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Control of Sclerotia of Rhizoctonia solani by a Sciarid Fly, *Phyxia scabiei*, in Soil

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Abstract

In field surveys of sugarbeet in Hokkaido, larvae of a Sciarid fly, Pnyxia scabiei (Hopkins) (Sciaridae), were often found feeding on sclerotia and hyphae of *Rhizoctonia solani* Kuhn AG-2-2, the causal agent of sugarbeet root rot. Population of the larvae increased rapidly with the root rot severity and occasionally up to 5,000 larvae per diseased root were counted. The larvae were found mostly on the lesions or in the soil within a distance of 2 cm from the root surface. When sclerotia were buried in the soil around the diseased or healthy roots of sugarbeet for 3 weeks in autumn in small plastic containers (10 or 250 μm mesh) sclerotial destruction occurred only in the 250 μm containers around the diseased roots, and larvae of *P. scabiei* were often seen inside these containers. Tests using sterilized or non-sterilized soil with sclerotia of *R. solani* revealed that when larvae were placed in a paper-pot, damping-off disease of sugarbeet could be prevented. However the disease was not suppressed by the dead larvae killed by isoxathion (insecticide) or propylene oxide. These results suggest that the insect reduces the sclerotial density of *R. solani* in soil leading to the decrease of the incidence of Rhizoctonia root rot of sugarbeet.

Discipline: Plant disease
Additional key words: biocontrol, sugarbeet, root rot, damping-off, feeding population dynamics

Introduction

There is a large variety of soil fauna, e.g. protozoa, nematodes, microarthropods or insects in cultivated soils. They are often referred to as "land plankton" by analogy with "sea plankton" and play a major role in the decomposition of plant debris. Mostly, these animals and soil microorganisms are interdependent of each other in the process of decomposition of organic residues[1]. Feeding behavior of soil animals may influence the population dynamics of root rot fungi through physical breakdown, perforation, transmission, inactivation or death of fungal structures in the intestine[2,3,5,18]. Some attempts have been made to control diseases by using small animals such as mycophagous nematodes[8], amoebae[15] and springtails (Collembola)[3,13-15] though mainly in the glasshouse using pot tests with an abnormally high population density of these animals. Little is known about the population dynamics of *R. solani* in association with soil animals in fields.

The incidence of Rhizoctonia root rot decreases during sugarbeet monoculture[6]. We observed that larvae of a mycophagous Sciarid fly, *Pnyxia scabiei*, aggregated on only the diseased roots and reduced fungal populations by feeding on the sclerotia of the anastomosis group (AG)-2-2 of *R. solani* in fields with sugarbeet root rot. In this paper the destruction and decrease in the number of sclerotia of *R. solani*, and the possibility of biological control by using larvae of mycophagous *P. scabiei* are reviewed.

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Decrease of sclerotial density of *Rhizoctonia solani* by a Sciarid fly

1) Feeding of larva on sclerotia[11]

Larvae of *P. scabiei* were transparent to opaque white, 1 to 5 mm long by 0.2 to 0.5 mm wide, with a black head, and fed on sclerotia of *R. solani* AG-2-2 with well-developed biting mouth-parts. Brown fragments of sclerotial cells passing through the intestine of the larvae were often observed under a dissecting microscope (Plate 1). When the fragments taken out from the intestine or excreta were placed on plain agar and incubated for a few days, no colonies grew out from them, suggesting that sclerotial cells died due to feeding by larvae. Larvae actively fed on sclerotia: 15 sclerotia of the AG-2-2 were almost completely eaten up by 30 larvae within 24 h in a 6 cm diameter petri dish. As the populations of the larvae increased, sclerotial destruction progressed (Table 1). Feeding on sclerotia took place even in soil; when 10 sclerotia and 30 larvae were added to a paper-pot (2 cm diameter by 13 cm height) containing non-sterilized nursery soil, sclerotia could not be recovered from the soil. Feeding on sclerotia occurred at 10 to 30°C, with the optimum temperature being 25°C. Larvae survived even at 5°C but not at 35°C.

Relationships between anastomosis groups of *R. solani* and severity of sclerotial destruction by larvae

Plate 1. Larva of *Pnyxia scabiei*
Scale: 0.5 mm.

