Colonization of Chestnut Blight Cankers by *Ceratocystis microspora* and *C. eucastaneae*

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ABSTRACT

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Ceratocystis microspora and *C. eucastaneae* commonly colonize chestnut blight cankers in eastern Kentucky. Both of these species failed to become established in living or dead tissue from any of the 25 hardwood species, including American chestnut, into which they were inoculated. *C. microspora* and *C. eucastaneae* also failed to become established in chestnut when coinoculated with virulent (V) and cytoplasmically hypovirulent (CH) isolates of *Endothia parasitica*. These species, however, naturally colonized 13-mo-old cankers induced by several V and CH isolates. *Ceratocystis* species may serve as surrogate fungal attractants for enhancing insect dissemination of CH isolates of *E. parasitica*. Growth and sporulation of *Ceratocystis* was reduced on a healthy bark extract (HBE) medium compared to those on a blighted bark extract (BBE) medium. In addition, growth of *Ceratocystis* was increased on Noble agar that previously supported mycelium of V or CH isolates of *E. parasitica*. Healthy bark extract contained antifungal compounds not detectable in BBE and contained much higher levels of condensed and hydrolyzable tannins than BBE. Establishment of *Ceratocystis* in blight cankers apparently is enhanced by the action of *E. parasitica* to modify inhibitory compounds in healthy bark, and to produce metabolites that directly stimulate *Ceratocystis*. The evidence suggested that *C. microspora* becomes established in chestnut blight cankers prior to *C. eucastaneae*.

Additional key words: Castanea dentata.

Species in the fungal genus *Ceratocystis* are responsible for several important hardwood diseases: eg, oak wilt (18,24); Dutch elm disease (17,30); and Ceratocystis canker of stone fruits (12,25), aspen (21,22), and *Platanus* spp. (8,37). The causal fungus in each case is vectored effectively by insects and is capable of colonizing freshly wounded tissue. Two species of *Ceratocystis*, *C. microspora* (Davids.) Davidson and *C. eucastaneae* Davidson, have been reported on cankers of American chestnut (*Castanea dentata* (Marsh.) Borkh.) caused by *Endothia parasitica* (Murr.) And. in North Carolina and Kentucky (10,11,27). Furthermore, *C. microspora* was reported to colonize cankers of *E. parasitica* on *Quercus virginiana* Mill. (9). Unlike the *Ceratocystis* species that incite the diseases mentioned above, both *C. microspora* and *C. eucastaneae* were observed only on necrotic tissue in old blight cankers (29).

Many species of *Ceratocystis* are closely associated with insects (23). Old chestnut blight cankers colonized by both *C. microspora* and *C. eucastaneae* were more attractive to insects than either young blight cankers without *Ceratocystis* or healthy chestnut bark (29). These earlier results suggested that establishment of *Ceratocystis* in cankers induced by sparsely-sporulating, cytoplasmically hypovirulent (CH) isolates of *E. parasitica* (2,16) might enhance insect dissemination of these curative isolates. The presence of *Ceratocystis* within CH cankers could increase the insect attractiveness of these cankers, with *Ceratocystis* acting as a surrogate fungal attractant. Insects have been reported to be carriers of both virulent (V) and CH isolates of *E. parasitica* (28). Thus, the association of *Endothia* and *Ceratocystis* may provide assistance to the spread of CH isolates within the native range of American chestnut.

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The objective of this study was to investigate the interrelationships in vivo and in vitro between these *Ceratocystis* species and *E. parasitica*.

MATERIALS AND METHODS

In vivo studies. Perithecia of *C. microspora* and *C. eucastaneae* are depicted in Fig. 1. Living stems in the field, as well as excised dead or dormant stems of 25 hardwood species, were screened for ability to support growth and sporulation of *C. microspora* and *C. eucastaneae*. The species tested were *Carya glabra* (Mill.) Sweet.; *Betula alleghaniensis* Britton; *B. lenta* L.; *Carpinus caroliniana* Walt.; *Fagus grandifolia* Ehrh.; *Castanea dentata*; *Quercus alba* L.; *Q. prinus* L.; *Q. rubra* L.; *Q. velutina* Lam.; *Q. coccinea* Meunch.; *Q. marilandica* Meunch.; *Liriodendron tulipifera* L.; *Sassafras albidum* (Nutt.) Nees; *Amelanchier arborea* (Michx. f.) Fern.; *Cercis canadensis* L.; *Robinia pseudoacacia* L.; *Rhus typhina* L.; *Acer saccharum* Marsh.; *A. rubrum* L.; *A. pensylvanicum* L.; *Aesculus octandra* Marsh.; *Nyssa sylvatica* Marsh.; *Cornus florida* L.; and *Oxydendrum arboreum* (L.) DC. Each species/isolate combination was replicated twice. Inoculation and incubation methods were those of Elliston (15).

