Fungi That Cause Cane Cankers on Thornless Blackberry in Ohio

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ABSTRACT

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Fusarium spp., *Alternaria* spp., *Epicoccum purpurescens, Cytospora* sp., *Pestalotiopsis* sp., *Leptosphaeria coniothyrium, Gnomonia rubi,* and *Botryosphaeria obtusa* were isolated from cane cankers on thornless blackberry in Ohio. Pathogenicity tests demonstrated that only *L. coniothyrium, G. rubi,* and *B. obtusa* were pathogenic. Each produced characteristic cankers on thornless blackberry and was recovered from diseased tissue after inoculation.

Several cultivars of thornless blackberry (*Rubus* spp., subgenus *Eubatus* hybrids) have been introduced in Ohio for evaluation. The cultivars Hull Thornless (Hull), Dirksen Thornless (Dirksen), and Thornfree have been planted commercially on a limited scale. The potential of these cultivars in terms of yield and fruit quality is great (3). In 1976, however, stem cankers appeared on most of the plants in an experimental planting at the southern branch of the Ohio Agricultural Research and Development Center (OARDC) at Ripley. Cankers were observed primarily on second-year canes (fruiting canes). In most cases, the portion of the cane above the canker either died or was extremely weak and did not produce marketable fruit. In 1977, a 1-acre commercial planting of Hull near Ripley had nearly 100% of the canes affected with these cankers and the grower destroyed the planting. Since then, several small plantings in southern Ohio have been removed because of this disorder. In 1981, research was initiated to determine the cause of these cankers.

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MATERIALS AND METHODS

Isolation. In May 1981, second-year canes (cultivars Hull and Thornfree) with cankers were collected at Ripley, OH. All canes were living and new growth was about 2 cm long. Cane sections (about 3 mm^2) that contained periderm and xylem were cut from the centers and margins of several cankers on each cultivar. Stem sections were surface-disinfested by soaking in a 5.25% sodium hypochlorite solution for 1 min, then rinsed in sterile distilled water. Cane sections were then placed on potato-dextrose agar (PDA), acidified to pH 4.5 with 85% lactic acid (APDA), and incubated under constant light at 24 C. As fungi emerged, hyphal tips were transferred to and purified on APDA in petri dishes, then stored on

APDA slants at 4 C. Isolates were transferred every 2 mo to maintain viability.

Identifications of pathogenic fungi were based on fructifications formed on diseased canes and in culture. For scanning electron microscopy, specimens of air-dried canes with fructifications were fixed to stubs, sputter-coated with about 150 Å of platinum using a Palaron E5100 coater, then viewed and photographed with an ISI 40 scanning electron microscope.

Pathogenicity tests. Three thornless blackberry cultivars (Dirksen, Hull, and Thornfree) were used in pathogenicity tests. Thornfree plants were inoculated in greenhouse tests, and plants of all cultivars were inoculated in the field. Plants in the greenhouse were grown in 4-L plastic pots that contained a mixture of soil (Wooster silt loam), peat, and perlite (2:2:1, v/v/v). Pots were watered daily as needed and weekly with 500 ml of 200 μg/ml of fertilizer (20-20-20, NPK). Pots were maintained under ambient conditions (20–30 C) throughout the experiment. Greenhouse inoculations were made on 15 December 1981 and 15 January 1982. Three 8-wk-old canes were inoculated with each fungal isolate. Field-grown plants were inoculated at Ripley. Three second-year canes and three first-year canes per cultivar were inoculated with each fungus on 15 April and 15 July 1982, respectively. Field inoculations were repeated on 18 April 1983.

Inoculum disks (3 mm in diameter) were cut from cultures grown on PDA for 15 days at 24 C. Three wounds (3 mm in diameter) were made 10 cm apart on each cane with a cordless electric drill. Wounds extended into the pith. One inoculum disk was inserted into each wound with a dissecting needle. Sterile agar disks were inserted into control plants. All wounds were covered with petroleum jelly to prevent desiccation. After 8 wk, canes were harvested. Canker length (length of discolored tissue beyond inoculation point) was rated as: 0 = none, 1 = discoloration less than 1 cm, 2 = discoloration from 1 to 3 cm, or 3 = discoloration longer than 3 cm. For greenhouse canes, a 2-cm section of cane extending down from the inoculation wound was cut into six approximately equal disks. Disks were surface-disinfested as described previously and plated serially on APDA. Six wedge-shaped sections were cut from field-grown canes at equal distances up to 2 cm below the inoculation wound and treated as described previously. Plates were incubated at 24 C for 7–10 days and recovery of fungi was recorded.