Table 1. Effect of *Pnyxia scabiei* larvae on the destruction of *Rhizoctonia solani* AG-2-2[a)]

No. of larvae	No. of sclerotia	Percentage of sclerotia destroyed[b)] by larval feeding after				
		1 day	2 days	3 days	4 days	6 days
0	15	0.0	0.0	0.0	0.0	0.0
5	15	3.3	33.3	66.7	66.7	100.0
10	15	13.3	26.7	60.0	73.3	100.0
20	15	46.7	76.2	100.0	100.0	
30	15	96.7	100.0			
50	15	70.0	100.0			

a): Larvae were fed with sclerotia in 6 cm petri dishes containing a moist filter paper and the dishes were kept at 18–20°C under dark conditions.
b): Sclerotia were counted as destroyed when 50% or more of their volume was eaten.

Table 2. Effect of anastomosis groups of *Rhizoctonia solani* on *Pnyxia scabiei* feeding on sclerotia[a)]

No. of larvae	*R. solani*			Percentage of sclerotia destroyed		
	Anastomosis group	Isolate	No. of sclerotia	Exp. 1	Exp. 2	Exp. 3
30	AG-1, IA	C-408	15	–	23.4	–
	AG-1, IB	Rh-121	15	10.0	6.6	0.0
	AG-2-1	SSa-1	15	3.4	6.6	0.0
	AG-2-2	Pf-28	15	100.0	100.0	100.0
	AG-3	ST-11-6	15	100.0	100.0	100.0
	AG-4	Rh-165	15	33.4	20.0	83.4
	AG-5	Rh-185	15	100.0	93.3	100.0

a): Larvae were fed with sclerotia in 6 cm petri dishes containing a moist filter paper and the dishes were kept at 18–20°C under dark conditions.

were investigated in petri dishes. Among the 6 anastomosis groups (AGs) tested, sclerotial destruction was severe in AG-2-2, AG-3 and AG-5, but less in AG-1 (culture type 1A and 1B), AG-2-1 and AG-4 (Table 2). Since there was no difference in the mycelial destruction severity among the 6 AGs when larvae were reared with mycelia, the significant difference in sclerotial destruction among the AGs may be related to the hardness or water content of the sclerotial cells rather than to the types or chemical components of the isolates of the AGs.

2) Aggregation of larvae on sclerotia[11]

Larvae of *P. scabiei* and sclerotia of *R. solani* AG-2-2 were placed, 20.5 cm apart from each other in a cylinder made of moist filter paper which was in close contact with the inner part of a stainless-steel cylinder, 5 cm in inner diameter by 30 cm in length covered with a lid. After 3 days of incubation at room temperature, larvae aggregated almost entirely on and around sclerotia. However, when no sclerotia were added, the larvae were randomly distributed on the filter paper. Subsequent experiments in a large petri dish revealed that more larvae aggregated on sclerotia and mycelia of *R. solani* grown on autoclaved barley grains and on pieces of diseased sugarbeet roots, but not on sterile barley grains or healthy roots. These data suggest that the larvae are attracted to sclerotia and mycelia of *R. solani* AG-2-2 as well as to the diseased roots of sugarbeet.

3) Sclerotial destruction in sugarbeet fields[11]

A large number of sclerotia of *R. solani* AG-2-2 are produced in soil around the diseased sugarbeet roots, and they play a major role as a source of infection in the outbreaks of root rot in the following summer[7]. An attempt was made to determine whether sclerotial destruction actually took place by feeding of *P. scabiei* larvae in fields. In autumn, sclerotia were placed in small plastic containers (30 mm outer diameter, 17 mm inner diameter, 5 mm thick) with 10 or 250 μm nylon mesh, then buried in soil around diseased or healthy roots of sugarbeet for 3 weeks. Sclerotial destruction occurred only in 250 μm containers around the diseased roots, and larvae of *P. scabiei* were often found inside these containers (Table 3). Around the diseased roots, all the sclerotia inside the 10 μm containers in which larvae could not enter were intact and not injured. On the other hand, no sclerotia were destroyed inside of the 10 and 250 μm containers around healthy roots of sugarbeet. These data suggest that the larvae of this insect are a causal factor of sclerotial destruction in fields after root rot of sugarbeet severely occurred, where sclerotia were produced abundantly in soil around the diseased roots. On the other hand, springtails which inhabit the rhizosphere of the diseased roots actively feed on mycelia of *R. solani* but never destroy sclerotial cells[3,13].

