Root Rot of *Brachychiton populneus* Seedlings Caused by *Lasiodiplodia theobromae*

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ABSTRACT

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Lasiodiplodia theobromae was the causal agent in the death of several hundred 18-mo-old Australian bottle trees (*Brachychiton populneus*) at a nursery in southern California. Disease became apparent after the plants, which had been shipped bare root from Israel, were repotted in California. The fungus infects the taproot, causing maceration and collapse of tissue and the eventual wilting and death of the plant. Inoculation of 1-cm-deep wounds with 10 μl of spore suspension (1×10^5 pycnidiospores per milliliter) consistently resulted in infection when wounds were wrapped in Parafilm after inoculation. Partial control of the disease was achieved by dipping bare roots in a benomyl suspension for 5 min before inoculation.

Several species of the plant genus *Brachychiton* are grown as flowering ornamental trees in semitropical areas of the United States. Native to Australia, the genus is a member of the plant family Sterculiaceae. *B. populneus* Schott & Endl., an evergreen member of the genus, is commonly known as the Australian bottle tree because of the distinctive shape of the heavy taproot and trunk. The specific epithet refers to the poplarlike shape of the leaves.

In the fall of 1990, several hundred 18-mo-old *B. populneus*, shipped bare root from Israel, wilted and eventually died after being replanted at a nursery in southern California. The taproots of the dead plants were blackened throughout and mummified. Many of the remaining seedlings had infected taproots, with long, softened depressions on the surface of the roots. Internally, infected tissue appeared macerated, soft, and discolored. Mucilage, present in mucilage ducts of healthy plants (5), was abundant in fresh lesions. Older lesions were dry, cottonlike, and green-gray to black. The firmer tissue surrounding the areas of macerated tissue was discolored. Mycelium was visible in the spaces created by the destruction of plant tissue (Fig. 1).

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. was shown to be the causal agent of the disease (10). A plurivorous, facultative wound pathogen of worldwide distribution, *L. theobromae* is also known as *Botryodiplodia theobromae* Pat. and, on citrus, as *Diplodia natalensis* Pole-Evans. The teleomorph, *Botryosphaeria rhodina* (Cooke) Arx, was not seen in these studies. The taxonomy of *L. theobromae* has been examined by Punithalingam (8,9) and Sutton (11). The isolates from *B. populneus* were identified as *L. theobromae* on the basis of the aggregated stromatic nature of the pycnidia, the absence of a true ostiole, the presence of paraphyses and simple conidiogenous cells, and the distinctive euseptate, striate phaeopycniospores.

Isolation of *Diplodia* sp. from stem cankers of *B. acerifolius* A. Cunn. ex F. v. Muell. has been reported in Florida (1). Because *Diplodia* sp. is a common synonym of *L. theobromae*, it is possible that the two diseases are caused by the same organism.

L. theobromae is generally considered to be a minor pathogen, usually infecting weakened or stressed hosts via wounds (9). Water stress is known to influence both severity and, under certain conditions, incidence of cankers caused by *L. theobromae* on sycamore (4) and dogwood (6). Because bare-root plants are potentially subjected to dehydration during storage and shipping, the effect of water stress on the disease was investigated.

These studies were designed to demonstrate the pathogenicity of *L. theobromae* on *B. populneus*, to test the ability of the fungicide benomyl to control the disease, and to determine if water stress predisposes the host to the disease.

MATERIALS AND METHODS

Seedling and bare root preparation. Seeds of *B. populneus* were cleaned with detergent, soaked in 0.525% sodium hypochlorite for 10 min, nicked with a razor blade, and soaked in deionized water for 3 days. The seeds were then planted in a mixture of moist vermiculite and perlite (1:1, v/v) in a loosely covered plastic tub. Seeds germinated within 1 wk at room temperature (22 C) and were transplanted into 3-L plastic pots filled with a mixture of UC soil mix and perlite (3:1, v/v). The UC mix contained peat and sand (1:1, v/v) plus 0.867 kg of treble superphosphate, 86 g of KNO_3, 86 g of K_2SO_4, and 1.3 kg of dolomite per cubic meter of mix. Once transplanted, the seedlings were watered daily with a dilute Hoagland's solution. The first trial was performed when the seedlings were 7–8 mo old and taproots were 4–6 cm in diameter.

Seedlings were bare-rooted by trimming the foliage, washing the potting mix from the roots, removing the relatively sparse side roots from the large, thick taproot, and cutting the taproot to a length of 10 cm. Such bare-root seedlings easily root when replanted, even after several days in storage.

