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Impact Statement

Ascospore Release of *Togninia minima*, Cause of Esca and Grapevine Decline in California

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Abstract
Esca and grapevine decline are important diseases affecting both young and mature grapevines worldwide. In California, these diseases are caused primarily by the fungal pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. Perithecia of *Togninia minima*, the newly-described teleomorph of *Phaeoacremonium aleophilum*, were produced by mating different strains in culture. Using a video camera, the process of emerging asci and release of ascospores from perithecia was filmed and presented herein for review. Furthermore, naturally occurring perithecia were discovered in infected California vineyards. These studies provide video documentation of the method of ascospore release of *Togninia minima* and suggest the importance of ascospore release in the disease cycle of esca in California vineyards.

Introduction
California's grape harvest accounts for nearly 91% of the total annual production in the United States. In 2003, the 882,000 total acres of grape varieties (819,000 bearing acres) were valued at over $2.3 billion, making it California's most valuable crop (2). Declines of both young grapevines (Petri disease) and mature grapevines (esca or black measles) cause significant damage to fresh, raisin, and wine grape varieties in California (20,21,22). Symptoms of Petri disease include poor vigor, stunting of plants, leaf chlorosis, and overall decline. Internally, the vascular tissue becomes dry and produces dark streaking in the woody cylinder. Esca is characterized by the presence of bright "tiger striped" patterns on the leaves of affected shoots, which can vary from one year to the next (Fig. 1). Affected fruit can exhibit superficial brown to purple spots on the berry skins (Fig. 2) or the fruit can wilt and die. Entire clusters can become affected, making fruit of table grape varieties unmarketable. Despite past research efforts, little information is available regarding disease epidemiology and management.

Fig. 1. Typical tiger-striped pattern of esca on the foliage of an infected Thompson Seedless grapevine in Madera Co., California.

Fig. 2. A Thompson Seedless grape bunch showing typical spotting and "measles" symptoms on the berries.

During the early 1990s a significant increase in the number of young grapevines exhibiting decline symptoms in California raised considerable concern. It was believed that the onset of these declines was associated with the large acreages of grapevines being planted with new phylloxera-resistant rootstocks (21,22). Scheck et al. speculated that the fungi causing declines have been present in California for many years but their occurrence and importance was only intensified after the extensive statewide planting boom of the 1990s (22). Furthermore, it is believed that these fungi are also well-adapted endophytes that are capable of living asymptomatically in grapevines for long periods of time without causing disease. It is when these vines are subjected to stressors such as poor cultural practices or lack of water that the fungi become pathogenic and cause disease (8,21).

On a worldwide basis, these diseases have been associated with a number of fungal pathogens (3,4). In California, the two most commonly isolated pathogens are *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, which are isolated from active water conducting tissue (16,20,21). Multiple species of *Phaeoacremonium* are cultured from grapevines in California, but *Phaeoacremonium aleophilum* is the most prevalent and is considered to be the primary pathogen of esca in California (16). The teleomorph for *Phaeoacremonium aleophilum* was recently identified as *Togninia minima*, and whose name will be used hereafter for this fungus (13,16,18,19). Aerially dispersed spores of *Phaeomoniella chlamydospora* and *T. minima* have been trapped in affected vineyards throughout California (5,6,7,15). Vaseline coated slides were attached to grapevine cordons, spurs, and trunks to collect fungal spores from these locations. Spore release of *Phaeomoniella chlamydospora* coincided strictly with rainfall events (5,7). In contrast, aerial born spores of *T. minima*, though usually associated with rainfall, were also occasionally trapped in the absence of precipitation (7,15). Spores of both *Phaeomoniella chlamydospora* and *T. minima* have been shown to be able to infect fresh pruning wounds up to 4 months after pruning (9). Furthermore, viable spores of *T. minima* were recovered from soil, standing water and decomposing debris from diseased vineyards (17). Viable pycnidiospores of *Phaeomoniella chlamydospora* have been shown to reside in pycnidia produced on pruning wounds 2 to 4 years after pruning (6). However, the source and type of *T. minima* spores has remained uncertain.

