Interactions of Collembola and Microflora of Cotton Rhizosphere

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ABSTRACT

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Proisotoma minuta and *Onychiurus encarpatus*, the most prevalent rhizosphere species of Collembola in Alabama cotton fields, feed on both plant pathogens and saprophytic fungi. These insects readily transported fungal spores and bacteria through sterilized soil, resulting in the colonization of dead organic matter, cotton rhizosphere, and live roots with various organisms, including *Fusarium oxysporum* f. sp. *vasinfectum*. *Rhizoctonia solani* was not transported in quantities sufficient to colonize substrates. The animals were most attracted to roots during periods when the soil was drying slowly. A potential role is postulated for Collembola that may be disease-related, ie, transmitting spores to the rhizosphere and reducing inoculum density by feeding on pathogen mycelia.

Additional key word: microarthropods

Kevan (12) pointed out that populations of soil microarthropods may be substantial and that Collembola and Acarina usually are the most abundant of soil-inhabiting animals exclusive of protozoa and nematodes. Most previous investigations of Collembola have dealt primarily with populations in grassland soils and forest litter (6,7,8); numbers were generally considered small in cultivated soils where organic matter was relatively low. Wallwork (21) has provided pertinent references to the distribution of Collembola and the effects of various agricultural practices on their activities. However, investigations of rhizosphere ecology have given virtually no attention to the influence of the immediate root zone and the possible interactions between arthropods and microflora.

Two species of Collembola, *Proisotoma minuta* Tullberg (Isotomidae) and *Onychiurus encarpatus* Denis (Poduridae), were predominant in Alabama cotton fields through a growing season and, by the extraction method used, were more abundant than the Acarina (22). Supplementary inorganic fertilization induced higher Collembola numbers, and populations were invariably greater in rhizosphere soil than 20 cm distant from roots. *P. minuta* was the more common of the two species.

Our investigation was designed to determine: (i) predominant genera of fungi and bacteria naturally transported by Collembola, (ii) interactions between rhizosphere fungi and the insects, (iii) migration of Collembola to cotton roots, (iv) role of the insects in the microbial colonization of organic matter and living cotton roots, and (v) effect of Collembola-transported rhizosphere microorganisms on cotton seedlings.

MATERIALS AND METHODS

Sample collection and extraction. Because the usual method of collecting rhizosphere soil, ie, lifting whole root systems and shaking or washing off soil, was not feasible for rapidly moving insects, a conventional soil sampling tube (2.2 cm i.d.) was used. The tube was inserted against a cotton plant stem parallel to the taproot to a depth of 20–25 cm, and the sample was ejected quickly into a plastic container. Parts of the lateral root system were obtained with each sample. Obviously, each sample contained some nonrhizosphere soil, but our previous study (22) showed striking differences in numbers of Collembola by this method; therefore, the term "rhizosphere soil" is retained here as distinguished from soil some distance from a root. Random samplings were made over four widely separated cotton fields of known fertilization and cropping histories in Alabama. Soils represented were Dothan sandy loam, Hartsells fine sandy loam, Decatur clay loam, and Lucedale sandy clay loam.

The Tullgren (20) extraction system (Fig. 1) was employed so that only living animals were collected. Glass funnels with 1,000–1,200 g of field soil were arranged in series under 40-W light bulbs positioned 19 cm above the soil; this maintained a surface temperature of 35 C. The funnel stem extended into a 50-ml glass collecting tube standing inside a 1-L Erlenmeyer flask, the stem end positioned a few millimeters above the surface of 10 ml of water in the tube. Each apparatus was wrapped in paper toweling to reduce water condensation on the surface of the glass funnel. As the soil slowly dried during a period of 5–7 days, the insects moved downward and dropped onto the water in the collection tube. They were then decanted onto a microsieve and refloated in a shallow dish of water for microscopic counting, washing, or transferring.

Isolation of Collembola-transported flora. Fungi were isolated from the body surface of unwashed insects, and both fungi and bacteria were isolated from the interior of washed insects. For surface isolations, 30 insects representing a mixture of the two species from each cotton field were picked individually from the surface of collection water, using a very fine dental root-canal file. They were then transferred to six sterile, glass petri dishes, five insects per dish. A thin layer of Ohio agar medium (11) was poured into the dishes, and after 6 days' incubation at 25 C, distinct fungal colonies growing from the insect bodies were counted. Transfers were made to potato-dextrose agar (PDA) and to Czapek-Dox agar (CDA).

For isolating internal organisms, 100 insects from the composite soil sample of each field were placed in a microsieve standing in a dish of sterile demineralized water that contained a few drops of Tween 20 (polysorbate). The insects were swirled in this solution for 10 min and rinsed in a stream of demineralized water for 25 min. They were then backwashed into a sterile tissue-grinding mortar tube (124 × 16 mm, 7-ml capacity) containing 2 ml of sterile water. The insects were slowly triturated by hand for 20 sec, and 0.2 ml of

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the suspension was delivered to each of five sterile petri dishes. Ohio agar medium, cooled to 50 C, was poured into the dishes and swirled before solidification. From the remaining suspension, 0.5 ml was delivered to 24.5 ml of sterile water, and 0.1 ml of this was delivered to five petri dishes. Thornton's standardized bacterial medium (11) was poured into these dishes, and all were incubated at 25 C for 5–7 days. Resulting colonies were counted, and fungi were transferred to PDA or CDA for identification. Bacterial genera were identified by standard procedures (5).

