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SELECTIVE CONSUMPTION OF DECOMPOSING WHEAT STRAW BY EARTHWORMS

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Summary—Three species of earthworm, *Lumbricus terrestris* L., *Aporrectodea longa* (Ude) and *Allolobophora chlorotica* (Savigny), were offered a choice of mixtures of soil and small wheat straw fragments which had been inoculated individually with six saprotrophic fungi. All earthworm species showed preferences between the six fungal species offered. Early straw decomposers, capable of utilizing water-soluble sugars and cellulose, were preferred in most cases to the lignin-decomposing fungi characteristic of the later stages of decomposition. The removal of fungal-inoculated straw pieces from the soil surface by *L. terrestris* followed the same pattern. The palatability of two wheat pathogens to *L. terrestris* was found to be similar to that of the preferred saprotroph. The implications of these findings for fungal abundance and dispersal in wheat fields are discussed.

INTRODUCTION

Considerable interest has recently been directed towards the role of the soil fauna in the shaping of microbial communities by microbial grazing, disturbance and dispersal, thereby influencing decomposition and nutrient cycling (Anderson and Ineson, 1984; Seastedt, 1984; Visser, 1985; Lussenhop, 1992). Mesofauna, such as Collembola, are known to selectively consume fungal species (e.g. Visser and Whittaker, 1977; Moore *et al.*, 1987; S. A. Moody, pers. observ.), altering the competitive balance between microorganisms (Parkinson *et al.*, 1979; Newell, 1984a; Klironomos *et al.*, 1992) and influencing decomposition rates in some cases (Newell, 1984b). Similar work on earthworms using the kinds of fungal-colonized resources that occur in the field has not yet been reported.

Earthworms have been shown to selectively consume different types of plant material (Darwin, 1881; Satchell and Lowe, 1967; Wright, 1972; Piearce, 1989), and to select different fungal species when offered on filter paper discs (Cooke, 1983). The presence of fungal propagules in the earthworm gut, and in cast material, has been known for some time (Day, 1950; Hutchinson and Kamel, 1956; Parle, 1963) and earthworms have been implicated in both the reduction and dispersal of soil-borne animal and plant fungal diseases (e.g. Keogh and Christensen, 1976; Hampson and Coombes, 1989), and the spread of beneficial groups such as mycorrhizal fungi (e.g. Gange, 1993). Recent work in Australia (Stephens *et al.*, 1993, 1994) has revealed that the earthworm *Aporrectodea trapezoides* (Duges) can reduce the severity of disease caused by two wheat root pathogens, *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker and *Rhizoctonia solani* Kühn. The authors speculated that fungal consumption by the earthworm was one possible explanation of the results.

The aim of our investigation was to examine the possibility that earthworms may selectively consume fungal species grown on wheat straw (*Triticum aestivum* L. cv. Slepjner) residues, potentially altering the competitive balance between fungal species. This work is of particular interest, as the recent government burning ban on straw residues (Environmental Protection Act, 1990; Statutory Instruments, 1991a,b) is likely to result in a larger amount of unwanted plant material being retained in the field as the cost of baling and transporting excess residues is often prohibitive (Prew and Lord, 1988).

Initially six fungal saprotrophs associated with wheat straw decomposition were offered to three species of earthworm commonly found in arable land. The fungal species, chosen for their varying abilities to degrade different parts of the straw residue, were *Mucor hiemalis* Wehmer (unable to degrade cellulose or lignin), *Fusarium lateritium* Nees, a *Trichoderma* sp., *Chaetomium globosum* Kunze (cellulose degraders), *Sphaerobolus stellatus* Tode and *Agrocybe gibberosa* (Fr.) Fay. (cellulose and lignin degraders). The study was later extended to include *Gaeumannomyces graminis* and *Pseudocercosporella herpotrichoides* (Fron) Deighton which are responsible for the ubiquitous wheat diseases, take-all and eye-spot, respectively.

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MATERIALS AND METHODS

Isolation of fungi

M. hiemalis, *F. lateritium*, *Trichoderma* sp., *C. globosum* and *S. stellatus* were obtained as described by Robinson *et al.* (1993). *G. graminis* and *P. herpotrichoides* were isolated by D. Hornby on 50% PDA and 50% tech. (No. 3) agar. All fungal cultures were maintained on potato dextrose agar (PDA; Oxoid) at 25°C in the dark, or at approx. 18°C in the light, depending on species.