The teleomorph of *Phaeoacremonium aleophilum* was only recently confirmed as *T. minima*, a heterothallic pyrenomycete in the Calosphaeriales, after perithecia were produced in vitro by crossing isolates of opposite mating types (13,16,18,19). Some researchers have speculated that ascal deliquescence, or dissolving of the ascospore sack to release its ascospores, is a likely mechanism for ascospore accumulation at the tips of perithecial necks within this group of fungi (Fig. 3) (10). This method of spore release seems more

favorable for insect-vectored fungi, such as the ophiostomatoid fungi (23), and does not address the issue of our spore trapping data associated with rainfall. The purpose of this research was to investigate the mechanism of ascospore release of *T. minima*.

Fig. 3. A group of *Togninia minima* perithecia produced in vitro by crossing isolates of opposite mating types. Ascospore accumulation can be seen at the neck tip of one perithecium.

Spore Release of *Togninia minima*

Perithecia of *T. minima* were produced in vitro as described previously by Rooney-Latham et al., using two California isolates (strains) of *T. minima* known to produce abundant perithecia in culture (Fig. 3) (16,18,19). Once perithecia matured and held sticky droplets at their ostioles, they were removed and dried in sterile petri dishes at room temperature (23 ± 3°C). After drying, the perithecia were rehydrated at room temperature in sterile distilled water and observed immediately using a stereomicroscope at 400×. Cinephotomicrography using a Sony DCR-TRV110E digital video camera was then used to document perithecial activity.

Shortly after rehydration, asci emerged through the ostiole and either gathered in masses at the ostiole or contracted and forcibly discharged ascospores. Forcible discharge usually occurred following emergence of the basal end of the ascus, or soon thereafter. Slow forwarding of the film speed showed that the duration of time, from when the asci began to exit the ostiole to forcible ascospore ejection, could be as fast as 4 seconds (Fig. 4). When collected and plated onto malt extract agar, ascospores germinated and produced colonies typical of *T. minima*. Asci were also stained using Calcofluor White and observed using fluorescence microscopy to view the apical apparatus of the asci (Fig. 5).

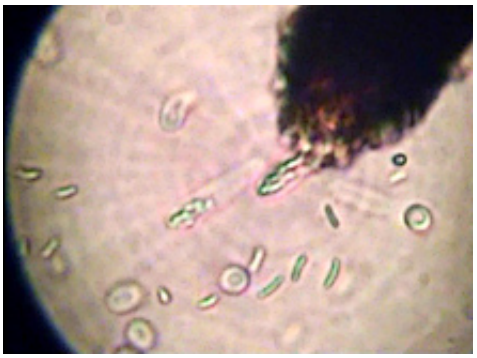

Fig. 4. Video documentation of ascospore release from perithecia of *Togninia minima*. (4.4 Megabytes, Windows Media Video format requiring a compatible program such as Windows Media Player.)

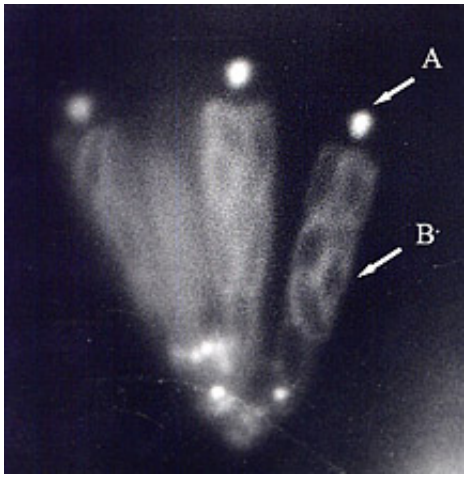

Fig. 5. Cluster of *Togninia minima* asci stained with Calcofluor White and viewed using fluorescence microscopy. (A) Apical ring that is likely shot or flipped off during forcible ascospore discharge. (B) Ascospores inside an intact ascus.

Discussion

Kenerley et al. demonstrated that ascospores of *Nemania serpens* (syn. *Hypoxylon serpens*) were capable of release by three different methods (11). Their work indicated that asci of perithecia produced in culture could either ooze ascospores in droplets from the ostioles after ascal deliquescence, forcibly discharge ascospores by thorough wetting and slow drying of perithecia, or passively release ascospores from asci that accumulate in mass, post-hydration. Similar observations were seen in our study. Forcible ascospore discharge from *T. minima* asci was only observed in perithecia that had been completely dehydrated, sufficiently remoistened, and then slowly redried. Furthermore, this process could be repeated multiple times from the same perithecium. It is likely that the ascospores seen on the tips of perithecia produced in culture were released by ascal deliquescence. This probably occurred inside the perithecia because droplets that had oozed from the ostiole often contained ascospores clumped in groups of eight; whereas when ascospores were forcibly discharged they were found to be non-clumped. Furthermore, empty asci were not seen in microscopic analysis of the droplets. Perhaps breakdown of the spore-bearing asci is also occurring among the asci that accumulate and do not forcibly discharge their spores after drying and remoistening.