Inoculum preparation. Cultures of a single pycnidiospore isolate of *L. theobromae* were grown on nonclarified V8 juice agar medium for 2 wk under constant fluorescent illumination. The medium was prepared by autoclaving a mixture of 200 ml of V8 juice, 2 g of $CaCO_3$, 15 g of agar, and 800 ml of deionized water. Initially cultures were

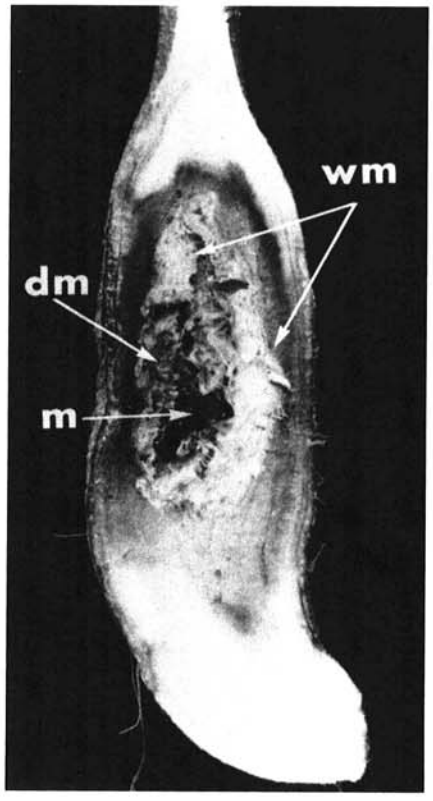

Fig. 1. Taproot (5 cm diameter) of a 7- to 8-mo-old *Brachychiton populneus* seedling 2 wk after inoculation on the left side with *Lasiodiplodia theobromae*. Dark mycelium (dm) is in the older portion of the lesion and white mycelium (wm), in the newer portion; m = mucilage.

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left unwrapped because wrapping with Parafilm inhibits pycnidium formation and sporulation. After 2 wk of growth under light, the cultures were wrapped with Parafilm and stored at 4 C in the dark.

To prepare the inoculum, pycnidia were crushed in distilled water and centrifuged at 8,000 rpm for 20 min to wet the hydrophobic mycelium and spore masses. The pellet was resuspended by shaking, and the resulting suspension was strained through a 45-μm screen to remove mycelium and hyphal fragments. The spores were then reconcentrated by centrifugation (8,000 rpm for 20 min) and used to prepare a suspension of 1×10^5 pycnidiospores per milliliter.

Treatments. Seedlings were subjected to one of three treatments: bare rooting only, bare rooting after 5 days without irrigation, and bare rooting followed by a 5-min dip in a suspension of benomyl (Tersan-1991 50WP, 0.59 g a.i./L).

Inoculations. Each bare-root seedling was wounded twice, once on each side of the taproot. The wounds were made by pushing a 100-μl plastic micropipette tip 1 cm into the taproot. One wound was left as a noninoculated control, and 10 μl of spore suspension was placed onto the other wound. Both wounds were wrapped with Parafilm, and the seedlings were stored in a loosely covered plastic tub in the laboratory at room temperature (22 C). Six days after inoculation, the taproot of each seedling was cut in cross section at both wound sites and examined for disease development.

The experiment was conducted three times, using 15 seedlings for each trial. Because each seedling was wounded twice (one inoculated wound and one noninoculated wound per seedling), a total of five wounds were evaluated for each of the six treatments per trial. Seedlings from the same planting were used for all three trials. Thus, the plants used in the third trial were 5 wk older than those used in the first trial.

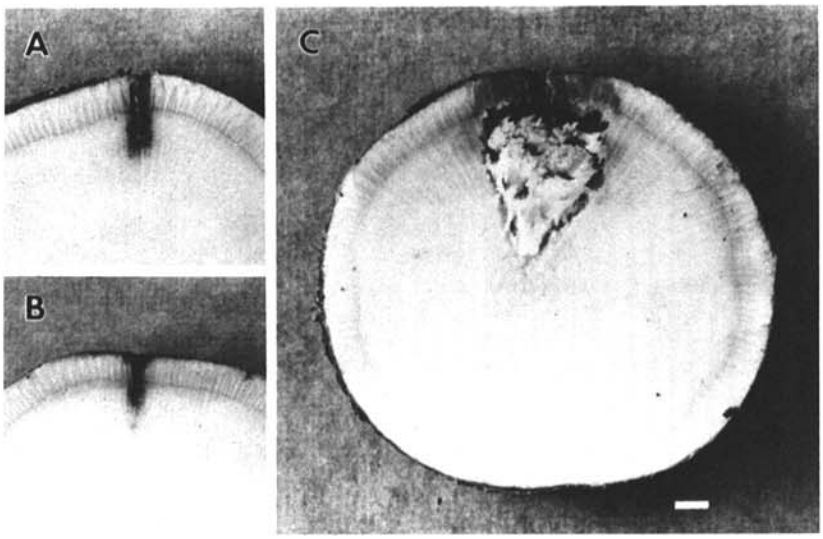

Fig. 2. Cross sections of taproots of *Brachychiton populneus* inoculated with *Lasiodiplodia theobromae:* **(A)** Arrested infection on taproot treated with benomyl before inoculation, **(B)** healed control wound, and **(C)** wedge-shaped lesion of active infection. Scale bar = 5 mm.

