineyards (36 ha) were infected by *Armillaria*, 2% of the cultivated vines exhibited disease symptoms and 0.25% of the vines died each autumn. The causal agent was almost exclusively *A. mellea* but, in two cases, *Armillaria gallica* was also isolated. The genetic diversity of the Italian population, according to IGS sequencing and somatic incompatibility testing, was very high, suggesting multiple pathogen introduction sites and the slow, vegetative underground spread of the fungus. Fungal clones were found within individual vineyards. Even if, within a particular area, the disease is spreading very slowly and crop losses are limited (one vine out of 400 dies each year), an effective agronomic plan for the prevention of infections should be implemented.
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1. Introduction

Armillaria mellea (Vahl:Fr.) P. Kumm. is the causal agent of root rots of several perennial woody plants (Fox, 2000), including grapevine (Baumgartner, 2004). *A. mellea* is a sapro-parasitic basidiomycete that can survive in the soil for long periods of time on wood and root debris in the absence of any living host. Genetic individuals, or genets, of *Armillaria* spp. are usually found in discrete patches that often include the root systems of several adjacent trees. Each dikaryotic individual is thought to arise from a unique mating event, and then grow vegetatively in an expanding territory over a long period of time (Smith et al., 1992).

Armillaria root disease of grapevines is an increasing problem in places such as California, where the clearing of forested hillsides for vineyard establishment is a widespread practice (Baumgartner, 2004), as well as in traditional grapevine-growing areas where, due to high land costs, grapes are immediately replanted without a suitable rotation interval (De Luca et al., 2003). After forest trees or old grapevines with *Armillaria* root disease have been removed, infected roots that remain in the ground serve as a source of inoculum for grapevines planted subsequently on the same site. In the Trentino region of northern Italy, where grapevine has been widely cultivated for centuries, and is currently the most

*Corresponding author. Tel.: +39 0461 615 239; fax: +39 0461 650 872.
E-mail address: ilaria.pertot@iasma.it (I. Pertot).

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important crop, covering about 10,000 hectares (Provincia Autonoma di Trento, 2006), *A. mellea* root rot is a severe and increasingly significant problem (De Luca et al., 2003).

On grapevine, *Armillaria* spp. infections cause reduced plant vigour, poor grape quality and, in the last stage of the disease, plant death (Pearson and Goheen, 1988; Aguin-Casal et al., 2004). In the vineyards, the disease appears in patches of different dimensions, suggesting that the disease spreads mainly by rhizomorphs in the soil (Baumgartner and Rizzo, 2002). A grapevine can survive for several years after infection; but once most of the roots have been killed by the fungus, the plant generally dies. In both red and white grapevine cultivars, root rot symptoms are linked to the premature reddening or yellowing of leaves in autumn.

Despite the considerable amount of information available on *A. mellea* in forests (Shaw and Roth, 1976; Ferguson et al., 2003), little information is available for grapevine. To date, no studies have been conducted on the distribution or spread of *Armillaria*, nor have any *Armillaria* isolates from Italian vineyards been characterized.

No chemical agents are available for use against *A. mellea*. A number of the common fungicides, sterol demethylation inhibitor fungicides, azoxystrobin, copper, fosetyl-Al, potassium phosphite, sodium tetrathiocarbonate and 2-(thiocyanomethylthiol) benzothiazole, were found to be ineffective against this pathogen (Aguin-Casal et al., 2006). Some experiments have indicated that certain fungicides (e.g. phenolic fungicides) are not only ineffective in reducing the incidence of infection, but may even lead to increases in the amount of root colonization. *Armillaria* mycelia survive the initial fungicide application protected inside wood and their growth is later stimulated. Additionally, many antagonistic soil-inhabiting microorganisms may be destroyed by the phenolic fungicides, giving a strong, competitive advantage to the surviving *Armillaria* (West and Fox, 2002). Only prudent agronomic practices, such as long rotations that allow enough time for the full degradation of all infected root debris (5–10-year rotation), can lead to a meaningful reduction in *Armillaria* spp. inoculum, thereby reducing the risk of infection of new vines (Galet, 1977). In Europe, viticulture is restricted to certain suitable areas. Therefore, it is almost impossible to relocate a vineyard to a new area and too expensive to have a 5–10-year rotation.

In order to precisely assess the status of the disease and the actual losses due to the disease and to plan prophylactic strategies for use across large areas, the estimation of disease incidence and developmental patterns is crucial. Analysis of aboveground symptoms usually results in an underestimation of the incidence of infection, and root sampling is both time-consuming and extremely expensive if performed exhaustively across a wide region. Moreover, the premature reddening or yellowing of leaves, typical of *Armillaria* infections, may also be due to other biotic (e.g. grapevine leafroll virus, other root rots, Esca disease, insect damage, etc.; Pearson and Goheen, 1988) or abiotic causes (e.g. water stress, incompatibility between rootstock and scion, etc.).

The aim of this research was a critical evaluation of different methods (and combinations of methods) for monitoring the incidence of *Armillaria* spp. infection across a wide viticultural region, namely the Piana Rotaliana region of northern Italy, which includes more than 2000 ha of contiguous vineyards. The dynamics of grapevine weakening and dieback were monitored in infected vineyards over a period of 4 years. We used molecular tools to identify the *Armillaria* species present in the Piana Rotaliana region and determined their patterns of grapevine colonization.

2. Materials and methods

2.1. Estimation of Armillaria spp. incidence across wide areas

This research was conducted in a grapevine-growing region of northern Italy (Trentino Province). Piana Rotaliana region is a flat area situated at the junction of two valleys (the Non and Adige valleys). Grapevine is almost the only crop present in this area and has been cultivated for centuries. The study included an area of 140.38 ha. Three approaches were employed for the estimation of the incidence of *Armillaria* spp., namely assessment of premature leaf discolouration through aerial photographs, a survey of growers (questionnaires) and sampling and isolation of *Armillaria* in symptomatic areas.

2.1.1. Identification of areas with premature leaf discolouration using aerial photographs

The main grapevine cultivars grown in Piana Rotaliana region are Teroldego Rotaliano and Lagrain. Both of these red cultivars are grown using the pergola trellis system, which provides a single or double side plane of canopy about 2 m above the ground. The predominant disease symptom visible on the aerial part of these plants is the premature reddening of leaves in autumn. The areas most likely to be infected were identified from aerial photographs of the entire area. These photographs were taken during the first week of October 2000, 3 weeks before the leaves generally change colour. Photographs were taken from a viewpoint on the nearby mountain (500 m above vineyard level). The photographs were processed using Corel Photo-Paint 12 to increase by four points the cyan–red channel and decrease the magenta–green and yellow–blue channels by four points, in order to accentuate the red areas of the vegetation and remove the reddishness due to late downy mildew infection of leaves. The analysis of red spots on these aerial pictures allowed for the identification of plants exhibiting premature reddening, including those suffering *Armillaria* spp. infection, as well as those suffering from other grapevine diseases and disorders that can cause premature reddening (e.g. grapevine

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