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

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Report to the Elvenia J. Slosson Endowment Fund. This is a final report for work performed from July 1, 2004 to June 30, 2005 (year 3 of a 3-year study).

SUMMARY OF ACCOMPLISHMENTS

The objective of our study was to clarify the cause of dieback in Raywood ash and develop recommendations for management of the problem. We have determined that *Botryosphaeria stevensii* is commonly associated with dieback and that this fungus can cause cankers when inoculated into healthy branches. Trees subjected to water stress under greenhouse conditions tend to develop longer lesions than well-watered trees, although the difference was not significant in all experiments. A field experiment has not confirmed a significant effect of water stress on canker development, perhaps because stress was not as severe as in the potted trees maintained in a greenhouse. We have shown that *B. stevensii* can infect pruning wounds but it is unknown if this is an avenue of infection under natural conditions or by what other means infections may become established. We have also isolated *B. dothidea* from symptomatic Raywood ash and confirmed its pathogenicity. Observations suggest that removal of dead branches and opening up of the canopy to reduce transpirational demand, coupled with irrigation can reduce the risk of damage from the dieback syndrome.

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. In our second year, we identified a second species, *Botryosphaeria dothidiea* (*Fusiccoccum* is the name for the anamorph) that was also associated with the dieback problem. Pathogens in the genus *Botryosphaeria* are generally considered to be opportunistic in that they are typically associated with growing conditions that are sub-optimal for the host plant.

Material and methods

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.

To mimic a more natural avenue for infection, one set of inoculations was conducted with a suspension of spores. The suspension was produced by crushing mature pycnidia (produced in culture) in water and counting and adjusting the number of spores to 10,000 spores per mL. To investigate the possibility of infection via pruning wounds and natural wounds, this spore suspension was introduced into such wounds on four established landscape trees. To create wounds more like what might occur naturally, leaves were removed from young branches and the resulting scars were inoculated. These branches were harvested one year after inoculation.

Because water stress is a well known predisposing factor in tree diseases (Ma et al., 2001) and because surveys 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 2004, 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) were made through the late summer and early fall.

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 three times.

Additionally, we established an experimental planting of 30 Raywood ash on the Davis campus. Differential irrigation treatments were applied to these trees during the summer of 2005. The trees were arranged in five blocks, two blocks receiving regular irrigation applied through the summer, and the other three blocks without irrigation. Each block contained six trees, three each on green ash and black ash rootstocks. When a depression in plant water potential was observed in the non-irrigated trees, both stressed and control trees were inoculated with *B. stevensii* and *B. dothidea* (on separate branches) by inserting colonized agar plugs into wounds as described above. On each of the thirty trees, two branches were inoculated with *B. stevensii* and two with *B. dothidea*. A fifth control branch on each tree was mock-inoculated (wounded but no fungi introduced).

These branches were evaluated in September, five weeks after inoculation, and the length of cankers was measured non-destructively. These branches will be left on the trees until 2006 to determine if dieback is apparent after they leaf-out in spring.

RESULTS AND DISCUSSION

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 allowed to incubate 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. As with the previous year's March inoculation, these did not produce cankers but did result in discoloration in the wood underlying and surrounding the wound.

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 addition to the many isolates of *Botryosphaeria stevensii* obtained from Raywood ash, isolations from six trees in three cities 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*). The isolate of *Fusicoccum* from ash that we have included in our pathogenicity tests has consistently been more aggressive, producing larger lesions than those caused by *Diplodia* (see below).

In October 2003, two established Modesto ash trees were inoculated with an isolate of *B. stevensii* from that host. On one tree, significant cankers developed on the three inoculated branches (mean length of 3.1 cm). On a second tree, callus developed within the wounds and two of the three branches had small cankers (mean length of 1.2 cm). Additional inoculations with the same isolate were conducted in September 2004. On one tree, cankers averaged 3 cm in length while on the second tree cankers averaged 7.1 cm, with the largest

canker measuring 11.7 cm. These results indicate that Modesto ash is susceptible to the Raywood pathogen.

In the experiment investigating various avenues of infection, trees were inoculated by application of spores to branches with fresh leaf scars or with pruning wounds. One year after inoculation, leaf scars did not result in canker development. In the case of pruning wounds inoculated with a spore suspension, *Botryosphaeria* appeared to colonize the wood back to next node. Only in one case did the necrosis of the cambium extend below the next lower branching point. However, the capacity of the pathogen to grow beyond this point might depend on the physiological status of the tree. We are therefore still uncertain by what means these fungi are able to initiate infections under natural conditions.

