Spread of Armillaria Root Disease in a California Vineyard

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The formation and expansion of groups of dead and dying grapevines (disease centers) caused by Armillaria root disease were tracked in a commercial vineyard in Sonoma County, California from 1998 to 2000. Approximately 50% of the vines that died in 1999 and 2000 were adjacent to vines that died in previous years, providing circumstantial evidence of vine-to-vine spread of root disease. To determine if symptomatic and dead vines were infected by vine-to-vine spread or by direct contact with partially decayed tree roots remaining from forest trees that inhabited the site prior to vineyard establishment, pneumatic soil excavation was used to expose the root systems of 30 vines within the oldest disease center. Root system excavation revealed infections on 26 of 30 excavated vines, 27 of which were in direct contact with decayed tree roots. No evidence of vine-to-vine spread was found and rhizomorphs were extremely rare. Therefore, the pattern of disease incidence was due to the patchy distribution of decayed tree roots belowground. Control efforts aimed at reducing vine-to-vine spread of Armillaria root disease in young vineyards, such as the one in this study, may be unnecessary, given the slow rate of spread of the pathogen.

Keywords: Armillaria mellea, Armillaria root disease, Vitis vinifera, grapes

Armillaria root disease is a chronic problem on grapevines and was first recorded in California in the 1880s [9]. The fungus that causes the disease, Armillaria mellea (Vahl. Fr.) P. Kumm., infects vine roots, killing the cambium and decaying underlying xylem. Symptoms include changes in leaf color, stunted shoots, dwarfed foliage, raisining of berries, and premature defoliation. Armillaria mellea is native to California, where it occurs on the roots of many forest tree species, such as Quercus kelloggii Newb., Q. agrifolia Née, Lithocarpus densiflorus (Hook & Arn.) Rehd., Arbutus menziesii Pursh, Umbellularia californica, and Pseudotsuga menziesii (Mirb.) Franco. [3, 4, 16].

Armillaria mellea can survive as a saprobe on woody host roots long after the host dies [15, 23]. Its vegetative fungal tissue (mycelium) decomposes cellulose, hemicellulose, and lignin for nutrients as it grows, thereby decaying the root wood. When forest trees with Armillaria root disease are cut down, any infected roots that remain belowground serve as a source of inoculum for grapevines planted in place of the trees. Infection occurs when grapevine roots come in direct contact with partially decayed tree roots and are colonized by A. mellea mycelium. Infection can also occur when grapevine roots contact A. mellea rhizomorphs, which are black, rootlike structures that contain mycelium that grow out from partially decayed roots and through the soil. Once grapevine roots are infected, they serve as a source of inoculum for neighboring vines whether they are living or dead.

Armillaria root disease occurs in circular patches in a vineyard (disease centers) that expand radially over time, which is believed to be due to spread of A. mellea between neighboring vines (vine-to-vine spread), either through direct root-to-root contact or via rhizomorphs. Research on the spread of Armillaria root disease has focused mainly on mapping the formation and distribution of disease centers in natural forests and forest tree plantations [18, 20, 27, 28]. Fewer studies are devoted to spread of Armillaria root disease in agricultural crops [17, 19, 25]. Expansion of disease centers among agricultural crops, such as orchard trees and grapevines, is assumed to be due to spread of the fungus from planted host to planted host. However, few studies have used examination of entire root systems as a means of inference.

The goal of this research was to assess the relative importance of different mechanisms of infection in the expansion of Armillaria root disease centers in a naturally infected, commercial vineyard. Belowground investigations were used to determine the extent of vine-to-vine spread of root disease through root contacts among neighboring vines, the importance of rhizomorphs in establishing infections, and the frequency of contact between infected vine roots and partially decayed tree roots. The relationship between aboveground symptoms of Armillaria root disease and extent of root system infection was also determined, as this information has never been documented.

