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

Principal Investigator: Thomas R. Gordon
Department of Plant Pathology,
UC Davis, One Shields Ave., Davis, CA 95616
Cooperating Personnel: Brenna J. Aegerter
Department of Plant Pathology,
UC Davis, One Shields Ave., Davis, CA 95616

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Raywood ash (*Fraxinus angustifolia* subsp. *oxycarpa* 'Raywood') is widely planted in parks, along streets, in lawns, and as a shade tree. Many cities in California currently recommend Raywood ash as a residential street tree. It has been touted as tolerant of drought and of many climates and soil types, and is generally pest-free, being less susceptible than other ash species to anthracnose and mistletoe. Notwithstanding this alleged wide adaptability to environmental conditions, a decline of Raywood ash in California has been observed for a number of years (Perry, 1997). The main symptom has been the dieback of multiple branches throughout the canopy (Figure 1). The effect is severe in many cases (Figure 2), although the extent to which the problem is associated with mortality has not been documented. Nonetheless, 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.

A number of decline problems have been documented on ash trees in other parts of the country. These problems have been attributed to pests such as tree-boring insects, pathogenic microbes, and adverse planting-site conditions. The lilac/ash borer (*Podosesia syringae*), a clearwing moth, can be a cause of branch dieback but it does not occur in all areas of California where Raywood ash dieback is observed. Other possible causes of ash dieback include the ash yellows phytoplasma, which is a problem on green ash and white ash in the north-central and northeastern United States (Sinclair and Griffiths, 1994), and Verticillium wilt. Based on the dieback symptoms observed in California, Vertcillium wilt seems the more likely cause, and Raywood ash is known to be susceptible to this disease. However, previous attempts to isolate the causal fungus have not been successful.

Objectives

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. The objectives of the first year of our study were to test for the presence of specific pathogens that might be responsible for branch dieback, and to look for an association of the dieback syndrome with various abiotic factor(s).

Results and discussion

Assays of affected trees for pathogens

Leaf and branch samples were obtained from symptomatic trees in 14 cities where Raywood ash is commonly grown. A variety of methods were used to assay for the presence of pathogenic microorganisms. Leaf petioles and stem pieces were plated on water agar to test for the presence of *Verticillium dahliae*. A genetic, PCR-based test was used for detection of phytoplasmas (causal agent of ash yellows disease) in symptomatic plant tissue. For the PCR test, leaves were taken from branches that had some dieback but which still had live foliage proximal to the dieback. DNA was extracted from leaf midribs and tested for phytoplasma DNA using a protocol developed by B. Kirkpatrick at UCD. One affected tree was assayed for the presence of the bacterium *Xylella fastidiosa* (Pierce's disease) using an antibody-based test (conducted in B. Kirkpatrick's lab). All tested samples were negative for Pierce's Disease, Ash yellows and Verticillium wilt.

For the majority of trees sampled, we assayed for fungal colonization of branches by 1) plating branch tissue pieces on water agar and/or 2) examining the bark with microscopy to detect fungal fruiting bodies. In June 2002, a visiting scientist from South Africa (M.J. Wingfield) suggested the symptoms resembled those caused by the fungal pathogen *Botryosphaeria*. Based on that suggestion, we have examined diseased branches for fruiting structures of this pathogen, which we have now positively identified in trees from 13 cities spread throughout the S.F. bay area and the Central Valley. In addition to Raywood ash, the fungus was also identified in association with branch dieback on evergreen ash (*Fraxinus uhdei*) and Modesto ash (*F. velutina* 'Modesto').

The fruiting structures of *Botryosphaeria* are formed within the bark, protruding slightly to the surface. By making thin sections of the bark just below the surface we can use the microscope to view the characteristic fruiting structures (pycnidia) (Figure 3) and the spores within them (Figure 4). The formation of these structures likely requires a number of months after death of the tissue, as well as an adequate amount of moisture. In most cases where fruiting structures were not present, the fungus has been identified based on its growth out of symptomatic tissue into an agar medium. These isolations are done from the margin between dead and living tissue (Figure 5).

