The Role of Ascospores and Conidia as Propagules in the Disease Cycle of *Hypoxylon mammatum*

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ABSTRACT

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Two lines of evidence, population structure and conidial germination properties, support the hypothesis that ascospores and not conidia disperse the fungus in the disease cycle of hypoxylon canker of aspen. Mycelial interactions, identified by the formation of a conidial interface between genetically different isolates, provided phenotypic markers for determining population structure in *Hypoxylon mammatum*. Single ascospore isolates from 14 asci from six perithecia found on one canker were tested for interactions. Each ascus contained four different interaction groups composed of identical twin ascospore isolates, indicating the heterothallic production of asci within perithecia. No isolates of the same group were found in interascus pairings. Mycelial isolations from cankers showed that each canker consisted of a single interaction type, suggesting its origin from a single spore. No isolates of the same type were found among cankers on adjacent trees, either in a wild clone or in a garden plantation, indicating that cankers caused by asexual propagation of the fungus were rare. Conidia produced by the mycelial interaction of two different isolates germinated 72–80%, as judged by germ tube emergence. Filtering conidial preparations through glass wool removed most of the hyphal fragments. Colony formation by these preparations was only 5% of the estimated number of conidia plated, but two to eight times more than the estimated number of hyphal fragments contaminating the preparations. Germinating conidia produced a few short hyphal branches on the surface of the agar medium and one long aerial hypha with branches at its tip, but no further development occurred. We concluded that conidia do not serve primarily as asexual propagules. We also observed two forms of conidiogenous cells, geniculate, and nodulose. The propensity to produce these differed among ascospore isolates. Conidial sizes varied significantly among isolates, and the conidia produced at the interface of opposed mycelia were usually significantly different from one or both of the parental strains.

Additional keywords: *Geniculosporium*, *Nodulisporium*, *Populus tremuloides*, vegetative compatibility.

Hypoxylon mammatum (Wahlenberg) J. H. Miller causes stem canker of *Populus tremuloides* Michx., probably the most important disease of aspen. The disease cycle is not well understood and even sources of inoculum (e.g., ascospores, conidia, or vegetative transfer by downy woodpeckers) (22) have not been clarified. Although it is commonly assumed that ascospores are the primary source of inoculum in nature (21), there is little evidence to support this hypothesis. A reliable

inoculation technique with ascospores was only recently developed for tissue culture plantlets (6), and no consistent technique using ascospores on trees or saplings has been reported (21). The possible role of conidia in the disease cycle has been controversial.

Ponomareff (23) first found the conidial stage of *H. mammatum*, but a clear description of conidial morphology has not been made. Most investigators obtained good germination of conidia (7,19,27,29), but Bagga et al (5) obtained less than 10% germination. These same authors also reported disparate results on the development of conidial germlings into independent mycelia. Thus, some claimed that mycelia developed readily from conidia (23,29), but others claimed that few conidial germlings formed mycelia (5,7,27). Rogers and Berbee (27) claimed that all colony development from conidial suspensions was from contaminating mycelial fragments.

In this paper, we examined the population structure of cankers in nature using mycelial interactions as phenotypic identifiers to determine whether nearby cankers were produced by asexual spread of the fungus. We also examined the biological properties of conidia produced in culture, including morphology and germination, to seek better understanding of their role in the disease cycle.

MATERIALS AND METHODS

Fungal strains and mycelial interactions. Perithecial stromata were collected from a canker on aspen clone B-50 (12) at Heiberg Memorial Forest, Tully, NY, placed on filter paper moistened with sterile distilled water in plastic petri plates, and incubated at 10 C in the dark. When ascospore discharge was evident on the petri dish lids, the stromata were washed with 3% H_2O_2 for 1 min followed by two rinses with sterile distilled water. We removed the tops of perithecia with a razor blade and aspirated the contents onto 8% water agar with a Pasteur pipette with the tip drawn to a fine bore. We separated and dissected individual asci and transfered the ascospores serially to 4% water agar that contained 100 mg L^{-1} each of filter-sterilized streptomycin sulfate and penicillin G added aseptically after autoclaving at 121 C.

