Effect of Soil Moisture and Texture on Transmission of the Nematophagous Fungus *Hirsutella rhossiliensis* to Cyst and Root-Knot Nematodes

E. C. Tedford, B. A. Jaffe, and A. E. Muldoon

Department of Nematology, University of California, Davis 95616-8668.

ABSTRACT

Tedford, E. C., Jaffe, B. A., and Muldoon, A. E. 1992. Effect of soil moisture and texture on transmission of the nematophagous fungus *Hirsutella rhossiliensis* to cyst and root-knot nematodes. Phytopathology 82:1002-1007.

The nonmotile spores of *Hirsutella rhossiliensis* adhere to and initiate infection of vermiform nematodes in soil. Transmission of spores to second-stage juveniles (J2) of *Heterodera schachtii* and *Meloidogyne javanica* was quantified in vials that contained 15–17 cm³ of loam, loamy sand, or sand in the absence of a host plant. Fungal inoculum was added to the soil at day 0 as colonized nematodes from which the fungus sporulated, and healthy J2 of *H. schachtii* or *M. javanica* were added after 14 days at 20 C and were extracted 66 h later. Transmission (measured as the probability [*P*] of a J2 acquiring at least one spore) to both nematodes and in all three soils was inversely related to soil matric potential between −80 and near 0 J kg⁻¹. Reduced transmission at high water

potentials appeared to reflect reduced sporulation of the fungus rather than reduced movement of nematodes. Transmission to *H. schachtii* was greater or equal to transmission to *M. javanica*. Transmission to both nematodes was greatest in loamy sand, intermediate in loam, and lowest in sand. The equation $P = 1 - e^{-tr \cdot col}$ (where col = number of colonized nematodes added per vial at day 0 and tr = transmission parameter) described transmission to *M. javanica*. This equation, which previously was found to describe transmission of *H. rhossiliensis* to *H. schachtii*, enabled comparisons among experiments and will facilitate the incorporation of transmission data into epidemiological models.

Additional keywords: biological control, endoparasitic, epidemiology, modeling, parameter estimation, soil water.

In the literature on epidemics caused by parasites in populations of nematodes and other invertebrate hosts, the process by which parasites contact hosts is termed "transmission" (1,9,11). As a prerequisite to infection, transmission is a critical component of epidemiological models but is the most difficult component to quantify and describe (1).

Many fungi that parasitize vermiform, soilborne nematodes are nonmotile. For these fungi, transmission is a function of the volume of soil traveled by the host nematode, hence the rate and duration of nematode movement (4,20,22). Other factors that influence the transmission of nonmotile parasites to nematodes include the number of infective propagules per unit of soil (4,9,20); the diameter of soil pores, host nematodes, and parasite propagules (9); and the attractiveness of the parasite to the host (14).

We are studying transmission of the nematophagous fungus *Hirsutella rhossiliensis* Minter & Brady to plant-parasitic nematodes to help understand the potential of this and similar fungi and bacteria as biological control agents. *H. rhossiliensis* produces nonmotile spores on phialides. Spores adhere to passing nematodes, and the germination tubes (one per spore) penetrate directly through the nematode's cuticle; only one spore is required for infection. The fungus grows throughout the nematode, kills it in a few days, and then sporulates from the cadaver (13).

Transmission of *H. rhossiliensis* to *Heterodera schachtii* Schmidt was substantially greater in a loamy sand than in a coarse sand, perhaps because of differences in soil water or texture (9). In the present study, we quantified the effects of soil water and texture on transmission of *H. rhossiliensis* to two important pests, the sugar beet cyst nematode, *H. schachtii*, and the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood.

MATERIALS AND METHODS

General. Three soils were used: a loam (46% sand, 36% silt, 17% clay, pH 7.0, 1.3% organic matter) from a fallow field in

Yolo County, California; a loamy sand (83% sand, 13% silt, 4% clay, pH 4.9, 0.2% organic matter) from a peach orchard in Merced County, California; and a coarse greenhouse sand (98% sand with 50, 37, and 13% coarse, medium, and fine sand, respectively; pH 8.1; <0.1% organic matter). The soils were heated for 2 h at 60 C to kill nematodes and any *H. rhossiliensis* in the soil, screened (with 5-mm-diameter openings for the loam and 2-mm-diameter openings for the loamy sand and sand), and air-dried. Data for moisture release curves (Fig. 1) were obtained with tension plates (3) and a pressure plate apparatus (16).

Cysts of *H. schachtii* were obtained from sugar beet (*Beta vulgaris* L. 'SSYl') pot cultures. Cysts were incubated on Baermann funnels, and healthy second-stage juveniles (J2) were collected every 2 h, aerated at 10 C, and used within 24 h. *M. javanica* was cultured on tomato (*Lycopersicon esculentum* Mill. 'UC 82') grown hydroponically (17). Juveniles less than 24 h old were collected from the hydroponic cultures, aerated at 10 C, and used within 24 h. *H. rhossiliensis* (isolate IMI 265748) was added to the soil in the form of colonized J2 of *H. schachtii* (9).

