Populations of Mycophagous Amoebae in Saskatchewan Soils

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ABSTRACT

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Populations of soil amoebae that feed on conidia of *Cochliobolus sativus*, the causal agent of common root rot of wheat and barley, were widespread in agricultural soils in Saskatchewan. They occurred mainly in the top 15 cm of soil, but some were also present to a depth of 30 cm. The largest population occurred in the fall and the smallest in the summer. Numbers of amoebae were negatively correlated with temperature, but they were not correlated with soil moisture. Estimates were based only on those amoebae that cause large perforations.

Recent studies have demonstrated that amoeboid organisms in soil perforate and feed on spores and mycelia of various fungi (1,8,13). These mycophagous amoebae or evidence of their activity has been found in soils from Canada (1,2), the United States (2,5), Australia (9), France (13), Scotland, and Holland (8). Some species of amoebae feed on spores by causing small perforations less than 1 μm in diameter (2), whereas others cause large perforations 1–7 μm in diameter (1,8,13). Those that cause large perforations are the most common (2). Although identification is difficult, several species have been implicated, namely *Arachnula impatiens* (10), *Leptomyxa reticulata* (8), *Vampyrella lateritia* (2), and *Thecamoeba granifera* subsp. *minor* (13). *Cashia mycophaga* has also been identified as a mycophagous amoeba that feeds by engulfing and completely digesting fungal hyphae rather than by causing perforations (12).

In most studies, the melanized conidia of *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur were used as bait to isolate the amoeboid organisms. *C. sativus*, imperfect state *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., syn. *Helminthosporium sativum* P.K. & B., is the main causal organism of common root rot in the Canadian prairies where the disease is estimated to cause an annual yield loss of 5.7% in spring wheat (6) and 10.3% in spring barley (11). Because soilborne conidia appear to be the main source of inoculum for common root rot infection, the presence and effect of the mycophagous amoebae in soil under field conditions are of particular interest. This study reports on a survey done to determine the distribution and population of spore-perforating amoebae in agricultural soils of Saskatchewan, their distribution in various soil profiles, and their seasonal population changes.

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MATERIALS AND METHODS

Soil collection. Soil was collected between 25 September and 17 October 1979 from 32 summer-fallowed fields chosen randomly in the agricultural area of Saskatchewan. Samples from 20 m inside each field were taken from five profiles: 0–5 and 5–10 cm, with a scoop; and 10–15, 15–20, and 20–30 cm, with a soil probe. In 1980, soil was collected from 27 May to 30 July from 174 summer-fallowed fields throughout the province. In each field, two samples of soil were taken with a soil probe. Each sample consisted of four cores that were taken at sites 30 m apart into the field commencing 30 m from the field edge. The second sample was collected parallel to the first and 30 m away. In 1981, soil samples were collected on 12 dates between 23 April and 28 October from 20 locations in an area encompassed by Saskatoon, Prince Albert, and North Battleford. The sampling procedure was the same as in 1980 except the distance between sites within a field was 20 m. Good success was achieved in collecting soil from the same sites each time by following the same route. Previous core holes were easily relocated if the field had not been disturbed between samplings.

Data from the Saskatchewan Research Council Meteorological Station at Saskatoon were used to obtain daily mean air temperatures and soil temperatures at the 5-cm depth. The temperatures were calculated as previous weekly means from the date of soil collection. In all 3 yr, the soil sample was 50–100 g. Soil was stored in plastic bags at 2 C until processed, which was usually within 2 wk. The soil was screened (5-mm sieve), and moisture was determined using a Cenco Moisture Balance.

Estimating numbers of amoeba. The method outlined by Anderson and Patrick (1) was used for estimating amoeba numbers. A serial dilution was made by mixing 5 g of soil in 45 ml of distilled water with a magnetic stirrer. One milliliter of a suspension holding $8–12 \times 10^4$ conidia per milliliter of *C. sativus* was added to 1-ml aliquots from the 10^{-1}, 10^{-2}, and 10^{-3} soil dilutions. A soil smear was prepared and scanned at ×100 to ×250 after five or more weeks of incubation at 22 C in vials measuring 19 × 48 mm with polyethylene stoppers to record the presence or absence of perforations (1–7 μm in diameter) in 100 conidia. Each dilution series was replicated five times, and numbers of amoebae were estimated by the most probable number method (7).