We cut cylinders of bark and wood with a 1.5-cm diameter cork borer from the margins of active cankers on the boles of 10 aspen ramets in a single clone in Pompey, NY, and from 29 aspen trees in the plantations at the Genetics Field Station, Tully, NY, and isolated mycelial growths from these. The cork borer was sterilized between samples with 0.5% NaHClO, prepared by dilution from commercial hypochlorite bleach. We took 10 samples from the circumference of each canker at Pompey and two samples from each canker at Tully. After surface sterilizing the samples with 0.5% NaHClO, we placed chips of wood on malt extract agar (MEA) that consisted of 2% Difco malt extract, 1.5% Difco-Bacto agar, and 40 mg L^{-1} each of filter-sterilized streptomycin sulfate and penicillin G. We identified mycelia as *H. mammatum* by mycelial characters and by morphology of conidia produced by mycelial interactions.

We tested mycelial interactions on a defined medium containing 3 mM proline as N source gelled with 1.5% Difco-Bacto agar (16). Circular plugs of agar (5 mm in diameter) containing mycelium were cut from the margins of growing colonies, placed 15 mm apart on the agar medium in 15 × 100 mm petri plates, and incubated at 28 C in the dark. We determined the reactions after 7–21 days depending on the growth rates of the colonies. Scoring of the reaction was based on colony mergence without noticable reaction, which indicated phenotypic identity, or on formation of a hyperbranched ridge of aerial mycelium-bearing conidia at the interface between two paired mycelia, which indicated phenotypic difference.

Conidial germination and form. Colony-forming ability of conidia was tested on 10 mM proline-defined medium gelled with 1.5% Difco-Bacto agar (PDM) (16). Cultures were incubated at 28 C in the dark for 2 wk. Some plates were incubated for more than 1 mo to check for late developing colonies. Conidia were produced by growing two different isolates of *H. mammatum* together to stimulate conidiogenesis by the mycelial interaction. Single ascospore isolates 50-52-2 and 30-99-1 were from the collection of D. H. Griffin (16), and 191-1 and 191-6 were from the collection of P. D. Manion, College of Environmental Science and Forestry, Syracuse, NY. Small pieces of mycelium were inoculated separately on PDM and incubated at 28 C for 7–10 days until the colonies were 15–20 mm in diameter. Twenty mycelial plugs of each isolate were taken from the growing margins with an 8-mm cork borer and macerated separately with 10 ml of sterile deionized water in a Sorvall Omnimixer (Newtown, CT). Aliquots, 0.5 ml each, of two strains were pipetted onto PDM, spread over the surface with a bent glass rod, and incubated at 28 C for 2 wk. Conidia were washed from the plates with sterile deionized water, filtered through glass wool packed into the stem of a small funnel, and the concentrations of conidia and mycelial fragments were determined by microscopic count with a hemacytometer. Germination of conidia was tested by plating undiluted preparations on PDM. We observed the plates microscopically at ×200 and scored germination as germ tube emergence.

Stromata on a canker from Pompey were the sources of single ascospore isolates for the study of conidial morphology. Ascospores came from each of three different asci of three different perithecia; isolates 1, 2, 5, and 6 were from one ascus, 11 and 12 from another, and 17, 19, and 22 from the third. Conidia were produced by the isolates alone and from the pairings done on MEA. We measured 50 conidia of each isolate alone and from conidial interfaces of each pairing at ×1,000 using oil immersion (Zeiss Neofluar numerical aperture = 1.3, Carl Zeiss, Germany). Analysis of variance and means separation by least significant difference was done with Minitab (28) on an IBM XT computer.

RESULTS

Mycelial interactions. Mycelia of selfed colonies and identical twin ascospores merged with no noticeable interaction (Fig. 1A). The initial interaction between genetically different isolates was dense hyperbranching on the submerged hyphae of one isolate just as contact between the two mycelia occurred (Fig. 1B). These photographs show the usual one-sided reaction, but two-sided reactions with hyperbranching on both isolates were seen occasionally. There was no clear zone of growth inhibition. A ridge of dense aerial mycelium formed above the hyperbranched region (Fig. 1C), and conidia developed on this mycelium (Fig. 1D).

