Investigation into the etiology of decline of Raywood ash in Northern California

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SUMMARY OF ACCOMPLISHMENTS

The objective of our continuing study is to clarify the contributions of *Botryosphaeria* and water stress to the dieback syndrome in Raywood ash. We continue to isolate *B. stevensii* from affected trees and have confirmed its pathogenicity to Raywood ash through inoculations of healthy branches. We have also isolated, from six trees, a second species of *Botryosphaeria* which has preliminarily been identified as *B. dothidea* and is also pathogenic to Raywood ash. To investigate the potential role of water stress, we have measured the water status of healthy and diseased landscape trees and found that trees with lower water potentials are more likely to be affected by dieback. In greenhouse trees artificially-inoculated with *B. stevensii*, water stress increased the frequency of canker formation and the rate of canker expansion. A field plot of Raywood ash has been established where experiments involving irrigation manipulation will be conducted on established trees.

Introduction

Raywood ash (*Fraxinus angustifolia* ‘Raywood’) is widely planted in parks, along streets, in lawns, and as a shade tree. While it has many desirable attributes, including freedom from some of the pests affecting other ash species, such as mistletoe and anthracnose, a branch dieback problem has been observed in California for a number of years (Perry, 1997). The main symptom has been the dieback of multiple branches throughout the canopy. We have found branch dieback in Raywood ash to be widely distributed in Northern California, affecting between 12% and 68% of trees within an area. Notwithstanding such widespread and severe disease problems, developers and municipalities continue to plant this variety. Many trees on public and private properties have been removed and others have been severely disfigured. Not only has this been costly to cities and homeowners but substantial canopy cover has been lost as well. Affected areas include the Central Valley and the San Francisco bay area.

The overall goal of our project is to identify the cause of the branch dieback problem in Raywood ash so that we might advise homeowners and municipalities on measures to manage affected trees and/or to avoid the problem in the future. In our first year of research, we identified a pathogenic fungus, *Botryosphaeria stevensii* (*Diplodia mutila* is the name for the anamorph) that is associated with dead branches. Pathogens in the genus *Botryosphaeria* are generally considered to be opportunistic in that they are typically associated with growing conditions which are sub-optimal for the host plant. The objective of our continuing study is to clarify the contributions of *Botryosphaeria* and water stress to the dieback syndrome in Raywood ash.

Material and methods

Samples of diseased plant tissue were surface sterilized in 1% sodium hypochlorite for 2 min. prior to plating on water agar amended with 200 ppm streptomycin. For identification, fungi were transferred to sterile pine needles upon which *Botryosphaeria* will produce fruiting structures. For confirmation of identification, the internal transcribed spacer (ITS) region of the ribosomal DNA was amplified and sequenced. DNA sequences thus obtained were compared to *Botryosphaeria* sequences available in the GenBank database.

Pathogenicity tests of candidate fungi were conducted by artificially inoculating branches on healthy trees. Inoculations were accomplished by removing a small piece of bark from a young branch and replacing it with a similarly sized piece of fungal-colonized nutrient agar. These tests were conducted both on established trees in the field as well as on young trees in the greenhouse. Fungi were re-isolated from diseased tissue as described above.

Because water stress is a well known predisposing factor in tree diseases (Ma et al., 2001) and because last year's incidence survey suggested that irrigation may affect the development of dieback, we initiated a series of studies designed to document an effect of water availability on branch dieback. In the fall of 2003, we used a pressure bomb to measure water potentials on established landscape trees that were either healthy or diseased. This included four trees in Newark, seven in Tracy and six in Rohnert Park. For each tree, an estimate of stem water potential was obtained by measuring water potentials of three leaves which had been sealed in plastic for at least 15 min. to allow them to equilibrate with the stem.

To complement this study, we attempted to demonstrate an effect of water stress on dieback by withholding water from established street trees. We identified a location in San Jose where 10-yr-old trees were being watered regularly by drip irrigation. Trees were rated for the percentage of dead canopy prior to initiation of differential irrigation treatments. Blocks of trees on the same irrigation valve were assigned to one of two treatments (irrigation on or off). Treatments were begun July 7th. Measurements of plant stress (water potential) will be made through the late summer and early fall and re-evaluations of die-back will be made in the spring (2005).

To evaluate the effect of water stress on infection and disease development under more controlled conditions, experiments were carried out on potted Raywood ash in the greenhouse. Trees were randomly assigned to one of two irrigation treatments (deficit or normal). Trees in each irrigation treatment group were inoculated with *B. stevensii*. Control trees were mock-inoculated (wounded but no fungi introduced). Water potential measurements were made one and 6 days after inoculation, just prior to watering when water stress should have been maximal. This experiment was conducted twice.

