COMPETITIVE HIERARCHY IN POST-FIRE ASCOMYCETES

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SUMMARY

Inability to compete directly with other members of the carbonicolous community is examined as a factor contributing to the stratification of fungal sporocarp appearance in a heterotrophic succession following prairie burning. Evidence is presented that late successional species of carbonicolous ascomycetes from a prairie burn are uniformly antagonistic to species appearing earlier in the sere. Rapidly developing species such as *Gelasinospora calospora, Podospora curvicolla, P. glutinans*, and *Sordaria fimicola* were inhibited by diffusable substance(s) produced by *Podospora pilosa*, a slower-growing and later-sporulating form. Rate of colony growth was not always related to the order of appearance of post-fire ascomycetes. The authors suggest that the evolutionary outcome of interference competition among post-fire-ascomycete populations is a pattern of competitive hierarchy in which late-successional species are increasingly capable of dominating earlier-appearing species.

A succession of fungal sporocarps following spring burning of a tallgrass prairies has been characterized by Wicklow (1975). It has recently been proposed (Zak and Wicklow, 1978b) that the biological cause(s) of this disturbance-mediated heterotrophic succession may be related to a combination of growth rate and minimal time required for sporocarp production among individual members of the carbonicolous-ascomycete community in burned soil (El-Abyad and Webster, 1968)

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and to an overall pattern of competitive hierarchy in which late-successional species are increasingly capable of dominating early-successional species (Horn, 1977). Studies of competitive interactions among microbes in the post-fire soil environment have emphasized exploitation or interference competition involving carbonicolous fungi and components of the pre-burn microflora (El-Abyad and Webster, 1968; Peterson, 1970; Wicklow, 1975; Widden and Parkinson, 1975). No one has looked at potential competitive interactions among the carbonicolous-ascomycete populations themselves. The purpose of this study was to examine relationships between cultural antagonism (interference competition), growth rate, and the order of appearance of sporocarps in burned prairie soils.

MATERIALS AND METHODS

Isolation of soil ascomycetes was accomplished by incubating samples of burned prairie soil (University of Wisconsin Arboretum, Madison, Wisc.) for 60 da in Petri-dish moist chambers. With the aid of a compound microscope (100 ×), the undersurfaces of the Petri-dish covers were carefully examined for discharged ascospores and the spores identified by associating them with mature ascocarps on the soil surface. To trigger germination, ascospores were heat activated by exposing the covers to aerated steam at 55 C for 60 sec. Activated spores were then collected from the lids, suspended in sterile distilled water and transferred to culture tubes. Portions (0.5 ml) of the spore suspension were distributed to solidified cornmeal-acetate germination medium (Olive, 1956) with streptomycin (30 μg/ml) added to eliminate bacteria and actinomycetes. Plates were incubated at room temperature for a maximum of 24 h and examined periodically for germinated ascospores. Using a microscalpel, individual germinated spores were removed in small 1–2-mm-square agar blocks to individual slants of cornmeal agar containing streptomycin. If no bacterial contamination was observed, the isolate was transferred to a second cornmeal-agar slant without streptomycin. Since cornmeal agar promotes sporocarp development in most of these fungi, it was often possible to identify isolates directly from these slants.

Growth rates were determined for all isolates by inoculating plates containing buffered potato-dextrose agar (*p*H 6.25) at three points and measuring colony diam at intervals of 3, 7, and 10 da. Growth rates per da were determined when colony diam reached 25 mm or at 10 da, whichever came first.

Evaluation of cultural antagonism was accomplished by inoculating

duplicate plates of buffered potato-dextrose agar with carbonicolous ascomycetes in all possible combinations. A nutrient-rich medium such as this insures that any inhibition of growth that occurs is not the result of depletion of nutrients by the antagonist (Dennis and Webster, 1971). Phosphate buffers were added to minimize the possibility that inhibition was occurring as a response to a change in *p*H. Potential antagonists were inoculated at the centers of Petri dishes; three test organisms were placed equidistant from one another and from the center. Fungi with rapid rates of growth were inoculated 4 da after the other fungi. Plates were incubated at room temperature under constant illumination for 3 wk.

Reaction types listed by Johnson and Curl (1972) were ranked and numerical values assigned according to the follow scheme:

Reaction type	*Points*
A. Mutual intermingling of the two organisms.	0
B. Mutual inhibition on contact; the space between the two colonies is small, but clearly marked.	1
C. Mutual inhibition at a distance.	2
D. Inhibition of one organism on contact; the antagonist continues to grow, unchanged or at a reduced rate, through the colony of the inhibited organism.	3
E. Inhibition of one organism at a distance; the antagonist continues to grow through the resulting clear zone at an unchanged or reduced rate.	4

An index of antagonism (IA) was calculated for each organism by examining cultural reaction(s) from all tested fungi and applying the following formula: IA = reaction type $B(n \cdot 1) + C(n \cdot 2) + D(n \cdot 3) + E(n \cdot 4)$; where the types of reactions elicited by a species on all potential competitors are assigned points and the totals for each category summed.