RESULTS AND DISCUSSION

The following fungi were isolated from naturally occurring cankers: *Alternaria* spp., *Botryosphaeria obtusa* (Schw.) Shoem., *Cytospora* sp., *Epicoccum purpurescens* Ehrenb. ex Schlecht., *Fusarium* spp., *Gnomonia rubi* (Rehm) Wint., *Leptosphaeria coniothyrium* (Fuckel) Sacc., and *Pestalotiopsis* sp. Only *B. obtusa, G. rubi,* and *L. coniothyrium* (anamorph *Coniothyrium fuckelii* Sacc.) were pathogenic. These three fungi produced characteristic cankers on inoculated canes in both field and greenhouse tests and all were consistently reisolated from diseased tissues. Length of cankers caused by each fungus varied among cultivars and time of inoculation; however, canker lengths produced by each fungus were uniform for a given cultivar and time of inoculation (Table 1). Similar results were obtained in 1983 tests. In all cankers with a rating of 1 (discoloration up to 1 cm), the fungus was isolated from apparently healthy tissue up to 2 cm beyond the margin of the canker.

All three fungi have been associated previously with *Rubus* spp. (9); however, only *L. coniothyrium* has been reported to cause cane blight on blackberry (1,2). Although *G. rubi* has commonly been associated with cankers on blackberries, its pathogenicity has not been demonstrated. *B. obtusa* has been reported on a variety of woody hosts under numerous synonyms. An association with *Rubus* has been cited by Shear et al (5) (as *Physalospora malorum* Peck) and by Stevens (7) (as *P. obtusa* (Schw.) Cooke). Stevens considered the fungus a sapro-

