A New Canker Disease of Apple Caused by *Leucostoma cincta* and Other Fungi Associated with Cankers on Apple in Michigan

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ABSTRACT

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Leucostoma cincta was found to be the cause of a newly recognized disease of apple in North America. The fungus was isolated from margins of cankers on trunks and branches of diseased trees and from spores taken from perithecia embedded in cankers. Key identifying characters were a perithecial stromata with a black delimiting conceptacle and a central pycnidium. The optimum temperature for growth of the fungus in culture was 20 C. Vegetative compatibility groups were demonstrated by pairing isolates on a modified cornmeal agar. A total of 21 vegetative compatibility groups were identified among 75 isolates from 48 trees. The vegetative compatibility groups did not segregate during conidiogenesis or ascosporogenesis. Other fungi associated with cankers on apple were *Valsa malicola*, *Botryosphaeria stevensii*, *B. obtusa*, *Nectria galligena*, *N. cinnabarina*, *Neofabraea malicorticis*, *Fusicoccum* sp., *Coniothyrium* sp., *Phomopsis* sp., and *Fusarium* sp. *L. cincta*, but not *V. malicola*, was pathogenic in inoculation trials conducted on cultivars Redchief and Empire apple trees and on cultivar Newhaven peach trees. In separate pathogenicity trials, *B. stevensii* caused greater canker formation on apple than *B. obtusa*, implicating this species for the first time in the canker complex on apple trees in Michigan.

Additional keywords: *Cytospora*, *Malus pumila*

In 1986 and 1987, a dieback of apple branches associated with cankers was noted by growers and extension personnel in several commercial apple (*Malus pumila* Miller) orchards in Michigan. Growers and extension agents had generally attributed the injury to black rot. However, an examination of dead and dying branches in two severely affected orchards in 1987 showed that black rot, caused by *Botryosphaeria obtusa* (Schw.) Shoemaker (=*Physalospora obtusa* (Schw.) Cooke) (imperfect state: *Sphaeropsis malorum* (Schw.) Cooke), was not associated with the active cankers. Perithecial stromata of *Leucostoma* were present on the cankers and single-ascospore isolates were identical in colony morphology to isolates obtained from infected host tissues at the canker margins. *Leucostoma* spp. were previously shown to cause a canker disease of apple in the German Democratic Republic (10).

The objectives of this study were to identify the *Leucostoma* sp. associated with cankers on apple trees and to establish its role in the etiology of the disease. Because there had been no systematic survey of canker diseases of apple in Michigan, other fungi found associated with cankers also were identified.

MATERIALS AND METHODS

Orchard canker survey and sample collection. In the summer of 1987 and the spring of 1988, apple orchards with dieback symptoms were visited and branches with cankers were collected and stored at 4 C in a cold room for 1-5 days. Unsolicited samples of cankers submitted by growers and extension agents also were examined. Fungal fruiting bodies present on the cankers were sectioned with a razor blade, mounted in lactophenol or water, and examined with a compound microscope. Identifications were based on fruiting body and spore characteristics using several keys and descriptions (2,3,7, 11,15-17). Voucher specimens were deposited in the Cryptogamic Herbarium, Department of Botany and Plant Pathology, Michigan State University.

Isolation of fungi. Cankers were surface-disinfested with 95% ethanol. They were flamed and the bark was removed to expose the underlying cambial and cortical tissues. Segments (2 × 4 mm) were cut from the margin of each canker and placed in petri plates on Difco potato-dextrose agar supplemented with 5 g/L of agar (PDA). The plates were incubated at 22-24 C and any resulting colonies were transferred to PDA and held at 22-24 C under ambient laboratory lighting.

When pycnidia or sporodochia were present, a mass of conidia from a single fruiting body was transferred to 2 ml of sterile water with a platinum transfer needle. If perithecia were present, the contents of a single perithecium were similarly transferred. Sporulation was induced when required by placing the samples in a moist chamber for 1-24 hr. Aliquots (0.1-0.4 ml) of the resulting conidial or ascospore suspension were plated on PDA, spread over the surface of the medium with a sterile glass rod, and incubated at 22-24 C for 24-48 hr. Single-spore isolates were obtained by transferring 4-16 germinated conidia or ascospores individually to PDA plates. Three single-ascospore isolates were deposited with the ATCC (64877, 64878, 64879).

Taxonomy of the *Leucostoma* sp. from apple. To identify the *Leucostoma* sp. associated with cankers on apple trees, we compared the morphology of our specimens with the descriptions of Gilman et al (7), Kern (11), and Urban (16). The color, shape, and size of the ectostromatic disk; the presence or absence of a pycnidium in the perithecial stroma; and the number of perithecial ostioles per stroma were determined using a dissecting microscope. Measurements were made with a mechanical caliper. The number, size, and arrangement of perithecia within the stroma, the size of asci and ascospores, and the thickness of the delimiting black conceptacle were determined with a compound microscope fitted with a calibrated ocular. Over 50 perithecial stromata were sectioned and examined and 300 ascospores were measured. The fruiting bodies of *Leucostoma* collected from apple also were compared with

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those collected from apricot (*Prunus armeniaca* L.), black cherry (*P. serotina* Ehrh.), peach (*P. persica* (L.) Batsch.), plum (*P. domestica* L.), and sour cherry (*P. cerasus* L.).

