Effects of Certain Solutes, Osmotic Potential, and Soil Solutions on Parasitism of *Criconemella xenoplax* by *Hirsutella rhossiliensis*

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Contribution 2081 of the South Carolina Agricultural Experiment Station. Partially funded through a cooperative agreement with USDA, ARS, Southeastern Fruit and Tree Nut Research Station, Byron, GA.
The authors thank M. J. Chapman for technical assistance and R. J. Cook and B. E. Michel for suggestions.
Accepted for publication 3 November 1982.

ABSTRACT

Jaffee, B. A., and Zehr, E. I. 1983. Effects of certain solutes, osmotic potential, and soil solutions on parasitism of *Criconemella xenoplax* by *Hirsutella rhossiliensis*. Phytopathology 73: 544-546.

Adult *Criconemella xenoplax* nematodes were inoculated with spores of the fungus, *Hirsutella rhossiliensis*, and incubated for 5 days in distilled water or in solutions adjusted to osmotic potentials of −0.3, −3, or −6 bars with KCl. Almost all nematodes in KCl solutions became infected, whereas those in distilled water did not. The effect of KCl was not due to the osmotic potential of the incubation solution because little or no infection occurred in solutions adjusted to −0.3, −3, or −6 bars with sucrose or polyethylene glycol 8000. All inoculated nematodes incubated in solutions adjusted to −6 bars with KCl, KNO_3, KH_2PO_4, $CaCl_2$, $Ca(NO_3)_2$, and $MgCl_2$ became infected; much less infection occurred in solutions containing either Na^+ or SO_4^{-2} (NaCl, $NaNO_3$, Na_2SO_4, K_2SO_4, and $MgSO_4$). Infection in extracts of saturated soil from five orchards was correlated with salt concentration as measured by electrical conductivity. Concentration or dilution of the soil extracts increased or decreased infection, respectively. The results indicate that parasitism of *C. xenoplax* by *H. rhossiliensis* is greatly influenced by the kind and concentration of ionic solutes in the ambient solution.

We recently reported (5) that the fungus *Hirsutella rhossiliensis* Minter and Brady was commonly isolated from dead *Criconemella xenoplax* (Raski) Luc and Raski extracted from peach orchard soils in South Carolina. Although parasitism of the nematode was observed in the laboratory, only 6% of adults and 25% of juveniles incubated in distilled or tap water were invaded. The percentage of nematodes parasitized was greatly increased if the nematodes were heat-stressed prior to inoculation. Because *C. xenoplax* is commonly found in sandy, well-drained soils, we hypothesized that low water potential might also predispose the nematode to infection by the fungus. In this paper, we report the influence of different incubation solutions on parasitism of *C. xenoplax* by *H. rhossiliensis*. The water potential of the solutions was adjusted with various ionic and nonionic solutes.

MATERIALS AND METHODS

H. rhossiliensis produces sticky spores that readily adhere to the cuticle of *C. xenoplax* (5). Adult female *C. xenoplax* on a fine-tipped nematode pick were touched to ~20 spores per nematode of *H. rhossiliensis* (ATCC 46487) grown on cornmeal agar for 2–4 wk at 25 C (5). Inoculated and uninoculated nematodes were incubated at 25 C for 5 days in 10 ml of test solution and then examined at ×430 and ×1,000 for signs of parasitism. In one experiment, a portion of the nematodes in solutions of KCl at −6 bars was transferred to −12 bars and then to −18 bars 24 and 32 hr after inoculation, respectively. Unless specified otherwise, all experiments were replicated three times with 15–25 nematodes per replication.