Role of water stress in the disease

In an attempt to demonstrate an effect of water stress on dieback, we arranged with the city of San Jose to withhold water from a set of street trees, while maintaining normal irrigation on a companion set of trees. All trees in the treatment area were rated for the extent of dieback at the inception of the experiment in the summer of 2004. Unfortunately, the irrigation treatments did not result in differential development stress between blocks of irrigated and non-irrigated trees. Measurements taken in September of 2004 revealed a comparable range of water potentials in all trees, regardless of treatment.

Experiments conducted in the greenhouse on potted trees demonstrated a significant impact of water stress on the development of cankers. In general, deficit-irrigated trees, which experienced minimum water potentials ranging from below -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 23 of 28 wounds. On the other hand, the normally irrigated trees, which experienced minimum water potentials ranging from -19 to -7 bars, typically exhibited good callus development, sometimes completely overgrowing the 6-mm diameter wounds. Cankers developed from 9 out of 28 wounds on non-stressed trees. In the first experiment, the mean canker length on inoculated, deficit-irrigated trees

was 3.6 cm compared to 1.4 cm for normally irrigated trees (Fig. 1). In a second experiment, there was little to no effect of irrigation treatment on canker development. In 2005, a third greenhouse experiment was conducted, with results intermediate between those of the first and second. In the third experiment, deficit irrigation resulted in cankers 74% longer than those on non-stressed trees (2.1 cm versus 1.2 cm, Fig. 1).

Comparing the water potential measured during the week after inoculation to the canker length (Fig. 2), we see a significant response in two out of the three experiments. The reasons for this discrepancy between experiments 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.

On the 30 trees inoculated in the field in August 2005, *B. stevensii* produced cankers 1.7 cm long on average, while those caused by *B. dothidea* were significantly larger ($P<0.0001$), averaging 2.9 cm. Mock inoculations never resulted in cankers. There was no effect of rootstock type on canker length ($P=0.114$).

The effect of irrigation treatment on canker length was not significant ($P=0.1651$), perhaps because withholding water was insufficient to induce significant stress. Water potential measurements in the field did not reach levels as low as those measured on deficit-irrigated trees in pots in the greenhouse. Estimated water potential of irrigated trees in the field experiment ranged from -15 to -9 bars while that of non-irrigated trees ranged from -24 to -9 bars. With deep root systems, these established trees are likely exploiting soil moisture at great depths and may be not suffer from a lack of irrigation to the extent that trees in other situations might.

Observation plots

Fourteen observation plots in thirteen cities were established in 2002 and revisited in 2003 and 2005. During the three-year study period, 10% of the 280 original trees were removed. Some of these were due to construction, however

many others were clearly removed due to the dieback problem. Of the 272 trees present during at least 2 years of the study, the dieback worsened in 25% of them while 52.6% remained in roughly the same condition. The remaining 22.4% improved over the course of the 3 years. This is attributable to pruning operations which removed dead branches.

We conducted a chi-square analysis of the association between the disease severity rating (based on the percent of the canopy killed) and the presence or absence of a functioning irrigation system. There were 279 trees from 14 plots included in this analysis. Among these trees there was a significant association between disease severity and irrigation (P=0.0015).

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*. Due to the wide geographical distribution of the dieback problem, 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 that are to be maintained, we suggest occasional deep watering in conjunction with thinning of the canopy to reduce transpirational demand. Raywood ash trees often have a multitude of scaffold branches emerging from the trunk and it may not be possible for the tree to supply all of these with adequate water. Dieback often appears first on branches associated with the central-most scaffold, which is perhaps an indication that it is last in line to receive the available water.

Although pruning experiments were not conducted as a part of this study, in the course of evaluating plots and collecting samples we observed a number

of trees that were pruned to remove dead branches. In many cases, additional pruning was done to further open up the canopy. In nearly all cases, the trees showed no further development of dieback in two subsequent years. Where new dieback did develop it remained quite limited during the observation period. These observations suggest that such pruning operations are likely to beneficial.

Outreach

We have extended information about this problem through direct contacts with city arborists and UCCE personnel. In addition 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 January 2005), and Petaluma (November 2004).

Literature Cited

Ma, Z., Morgan, D. P., Michailides, T. J. 2001. Effects of water stress on Botryosphaeria blight of pistachio caused by *Botryosphaeria dothidea*. Plant Disease 85:745-749.

Perry, Ed. 1997. Mysterious dieback in Raywood ash. Western Arborist 23:25.

Smith, H., Wingfield, M. J., Crous, P. W., and Coutinho, T. A. 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany. 62 (2):86-88.

Table 1. Number of established landscape trees categorized by disease severity and presence or absence of a functioning irrigation system.