To initiate most experiments, known numbers of *H. rhossiliensis*-colonized *H. schachtii* in 4.5 mM KCl were mixed into 26.1 g (dry weight) of loamy sand, 22.0 g of loam, or 25.0 g of sand to give a fungal inoculum and soil moisture level as indicated for each experimental treatment. On day 0, soil was packed into 25-ml plastic vials with holes in the bottom (18) to a volume of 15–17 cm³; the bulk density was 1.4, 1.5, or 1.6 for loam, loamy sand, or sand, respectively. Vials were covered with plastic lids, placed in a clear plastic box with moistened paper towels (moisture chamber), and incubated at 20 C for 14 days; this allowed time for the fungus to sporulate from the colonized nematodes (9). At day 14, assay nematodes (300–400 healthy J2 of either *H. schachtii* or *M. javanica*) in 0.5 ml of 4.5 mM KCl were added to the soil surface of each replicate vial. The assay nematodes were recovered by wet sieving (25-μm pore diameter) and centrifugal flotation (15) after 66 h, unless stated otherwise, and at least 60 nematodes per replicate were examined at 100–140× magnification. Transmission was evaluated as the percentage of recovered assay nematodes that acquired at least one spore of

H. rhossiliensis. In some experiments the number of spores per nematode with spores was determined; randomly selected J2 with spores were placed on a glass microscope slide, covered with a coverslip, and examined at 400× magnification (7).

Each experiment was completely randomized and conducted at least twice (trials 1 and 2) with six replicate vials per treatment (or level) per trial unless stated otherwise. Software from Statistical Analysis Systems (19) was used for statistical analysis. The SAS general linear models procedure was used to perform analysis of covariance (linear regression with trial as a covariate) and analysis of variance for a factorial design; data from trials were pooled if the effect of trial was not significant. Significance was determined at $\alpha = 0.05$. The nonlinear regression procedure was used to estimate parameters for an equation that described transmission of *H. rhossiliensis* to *M. javanica*.

Soil moisture and transmission in three soils. The labor required to extract and process samples precluded a factorial experiment involving three soils, six moisture levels, and two assay nematodes. Thus, six independent experiments, each with five moisture levels, were conducted with each combination of three soils and two assay nematodes. When *H. schachtii* was the assay nematode, the number of colonized *H. schachtii* added per vial at day 0 was 70 ± 5 or 51 ± 6 ($\bar{x} \pm$ SE for trial 1 or 2) in loam, 45 ± 4 or 53 ± 5 in loamy sand, and 57 ± 6 or 54 ± 4 in sand. When *M. javanica* was the assay nematode, the number of colonized *H. schachtii* added at day 0 was 246 ± 6 or 251 ± 7 in loam, 233 ± 10 or 211 ± 6 in loamy sand, and 221 ± 8 or 189 ± 4 in sand. More colonized *H. schachtii* were added when *M. javanica* was the assay nematode, because preliminary experiments indicated that transmission was greater to *H. schachtii* than to *M. javanica* (data not shown). Moisture levels were obtained by adding different volumes of 4.5 mM KCl with the colonized nematodes and encompassed "dry to wet" conditions for each soil based on subjective assessment. For each of the six experiments, the percentage of assay nematodes with spores was regressed on the percentage of water in the soil. The percentage of moisture indicated does not include the 0.5 ml of 4.5 mM KCl added with the assay nematodes.

Because soil moisture levels between 8 and 14% did not affect transmission in the loam (see Results), we tested a wider range of moistures in that soil. Vials were packed with loam containing 67 ± 7 or 76 ± 3 colonized *H. schachtii* (trials 1 or 2); soil moisture levels were 5, 8, 11, 14, 17, and 20%. It was difficult to uniformly distribute the water at the 5% level, and loam at 17 or 20% tended to clump when mixed and packed. Thus, estimates of matric potential at these moisture levels are only approximations. Vials were incubated and assayed with *H. schachtii* as described in the General section.

Effect of KCl on transmission. In the experiments described in the preceding paragraphs, soil moisture was adjusted by adding different volumes of diluted KCl. For example, 0.085–1.105 mg of KCl were added per vial to achieve a range of moistures in

the loamy sand (Fig. 2B). KCl was used to avoid potential detrimental effects of distilled water on the fungus and nematodes (12). Given previous data (7), this range in KCl was not expected to affect transmission, but the effect of KCl on transmission in loamy sand at 8.9% moisture was examined nevertheless. Loamy sand was used for this experiment because water affected transmission more in loamy sand than in loam or sand (see Results). Colonized nematodes (42 ± 6 or 38 ± 5 in trials 1 or 2) in 0.5 ml of 4.5 mM KCl were added to loamy sand moistened with 1.8 ml of water containing 0, 0.6, or 1.4 mg of KCl. Vials were incubated and assayed with *H. schachtii* as described in the General section.