Materials and Methods

Study site. The spread of Armillaria root disease was monitored in a commercial vineyard in Sonoma County, California.

from 1998 to 2000. The block was established on a 0.2 ha site cleared of mixed-hardwood forest, which included Q. kelloggii, Q. agrifolia, L. densiflorus, A. menziesii, U. californica, and P. menziesii. In summer 1990, all trees were removed and soil was cleared of surface roots. Dormant rootstocks of the rootstock 3309C (V. rupestris Schelle x V. riparia Michx.) were planted to a meter-by-meter spacing in spring 1991 and field-grafted to V. vinifera L. cv. Pinot noir in spring 1992. Vines were trained during the growing seasons of 1992 to 1994, with a unilateral cordon training system established to a fruiting wire at approximately 90 cm from the ground surface. Shoots were trained to two pairs of moveable catch wires to a maximum height of 90 cm from the cordon. From 1991 to 1996, vines were irrigated weekly from late spring through late fall. From 1997 to 2000, maintenance irrigations were used, which ranged from biweekly to twice per day depending on transpirational demand, from mid-August to late fall. In 1997, 191 dead vines were removed and replanted with new dormant rootstocks. The actual cause of death was not documented, although foliar symptoms consistent with Armillaria root disease were noted by a vineyard manager in 1996.

Aboveground observations. Annual surveys of vine status were conducted just before harvest from 1998 to 2000. Vines were categorized as symptomless, symptomatic, or dead. Vines that showed no foliar symptoms of root disease were labeled “symptomless.” “Symptomatic” vines showed one or more of the following symptoms: changes in leaf color, stunted shoots, dwarfed foliage, and rasming of berries. To verify that symptomatic and dead vines actually had Armillaria root disease, soil was removed from the base of their trunks and from their root collars (where main roots originate) and bark was removed from the exposed root collars to collect white sheets of fungal tissue (mycelial fans), decayed xylem, and/or rhizomorphs for identification. We did not examine the root collars of symptomless vines in an effort to allow Armillaria root disease to progress naturally. Armillaria mellea fruiting bodies that formed in or on the perimeter of the vineyard were also collected for identification.

Root system excavation. In Sept 2000, a pneumatic soil excavation technique was used to expose the root systems of 30 vines within the oldest disease center in the vineyard. Symptoms first appeared on vines in this disease center in 1997. With this soil excavation technique, air was delivered through an engineered air jet nozzle and a 3.2-cm-diameter hose at 93.5 hL/min at 7 kg/cm² (100 lb/in²) [26]. A 7 x 5 m area was excavated to a depth of approximately 0.6 m. Root systems were examined periodically during the excavation process for the presence of partially decayed tree roots and rhizomorphs. Following soil removal, root systems were individually examined for presence of infection. Exposed vine roots were sampled for mycelial fans, decayed xylem, and rhizomorphs, which were collected for identification.

All references to belowground observations refer to those made on the 30 vines in the excavated disease center. Vines in the excavated disease center that had infected root systems were characterized by the presence of mycelial fans, decayed xylem, and/or rhizomorphs. Vines in the excavated disease center with none of the above-listed signs of A. mellea were categorized as “not infected.”

Isolation and identification. Fungal tissue isolations from mycelial fans, decayed xylem, rhizomorphs, and fruiting bodies were made on water agar with benomyl 50WP (4 µg a.i./mL) and streptomycin sulfate (100 µg/mL) added after autoclaving. Tissues were aseptically removed from the laboratory from portions of mycelial fans and decayed wood that were previously not exposed. Rhizomorphs were scrubbed with cheesecloth, soaked in 95% ethanol for 10 min, and rinsed in sterile distilled water before plating on water agar. Isolation plates were incubated at 25°C in the dark and examined after 5 to 10 days. Subcultures of resulting colonies were transferred to 1% malt extract agar (MEA) with an overlay of sterile cellophane.

All cultures were identified using the PCR-based technique of Harrington and Wingfield [13]. Cultures were prepared for identification by growing mycelium on 1% MEA with an overlay of sterile cellophane. For most cultures, DNA was amplified directly from mycelium by gently scraping a pipette tip over the surface of the mycelium and dipping it into the PCR vial immediately before starting the reaction. For cultures that would not amplify directly from mycelium, DNA was extracted from mycelium collected from the top of the cellophane using the extraction technique of Cenis [!]. Restriction digests of PCR products were then done by adding Abl directly to the PCR vial and incubating it in a 37°C water bath for 2 hr. Restriction patterns were obtained by running restriction fragments on 3% agarose gels, followed by staining with ethidium-bromide.