Feeding and reproduction observations and tests. Populations of Collembola were maintained on PDA cultures of *Rhizoctonia solani* Kuhn in petri dishes kept in plastic bags at 100% relative humidity and incubated at 25 C. Insects were transferred to young cultures of the fungus every 6 wk. Preliminary microscopic observations were made on their movement, feeding, and reproduction in the presence of cultured fungi that produced no spores, small spores, or large spores; these were *R. solani*, *Trichoderma harzianum* Rifai, *Fusarium oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Snyder & Hansen, and *Curvularia lunata* var. *aeria* (Batista, Lima & Vasconcelos) Ellis. Some insects were placed on water agar, PDA, and CDA to observe their activities in the absence of fungi.

A study was then conducted to determine the influence of fungi in soil culture on survival and reproduction of Collembola. The fungi were *F. oxysporum* f. sp. *vasinfectum*, *Pythium aphanidermatum* (Edson) Fitzpatrick, *R. solani*, *C. lunata* var. *aeria*, *Penicillium herquei* Bainier & Sartory, *Aspergillus flavus* Link, and *T. harzianum*. A fertile sandy loam was screened through a 4.75-mm mesh sieve and dispensed in 100-g quantities into 40 large test tubes (20 × 150 mm). After the soil was sterilized by autoclave and amended with 10 ml of potato-dextrose broth (PDB), five soil tubes for each fungus and five control tubes without fungi were prepared aseptically. Tubes of soil were inoculated by delivering 2 ml of mycelial suspension of each fungus to the soil surface. The suspensions were prepared by chopping 35 disks (5 mm diameter) cut from the colony edge of 3-day-old cultures of the test fungi in 100 ml of sterile PDB for 10 sec in a semimicro mixer. Control tubes received 2 ml of PDB only.

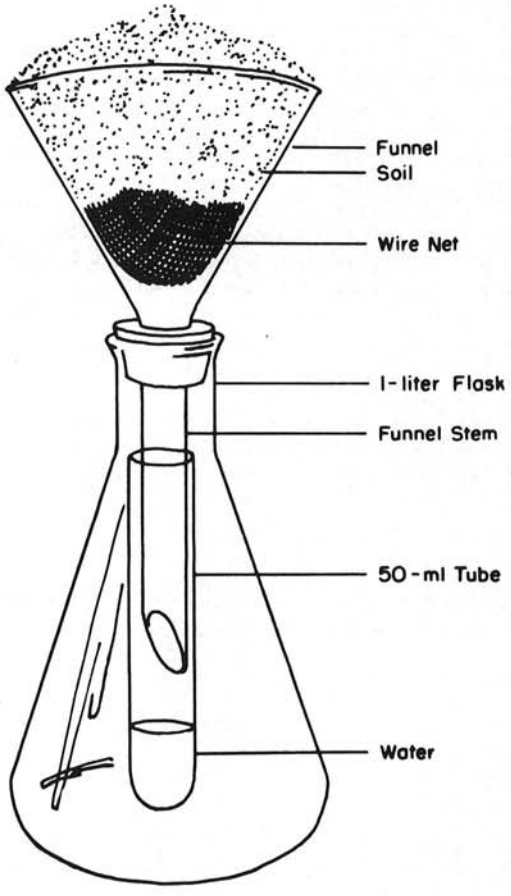


Fig. 1. Modified version of the Tullgren system for extracting live Collembola from soil. When placed under a 40-W light bulb, the soil dries slowly, forcing downward migration of the insects, which drop onto the water surface in a collection tube.

Collembola were extracted from field soil, and 100 mature insects of a single species, *P. minuta*, were placed in each soil tube. The cultures were incubated at 25 C in darkness for 6 wk. Soil from the tubes of each fungus and soil from control tubes then were pooled and processed through a smaller version of the extraction apparatus described previously; total numbers of Collembola extracted were recorded. Additional tubes of soil with and without fungi had been prepared for use in determining percent of soil moisture content and pH of a 1:1 soil-water mixture.

Test for attraction of Collembola to the rhizosphere. Two soil types, Dothan sandy loam and Lucedale sandy clay loam, were used. The soils were sieved (4.75-mm mesh), moistened lightly, and autoclaved. The soils were dispensed through a funnel into clear, 72 cm^2 polystyrene tissue culture flasks of 250-ml capacity, leaving a 2.5-cm air space at the top.