Effect of temperature on radial growth. Two monoconidial isolates (ASI17 and ASI86), three single-ascospore isolates (ASI26, ASI76, and ASI110), and one isolate from infected host tissue (ASI56) were selected to determine the optimum and maximum temperatures for growth of *Leucostoma* from apple. Petri plates containing a cornmeal-malt extract agar medium (Difco cornmeal agar amended with 5 g/L of Difco malt extract and 5 g/L of agar [CMEA]) were stabilized at 11, 15, 20, 24, 27, 29, 32, and 37 C for 24 hr before a 1-mm-diameter plug from the margin of an actively growing culture on CMEA was placed in the center of each plate. Each isolate was replicated five times at each temperature. The plates were wrapped with Parafilm (American Can Co., Greenwich, CT) and incubated in plastic bags in the dark at each temperature. Colony diameter measurements were made after 5 days with a mechanical caliper. Each colony was measured three times along axes separated by approximately 120 degrees. The 15 diameter measurements for each isolate-temperature combination were averaged and standard deviations were computed.

Vegetative compatibility. A procedure, similar to those described for *Leucocytospora kunzei* (13) and *L. persoonii* (Pers.) von Hohnel (imperfect state: *Leucocytospora leucostoma* (Pers.) von Hohnel) (8), was used to study vegetative compatibility in isolates of *Leucostoma* from apple. Mycelial plugs (1 mm in diameter) were cut from the margins of 1-wk-old colonies grown on CMEA at 20 C. The plugs were transferred to petri plates (100 × 15 mm) containing 25 ml of CMEA and placed 1 cm from each other in a grid pattern such that 21 plugs were situated on each plate. Each isolate was paired with itself and with each of the other isolates. The pairings were replicated twice. The plates were sealed with Parafilm and incubated in the dark at 20 C. Vegetative compatibility was determined by observing the reaction along the line of contact between the expanding colonies of the paired isolates after 3–4 wk. Isolates were considered vegetatively compatible if their colonies merged together uniformly upon contact and incompatible if a dark reaction line formed along the line of contact. A total of 75 isolates from 48 trees in nine apple orchards were paired. Of the 75 isolates, 45 were tissue isolates, 29 were single-ascospore isolates, and one was a single-conidium isolate. More than one isolate per tree was tested in 20 cases. Both tissue and single-ascospore isolates were tested from 10 trees.

The segregation of vegetative compatibility (v-c) groups through ascosporogenesis and conidiogenesis also was examined. Single-ascospore isolates, 8–12 isolates per perithecium, were paired in all possible combinations. Isolates from 10 perithecia collected from eight trees in four orchards were tested. Similarly, 15 single-conidium isolates from one pycnidium were paired. Vegetative compatibility also was tested between a single-ascospore and single-conidium isolate recovered from the same stromata.

Pathogenicity of *Leucostoma* sp. The pathogenicity of isolates of *Leucostoma* from apple was confirmed on young nursery trees (experiment 1) and on established orchard trees (experiment 2). In experiment 1, cultivars Empire and Redchief apple trees on M.111 rootstock were obtained from a commercial nursery (The Nursery Corp., Hartford, MI) and grown in 14-L metal or plastic cans containing 12 L of a commercial planting mix (Pro-Mix potting medium, Premier Brands, Inc., New Rochelle, NY). The trunk diameter of the trees was about 2 cm. The trees were grown outside in an open cold frame.

Inoculations were made in trunk tissue frozen 1–3 hr before inoculation by holding dry ice against the bark for 20–25 sec. The frozen area was circled with a grease pencil to aid in locating the site to be inoculated. There were no external signs of injury at the time of inoculation, but the cortical and cambial tissues were injured as indicated by a dark discoloration. A 4-mm-diameter plug (1 mm thick) of mycelium was placed under a 6-mm-square flap of bark cut to the depth of the cortical and cambial tissues. The bark was cut on three sides with the open end of the flap oriented toward the apex of the tree. Each inoculation site was wrapped with paper tape to prevent drying.

Three isolates of *Leucostoma* (ASI105, ASI106, and ASI108) from infected apple branch tissue in each of three orchards were tested. An isolate of *Valsa malicola* Urban (ASI203) also was tested. Inoculum disks were cut from the

Table 1. Fungi isolated from cankers on apple trees in 26 commercial orchards in Michigan

Fungal species[x]	Isolated from: Orchards[y] (no.)	Trees (no.)
Valsa malicola	15	26
Leucostoma sp.	11	43
Botryosphaeria obtusa	10	17
Fusicoccum sp.	6	9
Coniothyrium sp.	4	6
Phomopsis sp.	3	4
Fusarium sp.	2	4
Botryosphaeria sp.	2	3
Nectria galligena	1	2
N. cinnabarina	1	1
Neofabraea malicorticis	1	1
L. persoonii[z]	3	3
Pleurostromella sp.[z]	1	4
Diatrypella sp.[z]	1	1
Valsella sp.[z]	1	1

[x] Isolated from tissue and fruiting bodies unless otherwise noted.
[y] The column total is greater than 26 because more than one species was isolated from the same orchard.
[z] Isolated from fruiting bodies only.