Results and Discussion

Aboveground observations. The number of vines out of a total of 1,918 vines in the vineyard block that appeared symptomless, symptomatic, or dead on each of three survey dates is shown in Table 1. The percentage of dead vines (from a total of 1,918 vines) increased from 4.8% in 1998 to 11.1% in 2000. The percentage of symptomatic vines increased from 0.7% in 1998 to 7.5% in 2000. All symptomatic and dead vines were colonized by A. mellea, based on examination of their root col-

<table>
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<th>Table 1 Status of Pinot noir on 3309C in a Sonoma County vineyard with Armillaria root disease.</th>
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<td>Symptomaticc</td>
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bVines showed no symptoms of root disease. Infection with Armillaria mellea not confirmed.

cVines showed one or more of the following symptoms: changes in leaf color, stunted shoots, dwarfed leaves, raicing of borries. All were confirmed infected with A. mellea. Numbers reflect cumulative totals of symptomatic vines counted each survey year.
dAll were confirmed infected with A. mellea. Numbers reflect cumulative totals of dead vines counted each survey year.
The most common course of symptom development (red-dening of foliage, desication of leaves and berries, vine death) began after veraison and ended just before harvest. Rarely, the same series of symptoms occurred just before fruit set. The majority of vines that died became symptomatic only a few weeks before they died. Of the 121 vines that died in 1999 and 2000, 79 appeared symptomless one year before death (Table 7). Rapid symptom development immediately preceding death from Armillaria root disease has also been observed in young conifer seedlings [14], small hardwood trees [24], and saplings of several Eucalyptus species [8].

The distribution of symptomatic and dead vines is shown in Figure 1. Of the 45 vines that died in 1999, 20 were adjacent to vines that died before 1999. Of the 76 vines that died in 2000, 41 were adjacent to vines that died before 2000. Most dead and symptomatic vines were concentrated in the lower half of the vineyard, where the pattern of mortality was characterized by the formation of many small disease centers that coalesced into larger disease centers over the course of our three-year investigation.

Belowground observations. Prior to root system excavation, the foliar conditions of the 30 vines in the excavated section of the vineyard were as follows: 10 were dead, 8 were symptomatic, and 12 were symptomless. Replants, planted in 1997, accounted for four of the symptomless vines and one symptomatic vine. After their root systems were excavated, all dead and symptomatic vines were found to be infected (Table 3). Of the 12 symptomless vines, 8 were infected.

When vine status categories, as shown in Table 3, are ranked in order of increasing extent of root system colonization, the following list is obtained: symptomatic vines with yellow leaves, symptomless vines (excluding the four vines that were not infected), symptomatic vines with red leaves, dead vines. Dead vines showed the most advanced stage of root system colonization. The root collars of all 10 dead vines were completely covered by one continuous mycelial fan that encircled the entire base of the vine's trunk and extended from the root collar up to the soil line (average distance of 30 cm). Depth of xylem decay among dead vines varied from shallow (maximum depth of 5 mm) to deep (reached the pith).

Among symptomatic vines with red leaves, root system colonization was characterized by the presence of one continuous mycelial fan that covered the basal portion of the trunk (from the soil line to the root collar). Mycelial fans thereafter extended from the root collar to a distance of 50% of all main roots. Scattered, shallow areas of xylem decay (maximum depth of 5 mm) were found in the root collar and in main roots that were colonized.

Symptomatic vines with yellow leaves had small mycelial fans on their main roots or root collar, but it is unlikely that these small fans could cause foliar symptoms, as most of the vines we found with more heavily infected root systems showed no aboveground symptoms of root disease. It is possible that the three symptomatic vines with yellow leaves were suffering from some ailment other than Armillaria root disease, such as general water stress. It is also possible that these vines were simply weaker and, therefore, did not require extensive colonization to bring about symptoms.

