Soil pH and the Activity of a Pelletized Nematophagous Fungus

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Accepted for publication 27 November 2000.

ABSTRACT

Jaffee, B. A., and Zasoski, R. A. 2001. Soil pH and the activity of a pelletized nematophagous fungus. *Phytopathology* 91:324-330.

When formulated as assimilative hyphae in alginate pellets, the nematophagous fungus *Hirsutella rhossiliensis* was more active (i.e., parasitized more assay nematodes) in an acidic vineyard soil than in a neutral vineyard soil. To determine whether soil pH explained the difference, fungus activity was measured in soil from the neutral site that had been acidified (by adding sulfuric acid) and in soil from the acidic site that had been neutralized (by adding calcium hydroxide). As hypothesized, the activity of pelletized *Hirsutella rhossiliensis* was negatively correlated with soil pH. Maximum activity occurred at pH_{calcium chloride} 4.5, and activity gradually declined to near zero as the pH increased to 6.5

and rapidly declined to near zero as the pH dropped below 4.0. Assays performed on leached soil samples indicated that the effects of sulfuric acid and calcium hydroxide were largely due to pH rather than to specific ions or osmotic potential. The effect of pH, however, was indirect. Heating the neutral soil to 60°C for 2 h did not alter soil pH or electrical conductivity but increased fungus activity to levels equivalent to those in acidified soil. We conclude that, in these two soils, heat treatment or low soil pH suppresses soil organisms that otherwise interfere with growth of *Hirsutella rhossiliensis* from alginate pellets.

Additional keywords: fungistasis, *Heterodera schachtii*, plant-parasitic nematodes.

The soil fungus *Hirsutella rhossiliensis* has been formulated as a potential biocontrol agent against plant-parasitic nematodes (3, 24, 31), and one of these formulations, consisting of assimilative hyphae embedded and dried in alginate pellets, has performed well in laboratory microcosms (24). The pellets, however, did poorly in field microplots (16, 18). Because performance improved if the pellets were added to 60°C-treated rather than to non-heat-treated microplot soil, poor performance was attributed to antagonistic organisms. These putative antagonists of *Hirsutella rhossiliensis* pellets are not unique to our microplots; poor performance of pellets in non-heat-treated soil and good performance in heat-treated soil was also documented in three commercial tomato fields and in one commercial vineyard (12).

In contrast, pelletized *Hirsutella rhossiliensis* did well whether the soil was heat treated or not in a second commercial vineyard (12). This was surprising because the two vineyards seemed very similar. Both were mature vineyards near Lodi, CA, and both contained sandy, porous soil with very little organic matter. The soils, however, differed greatly in pH. Soil pH was nearly neutral where the pellets performed poorly and quite acidic where the pellets performed well. Jaffee (12) inferred that soil pH might explain why the formulation was more active in one vineyard than in the other.

This inference requires substantiation. First, we are uncertain whether *Hirsutella rhossiliensis* pellets actually perform better in the acidic than in the neutral soil because experiments at the two vineyards were by necessity independent. Consequently, soil temperature, soil water, pellet quality, and other factors were uncontrolled between sites, and statistical comparisons between vineyards were inappropriate (12). Second, the effect of pH on *Hirsutella rhossiliensis* pellets could be direct or could be mediated by other organisms, in particular, organisms antagonistic to *Hir-*

sutella rhossiliensis. Third, pH may be statistically but not mechanistically associated with causal factors.

In this study, we compared performance of pelletized *Hirsutella rhossiliensis* in soil from the two vineyards and determined whether soil pH affected pellet activity. We also attempted to determine whether the effect of pH was direct or indirect.

MATERIALS AND METHODS

Fungus pellets and biology. Alginate pellets containing hyphae of *Hirsutella rhossiliensis* (ATCC 46487) were prepared as described before (16, 24). Briefly, thin-walled hyphae rich in assimilated nutrients were grown in shake culture. The hyphae were rinsed free of medium, macerated in 1% sodium alginate, and pelletized in CaCl₂. Pellets were coated with sand (to prevent sticking), dried, and stored in glass vials at -1°C for 1 to 42 days. Pellet mass and diameter were 1.1 mg and 1.9 mm.