Fig. 1. Canker of apple caused by *Leucostoma cincta*. **(A)** Dieback of the central leader of a 12-yr-old cultivar Red Delicious tree following the complete girdling of the trunk by a canker. **(B)** Necrosis and flaking off of the outer bark around an old pruning wound on a branch of Top Red Delicious.

margins of 1-wk-old cultures grown on PDA. The controls were inoculated with disks of sterile PDA. The five treatments were inoculated in random order along the trunk on each of 12 trees (five inoculation sites per tree) on 24 April 1988. The cankers were measured after 1 mo and isolations were made from each canker on PDA. The colony morphology of the cultures was compared with the morphology of the original isolates.

In experiment 2, inoculations were made on eight 7-yr-old Redchief apple trees in an orchard near East Lansing, MI. The inoculation procedure was as described for experiment 1, except the tissues were not frozen. Inoculations were made with three isolates of *Leucostoma* (ASI32, ASI107, and ASI109) and an isolate of *V. malicola* (ASI204), with sterile PDA as the control. A total of 17 inoculations were made with each isolate and the control on 29 March 1988. Each inoculation was replicated twice on seven trees and three times on one tree. Cankers were measured after 2 mo.

To determine if the *Leucostoma* from apple was pathogenic to peach, two 6-yr-old cultivar Newhaven peach trees in the East Lansing orchard also were inoculated on 29 March 1988 with three isolates of *Leucostoma* (ASI32, ASI107, and ASI109). Each inoculation was replicated six times (three times per tree). Cankers were measured after 2 mo and isolations were made from each canker on PDA. The colony morphology of the cultures was compared with the morphology of the original isolates.

Pathogenicity of *Botryosphaeria* spp. The isolates were from 10 apple orchards and all except isolates from one orchard were recovered from cankers. Isolates NC080, NC085, and NC087 were supplied by T. B. Sutton and were isolated from apple fruit in North Carolina. The identification of the isolates and the number of septa per conidium were verified by examining conidia in pycnidia obtained from 12- to 14-day-old cultures grown on cellulose films placed on top of Difco oatmeal agar medium under continuous fluorescent illumination (1).

Unsprayed cultivar Golden Delicious apples were harvested on 7 September 1988, about 3 wk before the optimum harvest date for long-term storage. Fruit were selected for uniform size and ripeness. They were washed and surface-sterilized with a 70% ethanol solution. Inoculations were made by cutting into the side of the fruit midway between the blossom and stem end with a sterile 6-mm-diameter cork borer. Two inoculations, one on the front (blush) and one on the back side, were made on each of six apples per isolate. Mycelial plugs were cut from the margins of actively growing cultures on PDA and were inserted mycelial surface downward into the wounds. Plugs of PDA were inserted into the fruit used as controls. The plugs of apple tissue were reinserted into the wounds, the wounds were covered with a piece of transparent tape, and the fruit were incubated at 24 C in plastic bags for 7 days. The experiment was repeated on 19 September with fruit harvested on 7 September and stored in a cold room.

To test the pathogenicity of isolates to wood, 10-yr-old cultivar McIntosh trees on M.26 rootstock in an orchard near East Lansing, MI, were used. Branches (5–15 mm in diameter) on each of eight trees were inoculated on 1 September 1988. Each tree was inoculated with all 33 isolates from Michigan. A 6-mm-diameter plug of mycelium from actively growing cultures on PDA was inserted under a triangular flap of bark (6 mm long on two sides) cut with a sterile knife. The cuts extended to the wood and the plug was inserted with the mycelium against the bark. Sterile PDA was inserted as a control. Each inoculation site was wrapped with paper tape to prevent drying. The length of necrotic tissue under the bark was measured after 6 wk.

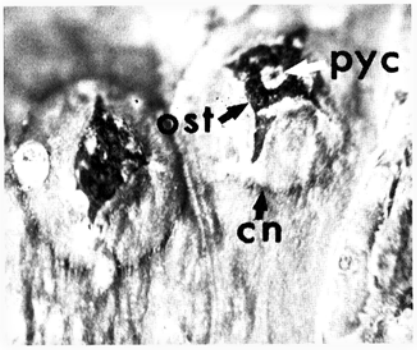

Fig. 2. Surface view of the perithecial stromata of *Leucostoma cincta* on apple bark. Note the central pycnidial opening (pyc) surrounded by the ostioles (ost) of the perithecia. The black conceptacle delimiting the stromata can be seen through the bark (cn).

