Susceptibility of Grapevine Pruning Wounds and Symptom Development in Response to Infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*

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ABSTRACT

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Fresh to 10-month-old pruning wounds on grapevine cvs. Thompson Seedless and Cabernet Sauvignon were inoculated with *Phaeoacremonium aleophilum* or *Phaeomoniella chlamydospora* at approximately 1×10^6 spores/ml. Successful infection was determined by isolation of the pathogen from necrotic margins of cankers or from vascular discoloration assessed 4 months after each inoculation date. Disease incidence decreased as the length of time between pruning and inoculation increased; however, wounds remained susceptible for up to 4 months. Vascular discoloration was significantly less extensive in the noninoculated control than in inoculated spurs. Reduction of shoot length in both cultivars varied depending on pruning wound age at the time of inoculation. In a separate study, inoculation of fresh pruning wounds of Cabernet Sauvignon resulted in successful infection regardless of the time of pruning from February to December.

Additional keywords: esca, young esca

Young esca and esca are important diseases of grapevine worldwide. Young esca, also known as Petri disease, young vine decline, and black goo, usually is associated with vines younger than 7 years old and is associated with infection and disease of the rootstock of grapevines (2,3,16,17,27). Symptoms of young esca include reduced trunk diameter, shortened internodes, stunted growth, and chlorotic or necrotic leaves (4,18,27). The most prominent internal symptom is the presence of brown to black spots or streaks in the xylem vessels of the woody cylinder (Fig. 1A). Esca, also known as black measles, commonly is associated with mature vines and shows a range of symptoms from severe to mild or chronic. The severe form, known as “apoplexy,” is characterized by sudden wilting and shedding of the leaves from all or part of the infected vine and shriveling of all fruit (17). Symptoms on chronically affected fruit appear as superficial brown to purple spots scattered over the surface of the berries (Fig. 1B). Symptoms on leaves consist of small chlorotic spots between the veins and along the margins. These spots may later coalesce and become necrotic, giving the leaves the characteristic “tiger-stripe” pattern (Fig. 1C). Shoots arising from infected

wood show stunting and tip dieback (Fig. 1D) (18,28).

Young esca has become a common disease of grapevines from 1 to 7 years old, mostly in California’s north-coast production area. The increased prevalence of the disease coincided with massive replanting of grapevines in Napa, Sonoma, and Mendocino Counties as a result of phylloxera (*Daktulosphaira vitifoliae* (Fitch)) infestation (30). The commonly planted rootstock

AXR1 was replaced with rootstocks that were resistant to phylloxera (6,12). However, these rootstocks apparently were more susceptible to the young esca pathogens (28–30). Although widespread in occurrence, vines showing decline due to young esca usually constitute a minor portion (1 to 5%) of a newly planted vineyard.

Likewise, the mild form of esca has increased in significance in California over the past 8 years. Symptoms that were not common on grapevines unless the vine was older than 10 years of age are now seen commonly on vines 1 to 6 years old. This symptom occurrence on young vines also coincided with the use of phylloxera-resistant cultivars.

The primary pathogen consistently associated with young esca in California has been *Phaeomoniella chlamydospora*, whereas the pathogen more closely associated with esca in California vineyards is *Phaeoacremonium aleophilum* as well as related species of *Phaeoacremonium* (5,7,14,19,27). *Phaeomoniella chlamydospora* (Chaetothyriales, Herpotrichiellaceae) produces pycnidia on exposed vascular tissue on cordons and spurs of grapevine (4,8) that may serve as a source of inoculum in infected vineyards. *Togninia minima* (Diaporthales) recently was confirmed

Fig. 1. Symptoms of esca on wood, leaves, fruit, and shoots. **A**, Grapevine with esca showing vascular discoloration of wood; **B**, Thompson Seedless grape bunch showing typical spotting and “measles” symptom on the berries; **C**, typical tiger-striped pattern of esca on a leaf of infected Thompson Seedless grapevine; and **D**, grapevine shoot with esca showing tip dieback (arrow).