4) Seasonal changes in the incidence of root rot disease and population of P. scabiei larvae in a sugarbeet field[11]

Relationship between root rot disease and population dynamics of the larvae was investigated in a sugarbeet field artificially-inoculated with *R. solani* AG-2-2 (Fig. 1). Larvae were first observed in soil around or on the diseased roots on July 21 (3 weeks after inoculation). Their number increased rapidly

Table 3. Destruction of sclerotia of *Rhizoctonia solani* AG-2-2 buried in the rhizosphere soil of sugarbeet with root rot by larvae of *Pnyxia scabiei* in fields

Field	Rhizosphere	No. of plants	Size of mesh (μm)	No. of sclerotia buried[a)]	Percentage of sclerotia destroyed
A	Diseased	2	10	40	0.0
		5	250	100	70.0
	Healthy	2	10	40	0.0
		3	250	60	0.0
B	Diseased	3	10	60	0.0
		4	250	80	22.5
	Healthy	3	10	60	0.0
		3	250	60	0.0
C	Diseased	3	10	60	0.0
		3	250	60	46.7

a): Sclerotia were placed in Matsumoto's plastic containers (30 mm outer diameter, 17 mm inner diameter, and 5 mm thick) with 10 or 250 μm nylon mesh.

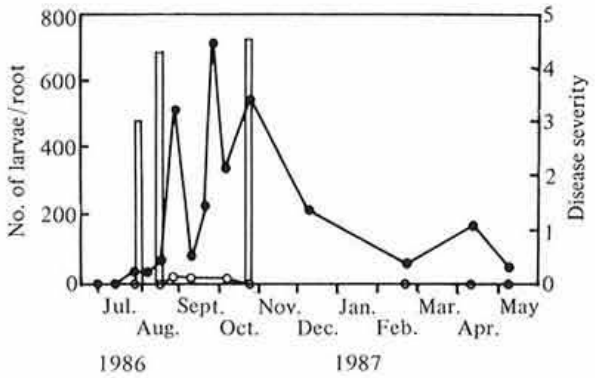

Fig. 1. Seasonal changes in the number of *Pnyxia scabiei* on and around diseased (●) or healthy (○) sugarbeet roots in a field
Rhizoctonia solani AG-2-2 was inoculated on July 1, 1986.
Disease severity (□) was rated on a scale of 0 (no symptoms) to 5 (fully rotted root).

Table 4. Relationships between root rot severity of sugarbeet and the number of *Pnyxia scabiei* larvae on and around diseased plants

Disease severity[a)]	No. of plants examined	No. of larvae /sugarbeet root	
		Mean	Range
0	4	1.5	0-4
1	6	20.8	1-75
2	5	8.4	3-14
3	3	371.0	141-586
4	3	996.3	155-2,482
5	5	2,344.8	268-5,752

a): Disease severity, estimated by an increase of visible symptoms on 6 degree rating scale, from 0 (no symptoms) to 5 (fully rotted root).

toward the end of August along with the root rot severity, then a high population density was maintained until the harvest of sugarbeet. In winter, the insects overwintered as larvae with a low population density. On the other hand, around healthy roots fewer larvae were observed throughout the year. Field surveys on disease severity and population of larvae at harvest time revealed that the number of larvae in sugarbeet fields increased rapidly along with the root rot severity (Table 4) and occasionally up to 5,000 larvae per dead root were counted. Therefore, the larvae of *P. scabiei* are almost completely dependent on the root rot incidence of sugarbeet.

5) Distribution of sclerotia and larvae in soil around diseased roots of sugarbeet[11]

Most of the larvae were distributed on the diseased roots or in the soil within a distance of 2 cm from the root surface. On the other hand, the number of sclerotia of *R. solani* AG-2-2 increased as the distance from diseased roots decreased, often up to 1,271 sclerotia per 100 g of dried soil within a 5 cm distance and 5 cm depth were counted. These findings suggest that the insect larvae play a significant role in the destruction of sclerotia in soil.