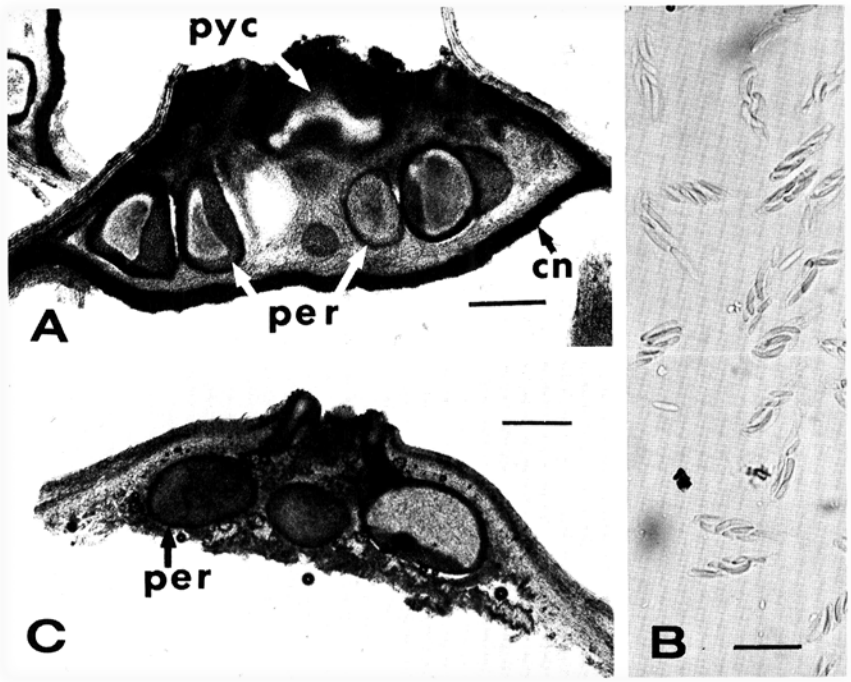

Fig. 3. Light photomicrographs of *Leucostoma cincta* and *Valsa malicola* from apple. **(A)** Cross section through a perithecial stroma showing the central simple pycnidium (pyc), circinately arranged perithecia (per), and the darkened conceptacle (cn) characteristic of *L. cincta*. Scale bar = 250 m. **(B)** Squash mount of asci and ascospores of *L. cincta* showing characteristic short, detached asci containing eight hyaline, allantoid ascospores. Scale bar = 40 m. **(C)** Cross section through a perithecial stroma of *V. malicola*, showing perithecia (per) and the absence of a delimiting conceptacle. Scale bar = 250 m.

RESULTS

Field symptoms and associated fungi. Nine genera of fungi were isolated from cankers on 120 apple trees in 26 orchards. The frequency of recovery of each fungus, in descending order by number of orchards, is given in Table 1. The fruiting bodies of an additional four genera were associated with cankers. However, these fungi were not isolated from the margins of cankered host tissue.

Leucostoma was associated with, and isolated from, cankers on 43 trees. Cankers were common on strains of cultivar Delicious, including Top Red, Miller Spur, Redchief, and some unidentified strains. Cankers also were found occasionally on Empire, Rome, and Spartan. The disease was common on 7- to 15-yr-old trees in good vigor. Cankers on the branches or trunk often caused a dieback of tissues distal to the canker (Fig. 1A). The cankers were covered with rough, scaly bark (Fig. 1B).

Cankers usually developed around pruning wounds or where branches were broken. Perithecial stromata were usually present on the surface of cankers that were more than 1 yr old.

In contrast to *Leucostoma*, *V. malicola*, *B. obtusa*, and *B. stevensii* (imperfect state: *Diplodia mutila* (Fries) Mont.) were associated with trees and branches in low vigor. *V. malicola* was common on dead apple branches, including the distal portion of branches killed by *Leucostoma*. Both the perfect and imperfect (*Cytospora schulzeri* Sacc. & Syd.) states were usually present. Frequently, pycnidia of *S. malorum* were intermingled with pycnidia of *C. schulzeri* on the same branch. Except for *Nectria galligena* Bres. and *Neofabraea malicorticis* Jacks., all of the remaining fungi were found associated with winter-injured or otherwise weakened branches.

Taxonomy of the *Leucostoma* sp. from apple. The taxonomic features of the *Leucostoma* associated with cankers on apple were consistent with those described for *L. cincta* (Fr.) von Hohnel (7,11,16). Viewed from the surface of the bark (Fig. 2), the perithecial stromata were round, 1.6–2.8 mm in diameter, very prominent, and delimited by a black conceptacle that was visible through the bark as a circumscribing line around the stromata. The ectostromatic disk was pale gray to tan and 0.6–1.1 mm in diameter. The black ostioles, 9–27 per perithecial stroma, were usually arranged circinately around a central pycnidial opening or remnant of a central pycnidium, and they sometimes obscured the outer portions of the convex disk. In cross sections through the conical perithecial stromata (Fig. 3A), the perithecia were 200–350 μm in diameter, arranged circinately around a central simple pycnidium, with necks centrally to laterally inserted through the ectostromatic disk. The black conceptacle delimiting the stromata was 30–80 μm thick. Asci (Fig. 3B) were clavate, eight-spored, 7–12 × 40–70 μm in size, detached, and free floating in the centrum at maturity. Ascospores were 2–3 × 14–22 μm in size, hyaline, aseptate, and allantoid. They matured from late autumn to early spring. The central pycnidial chamber was usually simple, with hyaline, aseptate, allantoid to ellipsoid conidia 1–2 × 5–7 μm. Pycnidial stromata alone were rarely encountered and it appeared that the pycnidia formed as an integral part of the developing perithecial stromata. Usually, pycnidia matured and released conidia before the maturation of the perithecia. The black conceptacle was always absent in cross sections of stromata of *V. malicola* (Fig. 3C) and this characteristic was used to distinguish *V. malicola* from *L. cincta*.