Based on the morphology of the fruiting structures and genetic analyses, Bernard Slippers and Mike Wingfield, plant pathologists at the University of Pretoria, South Africa, have identified our isolates as *Botryosphaeria stevensii* (=*Diplodia mutila*), a known pathogen of other trees (Sinclair et al., 1987).

Pathogens in the genus *Botryosphaeria* are generally considered to be opportunistic in that they are typically associated with predisposing stress factors such as drought (Ma et al., 2001). In certain plants, representatives of this genus have been shown to live as endophytes, being present in symptomless tissues and only causing disease under particular conditions. To determine if *Botryosphaeria* is present in healthy Raywood ash, we sampled healthy branches on affected trees as well as healthy nursery stock and nursery mother trees. Thus far, the fungus has not been isolated from any symptomless tissue.

Testing potential pathogens

Pathogenicity tests of candidate fungi were conducted by artificially inoculating branches on healthy, established 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. All three tested isolates of *Botryosphaeria* resulted in sunken cankers which affected the cambium and the underlying wood and contained characteristic fruiting structures. Although such cankers are not typical of what is observed on affected trees, these results do confirm the virulence of this fungus on Raywood ash and suggest it has a role in the etiology of branch dieback.

Leaves of healthy trees were also inoculated to determine if leaf infections might be an avenue by which the fungus gains entry into the tree. Preliminary results suggest that leaves do not become infected as we were unable to reisolate the fungus from inoculated leaves.

Survey of branch dieback incidence in Raywood ash

Fourteen areas were surveyed involving twenty-one municipalities across ten counties including Alameda, Contra Costa, Marin, San Joaquin, Santa Clara, Santa Cruz, Solano, Sonoma, Stanislaus and Yolo. In some cases, adjacent cities were combined for the purposes of the survey. The numbers of trees surveyed in each area ranged from 53 to 419; in total, 2,639 trees were evaluated. Trees with dead branches typical of the dieback syndrome were rated as diseased, whereas trees without dieback, or with dieback restricted to shade-suppressed branches were rated as healthy. Excluded from the counts were recently planted trees.

The dieback phenomenon appears to occur wherever Raywood ash is planted in Northern California, as no city we visited was free of the problem. Disease incidence within a survey area ranged from 11.5% to 68% (see Table 1). If we consider disease incidence by climate zone, it appears that more coastal locations exhibit higher incidences of the disease. However, the cities with higher incidences also are characterized by trees in more urbanized settings which may affect their susceptibility to disease. Many factors other than climate may contribute to differences between cities, such as the age distribution of trees, the quality of planting sites and the frequency of irrigation.

The incidence survey was not designed to evaluate these factors, but some insight into the effect of irrigation can be gained from a chi-square analysis to test for independence between tree condition (affected or unaffected) and planting environment (irrigated turf or not). Based on 2,261 trees for which we had planting site information, tree condition and planting site category were not independent (X^2= 6.022, d.f.=1, P= 0.0141), suggesting that growing conditions do affect disease incidence. Table 2 shows the results of the analyses of data from seven cities in which sufficient numbers of trees could be found in each category of planting site. In Davis, Fairfield, and Walnut Creek, planting site category and tree condition were independent, whereas in four cities closer to the coast these two factors were related (Table 2). This suggests that location influences the relative importance of irrigation as a factor in this disease, which may relate to the relative availability of ground water. If so, it implies that dieback is less likely to occur where trees have access to adequate moisture. However, further data collection, analyses and experimentation are needed to explore the relationship between irrigation and the disease.