Table 1. Disease reactions of bare-root *Brachychiton populneus* seedlings inoculated with *Lasiodiplodia theobromae*

Treatment[a]	Disease severity ratings[b]		
	Healed wound	Active infection	Arrested infection
Bare root only			
Control wounds	5/5/5	0/0/0	0/0/0
Inoculated wounds	0/0/0	5/5/4	0/0/1
Water stressed			
Control wounds	5/5/5	0/0/0	0/0/0
Inoculated wounds	0/0/0	5/5/5	0/0/0
Benomyl			
Control wounds	5/5/5	0/0/0	0/0/0
Inoculated wounds	0/0/0	1/1/3	4/4/2

[a] Bare root only = foliage was removed from 7- to 8-mo-old seedlings, potting mix was washed from roots, and taproots were cut to a length of 10 cm. Water stressed = water was withheld from seedlings for 5 days before bare rooting. Benomyl = bare roots were dipped in suspension of benomyl (0.59 g a.i./L) for 5 min.
[b] Number of wounds from trial 1/trial 2/trial 3.

RESULTS AND DISCUSSION

All wound sites fell into one of three classifications: healed wound, active infection, and arrested infection (Fig. 2, Table 1). Healed wound sites showed no signs of infection; discoloration and cell death were limited to a few cell layers surrounding the wound. All noninoculated control wounds were healed. Active infections were typified by wedge-shaped areas of softened, macerated tissue surrounded by areas of firmer, discolored tissue; mycelium was visible in spaces created by the collapse of infected tissue. All inoculated wounds on water-stressed bare roots and all but one on nonwater-stressed bare roots had active infections. Arrested infections had more discoloration and cell death surrounding the wound site than did the noninfected healed wounds, but little infection of healthy tissue occurred. Softening and maceration were not associated with arrested infections, and visible mycelium was limited to areas delineated by dead, blackened cells. Ten of the 15 benomyl-treated wound sites had arrested infections.

Seedlings that were left unwatered for 5 days were mildly wilted and produced less mucilage when cut than did nonwater-stressed seedlings. Lesions on bare roots from these seedlings were consistently larger and more extensive than those on nonwater-stressed bare roots. However, all active infections on water-stressed and nonwater-stressed bare roots were severe enough to eventually cause the death of the plants. Thus, whereas water stress appears to influence the rate at which the disease progresses once a bare root is infected, it is doubtful that the outcome of the infection, i.e., the death of the plant, is altered by the water status of the plant at the time of bare rooting and inoculation.

In many ways this disease resembles soft rot of yam caused by *L. theobromae*. Both diseases can be viewed as postharvest problems involving infection of the taproot during storage; both diseases cause soft rots involving the maceration of tissue in the early stages of pathogenesis. Several plant cell wall-degradative enzymes have been associated with rot of yam caused by *L. theobromae* (1,3,7,12). In particular, endopolygalacturonase was shown to play an important role in plant cell death and maceration of yam tissue (2,3,7). Such cell wall-degrading enzymes might also play a role in the maceration of *B. populneus* roots. Interestingly, endopolygalacturonase is known to be inhibited by oxidized plant-phenolic compounds (3). This correlates well with the observation that, in preliminary studies not described here, inoculated wounds left open to the air tended not to become infected, whereas inoculated wounds wrapped in Parafilm did. Wounded tissue also became discolored when left open to the air, and

wrapped wounded tissue did not. The wrapping of wounds would have also changed the relative humidity of the wound site, a factor shown to be important in the pathogenicity of the fungus on yam, cassava, and sweetpotato (13).

Anatomically, the wood of *Brachychiton* sp. contains abundant parenchyma that forms distinct vasicentric sheaths and wide rays (5). Additionally, the taproots of *B. populneus* have a disproportionate amount of parenchyma, which may function in water storage. Such tissue is more vulnerable to fungal invasion and enzymatic digestion than woody root tissue, so that the anatomy of the taproot may be an important factor in the susceptibility of the host to the pathogen.

ACKNOWLEDGMENTS

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