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Ecology and Epidemiology

Influence of Matric and Osmotic Water Potentials and Soil pH on the Activity of Giant Vampyrellid Amoebae

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ABSTRACT

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Perforation of conidia of *Cochliobolus sativus* by giant vampyrellid amoebae did not occur at soil matric water potentials higher (wetter) than −10 to −50 mbars or lower (drier) than −200 to −250 mbars. Perforation activity commenced with air entry into the soil, which occurred at about −50 mbars (or slightly drier) in both a silt and clay loam and at about −10 and −25 mbars in the same two soils, respectively, mixed (1:1, v/v) with coarse sand. In liquid culture, amoebae were highly active in 10% soil (Palouse silt loam) extract and in distilled water (0 bars), indicating that the flooding is not directly limiting to their activity. Perforation activity also tended to cease at lower matric potentials in the loams (−200 to −250 mbars) than in the loam-sand blends (−150 mbars), either because too few water-filled pores remained in the soils and blends at these matric potentials, or because the amoebae can no longer maintain adequate turgor at these matric potentials. In soils from 183 fields in Japan, perforation of conidia of *C. miyabeanus* was maximal in soils at pH 6.5–7.0 and at electrical conductivity (EC) values for salinity in the range from 400 to 800 μmho/cm, and it was nil in soils at pH 4.0 and having EC values >1,200 μmho/cm (= −426 mbars osmotic potential). In liquid culture, the trophozoites remained active in distilled water adjusted to about −450 mbars osmotic potential with KCl, and in 10% soil extract solution adjusted to −750 to −800 mbars osmotic potential with either KCl or $NaNO_3$. The different lower limits of osmotic potential for perforation activity in 10% soil extract than in distilled water probably indicate the lower limits at which the trophozoites can maintain turgor in a saline environment. Turgor may be lost and the trophozoites may therefore cease activity at even higher water potentials in nonsaline soils.

Additional key words: antagonists, biological control, soilborne plant pathogens.

Giant vampyrellid amoebae cause perforations and annular depressions (partial perforations) in the walls of pigmented spores (1,9–11) and hyphae (7,8) of plant pathogenic fungi. However, the effectiveness of these organisms as agents of biological control will depend on their ability to move in soil from one pathogen propagule to another. As shown for nematodes (12), aquatic fungi (5), and bacteria (6), the ability to move from site to site within the soil matrix depends on the size and continuity of water-filled pores. Amoebae can move through a Nuclepore filter provided the openings are 5.0 μm or greater (9); in soil, pores of this size and larger drain at a matric potential of about −600 mbars. In addition, amoebae in their trophozoite state may be more sensitive than bacteria or fungi to salts in soil; without a rigid cell wall, the trophozoite would be unable to maintain a high turgor (pressure) potential and hence also may be less able to make the internal osmotic adjustments necessary for maintenance of a turgor when moving between saline and nonsaline environments. Finally, amoebae, being strictly aerobic, are likely to become inactive in water-saturated soil if oxygen diffusion through the water-filled pores is too slow to meet their needs.

If amoebae are to be used as agents of biological control, either introduced or managed as residents in the soil (2), then the environmental parameters under which they function must be known. This work was undertaken to determine the influence of various physical and chemical properties of soils on the perforation activity of amoebae. A preliminary report has been published (3).

MATERIALS AND METHODS

Quantification of perforation activity. Perforation activity was quantified by counting the holes produced by amoebae in conidia of either *Cochliobolus sativus* (Ito et Kurib.) Drechs. ex Dast. or *C. miyabeanus* (Ito et Kurib.) Drechs. ex Dast. The conidia were collected dry from 2-wk-old cultures grown on potato-dextrose

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agar in the light at 20–25 C. They were distributed uniformly over a 13-mm-diameter Millipore filter by suspending them in sterile deionized water and then forcing 2–3 ml of the suspension through the filter mounted in a syringe. The Millipore filter was then sandwiched between two 25-mm-diameter Nuclepore filters (Nuclepore Corporation, Pleasanton, CA 94566) and buried in soil (9). The Nuclepore filters were joined at their outer edges by a thin ring of stopcock grease.