Establishment of long-term observation plots

Fourteen plots were established in thirteen cities. Each plot is comprised of 20 established trees. Where possible, trees in different types of planting sites were included (i.e., punch outs in sidewalks vs. in parks). For each tree, the following items were recorded: location (by street address and by GPS coordinates), tree height, tree diameter at breast height, and the number and condition of scaffold branches. Each major scaffold was rated for disease severity based on the percentage of the branch which was affected (0 = 0% dead, 1 = 1-10% dead, 2 = 10-50% dead, 3 = 50-80% dead, and 4 = >80% dead). The same scale was used to give each tree an overall rating. Tree structure was evaluated and each tree was assigned to a subjective category: 1 = good structure, 2 = poor structure or 3 = very poor structure, based upon the number of scaffolds at each branching point and the incidence of included bark. The presence of surface roots, mounding, or sprouting of the rootstock was noted for each tree. Overall tree health was evaluated subjectively based on foliage density, coloration, and the vigor of new growth. The presence and abundance of seeds were noted (1 = few to none, 2 = moderately abundant, or 3 = abundant).

The irrigation status of each tree was categorized based on the presence of a functioning irrigation system and the type and condition of groundcover. The quality of the planting site was rated based primarily on the proximity to concrete or asphalt. Soil type was determined from a soil survey map. The proximity of adjacent Raywood ash trees (next Raywood less than 100 feet away or not) was recorded. Foliage and bark were inspected for evidence of insects (wooly aphid, ash whitefly, ash mirid feeding damage, ash borer exit holes). Any evidence of pruning or other tree maintenance was noted. Where municipalities have records, the date of planting was included, as well as any other information available from the database (e.g. rootstock variety).

As seen in the incidence survey, trees in climate zone 17 appeared to be more affected than trees in other zones (Table 3). However, plots in zone 17 are in more highly urbanized settings which may be sub-optimal for the trees. The irrigation status of the tree appears to be an important factor as non-irrigated trees had, on average, a lower disease severity rating (Table 4).

The primary purpose of the plots is to observe the progression of the disease over time. To this end, the plots will be revisited annually.

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.

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.

Sinclair, Wayne A. and Griffiths, Helen M. 1994. Ash yellows and its relationship to dieback and decline of ash. Annual Review of Phytopathology 32:49-60.

Sinclair, W. A., Lyon, H. H., and Johnson, W. T. 1987. Diseases of trees and shrubs. Cornell University Press, Ithaca, NY.

City	Climate zone	Disease incidence
Fairfield	15	11.5%
Rohnert Park/Cotati	14	14.9%
Pleasanton	15	16.0%
Modesto	14	17.6%
Tracy	14	21.9%
Santa Cruz	17	22.6%
San Jose/Milpitas/Sunnyvale	15	29.0%
Newark	17	29.4%
Davis/Woodland	14	32.8%
Oakland/Berkeley/Emeryville	17	38.3%
Palo Alto	15	41.1%
Walnut Creek	15	41.9%
Livermore	14	48.1%
San Rafael/San Anselmo	17	68.0%
Average		26.5%

Table 1. Disease incidence (percentage of Raywood ash exhibiting branch dieback) within a given city. There were 2640 trees included in survey. Climate zones were identified according to maps provided in the Sunset Western Gardens book (2001 edition).

Area	Number of trees	chi-square	*P* value
Davis/ Woodland	134	0.227	0.6338
Fairfield	419	0.857	0.3546
Walnut Creek	86	3.355	0.0670
Rohnert Park/ Cotati	375	4.425	0.0354
Newark	248	5.108	0.0238
San Rafael/ San Anselmo	100	8.137	0.0043
San Jose and suburbs	297	8.227	0.0041

Table 2. Analysis of independence of tree status (affected versus unaffected) and site condition (irrigated turf versus other). *P* values below 0.05 indicate a significant correlation between tree status and site condition within the given area.

