Recovery of Fungi and Arthropods from Sclerotia of *Sclerotinia sclerotiorum* in Quebec Muck Soils

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ABSTRACT

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Baiting samples of muck soil from Quebec with sclerotia of *Sclerotinia sclerotiorum* revealed that *Trichoderma*, *Gliocladium*, *Penicillium*, *Sporidesmium*, *Rhizopus*, *Myxomycetes*, *Bradysia* (dark-winged fungus gnat), and *Onychiurus* sp. (springtails, Order Collembola) were present in the soils. The number of larvae of *Bradysia* was positively correlated with low soil pH, high levels of organic matter, and high levels of nitrate in the soil. There was no correlation between fungi or *Onychiurus* sp. recovered and any of the above soil parameters. In in vitro tests, sclerotia damaged by the feeding of the larvae of *Bradysia* had levels of mycelial germination of 0–30%, whereas undamaged sclerotia germinated at a rate of 95%. When sclerotia were buried at different depths in soil, and larvae or adults of *Bradysia* were placed on the soil surface, predation of sclerotia was greatest in the top 2 cm of soil. The larvae were recovered from as deep as 9 cm in the soil.

Sclerotinia spp. are well known as pathogens of crops grown in muck soil (20). However, surveys of the incidence of lettuce drop caused by *Sclerotinia sclerotiorum* (Lib.) de Bary in muck soils in Quebec indicated that the percentage of plants affected by *Sclerotinia* was only 0.06% (15). These relatively low levels of lettuce drop suggested that soils in this area were suppressive to *S. sclerotiorum*. Experiments were carried out to determine if there were any naturally occurring parasites or predators of *S. sclerotiorum* present in the muck soil region of Quebec. Initial observations suggested that the larvae of the dark-winged fungus gnat (*Bradysia* Winnertz) were feeding on the sclerotia and experiments were thus designed to study interactions between the larvae of *Bradysia* and the sclerotia. Portions of this work have been published previously (1,2).

MATERIALS AND METHODS

Baiting tests. Two hundred and fifty samples of muck soils (organic humic mesisols) from 25 sites near Ste-Clotilde, St-Remi, and St-Patrice-de-Sherrington, Quebec, were collected for baiting experiments designed to recover mycoparasites and predators of sclerotia of *S. sclerotiorum*. The samples were stored at 5 C in sealed plastic bags until use.

Sclerotia were produced on autoclaved carrot disks in 500-ml flasks by an isolate of *S. sclerotiorum* that had previously been recovered from diseased lettuce plants. Cultures were incubated at 22–24 C under ambient light conditions for 5 wk. Sclerotia were then separated from the carrots by washing in sieves (Tyler equivalent 16 mesh) under running tap water. Sclerotia were placed on filter paper, air dried, then stored at 5 C until needed. For baiting experiments, sclerotia (surface-sterilized in 2% sodium hypochlorite and rinsed in sterile water) were placed in nylon bags (2 × 3 cm), made from Nitex (Tetko, Elmsford, NY) monofilament screen cloth (17.224 mesh count per centimeter, opening in centimeter = 0.035). A 500-ml aliquot of each soil sample was placed in a 10-cm-diameter plastic pot and three bags (three sclerotia per bag) were then buried in each soil sample. The pots were placed in a growth chamber with a 14-hr photoperiod and temperatures of 21 (day) and 18 C (night). The soil was kept moist by watering with distilled water on alternate days. After 5–8 wk, the bags were recovered and the sclerotia removed. Sclerotia from one of the three bags were placed in 2% sodium hypochlorite for 2 min, rinsed twice in sterilized water, then plated on potato-dextrose agar (PDA), water agar (WA), or moist filter paper. Sclerotia from the remaining bags were placed directly onto the above media without pretreatment. After 5 days of incubation at 24 C, fungi growing out from the sclerotia were transferred to PDA and subsequently identified. The number of larvae of *Bradysia* and springtails (Order Collembola, *Onychiurus* Gervais sp.) were estimated by determining the number of each arthropod present on, or associated with, the sclerotia that had been placed on moist filter paper. Populations of *Bradysia* were ranked from 1 to 5, where 1 = no larvae on sclerotia, 2 = one to two larvae on the sclerotia, 3 = three to four larvae on the sclerotia, 4 = five larvae, and 5 = six or more larvae. Damage was defined as the percentage of the sclerotial rind consumed by the larvae. Population classes for *Onychiurus* sp. were ranked similarly, where 1 = no springtails on the sclerotia, 2 = one or two springtails present, 3 = three to four springtails present, 4 = five or six springtails present, and 5 = more than six springtails present. The soil samples were analyzed for pH and the amount of nitrate and ammonia present. Chi-square analyses (16) were performed to indicate relationships between number of larvae in the soil and these soil parameters.