On PDA, isolates of *L. cincta* from apple were appressed and suedelike, at first white and later light brown to gray. The colony margins ranged from lobate to entire. Colonies rarely covered the entire surface of the culture medium. Black pycnidia (1.0–1.5 mm), either singly or in stromatic groups, sometimes formed after several weeks. Conidial cirri, when present, were off-white to cream. Some isolates produced a diffusible light brown pigment that discolored the culture medium.

Two species of *Leucostoma*, *L. persoonii* and *L. cincta*, were identified on *Prunus* hosts. The specimens of *L. cincta* on *Prunus* were not identical to those on apple. The stromata were smaller and more elliptical on *Prunus* and contained fewer perithecia (5–14 vs. 9–27). Asci and ascospores were similar in size to those for *L. cincta* on apple. However, the ascospores of *L. cincta* on *Prunus* were mature at a different time of the year than those on apple. In March and April of 1988, most of the ascospores in perithecia of *L. cincta* collected from apple were mature, but very few of the ascospores in perithecia of *L. cincta* collected from *Prunus* were mature. On *Prunus*, pycnidia corresponding to *Leucocytospora cincta* (Sacc.) von Hohnel were common, whereas on apple, pycnidia were usually associated with perithecial stromata.

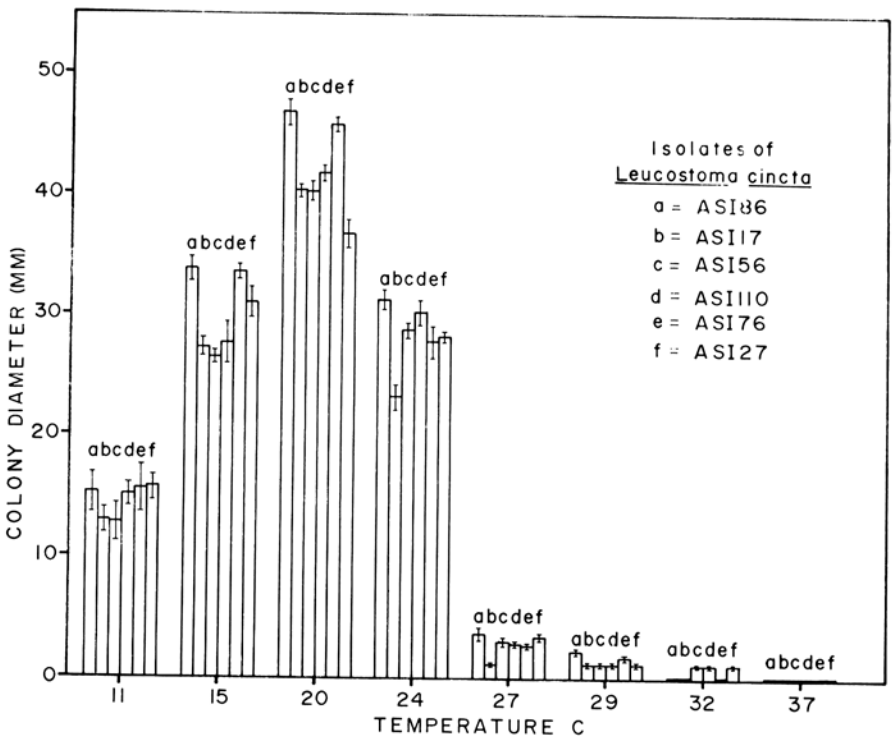

Fig. 4. Radial growth of six isolates of *Leucostoma cincta* from apple after 5 days. Isolates were grown on cornmeal-malt extract agar at eight temperatures in the dark. Values are the means of 15 measurements over five replications, three measurements per replication. Bars represent one standard deviation.

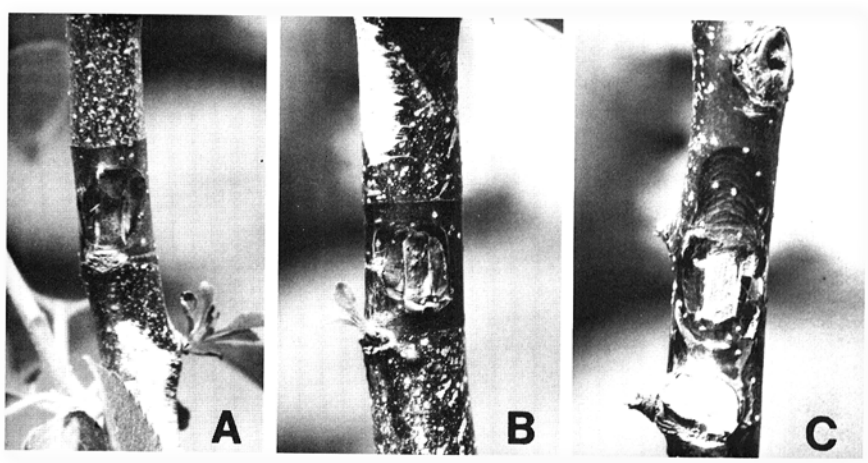

Fig. 5. Wound inoculations of 1-yr-old cultivar Empire apple trees after 1 mo. Inoculation sites were frozen with dry ice 3 hr before wound inoculation. Inoculation with **(A)** sterile PDA only, no discoloration; **(B)** *Valsa malicola*, colonization of frozen tissues only; and **(C)** *Leucostoma cincta*, colonization of tissues beyond the frozen tissue leading to canker formation.