Pairings of mycelia from ascospores originating from one ascus always showed the presence of four groups. Thirty-eight asci were dissected, and 71% of the ascospores germinated. From these, 14 asci with six or more viable ascospores were selected for further study. These asci came from six different perithecia. Six of the isolates were slow growing with mycelium densely covered with conidia. These isolates did not react in mycelial interaction tests. In intra-ascus pairings, mergence occurred only between those isolates that were selfed or paired with an identical twin. All other combinations and all interascus pairings resulted in the formation of a conidial interface in the demarcation zone.

Pairings of mycelial isolates from the margins of cankers always showed mergence between isolates originating from the same canker and conidial interfaces between isolates from different cankers. Fifty mycelial isolations were obtained from the 10 cankers from the wild clone in Pompey. We recovered two to seven hypoxylon isolates from the 10 samples taken from each canker. All isolates from a single canker merged on pairing with one selected as a tester, indicating the presence of only one interaction group within a canker. In contrast, all tester isolates paired between cankers showed the formation of a conidial interface. There was no duplication of mycelial interaction phenotypes among the canker population. Successful isolations of the fungus were made from 29 additional cankers in a plantation at Tully, and no cankers yielded isolates in the same mycelial interaction group as any other canker. Isolates from Tully were not tested against those from Pompey.

Conidial germination and form. Production of large numbers of conidia for germination experiments was obtained by the mixed culture of two isolates of *H. mammatum*. Washing of conidia from mycelial lawns resulted in dense suspensions of conidia ($\sim 10^4$ ml^{-1}). Isolates plated by themselves conidiated undetectably or very sparsely, suggesting that all, or nearly all, conidia produced on these plates were the product of the mycelial interaction. Conidial suspensions contained many hyphal fragments; however, filtration through glass wool removed most of the hyphae (Table 1). Conidial germination ranged from 72 to 80%. Germinated conidia produced several short hyphal branches and an aerial branch (Fig. 2). Frequency of colony formation of similar conidial preparations was considerably less than the germination frequency (Table 1). However, more colonies formed than the estimated numbers of hyphal fragments by a factor of two to eight.

Conidial formation on MEA was pleomorphic with both nodulose development of *Nodulisporium* and geniculate development of *Geniculosporium* (Fig. 3). Both types of conidiogenous cells occurred separately and together on the same hypha. Isolates 2, 5, 11, and 19 produced only geniculate conidiphores (Fig. 3B,C), isolates 1, 6, and 12 were mainly geniculate but with some nodulose conidiogenous cells, and isolates 17 and 22 were primarily nodulose with some geniculate conidiogenous cells (Fig. 3A). Mycelial interaction testing showed that isolates 1 and 6 and isolates 17 and 22 were identical twins. Conidiogenous cells formed at the conidial interface between opposed isolates were mostly geniculate, but some nodulose forms were evident.

Conidia from six isolates grown separately and from the conidial interfaces of several pairings varied significantly (Fig. 4). Those of isolate 17 (mostly nodulose) were significantly larger, in both length and width, than those of the other strains. Conidia produced at the interface of pairings were usually larger than those of the parental strains alone. Interface conidia were always longer than those of the parental strains, significantly so for pairings 12 + 5, 11 + 5, 19 + 2, and 17 + 2 (Fig. 4A). Widths of interface conidia were usually intermediate between the two isolates, but in three cases they were somewhat larger than both parents but not significantly so (Fig. 4B).