Production of larvae. Larvae of *Bradysia* (identified by A. Borkent, Biosystematics Research Institute, Agriculture Canada, Ottawa, Canada) were collected with a needle from senescing leaves of *Sclerotinia*-infected lettuce plants growing in containers of muck soil in the greenhouse. Larvae were cultured in vials on agar slants sprinkled with dry agar, and larvae were supplied with commercial baker's yeast as a food source (11).

Effect of larval population size on sclerotial survival. Various populations of larvae (1–2 days old) were placed on sclerotia on moist filter paper in a petri dish and their predatory activities were observed. Each treatment consisted of a single sclerotium per petri dish plus the appropriate (from 0 to 10) number of larvae. There

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were thus 11 treatments in total, with each treatment replicated 10 times. After 20 days of incubation at 24 C, sclerotia were placed on moist sterilized sand in glass jars to determine germinability. Sclerotia were rated as germinable if myceliogenic germination occurred within 2 mo of incubation at 15 C. Chi-square analyses were conducted to determine relationships between numbers of larvae and damage to sclerotia. The experiment was repeated three times.

Effect of sclerotial depth on predation. Muck soil collected from the Agriculture Canada Substation at Ste-Clotilde, Quebec, was steam-pasteurized for 30 min at 80 C and then air-dried. The experiment consisted of 11 treatments (five replicates per treatment), which varied from one another with respect to the depth of burial of a sclerotium in a 750-ml glass jar, containing 200 g of the air-dried soil. Depths of sclerotial burial were from 0 to 10 cm, at increments of 1 cm. To obtain a uniform soil compaction and the desired depth of burial, an amount of soil was placed in the jar and a weight of 1 kg was applied to the soil surface. A sclerotium was then placed on the soil surface and the remainder of the 200 g of soil was added. The soil was compacted again with the 1-kg weight. Water (250 ml) was then added to each jar. One hundred larvae (1–2 days after hatching) were placed on the soil surface in each jar and the jars were sealed with cheesecloth. All jars were placed in an insect cage held in a growth chamber with a 14-hr photoperiod and temperatures of 21 C (day) and 18 C (night). The soil was kept moist by spraying on alternate days with distilled water. After 15 days, the soil was removed from the jars and populations of larvae and pupae were determined. Soil was removed gradually from the jars by loosening the soil to a depth of 0.5 cm, then tilting and gently tapping the jar until the loosened soil was removed. The number of larvae and pupae in the removed soil was then determined. This procedure was repeated until all of the soil had been removed from the jar. Regression analyses were performed to determine the relationship between larval distribution in the soil and sclerotial depth. The experiment was repeated once.

A parallel experiment was established in which adult fungal gnats, rather than larvae, were added to the jars. Three female adults and five male adults were placed in each jar and the jars were sealed with cheesecloth. Each female laid about 50–80 eggs. After 20 days, the experiment was ended, and the numbers of larvae and pupae were determined as before. The experiment was repeated once.

RESULTS AND DISCUSSION

Fungi and certain arthropods were consistently associated with the sclerotia of *Sclerotinia sclerotiorum* in the soil baiting experiments. Tabulation of the fungi recovered from sclerotial baits indicated that *Trichoderma* occurred in 78% of the soil samples. Frequency of other fungi were: *Penicillium*, 38%; *Gliocladium*, 26%; *Rhizopus*, 16%; *Myxomycetes*, 4%; and *Sporidesmium*, 2%. Of the fungi recovered, *Trichoderma*, *Penicillium*, *Gliocladium*, and *Sporidesmium* have been recorded as antagonists of *Sclerotinia* spp. (3,8,9,18,19). Treatment of sclerotia with sodium hypochlorite before placing on agar did not affect recovery of fungi.

Many arthropods have been reported to be associated with the sclerotia of *Sclerotinia* spp. (5), but there have been no previous reports of larvae of *Bradysia* as predators of thalli in this genus. *Onychiurus* sp. also were associated with the sclerotia and were observed to feed on them.