The amount of perforation of conidia recovered from the soil was estimated under both light and scanning electron microscopy. For estimations by light microscopy, strips of double-stick tape were pressed against the Millipore filter, transferred with the adhering spores to a glass microscope slide, and mounted in a drop of cotton blue in lactophenol (9). For observations by SEM, Millipore filters bearing spores were air-dried, mounted on specimen stubs, and coated with gold. Examination was done with a scanning electron microscope. Three pictures were taken from each stub and perforated spores were counted from these pictures. In experiments with liquid cultures, the number of perforated spores and number of amoebae per either 25 or 50 fields (depending on the density) were determined under light microscopy (approximately ×200) by examining the fields at random.

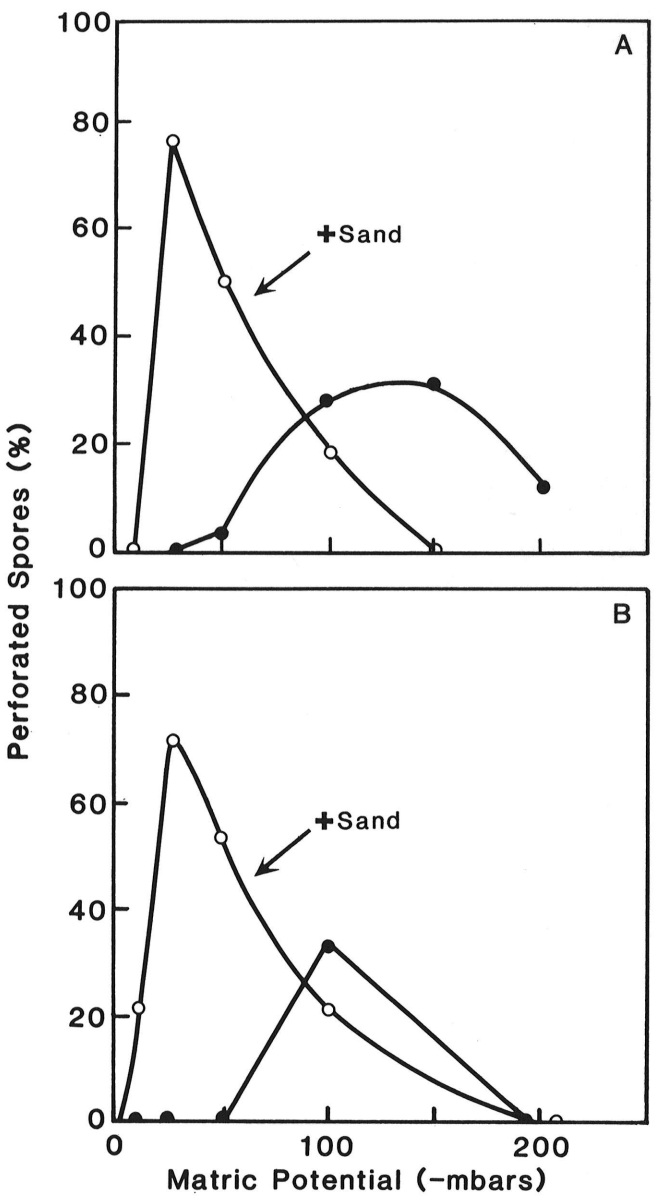

Fig. 1. Incidence of perforated spores of *Cochliobolus sativus* after burial in either **A**, a clay loam or **B**, a silt loam used either alone or blended with sand (1:1, v/v) and incubated for 4 wk at different matric water potentials.

Studies of the influence of soil matric potential on perforation activity. A clay loam from a wheat field near Moses Lake, WA, and a silt loam from a wheat field near Quincy, WA, were used for studies of the influence of soil matric potential. Each soil was air-dried, mixed in a twin-shell blender, passed through a 4-mm (5-mesh) sieve, and stored in a cool, dry place. Each soil was used unmodified (except for the mixing and sieving) or as a blend with plaster sand (1:1, v/v), to modify its texture and hence its pore size distribution (4).