Attempts to establish *Ceratocystis* in living chestnut stems were made by coinoculation of these species with or without *E. parasitica* into chestnut stems in September 1979. Inoculated stems were at least 4 cm in diameter at breast height (1.37 m) and free of visible blight symptoms. Each stem received five different inoculations, as follows: 6 mm-diameter disks from potato-dextrose agar (Difco) cultures of *C. microspora* or *C. eucastaneae* and of a V (EP 29) or CH (EP 27-9) isolate of *E. parasitica* placed into overlapping cork borer wounds (6 mm in diameter); a slurry of the above placed into a single cork borer wound; agar disk of the isolate of *E. parasitica* alone in a cork borer wound; agar disk of the *Ceratocystis* species alone in a cork borer wound; and agar disk alone in a cork borer wound. The inoculations with a given pair of isolates of *Ceratocystis* and *Endothia* were repeated on two stems. Inoculation sites were covered with masking tape to prevent rapid desiccation of the agar.

Ceratocystis microspora and *C. eucastaneae* were inoculated singly and in combination into 13-mo-old V and CH cankers in September 1980. Mycelium of *Ceratocystis* in agar disks was placed into each of four cork borer holes spaced 1 cm from the original inoculation site at the canker center. For coinoculations, each species of *Ceratocystis* was inoculated into two of the four sites. All inoculation sites were within the canker perimeter. Inoculations were covered with masking tape to retard drying of the agar disk. A similar set of uninoculated, nonwounded V and CH cankers served as controls. A duplicate experiment was planned for April 1982 on V and CH cankers that had been initiated in March 1981. Perithecia of *Ceratocystis*, however, were established naturally in a number of cankers at the time of the planned April 1982 inoculation. Six of the colonized cankers were excised and dissected upon return to the laboratory. These cankers had been initiated by six different V and CH isolates. The numbers of perithecia of *C. microspora* and *C. eucastaneae* were counted, and the distribution of perithecia within each canker was recorded.

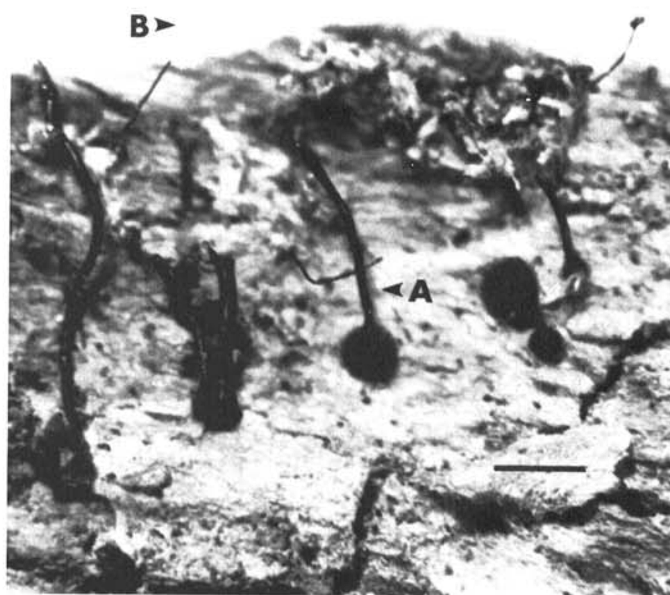

Fig. 1. Perithecia of *Ceratocystis microspora* (A) and *C. eucastaneae* (B) on blighted chestnut bark. Scale bar represents 1 mm.

In vitro studies. Both *Ceratocystis* species were grown on Noble agar (Difco) containing 50% aqueous extract of either blighted (BBE) or healthy (HBE) chestnut bark. Preliminary results indicated that growth and sporulation of *Ceratocystis* did not differ significantly on media prepared from chestnut bark of different genotypes or developmental stages. Bark extract media were prepared as follows. Twenty grams of dried bark were ground in a Wiley mill to pass a 1.2-mm (20/2.54 cm mesh) screen. Bark meal was extracted with 400 ml of double-deionized water for 1 hr at 25 C. After initial filtration, 125 ml of the extract was sterilized by filtration (0.45 μm pore size). The sterile extract was mixed with 125 ml of autoclaved, 4% Noble agar to give a final agar concentration of 2%, and then dispensed into petri dishes. Disks (4 mm in diameter) from PDA cultures of *Ceratocystis* were placed in petri dishes which then were incubated under standard conditions (16 hr of light daily, 24 C). Each combination of *Ceratocystis* and a medium was replicated six times. Colony diameter and perithecial production were measured after 20 days.