Table 2. Pathogenicity of isolates of *Leucostoma cincta* and an isolate of *Valsa malicola* from apple on 1-yr-old cultivars Redchief and Empire apple trees following wound inoculation to artificially frozen trunk tissues

Isolate	Redchief					Empire				
	Canker initiation[v] (%)	Canker length (mm)[w]			Fungus reisolated[x]	Canker initiation[v] (%)	Canker length (mm)[w]			Fungus reisolated[x]
		Minimum	Maximum	Mean			Minimum	Maximum	Mean	
L. cincta										
ASI105	91.7	12	68	38	10/11	33.3	19	24	21	4/4
ASI106	83.3	20	47	33	10/10	41.7	19	65	37	5/5
ASI108	25.0	wc[y]	22	16	3/3	8.3	wc	20	20	1/1
V. malicola										
ASI203	0	wc	wc	wc	...[z]	0	wc	wc	wc	...
Control	0	0	0	0	...	0	0	0	0	...

[v] Number of inoculations resulting in cankers per total number of inoculations.
[w] Linear extension of cankers after 1 mo.
[x] Number of cankers, out of the total number of cankers formed, from which *L. cincta* was reisolated.
[y] Wound caused by freeze injury colonized but no canker expansion beyond the freeze injury.
[z] No cankers present for reisolation.

Table 3. Pathogenicity of isolates of *Leucostoma cincta* and *Valsa malicola* from apple on 7-yr-old cultivar Redchief apple trees following wound inoculations

Isolate	Canker initiation[x] (%)	Canker length (mm)[y]		
		Minimum	Maximum	Mean
L. cincta				
ASI109	76.5	15	44	21
ASI32	94.1	17	30	25
ASI107	41.2	12	20	16
V. malicola				
ASI204	0	wc[z]	wc	wc
Control	0	0	0	0

[x] Number of inoculations resulting in cankers per total number of inoculations.
[y] Linear extension of cankers after 2 mo.
[z] Wound flap colonized but no canker expansion.

Isolates of *Leucostoma cincta* from *Prunus* were appressed and feltlike, at first white and later light gray, on PDA. The colony margins were entire and the colonies usually covered the surface of the culture medium. Light-colored pycnidia (1.0–2.0 mm) occurred singly on most plates. No conidial cirri formed.

The two species of *Botryosphaeria* encountered in this survey were distinguished based on their colony morphology and on the shape and septation of the conidia. On PDA, the mycelium of colonies of *B. obtusa* were initially white and darkened with age to dark gray or black, whereas colonies of *B. stevensii* remained white to off-white. Both species produced pycnidia in culture. Conidia produced by *B. obtusa* were oblong (10 × 25 μm), echinulate, tan at maturity, and aseptate. Conidia produced by *B. stevensii* were elliptical (14 × 25 μm), smooth-walled, brown to dark brown at maturity, and often with a median septum.

Effect of temperature on radial growth. The optimum temperature for radial growth of isolates of *L. cincta* from apple was 20 C (Fig. 4). Good growth occurred at 11, 15, and 24 C. Growth was reduced by about 90% at 27 C as compared with 24 C. Three isolates failed to grow at 32 C and no isolate grew at 37 C.

Vegetative compatibility. Colonies of *L. cincta* grew uniformly from the mycelial plugs and the black reaction line characteristic of the incompatible response was evident in about 3 wk. Self-pairings used as controls were always compatible. V-c groups did not change through ascosporogenesis or conidiogenesis. In all cases, pairings among single-ascospore isolates from the same perithecium or single-conidium isolates from the same pycnidium were compatible. A compatible reaction also was observed upon pairing a single-conidium and a single-ascospore isolate from the same stromata. In eight of 10 cases, single-ascospore isolates and tissue isolates from the same canker belonged to the same v-c group and in the two remaining cases to different v-c groups. Among 75 isolates from 48 trees, 21 v-c groups were identified. As many as five v-c groups were detected in a single orchard. However, in each of the seven orchards where more than one tree was surveyed, at least two trees yielded isolates in the same v-c group. Isolates from different orchards generally belonged to different v-c groups. Only two v-c groups contained isolates from different orchards. One isolate was compatible with isolates in two separate v-c groups that were incompatible when paired to each other.

Pathogenicity of *Leucostoma* sp. In experiment 1, the three isolates of *L. cincta*, but not the isolate of *V. malicola* or the control, incited canker formation (Fig. 5A,B). The most virulent isolates on Redchief and Empire were ASI105 and ASI106 (Table 2). More cankers were initiated on Redchief than on Empire. The cankers were similar in appearance to those observed in the field. *V. malicola* colonized and frequently produced pycnidia on freeze-damaged tissue but not on the surrounding healthy tissue. The isolates of *L. cincta* recovered from the cankers were identical in colony morphology to the original isolates.