RESULTS

Based on the presence of perforated conidia, amoebae occurred in all profiles in 1979 (Table 1). More amoebae occurred in the top 15 cm of soil than below 15 cm. Also, 50% of locations had amoebae in at least one profile in the top 15 cm, whereas only 25% of the locations had activity in profiles below 15 cm.

Table 1. Number of spore-perforating amoebae in soil profiles collected from 32 locations in 1979

Profile (cm)	Amoebae per gram of dry soil		Locations with amoebae (%)
	Range	Mean ± SE	
0–5	0–60	3.2 ± 1.95	28
5–10	0–50	4.5 ± 2.01	34
10–15	0–30	2.9 ± 1.23	31
15–20	0–14	0.8 ± 0.46	16
20–30	0–9	0.9 ± 0.40	19

Table 2. Number of spore-perforating amoebae in soil from fields categorized on the basis of soil texture in 1980

Soil texture	Fields (no.)	Amoebae per gram of dry soil[z] (mean no.)
Sandy loam	9	0.4 ab
Light loam	22	2.5 ab
Loam	82	2.2 ab
Silty loam-clay loam	12	3.8 a
Silty clay	9	2.5 ab
Clay	30	1.1 b
Heavy clay	10	1.1 ab

[z]Values followed by the same letter do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

In the 1980 study of 174 locations, numbers of amoebae followed a Poisson distribution as determined by the Kolmogorov-Smirov test for goodness of fit. The range was estimated as 0–39 amoebae per gram of dry soil, and the mean number was 2.0. Eighty-one of 174 fields showed no evidence of activity. The correlation coefficient of amoeba numbers and soil moisture was not significant ($n = 174$, $r = 0.055$, $P > 0.05$).

The grouping of locations in 1980 according to soil texture shows that amoeba numbers were higher in the medium (loam) than in the coarse (sandy) or very fine (clay-heavy clay) textured soils (Table 2). The grouping of locations according to soil color shows that gray and brown soils had fewer amoebae than degraded black, black, and dark brown soils (Table 3). Although the grouping of locations by soil textures and colors showed some differences between groups, there were also significant differences for locations within groups. This indicates that amoeba numbers varied a great deal between locations within a group.

In 1981, the estimated number of amoebae per gram of dry soil varied from 0 to 89. The population size decreased in summer, with the lowest mean value over the 20 locations of 0.7 being recorded for soil sampled on 14 July (Fig. 1). Numbers of amoebae increased in the fall, with the highest mean reading for all locations of 12.6 being recorded for soil sampled on 6 October.

Soil moisture varied from 2.9 to 24.8% for all locations; for the average of the 20 locations, soil moisture varied from 10.6 to 17.6% (Fig. 1). These levels were usually between field capacity and wilting point of the soil, but for some soils at some dates, the moisture levels were below the wilting point. High numbers of amoebae were not associated with high soil moisture levels. In September, when the moisture level (average of all soils) was the lowest, amoeba numbers were increasing. The correlation coefficient between percentage of moisture and amoeba numbers was not significant whether all samples were used ($n = 240$, $r = -0.060$, $P > 0.05$) or whether only means at each sampling date were used ($n = 12$, $r = -0.145$, $P > 0.05$).

There was an inverse relationship between both air and soil temperature and numbers of amoebae (Fig. 1). Based on the means of the 12 sample dates, the correlation coefficient between numbers of amoebae per gram of dry soil and the variables of daily mean air temperature, soil temperature at 5 cm at 0900 hr, and soil temperature at 5 cm at 1600 hr was −0.624, −0.652, and −0.635, respectively. These values were all significant ($P < 0.05$).

All fields selected in the spring of 1981 had been cropped to a cereal in 1980. The cropping practices for these fields in 1981 varied; 10 fields were left fallow (no crop) and 10 fields were cropped (six wheat, two barley, one sweet clover, and one grass). Amoeba numbers were lower in fallow than in cropped fields during the summer (Fig. 2). Nevertheless, as indicated in Figure 1, amoeba numbers were highly variable, and this is reflected in the large standard errors.