The effect of *E. parasitica* on growth of *Ceratocystis* was investigated by placing 4-mm-diameter PDA plugs of selected V (EP 29) and CH (EP 27-9, EP 88, and EP 234) isolates on cellophane (Du Pont 193 PUDO) disks (9 cm in diameter) that were autoclaved wet and placed immediately on Noble agar. Isolates EP 27-9, EP 88, and EP 234 exhibited high, moderate, and low virulence, respectively, when inoculated into stems of American chestnut (26). When mycelium of these isolates had grown near but not past the edge of the cellophane disk (after 5–7 days), cellophane with mycelium was removed from the agar surface and replaced with a plug of either *Ceratocystis* species on a second cellophane

TABLE 1. Presence of *Ceratocystis* perithecia 2 mo after inoculation of *Ceratocystis microspora* (CM) and *C. eucastaneae* (CE) into 13-mo-old cankers induced by virulent (V) or cytoplasmically hypovirulent (CH) isolates of *Endothia parasitica* in September 1980

Isolate	Origin[a]	V/CH	Virulence[b]	*Ceratocystis* species inoculated	Perithecia of *Ceratocystis*
EP 29	CT	V	high	CM + CE	–
EP 516[d]	CT	V	high	CM + CE	+[c]
EP 287[d]	CT	V	high	CE	+
EP 293[d]	CT	V	high	CE	–
E 2/73	KY	V	high	CM + CE	–
MV 1[e]	KY	V	moderate	CM	–
TK 1[e]	KY	V	moderate	CE	–
ORL 1[f]	KY	V	high	CM	+
EP 27-9	Italy	CH	high	CE	+
EP 103	VA	CH	high	CM	+
EP 431	Italy	CH	high	CM + CE	–
EP 405	Italy	CH	moderate	CM	–
EP 88	MI	CH	low	CM	–
EP 433	Italy	CH	low	CM + CE	–
EP 419	Italy	CH	low	CE	–
EP 50	Italy	CH	low	CE	–
EP 49	Italy	CH	low	CM	–
EP 47	Italy	CH	low	CM + CE	–
EP 454	Italy	CH	low	CM + CE	+[c]
EP 518	MI	CH	low	CM	–
EP 524	MI	CH	low	CE	–
EP 515	France	CH	low	CM + CE	–
EP 234	TN	CH	low	CM	–

[a] Isolates not from Kentucky were provided by J. E. Elliston, Connecticut Agricultural Experiment Station.
[b] High = canker areas >100 cm^2, moderate = canker areas 50–100 cm^2, and low = canker areas <50 cm^2. Levels of virulence were determined from 13-mo-old cankers.
[c] Perithecia of both *Ceratocystis* species observed.
[d] Methionine-requiring auxotrophic isolate.
[e] Obtained from large, surviving chestnut trees.
[f] Obtained from basal *E. parasitica* canker on *Quercus coccinea*.

disk. Plates containing *Ceratocystis* growing on cellophane-covered Noble agar served as controls. Cultures were incubated under standard conditions. Each combination of *Ceratocystis* and *Endothia* was replicated six times. Results were recorded after 20 days. The effect of *Ceratocystis* species on growth and sporulation by *E. parasitica* were studied by reversing the order of culturing these genera on cellophane-covered Noble agar.

Aqueous extracts of blighted and healthy chestnut bark were chromatographed by TLC on cellulose. The solvent system was *n*-butanol:acetic acid:water (5:1:4, v/v; hyperphase). Air-dried chromatograms were sprayed with a suspension of *Cladosporium cucumerinum* conidia in a nutrient solution (1) and incubated under a water-saturated atmosphere in a sealed glass container at room temperature. White areas on the chromatogram surrounded by dark mycelium of *C. cucumerinum* indicated the presence of compounds with antifungal activity.

Blighted (BB) and healthy (HB) bark meal were analyzed for gallitannins (20), ellagitannins (4,5), and condensed tannins (19). Bark samples were of identical genotype and developmental stage.