Each soil and soil-sand blend was placed as a layer about 2 cm deep on a 9-cm-diameter fritted glass plate of fine porosity contained in a Büchner-type funnel. The fritted glass plate served as a tension plate. Nuclepore/Millipore sandwiches with conidia of *C. sativus* were buried flat and about 1 cm deep in the soil layer. Tygon tubing was connected to the funnel, filled (including the space inside the funnel beneath the plate) with air-free water, and supported as a "U"to pull a suction. When the soil or soil-sand blend was saturated (10–15 min), the tube was lowered to produce tensions (height differences measured to the level of the sandwiches in the soil) of 10, 25, 50, 100, 150, and 200 cm. Since 1,020 cm of water equals 1 bar matric potential, these tensions corresponded approximately to the matric potential in millibars. The units were incubated at about 25 C for 4 wk, during which each funnel was covered with loosely fitting aluminum foil to retard evaporation from the soil. Soil water contents of each soil and blend at each matric potential were determined at the end of the experiment by oven drying samples for 24 hr at 105 C.

Studies of the influence of osmotic potential on perforation activity. Perforation activity was measured in either distilled water or 10% soil extract contained in petri dishes as a thin (1.0 mm thick) layer and adjusted to different osmotic potentials between 0 and about −1,000 mbars with either KCl or $NaNO_3$. Fresh conidia of either *C. sativus* or *C. miyabeanus* were added along with trophozoites and cysts of amoebae and incubated in the dark for 2 wk at 25 C.

Studies of the relationship of pH and soil salinity. Soils were collected from 183 fields located in 37 prefectures of Japan and representing a great diversity of soil textures and cropping systems. The soils were processed to determine pH of a 1:1 suspension in 0.01 M $CaCl_2$, electrical conductivity (EC) (μmho/cm), and activity of amoebae based on perforation of conidia of *C. miyabeanus* after burial in soil (Millipore/Nuclepore sandwich method) for 4 wk at 25 C.

RESULTS

Influence of soil matric potential. Spores of *C. sativus* were not perforated by amoebae in any of the two soils or soil-sand blends at 0 bars matric potential (Fig. 1A and B). As the soil pores were drained in response to decreasing matric potential, perforations occurred in greatest frequency in the two soil-sand blends at −25 mbar, and in the two soils at −100 to −150 mbars. Some perforations occurred in the silt loam-sand blend but not in the clay loam-sand blend at −10 mbar. The frequency of perforation was progressively less in both blends as the matric potential dropped below −25 mbars. No perforations occurred in either soil until the matric potential was below −50 mbars, but −200 mbars was again too dry for perforation in the soils. The water contents for these matric potentials and the ranges within which perforation activity occurred in the soils and soil-sand blends are given in Fig. 2A and B.

Influence of osmotic potential. Perforation activity with spores of *C. sativus* was consistently greater in 10% soil extract solution plus KCl than in distilled water plus KCl at each osmotic potential (Table 1). Perforation of conidia of *C. sativus* was prevented, or nearly so, between about −225 and −450 mbars with distilled water as the basal liquid, and between about −450 and −900 mbars with 10% soil extract as the basal liquid (Table 1). In a second study with only 10% soil extract as the basal liquid, perforation of conidia of *C. miyabeanus* and the number of amoebae were progressively less down to a lower limit of −750 to −800 mbars (Fig. 3).

Influence of soil pH and salinity. Of the 183 soil samples collected from fields in Japan, amoeboid activity (based on perforations of conidia of *C. miyabeanus*) was detected in 105 of the samples and related to both the pH and the electrical conductivity of the soils (Figs. 4 and 5). Perforation activity was maximal in soils at about pH 7.0 and was not detected in soils at either pH 4.0 or 8.5 (Fig. 4). Perforation activity was maximal in soils having EC values in the range from 600 to 900 μmho/cm (−213 to −320 mbars) and was not detected in soils that registered EC values >1,200 μmho/C (osmotic potential about −500 mbars) (Fig. 5).