Transmission as affected by soil, species of nematode, and time. To compare the effects of soil and species of assay nematode on transmission, we used one moisture level for each soil; the selected moisture levels were near optimum for transmission in each soil based on other experiments described in this study. Vials were packed with loam (11.0% moisture, $-1,400$ J kg^{-1}), loamy sand (6.9% moisture, -28 J kg^{-1}), or sand (3.0% moisture, -68 J kg^{-1}). In trials 1, 2, and 3, vials contained 105 ± 3, 78

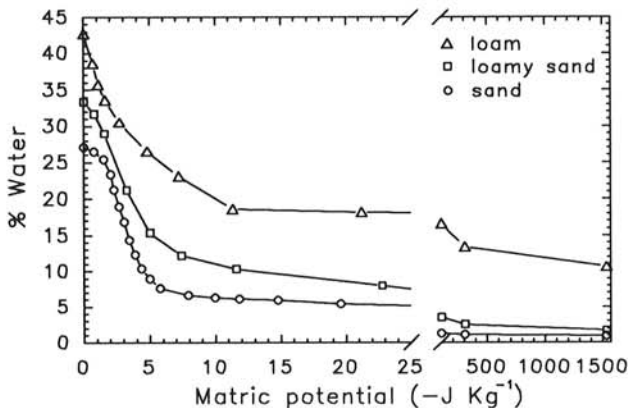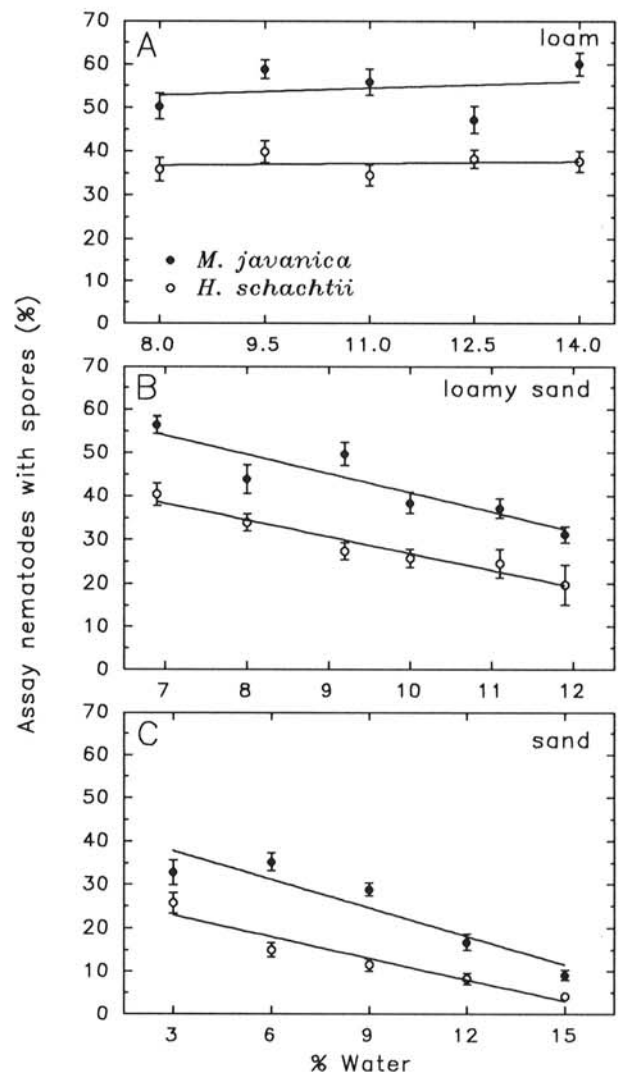**Fig. 2.** Effect of soil moisture on transmission of spores of *Hirsutella rhossiliensis* to assay nematodes (*Heterodera schachtii* or *Meloidogyne javanica*) in three soils. The fungus was added to soil in vials in the form of *H. rhossiliensis*-colonized nematodes. After 14 days at 20 C, assay nematodes were added to each vial (one species per vial). After 66 h, assay nematodes were recovered and examined for spores. Transmission in **A**, loam, **B**, loamy sand, and **C**, sand. Each value is the mean ± SE of 12 replicates (pooled data from trials 1 and 2). Experiments with *M. javanica* and *H. schachtii* were conducted at different times and with different numbers of colonized nematodes; data are presented in one figure to simplify presentation.

Fig. 1. Moisture release curves for loam, loamy sand, and sand (1 J kg^{-1} = 1 kPa = 0.01 bar). Percent water was calculated as grams of water per 100 g of dry soil.

± 6, and 98 ± 8 colonized *H. schachtii*. Vials were incubated and were assayed with *H. schachtii* and *M. javanica* as described in the General section.