Consumption of a fungal inoculated soil–straw mixture

Wheat straw and soil were collected from a field site at the Lancashire College of Agriculture, Preston, U.K. Internodal sections of straw, stubble left standing after harvest, were ground (<250 μm) in a hammer mill. A "bait material", adapted from von Törne (1990), was prepared using 10.5% (w/w) powdered straw, 40.5% washed, finely sieved (89 μm) soil and 49% distilled water. Once prepared the bait material was spread evenly in a thin layer on the bottom of a glass Petri dish and autoclaved.

Fungal species were added to the bait material either as five drops of spore–mycelial suspension (*M. hiemalis*, *F. lateritium*, *Trichoderma* sp. and *C. globosum*), or as five 3-mm dia agar plugs (*S. stellatus* and *A. gibberosa*) and incubated at 25°C for 10 days. When the experiment was repeated the acceptability of the wheat pathogen *P. herpotrichoides* was compared to that of *F. lateritium*, *A. gibberosa* and an uninoculated control. *P. herpotrichoides* was inoculated onto the bait material as a spore–mycelial suspension. As various inoculation attempts were unsuccessful *G. graminis* was not used.

The bait–fungal material was inserted into holes in 14 cm long plastic strips (bait lamellae) which had been sterilized by soaking in 99% alcohol, each strip having a row of 16 holes of 1 mm spaced evenly down 70% of its length (adapted from von Törne, 1990). Lamellae, held in position by a centrally placed cork, were arranged radially in random sequence on the bottom of a plastic bowl (dia 30 cm, height 12 cm) lined with moist paper tissue. Each bowl contained one lamella baited with each fungal species and one with uninoculated bait material.

Initially three earthworm species, *Lumbricus terrestris* L., *Aporrectodea longa* (Ude) and *Allolobophora chlorotica* (Savigny), were separately offered fungal inoculated bait material; in the succeeding trial only *L. terrestris* was used. After rinsing in tap water four adults were added to each bowl in the experiments using *L. terrestris* and *Ap. longa*, 10 adults in the case of *A. chlorotica*. Each trial comprised 20 replicates, with bowls being kept in the dark at room temperature (approximately 18°C). The number of baits taken from each lamella was recorded daily for 4 days.

Consumption of fungal-covered straw pieces

Straw pieces were made by cutting internodal sections of stubble into 1 cm lengths and splitting these lengthwise to form two equal-sized pieces (width approximately 4 mm). A small hole was burnt in the centre of each straw piece using a hot wire, allowing a small piece of coloured cotton thread to be tied around each piece to facilitate the identification of its inoculum (lack of inoculum for the control). A test to determine whether *L. terrestris* showed a preference for any of the thread colours revealed no significant discrimination.

Straw pieces marked with thread were autoclaved, inoculated with a spore–mycelial or mycelial suspension, a 3 mm dia agar plug in the case of *G. graminis*, and incubated at 25°C for 10 days. Controls comprised straw pieces treated with sterile distilled water. In the initial experiment 14 straw pieces, two per treatment, were placed in randomized sequences on the surface of sieved (1 cm mesh) field soil in each of 21 plant pots (15 cm dia, 15 cm deep) containing two adult *L. terrestris*. The pots were covered and kept in the dark at 14°C for 16 h, followed by 10°C for 8 h, each day. In the second trial five *L. terrestris* were placed in each of 10 bowls (30 cm dia, 12 cm deep) filled with sieved field site soil. Six straw pieces of each type were placed randomly on the soil surface and each bowl was covered and kept in the dark at approximately 18°C. Numbers of each type of straw piece taken were recorded daily for 7 days in each experiment.

Statistical analysis

Only the final set of readings (day 4) for soil–straw mixtures were examined statistically. Data were square-root transformed and compared using ANOVA and Tukey's HSD comparison of means (Minitab). The data obtained for straw pieces can be treated as discrete time-survival data (Aitkin *et al.*, 1987) or interval-censored survival data (Collett, 1993), and were modelled assuming a binomial distribution with a logistic link, with effects for day and fungus being included (GLIM 4; Francis *et al.*, 1993). Differences between fungi were assessed by examining the differences between their parameters in the model. When these differences are divided by their standard error, the resulting statistic has a Normal distribution (e.g. Collett, 1991, p. 59).

RESULTS AND DISCUSSION

In the experiments involving the consumption of fungal-inoculated bait material replicates were only included in the analysis when $>5\%$ of the baits available had been consumed. This resulted in 12 replicates being used when preferences were examined for *A. chlorotica* (Table 1) and 15 replicates when *L. terrestris* was offered *P. herpotrichoides*, *F. lateritium*, *A. gibberosa* and a control (Fig. 1).