Table 3. Number of spore-perforating amoebae in soil from fields categorized on basis of soil color in 1980

Soil color	Fields (no.)	Amoebae per gram of dry soil[z] (mean no.)
Gray	8	0.8 ab
Degraded black	17	2.6 ab
Black	49	2.7 a
Dark brown	65	2.1 ab
Brown	35	1.0 b

[z]Values followed by the same letter do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

DISCUSSION

Amoebae, which cause large perforations in *C. sativus* conidia, were widespread in agricultural soils in Saskatchewan. There was no indication of an unusual or uneven distribution, but fewer amoebae occurred in brown soils, which are located in the southwestern region. Amoebae were present mainly in the top 15 cm of soil, but they were also found down to a 30-cm depth. In soils sampled in Ontario, amoebae were most numerous in the top 5 cm, but they were also found to a depth of 20 cm (1).

Populations of spore-perforating amoebae showed seasonal fluctuations,

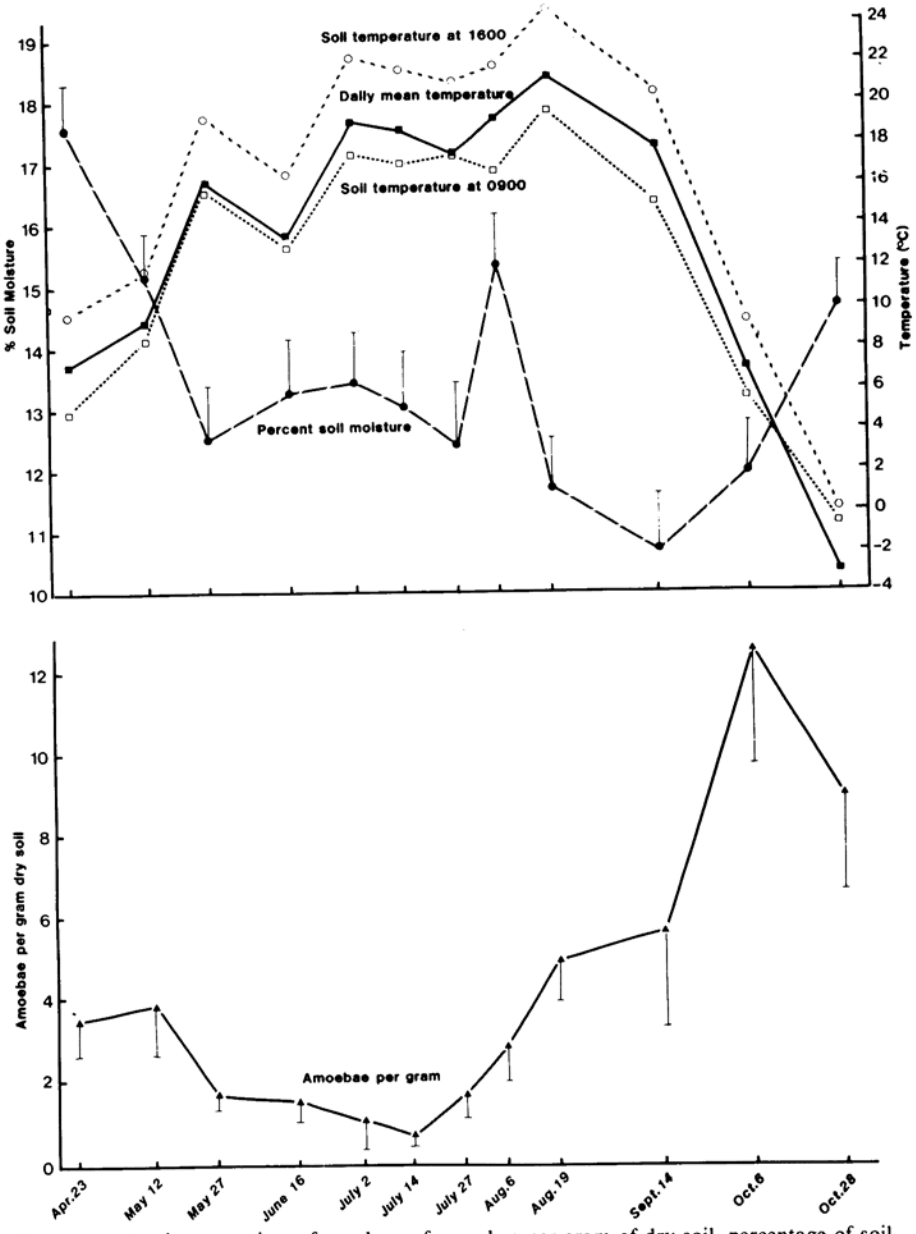

Fig. 1. Fluctuation over time of numbers of amoebae per gram of dry soil, percentage of soil moisture, and previous weekly means of daily mean temperature and of soil temperature at a 5-cm depth at 0900 and 1600 hours in 1981. Bars represent half the standard error of the mean of 20 locations.