When placed in soil, these pellets produce thick-walled "external hyphae" that radiate into the soil and bear phialides. Each phialide bears one conidium. When touched by a passing vermiform nematode, the conidium adheres to the nematode, detaches from the phialide, and within approximately 24 h penetrates the nematode's cuticle via a germ tube. After the fungus consumes the nematode and converts it into assimilative hyphae, the process repeats as external hyphae, bearing phialides and conidia, grow from the parasitized nematode and into the soil. In vitro at 20°C, one pellet produces approximately 100,000 conidia in 2 weeks (35). The fungus is specialized for parasitism of nematodes and has little or no competitive saprophytic ability (13).

Soils. The soils were obtained from two mature vineyards, designated site 4 and site 5 as in a previous paper (12). According to the soil survey (29), site 4 contains Tokay fine sandy loam (coarse-loamy mixed, thermic Typic Haploxeroll) and site 5 contains Kingdon fine sandy loam (coarse-loamy mixed, thermic Typic Argixeroll). However, Jaffee (12) found the soils to be loamy sands rather than sandy loams (Table 1). In agreement with the soil survey, soil pH was nearly neutral at site 4 but acidic at

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Publication no. P-2001-0122-02R

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site 5 (Table 1). Other characteristics described in Table 1 were determined with standard methods and by the Division of Agriculture and Natural Resources Laboratory (University of California, Davis).

Soil was collected from the 5 to 20 cm depth adjacent to vines. Site 4 soil contained the root-knot nematode, *Meloidogyne* spp., and the ring nematode, *Criconebella xenoplax*. Site 5 soil contained *Meloidogyne* spp. and the dagger nematode, *Xiphinema index*. Neither soil contained the cyst nematode, *Heterodera schachtii*. Because antagonists of *Hirsutella rhossiliensis* might decline with storage, soil was stored at 10°C (except as noted) and used within 14 days of collection.

To remove putative antagonists for some experiments, soil was placed in a glass 500-ml beaker, sealed with aluminum foil, and placed in an oven at 65°C. When the center of the soil mass increased to 60°C, the oven temperature was reduced to 60°C. After 2 h at 60°C, the soil was removed from the oven. Cooled soil was placed in an open plastic bag on the laboratory bench. After 4 to 6 h (to allow potentially toxic volatiles to escape), the soil was mixed, sealed in the plastic bag, and maintained at 22°C (to allow the surviving organisms to recolonize). After 2 to 7 days, soil water content was adjusted and the soil was used for experiments.

Vial bioassay for *Hirsutella rhossiliensis* activity. The following bioassay was used for all experiments. After soil was treated and soil water was adjusted, 1.5 cm³ of soil was placed in a 25-ml plastic vial, and one pellet was placed centrally on the surface of the soil. Additional soil was added and tamped so the pellet was 3 mm from the bottom of a soil column (height 3.5 cm, diameter 2.5 cm, volume 17 cm³; soil dry mass 24.0 g). Preliminary experiments indicated that this positioning of the pellet usually resulted in less than 50% of the assay nematodes acquiring conidia and thereby increased the ability of the assay to detect treatment differences. Detecting treatment differences becomes difficult when more than 50% of the assay nematodes acquire conidia, because the relationship between percentage of acquisition and fungus density is nonlinear (it saturates) when the percentage exceeds 50% (35). Saturation often occurs when the pellet is placed closer to the soil surface, where assay nematodes are added. Each vial was sealed with a plastic lid (the lid had a 2-mm-diameter hole for gas exchange) and placed in a moisture chamber.

After 14 days in the dark at 20°C, 500 assay nematodes (second-stage juveniles [J2] of *Heterodera schachtii*) in 0.25 ml of 4 mM KCl were added to the surface of the soil in each vial. The vials were sealed and returned to 20°C. After 2 days, nematodes were extracted by wet sieving and sucrose centrifugation (22). The nematode suspension from each vial was adjusted to 10 ml, and 2 ml (containing approximately 50 assay nematodes) was examined at 70× magnification with a dissecting microscope. The number of assay nematodes with at least one adhering conidium of *Hirsutella rhossiliensis* was determined. In some experiments, other nematodes in the sample were identified to trophic group (40).