Table 1. Mean number ±SE of soil–straw baits, inoculated with different saprotrophic fungal species, taken by *L. terrestris*, *Ap. longa* and *A. chlorotica**

	L. terrestris	*Ap. longa*	*A. chlorotica*
F. lateritium	12.8 ± 0.6(a)	7.9 ± 1.1(a)	7.4 ± 1.5(a)
Control	12.0 ± 0.6(ab)	6.9 ± 1.1(a)	3.9 ± 1.0(ab)
M. hiemalis	11.8 ± 1.0(ab)	6.4 ± 1.0(ab)	4.5 ± 1.5(ab)
Trichoderma sp.	10.9 ± 0.8(ab)	7.0 ± 1.1(a)	5.2 ± 1.9(ab)
C. globosum	9.2 ± 1.1(bc)	4.4 ± 0.7(abc)	0.6 ± 0.3(b)
A. gibberosa	5.8 ± 0.8(cd)	2.2 ± 0.6(c)	3.3 ± 1.2(ab)
S. stellatus	3.6 ± 0.8(d)	3.0 ± 0.8(bc)	4.8 ± 1.6(ab)

*Means, within each experiment, have different letters where significantly different, $P < 0.05$, Tukey's HSD comparison of means.

In the initial experiment *F. lateritium* inoculated bait was taken most often by all three earthworm species, with *Trichoderma* sp. and *M. hiemalis* being consumed slightly less frequently (Table 1). The preferences recorded for *L. terrestris* and *Ap. longa* were very similar, with the two basidiomycetes, *S. stellatus* and *A. gibberosa* being taken significantly less often than *F. lateritium* and *Trichoderma* sp., in both cases ($P < 0.05$). *A. chlorotica* differed in that bait inoculated with *C. globosum* was taken least often, while *S. stellatus* was the third most consumed fungal species. When the experiment was repeated to include *P. herpotrichoides*, very similar results were obtained, with *F. lateritium* again being taken significantly ($P < 0.05$) more often than *A. gibberosa* (Fig. 1). In this case, however, the wheat pathogen, *P. herpotrichoides*, was ranked higher than *F. lateritium*, with slightly more baits being taken by day 4.

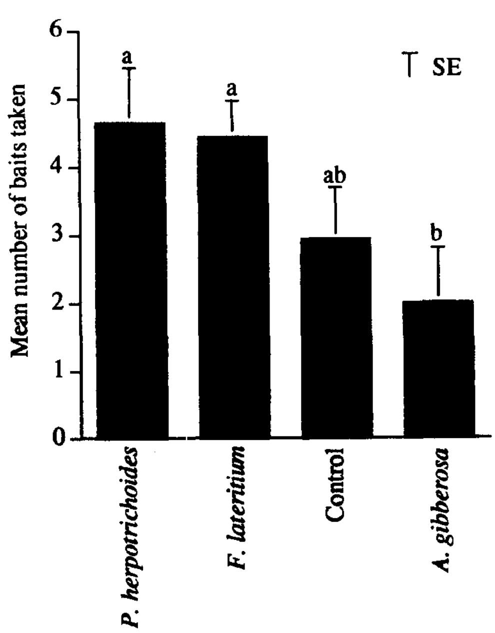

Fig. 1. Preferences shown by *L. terrestris* for soil–straw baits inoculated individually with one pathogenic and two saprotrophic fungal species. Means have different letters where significantly different, $P < 0.05$, Tukey's HSD comparison of means.

Very similar results were obtained when *L. terrestris* was offered fungal-covered straw pieces. *F. lateritium* inoculated material was taken most often as indicated by cumulative totals over all 21 pots [Fig. 2(a)]. *S. stellatus*, the only fungus to show a marked deviation from the rank of preference found for *L. terrestris* in the bait material experiment, was taken third after *Trichoderma* sp.. Straw pieces inoculated