A series of extraction funnels were filled with field soil, and each funnel stem was fitted into the mouth of a tissue culture flask so that insects fell directly onto the soil during a 5-day extraction period under lights. The funnels were removed, and a single germinated cotton seed was planted in each flask against the flat side. The seeds were acid-delinted, nontreated *Gossypium hirsutum* L. 'Stoneville 213' that had been surface-disinfested in a mixture of 2% sodium hypochlorite and 70% alcohol (20:1, v/v) for 30 min, then washed and germinated on CDA to select noncontaminated seedlings. The flasks were wrapped with aluminum foil to darken the developing root area and slanted at a 45° angle to promote root growth along the side. Flasks were capped until the seedlings became established (4 days). The caps were then removed, and the flasks were positioned under a combination of incandescent and fluorescent lights (about 6 klux) automatically controlled for 10-hr exposure alternating with a 14-hr dark period; constant light-dark air temperatures at the plant leaf surface were 27 and 25 C, respectively.

Six days after the seeds were planted, 10 ml of sterile demineralized water was added to eight flasks, four of each soil type; 5 ml was delivered on opposite sides of each plant with a 10-ml syringe equipped with a 14-gauge cannula 10 cm in length. The flasks were watered at weekly intervals throughout the 5 wk of the experiment. Water was not added to the other eight flasks, and the plants were allowed to wilt as the soil slowly dried. After the fifth week, the number of Collembola in the rhizosphere (surface of roots to within 5 mm of roots) was compared with that in nonrhizosphere soil. Two counts were made 48 hr apart in three stereomicroscope fields of 2.5 cm diameter on each flask.

Role of Collembola in microflora colonization of organic matter, rhizosphere, and rhizoplane. Two tests were devised to determine the insects' role in initiating colonization of fresh organic matter and of cotton rhizosphere or rhizoplane by microorganisms transported through soil. Collembola exposed to specific fungi were used in the first test, and field-collected insects with their natural microflora were used in the second.

Fertile sandy loam was air-dried, screened, moistened, and sterilized by autoclaving. Air-dried soybean stem sections (8–10 mm) were soaked in PDB for 24 hr and sterilzied. Soil tubes were prepared by aseptically placing the moistened sterilized soil and 30 stem sections in alternating layers in 20 × 150-mm glass tubes. The tubes, filled to two-thirds capacity, were closed with Styrofoam plugs; final soil moisture was 12% by weight. Cotton seeds were surface-disinfested as before and germinated on CDA, then transplanted into the tubes (one seedling per tube) when the radicles were about 1 cm long.

For the first test, sterile 1-ml beakers containing a thin layer of moist sterile soil were used to introduce Collembola into the tubes. Insects in this test were collected from 3-wk-old petri dish cultures of *F. oxysporum* f. sp. *vasinfectum*, *A. flavus*, and *T. harzianum*

where they had been placed when the cultures were young. Each beaker contained 100 insects from both species. After 48 hr of seedling growth, the beakers were placed bottoms down at an angle of about 30° in three of the large soil tubes so that as the beaker soil dried the insects migrated into the moist soil of the tubes (Fig. 2). Four soil tubes with seedlings and beakers of sterile soil without insects were used as controls.

In the second test, to determine the natural microflora that may be insect-transported to organic matter and roots, 5-ml beakers with sterile moist soil were used instead of water blanks as collecting vessels in the extraction apparatus. The beakers were placed on sterilized soil in 10 tubes, each tube containing 30 soybean stem sections and a germinated cotton seed. Because the insects were expected to contaminate the beakers of soil, controls were included to test for air-current contamination from beakers to tube soil. For this purpose, field soil in some beakers was air-dried to free it of insects, then remoistened and placed in 10 soil tubes. All tubes were wrapped in aluminum foil to keep roots in darkness.

After 7 days' incubation under a 10- and 14-hr light-dark regimen, the buried stem sections were recovered from each tube and tapped to remove loose soil particles. Fifteen of the sections from each tube of the first test were plated directly on acidified PDA, and 15 were washed serially in demineralized water and plated either on moist sterilized sandy loam or on water agar containing 2.5 μg of streptomycin sulfate per milliliter. After 3 days' incubation at 25 C, fungi growing from the sections were identified. All stem sections of the second test were washed, plated on streptomycin agar, and examined after 15 hr for *R. solani* and *Pythium* spp. and again at 3 days for other fungi.

To determine rhizosphere colonization, plant tops were cut off at the soil line, the root systems were removed gently, and loose soil was shaken off. Each root system was placed in 20 ml of sterile demineralized water in 50-ml Erlenmeyer flasks on a magnetic stirrer. The soil-water-root suspension was stirred for 5 min, then 0.5-ml quantities were removed from the vortex by pipette and delivered into five sterile petri dishes; Ohio agar medium was poured and swirled to mix. For bacteria, 0.1 ml of suspension was plated in Thornton's standardized agar. Dishes were incubated at 25 C for 5–7 days, and numbers of colonies per gram of soil were calculated on a dry-weight basis.

