

Botryodiplodia Trunk Lesions in Texas Citrus

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ABSTRACT

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The causal agent of a trunk disease of young citrus trees in South Texas was investigated. The disease was characterized by gray to tan lesions of the inner wood, copious gumming, and a consistent association with freeze-damaged tissue. Symptoms of the disease were reproduced with two isolates of *Botryodiplodia theobromae*, but 10 other isolates of the fungus were apparently avirulent. To determine the relative ability of the 12 isolates to decay citrus wood, sterilized blocks of citrus wood were inoculated, incubated for 16 wk, and weighed. One of the two virulent isolates reduced the weights of the blocks by 12.5%; all other isolates reduced their weights by about 4%.

Additional key words: *Diplodia natalensis*

A severe freeze in December 1983 destroyed more than 40,000 acres of citrus in the Lower Rio Grande Valley of Texas (LRGV) (5). To produce replacement trees as soon as possible, some nursery workers budded onto shoots of freeze-damaged sour orange seedlings that had resprouted from below ground level. When the dead tops of the rootstock seedlings were removed flush with the soil surface, a dead portion of the stem often remained below ground. As a result, the pruning wounds healed slowly and incompletely and dead stubs often persisted on the rootstocks for as long as 18 mo. In 1985, trunk lesions of the live wood adjacent to the dead sour orange stubs appeared in several nurseries. In a few cases, the lesions girdled and killed the young trees. This paper identifies the causal agent of this disease and provides proof of its pathogenicity.

MATERIALS AND METHODS

Wood samples from lesions on young trees in various stages of decline in three LRGV nurseries were collected in 1985 to isolate and identify possible agents of the decline. All of the diseased trees were budded in the spring or summer of 1984 on sour orange shoots that had resprouted from seedlings killed back to the soil line by the 1983 freeze. Isolations were made from six to eight trees in each nursery. Chips of wood cut from the margins of the trunk lesions were soaked in a 0.5% solution of sodium hypochlorite for 5 min, blotted dry on sterile filter paper, and aseptically transferred to petri dishes of potato-dextrose agar (PDA). Plates were incubated in the light (two

fluorescent Sylvania bulbs, 40W) at 22–25 C. After 8–10 days, single spores from all fungus cultures were transferred to culture tubes and incubated as before to provide pure cultures for identification and inoculation studies.

Inoculation trials. One-year-old grapefruit scions (*Citrus paradisi* Macf. cv. Ruby Red) budded on sour orange (*C. aurantium* L.) rootstock in an orchard at the Citrus Center were inoculated with 12 isolates of *Botryodiplodia theobromae* Pat. (*Diplodia natalensis* Pole Evans) isolated from wood chips from the three nurseries under study. Inoculum consisted of 5-mm-diameter plugs cut from 10-day-old cultures of *B. theobromae* on PDA. The inoculum for all experiments was isolated from trunk lesions no more than 12 wk before the inoculation trials were initiated. One plug from a culture of each isolate was placed in a wound made with a sterilized cork borer drilled past the bark of the trunks of 10 trees. The wounds were made about 5 cm above the bud unions of the trees. Controls received sterile PDA. The sites of the inoculations were wrapped with plastic tape to prevent desiccation. Thirty days later, the inoculations were examined for symptoms. Pieces of wood from the margins of lesions were plated on PDA to isolate any suspected pathogenic fungi.

Twelve-month-old sour orange, pineapple sweet orange (*C. sinensis* (L.) Osbeck), grapefruit, or Troyer citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) seedlings growing in 15-cm-diameter pots in the greenhouse were also inoculated with the 12 isolates of *B. theobromae*. The seedlings were inoculated by the procedure described before. Treatments were replicated six times. The diameters of the lesions were measured 3 wk after inoculation.

Wood decay. The ability of the various isolates of *B. theobromae* to decay citrus wood was determined. Blocks of grapefruit heartwood (3.0 × 3.0 × 0.7 cm)

were cut from the trunks of healthy 20-year-old trees, dried in a force-draft oven at 40 C for 2 wk, weighed, and sterilized in 110 C steam for 15 min. The blocks were then placed on V-shaped glass rods placed in petri dishes of PDA so that the wood was not touching the agar. An agar plug 5 mm in diameter of each isolate of *B. theobromae* was placed in each dish. Two common saprophytes of citrus wood, *Phomopsis* sp. and *Colletotrichum* sp., which were isolated from dead grapefruit wood, were also included in this study. Each treatment was replicated six times. After the plates were incubated for 16 wk at 27 C, the blocks were removed and gently brushed with a 2% solution of benzalkonium chloride to remove mycelia on the wood surfaces. The blocks were then dried again at 40 C for 2 wk and reweighed. The loss of weight from the blocks was adjusted according to the weight loss of the uninoculated blocks.

RESULTS

About 55% of the 400 wood chips collected from the margins of the lesions yielded *B. theobromae*. Species of *Colletotrichum*, *Phomopsis*, *Nigrospora*, and *Fusarium* were isolated from less than 7% of the chips. Characteristics of cultures of *B. theobromae* on PDA varied among the isolates. Cultures ranged from light gray to black, aerial mycelium was usually abundant but almost nonexistent in two isolates, and the spore-forming ability of the isolates varied. There was no apparent relationship between culture characteristics, including growth rates, of the isolates and their virulence.