Because transmission is related to the volume of soil traveled by host nematodes, and this volume is probably related to the total time of travel, we examined the effect of time on transmission. Fifty vials were packed with sand (3.0% moisture) containing colonized *H. schachtii* (115 ± 7 or 132 ± 3 for trials 1 or 2). After 14 days at 20 C, assay nematodes (either *H. schachtii* or *M. javanica*) were added to all vials. Five replicate vials for each species of assay nematode were extracted daily (days 15–19). All of the extracted assay nematodes were counted, and the percentages of transmission and extraction efficiency were calculated. Every nematode with at least one spore was picked into a drop of water on a glass slide, covered with a coverslip, and observed at 400× magnification to determine the number of spores per nematode.

Nematode motility. To determine whether soil water affected transmission via nematode motility, vials were packed with loamy sand containing no *H. rossiliensis*; moisture levels were 6.9, 8.0, 9.2, 10.0, 11.1, or 11.9%. After 14 days at 20 C, 34 ± 6 or 33 ± 5 (trials 1 or 2) *H. schachtii* J2 in 0.5 ml of 4.5 mM KCl were added to the surface of the soil in each vial. After 16 h, one germinated cabbage seed (*Brassica oleracea* L. 'Chieftain Savoy') was planted 0.5 cm deep in each vial. Vials were placed in a moisture chamber under fluorescent lights (12-h photoperiod) at 24 ± 3 C. After 3 days, roots were removed from soil, measured, and stained (2), and the number of *H. schachtii* per root was determined. There were five replicate vials per water level in each trial.

Differences in nematode movement may affect transmission to the two nematode species. In order to compare motility of these nematodes, we evaluated the percentage of nematodes that penetrated roots of host plants. Loamy sand (9.2% moisture) containing no *H. rossiliensis* was packed into vials. Vials were placed in a moisture chamber at 20 C. On day 12, freshly hatched J2 of *H. schachtii* or *M. javanica* were added to the top or the bottom of each vial (50 ± 4 or 59 ± 4 *H. schachtii* J2 and 57 ± 3 or 59 ± 5 *M. javanica* J2 in trials 1 or 2). On day 14, one germinated cabbage or tomato seed was planted in each vial. Vials were placed in a moisture chamber under fluorescent lights (12-h photoperiod) for 5 days at 25.5 ± 3.5 C. Roots were removed from the soil and stained (2), and the nematodes within the roots were counted. There were 10 replicate vials for each combination of nematode, plant, and site of inoculation; however, the combination of *H. schachtii* and tomato was not included.

Transmission equation. Jaffee et al (11) described transmission

of *H. rossiliensis* to *H. schachtii* in soil in vials with the following equation:

$$P = 1 - e^{-bs} \qquad (1)$$

where P = the probability that a nematode will contact at least one spore after 66 h at 20 C, e = 2.7183, s = thousands of spores per vial, and b (transmission parameter) = the relative volume of soil traveled by the nematode. In previous uses of transmission equation 1 (9,11), each colonized *H. schachtii* added to soil at day 0 was assumed to produce the same number of spores (115 per nematode) in soil as in moisture chambers; hence, $s = (col \times 115)/1,000$, where col = number of colonized nematodes added per vial. Because sporulation may be influenced by soil moisture, we chose not to make this assumption. In the present study, we replaced s with col and b with tr:

$$P = 1 - e^{-tr \cdot col} \qquad (2)$$

Although equations 1 and 2 describe identical curves, equation 2 recognizes the difficulties in separating factors affecting transmission. Equation 2 was used to calculate tr in some experiments.

To determine whether equation 2 described transmission of *H. rossiliensis* to *M. javanica* in sand, vials were packed with sand (6.0% moisture) containing a range in number of colonized *H. schachtii* and were incubated and assayed with *M. javanica* as described in the General section. There were four replicate vials per level of colonized nematodes.

RESULTS

Soil moisture and transmission in three soils. Transmission (the percentage of assay nematodes with spores) of *H. rossiliensis* to either *H. schachtii* or *M. javanica* in loam was not affected by soil moistures between 8 and 14% (Fig. 2A); transmission to *H. schachtii*, however, was suppressed outside that range (Fig. 3). In loamy sand and sand, transmission to both nematodes was greatest at the lowest moisture levels tested and decreased
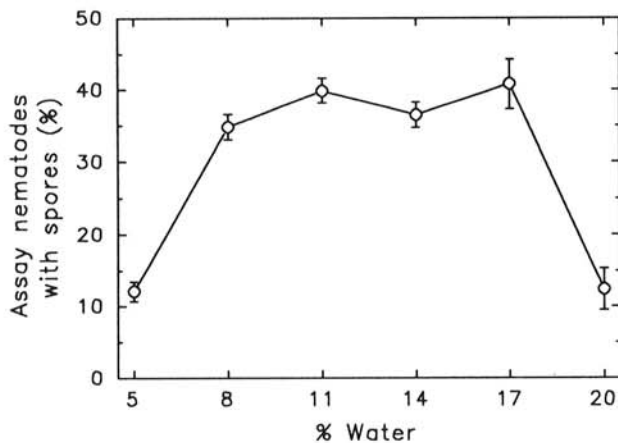

