INFLUENCE OF INFECTION COURT, HOST VIGOR, AND CULTURE FILTRATES ON CANKER PRODUCTION BY BOTRYODIPLODIA THEOBROMAE CONIDIA IN SYCAMORE

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ABSTRACT

Some of the factors that influence canker development in American sycamores inoculated with Botryodiplodia theobromae conidia were determined. A combination of B. theobromae culture filtrates and conidia resulted in 100% canker production when introduced into stem wounds; however, a combination of Cephalosporium diospyri culture filtrates and B. theobromae conidia resulted in no canker production in healthy sycamores. Inoculations with conidia without fungous filtrates resulted in canker development at wounds 0.5 cm below pruned twig terminals but not at wounds on branches with intact terminals. Conidial inocula also induced canker development in Cephalosporium-wilted and water-stressed sycamores. Cankers resulting from conidial inoculations are favored by stress in American sycamores.

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Botryodiplodia theobromae Pat. causes cankers and dieback in sycamores (Platanus occidentalis) growing in plantations (9,10) and natural stands (2, 3, 12). Many sycamores in east Texas have been killed by a combination of a wilt caused by Cephalosporium diospyri Crandall, and B. theobromae infections (4, 5).

Botryodiplodia theobromae has a wide host range and geographic distribution (11, 13). Its ascogenous stage, Physalospora rhodina (Berk. & Curt.) Cke., has been reported on Citrus spp. and other species (13) but not on sycamore. It is therefore believed that conidia are the major, and perhaps only, spore type for spread in sycamore.

Preliminary investigations indicated that B. theobromae conidial inocula could not easily induce canker development in vigorous American sycamores. The objective of this study was to determine some of the required conditions for canker and dieback induction resulting from conidial inoculation of this species. The influence of infection court, culture filtrates, water-stress, and Cephalosporium wilt on canker production by conidia was investigated.

MATERIALS AND METHODS

Live sycamore twigs (1-2-cm diam) were cut into 4-6-cm lengths, placed in 125-ml Erlenmeyer flasks, autoclaved for 20 min at 1.05 kg/cm^2, and seeded with mycelium from a single-spore culture of a hypervirulent strain of B. theobromae. Mature conidia were produced after 2-3 weeks and were collected by rinsing with sterile distilled water 1-2 hr before inoculation. The hypervirulent strain of B. theobromae used in this study was capable of producing cankers in vigorous sycamores within 4 days after inoculation with mycelium in agar.

Fresh and old wounds on 2-year-old sycamores were compared as infection courts for conidia during the summer of 1975. Stems were surface-sterilized with 70% ethanol and 1-2-cm incisions were made through the outer xylem with a razor blade. Three incisions (approximately 20 cm apart) were made on each stem. Water-suspended conidia (200-300 per wound) were placed onto fresh and 4-week-old wounds. Controls received sterile distilled water only. Each inoculated wound was covered with sterile water-saturated cotton balls and sealed with clear plastic and masking tape. Initially, 15 inoculations were made for each wound type; two additional inoculation experiments were performed on different trees at 1-week intervals to give 45 inoculations for each type of wound.

Cell-free culture filtrates of B. theobromae and C. diospyri were tested as stimuli for canker production by conidia in sycamore. Filtrates were produced by growing B. theobromae and C. diospyri separately in 0.8% sycamore wood-chip broth for 15 days on a gyratory shaker (100 rpm) at 28-30° C. Then broth was separated from mycelium by centrifugation and filtration through 0.5-μm Millipore filters. Two-year-old pot-planted sycamores were inoculated by

making 1-2-cm incisions through the bark and placing 200-300 conidia onto each wound. Botryodiplodia theobromae filtrates or C. diospyri filtrates (in cotton balls saturated with broth) were applied over 12 inoculated wounds for 5 days.

The ability of conidial inoculations to result in canker development was evaluated on different types of artificial infection courts on 12-yr-old disease-free plantation sycamores in August 1975. Infection courts were: 1) stem wounds made by tangential cuts through outer xylem 0.5 cm below freshly pruned twig terminals, 2) wounds made by cutting through outer xylem of current-year twig growth, 3) wounds made through xylem of second-year twig growth, 4) wounds made by excising leaf buds, and 5) unwounded leaf buds. Twenty infection courts of each type were inoculated with conidia and 20 of each with mycelium in agar.

Canker production resulting from conidial inoculations in water-stressed sycamores and a wilting sycamore was investigated also. Pot-planted sycamores were water-stressed by withholding water from them (4-5 days) until incipient wilt symptoms were observed, and then inoculated with conidia. The inoculated sycamores were maintained in a greenhouse at 28-30° C. A branch on a 15-year-old sycamore that had exhibited recurrent Cephalosporium wilt symptoms, but no cankers or dieback, was inoculated in June 1975 by excising its terminal and placing conidia on the wounded surface. Canker and dieback development in the artificially inoculated twig in addition to uninoculated twigs and branches on the same tree were observed closely at 1-week intervals for 3 months.

RESULTS

Cankers were not produced at any of the 90 fresh and 4-week-old wounds on healthy sycamores inoculated with B. theobromae conidia. The fungus was isolated from 93% of the inoculated fresh wounds and 44% of the inoculated old wounds, however, after 6 months. The fungus was also isolated from 74% of the stem sections 0.5 cm or more above scars left by inoculating fresh wounds and 37% of the stem sections above scars left by inoculation of old wounds. Inoculated sycamores were colonized by the fungus but no cankers developed.