RESULTS

In vivo studies. Both *C. microspora* and *C. eucastaneae* failed to become established in either living or dead tissue of any of the 25 hardwood species that were inoculated.

Observations up to 12 mo after inoculation failed to show establishment of either species of *Ceratocystis* in healthy chestnut stems when inoculated singly or coinoculated with *E. parasitica*. Areas of cankers initiated by EP 29 (V) or EP 27-9 (CH) were unaffected by coinoculation with either *Ceratocystis* species.

Results of inoculations of 13-mo-old V and CH cankers with *Ceratocystis* in September 1980 are presented in Table 1. Perithecia of *Ceratocystis* species were observed within 2 mo in cankers incited by EP 27-9, EP 516, EP 103, EP 287, EP 454, and ORL 1. These perithecia were not observed in similar cankers that were not inoculated with *Ceratocystis*. Perithecia of *Ceratocystis* were produced under laboratory conditions on bark disks removed from cankers 2 mo after inoculations with *Ceratocystis* but were not produced on bark disks from uninoculated control cankers. Perithecia of *Ceratocystis* were observed in all inoculated and control cankers in the study area after 2 yr.

Perithecia of *Ceratocystis* already were present naturally in 13-mo-old V and CH cankers that were to be inoculated in April 1982. Therefore, the inoculation was not conducted. Observations of perithecia of *Ceratocystis* in these cankers are summarized in Table 2. Perithecia of *Ceratocystis* were observed in cankers incited by both V and CH isolates of *E. parasitica*. Canker dissections revealed that perithecia of *C. microspora* greatly outnumbered those of *C. eucastaneae* (30:1). In many instances, cavities were formed between the raised bark of canker surfaces and the underlying wood. When present, perithecia usually were found within these cavities. Some perithecia were on external canker surfaces; these were associated with holes or cracks in the cankered tissue.

TABLE 2. Natural establishment of *Ceratocystis microspora* and *C. eucastaneae* in 13-mo-old cankers induced by virulent (V) or cytoplasmically hypovirulent (CH) isolates of *Endothia parasitica* in April 1982

Isolate	V/CH	Virulence[a]	Perithecia of *Ceratocystis*
EP 29	V	high	+
EP 27-9	CH	high	+
EP 103	CH	high	+
EP 431	CH	high	+
EP 405	CH	high	+
EP 49	CH	moderate	+
EP 88	CH	low	+
EP 433	CH	low	−
EP 419	CH	low	+
EP 50	CH	low	−
EP 47	CH	low	+
EP 454	CH	low	−
EP 518	CH	low	−
EP 524	CH	low	−
EP 515	CH	low	−
EP 234	CH	low	−

[a] High = canker areas >100 cm^2, moderate = canker areas 50–100 cm^2, and low = canker areas <50 cm^2. Levels of virulence were determined from 13-mo-old cankers.

In vitro studies. Colony diameters for both *C. microspora* and *C. eucastaneae* on HBE were reduced significantly below those on BBE (Fig. 2). A significant reduction in perithecial production on HBE compared to that on BBE was exhibited by *C. eucastaneae* but not by *C. microspora* (Fig. 2).

Diameter growth by both *Ceratocystis* species increased when they were grown on Noble agar that previously supported V or CH isolates of *E. parasitica* (Fig. 3). When the order of culturing these genera on cellophane-covered Noble agar was reversed, diameter growth and pycnidial production of *E. parasitica* were reduced substantially on media that previously supported *C. eucastaneae*. Much smaller reductions were obtained when EP 29 was grown on Noble agar that previously supported *C. microspora* (Fig. 4).

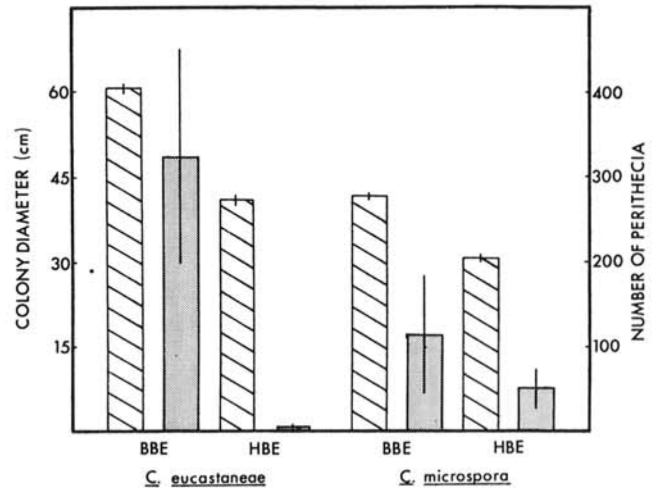

Fig. 2. Colony diameter (hatched bar) and perithecial production (stippled bar) by *Ceratocystis eucastaneae* and *C. microspora* grown 20 days on Noble agar amended with aqueous extracts from blighted (BBE) or healthy (HBE) chestnut bark. Vertical lines delimit standard errors of means for six colonies.