Additionally, we have established an experimental planting of 30 Raywood ash on the Davis campus. These trees are now two years in the ground, well-established, and differential irrigation treatments will be applied to these trees in the spring of 2005. When a depression in plant water potential is observed in the under-watered trees, both stressed and control trees will be inoculated with *B. stevensii* and/or *B. dothidea* as described above.

RESULTS AND DISCUSSION

In the second year of our study, we continued to sample affected trees, to document the association of pathogenic fungi with the problem. In particular, we conducted isolations tailored towards the recovery of both *Botryosphaeria* and *Verticillium dahliae*. We continue to isolate *Botryosphaeria* from many, though not all, branches affected by dieback. *Verticillium dahliae*, a vascular wilt pathogen, has not yet been isolated from any samples. In November 2003, six severely diseased trees in Modesto and Tracy were removed by city crews, allowing us to examine the entire tree including the trunk and all major branches. No vascular discoloration (typical of *Verticillium* infections) was observed and isolations from the previous two years wood (two outermost growth rings) did not yield *Verticillium*. Wood discoloration was observed, however, in conjunction with necrotic bark typical of *Botryosphaeria* infections, and in the case of one tree, discoloration was observed deep within the wood of the base of the trunk. *Botryosphaeria* was isolated from this trunk, and from wood underlying necrotic bark from many branches.

Studies of *Botryosphaeria* in other plant hosts have shown it can infect without causing symptoms, and may remain latent for some period of time before causing disease (Smith et al., 1996). For this reason, we are investigating the potential for this fungus to exist as an endophyte in Raywood ash, that is, without causing symptoms. Thus far, no isolations from healthy branches of affected trees have yielded *Botryosphaeria*, indicating that if this fungus does have endophytic potential then at least these capabilities are not extensively expressed in Raywood ash. Alternatively, the fungus may be present, but with a very limited distribution within symptomless trees, making it difficult to detect.

In addition to the many isolates of *Botryosphaeria stevensii* obtained from Raywood ash, isolations from six trees in three cities have yielded a second *Botryosphaeria* species which differs morphologically from the previously isolated strains. Based on morphology and molecular sequence data, these isolates were identified as *Botryosphaeria dothidea* (anamorphic name is *Fusicoccum*, see Fig. 1 & 2). These isolates appear to be related to the isolates which cause panicle blight of pistachio (Michailides, 2002; Smith et al., 2001). *B. dothidea* is a known canker pathogen of ash (Sinclair, 1987).

Pathogenicity of *Botryosphaeria* to Raywood ash and other woody plants

In October of the first year of our study, three isolates of *B. stevensii* were tested for pathogenicity on healthy trees. All three isolates of *Botryosphaeria* resulted in sunken cankers (Table 1) which affected the cambium and the underlying wood and contained characteristic fruiting structures.

In March 2003, a second group of three trees was inoculated with eight fungal isolates. One set of these branches was incubated for 4 months, a second set for 6 months, and a third set for one year. These inoculations, which included one of the previously tested isolates, did not produce cankers. Instead, callus developed around the 8-mm diameter wound site and the cambium was not necrotic. However, the underlying wood was discolored and colonized by *Botryosphaeria*. This discoloration extended as far as 1 cm axially away from the inoculation site, and in some cases was quite deep, involving the pith. In October 2003, a third group of three trees was inoculated with six isolates as described above, except that two wound sizes were used (3.5-mm and 6-mm diameters). These were incubated for five months. The cankers resulting from these inoculations were smaller than those from the previous year, however the wounds made had also been smaller (Table 1). In March 2004, a fourth group of trees was inoculated. These inoculations are currently incubating.

The difference in results between tests is likely attributable to the time of year the inoculations were performed (October versus March), which may impact both the fungus and the host response. This could be an indication that infections during late winter/early spring can give rise to latent infections.

In October 2003, two established Modesto ash were inoculated with an isolate of *B*. *stevensii* from that host. On one tree, significant cankers developed on the three inoculated branches (mean of 31 mm). On a second tree, callus developed within the wounds and two of the three branches had small cankers (mean 12.5 mm).

In June 2003, leaves of healthy trees were inoculated with a spore suspension to determine if leaf infections might be an avenue by which the fungus gains entry into the tree. We were unable to recover the fungus from any of these leaves or twigs after incubation periods of 5 weeks, 15 weeks, or 16 months.