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as the sexual stage of *Phaeoacremonium aleophilum* (25). Previous work showed that these pathogens generally are ubiquitous in California vineyards; the pathogens were found in vineyards from 10 to 80 years old and were associated with black or brown vascular streaking in cordons and spurs. The pathogens are known to be transported in nursery plants; however, the incidence is generally low, somewhere between 1 and 50%. It has been shown that the pathogens are present in asymptomatic, infected, propagating material (1,5,8,15). The widespread occurrence, prevalence, and distribution of infected plants in vineyards suggest other possible modes of transmission.

Routine isolations from symptomatic samples submitted for diagnosis and collected from vineyards statewide yielded cultures of both organisms. When isolations were made from diseased younger vines (<7 to 8 years old), pathogens were associated primarily with the rootstock. In older vines, the pathogens were detected frequently in the scion, in close proximity to pruning wounds of spur-pruned or cane-pruned vines (15,27). Earlier work showed that pruning wounds could serve as avenues for ingress and subsequent infection by the two pathogens (6,13,15); however, data on effects of infection were lacking. Perithecia of *T. minima* also were discovered on grapevine exhibiting symptoms of esca in vineyards located throughout California (9,10,23). Ascospores are rain splash dispersed and airborne dispersal may account for recovery of *P. aleophilum* propagules in proximity to diseased vines (21,23,24). Though all *Phaeoacremonium* spp. are pathogenic on grapevine, *P. aleophilum* (teleomorph: *T. minima*) is the most prevalent species (9,10,20). The importance of pruning wounds is further emphasized by the recent finding that aerial propagules of *Phaeoacremonium* spp. were present in infected vineyards in multiple counties throughout the state of California during winter and spring. *P. aleophilum* also was found in symptomatic berries (11), indicating that berries can become infected during the time when aerial conidia are present in the summer. In addition, many trapping events were correlated with rainfall, and it was concluded these pathogens are dispersed as airborne inoculum during rainfall events. (5,7,16,22,24). The present study was conducted to determine the duration of susceptibility of pruning wounds and the effect of time of pruning on susceptibility of fresh wounds to infection by two isolates of *P. aleophilum* and an isolate of *Phaeomoniella chlamydospora*.

MATERIALS AND METHODS

P. chlamydospora isolate P99.16 and *Phaeoacremonium aleophilum* isolate P99.10 were obtained from grapevines that were diagnosed with young esca or Petri

Fig. 2. Black streaking in vascular tissue of inoculated grapevine shoots. A and B, Streaking vascular discoloration caused by *Phaeomoniella chlamydospora*. C, Vascular discoloration of wood caused by *Phaeoacremonium aleophilum*.

Fig. 3. Percentage of infected wound based on fungal reisolation of A, Thompson Seedless and B, Cabernet Sauvignon grapevines as affected by age of pruning wound at inoculation with *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*.

disease in California. The isolates were maintained in our culture collection on potato dextrose agar (PDA) slants stored at 4°C or on sterilized filter paper stored at -18°C. For preparation of inoculum, cultures were grown on PDA amended with tetracycline at 1 mg/liter (PDA-Tet) in the laboratory at about 24°C for 2 weeks. The culture was flooded with sterile distilled water and the colony rubbed with a sterile glass rod. Then, the wash containing the conidia was filtered through four layers of cheesecloth to remove mycelial fragments. The concentration of the spore suspension was adjusted to approximately 1×10^6 spores/ml using a hemacytometer.