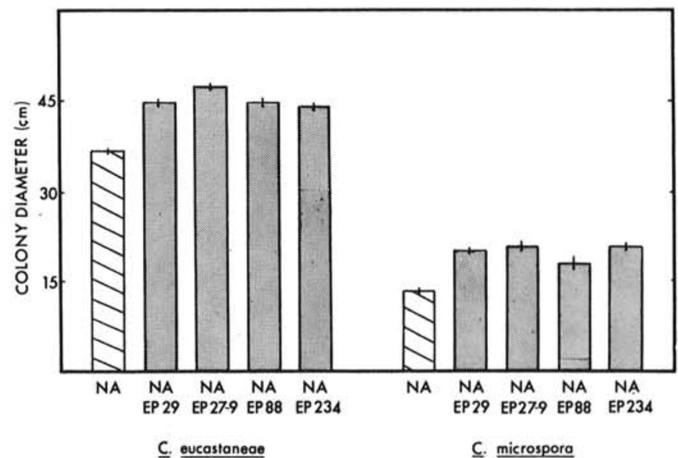

Fig. 3. Colony diameters of *Ceratocystis eucastaneae* and *C. microspora* grown 20 days on Noble agar (NA) alone and on NA that previously supported growth by virulent (EP 29) or cytoplasmically hypovirulent (EP 27-9, EP 88, and EP 234) isolates of *Endothia parasitica*. Vertical lines delimit standard errors of means for six colonies.

Chromatogram bioassays with *C. cucumerinum* indicated the presence of antifungal compounds in HBE but not in BBE or water controls (Fig. 5). Four major zones of inhibition were detected: R_f = 0.32, 0.24, 0.12, and 0.03.

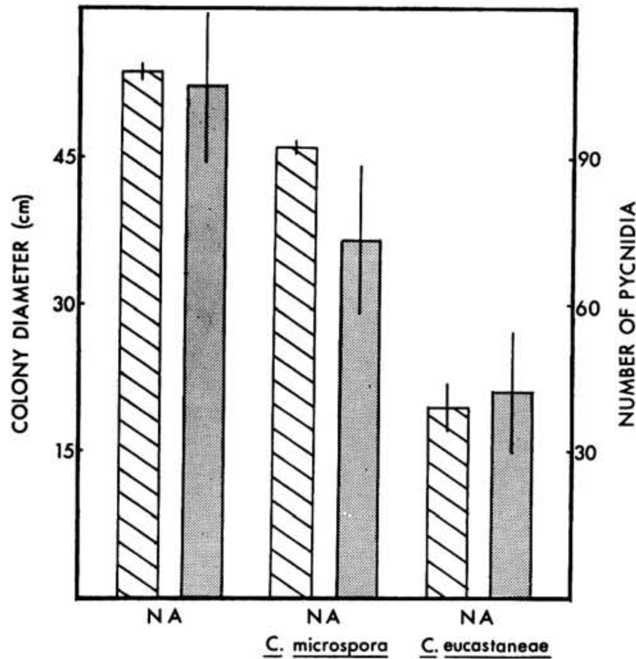

Fig. 4. Colony diameter (hatched bar) and pycnidial production (stippled bar) by *Endothia parasitica* grown 20 days on Noble agar (NA) alone and NA that previously supported *Ceratocystis microspora* or *C. eucastaneae*. Vertical lines delimit standard errors of means for six colonies.