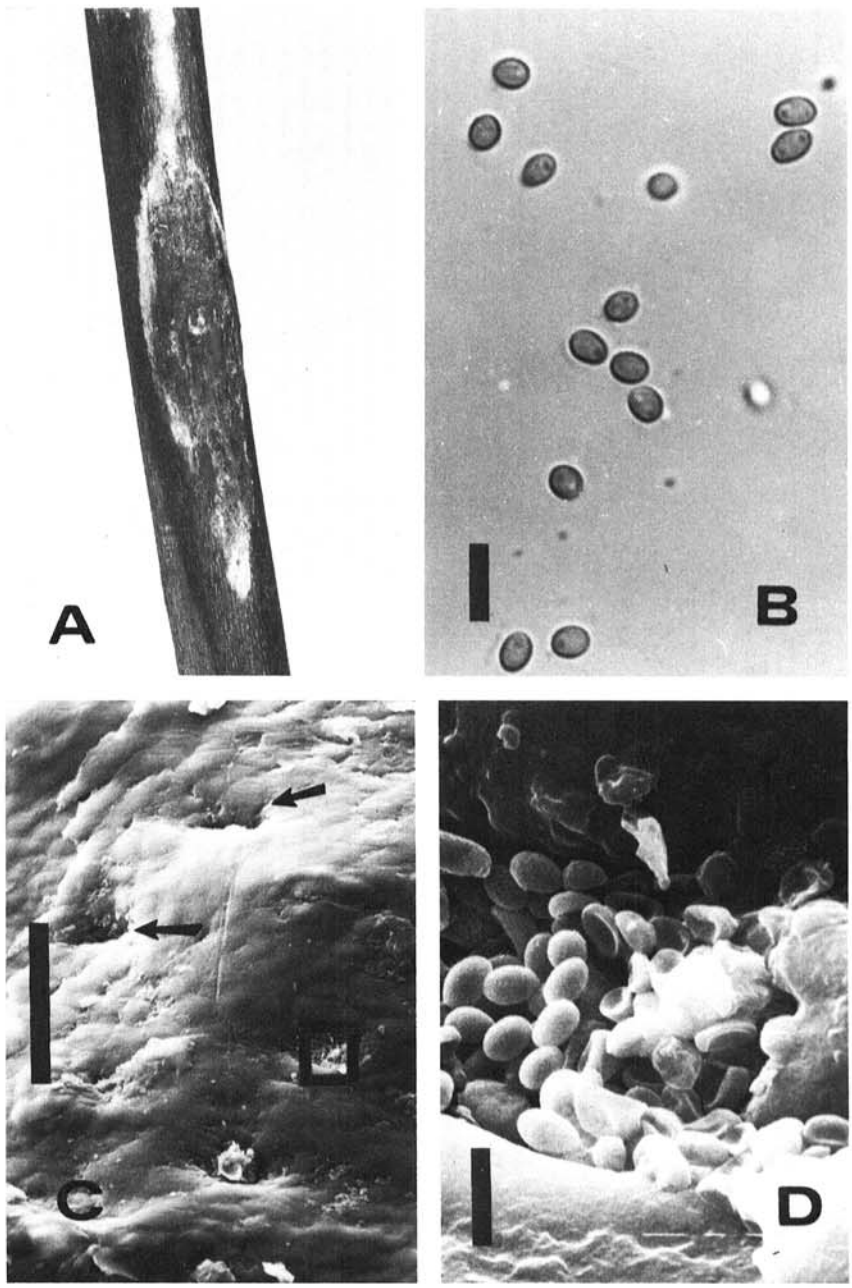

Fig. 1. Canker on thornless blackberry (cultivar Hull Thornless) caused by *Leptosphaeria coniothyrium.* **(A)** Typical symptoms on second-year cane. **(B)** Conidia of *L. coniothyrium* observed with light microscope. Bar = 10 μm. **(C)** Scanning electron micrograph of canker with ostioles of *L. coniothyrium* (arrows). Bar = 100 μm. **(D)** Enlarged inset from C showing conidia. Bar = 10 μm.

phyte invading plants damaged or killed by wind or other pathogens (7).

Under natural conditions in the field, all three fungi were commonly found on the same cane, and cankers produced by each fungus were difficult to distinguish. Typical cankers caused by *L. coniothyrium* were initially dark red to reddish purple with dark purple irregular borders. Eventually, the cankers turned gray (Fig. 1A). Those produced by *G. rubi* were whitish gray but did not typically have distinct borders (Fig. 2A). Cankers produced by *B. obtusa* had dark brown centers with regions of lighter brown or gray scattered throughout. These cankers also had very dark brown to purple irregular borders (Fig. 3A).

Table 1. Canker length ratings on three thornless blackberry cultivars inoculated in the greenhouse or field with *Leptosphaeria coniothyrium, Gnomonia rubi,* and *Botryosphaeria obtusa*

	Greenhouse (15 Dec. 1981)	Field (15 Apr. 1982, first-year canes)			Field (15 Jul. 1982, second-year canes)		
Fungus	TF[y]	TF	D	H	TF	D	H
Leptosphaeria	2[z]	3	2	2	3	2	3
Gnomonia	1	1	1	1	3	2	2
Botryosphaeria	2	1	1	2	3	1	2

[y] Cultivars inoculated: TF = Thornfree, D = Dirksen Thornless, and H = Hull Thornless.
[z] Canker rating system: 0 = no discoloration, 1 = discoloration less than 1 cm, 2 = discoloration from 1 to 3 cm, and 3 = discoloration more than 3 cm beyond point of inoculation. Rating values represent the mean of three inoculations on each of three canes per cultivar and date of inoculation.