Inoculation trials. Infections with symptoms typical of those seen in the commercial nurseries (tan to gray wood discoloration and copious gumming)

Table 1. Development of trunk lesions in 1-year-old field trees inoculated with various isolates of *Botryodiplodia theobromae*^y

| Inoculum | No. of lesions in 10 inoculations | Av. lesion diameter ^z (mm) |
|-------------------------------|-----------------------------------|---------------------------------------|
| Sterile agar | 0 | 0.0 a |
| Isolate | | |
| R-02 | 9 | 38.5 c |
| G-01 | 7 | 36.3 c |
| B-05 | 2 | 2.8 b |
| B-03 | 1 | 1.9 ab |
| All other isolates (12 total) | 0 | 0.0 a |

^y Inoculum was placed in wounds made on the grapefruit scion about 6 cm above the sour orange rootstock.

^z Mean separation by Duncan's multiple range test ($P = 0.01$).

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Table 2. Development of stem lesions in 10-mo-old citrus seedlings inoculated with various isolates of *Botryodiplodia theobromae*

| Inoculum | Lesion diameter (mm) ^z | | | |
|----------------------------------|-----------------------------------|--------------|------------|-----------------|
| | Sour orange | Sweet orange | Grapefruit | Troyer citrange |
| Sterile agar | 0 a | 0 a | 0 a | 0 a |
| Isolate | | | | |
| R-02 | 54 c | 37 d | 53 b | 11 c |
| G-01 | 48 c | 17 c | 62 b | 4 b |
| B-05 | 7 b | 5 b | 0 a | 0 a |
| B-03 | 0 a | 6 b | 2 a | 0 a |
| R-01 | 2 a | 0 a | 4 a | 0 a |
| All other isolates (12 total) | 0 a | 0 a | 0 a | 0 a |

^zMean separation within columns by Duncan's multiple range test ($P = 0.01$).

were reproduced in the field trees inoculated with 2 of the 12 isolates of *B. theobromae* (Table 1). Most of the isolates failed to cause trunk lesions or were only slightly active. *B. theobromae* was reisolated from 17 of the 19 lesions on the field trees.

The same two isolates that were pathogenic on the field trees caused significant lesion development on various seedlings in the greenhouse (Table 2). Symptoms were similar to those seen in the field, but the lesions spread faster on the greenhouse plants than on field trees. Small lesions developed on the greenhouse seedlings with a few isolates of *B. theobromae*, but seven of the isolates were apparently avirulent. Troyer citrange seedlings were relatively resistant to infection. One grapefruit and two sour orange seedlings were girdled and killed as a result of the inoculations.

Wood decay. One of the two virulent isolates of *B. theobromae* (isolate G-01) reduced the weights of the blocks by 12.5%; all other isolates reduced the weights of the blocks by an average of about 4%. The two other fungi, *Phomopsis* sp. and *Colletotrichum* sp., also reduced the weights of the blocks by about 4%.

DISCUSSION

B. theobromae is a common facultative

parasite of citrus tissues. Its pathogenicity on stored citrus fruit is well known (1), and it has been implicated in several wood diseases of citrus. For example, *B. theobromae* is apparently involved in a twig disease of Robinson tangerine (4), a trunk disorder of Tahiti lime (3,7), and Rio Grande gummosis (2). Having satisfied Koch's postulates in our experiments, we have associated *B. theobromae* with another disorder of citrus wood.

The lesions on the young trees growing in nurseries in South Texas were always associated with dead wood on the rootstock. The fungus may need to grow saprophytically on the dead wood to increase its inoculum potential before it can cause significant damage to the healthy tissue. The area around the dead wood, which did not heal properly because of the nature of the wound, was the apparent entry into the host. There is also an association between dead wood and disease incidence in Rio Grande gummosis (2). Fawcett (3) suggested that *B. theobromae* can invade wood tissue much more rapidly than it can invade bark tissue, which prevents colonization of the wood. This theory is consistent with our observations that the incidence of disease in the nurseries was limited to trees with exposed wounds left from freeze-damaged tissue.

The variable culture characteristics of

the different isolates of *B. theobromae*, which have been studied elsewhere in detail (8), are apparently very common in this fungus. Virulence among the different isolates was also extremely variable. Most of our 12 isolates of *B. theobromae* showed a low degree of virulence or were possibly avirulent. Of the two isolates that were virulent on citrus seedlings and young trees, one did not have the ability to decay sound citrus wood to any greater extent than the isolates of *B. theobromae* with low virulence or the two common saprophytes. Only one isolate had the ability to utilize substances in the citrus wood not available to the other fungi.

The reason for the variability among the isolates of *B. theobromae* is not known. Recently, Mathews and Dodds (6) reported a negative relationship between virulence and the occurrence of double-stranded RNA in many isolates of *B. theobromae* from grape lesions. A similar mechanism may explain the variability among isolates of *B. theobromae* from citrus wood.

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