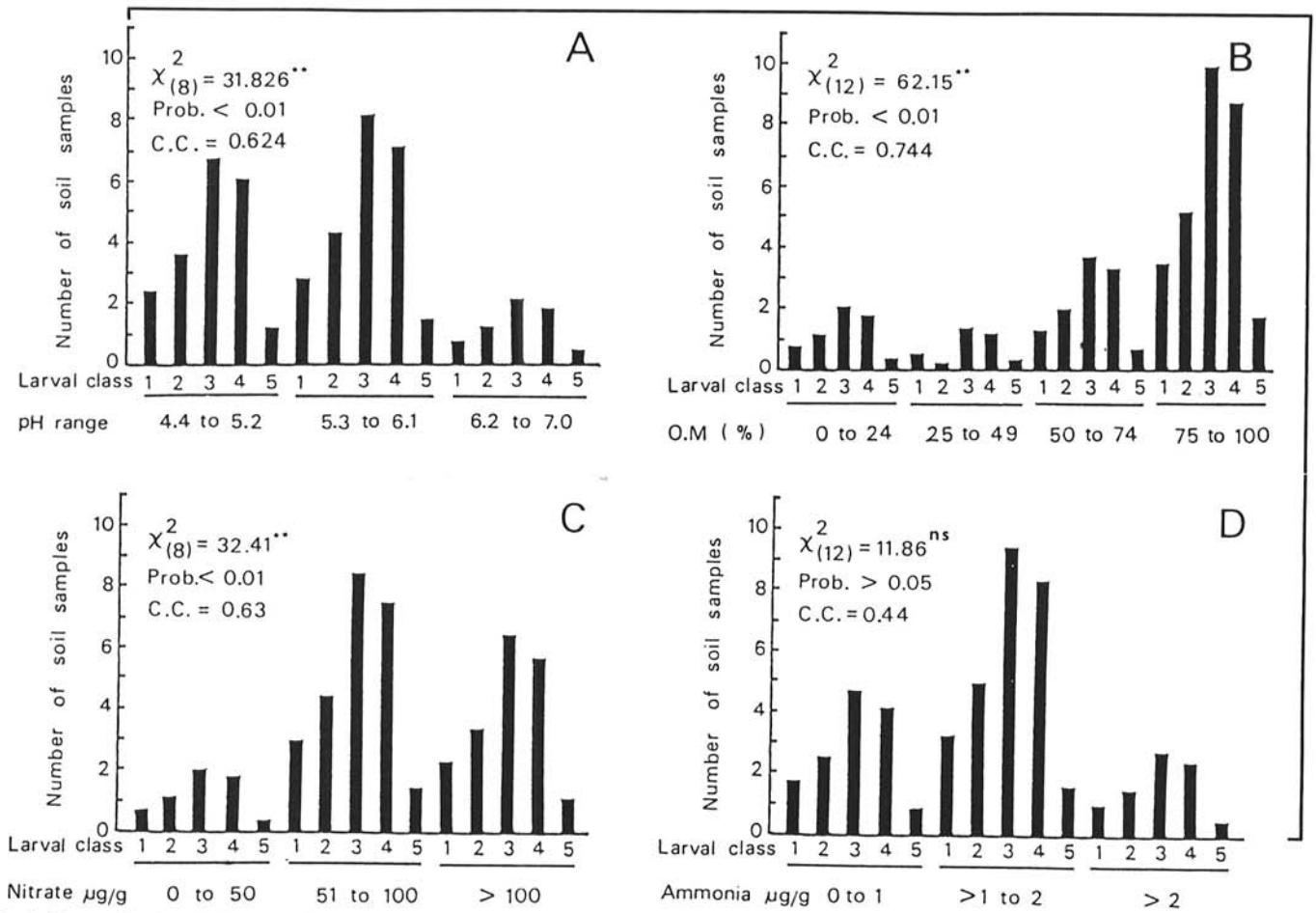

Fig. 1. Expected values (chi-square distributions) for relationships between populations of *Bradysia* and **A,** Soil pH; **B,** Organic matter content; **C,** Soil nitrate content (µg/g of soil); and **D,** Ammonia content (µg/g of soil). Larval class was defined as follows: 1 = no larvae on sclerotium; 2 = one or two larvae on sclerotium; 3 = three or four larvae; 4 = five larvae; and 5 = six or more larvae on sclerotium. Contingency coefficient (C.C.). Calculated χ^2 values are indicated with the appropriate degrees of freedom.