Fig. 3. Effect of soil moisture in loam on transmission of spores of *Hirsutella rossiliensis* to *Heterodera schachtii*. Vials were packed with loam containing 67 or 76 (trials 1 or 2) *H. rossiliensis*-colonized nematodes. After 14 days at 20 C, assay nematodes were added to each vial. After 66 h, assay nematodes were recovered and examined for spores. Each value is the mean ± SE of 12 replicates (pooled data from trials 1 and 2).
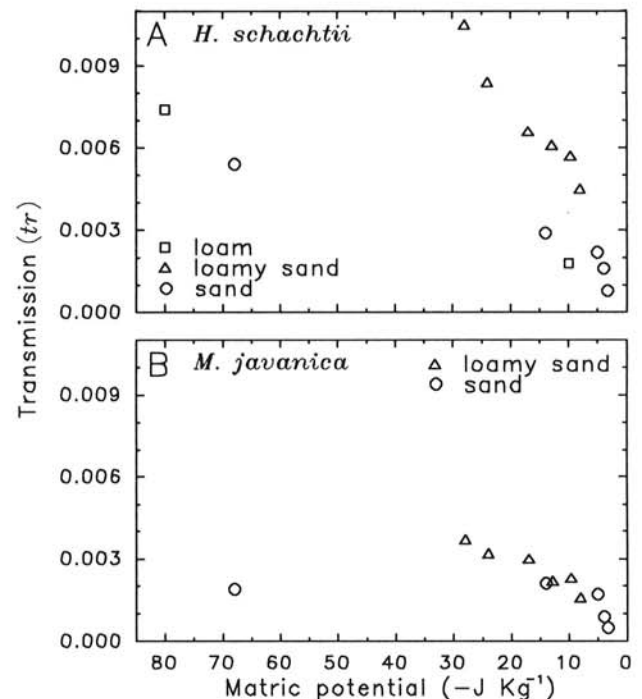

Fig. 4. Relationship between matric potential and transmission of spores of *Hirsutella rossiliensis* to **A**, *Heterodera schachtii* or **B**, *Meloidogyne javanica*. Transmission is expressed as the parameter tr from the equation $P = 1 - e^{-tr \cdot col}$. Estimates of tr and matric potentials are based on data from Figures 1–3. Transmission to *M. javanica* in loam at potentials greater than −80 J kg⁻¹ was not determined.

TABLE 1. Percentage of assay nematodes (*Heterodera schachtii* vs. *Meloidogyne javanica*) with spores of *Hirsutella rhossiliensis* and transmission parameter (*tr*) values as affected by soil[a]

Soil	Water (%)	−J kg⁻¹ [d]	Assay nematodes with spores (%)[b]		*tr* values[c]	
			H. schachtii	*M. javanica*	*H. schachtii*	*M. javanica*
Loam	11.0	<1,360	46 ± 2	37 ± 1	0.0067 ± 0.0001	0.0050 ± 0.0006
Loamy sand	6.9	28	50 ± 2	52 ± 3	0.0075 ± 0.0007	0.0078 ± 0.0005
Sand	3.0	68	26 ± 2	28 ± 2	0.0032 ± 0.0005	0.0035 ± 0.0002

[a] Vials were packed with loam, loamy sand, or sand containing 105, 78, or 98 (trials 1, 2, or 3, respectively) *H. rhossiliensis*-colonized nematodes. After 14 days at 20 C, assay nematodes were added to each vial (one species per vial). After 66 h, assay nematodes were recovered and examined for spores.
[b] Values are the means ± SE of 18 replicate vials (pooled data from trials 1, 2, and 3).
[c] Values (± SE) of *tr* were calculated using equation 2 (see Materials and Methods).
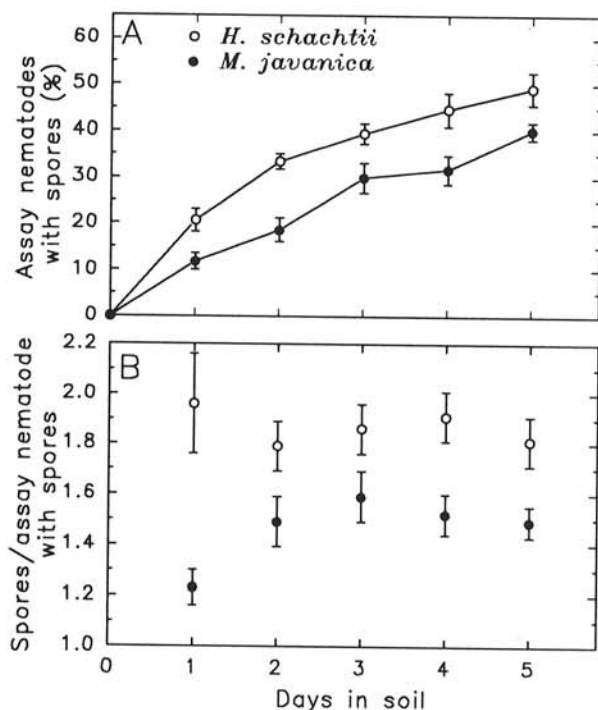[d] Moisture levels were near optimum for transmission.