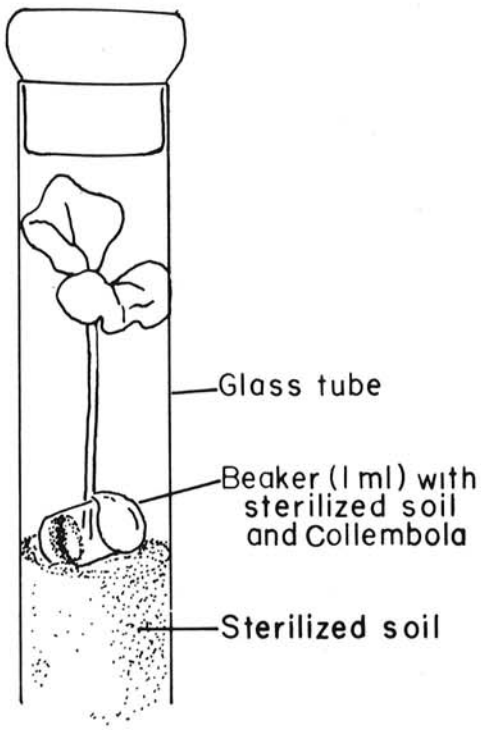


Fig. 2. Tube method of culturing cotton seedlings for tests with Collembola; the insects migrated from a sterile 1-ml beaker into the tube soil.

The root systems from the tubes were washed serially in four changes of demineralized water, then examined for injury. After the root-shoot length was measured, small sections of root were cut and plated on acidified PDA to determine rhizoplane colonization.

Test for transmission of Rhizoctonia mycelium. Tests were made to determine whether Collembola can transmit mycelium of *R. solani* through soil to dead organic matter and to cotton seedling roots. *F. oxysporum* f. sp. *vasinfectum* was included for reference.

Sterile soil tubes with buried soybean stem sections and clean, germinated cotton seed were prepared as before. Insects used were a mixture of the two species that had been feeding on 2-wk-old cultures of the two pathogens. Fifty insects from each source were placed in 1-ml beakers of moist sterile soil, and the beakers were laid on the tube soil surface as previously described; 10 tubes were prepared for each fungus. Ten tubes with beakers of sterile soil without insects were used as controls. The tubes, with root systems in darkness, were incubated for 14 days under alternating light and dark, and colonization of organic matter, rhizosphere, and rhizoplane was determined; roots were washed and examined for disease or other injury.

All tests described were repeated one or more times.

RESULTS

Fungi and bacteria isolated from field Collembola. Relative numbers of fungi and bacteria isolated from the body surface or interior of Collembola varied considerably between fields or plots for specific sampling dates, and no consistent trends were established to suggest influence of rhizosphere, fertilization (NPK), or soil type (data not presented).

Common fungi, primarily those producing small spores in abundance, were most frequently isolated from the insect bodies, both externally and internally (Table 1). *Penicillium* spp. comprised the highest percentage, followed by species of *Aspergillus*, *Trichoderma*, *Verticillium*, and *Cladosporium*. Species of *Fusarium*, *Alternaria*, *Cunninghamella*, and *Chaetomium* were infrequently isolated. *Acromobacter* and *Pseudomonas* spp. provided 54 and 43%, respectively, of the bacteria isolated from the interior of the insects.

Influence of fungi on activities of Collembola. The insects moved rapidly and easily among the hyphae of *R. solani* and of sparsely sporulating young cultures of other fungi. They survived on *Rhizoctonia* cultures for up to 5 mo and had a decided feeding preference for young hyphae rather than older mycelial strands or aggregations. Eggs in masses of 3–35 (Fig. 3) were produced within 48 hr after introducing adult Collembola into the fungal cultures. Abundant spores of *Trichoderma*, *Penicillium*, and *Aspergillus* spp. impeded Collembola activity; many conidia were visible on the

TABLE 1. Genera of fungi and bacteria isolated from the body surface and from the interior portion of field-collected Collembola[a]

	Percentage of total isolates	
Genus	Body surface	Body interior
Fungi		
Penicillium	60	47
Aspergillus	10	8
Trichoderma	9	12
Verticillium	9	11
Cladosporium	7	15
Fusarium	2	3
Alternaria	1	0
Cunninghamella	1	4
Chaetomium	1	0
Bacteria		
Acromobacter	...	54
Pseudomonas	...	43
Enterobacter	...	3

[a]Surface fungi were isolated from 30 Collembola (mixed species) from each of four locations in Alabama, and internal organisms were isolated from 100 animals representing each location. Bacteria were not included in the surface isolations.

insect bodies. Mature spores of *Verticillium* sp., *F. oxysporum* f. sp. *vasinfectum*, and *C. lunata* var. *aeria* were picked up readily by body hairs and antennae and transported, but immature spores did not detach readily. Collembola on water agar, PDA, or CDA survived and reproduced in the absence of specific fungi. They were observed eating the media, leaving shallow trenches behind. Bacteria and fungi from their bodies eventually developed over the agar, apparently providing a further food source. Species of *Penicillium*, *Aspergillus*, *Trichoderma*, and an unidentified fungus colonized and sporulated on dead insects (Fig. 4) and on exuviae.