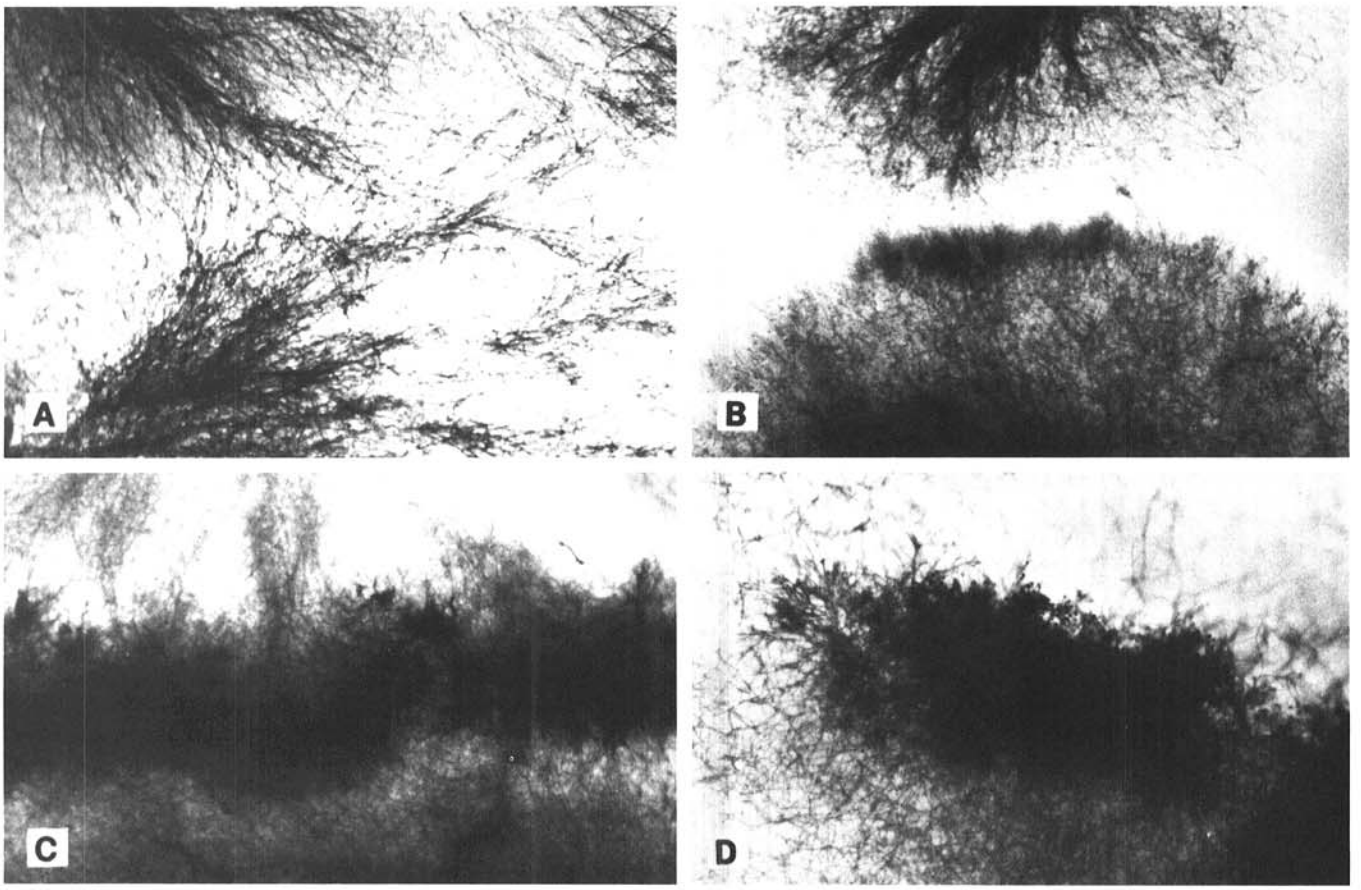

Fig. 1. Interaction morphology of mycelia of the same and different mycelial interaction groups in *Hypoxylon mammatum*. **A,** merging of mycelia from a selfed inoculation (×50). **B,** formation of the interaction interface of submerged mycelium at the time of first observation (×4). **C,** interaction interface of submerged mycelium 1 wk later (same culture). Note the lack of a cleared zone of inhibition (×4). **D,** conidia on aerial mycelium on one side of the interaction interface (×50).

TABLE 1. Colony-forming abilities of conidia produced by different pairings of mycelia of *Hypoxylon mammatum*

Paired isolates	Number per plate[a]		Colonies ± SD[b]	Colonies as percentage of particles plated	
	Spores	Hyphae		Spores	Hyphae
191-6 + 50-52-2	150	3.9	8 ± 4.6	5.3	205
191-1 + 30-99-1	570	3.4	27 ± 15.2	4.7	794
191-1 + 191-6	176	1.7	9 ± 2.8	5.1	529

[a]Number of replicate plates for each pairing were six for 191-6 + 50-52-2, three for 191-1 + 30-99-1, and two for 191-1 + 191-6.
[b]Standard deviation.