6) Distribution of the insect in fields cultivated with several crops[10]

A field survey showed that *P. scabiei* was widely distributed with high population densities in infested sugarbeet fields with root rot disease, in Hokkaido. When potato tuber slices were buried in field soils for 3 weeks from late July through early August, larvae were trapped from fields where 9 crops were cultivated: corn, alfalfa, barley, oat, potato, soybean, adzuki-bean, kidney bean and radish, with the exception of the wheat field, but the density of their populations was low. Before spring plowing, a few larvae were trapped from fields after cultivation of sugarbeet or corn. These results suggest that the insect is usually saprophagous and ubiquitous, with low population densities.

7) Development of the insect[10]

The larva of *P. scabiei* has four instars. When larvae were reared with *R. solani* AG-2-2 cultured on barley grains, the duration from egg to adult was 39, 29, 19 and 14 days at temperatures of 10, 15, 20 and 25°C, respectively. A female adult deposited 46 to 97 eggs, then died within 10 days after oviposition. The insects overwintered as larvae and five to six generations may be repeated in a year. Hence, it is considered that the populations of larvae increase rapidly in sugarbeet fields with root rot disease.

Possibility of promoting the biocontrol of Rhizoctonia root rot and damping-off diseases of sugarbeet by the Sciarid fly

1) Suppression of damping-off of seedlings of sugarbeet by the insect[12]

Since the larvae of *P. scabiei* can feed on and break down sclerotia, they are presumably useful for suppressing the sclerotia density of *R. solani* in soil. Five to ten sclerotia of AG-2-2 and 10 to 30 larvae were added to a paper-pot containing sterilized or non-sterilized soil at the time of sugarbeet seeding, and the possibility of suppressing damping-off disease was examined. One month after the inoculation, in

Table 5. Effect of *Rhizoctonia solani* AG-2-2 and *Pnyxia scabiei* larvae on sugarbeet seedlings in paper-pots with non-sterilized soil[a)]

Treatment[b)]	Exp. I[c)]			Exp. II[c)]		
	No. of plants[d)]	Percentage of emergence	Percentage of damping-off	No. of plants	Percentage of emergence	Percentage of damping-off
NSS	45	4.4	100.0	60	10.0	100.0[e)]
NSS + PL	45	53.3	37.5	48	41.7	45.0
NSS + RS	45	42.2	73.6	60	43.3	84.6
NSS + RS + PL	45	40.0	38.9	60	56.7	44.1

a): Paper-pot; 2 cm in diameter, 13 cm in height.
b): NSS; non-sterilized soil, PL; *P. scabiei* (20–30 larvae/pot), RS; *R. solani* (10 sclerotia/pot).
c): Data, one month after inoculation.
d): Three seeds of sugarbeet, monogermic, were sown in a pot.
e): Isolation frequencies of the pathogen from diseased plants were 80.5% for *Pythium* spp., 2.4% for *R. solani*, 2.4% for *Aphanomyces* sp. and 14.7% for others, respectively.

the artificially-infested soil, the addition of the larvae markedly reduced the occurrence of damping-off in comparison with the control not exposed to the larvae (Table 5). On the contrary, the disease was not suppressed by dead larvae killed by isoxathion (insecticide) or propylene oxide. Subsequent tests with non-sterilized soil, naturally infested with *Pythium* spp. or supplemented with sclerotia of *R. solani* AG-2-2, revealed that the added larvae in a paper-pot provided a significant protection of the seedlings against the disease (Table 6).

The extent of the suppression of the damping-off incidence by the larvae was different among the AGs of *R. solani*: the disease severity was reduced in AG-2-2 but not in AG-1 (culture type IB) and AG-4. These results are in agreement with the observation that larvae prefer to feed on sclerotia of AG-2-2 than on those of AG-1 and AG-4.