Osmotic potentials of the incubation solutions were adjusted with KCl, $CaCl_2$, $MgCl_2$, NaCl, KNO_3, $Ca(NO_3)_2$, $NaNO_3$, K_2SO_4, Na_2SO_4, $MgSO_4$, KH_2PO_4, sucrose, or polyethylene glycol 8000 (formerly 6000 = PEG 8000). Concentrations of PEG 8000 required to obtain different osmotic potentials were determined by using the equation: osmotic potential = $1.29\ [PEG]^2 T - 140\ [PEG]^2 - 4[PEG]$; in which [PEG] = grams of PEG 8000 per gram of H_2O and T = temperature (C). This equation was derived by B. E. Michel (*personal communication*) and is an adjustment of a previously published equation (7). Required concentrations of all other solutes were determined with equations provided by Scott (11) and osmotic coefficients and water activity values published by Robinson and Stokes (10).

Soil samples from four peach orchards were collected in winter, 1982, before fertilizer was applied. A soil sample from a fifth orchard was collected in spring, 1982, after fertilization. Nematodes were incubated in extracts from soil saturated with distilled water (saturation extract [9]) and in saturation extracts that were diluted 2 or 4× with distilled water or concentrated 2× in a rotary vacuum evaporator at 55 C. Experiments in undiluted soil extracts were repeated three times and experiments in the diluted or concentrated extracts, twice.

Electrical conductivity of soil extracts and KCl solutions was measured with a conductivity bridge (Model 31; Yellow Springs Instrument Company, Yellow Springs, OH). The pH of all solutions was also measured.

RESULTS

Effects of KCl, $CaCl_2$, sucrose, or PEG 8000 on parasitism. All inoculated nematodes incubated in solutions adjusted to −6, −12, or −18 bars with KCl were penetrated, infected, killed, and colonized (filled with hyphae) (Table 1). Inoculated nematodes in

TABLE 1. Mortality of *Criconemella xenoplax* inoculated with *Hirsutella rhossiliensis* and incubated in solutions adjusted to 0, −6, −12, or −18 bars with KCl [a]

	Mortality (%) at water potential (bars)			
C. xenoplax	0	−6	−12	−18
Inoculated	0 ± 0[b]	100 ± 0	100 ± 0	100 ± 0
Uninoculated	0 ± 0	2 ± 5	10 ± 13	37 ± 15

[a] Adult *C. xenoplax* were uninoculated or inoculated with *H. rhossiliensis* (~20 spores per nematode) and incubated in test solutions at 25 C for 5 days. A portion of the nematodes in −6 bars KCl was transferred to −12 bars and then to −18 bars KCl at 24 and 32 hr after inoculation, respectively.

[b] Percent nematodes dead ± SD. Each value is the mean of three replications (15–25 nematodes per replication). Death of inoculated nematodes was always associated with fungal infection.

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distilled water and uninoculated controls were not parasitized, but mortality of uninoculated nematodes increased as osmotic potential decreased (Table 1).

In KCl solutions at higher potentials (−0.3, −3, and −6 bars), the percent penetration also was high (Table 2), but the percent colonized nematodes increased from 10 to 100% as osmotic potential decreased from −0.3 to −6 bars. Uninoculated nematodes appeared healthy and active in distilled water and in KCl solutions at −0.3 or −3 bars. Approximately 75% of uninoculated nematodes incubated in KCl solutions at −6 bars appeared healthy; the remainder moved only if gently touched with a fine wire.

Similar results were obtained with $CaCl_2$, but there was much less parasitism in sucrose solutions and none in PEG 8000 solutions (Table 2). However, all nematodes killed by exposure to 60 C for 30 min before inoculation were penetrated and colonized if incubated in sucrose or PEG 8000 solutions (Table 2).

Parasitism as affected by other solutes. We investigated parasitism in solutions adjusted to −6 bars with the following salts: KCl, KNO_3, K_2SO_4, KH_2PO_4, $CaCl_2$, $Ca(NO_3)_2$, NaCl, $NaNO_3$, Na_2SO_4, $MgCl_2$, or $MgSO_4$. Infection was 100% in all solutions except those containing Na^+ or SO_4^{-2} (Table 3). Less than 5% death occurred among uninoculated controls.