The fact that asexual fruiting structures of *T. minima* have not been identified, along with the recent finding that *T. minima* perithecia are commonly produced on dead and decaying vascular tissue of naturally infected grapevines (*unpublished*), lead us to speculate that ascospores are the spore type being trapped in infected vineyards. Dried perithecia produced in vitro require wetting for ascospore discharge and this explains why the majority of spore release in vineyards occurs after rainfall events. Grapevine pruning in California typically occurs during the dormant season from December through early March. Furthermore, it is during these months when the majority of the yearly precipitation total occurs, suggesting ascospore release primarily occurs during the pruning season (7,15). Pathogenicity tests conducted at times coincident with vineyard pruning indicate that grapevine pruning wounds are susceptible to infection by conidia of *T. minima* (9), suggesting that pruning wounds and injuries made on cordons and trunks are likely the sites of which new infections occur each year.

Spore release documented in the absence of rainfall may be due to local spread of ascospores from deliquesced asci that have oozed out and accumulated at the ostiole. We speculate that local spread and infection result from vineyard irrigation practices or insects. Spores of *T. minima* have been successfully isolated from drip irrigation puddles under grapevines in California, but were not isolated directly from irrigation water as it passed through the emitter (17). This suggests that *T. minima* inoculum resides at the soil level, and that this inoculum may be the source for water-splashed infections resulting from various cultural practices. It is possible that insects may also be vectors for new grapevine infections. Eskalen et al. reported the presence of *T. minima* propagules on mites and termites from naturally infected grapevines (*unpublished*). Additionally, Kubatova et al. reported that *Phaeoacremonium rubrigenum*, another species of *Phaeoacremonium* occasionally isolated from declining grapevines, can be isolated from bark beetles (*Scolytus intracatus* and *Leperisinus fraxini*) and their galleries on *Quercus* and *Fraxinus* spp. in the Czech Republic (12). Different bark beetle species occur throughout California, many of which can cause significant damage to woody hosts. Their occurrence and importance on grapevines, however, is not well studied.

We postulate that perithecia of *T. minima* most likely form during the dry summer months in California, due to their high temperature requirement for growth (16). At maturity and under high relative humidity, these structures probably ooze ascospores, which are spread by site-specific irrigation splashing, insects, or both. This would account for the recovery of propagules in California vineyards in the absence of rainfall (7,15). These perithecia probably overwinter and release the primary inoculum (ascospores) when rainfall resumes, either through forcible or passive ascospore release. Ascospores trapped during these

precipitation events may come from perithecia that have developed on other hardwood hosts on which *T. minima* has been identified (1). However, the association of spores and perithecia of *T. minima* on grapevines indicate that inoculum probably comes from within the vineyard. Previous research has documented perithecial formation on xylem and pith tissue of naturally infected, moist incubated grapevine pieces, confirming that both mating types co-exist within the same grapevine (14,16). Furthermore, perithecia were recently identified on old pruning wounds and cracks of dead vascular tissue on standing grapevines in many California vineyards (*unpublished*).

This work documents both the presence of perithecia on grapevines in California, further details of which will be published at a later date, and the actual mechanism of ascospore release of *T. minima*. These findings lead us to conclude that ascospores are important in the disease cycle of vine decline and esca in California. The fact that perithecia release ascospores multiple times and via different mechanisms is important in our understanding of the epidemiology of esca and Petri disease. These results suggest that aerial spores may be more prevalent and important in the infection process than previously thought and may offer clues for the development of effective management strategies. This work presents the first evidence of ascospore release of an elusive group of fungi and provides clear video documentation of this occurrence.