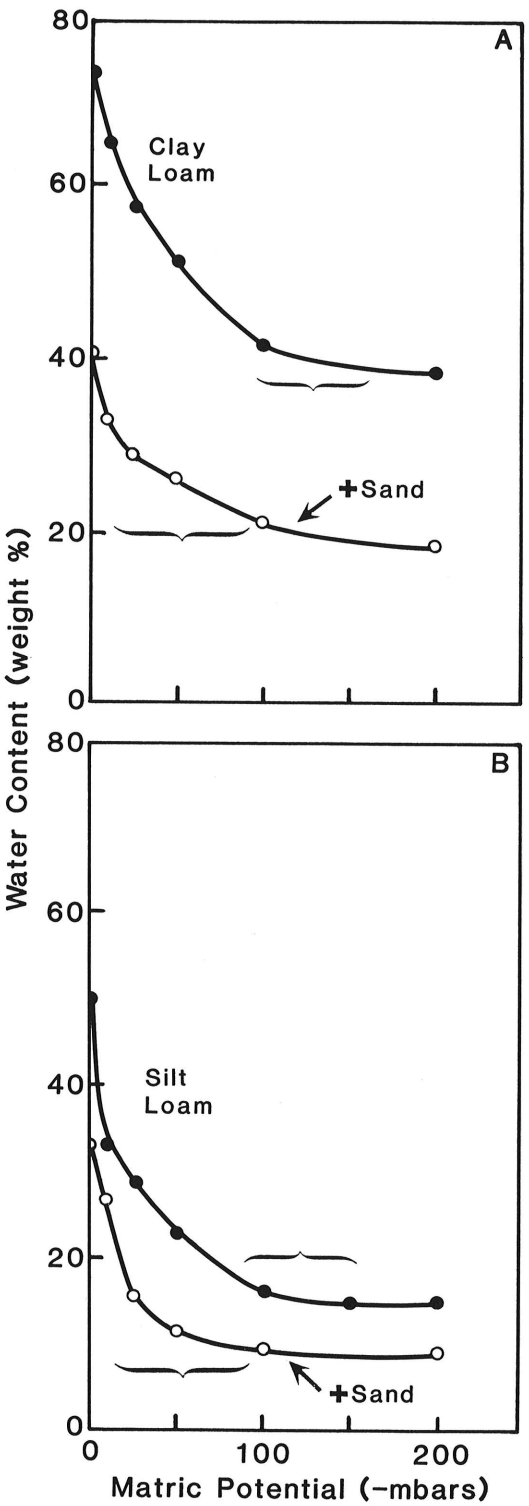

Fig. 2. The matric potential:water content relationships for **A,** a clay loam and **B,** a silt loam used either alone or blended with sand (1:1, v/v). The brackets indicate the range of matric potentials within which amoebae perforated spores of *Cochliobolus sativus*.

DISCUSSION

Based on the frequency of perforations of conidia of *C. sativus*, amoebae are active within a narrow range of soil matric potentials, namely, drier than −10 to −50 mbars but wetter than −150 to −250 mbars, depending on soil texture. The matric potential at field capacity is −300 to −350 mbars for most silt and clay loams, and about −100 to −200 mbars for sandy soils. It appears that biocontrol with amoebae should not be expected while the soil is flooded, nor should it be expected while the soil is at field capacity or drier than field capacity. From results of experiments with liquid media adjusted to different osmotic potentials with salts, and from data on the occurrence of amoeboid activity in the soil samples collected from throughout Japan, it appears that biocontrol by

TABLE 1. Perforation of spores of *Cochliobolus sativus* and numbers of vampyrellid amoebae in distilled water or 10% of soil extract solution amended with increasing concentrations of KCl to decrease the osmotic water potentials

Basal liquid	KCL (m × 10^3)	Osmotic potential[b] (−mb)	Amoebae per 50 fields (no.)	Perforated conidia (%)
Dist. water		0	1,155	92.1
	1	45	885	79.4
	3	133	412	68.5
	5	223	131	35.8
	10	446	33	4.8
	20	892	28	4.5
	30	1,328	32	4.2
Soil extract		0	2,024	96.3
	1	45	950	95.6
	3	133	804	81.9
	5	223	789	87.2
	10	446	420	68.5
	20	892	38	6.2
	30	1,328	30	6.7

[a]The liquid was contained in petri dishes as a layer about 1 mm thick and incubated for 2 wk in darkness at 25 C.
[b]Osmotic potential was calculated on the basis of the KCl concentration. Values for KCl added to 10% soil extract solution were probably slightly lower than given, owing to the dilute salts extracted from the soil.