In experiment 2, the three isolates of *L. cincta* produced cankers, whereas the isolate of *V. malicola* did not (Table 3). The wound flaps in the control remained healthy, whereas those inoculated with *V. malicola* were colonized by the fungus. The cankers caused by *L. cincta* on the inoculated trees were similar to those observed on naturally infected trees in commercial orchards.

The three isolates of *L. cincta* from apple were pathogenic on peach. All 18 inoculations resulted in sunken cankers that ranged in size from 23 × 15 to 60 × 25 mm. Gummosis was common. Isolates recovered from the cankers produced on peach were identical in colony morphology to the original isolates from apple.

Pathogenicity of *Botryosphaeria* spp. No lesions developed in wounds on apples where sterile PDA was inserted. Lesions were evident after 4 days on apples inoculated with the 33 isolates recovered from apple orchards in Michigan and extensive rotting of tissue was noted after 7 days. Only five isolates (B20, C12, C13, G24, G25) produced lesion diameters equal to or larger than lesion diameters for three isolates from North Carolina ($P = 0.01$) (Table 4). Similar results were obtained when the experiment was repeated.

Isolates of *B. stevensii* developed significantly ($P = 0.01$) larger cankers within 6 wk than isolates of *B. obtusa*

(Table 4). Lesions produced by many isolates of *B. obtusa* did not extend beyond the wounded bark.

DISCUSSION

Leucostoma canker was identified as a new disease of apple trees in North America, specifically in Michigan. Previously, *L. cincta* was identified on apple in the German Democratic Republic, where it was the second most frequent cause of bark damage on apple after *Cytospora personata* Fr. (10). We did not detect this latter species in Michigan, nor has it been reported in other areas of North America. *Valsa* or *Cytospora* spp. have been reported as minor pathogens of apple in the United States (6,12,14), but it is unlikely that *L. cincta* was involved in these studies. The species were either not identified (6) or were identified as *L. persoonii* (12,14), and none of the isolates were pathogenic to apple trees (6,12). We also found fruiting bodies of *L. persoonii* associated with dead apple branches, but the fungus was never isolated from canker margins. Kastirr and Ficke (10) concluded that *V. malicola* was not pathogenic on apple in the German Democratic Republic, which agrees with our results with two isolates of *V. malicola*.

Beyond the fact that *L. cincta*, *B. obtusa*, and *B. stevensii* were identified as canker pathogens in Michigan apple orchards, there was little relationship among the three pathogens. We observed a greater loss of bearing surface in orchards with *L. cincta* than in orchards with *Botryosphaeria*. Isolates of *L. cincta* were obtained primarily from orchards located in counties with significant apple production, whereas isolates of *B. obtusa* and *B. stevensii* were obtained primarily from orchards located in counties where apple production was minor. *L. cincta* was associated with vigorous trees, and *B. obtusa* and *B. stevensii* with weak trees or following winter injury, sunburn, or infection by the fire blight pathogen. The more aggressive *B. stevensii* was recovered from two orchards located outside of the primary apple-producing region along Lake Michigan.

In addition to *L. cincta*, *Nectria galligena* and *Neofabraea malicorticis* were isolated from cankers on vigorous trees, and isolates of both fungi were pathogenic when inoculated into branches of 10-yr-old McIntosh apple trees (A. L. Jones, *unpublished*). The other fungi we identified, *V. malicola*, *N. cinnabarina*, *Fusicoccum* sp., *Phomopsis* sp., *Coniothyrium* sp., and *Fusarium* sp., were all isolated from cankers on trees in a weakened condition. These fungi were probably not primary pathogens, but we did not attempt to check the pathogenicity of each fungus. *V. malicola* was ubiquitous in its distribution and appears to be a prominent saprophyte on dead apple branches.

Several lines of evidence support the identification of the *Leucostoma* associated with cankers on apple as *L. cincta*. A key taxonomic feature of the species is the occurrence of a central, simple pycnidium in the perithecial stroma (11,16). A central pycnidium was observed in virtually all of the perithecial stromata examined. Measurements of the ascospores, asci, perithecia, and the thickness of the black conceptacle delimiting the stromata were within the limits reported for this species (7,11,16). The optimum temperature for growth has been used in studies on peach to distinguish isolates of *L. cincta* from those of *L. persoonii* (4,5,8,9). The optimum of 20 C for growth of six isolates of *Leucostoma* from apple matches the optimum generally accepted for *L. cincta* (4,9) and is well below the optimum of 28–30 C for *L. persoonii* (4,5,9). The isolates of *L. cincta* from apple were also pathogenic to peach.