Cell-free B. theobromae cultural filtrates applied over 12 wounds inoculated with conidia resulted in 100% canker production after 5 days, but cell-free C. diospyri filtrates applied over 12 inoculated wounds resulted in no canker production. Cankers increased in size even after the B. theobromae filtrates were removed and pycnidia were produced on the cankers after 3 weeks. Necrosis of succulent leaves on sycamore cuttings placed in cell-free B. theobromae filtrates indicated that the fungus probably produced phytotoxin(s). Necrosis did not occur in leaves on cuttings placed in C. diospyri filtrates or plain broth.

Plantation sycamores did not develop cankers where wounded or unwounded leaf buds were inoculated. Botryodiplodia theobromae mycelium in agar induced canker development in wounds on current-year growth, second-year growth, and twigs with pruned terminals, but inoculations by conidia resulted in cankers only on twigs with pruned terminals (Table 1). B. theobromae was isolated from canker-free twigs and wounded leaf buds, however, that were inoculated with conidia. Cankers resulting from inoculations with conidia and mycelium on twigs with pruned

Table 1. Canker production by conidia and mycelium of a hypervirulent strain of Botryodiplodia theobromae in different types of artificial infection courts on sycamore twigs.

Types of infection courts	Type of inoculum[a]	Percent cankered[b]	Average canker length (cm)
Wounds[c] 0.5 cm below pruned twig terminal	Mycelium	100	26.3
	Conidia	100	24.5
Wounds on current-year growth	Mycelium	100	4.3
	Conidia	0	--
Wounds on second-year growth	Mycelium	100	3.8
	Conidia	0	--
Wounds on leaf buds	Mycelium	0	--
	Conidia	0	--
Unwounded leaf buds	Mycelium	0	--
	Conidia	0	--

[a]Mycelial inoculum included agar from a 2-week-old culture of the fungus and conidial inoculum included 200 water-suspended spores per inoculation.
[b]Twenty inoculations were made for each test.
[c]Wounds were made by cutting into outer xylem.

terminals were similar in size and much larger than those produced on twigs with intact terminals (Table 1). A few twigs with pruned terminals were killed by the fungus, but most developed cankers of about the same width (average 1 cm) as the inoculation wounds. When cankers extended down the inoculated twig to lateral shoots, they did not penetrate the shoots but terminated at the shoot intersections.

Cankers resulted from inoculations by conidia in all 20 inoculation sites on water-stressed sycamores after 5 days; some of the cankers were 10 cm long but the average length was 4.7 cm. None of the sycamores was killed by the fungus even though extensive dieback occurred. B. theobromae sporulated on the cankers after 3 weeks.

The inoculated branch on a sycamore tree with Cephalosporium wilt was killed back to the main stem and was covered with B. theobromae conidia after 3 weeks. Cankers did not extend from the dead inoculated branch into the main stem of the tree, but conidia produced on the dead branch apparently served as inoculum for spread of the fungus to other parts of the tree. New cankers and dieback were initiated each week in small twigs and branches; cankers advanced downward through larger branches toward the main stem. Branches defoliated by wilt were killed by B. theobromae before those that were not defoliated. The main stem apex developed dieback during July 1975 and was killed to the root collar after 6 weeks. Botryodiplodia theobromae could be isolated from any part of the tree affected by cankers and dieback but could not be isolated from roots which resprouted during the spring of 1976.

DISCUSSION

Fresh and old wounds inoculated with conidia of B. theobromae did not develop cankers even though colonization of stems was frequent. The same response to inoculation has been observed in sycamores inoculated with a hypovirulent strain of the fungus (4, 6, 9). Fresh wounds are better infection courts than old wounds. Callus formation and colonization of exposed wounds by other fungi before they were exposed to B. theobromae were partially responsible for less frequent colonization through old wounds than through fresh wounds.

Mycelial inoculum has been used repeatedly in studies involving the pathogenicity of B. theobromae in sycamores (2, 3, 4, 5, 6, 9). One study, however, showed that mycelial inoculum induced dieback in cashew but conidial inoculum did not (8). The most important difference between conidial and mycelial inocula appears to be the presence of metabolites, including phytotoxin(s), produced in culture media. Inoculum consisting of B. theobromae conidia along with culture filtrates of the fungus were as effective as mycelium with agar in inducing cankers in healthy sycamores. Because B. theobromae conidia are often released in columnar conidial clumps on sycamore cankers and are disseminated by wind (7) and insects (3), I believe that clumps of conidia are responsible for inducing many of the naturally occurring cankers. A germinating clump of conidia might produce enough metabolites to influence canker production; however, factors other than excessive metabolite production by germinating clumps of conidia appear to be more important in influencing canker production.

Canker production by B. theobromae in sycamore is greatly stimulated by stress (4, 5, 12). Stress was involved in each case where conidia alone induced cankers in this study. Because conidia are responsible for fungus spread, the requirements for successful conidial inoculations help explain why Botryodiplodia cankers and dieback were observed almost exclusively on wilting sycamores in College Station, Texas (5). Sycamores, stressed by wilt and water deficits, are highly vulnerable to infection and subsequent canker development from conidial inoculations during summer months. Individual twigs can be stressed by pruning their terminals, and cankers from conidial inoculations can also develop. If tree vigor is maintained and branch stubs are not made to allow infection and subsequent inoculum build-up, Botryodiplodia cankers should be minimized. Because mature conidia can remain viable on dead bark from one season to the next (1), dead limbs should be removed to minimize the source of inoculum for new infections during times of stress.

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