DISCUSSION

The mycelial interactions that we observed were similar in some respects to vegetative compatibility that occurs in both sexually and asexually reproducing fungi. Vegetative compatibility may be most clearly seen in vitro as a zone of inhibition with a conidial interface (2,25). The genetic control of incompatible interactions in vegetative compatibility systems is heterogenic and allelic, and an incompatible reaction requires that both mycelia contain different alleles in at least one of several loci (3,8). Identification of vegetative compatibility phenotypic differences in wild populations of fungi has been used to investigate population structure from which one can infer the relative extents of sexual and asexual propagation of the fungi in nature (1,24). Vegetative compatibility-like mycelial reactions have been shown previously in *H. mammatum* (5,7,13,18,29). The interaction that we found was heterogenic, that is, genetic differences between two paired isolates resulted in the conidial interface reaction. The polygenic nature of this phenomenon was indicated by the results of the ascus dissections. The role of these interactions in controlling heterocaryosis has not been demonstrated for *H. mammatum*.

Bier (7) described the responses of interascospore mycelial pairings in detail showing that four different groups of spores occurred from each ascus, although the vegetative compatibility phenomenon was not recognized until later (15). We confirmed Bier's observations indicating a heterogenic interaction, although the response we observed was different. We made use of this technique to examine the genetic structure of *H. mammatum* populations. Our data show that the mycelial interaction phenotypes clearly identify genotypic identity at several genetic loci and contrastingly, genotypic differences at these same loci. In this sense, and in the stimulation of conidiation, the phenomenon that we observed is similar to vegetative compatibility.

Although many investigators assume that ascospores are the principal agents of spread of this fungus (21,27), little evidence for this hypothesis has been obtained. Several investigators suggested that conidia or mycelial fragments also function in dispersal (4,17,22). Our results, which showed that each canker consisted of a single mycelial interaction phenotype and that no neighboring cankers shared phenotypes, argues for the nearly exclusive functioning of ascospores as inoculum in nature. This exclusivity is even more pronounced than that shown for *Leucostoma persoonii* on peach (1), and it is very different from the extensive asexual propagation shown for *Leucocytospora kunzei* on spruce where fungi from cankers on the same tree and among closely spaced trees belonged to only one or very few vegetative compatibility groups (24).

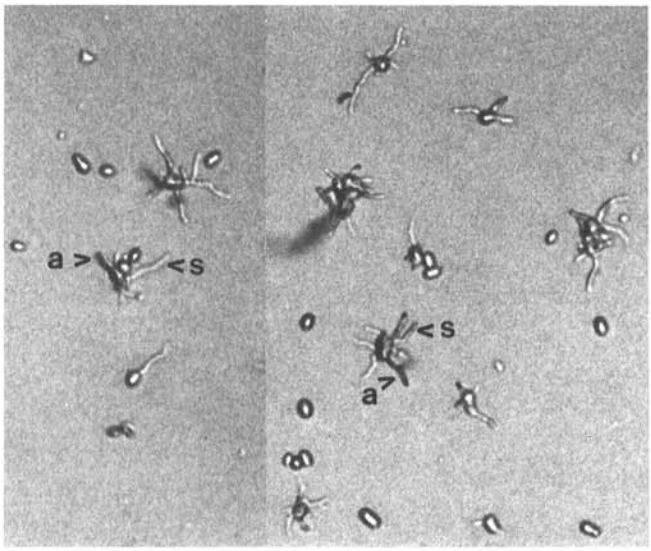

Fig. 2. Germinating conidia of *Hypoxylon mammatum* with short surface hyphae (s) and aerial hypha (a) (×300).

There are two possible explanations for the results from the spore germination and colony formation experiments. The first is that these results represent the true colony formation ability of conidia, and the small hyphal fragments in the conidial suspensions were not viable or contributed insignificantly to the numbers of colonies. The second explanation is that all colonies developed from hyphal fragments, and the number of fragments was underestimated by the hemacytometer counts to variable extents. The counts of hyphal fragments were low so that accuracy was questionable. Furthermore, only very small fragments, frequently little bigger than conidia, were in the preparations. Such fragments were difficult to distinguish from conidia at ×100. We have no information about the viabilities of these fragments. It is possible that such small hyphal fragments were not viable. The colony frequencies were always a relatively constant proportion (5%) of the plated conidia, suggesting that this was a consistent property of conidia. Whichever explanation for the colonies formed by conidial suspensions is true, one thing is clear, viability of conidia estimated by germ tube production greatly exceeded their ability to continue growth and form normal mycelial colonies in vitro. Some authors reported extensive colony formation from conidia (23,29), but our results agree with those of Bier (7) and of Bagga et al (5), while the observation of Rogers and Berbee (27), that no colony formation occurred from otherwise germinable conidia, was even more extreme. Clearly the interference of growth by small hyphal fragments must be controlled in experiments with conidia of this fungus.

