Ecology and Epidemiology

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

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ABSTRACT


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 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 are 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 Winnetz) 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 mull soils) from 25 sites near Ste-Clotilde, Ste-Remi, and St-Patrice-de-Sherrington, Quebec, were collected for baiting experiments designed to recover mycopathogenic 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 X 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 250-ml conical flasks containing 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 Collombola, 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 to 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 were performed to determine 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 fungal 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
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-Clothilde, 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 with 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 200g 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. (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 *Bradyssia* as predators of thalli in this genus. *Onychiurus* sp. also were associated with the sclerotia and were observed to feed on them.
Brady sia 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, Brady sia panpora, 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 0.4 to 8.7%, 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 Brady sia 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 Brady sia 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 Brady sia 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
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 1 but were highly voracious and gregarious during instar stages II, III, and IV. Newly hatched larvae (instar stage 1) 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 tunneled 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 sclerotal damage and larval numbers indicated a highly significant

<table>
<thead>
<tr>
<th>Number of larvae per sclerotium</th>
<th>Days taken by larvae to consume sclerotium</th>
<th>Mean percent damage</th>
<th>Myceliogenic germination of sclerotium on moist sand (%)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>...</td>
<td>0.0</td>
<td>95</td>
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<td>1</td>
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<td>5.8</td>
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*Observations made over a period of 20 days on 10 replicates.

** indicates that sclerotia were not completely consumed after 20 days.

Damage determined by visually assessing the amount of the sclerotal consumed by the larvae. For the relationship between larval numbers per sclerotium and damage, \( \chi^2 \) calculated = 192.3 \( P < 0.001 \).

Fig. 3. Effect of soil depth on recovery of larvae of *Bradysia*, where "0" indicates larval populations (including pupae) at various depths when adult gnats were added to the soil surface, and "1" 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, \( Y = \text{percent larvae recovered (dependent variable)}; b_0 = Y \text{ intercept}; b_1 = \text{slope of regression line}; X = \text{depth of sclerotia in soil (independent variable)} \).