The experiments were conducted on mature vines at the University of California Field Research Station in Davis. The effect of the age of the pruning wound on susceptibility was determined by inoculating pruning wounds made on 1-year-old wood on 25- and 10-year-old grapevines (*Vitis vinifera* cvs. Thompson Seedless and Cabernet Sauvignon, respectively). Wounds were inoculated at various times after pruning. Ten spur positions from each grapevine were pruned in February of 2000 and the experiment was repeated in 2001 on dif-

ferent grapevines. Wounds were inoculated immediately after pruning or at monthly intervals thereafter for a period of 10 months; thus, wounds were from 0 to 10 months old at the time of inoculation. Inoculation was done by depositing 0.1 ml of a spore suspension with 10^6 conidia/ml on the pruning wound using a micropipette. Control vines were treated with sterile distilled water. Four months after each inoculation date, the spurs were excised about 15 cm below the point of inoculation and brought to the laboratory for measurement of the extent of vascular discoloration and for reisolation of the pathogen. Extent of vascular discoloration and streaking were measured from the point of inoculation and fragments of necrotic tissue were cultured on PDA-Tet agar media and incubated at room temperature ($24 \pm 2^\circ\text{C}$) for 10 to 14 days. Typical mycelial growth was transferred to new PDA-Tet in an attempt to obtain pure cultures. The fungus was identified by colony size, shape, color, and growth rate and by spore morphology. In a second experiment, the susceptibility of fresh wounds made on green and dormant shoots at different times of the year was investigated. Shoots

were pruned to two buds in April, June, August, September, and November and immediately inoculated as described above. Isolations were made from the necrotic margins of the vascular discoloration 4 months after each inoculation date.

All tests were established in a completely randomized design using eight replications per treatment. Similar results were obtained in each of the 2 years of tests; hence, data were pooled for analysis. Differences between treatments were determined by analysis of variance in SAS and means were separated using the Waller-Duncan k-ratio *t* test.

RESULTS

Artificially inoculated pruning wounds were successfully colonized by *P. aleophilum* and *Phaeomoniella chlamydospora* (Fig. 2A, B, and C). The susceptibility of the wood generally declined as the length of time between pruning and inoculation increased (Fig. 3). In both Thompson Seedless and Cabernet Sauvignon, infection was highest in fresh wounds and remained high for up to 2 months after pruning. There was a sharp decline in infection after the second month but a small percentage of wounds remained susceptible for up to 4 months. No infection resulted from inoculations of 5-month-old or older pruning wounds. Water-inoculated control wounds were not infected.

The extent of vascular discoloration was greater in Thompson Seedless than in Cabernet Sauvignon, regardless of treatment (Fig. 4). Extent of vascular streaking in the controls ranged from 0.83 to 1.32 cm for Thompson Seedless and from 0.62 to 0.90 cm for Cabernet Sauvignon. In both cultivars, vascular discoloration was significantly less extensive in the water-treated control than in spurs inoculated with either of the two pathogens. Spurs inoculated with the pathogens varied in the extent of vascular discoloration but the differences were not always significant. In Thompson Seedless, vascular streaking was more extensive with *Phaeoacremonium aleophilum* (8.3 cm) than with *Phaeomoniella chlamydospora* (7.0 cm). Similar trends were observed for Cabernet Sauvignon with 6.5 and 5.7 cm of discoloration for *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*, respectively. In all cases, vascular discoloration had extended down to or beyond the basal bud, indicating that the pathogens moved quickly in grapevine wood.

Growth of new shoots was suppressed when growing from spurs inoculated with any of the pathogens (Fig. 5). In Thompson Seedless, reduction in shoot length, depending on pruning wound age, ranged from 33 to 55% in spurs inoculated with *P. chlamydospora* and 40 to 50% in spurs inoculated with *Phaeoacremonium aleophilum*. In Cabernet Sauvignon, shoot length was reduced by 26 to 60% in spurs

Fig. 4. Length of vascular streaking in shoots of A, Thompson Seedless and B, Cabernet Sauvignon grapevines inoculated with *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*.

inoculated with *Phaeoacremonium aleophilum* and 25 to 42% in spurs inoculated with *Phaeoacremonium aleophilum*.

Fresh pruning wounds were susceptible to infection regardless of the month they were inoculated (Fig. 6), with an incidence of infection between 23 and 50%. Degree of infection did not differ significantly between organisms or month of inoculation.