Bier (7) also observed that conidia collected from natural cankers germinated readily in hanging drop cultures, but only a few ever developed into mycelia. The poor colony-forming capabilities of conidia in vitro suggests that they may not play an important role in spread of the fungus. Microconidia of *Neurospora crassa* also gave low frequencies of colony formation, ranging from 1 to 20% among preparations (11). Dodge demonstrated that microconidia of *N. sitophila* can act as spermatia, but he also claimed that microconidia germinated and grew into mycelia, although outgrowth of mycelium after germination was considerably delayed compared to macroconidia or mycelial fragments (9). Microconidia of *Sclerotinia gladioli* did not yield "appreciable growth through germination," but they did act to spermatize apothecial initials (10). A possible role for conidia of *H. mammatum* as spermatizing elements has not been demonstrated.

The hypothesis that conidia may be effective for infection of aspen implies that the continued outgrowth of the conidial germ tubes requires special nutrients or conditions supplied by the host that are not required in vitro either for germination or for mycelial growth. It seems more likely that the conidia of *H. mammatum* are comparable to the microconidia of *N. crassa* and *Sclerotinia* and serve primarily as spermatia. What limits the conidial germ tubes from developing the normally unlimited growth pattern of the mycelium is not known.

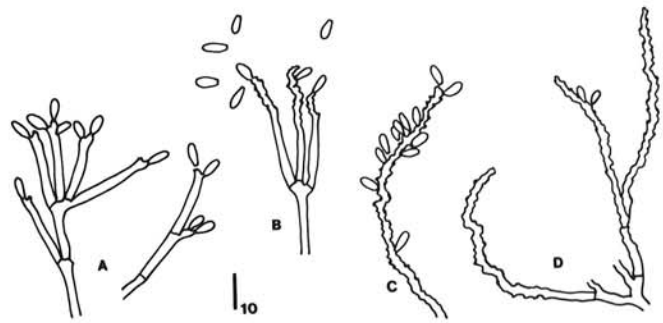

Fig. 3. Development of conidiogenous hyphae of *Hypoxylon mammatum*. **A,** nodulose development from strain 17 at 16 wk. **B,** geniculate development from strain 2 at 9 wk. **C,** geniculate development from strain 11 at 10 wk. **D,** geniculate development from interaction of strains 5 and 19 after 15 wk. All ×1,000; bar = 10 μm.

The variation in conidiogenesis that we observed suggests separate genetic control of these forms during *H. mammatum* development. While Bier observed only nodulose conidiogenous cells (7), Ponomareff illustrated both nodulose and geniculate forms without comment on the variation (23). Some species of *Hypoxylon* have nodulose conidiogenous cells and others have geniculate conidiogenous cells (14,20), but *Rosellinia subacutta* (synonym *Hypoxylon subacutum*) is the only other species to have both types (14,20,26). Among the nine monoascospore isolates from three asci, only two isolates had nodulose anamorphs. The geniculate anamorph was more prevalent.

The interactions between strains in the interface between opposed mycelia clearly stimulated conidial development. The significant differences in size, especially the tendency in the interface to produce conidia larger than either parent, suggests a genetic interaction that might be the result of heterocaryosis. The inability of these conidia to form mycelia after germination means that we cannot readily determine the genetic properties of the conidia.