Heterodera schachtii was used to assay for *Hirsutella rhossiliensis* because *Heterodera schachtii* was not naturally present in the soils (if it were, the time spent in the soil and hence the probability of acquiring conidia would be variable and unknown). Moreover, *Heterodera schachtii* is quite susceptible to *Hirsutella*

rhossiliensis, and large numbers of vigorous and uniform J2s are easily obtained. *Heterodera schachtii* was cultured on sugar beet in the greenhouse. To obtain J2s, cysts were placed on Baermann funnels. The J2s collected after 6 h were discarded and the funnels drained and covered with plastic to maintain moisture. After 15 h, the funnels were filled with water and J2s were collected every 30 min. The J2s were stored with aeration at 10°C for 4 to 30 h.

Comparison of soils from sites 4 and 5 (experiment 1). We first hypothesized that *Hirsutella rhossiliensis* would sporulate better in non-heat-treated soil from site 5 than site 4. We also hypothesized that heat treatment would enhance sporulation in site 4 soil but not in site 5 soil. Soil was collected from both sites on the same day. Half the soil from each site was heated and half was not. Soil water content was adjusted to 11.0% (grams of water per 100 g of dry soil), and the vial bioassay was performed.

Experiment 1 was performed three times (trials 1, 2, and 3), and soil for each trial was collected on different dates. Because site 5 soil seemed to compress more than site 4 soil in trials 1 and 2, we determined, in trial 3, whether source of soil, heat treatment of soil, or water content (11.0 versus 9.0%) affected bulk density. We reasoned that reducing the water content would reduce the tendency for site 5 soil to compress. The volume of the soil in each vial was determined by measuring the soil column's height and measuring the volume of a similar column of water.

There were 8 (trial 1 and 2) and 10 (trial 3) replicate vials for each combination of soil and soil treatment. Four additional vials of non-heat-treated soil from each site were included for each trial; these vials did not receive pellets and therefore provided information on resident levels of *Hirsutella rhossiliensis*.

Decreasing the pH of site 4 soil (experiment 2). We hypothesized that reducing the pH would increase the activity of pelletized *Hirsutella rhossiliensis* in site 4 soil. Non-heat-treated soil was mixed and divided into four lots. Each lot received 0, 1, 2, or 4 ml of 0.5 M H₂SO₄ per 100 g of soil (dry weight equivalent); the acid was supplemented with distilled water so the total volume added per lot was 4 ml. After acid was added, the soil was mixed. Because adding acid not only reduces soil pH but also increases the salinity of the soil solution (the acid releases cations from the surface of soil particles) (10), we examined the effect of soil leaching. In trials 1 and 2, however, the soil was not leached. It was mixed and adjusted to 9.0% water content. After 2 weeks at 22°C, the soil was packed into vials, and the bioassay was performed. At the time of packing, electrical conductivity of a saturated extract (36) was determined with a conductivity bridge and soil pH was determined with a pH meter; to measure pH for this and all other experiments, soil was wetted to a paste with 0.01 M CaCl₂ (34).

In trials 3 to 5 of experiment 2, the soil was leached in the field before it was assayed in vials. In trial 3, the soil, after receiving the different levels of acid, was packed into PVC pipe (5.1-cm diameter and 5.1 cm long, 103 cm³ of soil per pipe). There were six replicate pipes for each acid level. These were buried with open ends oriented vertically approximately 15 cm deep in each of six blocks in the vine row of site 4. After 16 days and 10 cm of rain (January 2000), the pipes were recovered. According to the Integrated Pest Management Database (University of California,

TABLE 1. Physical and chemical characteristics of untreated soils from two vineyards^a

Site	pH		%					cmol/kg		mg/kg		
	(CaCl ₂) ^b	(Water) ^c	Organic matter ^c	Sand ^c	Silt ^c	Clay ^c	N ^b	Ca ^b	Mg ^b	Mn ^b	Al ^b	P ^b
4	6.5	7.0	0.4	81	15	4	0.037	400	78	4	nd	57
5	4.1	5.1	0.7	81	15	4	0.059	206	17	21	12	138

^a pH of a 0.01 M CaCl₂-saturated or water-saturated soil paste. Al (extractable), Mn (DTPA extractable), P (acid-ammonium fluoride extractable), Ca and Mg (exchangeable using 1 M KCl), N indicates total Kjeldahl N, and nd = not detected.