Fig. 5. Transmission of spores of *Hirsutella rhossiliensis* to assay nematodes (*Heterodera schachtii* or *Meloidogyne javanica*) as affected by number of days assay nematodes were in soil. Vials were packed with sand (3.0% moisture) containing 115 or 132 (trials 1 or 2) *H. rhossiliensis*-colonized nematodes. After 14 days at 20 C, assay nematodes were added to each vial (one species per vial). After 1–5 days, assay nematodes were recovered and examined for spores. Each value is the mean ± SE of 12 replicate vials (pooled data from trials 1 and 2). **A,** Transmission. **B,** Number of spores per assay nematode with spores.

linearly ($r^2 > 0.49$) with increasing soil moisture (Fig. 2B and C).

Data from Figures 2 and 3 were used to determine the relationship between transmission (expressed as *tr*) and matric potential (estimated from Fig. 1). As matric potentials approached 0 J kg⁻¹, *tr* decreased (Fig. 4). The relationship between matric potential and transmission, however, varied with soil and nematode species.

Effect of KCl on transmission. The amount of KCl added per vial did not effect transmission to *H. schachtii* in loamy sand; the percentage of assay nematodes with spores was 23 ± 2, 22 ± 3, and 22 ± 3 in vials that received 0.0, 0.6, and 1.4 mg of KCl, respectively.

Transmission as affected by soil, species of nematode, and time. A significant soil-by-nematode interaction was detected, because transmission was greater to *H. schachtii* than to *M. javanica* in loam but was equivalent in loamy sand and sand (Table 1). For both nematodes, transmission was greatest in the loamy sand, intermediate in the loam, and lowest in the sand (Table 1).

Transmission to *H. schachtii* and *M. javanica* increased over 5 days (Fig. 5A). Transmission was higher for *H. schachtii* than

TABLE 2. Percentage of nematodes in cabbage or tomato roots as affected by species of nematode, host plant, and site of inoculation (top or bottom of vial)[a]

Nematode	Trial[b]	Nematodes in roots (% of total added per vial)			
		Cabbage		Tomato	
		Top	Bottom	Top	Bottom
Meloidogyne javanica	1	38 ± 4	33 ± 2	50 ± 4	41 ± 2
Heterodera schachtii	1	34 ± 4	34 ± 4	...	...
M. javanica	2	29 ± 6	25 ± 3	58 ± 7	50 ± 4
H. schachtii	2	36 ± 2	56 ± 7	...	...

[a] Vials were packed with loamy sand at 9.2% water and contained no *Hirsutella rhossiliensis*, *M. javanica*, or *H. schachtii*. After 12 days at 20 C, 50–59 *M. javanica* or *H. schachtii* were added to soil at the top or the bottom of each vial. After 2 days, one germinated cabbage or tomato seed was planted in each vial. After 5 days roots were stained, and nematodes within roots were counted.
[b] Data from trials 1 and 2 are presented separately because of significant nematode-by-trial and plant-by-trial interactions.

for *M. javanica* on all sampling dates (Fig. 5A). The number of spores per nematode with spores was higher for *H. schachtii* than for *M. javanica* (Fig. 5B). The extraction efficiency (number of assay nematodes recovered divided by the number added × 100) was 45% for both nematodes and did not change over time.

Nematode motility. Motility of *H. schachtii*, estimated as the percentage of J2 that penetrated roots, was high (range = 39–48%) and was unaffected by water content in loamy sand; root lengths also were unaffected by water content (data not shown). In the experiment in which the motilities of *H. schachtii* and *M. javanica* J2 were compared, data from trials 1 and 2 were not pooled, because of significant nematode-by-trial and plant-by-trial interactions. Infection of roots was similar for *H. schachtii* and *M. javanica* in trial 1, but was greater for *H. schachtii* than for *M. javanica* in trial 2 (Table 2). The location of inoculation (top or bottom) did not affect the number of nematodes in the roots. More *M. javanica* penetrated tomato roots than cabbage roots in both trials.

Transmission equation. Transmission of *H. rhossiliensis* to *M. javanica* in sand at 6.0% soil moisture was described ($r^2 = 0.87$, 0.93, and 0.94 in trials 1, 2, and 3, respectively) by equation 2. However, estimated values of *tr* were quite variable (0.0053, 0.0017, and 0.0006 in trials 1, 2, and 3, respectively); data from trial 2 are presented graphically (Fig. 6A). The number of spores per assay nematode with spores increased with increasing numbers of colonized nematodes added per vial (Fig. 6B).

DISCUSSION

Soil water status substantially affected transmission of *H. rhossiliensis* to *H. schachtii* and *M. javanica* in loam, loamy sand, and sand. Because water can greatly affect movement of nematodes (23), we suspected that changes in nematode motility accounted for at least some of the response to water. Surprisingly,

water levels that affected transmission did not affect motility of *H. schachtii* in loamy sand, according to results of a root-penetration assay. Alternatively, water may have affected spore adhesiveness or spore density (via spore production and/or spore mortality).