To determine if the Raywood ash strain also affects woody plants in other genera, an isolate of *B*. *stevensii* from Raywood ash was inoculated into stems of potted plants of oak, tan oak, pistachio, peach, apple, grape and three conifers. This isolate caused substantial cankers on oak and apple, known hosts of *B. stevensii*, and pistachio and peach, not previously reported as hosts, but did not cause disease on tan oak, grape, or any of the conifers.

In the greenhouse, eight *Botryosphaeria* isolates from ash (7 isolates of *B. stevensii* and one isolate of *B. dothidea*) were inoculated into wounds on potted trees. Of these isolates, the *B. dothidea* isolate produced the longest lesion, significantly longer than the lesions produced by *B. stevensii***,** suggesting that it is more aggressive (Fig. 2).

Role of water stress in the disease

In comparing healthy and diseased landscape trees in three cities, the healthy trees showed no visible indications of water stress and had relatively high water potentials (mean = -11.0 bars). Trees with dieback were more variable in appearance; some appeared generally stressed (with premature fall color) but others did not, and their water potentials were generally lower (mean = -16.2 bars). Thus, on average, trees with lower water potentials appear more likely to be affected by dieback (Fig. 3). However, the relationship is not clear cut, because some trees with dieback had water potentials comparable to healthy trees. Our hypothesis is that such trees experienced water stress previously, but recovered because the loss of canopy due to dieback reduced transpirational demand to a level more compatible with available soil moisture. It is also possible trees with dieback are receiving more water through irrigation now than they did in the past (when water stress may have occurred).

From the two experiments conducted in the greenhouse on potted trees, we demonstrated a significant impact of water stress on the development of cankers. Deficit-irrigated trees, which experienced minimum water potentials ranging from -40 to -22.4 bars, did not develop callus at the wound sites. On stressed trees where the fungus was introduced, significant cankers developed from 14 out of 16 wounds. On the other hand, the normally irrigated trees, which experienced minimum water potentials ranging from -11.4 to -8.5 bars, exhibited good callus development, often completely overgrowing the 6-mm diameter wounds. Cankers developed from 6 out of 14 of wounds on non-stressed trees. The mean lesion length on inoculated, deficit-irrigated trees was 24.3 mm compared to 13.3 mm for normally irrigated trees and these differences were correlated with measured water potentials (Fig. 4). The impact of water stress on lesion length was clear-cut in the first trial, but not in the second. The reasons for this discrepancy are not known, but might reflect more severe water stress in the second trial. It is possible that drying of the colonized tissue was great enough to curtail growth of the pathogen.

Observation plots

Fourteen observation plots in thirteen cities were established in 2002 and revisited in 2003. Because the disease is not progressing quickly in these trees, we decided to delay reassessment of these plots until 2005.

Management recommendations

Based on our observations and those of cooperating arborists, we feel that Raywood ash may not possess the degree of drought tolerance which has been attributed to it. Furthermore, it appears that this variety's suitability for highly urbanized conditions has not been adequately demonstrated. We hypothesize that stresses associated with sub-optimal site conditions predispose Raywood ash to damage by *Botryosphaeria*. Until more is known about this disease, we recommend that Raywood ash not be planted in California. The varieties of green ash (*Fraxinus pennsylvanica*) planted in California appear not to suffer from this problem and might be an appropriate alternative to Raywood in some situations.

For existing plantings of Raywood ash which are to be maintained, we suggest occasional deep watering in conjunction with thinning of the canopy to reduce transpirational demand.

Outreach

Our study has generated considerable interest from public and private arborists and from homeowners who are all eager to know the cause of this disease and how they might manage this problem. We have attended professional meetings to gather information about the problem from arborists and to disseminate the findings of our research. Thus we have made presentations at regional meetings of the Western Chapter of the International Society of Arboriculture in San Francisco (July 2002), Modesto (July 2003), and Milpitas (December 2003), and in other continuing education formats in Monterey (March 2003), San Rafael (December 2003), and Petaluma (November 2004).