Bradysia sp. (dark-winged fungus gnat) belongs to the family Sciaridae (Diptera). Steffan (17) gave a generic revision of the family. Binns (4) reported that the larvae of fungus gnats prefer media with a high concentration of organic nitrogen for their growth and were attracted towards such media. He also reported that the mushroom fungus gnat, *Bradysia paupera*, was attracted to ammonia, which was released from newly steamed soil by bacterial action. In our study, pH of the soils sampled ranged from 4.4 to 6.8, with most of the samples in the range of 4.8–6.0. The percent organic matter ranged from 6.4 to 86.7%, and nitrate and ammonia levels were 29.5–244.5 μg/g and 0.2–4.5 μg/g of soil, respectively. The number of larvae of *Bradysia* recovered per sclerotium varied from 0 to 8, and the number of springtails recovered per sclerotium varied from 0 to 17. When chi-square tests were conducted (Fig. 1), we found that soil pH, percent organic matter, and soil nitrate were significantly ($P < 0.01$) related to the population of larvae of *Bradysia* in the soil. Contingency coefficients were 0.62, 0.74, and 0.63, respectively, indicating strong relationships. The results for these three factors indicate that the populations of larvae of *Bradysia* were highest in soils with pH between 4.4 and 5.2, organic matter content greater than 75%, and nitrate levels of more than 100 μg/g soil. The amount of ammonia found in these soils was not correlated with the number of larvae found. This could be due to the volatility of ammonia, which results in its rapid loss from soil. No consistent relationship was observed between soil factors and the number of springtails or fungi recovered. It has been reported that springtails are associated with phytopathogenic fungi (7), and they may play a role in the transfer of spores of certain mycoparasitic fungi (14).

When newly hatched larvae were placed on sclerotia, the age and number of larvae per sclerotium were directly related to the time required for the consumption of the sclerotia. It was observed that