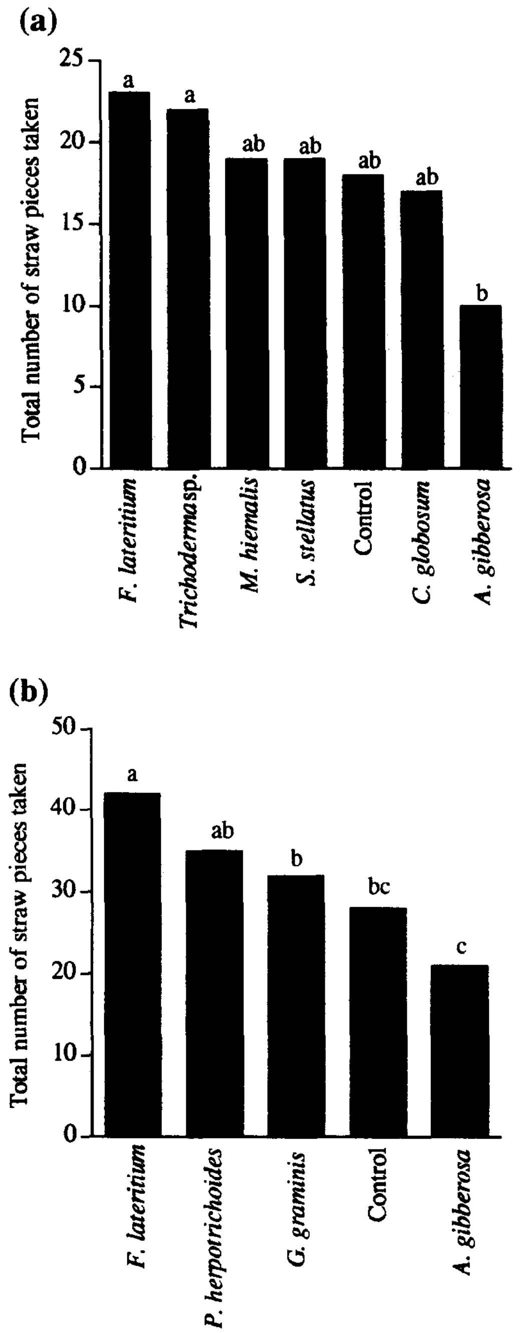

Fig. 2. Total number of straw pieces, inoculated with (a) different saprotrophic fungal species and (b) two saprotrophic and two pathogenic fungal species, taken by *L. terrestris* by day 7. Species with different letters are significantly different in the discrete time model, $P < 0.05$.

with *F. lateritium* or *Trichoderma* sp. were significantly preferred ($P < 0.05$) to those inoculated with *A. gibberosa*, the least taken of the fungal species examined. When the experiment was repeated using the two wheat pathogens, *F. lateritium* was again significantly preferred to *A. gibberosa* ($P < 0.05$), as was *P. herpotrichoides*, which was taken almost as frequently as *F. lateritium* [Fig. 2(b)]. The second pathogen studied, *G. graminis*, was also taken significantly more often than *A. gibberosa*, but significantly less frequently than *F. lateritium* ($P < 0.05$).

The reason why earthworms consume organic matter colonized by some fungal species in preference to that colonized by others could include: (1) digestion of fungal tissues as a nutritional supplement (Piearce, 1978; Flack and Hartenstein, 1984; Dash *et al.*, 1986), possibly enhancing the C-to-N ratio found in plant tissue (Satchell, 1983); (2) digestion of fungal tissues as the principle source of nourishment (Morgan, 1988); or (3) detoxification or other beneficial conditioning of plant residues (Satchell and Lowe, 1967; Wright, 1972; Cooke and Luxton, 1980). The effects of such selectivity would be that the fate of heavily-grazed fungal species may be determined by their ability to survive passage through the earthworm gut, with species which are destroyed being reduced in number within the soil matrix, and those able to survive gut passage being dispersed by earthworm movement, enhancing their spread to new areas. Species which are rejected by earthworms may grow unhindered, but the possibility of their dispersal by earthworm activity will be low. Suggestions that preferential consumption of fungal species by earthworms may be occurring in the field have been made by several authors (e.g. Dash *et al.*, 1986; Striganova *et al.*, 1989) after examination of the contents of the alimentary tracts of earthworms and comparison with the surrounding soil. Such preferential consumption of saprotrophic fungi by earthworms could ultimately influence the rate of organic matter decomposition as noted by Newell (1984b) for Collembola. Consumption of pathogenic species could enhance or reduce the incidence of disease in successive crops.

It is possible that, with sufficient knowledge about earthworm microbial interactions, earthworm populations could be managed to provide soils in which crop productivity is increased and disease severity is decreased. Similar suggestions have been made by Doube *et al.* (1994), who have shown that earthworms can be used to transport *Rhizobium*, causing increased colonization and nodulation of legume roots, and *P. corrugata* 2140R, a biocontrol agent for *G. graminis*.

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