TABLE 2. Influence of fungi on survival and reproduction of *Proisotoma minuta* in soil cultures

Fungal cultures	No. of Collembola[a]
Fusarium oxysporum f. sp. *vasinfectum*	197 a
Pythium aphanidermatum	143 b
Rhizoctonia solani	129 b
Curvularia lunata var. *aeria*	126 b
Penicillium herquei	103 c
Aspergillus flavus	94 cd
Trichoderma harzianum	83 cd
Control	70 d

[a]Initially, 100 Collembola were added to each tube of each fungal culture and control; numbers are averages of three tube cultures per fungus. Numbers followed by different letters are significantly different according to Duncan's multiple range test ($P = 0.05$).

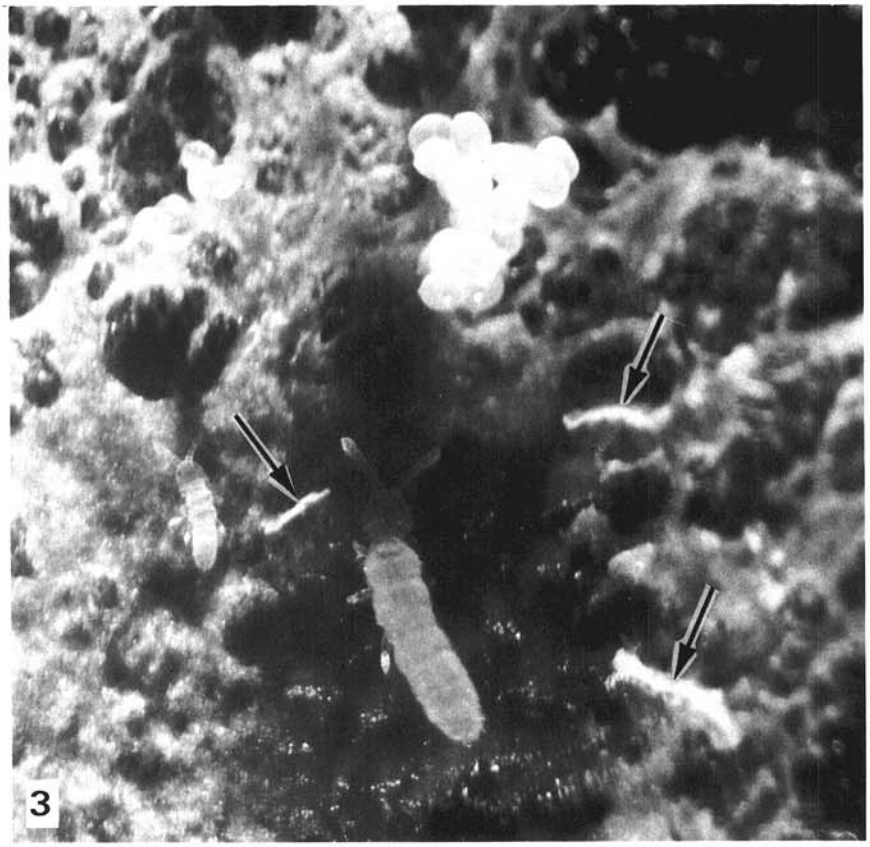
F. oxysporum f. sp. *vasinfectum* was most favorable for survival and reproduction of *P. minuta* in soil tube cultures; numbers nearly doubled during 6 wk in the presence of this fungus (Table 2). Significant ($P = 0.05$) increases also occurred in soil with *P. aphanidermatum*, *R. solani*, or *C. lunata* var. *aeria* but not in that with *P. herquei*, *A. flavus*, or *T. harzianum*; soil with the latter fungus had a 17% lower yield. Of *P. herquei*, *A. flavis*, and *T. harzianum*, only *P. herquei* supported a population significantly higher than that in control tubes without fungi, which showed a 30% decrease. Soil moisture was relatively constant (10.2–11.2%), and soil pH (6.1–6.6) was not a limiting factor in survival and reproduction of Collembola.