City	Climate zone	Disease severity
Fairfield	15	0.26
Rohnert Park	14	0.45
Walnut Creek	15	0.5
Tracy	14	0.63
Modesto-1	14	0.68
Modesto-2	14	0.74
Berkeley	17	0.75
Livermore	14	0.85
San Jose	15	1.15
Palo Alto	15	1.17
Davis	14	1.2
Newark	17	1.2
Oakland	17	1.35
San Rafael	17	1.55

Table 3. Disease severity in observational plots in 2003. Values are means of 20 observations where the trees were rated based on the percent of the existing canopy that was dead (the scale is 0 = 0% dead, 1 = 1-10% dead, 2 = 10-50% dead, 3 = 50-80% dead, and 4 = >80% dead).

Irrigation status	Disease severity
Irrigated	0.75
Non-irrigated	1.19

Table 4. Mean disease severity of trees by irrigation status. Values are means of 188 and 92 observations for irrigated and non-irrigated trees, respectively.

Figure 1. Raywood ash with branch dieback scattered throughout the canopy.

Figure 2. Raywood ash with extensive branch dieback.

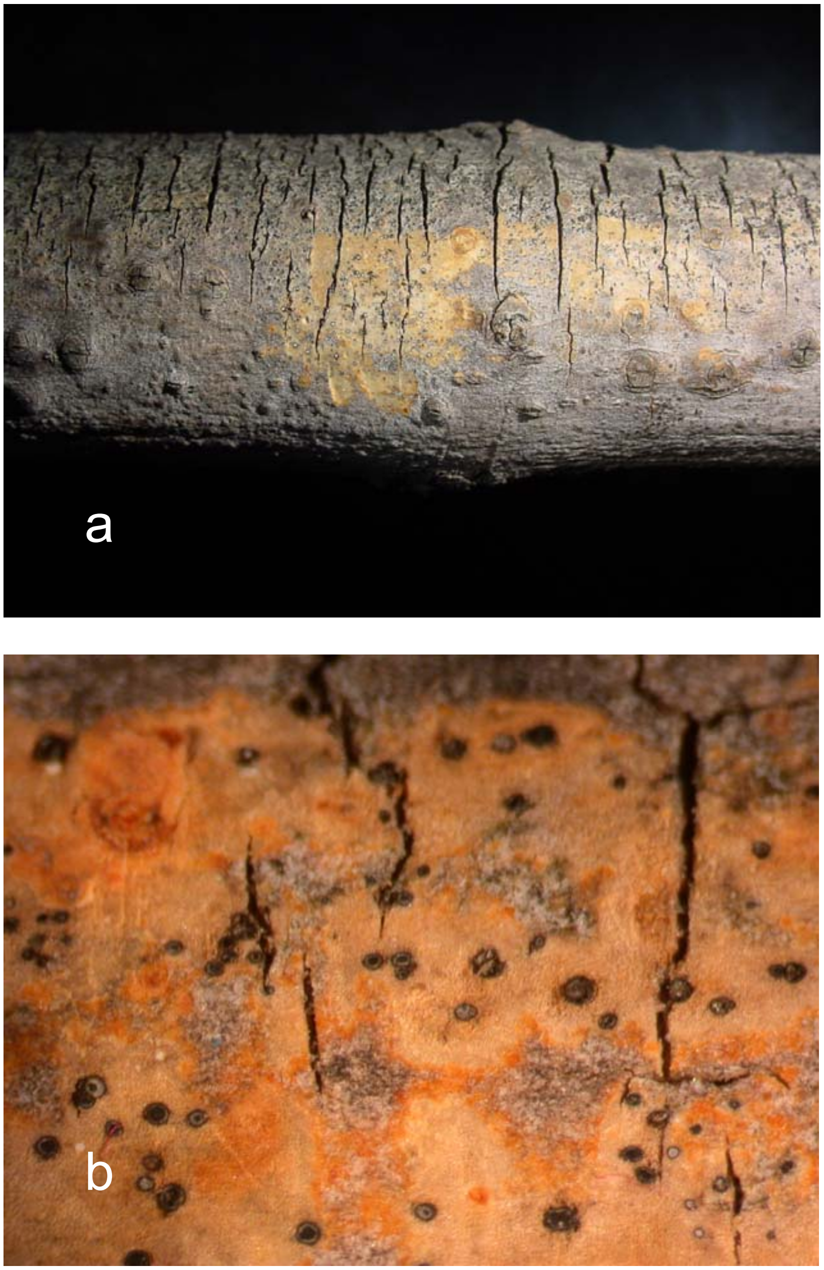

Figure 3. a) Raywood ash branch with bark removed to reveal dark fruiting structures (pycnidia) of *Botryosphaeria stevensii* and b) close-up of pycnidia in bark.

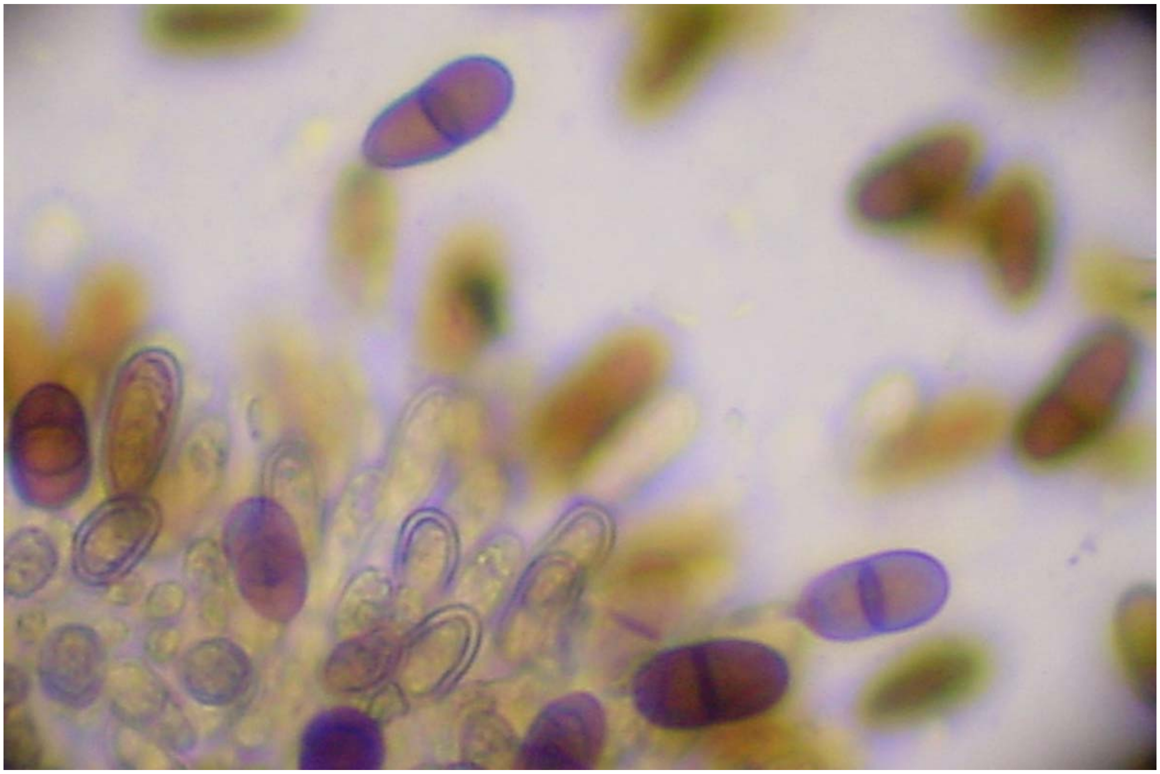

Figure 4. Spores (conidia) of *Botryosphaeria stevensii* viewed under the microscope.

Figure 5. Margin of affected and healthy cambium tissue. *Botryosphaeria stevensiil* can be isolated from this area.