Several v-c groups were identified among the isolates of *L. cincta* from apple, a feature found in both *L. kunzei* (13) and *L. persoonii* (8). No segregation of v-c groups was observed among single-ascospore isolates from the same perithecium, indicating that *L. cincta* can be self-fertile (homothallic). This is in contrast to results obtained with *L.*

Table 4. Decay of fruit of cultivar Golden Delicious after 4 days and size of cankers on branches of cultivar McIntosh apple trees 6 wk after inoculation with *Botryosphaeria* spp. isolated from 10 orchards in Michigan

Isolate	Source	Cultivar	Septa per conidium[s] (no.)	Lesion diameter[t] (cm)	Canker length[u] (cm)
A03[v]	Canker	Northern Spy	1	1.89 defg	6.32 a
A06	Canker	Northern Spy	1	1.77 efghi	6.20 a
A01	Canker	Northern Spy	1	1.82 defghi	5.93 a
A28	Canker	Northern Spy	1	1.62 ghij	5.66 a
C11	Canker	Red Delicious	1	1.90 defg	5.66 a
A02	Canker	Northern Spy	1	1.88 defg	5.21 a
A04	Canker	Northern Spy	1	1.85 defgh	5.09 a
C10	Canker	Red Delicious	1	1.88 defgh	4.59 a
A05	Canker	Northern Spy	1	1.62 ghij	4.32 ab
B07	Canker	Spartan	0	1.64 ghij	2.55 bc
G25	Canker	bt[w]	0	2.91 a	2.46 bc
F18	Canker	Red Delicious	0	1.55 hijk	1.47 c
B20	Canker	Spartan	0	2.10 cd	1.40 c
C13	Canker	Red Delicious	0	2.26 bc	1.35 c
G22	Canker	bt	0	1.89 defg	1.33 c
C12	Canker	Red Delicious	0	2.03 cdef	1.31 c
D15	Canker	cu[x]	0	1.79 defghi	1.16 c
H26	Canker	Red Delicious	0	1.06 mn	1.11 c
E17	Canker	Red Delicious	0	1.51 ijk	1.04 c
G24	Canker	bt	0	2.09 cde	1.00 c
G23	Canker	bt	0	1.84 defgh	0.93 c
J32	Fruit	Idared	0	1.28 klm	0.88 c
J30	Fruit	Idared	0	1.38 jkl	0.84 c
J29	Fruit	Idared	0	1.43 jkl	0.82 c
B08	Canker	Spartan	0	1.79 defghi	0.75 c
G01	Canker	bt	0	0.97 n	0.73 c
J33	Fruit	Idared	0	1.32 jklm	0.66 c
D16	Canker	cu	0	1.77 efghi	0.61 c
D14	Canker	cu	0	1.75 fghi	0.57 c
I27	Canker	Spartan	0	1.13 lmn	0.54 c
B09	Canker	Spartan	0	1.34 jklm	0.54 c
F19	Canker	Red Delicious	0	1.42 jkl	0.44 c
J31	Fruit	Idared	0	1.35 jklm	0.43 c
NC080[y]	Fruit		0	2.47 b	nd[z]
NC085	Fruit		0	2.24 bc	nd
NC087	Fruit		0	2.22 bc	nd

[s] Isolates with conidia lacking septa were identified as *B. obtusa*, those with septate conidia predominating were *B. stevensii*.

[t] Values are the means of two inoculations per fruit with six replications. Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$).

[u] Values are the means of eight single-tree replications. Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$).

[v] Letter preceding each isolate number codes for the orchard of origin. The location of the orchards by county were as follows: A = Eaton, B = Mason, C = Clinton, D = Leelanau, E,J = Kent, F = Oakland, G = Kalamazoo, H = Maskegon, and I = Calhoun.

[w] Isolated from shoots around the base of trees killed by low winter temperatures.

[x] Cultivar unknown.

[y] Isolates preceded by NC were supplied by T. B. Sutton and were isolated from orchards in North Carolina.

[z] nd = Not done.

persoonii, where segregation of v-c groups occurred during ascosporogenesis (8). However, 21 v-c groups were detected among the 75 isolates of *L. cincta* tested. Therefore, there is some mechanism for gene exchange and segregation, indicating the species also may function heterothallically. Further work is required to address the nature of the mating behavior of *L. cincta*. V-c groups did not segregate during conidiogenesis, in agreement with results reported for *L. kunzei* (13) and *L. persoonii* (8).

Not only was *L. cincta* associated with cankers on apple in Michigan, but Koch's postulates were completed to demonstrate its pathogenicity. Inoculation studies and field observations indicate that several varieties of Delicious were susceptible to Leucostoma canker. Cankers caused by *L. cincta* on naturally infected apple trees were consistently associated with pruning wounds, suggesting that wounds may be required before infection can occur.

Although specimens from apple and *Prunus* were consistent with the description for *L. cincta*, differences were noted between the two groups. The ascospores appear to mature at a different time of year. The asci and ascospores of *L. cincta* on *Prunus* were immature, whereas the asci and ascospores of *L. cincta* from apple and of *L. persoonii* on *Prunus* and on apple were fully mature in March and April. In culture, growth of isolates of *L. cincta* from apple was restricted, slower, and more dense than growth of isolates from *Prunus*. The genus *Leucostoma*, because of the difficulty in separating the large number of important plant pathogenic species, particularly when the perfect state is not found, is in need of further work and possible revision. Due to the variability of *Leucostoma* species, both in fruiting body morphology (11,16) and in colony morphology in culture (8,9,18), a combination of taxonomic, host range, vegetative compatibility, and molecular taxonomy studies are warranted.

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