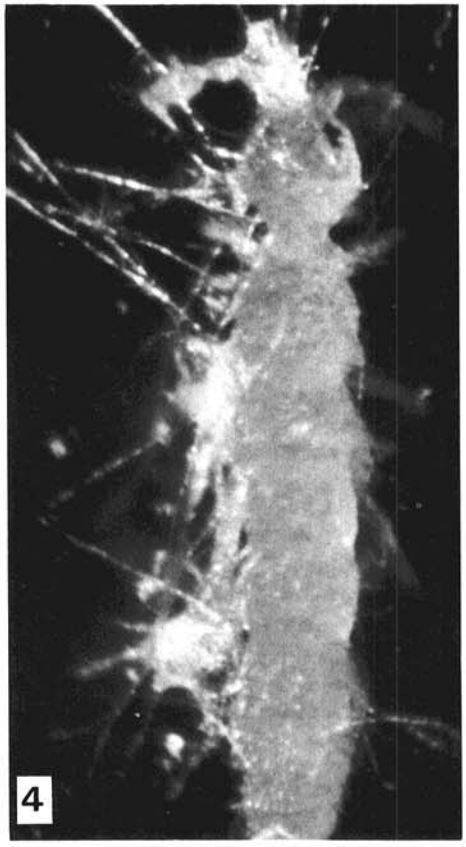
Attraction to rhizosphere in wet and drying soil. Field-collected insects in flasks of drying soil had a greater affinity for the root zone than did those in soil with adequate and evenly distributed moisture (Table 3). This movement to the root area was observed in both

TABLE 3. Influence of soil moisture on attraction of Collembola to cotton rhizosphere in vitro[a]

Soil type	Treatment	Soil moisture (%)	No. of Collembola	
			Rhizosphere	Nonrhizosphere
Dothan sandy loam	Watered	7.11	6	118
	Not watered	4.00	19	1
Lucedale sandy clay loam	Watered	7.04	10	78
	Not watered	4.79	56	9

[a]Collembola on roots or within 5 mm of a root were considered in the rhizosphere. Values are total numbers from two counts made 48 hr apart; a count consisted of three stereomicroscopic fields (2.5 cm diameter) per root system in each of four flasks. Compared values for rhizosphere vs. nonrhizosphere and watered vs. not watered were significantly different in both soils according to $\times^2$ analysis ($P < 0.001$).





Figs. 3 and 4. *Proisotoma minuta* in petri dish cultures. **3)** Egg mass, grazing young insect, and exuviae (arrows) on culture of *Rhizoctonia solani* (×35). **4)** Dead insect on water agar, colonized by unidentified fungus (×100).

Dothan sandy loam and Lucedale sandy clay loam. Insects in constantly wet soil often made brief contact with roots but then moved on, becoming randomly dispersed. Groups of insects were frequently seen at root tips in unwatered soil. Necrotic areas on roots were observed occasionally, but root systems as a whole were not affected adversely.

Colonization of dead organic matter and rhizosphere or roots. Laboratory-reared Collembola were maintained on pure cultures of *A. flavus*, *T. harzianum*, and *F. oxysporum* f. sp. *vasinfectum* for 14 days, then transferred to tubes of sterile soil containing buried soybean stem sections and aseptically grown cotton seedlings. After 7 days, insects were distributed throughout the columns of soil, and fungi were recovered from stem sections, rhizosphere soil, and root tissue. All three fungi were recovered from 100% of both buried stem sections and plated root sections; relative numbers of propagules per gram of dilution-plated rhizosphere soil were 88,000, 74,000, and 55,000 for *A. flavus*, *T. harzianum*, and *F. oxysporum* f. sp. *vasinfectum*, respectively. During the short incubation period allowed for cotton growth in the tubes, no injury or disease symptoms occurred on any of the seedlings.

Collembola collected from field soil and introduced into sterile soil tubes migrated to the soybean stem substrates and cotton roots, which became colonized by microorganisms apparently carried in by the insects (Table 4). *Fusarium*, *Penicillium*, and *Trichoderma* spp. were recovered from a high percentage of colonized soybean stem and cotton root sections plated on streptomycin water agar. Rhizosphere soil yielded over 60,000 colonies of these fungi per gram of soil. No evidence was found to indicate animal transport of either *Rhizoctonia* or *Pythium* to the dead organic substrates or to roots. Bacteria consistently were present in large numbers in rhizosphere soil. Plants exposed to field-collected animals invariably had cleaner, more fully developed root systems and were on the average 9.3 cm longer in total root-shoot length than plants maintained in sterile soil (Fig. 5); no significant root lesions or other injuries were noted.

Rhizoctonia transmission test. When Collembola were allowed to feed on pure cultures of *R. solani*, then introduced into sterilized soil with cotton seedlings and buried soybean stem sections, the pathogen was not recovered from either roots or dead organic matter. *F. oxysporum* f. sp. *vasinfectum*, which was tested similarly, was readily recovered from rhizosphere, roots, and stem sections. No root injury was observed.

DISCUSSION

Our studies show that two species of Collembola, *P. minuta* and *O. encarpatus*, feed on both plant pathogenic fungi and common saprophytes and that various fungi affect their activities and reproduction. The ready transport of bacteria and small-spored fungi on and in their bodies makes these insects potentially important in initiating rhizosphere and rhizoplane colonization by pathogens or antagonistic organisms. Initiating organic matter colonization by pathogens near roots may contribute to buildup of sufficient inoculum density for infection (4). Smith (19) pointed out that a small undetectable inoculum level may lead to *Fusarium* wilts.