Nothing is known about the effects of soil water on adhesiveness or mortality of *H. rhossiliensis* spores. This fungus, however, does not sporulate when submerged (10). Because phialides of *H. rhossiliensis* are 30 μm long and grow perpendicularly to the substrate, we assume that sporulation does not occur unless "critical" pores (those with diameters ≥ 30 μm) are drained. Given the moisture release curves (Fig. 1) and the theoretical relationship between pore diameter, matric potential, and drainage (5), 30-μm pores will drain at approximately 20, 11, and 7% soil moisture for the loam, loamy sand, and sand, respectively. These water levels generally coincide with those that affected transmission, and this provides some indirect evidence that the observed effect of water on transmission resulted from changes in sporulation.

Our data on the effects of water on transmission are consistent with field and laboratory observations. In peach orchards in California, *H. rhossiliensis* parasitized more nematodes in the drier soil between irrigation furrows than in the wetter soil adjacent to irrigation furrows (6). Higher levels of *H. rhossiliensis* have been found in sandy, well-drained orchard soils (6) than in heavier soils used for sugar beets (8). Finally, transmission of *H. rhossiliensis* to *H. schachtii* in loamy sand was higher at 10.3% (11) than at 11.9% soil moisture (9), and transmission to *Pratylenchus penetrans* in sand was inversely related to soil moisture (21).

Soil and nematode species also affected transmission. At near optimum water levels, transmission to both *H. schachtii* and *M. javanica* was greater in the finer-textured soils than in the coarsely textured sand. This difference may be due to the effect of pore size distribution on the probability of nematodes contacting spores (9,23), but unknown chemical and biological factors cannot be eliminated. The effect of species of assay nematode was not consistent. Transmission was greater to *H. schachtii* than to *M. javanica* in some trials (Figs. 4 and 5) but equivalent in others (Table 2). In a previous study (11), transmission was much greater to the entomogenous nematode *Heterorhabditis bacteriophora* Poinar than to *H. schachtii*, probably because *H. bacteriophora* is larger and more active (22). In contrast, the size and activity of *H. schachtii* and *M. javanica* are relatively similar, and penetration of roots by these nematodes was not very different in loamy sand. Because *H. schachtii*, in contrast to *M. javanica*, often attains high numbers in heavy soils, the higher transmission to *H. schachtii* in loam was expected.

The parameter *tr* from equation 2 enabled comparisons among experiments and will facilitate incorporation of data from this study into a model that describes nematode–fungus population dynamics (11). However, the equation should be used with caution. First, it may not describe transmission under all environmental conditions. Second, it simplifies time as a variable, in that transmission is measured after 66 h, for reasons discussed before (9). In the field, nematodes may be exposed to the fungus for a few minutes or for hundreds of hours. Nevertheless, the equation is biologically reasonable (11) and has described transmission of *H. rhossiliensis* to *H. schachtii* in loamy sand at two water levels (9,11), to *H. schachtii* in silty clay (9), to *Heterorhabditis bacteriophora* in loamy sand (11), and to *M. javanica* in sand (Fig. 6).

Our data on soil moisture have highlighted an important deficiency in our bioassay. Because environment may affect both the assay nematodes and the fungus, changes in transmission with environment are difficult to interpret. A direct assay for spores of *H. rhossiliensis* is needed to separate and understand factors affecting transmission; for example, a direct assay could be used to determine whether soil moisture affects sporulation and or spore mortality. A direct assay also could be used to quantify the fungus in soil samples; such samples are usually mixed, and mixing causes underestimation of the fungus based on bioassay (18).
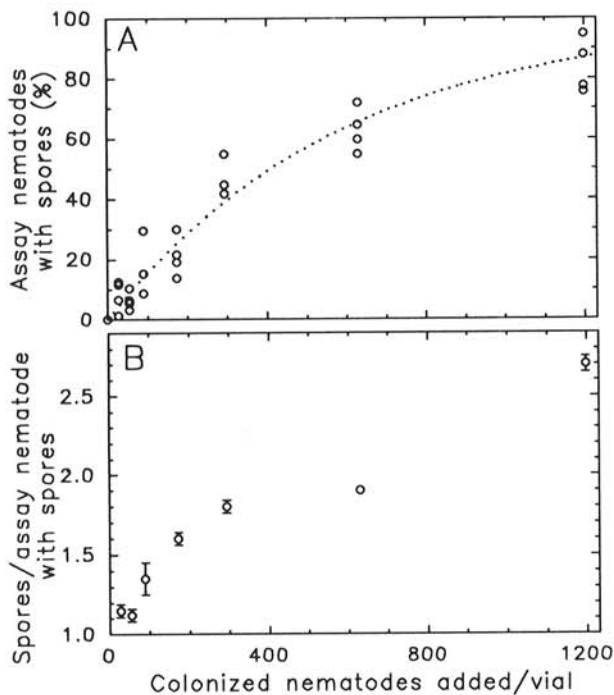