Since the larvae depend on sclerotia or root rot of sugarbeet, the suppression of root rot due to larvae may be achieved by naturally occurring larvae. Residues collected from dead roots and soils around them by the sieving-floatation technique in water were mixed with soil near the roots of 2-month old sugarbeet plants. When root residues including 1,000 larvae of *P. scabiei* were added to a pot, 23 cm in diameter, they markedly suppressed root rot disease in comparison with the residues lacking larvae and heated ones (at 100°C for 10 min) (Table 7, unpublished). When the residues in which the insects were killed by the insectcide isoxathion were added to soil, the incidence of root rot disease decreased though its severity was higher than when raw residues were added. This difference may be related to the feeding of the larvae on the root rot pathogen.

Table 6. Effect of *Rhizoctonia solani* AG-2-2 and *Pnyxia scabiei* larvae on sugar beet seedlings in paper-pots with sterilized soil[a)]

Treatment[b)]	No. of plants[c)]	Percentage of emergence	Percentage of damping-off[d)]
SS	90	94.4	1.2
SS + PL	90	93.3	2.4
SS + RS	90	70.0	42.9
SS + RS + PL	90	87.6	6.3
SS + RS + DPL-1	90	64.4	56.9
SS + RS + DPL-2	90	77.8	44.3

a): Paper-pot; 2 cm in diameter, 13 cm in height.
b): SS; sterilized soil, PL; *P. scabiei* (15 larvae/pot), RS; *R. solani* (5 sclerotia/pot), DPL-1; dead larvae killed by isoxathion, DPL-2; dead larvae killed by propylene oxide.
c): Three seeds of sugar beet, monogermic, were sown in a pot.
d): Data, one month after inoculation.

2) *Influence of the mycophagous Sciarid fly on stands and growth development of several plants*[10)]

In artificial feeding tests in a pot with an abnormally high population of larvae, the insect inhibited not only the growth development of bean, adzuki-bean and cucumber but also wheat germination. Light inhibition of the germination of sugarbeet and spinach as well as of the growth of corn was observed. It remains to be determined whether such a damage occurs in fields, because the insect has not been reported as a pest in Japan so far. Ten to 30 larvae per pot, which were effective in the control of damping-off of sugarbeet, never affected adversely germination and growth of the plants.

Table 7. Suppression of root rot of sugarbeets by root residues[a] with larvae of *Pnyxia scabiei*

R. solani AG-2-2	Treatment of residues[b]	No. of plants	Percentage of diseased plants	Disease severity[c]
Inoculated	No residues	12	100.0	3.0
Inoculated	Heated at 100°C, 10 min	12	100.0	2.6
Inoculated	Treated with insecticide[d]	12	91.7	1.3
Inoculated	Not sterilized	12	33.3	0.4

a): Residues were collected from soil around and on heavily rotted sugarbeet roots by sieving-floatation technique in water.
b): The number of *P. scabiei* in residues was 1,000 larvae/pot.
c): Disease severity; 0 (healthy) - 5 (dead).
d): Larvae of *P. scabiei* were killed by the insecticide isoxathion.

Conclusion

Sclerotia of *R. solani* AG-2-2 are produced abundantly in soil around or on rotten roots in fields of sugarbeet. On the other hand, populations of *Pnyxia scabiei* increase rapidly along with the severity of the disease and the larvae which feed on sclerotia produced in soil around or on the diseased roots can induce sclerotial destruction. Sclerotia play a major role as a source of inoculum in the following summer. However, large-sized sclerotia display a higher saprophytic survival than small ones[9]. Even if sclerotia are only partly eaten by larvae, their leftover fragments are presumably less important as infection sources. Though, experimentally, the larvae were able to reduce the incidence of damping-off as well as root rot of sugarbeet, the relative contribution of the insect larvae to the suppression of root rot disease in fields of sugarbeet with monoculture remains unsolved.

The insect can be considered to be a kind of natural enemy against pathogens. On the other hand, it may damage some crops. In an early report, *P. scabiei* larvae[4] were incriminated in deep-pitted scab of potato. However, in 1976 Tamaki et al[16] reevaluated the role of this insect and found that it did not feed on healthy potato but aggregated on scab lesions and fed only on the pathogen. In Japan, crop damage by this species has not been recorded yet in fields. However, more attention should be paid to the fact that this insect is an indoor pest in houses[17].

To develop a method of control of the pathogens, the role of insects in the disinfection of heavily infested soils with *R. solani* AG-2-2 should be considered.

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