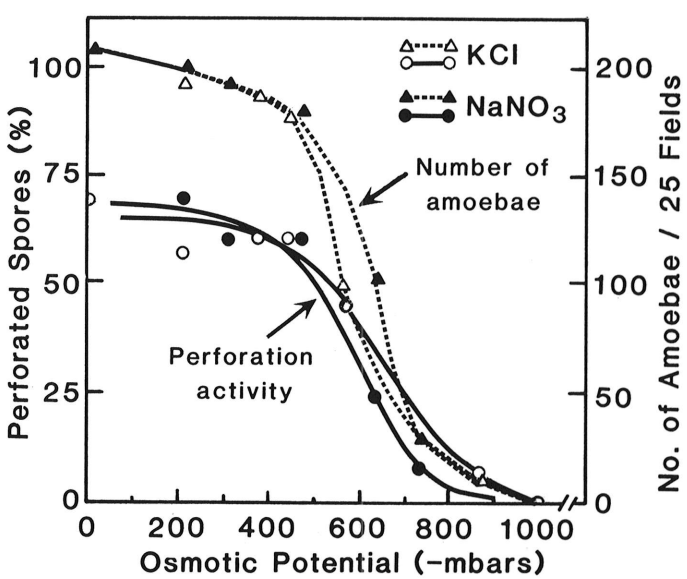

Fig. 3. Number of amoebae (counted under light microscopy at ×200) and the percentage of perforated spores of *Cochliobolus miyabeanus* in 10% soil extract solution adjusted to different osmotic water potentials with either KCl or $NaNO_3$.

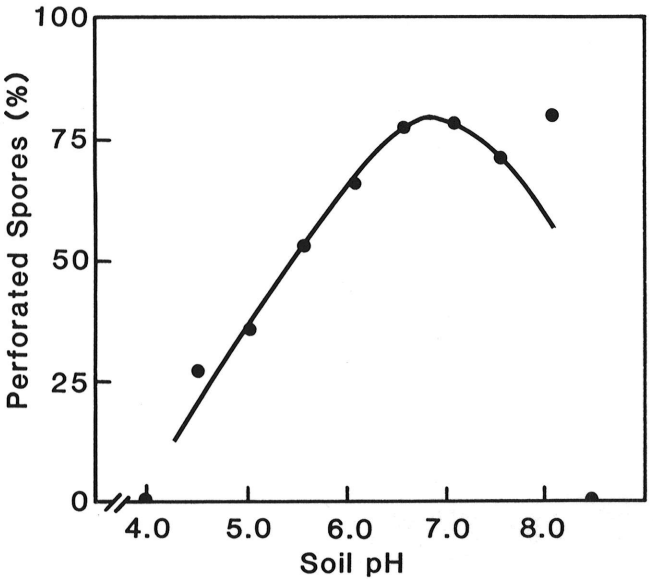

Fig. 4. Relationship of spore perforation by amoebae to soil pH. Spores of *Cochliobolus miyabeanus* were buried in each of 183 soils collected from locations throughout Japan. Each data point is a mean for all soils at that pH (determined in 1:1 suspension in 0.01 M $CaCl_2$) and grouped by half-unit pH increments (4.5, 5.0, 5.5, etc.).