Parasitism in soil extracts. The salt concentration in five peach orchard soils was estimated from electrical conductivity values of soil extracts. The conductivity of saturation extracts ranged from 0.40 to 2.54 mmhos/cm (Table 4). The extract with the highest conductivity was obtained from a soil (designated "soil A") collected in spring, approximately 1 mo after orchard fertilization, and it supported the highest level of parasitism. The extract from soil E, collected prior to orchard fertilization, had the lowest conductivity and supported the lowest level of parasitism. The other soil extracts (from soils B, C, and D) were intermediate in conductivity and supported an intermediate level of parasitism (Table 4). The percent infected and colonized nematodes increased when the extract was concentrated 2× and generally decreased when the extract was diluted 2× (Fig. 1). Similar results were obtained when a KCl solution (−0.3 bars) was concentrated or diluted 2×. Dilution of the extract from soil A did not substantially reduce the percent infection (Fig. 1A) but did reduce the percent colonization (Fig. 1B). There was no correlation between the level of parasitism and the pH of soil extracts or the pH of other solutions tested.

DISCUSSION

Lowering the water potential of the incubation solutions increased parasitism only if the water potential was adjusted with certain ionic solutes. PEG 8000 and most salts that contained Na^+ or SO_4^{-2} did not stimulate parasitism. Thus, the increased parasitism cannot be explained solely by the water potential of the incubation solution.

It is unlikely that the increased parasitism resulted from injury to the nematodes in ionic solutions. Uninoculated nematodes in solutions adjusted to −0.3 or −3 bars with KCl were not visibly injured or stressed, whereas almost all inoculated nematodes in these solutions were parasitized. Plant parasitic nematodes usually are not affected by osmotic potentials greater than −10 bars (8).

Osmotic potentials of about −0.3 bars are common in soils where plant parasitic nematodes occur. For example, the mean osmotic potential of saturation extracts from six nonsaline soils was −0.5 bars (1), and that of a similar extract from a California avocado orchard was −0.3 bars (13). In our study, osmotic potentials of saturation extracts from five peach orchards, all of which support high populations of *C. xenoplax* (B. A. Jaffee, *unpublished*), ranged from −0.1 to −0.9 bars.

Stimulation of fungi by addition of solutes to conventional media was discussed recently (2). The similar patterns of response of most fungi to different solutes at similar osmotic potentials suggest that the solutes affect microorganisms primarily by means of osmotic potential (2,3). As previously discussed, we observed different responses when different solutes were used to adjust water potential from −0.3 to −6 bars (Table 2). Although the reasons for the observed differences remain unclear, it is possible that the increased parasitism resulted from fungal stimulation by the solutes and that such stimulation may depend on the uptake of the solutes by the fungus (2,3).

The low level of infection in distilled or tap water may be explained by the limiting or adverse effect of pure water on some fungi and bacteria (4,14). The high level of penetration and colonization of heat-killed or heat-stressed nematodes in distilled water (5) may be due to impairment of the nematode's defense mechanisms (eg, a reduction in cuticle integrity) or to stimulation

TABLE 2. Penetration of *Criconemella xenoplax* by *Hirsutella rhossiliensis* in solutions adjusted to 0, −0.3, −3, or −6 bars with KCl, $CaCl_2$, sucrose, or PEG 8000[a]

Solute	Nematode treatment[b]	Nematodes penetrated (%) at water potential (bars) 0	−0.3	−3	−6
KCl	None	0 ± 0[c]	95 ± 5	100 ± 0	100 ± 0
$CaCl_2$	None	0 ± 0	48 ± 14	100 ± 0	100 ± 0
Sucrose	None	0 ± 0	16 ± 16	20 ± 19	29 ± 23
Sucrose	Heat-killed	100 ± 0	100 ± 0	100 ± 0	100 ± 0
PEG 8000	None	0 ± 0	0 ± 0	0 ± 0	0 ± 0
PEG 8000	Heat-killed	100 ± 0	100 ± 0	100 ± 0	100 ± 0