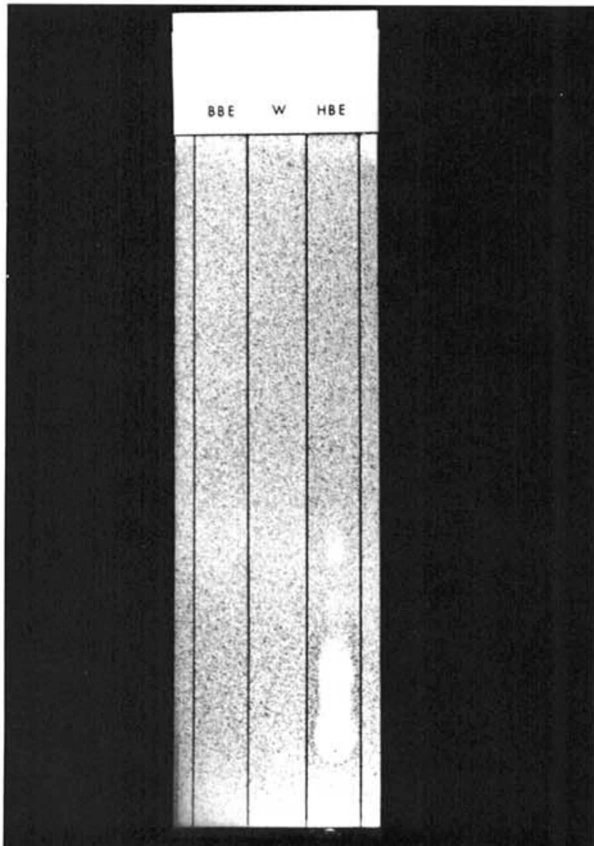

Fig. 5. Thin layer chromatogram of aqueous extracts from blighted (BB) and healthy (HB) chestnut bark and water controls (W) bioassayed with *Cladosporium cucumerinum*.

Results of assays of blighted and healthy bark for condensed and hydrolyzable tannins are given in Fig. 6. Blighted bark meal contained markedly less condensed and hydrolyzable tannins than did healthy bark meal. The standard used for gallitannin analysis was gallic acid. Ellagitannins are expressed as milliequivalents of hexahydroxydiphenoyl glucose (HHDPG) by using the molar absorption coefficient given by Bate-Smith (5). Condensed tannins are expressed as milliequivalents of perlargonidin, an anthocyanin from strawberry, by using the molar absorption coefficient of Sondheimer and Kertesz (34). Levels of tannins are expressed as milliequivalents of standards per 0.05 g of dried bark meal.

DISCUSSION

Numerous studies have described sequences of microorganisms that precede decay fungi (31,32,36). One of the best understood succession sequences involves *Phialophora melinii* and its role in the decay of sugar maple by *Fomes connatus*. *P. melinii* is isolated frequently from discolored tissue in advance of *F. connatus* (36), and it has been suggested that *P. melinii* can utilize or modify phenols in healthy tissue that may be inhibitory to *F. connatus* (33,35).

A similar series of events may be involved in colonization of blight cankers by *Ceratocystis*. Establishment of *Ceratocystis* apparently is enhanced by a 2-fold action of *E. parasitica*: modification of compounds in healthy bark that may be inhibitory to *Ceratocystis* (Figs. 2 and 5), and production of metabolites that directly stimulate *Ceratocystis* (Fig. 3). Although the identity of the inhibitory compound(s) in HBE was not determined, drastic reductions of tannin levels in blighted bark suggest that the antifungal activity observed on chromatograms may be due in part to high tannin levels in healthy chestnut bark. Similar reductions in tannin levels of blighted bark have been reported (6,7), and support conclusions that *E. parasitica* is capable of using large amounts of tannins as a nutrient source (7,14). However, both *C. microspora* and *C. eucastaneae* are capable of limited growth on media containing healthy bark meal or 0.5% tannic acid (*unpublished*). This may suggest that factors other than or in addition to tannins are involved in this growth inhibition by HBE.

Increased colony diameter growth by both *C. microspora* and *C. eucastaneae* on BBE compared to that on HBE (Fig. 2) also may be due in part to increased availability of nutrients in BBE. Thin-layer chromatograms developed in various solvent systems revealed marked differences between water-soluble components of blighted and healthy chestnut bark (Fig. 5 and *unpublished*). Similar results have been reported by Barnett (3) in a comparative study of phenolic compounds in *Castanea*.