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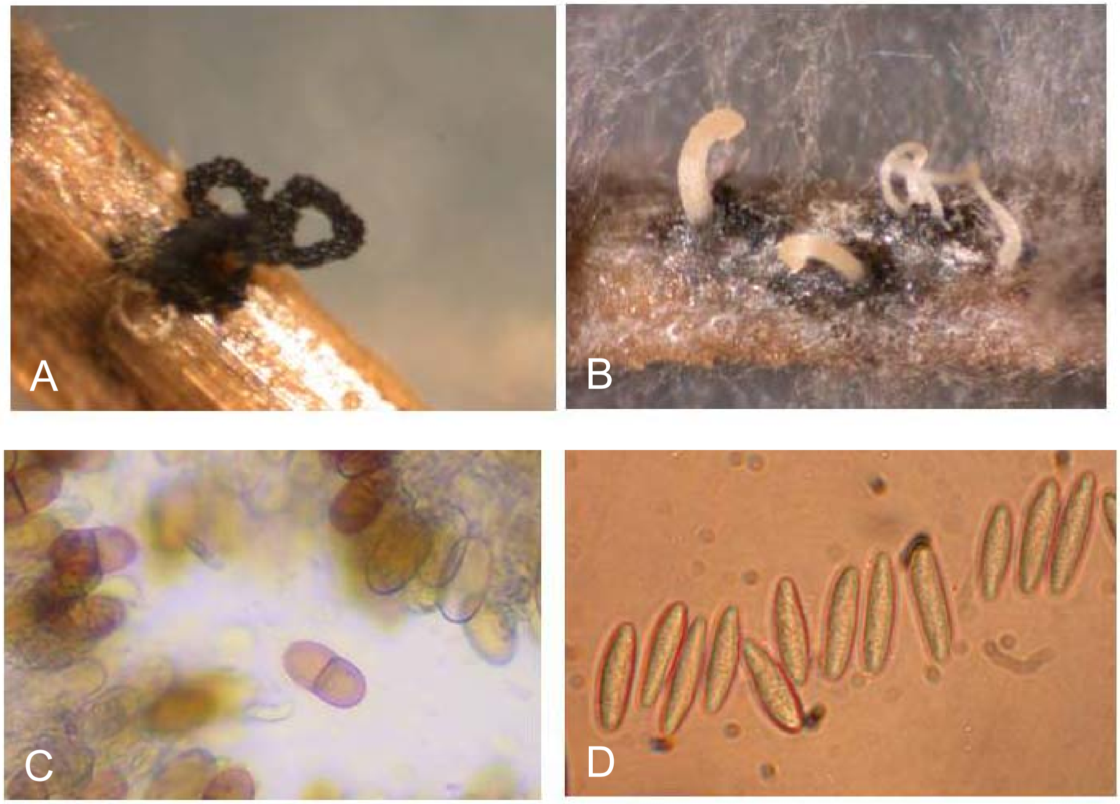

Fig. 1. Fruiting structures and spores used in the identification of *Botryosphaeria* species isolated from diseased Raywood ash trees. Pycnidia (A) extruding dark spores (C) characteristic of a *Diplodia* anamorph and pycnidia (B) with the light-colored spores (D) characteristic of a *Fusicoccum* anamorph.

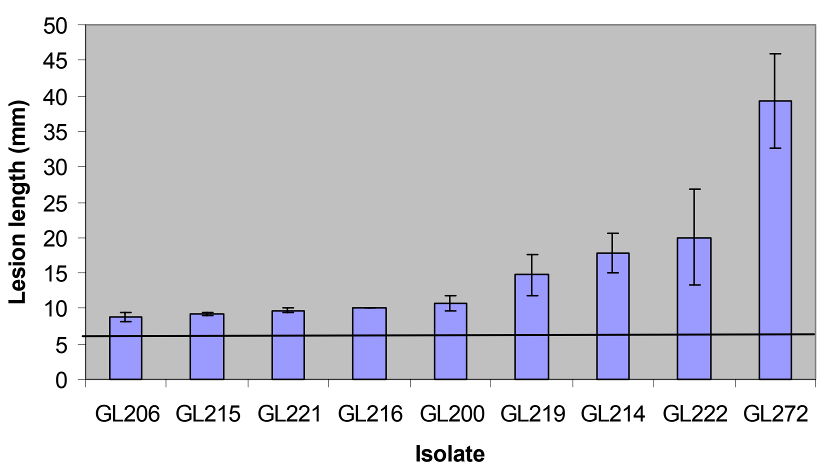

Fig. 2. Pathogenicity of *Botryosphaeria* isolates from ash. The horizontal line indicates the size of the original wound (and therefore the minimum possible lesion size of 6 mm diameter). Isolates GL206-GL222 are all representatives of the species *Botryosphaeria stevensii*, while GL272 is a species of *Fusicoccum*.