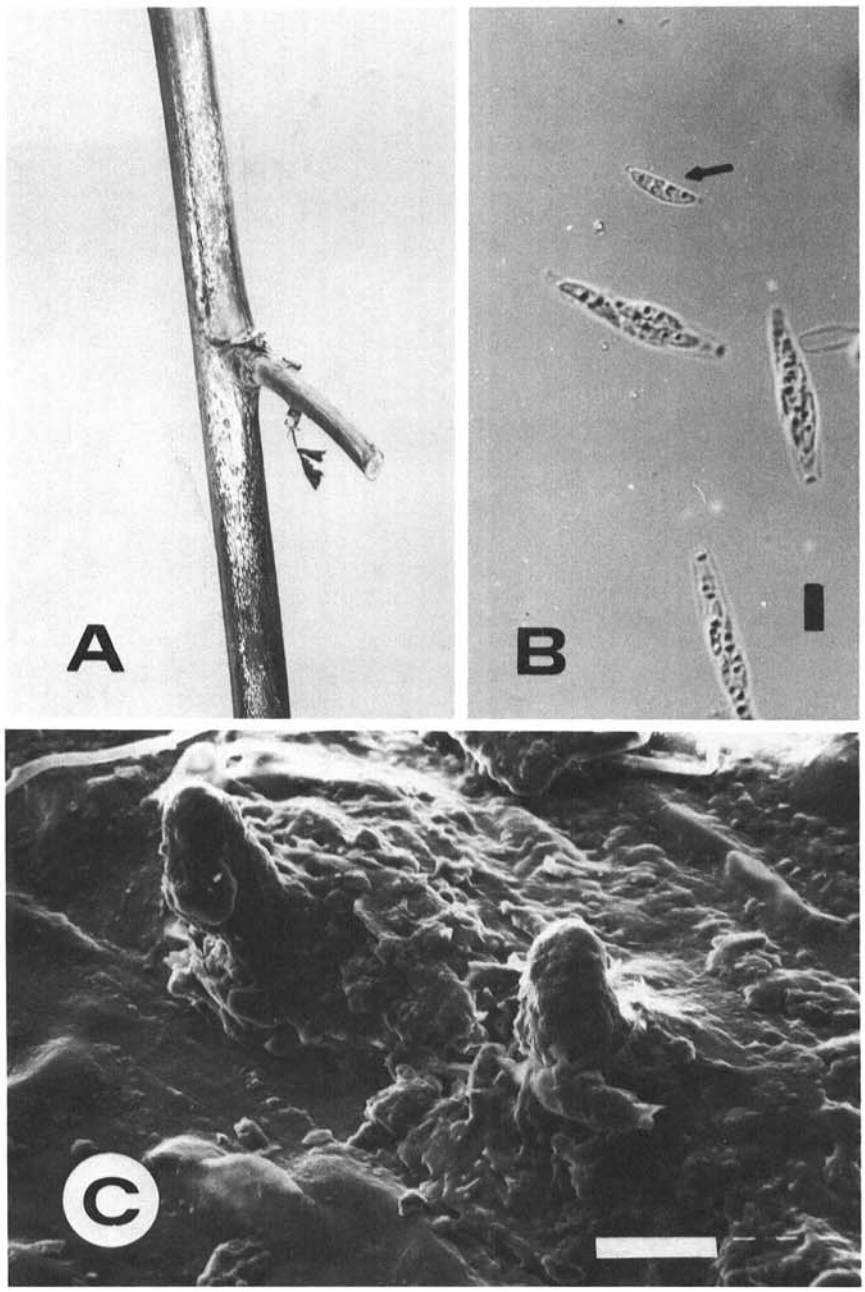

Fig. 2. Canker on thornless blackberry (cultivar Hull Thornless) caused by *Gnomonia rubi.* **(A)** Typical symptoms on second-year cane. **(B)** Asci and ascospore (arrow) of *G. rubi.* Bar = 10 μm. **(C)** Scanning electron micrograph of ascomata on cane. Bar = 10 μm.

Ascomata (perithecia) of *G. rubi* and conidiomata (pycnidia) of *B. obtusa* and *L. coniothyrium* appeared in the centers of the cankers. Superficially, these fructifications were similar in appearance: subepidermal, globose to flask-shaped, with a single locule. Nevertheless, the three fungi could be readily distinguished by careful observation (×10) of fruiting structures or by microscopic examination of spores. Fructificatons and spores produced by each fungus on the canes (Figs. 1–3) were nearly identical to those produced in pure culture. *G. rubi* produced solitary ascomata that had distinct beaks at maturity (Fig. 2C). Asci containing four two-celled ascospores were readily released from the mature ascomata in squash preparations (Fig. 2B). Pycnidial conidiomata of *B. obtusa* were either solitary or in linear clusters (Fig. 3C). They were erumpent but lacked the distinct beaks characteristic of *G. rubi.* Large (about 24 × 10 μm) dark conidia appeared rough-textured when observed by the light miscroscope (Fig. 3B) (6) but were smooth when examined with the scanning electron microscope (Fig. 3D). Occasionally, two-celled conidia were observed. The conidiogenous cells of this fungus are holoblastic with percurrent annelidic proliferations characteristic of the anamorph genus *Sphaeropsis* Sacc. and different from those of the *Diplodia* Fr. and *Fusicoccum* Corda anamorphs of other common *Botryosphaeria* spp. (8). This fungus is distinct from *B. dothidea* (Moug.:Fr.) Ces. & de Not., which is causing a similar type of problem on thornless blackberry in Maryland (J. L. Maas, *personal communication*). Although pycnidial conidiomata of *L. coniothyrium* were easily observed in cankers, in contrast to the other fungi, they were entirely subepidermal, globose to slightly flattened, with very short, nonprotruding papilla (Fig. 1C). Conidia (Fig. 1B,D) were smooth and considerably smaller (about 4 × 2.5 μm) than those of *B. obtusa.*