C. microspora consistently was less affected by components of HBE in vitro than was *C. eucastaneae*. This suggests a colonization sequence in which *C. microspora* may become established earlier in blight canker development than does *C. eucastaneae*. This is supported by observations of many more perithecia of *C. microspora* than of *C. eucastaneae* in young blight cankers that were colonized naturally by these species. Observations of blight

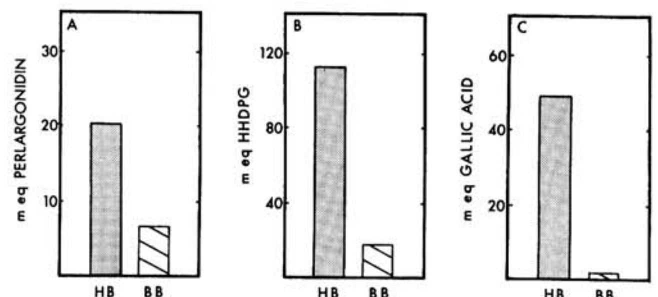

Fig. 6. Levels of **A,** condensed tannins, **B,** ellagitannins (hydrolyzable), and **C,** gallitannins (hydrolyzable) in blighted (BB) and healthy (HB) chestnut bark meal expressed as milliequivalents of standards per 0.05 g of dried bark.

cankers >2 yr old indicate that the number of perithecia produced by *C. eucastaneae* increases greatly during the later stages of canker development (26).

Both V and CH cankers expanded very slowly during winter (26). Furthermore, field inoculations with V and CH isolates during winter months were not successful (13). These results suggest a seasonal effect on both initiation and enlargement of chestnut blight cankers. The natural establishment of *Ceratocystis* in 13-mo-old blight cankers that were initiated in March 1981 but not in similarly aged cankers that were initiated in August 1979 may indicate a seasonal effect also on blight canker colonization by *Ceratocystis* species. In areas where *Ceratocystis* occurs naturally, inoculations of CH isolates of *E. parasitica* for dissemination studies should be performed in spring to enchance rapid colonization of the resultant cankers by *Ceratocystis*. Artificial introduction of *Ceratocystis* species into CH cankers may be used to increase the attractiveness of these cankers to insects in areas where *Ceratocystis* normally may not be encountered.