The evidence we have presented supports the hypothesis for distinctive roles for ascopores and conidia in the disease cycle of *H. mammatum*. The mycelial interaction phenotypes clearly identified genetic differences among strains of the fungus and allowed the demonstration that all cankers tested consisted of a single resident mycelial phenotype, implying that each canker originated from a single spore infection. These data also demonstrated that each canker differed phenotypically, and by implication genetically, from its neighbors. From this we concluded that ascospores have an exclusive role in the dispersal of the fungus, and asexual propagules such as conidia and mycelial fragments are rarely involved. This conclusion was supported by the observation that viable, germinable conidia were at best only weakly capable of asexual propagation of the fungus. We suggest that their primary role is as spermatia, but have not demonstrated this function directly. The conclusion that each canker is composed of a single genetic type but that asci are the product of a heterothallic sexual system requires a mobile fertilization mechanism. This could easily be provided by the conidia functioning as spermatia. The morphological observations demonstrating two forms of conidial formation raise the question of whether there are functional differences among the conidia. Another interesting question raised by our observations is, what prevents conidial germlings from continuing to develop into normal mycelia? Additional study is needed to answer these questions.

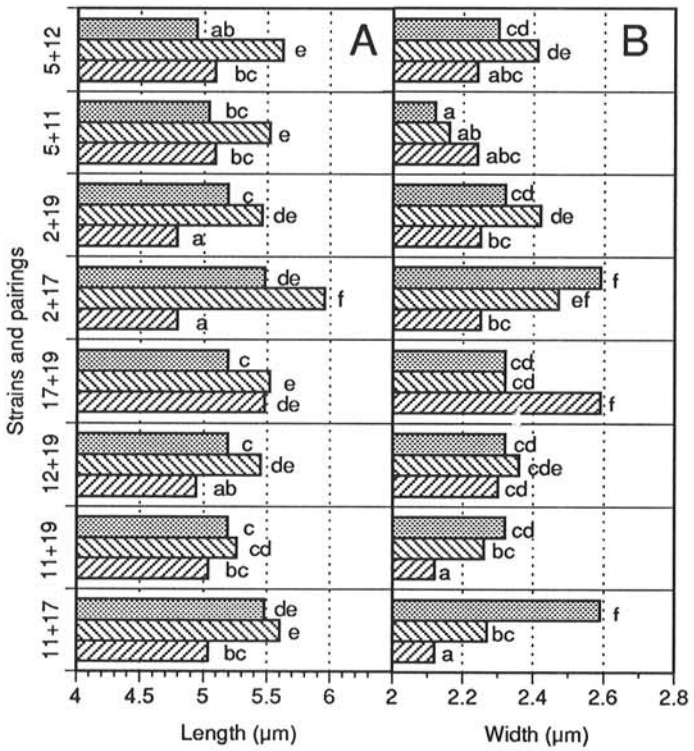

Fig. 4. Conidial size variation among strains and pairings of *Hypoxylon mammatum*. In each group of three bars, conidia from one isolate is on the top and the other is on the bottom. Conidia from the interface are in the middle. Bars with the same letter are not significantly different, $P > F \leq 0.05$, $n = 50$. **A,** length, least significant difference (LSD) = 0.227 μm. **B,** width, LSD = 0.125 μm.