Bioassays for antibiotic production in *Podospora pilosa* included the dialysis-membrane procedure and the culture-filtrate/paper-disc method. In the first, *Podospora pilosa* was inoculated onto a 30-mm-square sheet of dialysis tubing placed in the surface of buffered PDA in Petri dishes (Singh and Webster, 1973). The dialysis tubing was sterilized by autoclaving. Plates were incubated for 10 da at room temperature to allow the fungal metabolites to diffuse through the membrane into the agar. On the 10th da, the dialysis tubing and adhering fungal colony were removed and a test organism was point inoculated in the center of the zone previously occupied by *P. pilosa*.

The test fungi chosen were *Podospora curvicolla* and *Sordaria fimicola*, both of which appeared early in the successional sere, and both of which were inhibited at a distance by *Podospora pilosa*. *Iodophanus carneus* (Pers. ex Pers.) Korf, a rapidly growing discomycete recorded from burned soil (Peterson, 1970), also was tested and *Podospora pilosa* was examined for sensitivity to its own metabolites. Controls consisted of plates that had not been exposed to *P. pilosa*.

The production of metabolites in liquid medium was investigated using the following procedure. Four 250-ml flasks containing 100 ml of buffered potato-dextrose liquid (PDL) were inoculated with 2 ml of an aqueous suspension of blended mycelium harvested from cultures of *P. pilosa* growing on agar slants. Following incubation on a rotary shaker for 7 da at room temperature (24 C), culture filtrate was passed first through Whatman filter paper, then fiber glass, and sterilized by millipore filtration.

Preliminary isolation of antibiotic substances involved chloroform extraction of culture filtrate (Singh and Webster, 1973). Following evaporation of the chloroform, the remaining residue was redissolved in 5 ml of chloroform. Analytical-grade filter-paper discs (12.7 mm diam) were briefly submerged in this concentrated solution, air dried, and placed in the center of plates containing buffered potato-dextrose agar. After 24 h at room temperature, the discs were removed and a test organisms was inoculated at the position occupied by the paper disc. Extracted culture medium (PDL) which had not been inoculated with *P. pilosa* served as the control. Plates were prepared in triplicate and colony diam of the test organisms were measured after 1, 2, 3 and 4 da.

RESULTS

Growth rate, type of antagonistic response, and activity index were determined for seven carbonicolous ascomycetes from a tallgrass prairie (TABLE I). Species developing sporocarps within the first 2 wk (*Coniochaeta discospora, Sordaria fimicola, Gelasinospora calospora, Podospora glutinans*) grew at 2.3–14.3 mm/da) on potato dextrose agar while *Sporormiella pilosella* and *Podospora pilosa*, both appearing during the 6th wk of incubation, had average growth rates of 2.3 and 1.7 mm/da, respectively. The observation that *C. discospora* and *P. glutinans* had comparatively slow rates of growth when contrasted with *S. fimicola* and *G. calospora* argues against the hypothesis of growth-rate correlation with pattern of fungal sporocarp appearance. However, examination of the abilities of each species in the sere to inhibit the

growth of other populations shows that, in culture, the late-appearing species *S. pilosella* and *P. pilosa* are capable of inhibiting all of the earlier-appearing species. Both of these species had an index of antagonism of 21, while earlier-appearing species showed little or no antagonism to other members of the post-fire community (IA = 0–2).

Results of the dialysis-membrane assay for evidence of biological activity by *P. pilosa* are presented in TABLE II. The percent growth inhibition of *Iodophanus carneus, Podospora curvicolla,* and *Sordaria fimicola,* three early-appearing post-fire ascomycetes, was considerable. Forty-eight h after inoculation the test organisms, including *P. pilosa,* showed between 50–92% cultural inhibition relative to the control. Following this initial retardation in colony growth rate, *S. fimicola* matched the control by da 3, while the other test organisms were restricted throughout the 4-da test period. These results suggest that one or more low-molecular-weight compound(s), small enough to diffuse through a dialysis membrane, are toxic to the test organisms as well as to the fungus producing them. It is unlikely that depletion of nutrients in the test medium by *P. pilosa* caused inhibition of growth since a rich medium was used for short incubation periods.