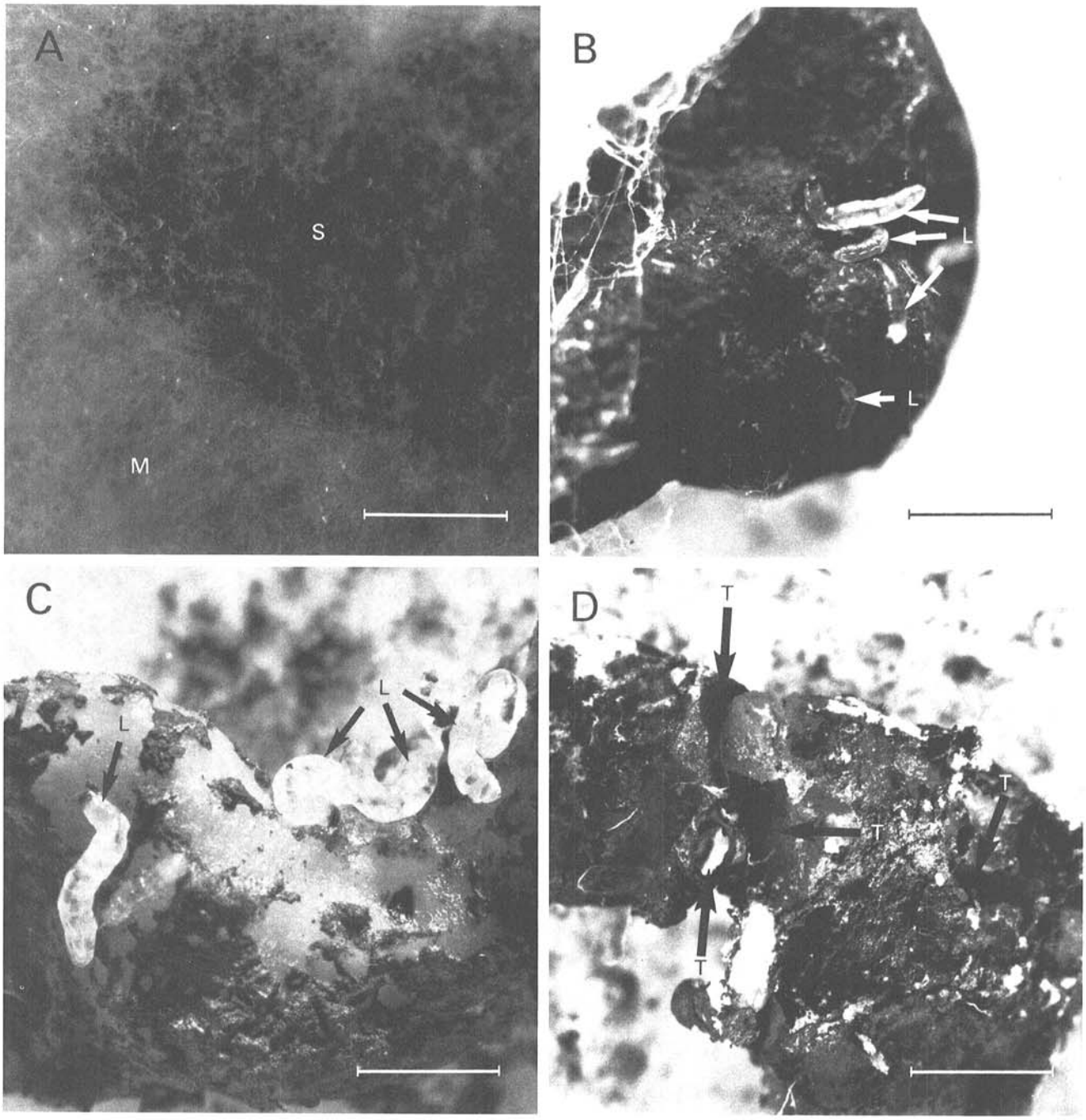

Fig. 2. Predation of sclerotia of *Sclerotinia sclerotiorum* by larvae of *Bradysia*. **A,** Sclerotium (S) to which no larvae (L) had been added. Note extensive mycelial growth (M) that partially obscures sclerotial rind. **B,** Early stages of predation by larvae. Note that most mycelium has been consumed by the larvae. **C,** Extensive surface grazing by larvae. Much of the rind has been removed, exposing the cortex tissues. **D,** Larvae are actively tunnelling (T) through the sclerotium. Scale = 2 mm.

when the larvae of *Bradysia* were placed with sclerotia on moist filter paper in petri dishes, they were slightly or moderately voracious during instar stage I but were highly voracious and gregarious during instar stages II, III, and IV. Newly hatched larvae (instar stage I) did not show much feeding activity for the first 5 days, but instead moved around on the surface of the moist filter paper. Subsequently, the larvae aggregated around and under sclerotia and began feeding on them (Fig. 2). For the next 10 days, predation was at its maximum. Larvae repeatedly tunnelled through a sclerotium until only remnants remained.

The results presented in Table 1 indicate that the amount of damage observed depended on the number of larvae per sclerotium. A chi-square analysis of the interaction between sclerotial damage and larval numbers indicated a highly significant ($P < 0.005$) relationship. It appears, therefore, that larvae do not actively repel one another from the sclerotia. The damage caused by only one larva was about 5–7%, whereas the damage caused by three larvae was estimated at between 25 and 40%. If more than 25% of the sclerotial rind had been destroyed, the sclerotium usually did not germinate.

TABLE 1. Effect of larvae of *Bradysia* on viability of sclerotia of *Sclerotinia sclerotiorum*

Number of larvae per sclerotium[a]	Days taken by larvae to consume sclerotium[b]	Mean percent damage[c]	Myceliogenic germination of sclerotium on moist sand (%)
0	...	0.0	95
1	...	5.8	30
2	...	12.5	10
3	...	32.5	0
4	...	45.0	2
5	...	54.0	0
6	17	74.0	0
7	15	86.0	0
8	11	90.0	0
9	11	90.0	0
10	11	90.0	0

[a] Observations made over a period of 20 days on 10 replicates.
[b] "..." indicates that sclerotia were not completely consumed after 20 days.
[c] Damage determined by visually assessing the amount of rind of the sclerotia consumed by the larvae. For the relationship between larval numbers per sclerotium and damage, χ^2 calculated = 192.3 ($P < 0.001$).