LITERATURE CITED

1. Allen, E. H., and Kuć, J. 1968. α-Solanine and α-chaconine as fungitoxic components in extracts of Irish potato tubers. Phytopathology 58:776-781.
2. Anagnostakis, S. L. 1982. Biological control of chestnut blight. Science 215:466-471.
3. Barnett, P. E. 1972. A comparative study of phenolics in chestnut (*Castanea*), and their relationships with resistance to *Endothia parasitica*. Ph.D. dissertation. University of Tennessee, Knoxville. 145 pp.
4. Bate-Smith, E. C. 1972. Detection and determination of ellagitannins. Phytochemistry 11:1153-1156.
5. Bate-Smith, E. C. 1972. Ellagitannin content of leaves of *Geranium* species. Phytochemistry 11:1755-1757.
6. Bazzigher, G. 1955. Über tannin- und phenolspaltenede Fermente von *Endothia parasitica*. Phytopathol. Z. 24:265-282.
7. Cook, M. T., and Wilson, G. W. 1915. The influence of the tannin content of the host plant on *Endothia parasitica* and related species. Bot. Gaz. 60:346-361.
8. Crone, L. J., and Bachelder, S. 1961. Insect transmission of canker stain fungus, *Ceratocystis fimbriata* f. *platani*. Phytopathology 51:576.
9. Davidson, R. W. 1969. *Ceratocystis microspora* associated with *Endothia parasitica* cankers on live oak in Virginia. Colorado-Wyoming Acad. Sci. 6:16.
10. Davidson, R. W. 1978. A new species of *Ceratocystis* on *Endothia parasitica* cankers of American chestnut. Mycologia 70:856-858.
11. Davidson, R. W., and Kuhlman, E. G. 1978. A species of *Ceratocystis* closely associated with *Endothia* cankers on American chestnut in eastern United States. Mycologia 70:853-855.
12. DeVay, J. E., Lukezic, F. L., English, H., Trujillo, E. E., and Moller, W. J. 1968. Ceratocystis canker of deciduous fruit trees. Phytopathology 58:949-954.
13. Double, M. L. 1982. Bimonthly inoculations of virulent and hypovirulent isolates of *Endothia parasitica*. Page 226 in: Proc. U.S. Dep. Agric., For. Serv., American Chestnut Coop. Mtg., Morgantown, WV. H. C. Smith and W. L. MacDonald, eds. West Virginia University Press, Morgantown.
14. Elkins, J. R., Lawhorn, Z., and Weyand, E. 1982. Utilization of chestnut tannins by *Endothia parasitica*. Pages 141-144 in: Proc. U.S. Dep. Agric. For. Serv., American Chestnut Coop. Mtg., Morgantown, WV. H. C. Smith and W. L. MacDonald, eds. West Virginia University Press, Morgantown.
15. Elliston, J. E. 1979. Pathogenicity and sporulation of normal and diseased strains of *Endothia parasitica* in American chestnut. Pages 95-100 in: Proc. American Chestnut Symp. W. L. MacDonald, F. C. Cech, J. Luchok, and C. Smith, eds. WV Agric. Exp. Stn. and U.S. Dep. Agric. For. Serv. West Virginia University Press, Morgantown.
16. Elliston, J. E. 1981. Hypovirulence and chestnut blight research: Fighting disease with disease. J. For. 79:657-660.
17. Gibbs, J. N. 1978. Intercontinental epidemiology of the Dutch elm disease. Annu. Rev. Phytopathol. 16:287-307.
18. Gibbs, J. N., and French, D. W. 1980. The transmission of oak wilt. U.S. Dep. Agric. For. Serv., Res. Pap. NC-185. 17 pp.
19. Govindarajan, V. S., and Matthew, A. G. 1965. Anthocyanidins from leucoanthocyanidins. Phytochemistry 4:985-988.
20. Haslam, E. 1966. Chemistry of vegetable tannins. Academic Press, London. 179 pp.
21. Hinds, T. E. 1972. Ceratocystis canker of aspen. Phytopathology 62:213-220.
22. Hinds, T. E. 1972. Insect transmission of *Ceratocystis* species associated with aspen cankers. Phytopathology 62:221-225.
23. Hunt, J. 1956. Taxonomy of the genus *Ceratocystis*. Lloydia 19:1-59.
24. Jewell, F. F. 1956. Insect transmission of oak wilt. Phytopathology 46:244-257.
25. Moller, W. J., and DeVay, J. E. 1968. Insect transmission of *Ceratocystis fimbriata* in deciduous fruit trees. Phytopathology 58:1499-1508.
26. Russin, J. S. 1983. Sporulation and dissemination of cytoplasmically hypovirulent isolates of *Endothia parasitica*. Ph.D. dissertation. University of Kentucky, Lexington. 116 pp.
27. Russin, J. S., and Shain, L. 1981. Studies on surrogate fungal attractants for insect dissemination of hypovirulent strains of *Endothia parasitica*. Phytopathology 71:902.
28. Russin, J. S., and Shain, L. 1983. Insects as carriers of virulent and hypovirulent isolates of *Endothia parasitica*. Phytopathology 73:837.
29. Russin, J. S., Shain, L., and Nordin, G. L. 1982. Sporulation and dissemination of hypovirulent strains of the chestnut blight fungus. Pages 40-48 in: Proc. U.S. Dep. Agric. For. Serv., American Chestnut Coop. Mtg., Morgantown, WV. H. C. Smith and W. L. MacDonald, eds. West Virginia University Press, Morgantown.
30. Schreiber, L. R., and Peacock, J. W. 1979. Dutch elm disease and its control. U.S. Dep. Agric. For. Serv., Agric. Inf. Bull. 193. 13 pp.
31. Shigo, A. L. 1972. Succession of microorganisms and patterns of discoloration and decay after wounding in red oak and white oak. Phytopathology 62:256-259.
32. Shigo, A. L. 1976. Microorganisms isolated from wounds inflicted on red maple, paper birch, American beech, and red oak in winter, summer, and autumn. Phytopathology 66:559-563.
33. Shortle, W. C., Tattar, T. A., and Rich, A. E. 1971. Effects of some phenolic compounds on the growth of *Phialophora melinii* and *Fomes connatus*. Phytopathology 61:552-555.
34. Sondheimer, E., and Kertesz, Z. I. 1948. The anthocyanin of strawberries. J. Am. Chem. Soc. 70:3476-3479.
35. Tattar, T. A., and Rich, A. E. 1973. Extractable phenols in clear, discolored, and decayed woody tissues and bark of sugar maple and red maple. Phytopathology 63:167-169.
36. Tatter, T. A., Shortle, W. C., and Rich, A. E. 1971. Sequence of microorganisms and changes in constituents associated with discoloration and decay of sugar maples infected with *Fomes connatus*. Phytopathology 61:556-558.
37. Walter, J. M., Rex, E. G., and Schreiber, R. 1952. The rate of progress and destructiveness of canker stain of plane trees. Phytopathology 42:236-239.