Disease rating (% dieback)	Irrigation	
	Some	None
None	52	16
1-10%	74	29
11-50%	55	39
51-80%	5	6
>80%	1	2

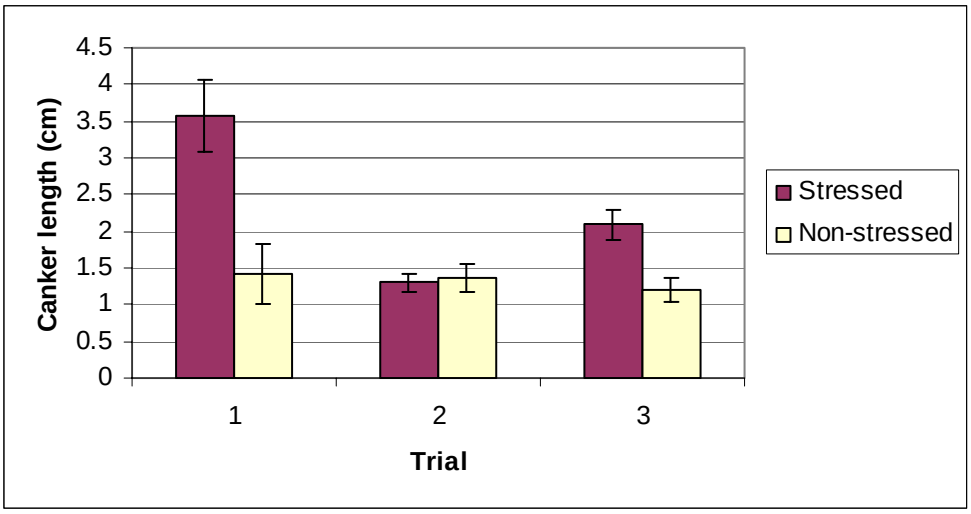

Fig. 1. Effect of a differential irrigation treatment on canker length on artificially-inoculated trees in a greenhouse.

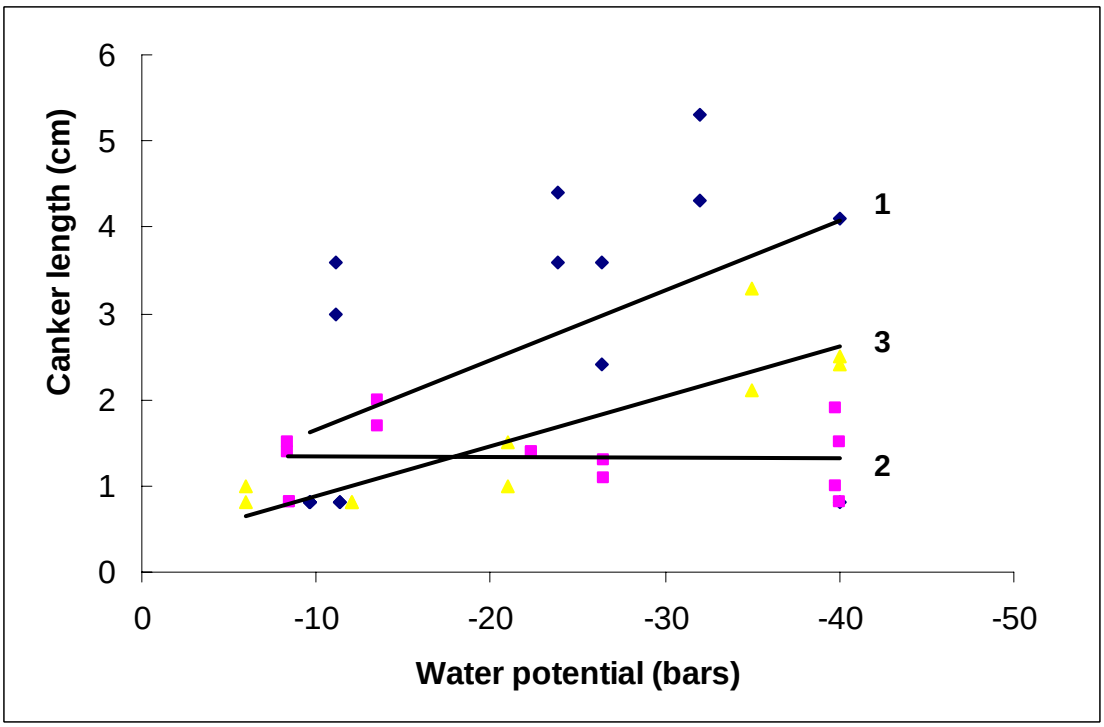

Fig. 2. Pathogenicity of *Botryosphaeria stevensii* to greenhouse Raywood ash as a function of water stress measured six days post-inoculation. Trees were subjected to differential irrigation to achieve stress and non-stress treatments. Each set of points represents data from one experiment (numbered 1 to 3).