[a] Adult *C. xenoplax* were inoculated with *H. rhossiliensis* (~20 spores per nematode) and incubated in test solutions for 5 days at 25 C. PEG 8000 = Polyethylene glycol 8000.
[b] Nematodes were untreated or killed (30 min at 60 C) prior to inoculation.
[c] Percent nematodes penetrated by *H. rhossiliensis* ± SD. Each value is the mean of three replications (15–25 nematodes per replication).

TABLE 3. Percent infection of *Criconemella xenoplax* by *Hirsutella rhossiliensis* in solutions adjusted to −6 bars with certain ions[a]

Cations	Nematodes infected (%) in solutions containing anions Cl^-	NO_3^-	SO_4^{-2}	$H_2PO_4^-$
K^+	100 ± 0[b]	100 ± 0	18 ± 18	100 ± 0
Ca^{+2}	100 ± 0	100 ± 0	NT[c]	NT
Na^+	47 ± 37	0 ± 0	0 ± 0	NT
Mg^{+2}	100 ± 0	NT	8 ± 4	NT

[a] Adult *C. xenoplax* were inoculated with *H. rhossiliensis* (~20 spores per nematode) and incubated in test solutions at 25 C for 5 days.
[b] Percent nematodes infected ±SD. Each value is the mean of three replications (15–25 nematodes per replication).
[c] NT = not tested.

TABLE 4. Infection and colonization of *Criconemella xenoplax* by *Hirsutella rhossiliensis* in soil extracts[a]

Soil	Extract conductivity[b]	Osmotic potential[c]	Infected[d]	Colonized[e]
A	2.54 ± 0.49	−0.9	100 ± 0	77 ± 24
B	1.20 ± 0.38	−0.4	62 ± 30	22 ± 17
C	0.98 ± 0.05	−0.4	55 ± 32	20 ± 17
D	0.93 ± 0.03	−0.3	58 ± 20	22 ± 17
E	0.40 ± 0.03	−0.1	24 ± 9	7 ± 6

[a] Adult *C. xenoplax* were inoculated with *H. rhossiliensis* (~20 spores per nematode) and incubated at 25 C for 5 days in saturation extracts from five peach orchards. Soil A was collected in spring, approximately 1 mo after fertilizer was applied, whereas other soils were collected in the winter, prior to fertilization.
[b] mmhos/cm ±SD. Each value is the mean of three replications.
[c] Osmotic potentials (bars) of saturation extracts were calculated from the conductivity values according to Richards (9).
[d] Percent nematodes containing *H. rhossiliensis* infection hyphae ±SD. Each value is the mean of three replications (20–25 nematodes per replication).
[e] Percent nematodes with body filled by *H. rhossiliensis* hyphae ±SD. Each value is the mean of three replications (20–25 nematodes per replication).