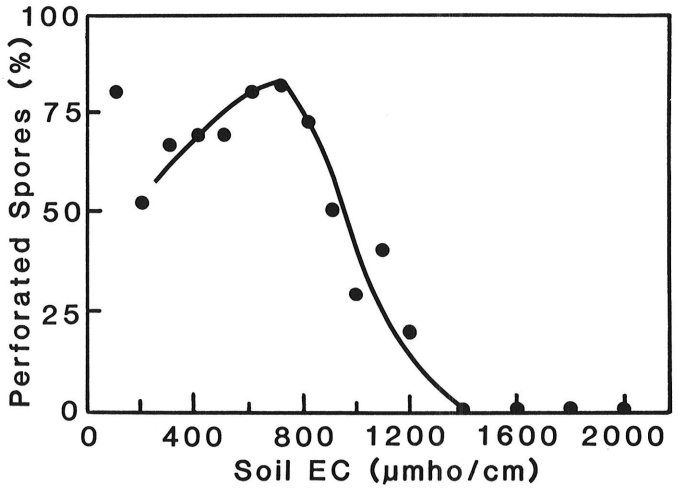

Fig. 5. Relationship of spore perforation by amoebae to electrical conductivity (EC, as μmho/cm). Spores of *Cochliobolus miyabeanus* were buried in each of 183 soils collected from locations throughout Japan. Each data point is a mean for all soils grouped according to 50-unit EC values (300, 350, 400, etc.).

these organisms should not be expected in either saline (>1,200 μmho/cm), acid (pH 4.0–5.0), or alkaline (higher than pH 8.5) soils.

The absence of activity in very wet (or flooded) soils probably relates to the lack of oxygen for the amoebae. That amoebae were active in a thin layer of distilled water or 10% soil extract solution in a petri dish (0 bars) demonstrates that high water potentials are not directly limiting to their activity. The moisture release curves (Fig. 2A and B) provide further support for this conclusion; in all four soils and soil-sand blends, perforations did not occur at matric potentials above (wetter than) those required to permit air entry.

The cessation of perforation activity at the lower matric potentials is more difficult to interpret. Accepting that the giant amoebae cannot move through pores smaller than 5 μm in diameter (9), then −600 mbar (the matric potential required to drain pores larger than 5 μm) can be taken as an estimate of the lower physical limit for movement of amoebae in soil. However, perforation activity ceased in the soil-sand blends at matric potentials drier than −150 to −200 mbar and in the loams at matric potentials drier than −200 to −250 mbars. Some factor other than size of the pore necks still filled with water must account for cessation of their activity at these matric potentials.

Probably the frequency of pores still holding water becomes limiting at −150 mbars in soil-sand blends and at −200 to −250 mbars in the silt loams (4). This could explain why perforation activity ceased at higher matric potentials in the soil-sand blends (−150 to −200 mbars) than in the soils (−200 to −250 mbars) in which a larger proportion of the total pore space is in the smaller size ranges. The physical environment may be suitable for movement down to only −150 to −200 mbars in soil-sand blends but down to −200 to −250 mbars in the soils.

The lower limit of osmotic potential for perforation activity gives an approximation of the internal osmotic potential of the protoplasm of trophozoites, and hence also the turgor potential of the trophozoites. The different values (−450 versus −750 to −800 mbars) of osmotic potential in distilled water and 10% soil extract at which perforation activity ceased are difficult to explain, but these may reflect uptake by the trophozoites of certain ions from the soil extract solution. An uptake of ions could help to lower the internal osmotic potential of the trophozoite and maintain turgor at slightly lower external osmotic potentials. It is also likely that some salt (eg, KCl) will have entered the trophozoites in the different solutions amended with salts, lowering their internal osmotic potential and enabling maintainance of turgor at lower external osmotic potentials than otherwise could have been endured. If this was so, then the turgor potential of an amoeboid cell in a nonsaline soil (eg, the silt loams and silt loam-sand blends used in part of this study) is probably less than +450 mbars, the lower limit of osmotic potential when distilled water was the solvent. Conceivably, it could even be as little as +200 to +250 mbars, in which case the absence of perforation activity at soil matric potentials drier than −200 to −250 mbars (and where uptake of salt would be minimal) could be the result of inadequate turgor. The absence of perforation activity by amoebae in soils from Japan having EC readings for salinity at >1,200 μmho/cm (−450 mbars osmotic potential) is also a likely response to inadequate turgor in the trophozoites.

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