Fig. 6. Transmission of spores of *Hirsutella rhossiliensis* to *Meloidogyne javanica* (assay nematodes) in sand as affected by the number of *H. rhossiliensis*-colonized *Heterodera schachtii* added per vial. Vials were packed with sand (6.0% moisture) containing 0 to 1,200 colonized nematodes. After 14 days at 20 C, assay nematodes were added to each vial. After 66 h, assay nematodes were recovered and examined for spores. Data are from trial 2. **A,** Transmission. Each value represents one replicate vial. $Y = 1 - e^{-tr \cdot x}$, $tr = 0.0017$, $r^2 = 0.93$. **B,** Number of spores per nematode with spores. Each value is the mean ± SE of four replicate vials.

LITERATURE CITED

1. Anderson, R. M., and May, R. M. 1981. The population dynamics of microparasites and their invertebrate hosts. Philos. Trans. R. Soc. London B. 291:451-524.
2. Byrd, D. W., Jr., Kirkpatrick, T., and Barker, K. R. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. J. Nematol. 15:142-143.
3. Duniway, J. M. 1975. Formation of sporangia by *Phytophthora drechsleri* in soil at high matric potentials. Can. J. Bot. 53:1270-1275.
4. Gaspard, J. T., and Mankau, R. 1987. Density-dependence and host-specificity of the nematode-trapping fungus *Monacrosporium ellipsosporum*. Rev. Nematol. 10:241-246.
5. Griffin, D. M. 1972. Pages 76-80 in: Ecology of Soil Fungi. Syracuse University Press, Syracuse, NY. 193 pp.
6. Jaffee, B. A., Gaspard, J. T., and Ferris, H. 1989. Density-dependent parasitism of the soil-borne nematode *Criconemella xenoplax* by the nematophagous fungus *Hirsutella rhossiliensis*. Microb. Ecol. 17:193-200.
7. Jaffee, B. A., and Muldoon, A. E. 1989. Suppression of cyst nematode by natural infestation of a nematophagous fungus. J. Nematol. 21:505-510.
8. Jaffee, B. A., Muldoon, A. E., Anderson, C. E., and Westerdahl, B. B. 1991. Detection of the nematophagous fungus *Hirsutella rhossiliensis* in California sugar beet fields. Biol. Control 1:63-67.
9. Jaffee, B. A., Muldoon, A. E., Phillips, R., and Mangel, M. 1990. Rates of spore transmission, mortality, and production for the nematophagous fungus *Hirsutella rhossiliensis*. Phytopathology 80:1083-1088.
10. Jaffee, B. A., Muldoon, A. E., and Tedford, E. C. 1992. Trap production by nematophagous fungi growing from parasitized nematodes. Phytopathology 82:615-620.
11. Jaffee, B., Phillips, R., Muldoon, A., and Mangel, M. 1992. Host-parasite dynamics in soil microcosms. Ecology 73:495-506.
12. 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.
13. Jaffee, B. A., and Zehr, E. I. 1983. Sporulation of the fungus *Hirsutella rhossiliensis* from the nematode *Criconemella xenoplax*. Plant Dis. 67:1265-1267.

14. Jansson, H.-B. 1982. Predacity by nematophagous fungi and its relation to the attraction of nematodes. Microb. Ecol. 8:233-240.

15. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.

16. Klute, A. 1986. Water retention: Laboratory methods. Pages 635-662 in: Methods of Soil Analysis. Part I, Physical and Mineralogical Methods. 2nd ed. A. Klute, ed. American Society of Agronomy/ Soil Science Society of America, Madison, WI.

17. Lambert, K. N., Tedford, E. C., Caswell, E. P., and Williamson, V. M. 1992. A system for continuous production of root-knot nematode juveniles in hydroponic culture. Phytopathology 82:512-515.

18. McInnis, T. M., and Jaffee, B. A. 1989. An assay for Hirsutella rhossiliensis spores and the importance of phialides for nematode inoculation. J. Nematol. 21:229-234.

19. SAS Institute. 1985. SAS/STAT Guide for Personal Computers. Version 6 ed. SAS Institute, Cary, NC.

20. Stirling, G. R., Sharma, R. D., and Perry, J. 1990. Attachment of Pasteuria penetrans spores to the root knot nematode Meloidogyne javanica in soil and its effects on infectivity. Nematologica 36:246-252.

21. Timper, P., and Brodie, B. B. Infection of Pratylenchus penetrans by nematode-pathogenic fungi. J. Nematol. (In press.)

22. Timper, P., Kaya, H. K., and Jaffee, B. A. 1991. Survival of entomogenous nematodes in soil infested with the nematode-parasitic fungus Hirsutella rhossiliensis (Deuteromycotina: Hyphomycetes). Biol. Control 1:42-50.

23. Wallace, H. R. 1958. Movement of eelworms. I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, Heterodera schachtii Schmidt. Ann. Appl. Biol. 46:74-85.