Of the eight symptomless vines with infected root systems, three vines had root systems that were colonized to the same extent as the root systems of symptomatic vines with red leaves, while five vines only had mycelial fans on 25% of their main roots (their root collars were not infected). All eight symptomless vines with infected root systems had scattered, shallow areas of xylem decay (maximum depth of 5 mm) beneath mycelial fans in the root collar and/or in the main roots.

Our comparisons of aboveground symptoms of Armillaria root disease to extent of root system colonization are similar to observations made on Prunus armeniaca L. [12], Malus sylvestris Mill. [19], and Citrus species [5]; foliar symptoms of Armillaria root disease do not appear until one-half to three-

| Table 3 Characteristics of Armillaria root disease of 30 vines excavated in a Pinot noir vineyard on 3309C, with respect to vine status, Sonoma County. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Vine status*                    | Symptomatic (n=8)               | Yellow leaves (n=3)             | Red leaves (n=5)                |
| Infection characteristics       | (n=12)                         | (n=10)                         | (n=5)                           |
| Not infected                    | 4                              | 0                              | 0                               |
| Infected                       | 8                              | 10                             | 3                               |
| Root collar                     | 3                              | 10                             | 1                               |
| Main roots                      | 8                              | 10                             | 2                               |

*Recorded on 6 Sept 2000, based on aboveground observations. Symptomless vines showed no foliar symptoms of root disease. Symptomatic vines were placed in one of two status categories, based on the color of their leaves. Symptomatic vines with yellow leaves also had stunted shoots. Symptomatic vines with red leaves also had raised berries.

*Recorded on 11 Sept 2000, based on belowground observations made following root system excavation.

*Among infected symptomless vines, three vines had both infected root collars and infected main roots, while five vines only had infected main roots. All infected dead vines and infected symptomatic vines with red leaves had both infected root collars and infected main roots. Among infected symptomatic vines with yellow leaves, one vine only had an infected root collar, while the other two only had infected main roots.

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**Table 2 Changes in status of Pinot noir on 3309C from 1998 to 2000 in a Sonoma County vineyard with Armillaria root disease.**

<table>
<thead>
<tr>
<th>Vine status*</th>
<th>Survey year interval</th>
<th>1998 to 1999</th>
<th>1999 to 2000</th>
</tr>
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<tbody>
<tr>
<td>Symptomless to symptomatic</td>
<td>102</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Symptomless to dead</td>
<td>39</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Remained symptomatic</td>
<td>7</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Symptomatic to dead</td>
<td>6</td>
<td>36</td>
<td></td>
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</tbody>
</table>

*Recorded on 20 Aug 1998, 13 Aug 1999, and 6 Sept 2000, based on aboveground observations. First listed status corresponds to observations made in first listed year of the survey year interval. Second listed status (after "to") corresponds to observations made in second listed year of the survey year interval.
Figure 1: Distribution of dead and symptomatic Phylloxera on 3309C with Armillaria root disease in a Sonoma County vineyard. Annual surveys of vine status, based on aboveground observations, were done in each of three survey years: (A) 1998, (B) 1999, and (C) 2000. Each square (1 m² in area) represents the location of an individual grapevine. Square contents correspond to vine status: symptomless (Armillaria mellea infection not confirmed), symptomatic (infection confirmed), dead (infection confirmed), and replants planted in 1997.

quarters of the main roots of a host are colonized by *A. mellea*. It is likely that the eight symptomless vines with infected root systems, had they not been destructively sampled as a result of this study, would have shown symptoms of Armillaria root disease. The presence of a mycelial fan at the root collar, a characteristic of all symptomatic vines with red leaves and three of the symptomless, infected vines that we investigated, signifies that the fungus has destroyed some of the underlying cambium and is in the process of girdling the host's trunk [29].