In slowly drying soil, Collembola migrated to the rhizosphere and root surface of cotton seedlings. Therefore, these insects probably are more abundant and active in the root zone as field soil dries between periods of rainfall. Exudation of sugars, amino acids, and other components by roots may be greatly increased in reversibly wilted plants (17). Thus, a higher water potential and greater availability of a microflora food source for the insects would be expected in the root zone during drought.

The intense feeding of these animals on young fungal hyphae conceivably could also reduce the inoculum level of pathogens such as *R. solani*, *F. oxysporum* f. sp. *vasinfectum*, and *Pythium* spp. around cotton roots. Coleman and McGinnis (10) used buried isotope-labeled fungal mycelium in field soil to show that most of the food source was consumed by Collembola rather than by mites. Predation of nematodes by species of Collembola, including the genus *Onychiurus*, has been observed (3), reflecting their unspecialized feeding habits and capacity to destroy other components of the rhizosphere biota.

Collembola regularly molt, and the exuviae, along with dead insects, provide additional substrates for colonization and fruiting by fungi. As early as 1938, Kubiena (13) referred to fungi fruiting in soil on exuviae and dead bodies of fauna; we frequently observed such fruiting of several common hyphomycetes on insects both in agar cultures and in soil tubes.

Though cotton seedling roots in sterile soil tubes were readily colonized by bacteria and fungi introduced on the bodies of field-collected Collembola, root injury during the 14-day experimental period was observed infrequently; small root lesions appeared

TABLE 4. Colonization of sterilized soybean stem sections and live cotton roots by fungi transported through sterilized soil by field-collected Collembola

Fungal genus	Soybean sections colonized[a] (%)	Root pieces yielding fungi[a] (%)
Fusarium	84	86
Penicillium	63	61
Trichoderma	56	42
Phoma	28	17
Aspergillus	12	13
Rhizopus	7	...
Alternaria	7	8
Rhizoctonia	0	0
Pythium	0	0

[a]Total number of stem sections or root pieces plated = 100.

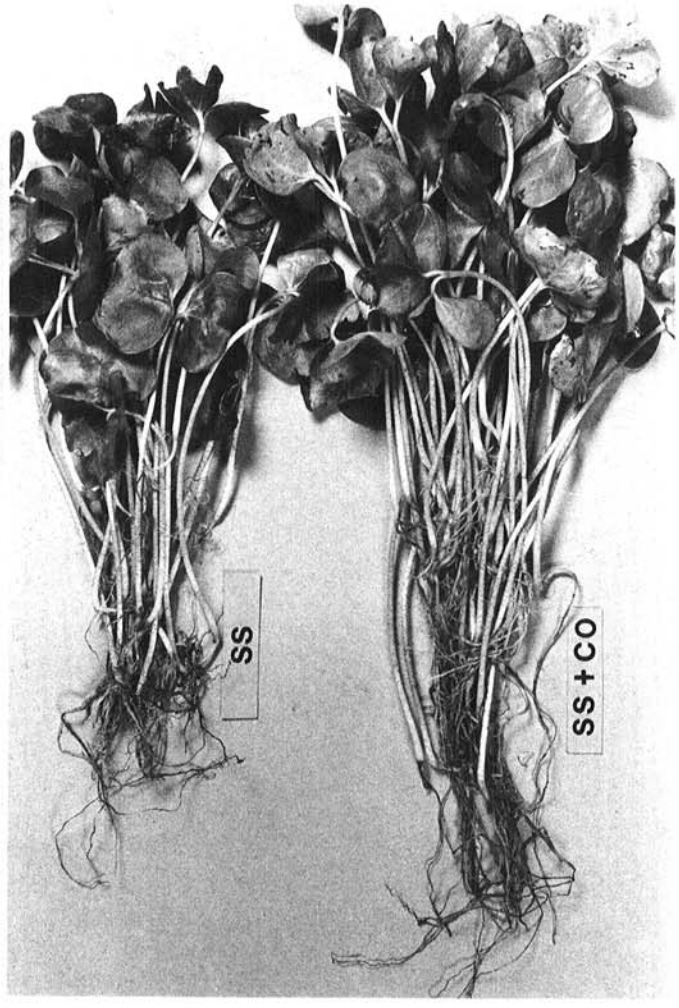


Fig. 5. Cotton seedlings grown in sterilized soil (SS) compared with those grown in sterilized soil plus Collembola (SS + CO).

occasionally in slowly drying soil when tissue culture flasks were used. Microbial colonization of roots by field-collected Collembola in soil tubes invariably resulted in plants of greater total root-shoot length and cleaner root systems than when plants were not exposed to the insects. Enhancement of plant growth has been attributed to increased nutrient or growth factor availability and uptake resulting from nonmycorrhizal microbial activity in the rhizosphere and rhizoplane (2,17). It is unlikely that a mycorrhizal association was established in the short term of our experiment. In view of the work of Rovira and Bowen (16), plant stimulation could be due to microbial destruction of plant-growth inhibiting toxins formed by heat sterilization (autoclaving) of the soil used.