A chloroform extract of cell-free culture filtrate was used in a paper-disc bioassay procedure to test for growth inhibition of *Iodophanus*

TABLE I

EVALUATION OF CULTURAL ANTAGONISM AMONG CARBONICOLOUS ASCOMYCETES

Taxa	Initial appearance (wk)[a]	Growth rate, mm per da	distributed according to reaction type[b]				Index of antagonism[c]
			B	C	D	E	
Coniochaeta discospora (Auersw.) Cain	2	2.3	0	0	0	0	0
Sordaria fimicola (Rab.) Ces. & DeNot.	2	14.3	1	0	0	0	1
Gelasinospora calospora (Mout.) C. & M. Moreau	2	14.3	1	0	0	0	1
Podospora glutinans (Cain) Cain	2	3.1	0	0	0	0	0
Podospora curvicolla (Wint.) Niessl	3	3.3	2	0	0	0	2
Sporormiella pilosella (Cain) Ahmed & Cain	6	2.3	1	0	0	5	21
Podospora pilosa (Mout.) Cain	6	1.7	1	0	0	5	21

[a] From Zak and Wicklow (**1978b**).

[b] Reaction types after Johnson and Curl (**1972**); see text.

[c] Index of Antagonism (IA) = reaction type $B(n \cdot 1) + C(n \cdot 2) + D(n \cdot 3) + E(n \cdot 4)$; values obtained by examining all possible combinations of species listed in this table; see text.

TABLE II

ANTIBIOTIC ACTIVITY OF *Podospora pilosa* AS DETERMINED BY DIALYSIS MEMBRANE AND CULTURE FILTRATE/PAPER-DISC BIOASSAY[a]

	Test organism			
Da	*Iodophanus carneus* (%)	*Sordaria fimicola* (%)	*Podospora curvicolla* (%)	*Podospora pilosa* (%)
Dialysis-membrane bioassay:				
1	100	82	0	0
2	92	71	71	50
3	89	0	73	71
4	85	0	67	60
Chloroform extract of cell-free culture filtrate/paper disc bioassay:				
1	100	100	—	—
2	71	27	—	—
3	70	13	—	—
4	60	0	—	—

[a] Growth inhibition (%) = [colony diam (control)-colony diam (+ *P. pilosa*)/ colony diam (control)] × 100.

carneus and *Sordaria fimicola* (TABLE II). The inhibitory effect again was only temporary in the case of *S. fimicola*, since by da 4, colony growth equalled that of the control. Inhibition of *I. carneus* by da 4 remained significant (60%).

DISCUSSION

Fungal successions based on patterns of sporocarp development may not reflect actual changes in the dominance patterns of the fungal populations. Harper and Webster (1964) elegantly demonstrated that initiation of vegetative development among coprophilous fungi often is simultaneous with the incubation of moist feces and the interval required for sporocarp development may be an inherent feature independent of competitive interaction. El-Abyad and Webster (1968) suggested that the same phenomenon may be taking place following burning, the order of appearance of pyrophilous discomycetes in burned-over soil also being related to rate of reproductive maturation in individual species. Our results, based on colony growth rates on a solidified culture medium, show that growth rate is not the only factor determining timing of sporocarp development. *Coniochaeta discospora*, an early successional species following grassland fires, had a comparatively slow rate of growth equivalent to that of two later-appearing forms, *P. pilosa* and *S. pilosella*.

Interference competition was examined as a mechanism-producing structure in the carbonicolous ascomycete community. Hyphomycete dominance at levels approximating those in pre-fire soils probably defines the upper limit to the extent of any post-fire successional sere (Peterson, 1970; Wicklow, 1975). However, in the interval during which carbonicolous fungi develop sporocarps in prairies, interactions among post-fire colonists may have some effect in determining structure, composition, and developmental pattern in the ascomycete community. Our results indicate that two species appearing late in the successional sequence on burned prairie soil, *P. pilosa* and *S. pilosella,* were able to restrict colony growth of all of five earlier-appearing post-fire colonists tested. *Podospora pilosa* produces a diffusable metabolite(s) capable of inhibiting cultural growth of rapidly developing post-fire ascomycetes. We did not evaluate the biological activity of these fungal isolates toward soil hyphomycetes representing pre-burn dominants. It is possible that antagonism associated with slow growth and late appearance also is expressed in competitive interactions with soil hyphomycetes during recolonization of the burned soil surface. Whether antibiotics are produced by *P. pilosa* in burned soil remains to be shown. However, sensitivity of *P. pilosa* to its own antibiotics would seem to argue against the advantage of the ability to produce antibiotics as a mechanism of interference competition.

Antibiosis is one of many factors believed to play a role in determining the course of a microbial succession and ultimate community composition (Park, 1967; Christensen, 1969). Early colonists of substrates often exhibit a low tolerance to biologically formed inhibitors. Barton (1960) suggests, for example, that it is primarily sensitivity to antagonism that restricts *Pythium mamillatum* Meurs to the role of a pioneer colonist. Patterns of community development among carbonicolous ascomycetes may also be controlled in part by production and sensitivity to antibiotics. The evolutionary product of fungal antagonism among populations of carbonicolous ascomycetes appears to be a competitive hierarchy. This mechanism of succession contributes to increasing species richness by fostering distribution of finite resources among numerous post-fire colonists.

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