Spread of Armillaria root disease. The locations of 35 partially decayed tree roots and four partially decayed grapevine roots that were still in place after excavation were mapped with respect to the nearest excavated vine (Figure 2). Partially decayed grapevine roots were the remains of infected root systems of three vines that were killed by Armillaria root disease prior to 1991 and subsequently replanted. Of the 35 partially decayed tree roots, 29 were smaller than 4 cm in diameter and 30 cm long. The approximate dimensions of the largest piece were 100 x 20 x 30 cm. *Armillaria mellea* was identified from all four partially decayed grapevine roots and 33 of the partially decayed tree roots.

Of the 26 infected vines in the excavated section of the vineyard, 23 were in direct contact with partially decayed tree roots (see Figure 3A for an example). Infections on the vines in direct contact with partially decayed tree roots were characterized by the presence of a mycelial fan at the point of contact between the vine root and the partially decayed tree root. A mycelial fan was found on the root of a symptomatic replant where it contacted a partially decayed grapevine root. For heavily infected root systems (all 10 dead vines, all 5 symptomatic vines with red leaves, and 3 of the symptomless vines with infected roots) the mycelial fan extended to the root collar, 0.3 m above the root collar (to the soil line), and thereafter 0.1 to 0.6 m down one or more other main roots.

Root system excavation revealed the presence of root overlap (roots in direct contact with other roots) between neighboring vines (Figure 3B). No root grafts (solid connections between adjacent root systems) were found. Overlap of main roots among the 30 excavated vines occurred within and between rows. Most

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**Figure 2** Aboveground and belowground observations of Armillaria root disease in an excavated section of a Sonoma County vineyard. Each square (1 m² in area) represents the location of a grapevine. The following letters represent aboveground observations: S, symptomless; D, dead; S, symptomatic; S, R, symptomatic replant. Underlined letters represent grapevines with infected root systems. The location of partially decayed tree roots relative to the root collar of each excavated vine (the center of each square) is represented by a dot. Partially decayed grapevine roots are represented by a cross. Squares with no letters represent root systems that were removed following vine death in 1998 and 1999.

**Figure 3** Exposed root systems of Pinot noir on 3309C with Armillaria root disease in a Sonoma County vineyard. (A) Partially decayed tree root (see arrow) found in soil under infected grapevine root system. (B) Four adjacent root systems with overlapping roots. Ruler is 1 m. Prior to excavation of the surrounding soil in Sept 2000, status of each vine was as follows (clockwise from upper left): symptomless, dead (confirmed infected with *A. mellea*, Aug 2000), symptomless, symptomatic. Root system excavation revealed infections on all but the lower right vine. Although all four root systems had overlapping main roots (see arrow), infections occurred due to direct contact with partially decayed tree roots (see lower left).
overlap was among one to three main roots of neighboring vines and was concentrated approximately 0.6 m away from the root collar (all root collars were 1 m apart). Mycelial fans were absent from all points of root overlap between neighboring infected vines.

Rhizomorphs were found growing within the bark of only one infected vine root and they did not appear to extend into the soil. This infected root was in direct contact with a partially decayed tree root and was heavily decayed at the point of contact. No rhizomorphs were found on partially decayed tree or grapevine roots or in the soil.

We did not find sources of inoculum for the three infected vines that were not in direct contact with partially decayed tree roots. Their infections were identical, in terms of mycelial fan coverage, to that of infected vines in direct contact with partially decayed tree roots. Partially decayed tree roots were found in the 21 m³-pile of soil that was removed from the site. It is possible that partially decayed tree roots were the source of inoculum for these infections, but that the force of the high-pressure air (7 kg/cm² or 100 lb/in²) blew the pieces away during excavation.

Based on the distribution of mycelial fans on individual root systems in the excavated section of the vineyard and the presence of abundant partially decayed tree roots that all but three of the infected vines were in direct contact with, none of the infected vines appear to have become infected by simply being in direct contact with the infected roots of a neighboring vine. Expansion of the disease center in the excavated section of the vineyard over the course of this research was not due to spread of A. mellea between neighboring vines, but from spread of the fungus directly from many scattered and partially decayed tree roots to multiple grapevines.