Second-year fruiting canes appeared to be more susceptible than first-year primal canes (Table 1). In most field plantings where disease was severe, second-year canes were heavily infected and nonproductive, whereas first-year canes often appeared healthy. These observations indicate the possibility of predisposition to infection resulting from winter injury (4) or that first-year canes may be inherently resistant and become more susceptible as they change from the vegetative to the reproductive state.

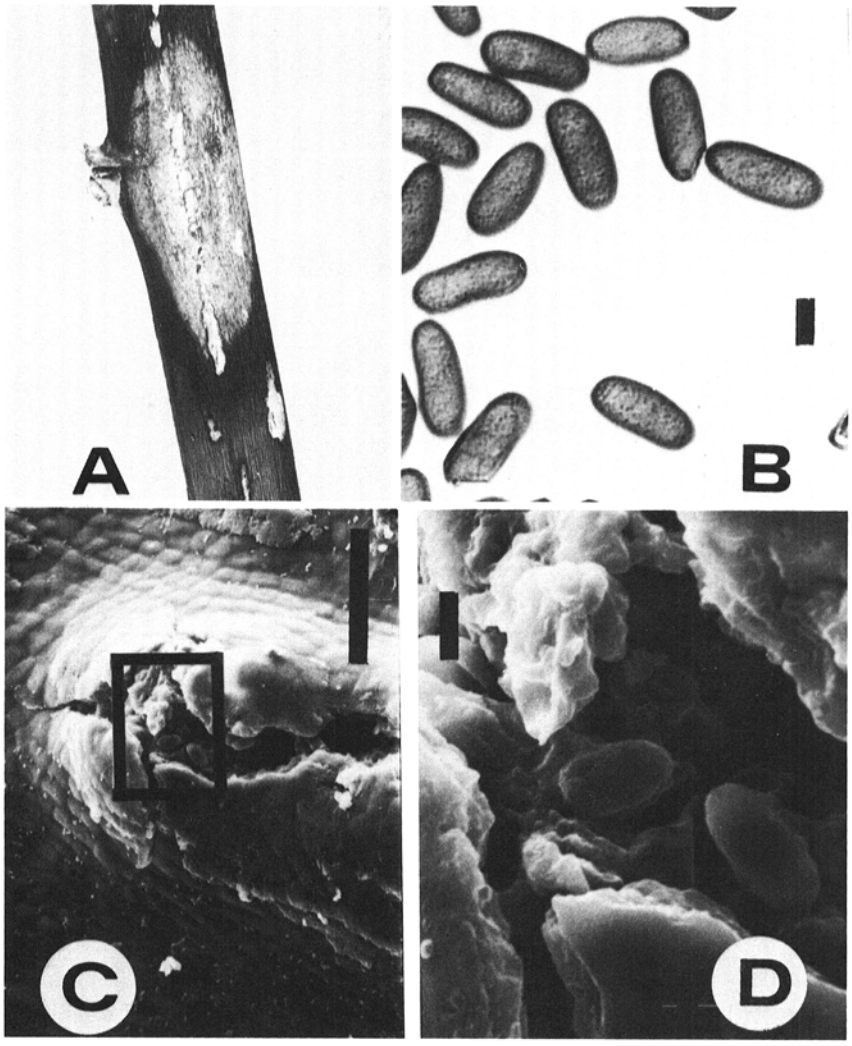

Fig. 3. Canker on thornless blackberry (cultivar Hull Thornless) caused by *Botryosphaeria obtusa*. **(A)** Typical symptoms on second-year cane. **(B)** Conidia of *B. obtusa* observed with light microscope. Bar = 10 μm. **(C)** Scanning electron micrograph of conidiomata in canker. Bar = 100 μm. **(D)** Enlarged inset from C showing conidia. Bar = 10 μm.

Although the effects of winter injury were not studied, several observations have indicated that they may be an important factor in disease development. Further studies are needed to determine the effects of winter injury on disease development. Commercial production of these cultivars of thornless blackberry in Ohio will not be possible until effective means to control these canker diseases are developed.

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