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Literature Cited

1. Berlese, A. N. 1900. Icones fungorum omnium hucusque cognitorum. Patavia 3:9-21.
2. California Agricultural Statistics Service. 2003. California Agricultural Resource Directory 2002. Calif. Dep. Food and Agric., Sacramento, CA.
3. Crous, P. W., and Gams, W. 2000. *Phaeomoniella chlamydospora* gen. et comb. Nov., a causal organism of Petri grapevine decline and esca. Phytopathol. Mediterr. 39:112-118.
4. Crous, P. W., Gams, W., Wingfield, M. J., and van Wyk, P. S. 1996. *Phaeoacremonium* gen. nov. associated with wilt and decline disease of woody host and human infection. Mycologia 88:786-796.
5. Eskalen, A., Latham, S. R., and Gubler, W. D. 2004. Spore release of *Phaeomoniella chlamydospora* with grapevine cordons in California.. Phytopathology 94:S28.
6. Eskalen, A., Rooney, S. N., and Gubler, W. D. 2002. First report of the pycnidial state of *Phaeomoniella chlamydospora*, a causal agent of black measles (esca) and Petri disease in California vineyards. Phytopathology 92:S24.
7. Eskalen, A., and Gubler, W. D. 2001. Association of spores of *Phaeomoniella chlamydospora, Phaeoacremonium inflatipes* and *Phaeoacremonium aleophilum* with grapevine cordons in California. Phytopathol. Mediterr. 40:S429-432.
8. Ferreira, J. H., van Wyk, P. S., and Calitz, F. J. 1999. Slow dieback of grapevine in South Africa: stress-related predisposition of young vines for infection by *Phaeoacremonium chlamydosporum*. S. Afr. J. Enol. Vitic. 20:43-46.
9. Gubler, W. D., Eskalen, A., Feliciano, A. J., and Khan, A. 2001. Susceptibility of grapevine pruning wounds to *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. Phytopathol. Mediterr. 40:S482-483.
10. Hausner, G., Eyjolfsdottir, G., and Reid, J. 1991. Two additional species of the genus *Togninia*. Can. J. Bot. 70:724-734.
11. Kenerley, C. M., and Rogers, J. D. 1976. On *Hypoxylon serpens* in culture. Mycologia 68:688-691.
12. Kubatova, A., Kolarik, M., and Pazoutova, S. 2004. *Phaeoacremonium rubrigenum*, hyphomycete associated with bark beetles found in Czechia. Folia Microbiol. 49:99-104.
13. Mostert, L. P., Crous, P. W., Groenwald, J., Gams, W., and Summerbell, R. C. 2003. *Togninia* (Calosphaeriales) is confirmed as the teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility and DNA phylogeny. Mycologia 95:646-659.
14. Pascoe, I. G., Edwards, J., Cunnington, J. H., and Cottral, E. H. 2004. Detection of the *Togninia* teleomorph of *Phaeoacremonium aleophilum* in Australia.

Phytopathol. Mediterr. 43:51-58.
15. Rooney-Latham, S., Eskalen, A., and Gubler, W. D. 2004. Ascospore discharge and occurrence of *Togninia minima* (anamorph=*Phaeoacremonium aleophilum*) in California vineyards. Phytopathology 94:S57.
16. Rooney-Latham, S., Eskalen, A., and Gubler, W. D. 2005. Teleomorph formation of *Phaeoacremonium aleophilum*, cause of esca and grapevine decline in California. Plant Dis. 89:177-184.
17. Rooney, S. N., Eskalen, A., and Gubler, W. 2001. Recovery of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from soil and grapevine tissues. Phytopathol. Mediterr. 40:S351-356.
18. Rooney, S. N., Eskalen, A., and Gubler, W. D. 2003. First report of the teleomorph of *Phaeoacremonium* spp., the cause of esca and decline of grapevines. Phytopathology 93:S129.
19. Rooney Latham, S., Eskalen, A., Gubler, W., Harrington, T. C., and McNew, D. 2004. Further investigations into the first report of the teleomorph of *Phaeoacremonium aleophilum*. Phytopathol. Mediterr. 43:145.
20. Scheck, H. S., Vasquez, S. J., and Gubler, W. D. 1998. First report of three *Phaeoacremonium* spp. Causing young grapevine decline in California. Plant Dis. 82:590.
21. Scheck, H. S., Vasquez, S. J., Fogle, D., and Gubler, W. D. 1998. Grape growers report losses to black foot and grapevine decline. Calif. Agri. 52:19-23.
22. Scheck, H. S., Vasquez, S. J., Gubler, W. D., and Fogle, D. 1998. Young grapevine decline in California. Practical Winery and Vineyard (May/June):32-38.
23. Wingfield, M. J., Seifert, K. A., and Webber, J. F. 1993. *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. American Phytopathological Society, St. Paul. MN.