Our findings are in contrast to those of Rizzo et al. [25], who concluded that the spread of Armillaria root disease in a northern California pear (Pyrus communis) orchard was primarily due to rhizomorph growth from partially decayed forest tree roots to pear roots and, to a lesser extent, from infected pear roots to neighboring pear roots. Possible reasons for the rarity of rhizomorphs in the vineyard we studied include lack of a suitable soil environment [22] and/or lack of a sufficient base of specific nutrients required for rhizomorph formation [11, 21].

Conclusions

Our results imply that vine-to-vine spread of Armillaria root disease through rhizomorph growth or direct contact between neighboring vines may take more than 10 years to occur. Assuming our results are not unique to the vineyard we examined, it may be prudent to select Armillaria root disease control treatments based on the age of a vineyard. Obviously, the most effective control of Armillaria root disease is preplant removal of partially decayed tree roots. If the vineyard we examined was thoroughly cleared before vines were planted, a reduction in partially decayed tree roots may have lowered the rate at which vines became infected. This site was forested immediately prior to vineyard conversion. Soil was not ripped after clearing, but merely cleared of roots found on the soil surface. Based on the distribution of dead and symptomatic vines in the block (Figure 1), and the assumption that they were all infected by direct contact with partially decayed tree roots, there were likely multiple A. mellea-infected trees on the site before vineyard conversion.

There are few available postplant control treatments for Armillaria root disease. One option is to remove dead/dying vines and chemically treat the soil with methyl-bromide or sodium tetraphiocarbonate (Enzone®, Entek Inc., Elkridge, MD) on a spot basis (at labeled preplant rates) and/or mechanically remove buried partially decayed tree roots before replanting. Another option is to annually apply sodium tetraphiocarbonate (at labeled postplant rates) to the entire infected block, in an effort to kill rhizomorphs that may grow out into the soil from partially decayed tree roots or from infected vine roots. This second option would be ineffective in the young vineyard we examined because roots were not developing infections from rhizomorphs.

Few therapeutic treatments (products applied to a diseased plant in an attempt to prolong its life) are available for control of Armillaria root disease on infected vines. Examples include Vesta™ and LiquiComp™ (Biologically Integrated Organics, Inc., Fresno, CA), which are microbial products that are registered as soil inoculants. The success of a therapeutic treatment depends on how much damage a vine has already suffered, in addition to the ability of a treatment to decrease further colonization of healthy root tissue by A. mellea. Based on our observations, a symptomatic vine already has extensive mycelial fan coverage on its root collar and on some of its main roots. Although some of the cambium at the root collar of a symptomatic vine may function, the vine likely has limited capacity for new root growth. Efforts aimed at treating symptomless, infected vines neighboring symptomatic vines may be more valuable, assuming that their root collars are not yet infected.

Vine-to-vine spread of Armillaria root disease could, potentially, be more severe in meter-by-meter plantings, such as the one examined for this study, than in vineyards with less dense spacings. Obviously, more root contacts between neighboring plants increase the likelihood of vine-to-vine spread of root disease. In meter-by-meter plantings, root contacts exist among all adjacent vines. Therefore, spread of root disease is not limited to within rows. Grapevines in meter-by-meter plantings tend to have higher root densities than those with wider spacing [1]. Higher root densities are correlated with a more rapid rate of soil water depletion, which can lead to higher water stress among narrowly spaced vines during hot, dry weather [2] and the need for more frequent irrigation. Meter-by-meter vineyards generally require more water than vineyards with wider spacings, providing a nearly continuous, uniform soil moisture distribution. The relationship between wet soil conditions and Armillaria root disease has been noted [10, 17, 25]. Irrigation in the meter-by-meter vineyard investigated for this study begins after veraison (approximately 16 weeks after budbreak) because of high annual precipitation in this coastal location. However, the vineyard was irrigated weekly throughout the entire growing season during the first five years of establishment. Studies on the effects of drying on mycelial growth of A. mellea and
other wood-decay fungi show a direct relationship between growth rate and substrate water content [6,30]. Therefore, higher soil moisture content due to increased irrigation in meter-by-meter plantings could contribute to increased severity of Armillaria root disease, given the presence of sufficient partially decayed tree roots and time.

**Literature Cited**