DISCUSSION

Data over two seasons showed that pruning wounds remained susceptible to infection by *Phaeoacremonium aleophilum* and *Phaeoacremonium aleophilum* for up to 4 months after pruning. Even though a significant decrease in susceptibility occurred after 2 months, any amount of infection is considered important because this may play a significant role as a source of inoculum for further spread of the disease in subsequent years. In Southern France, pruning wounds remained susceptible for 9 to 12 weeks when pruning was done in December or January and shorter than 9 weeks when pruning was done later in the season (9). Whether the difference in the susceptibility period in France and California could be attributed to environmental or other factors (e.g., cultural practices and plant age) is not clear at the present time.

Our results showed that fresh pruning wounds were susceptible to infection throughout the growing season. The importance of this finding is further emphasized by the results of our spore-trapping studies (5,7), in which spores of *P. aleophilum* and *Phaeoacremonium aleophilum* were trapped throughout the year in vineyards from the northern California coast to the southern San Joaquin Valley (5). The susceptibility of fresh wounds and availability of spores throughout the growing season in addition to the long duration of susceptibility of pruning wounds make management of young esca and esca extremely difficult.

No infection occurred in the noninoculated control spurs. Because pruning wounds were not protected, it is assumed that no airborne conidia, or at least no significant numbers of airborne conidia, were present in the experimental vineyards during the 2 years of the study. The vineyards used in this study have no history of young esca or esca. Recently, however, about 3 years after the study was initiated, propagules of *Phaeoacremonium aleophilum* and *Phaeoacremonium aleophilum* were trapped in these vineyards, and symptoms of esca on leaves and fruit have appeared on some inoculated vines of Thompson Seedless.

Although planting material can become infected in nurseries, it has been shown by this study that pruning wounds are potential infection courts and can explain the common occurrence of the disease in spite

Fig. 5. Length of first shoot on the spur of A, Thompson Seedless and B, Cabernet Sauvignon grapevines that previously were inoculated with *Phaeoacremonium aleophilum* or *Phaeoacremonium aleophilum*.

Fig. 6. Effect of time of pruning or inoculation on susceptibility of current season's spurs of Cabernet Sauvignon grape to infection by *Phaeoacremonium aleophilum* or *Phaeoacremonium aleophilum*. Spurs were inoculated immediately after pruning. Control spurs were not infected.

of the low percentages of infected planting materials. Infection of grapevines with these endophytic pathogens will be only a matter of time even when planting disease-free plants because inoculum has been shown to be present in adjacent older vineyards that show high numbers of pycnidia of *P. chlamydospora* and perithecia of *T. minima*. Esca itself appears to be the result of pruning wound infection, whereas young esca or Petri disease occurs as a result of infection of the rootstock that occurred in nurseries.

Recently, perithecia of *T. minima* (22,23,25), *T. fraxinopennsylvanica* (9), and *T. viticola* (10) were found on standing vines in the field on dead vascular tissue in deep cracks along the trunks and cordons (9,10,20,22,23,25,26). They also were seen on the surface of 7- to 10-year-old decayed pruning wounds. Perithecia of *T. fraxinopennsylvanica* and *T. viticola* also were found on ash trees both in the vicinity of vineyards and on city trees (*Fraxinus latifolia*) in California. This host range illustrates the potential importance for inoculum from these trees that commonly grow in the vicinity of vineyards. Pruning wounds are the most obvious port of entry into grapevines for these pathogens. Several studies have shown that *Phaeoacremonium aleophilum* can infect pruning wounds inoculated with conidia readily (15,16) and that pruning wounds were particularly vulnerable to colonization when pruning was done early in the season. However, this is the first study of these pathogens to show stunting of new shoot growth and foliar and fruit infection in the season following infection. Our results indicate that symptom expression of this discontinuous disease occurs in the year of new infections. As endophytes with the capability of being pathogenic under conditions of stress (11), these fungi are quickly walled off with gums and tyloses in the xylem and, therefore, are considered to be short-lived in terms of their pathogenic nature.

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