Collembola can carry mycelial fragments as well as spores in their gut (1,9,14), but our studies revealed no evidence of insects transporting sufficient quantities of a nonsporulating pathogen (*R. solani*) to initiate organic matter or root colonization. At the same time, *F. oxysporum* f. sp. *vasinfectum* and *Verticillium* sp. were readily transmitted, along with other small-spored fungi. It is not known at this time whether the insects can transport chlamydospores or whether they may harbor viable fragments of microsclerotia, as reported for the bulb mite (15). Soilborne mites also have been implicated in the occurrence of pod rot disease of peanuts caused by *Pythium myriotylum* Drechs. (18).

Our investigation shows that microarthropods, specifically Collembola, no longer can be ignored in studies of rhizosphere ecology. We suggest that they may contribute to determining the quantitative and qualitative nature of the rhizosphere microflora by transporting propagules and by feeding on mycelia. These activities might affect the competitive advantage of a pathogen at the root surface and influence disease incidence.

LITERATURE CITED

1. ANDERSON, J. M., and I. N. HEALEY. 1972. Seasonal and interspecific variation in major components of the gut contents of some woodland Collembola. J. Anim. Ecol. 41:359-368.
2. BARBER, D. A. 1968. Microorganisms and the inorganic nutrition of higher plants. Annu. Rev. Plant Physiol. 19:71-88.
3. BOOSALIS, M. G., and R. MANKAU. 1965. Parasitism and predation of soil microorganisms. Pages 374-391 *in* K. F. Baker and W. C. Snyder (eds.). Ecology of Soil-borne Plant Pathogens. University of California Press, Berkeley. 569 pp.
4. BOWEN, G. D., and A. D. ROVIRA. 1976. Microbial colonization of plant roots. Annu. Rev. Phytopathol. 14:121-144.
5. BUCHANAN, R. E., and N. E. GIBBONS. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams and Wilkins, Baltimore, MD. 1,246 pp.
6. BUND, C. F. van de. 1970. Influence of crop and tillage on mites and springtails in arable land. Neth. J. Agric. Sci. 18:308-314.
7. BUTCHER, J. W., R. SNIDER, and R. J. SNIDER. 1971. Bioecology of edaphic Collembola and Acarina. Annu. Rev. Entomol. 16:249-288.
8. CHRISTEN, A. A. 1974. Collembola and their fungal associates. M.S. thesis, Washington State University, Pullman. 33 pp.
9. CHRISTIANSEN, K. 1964. Bionomics of Collembola. Annu. Rev. Entomol. 9:147-178.
10. COLEMAN, D. C., and J. T. McGINNIS 1970. Quantification of fungus–small arthropod food chains in soil. Oikos 21:134-137.
11. JOHNSON, L. F., and E. A. CURL. 1972. Methods for Research on the Ecology of Soil-borne Plant Pathogens. Burgess Publ. Co., Minneapolis, MN. 247 pp.
12. KEVAN, D. K. McE. 1965. The soil fauna—its nature and biology. Pages 33-51 *in* K. F. Baker and W. C. Snyder (eds.). Ecology of Soil-borne Plant Pathogens. University of California Press, Berkeley. 569 pp.
13. KUBIENA, W. L. 1938. Micropediology. Collegiate Press, Ames, IA. 234 pp.
14. POOLE, T. B. 1959. Studies on the food of Collembola in a Douglas fir plantation. Proc. Zool. Soc. Lond. 132:71-82.
15. PRICE, D. W. 1976. Passage of Verticillium albo-atrum propagules through the alimentary canal of the bulb mite. Phytopathology 66:46-50.
16. ROVIRA, A. D., and G. D. BOWEN. 1966. The effects of microorganisms upon plant growth. II. Detoxication of heat-sterilized soils by fungi and bacteria. Plant Soil 25:129-142.
17. ROVIRA, A. D., and C. B. DAVEY. 1974. Biology of the rhizosphere. Pages 153-204 *in* E. W. Carson (ed.). The Plant Root and Its Environment. University Press of Virginia, Charlottesville. 691 pp.
18. SHEW, H. D., and M. K. BEUTE. 1978. The role of soil-borne mites in Pythium pod rot of peanut. Proc. Am. Phytopathol. Soc. 3:342 (Abstr.).
19. SMITH, S. N. 1970. The significance of populations of pathogenic Fusaria in soil. Pages 28-30 *in* T. A. Toussoun et al (eds.). Root Diseases and Soil-borne Pathogens. University of California Press, Berkeley. 252 pp.
20. TULLGREN, A. 1917. En enkel apparat för automatiskt vittjande av sallgods. Entomol. Tidskr. 38:97-100.
21. WALLWORK, J. A. 1976. The Distribution and Diversity of Soil Fauna. Academic Press, New York. 355 pp.
22. WIGGINS, E. A., E. A. CURL, and J. D. HARPER. 1979. Effects of soil fertility and cotton rhizosphere on populations of Collembola. Pedobiologia 19: (In press).