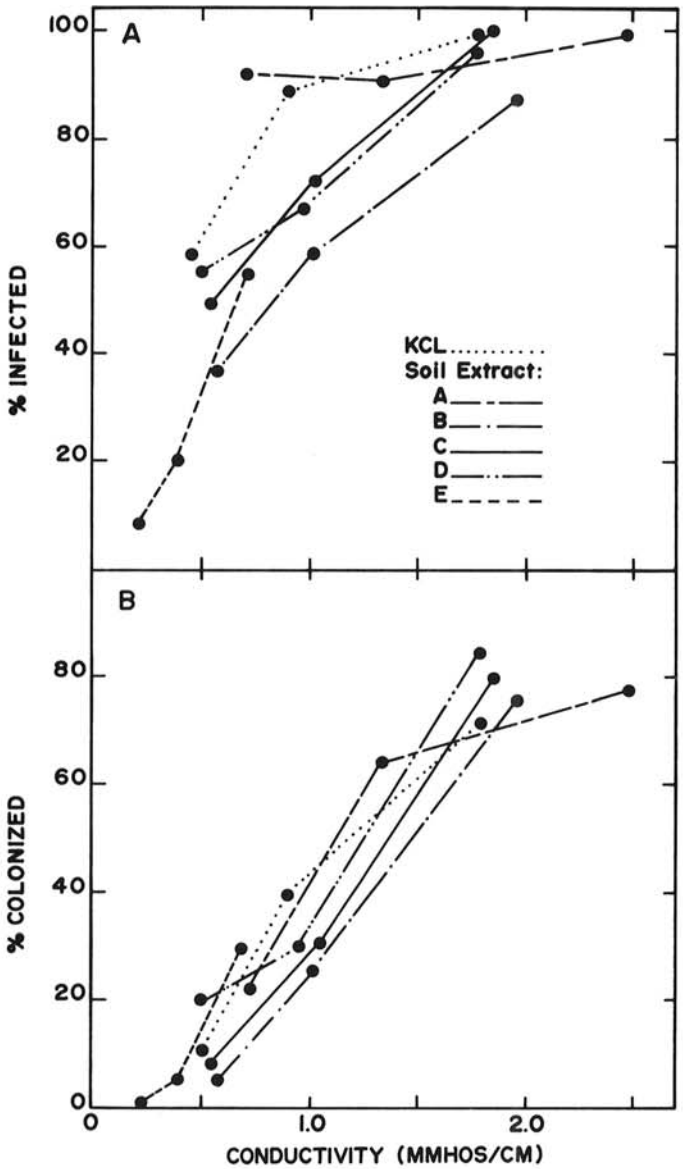

Fig. 1. Parasitism of *Criconemella xenoplax* by *Hirsutella rhossiliensis* in untreated, diluted, or concentrated soil extracts. Adult *C. xenoplax* were inoculated with *H. rhossiliensis* (~20 spores per nematode) and incubated for 5 days at 25 C in 10 ml of extract from five peach orchard soils (designated A-E). The saturation extract from soil A was untreated (point with highest conductivity) or diluted 2 or 4×. For other soils, the saturation extract was untreated (intermediate point), diluted 2×, or concentrated 2× at 55 C in a rotary vacuum evaporator. KCl solutions (−0.3 bars, 2× diluted, or 2× concentrated) were also tested. **A,** Percentages of nematodes infected. Nematodes were considered infected if the fungus penetrated the cuticle and formed infection hyphae. **B,** Percentages of nematodes colonized. Nematodes were considered colonized if filled with fungal hyphae. Uninoculated nematodes (controls) were not infected and remained alive. Each value is the mean of two replications (20–25 nematodes per replication).

of the fungus by ions released from the injured nematode.

Of the ions tested, only Na^+ and SO_4^{-2} failed to stimulate parasitism. Inhibition of growth by Na^+ has been described for other fungi (6). Sung and Cook (14) observed greater sporulation of *Fusarium roseum* with KCl compared to NaCl at 10 and 25, but not at 30 C. Sterne (12) reported that $MgSO_4$ inhibited *Phytophthora cinnamomi* but the inhibition was attributed to the Mg^{+2} ion.

The percent nematodes infected and colonized in saturation extracts from five peach orchard soils was correlated with salt concentrations as estimated by electrical conductivity (Table 4, Fig. 1). If the degree of parasitism in soil is determined in part by salt concentration, parasitism might be increased by application of certain fertilizers at certain times. However, it is possible that soil solutions in the field, which at field capacity probably are 2–4× more concentrated than saturation extracts (9), always contain sufficient ions to support infection of inoculated nematodes. Inoculum levels (number of spores adhering to each nematode), temperature, and other unknown factors probably interact with salt levels to affect parasitism of *C. xenoplax* by *H. rhossiliensis*.

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