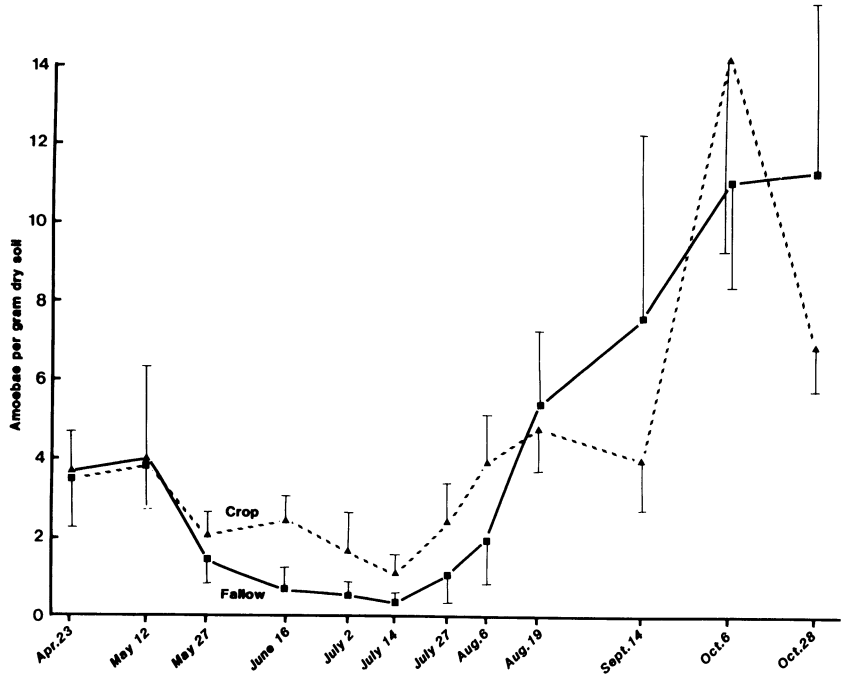

Fig. 2. Numbers of amoebae per gram of dry soil in summer-fallowed (bare) and in cropped fields in 1981. Bars represent half the standard error of the mean of 10 locations.

with the largest numbers occurring in the fall and the smallest in summer. These results are similar to those reported by Cutler et al (3), who did daily counts of amoeba numbers in soil over a season. A fall peak in population has also been recorded in soils in Europe and in Russia (15) and in a freshwater pond in the United States (16). In contrast, Singh and Crump (14) found no seasonal fluctuation in the total amoeba population in soil.

In the present study, numbers of mycophagous amoebae were not correlated with soil moisture where moisture levels were generally between field capacity and wilting point. Cutler et al (3) also found no significant effect of soil moisture on amoeboid numbers when soil moisture varied from 12 to 22%. Cutler and Dixon found that active forms of common soil amoebae were present at moisture levels above 13% in a soil with a water-holding capacity of 37.9% (4). Below this moisture level, amoebae were present only as cysts. From this they postulated that soil moisture would limit amoeboid activity when it was one-sixth to one-fifth of the water-holding capacity. However, Old and Patrick (10) suggested that mycophagous amoebae will not be very active in soil drier than field capacity (about −300 mbar) and that most activity would be between −25 and −100 mbar, which is at soil moisture levels greater than field capacity. Although one would assume that soil moisture is a major limiting factor for amoebal activity, this view is not supported by the data from the present field study and from that of Cutler et al (3). Numbers of amoebae fluctuate even when soil moisture is relatively low.

Numbers of spore-perforating amoebae were correlated negatively with soil temperature and with mean air temperature. Also, amoeba numbers were higher in cropped fields than in summer-fallowed (bare) fields during the summer when soil temperatures would be expected to be higher in summer-fallowed than in cropped fields.

In this study, numbers of amoebae were estimated only indirectly by recording the frequency of conidia that showed perforations. This technique was considered appropriate because previous studies (1,2,8) have shown that perforations in spores are caused by amoebae. Pussard et al (13) used a different technique to estimate the population of amoebae. Also, only the population of amoebae that cause large perforations was determined. The small perforations that are reportedly caused by other species could not be detected at the magnifications used. However, the species that cause large perforations were more common (2).

This survey provides a basis for further investigation into the role of moisture and temperature on mycophagous amoeboid activity in soil.

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