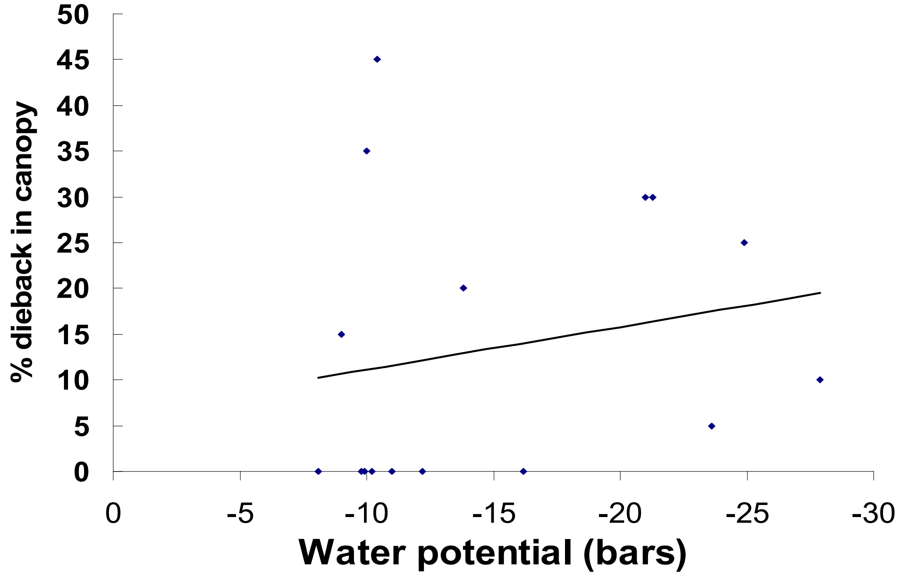

Fig. 3. Relationship between tree water status and dieback in Raywood ash in landscape plantings.

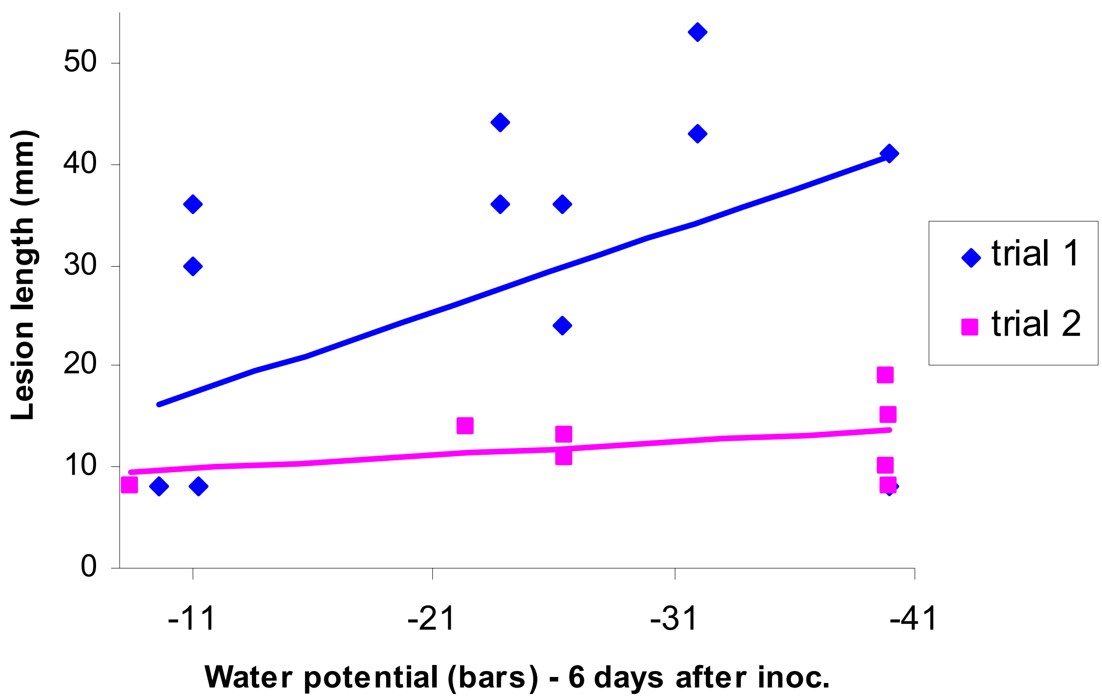

Fig. 4. Pathogenicity of *Botryosphaeria stevensii* to greenhouse Raywood ash as a function of water stress measured six days post-inoculation. Trees were subjected to differential irrigation to achieve stress and non-stress treatments.

Fig. 5. Response of non-stressed (above) and stressed (below) trees to wounding with a 6-mm diameter cork borer and inoculation with *Botryosphaeria stevensii*.

Table 1. Canker development from artificial inoculations on established landscape(?) trees. Values are the mean of 2 to 4 replicate inoculations.

Isolate	**Growth of canker beyond wound (mm)**	
	October 2002 test	October 2003 test
GL214	25.5	10
GL215	16	6.25
GL216	18	4
GL206	-	4.5
GL219	-	7
GL221	-	4.9