LITERATURE CITED

1. Adams, G., Hammar, S., and Proffer, T. 1990. Vegetative compatibility in *Leucostoma persoonii*. Phytopathology 80:287-291.
2. Anagnostakis, S. L. 1977. Vegetative incompatibility in *Endothia parasitica*. Exp. Mycol. 1:306-316.
3. Anagnostakis, S. L. 1984. The mycelial biology of *Endothia parasitica*. II. Vegetative incompatibility. Pages 499-507 in: The Ecology and Physiology of the Fungal Mycelium. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, Cambridge.
4. Anderson, G. W., and Martin, M. P. 1981. Factors related to incidence of hypoxylon canker in aspen and survival of cankered trees. For. Sci. 27:461-476.
5. Bagga, D. K., Smalley, E. B., and Berbee, J. G. 1974. Conidial stage of *Hypoxylon pruinatum*: Factors influencing pillar production and conidial germination. Phytopathology 64:411-413.
6. Bélanger, R. R., Manion, P. D., and Griffin, D. H. 1989. *Hypoxylon mammatum* ascospore infection of *Populus tremuloides* clones: Effects of moisture stress in tissue culture. Phytopathology 79:315-317.
7. Bier, J. E. 1940. Studies in forest pathology. III. Hypoxylon canker of poplar. Can. Dept. Agric. Tech. Bull. 27: 1-40.
8. Brasier, C. M. 1984. Inter-mycelial recognition systems in *Ceratocystis ulmi*: Their physiological properties and ecological importance. Pages 451-497 in: The Ecology and Physiology of the Fungal Mycelium. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, Cambridge.
9. Dodge, B. O. 1932. The non-sexual functions of microconidia of *Neurospora*. Bull. Torrey Bot. Club 59:347-360.
10. Drayton, F. L. 1932. The sexual function of the microconidia in certain discomycetes. Mycologia 24:345-348.
11. Ebbole, D., and Sachs, M. S. 1990. A rapid and simple method for isolation of *Neurospora crassa* homokaryons using microconidia. Fungal Genet. Newsl. 37: 17-18.
12. Falk, S. P. 1987. Sensitivity to *Hypoxylon mammatum* toxic extracts and the incidence of hypoxylon stem canker in naturally occurring clones of *Populus tremuloides*. Ph.D. thesis. State University of New York, College of Environmental Science and Forestry, Syracuse, NY. 217 pp.
13. French, J. R. 1973. Host-pathogen relationships in hypoxylon canker of quaking aspen. M.S. thesis. State University of New York, College of Environmental Science and Forestry, Syracuse, NY. 51 pp.
14. Greenhalgh, G. N., and Chesters, C. G. C. 1968. Conidiophore morphology in some British members of the Xylariaceae. Trans. Br. Mycol. Soc. 51:57-82.
15. Gregory, P. H. 1984. The fungal mycelium—An historical perspective. Pages 1-22 in: The Ecology and Physiology of the Fungal Mycelium. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, Cambridge.
16. Griffin, D. H., Quinn, K., and McMillen, B. 1986. Regulation of hyphal growth rate of *Hypoxylon mammatum* by amino acids: Stimulation by proline. Exp. Mycol. 10:307-314.
17. Gruenhagen, R. H. 1945. *Hypoxylon pruinatum* and its pathogenicity on poplar. Phytopathology 35:72-89.
18. Hsu, B. C. 1975. Morphological variation and distribution of identical twin ascospores in *Hypoxylon mammatum* tetrads. M.S. thesis. State University of New York, College of Environmental Science and Forestry, Syracuse, NY. 52 pp.
19. Hubbes, M., and D'Astous, R. 1967. Factors affecting the germination of spores of *Hypoxylon pruinatum*. Can. J. Bot. 45:1145-1153.
20. Jong, S. C., and Rogers, J. D. 1972. Illustrations and descriptions of conidial states of some *Hypoxylon* species. Wash. Agric. Exp. Stn. Tech. Bull. 51 pp.
21. Manion, P. D., and Griffin, D. H. 1986. Sixty-five years of research on hypoxylon canker of aspen. Plant Dis. 70:803-808.
22. Ostry, M. E., Daniels, K., and Anderson, N. A. 1982. Downy woodpeckers—A missing link in a forest disease cycle. Loon 54:170-175.
23. Ponomareff, N. V. 1938. The conidial stage of *Hypoxylon pruinatum*. Phytopathology 28:515-518.
24. Proffer, T. J., and Hart, J. H. 1988. Vegetative compatibility groups

in *Leucocytospora kunzei*. Phytopathology 78:256-260.
25. Rayner, A. D. M., Coates, D., Ainsworth, A. M., Adams, T. J. H., Williams, E. N. D., and Todd, N. K. 1984. The biological consequences of the individualistic mycelium. Pages 509-540 in: The Ecology and Physiology of the Fungal Mycelium. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, Cambridge.
26. Rogers, J. D. 1985. *Hypoxylon duranii* sp. nov. and the anamorphs of *H. caries*, *H. papillatum*, and *Rosellinia subiculata*. Mycotaxon 23:429-437.
27. Rogers, J. D., and Berbee, J. G. 1964. Developmental morphology of *Hypoxylon pruinatum* in bark of quaking aspen. Phytopathology 54:154-162.
28. Ryan, T. A., Joiner, B. L., and Ryan, B. F. 1988. Minitab reference manual. Duxbury Press, Boston. 341 pp.
29. Sharland, P. R., and Rayner, A. D. M. 1989. Mycelial interactions in outcrossing populations of *Hypoxylon*. Mycol. Res. 93:187-198.