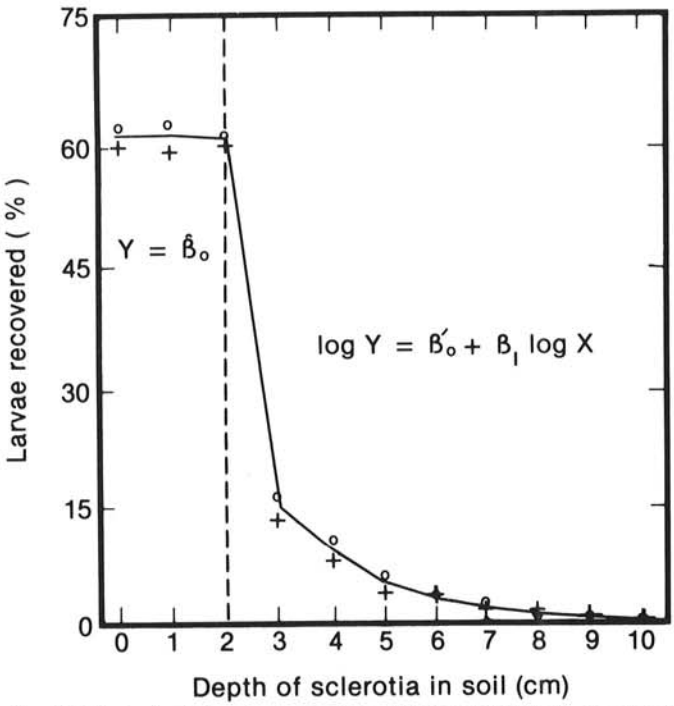

Fig. 3. Effect of soil depth on recovery of larvae of *Bradysia*, where "o" indicates larval populations (including pupae) at various depths when adult gnats were added to the soil surface, and "+" indicates larval populations (including pupae) obtained when 1–2-day-old larvae were added to the soil surface. The curve was determined by combining data obtained from adult and larval tests. In the equation shown above, Y = percent larvae recovered (dependent variable); β_0' = Y intercept; β_1' = slope of regression line; X = depth of sclerotia in soil (independent variable).

Larvae added to the soil in the sclerotial depth experiment were observed to move very actively on the surface. The majority of larvae recovered were present in the top 0–2 cm of the soil, but a few were recovered from as deep as 8 or 9 cm in soil (Fig. 3). Pupae produced by larvae were present in the top 1 cm of the soil. In related greenhouse experiments, the larvae and pupae also were found to be in the top 0–2 cm of soil (1). When simple linear regression analysis was employed on raw and logarithmically transformed data from the sclerotial depth experiment, goodness of fit could not be demonstrated. A logistic curve was obtained when the mean percent larvae from each treatment was plotted against depth of the sclerotium in soil (Fig. 3). To test the logistic nature of the response, the curve was split into two sections; the first section from depths 0–2 cm, which exhibited linearity with no response, and the second from 2–10-cm section of the curve was found to fit a simple linear response, indicating that the relationship between the dependent and independent variables was multiplicative. Correlation coefficients (r) of the transformed data indicated that there was a negative relationship between depth of the sclerotium and percent larvae recovered. For treatments in which adult fungus gnats were added, r was -0.83 ($P < 0.01$), whereas, for treatments in which larvae were added, r was -0.88 ($P < 0.01$). The two groups of treatments (larvae and adults) were not significantly different from one another.

Fungus gnat larvae have been classified as minor greenhouse pests (6,21) and as vectors of fungi such as Fusarium in alfalfa and red clover (12) and *Verticillium albo-atrum* in greenhouse-grown alfalfa (10). In field situations, however, the larvae do not apparently cause any damage, unless plants are already diseased (13). Larvae of *Bradysia* sp. may play an important role in the rate of natural destruction and lysis of sclerotia. In related experiments, when sclerotia were buried at depths of 0–4 cm, carpogenic germination (stipe formation followed by apothecial production) took place (2). At depths greater than 4 cm, stipe formation occurred, but the stipes did not emerge from the soil. Our results indicate that larvae of *Bradysia* prefer to remain in the top few centimeters of the soil and feed on sclerotia in that region. This feeding habit of the larvae, in conjunction with the activity of potential mycoparasites such as *Trichoderma*, *Gliocladium*, *Penicillium*, and *Sporidesmium*, may provide an explanation for the low incidence of lettuce drop in muck soils in Quebec.

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