^b Soil samples were collected in March 2000.

^c Soil samples were collected in 1998, and data were published previously (12).

Davis), soil temperatures at 15 cm depth during leaching ranged from 6 to 12°C; this was true for all trials involving leaching in experiments 2 and 3. The soil for each treatment in trial 3 was combined. Soil receiving no acid was divided into two lots; one lot was heated to 60°C for 2 h and the other was not heated; the nonacidified soil was heat-treated to determine whether reducing pH could substitute for heat treatment. Soil water was adjusted to 9.0%. Within 2 weeks of soil recovery from the field, soil pH and electrical conductivity were measured and the vial bioassay was performed.

Trials 4 and 5 were identical to trial 3 except that soil was leached in fiberglass bags buried in January 2000 between vine rows rather than in rows (to avoid treatment with herbicide). Each bag (approximately 30 cm long, 20 cm wide, 2 cm thick, and with pore openings of approximately 1.3×1.3 mm) contained approximately 900 g of soil. There was one replicate bag per treatment per trial. When bags were recovered (after 57 days for trial 4 and 67 days for trial 5), approximately 28 cm of rain had fallen on the vineyard. There were 10 replicate vials per treatment in each trial of experiment 2.

Increasing the pH of site 5 soil (experiment 3). We hypothesized that increasing the pH of site 5 soil would reduce the activity of pelletized *Hirsutella rhossiliensis*. The pH of site 5 soil at 9.0% water content was increased by adding $\text{Ca}(\text{OH})_2$ (0, 16.8, 33.2, 66.8, 133.2, or 200.0 mg/100 g of soil). In trials 1 and 2, the soil was not leached but was incubated at 22°C in the laboratory. After 2 weeks, the pH and electrical conductivity of the soil were determined and the vial bioassay for *Hirsutella rhossiliensis* activity was conducted. In trials 3 and 4, soil treated with 0, 100, or 200 mg of $\text{Ca}(\text{OH})_2$ per 100 g of soil was placed in fiberglass bags and buried 15 cm deep in the vine row of site 5 (January 2000). After 40 days and 25 cm of rain (trial 3) and 55 days and 28 cm of rain (trial 4), the bags of soil were recovered. Soil pH and electrical conductivity were measured, and the vial bioassay was performed. There were 10 replicate vials per treatment in each trial of experiment 3.

Statistical analyses. The SAS general linear models procedure (release 7.00, SAS Institute, Cary, NC) was used throughout. The dependent variable for all experiments was the percentage of assay nematodes with *Hirsutella rhossiliensis* conidia; percentages were arc sine transformed before analyses except for regressions. For experiment 1, we first determined whether the percentage of assay nematodes with conidia differed in non-heat-treated soil from site 4 versus site 5. We then performed a factorial analysis to detect statistical interactions between source of soil and heat treatment (trials 1 and 2) and between source of soil, heat treat-

ment, and water content (trial 3). For experiments 2 and 3, linear regression was performed, with soil pH or electrical conductivity as the independent variable. Significance was determined at $P \leq 0.05$.

RESULTS

Comparison of soils from sites 4 and 5 (experiment 1). Although the effect of trial was significant, all trials of experiment 1 exhibited the same pattern. In non-heat-treated soil, the percentage of assay nematodes with conidia was always less in site 4 than in site 5 soil (Fig. 1). Whereas heat treatment always increased fungus activity in site 4 soil, it had no effect on activity (Fig. 1A) or decreased activity (Fig. 1B through D) in site 5 soil. Thus, the statistical interaction of heat treatment and soil source was significant.

When soil water was 11.0% in trial 3, bulk density (gram of dry soil per cubic centimeter of soil, mean $\pm$ SE) was greater with site 5 soil than with site 4 soil (1.46 ± 0.01 versus 1.41 ± 0.01); greater in heat-treated than in non-heat-treated site 5 soil (1.48 ± 0.01 versus 1.44 ± 0.01); and similar in heat-treated and non-heat-treated site 4 soil (1.42 ± 0.01 versus 1.41 ± 0.01). When soil water content was 9.0% however, bulk density was 1.40 ± 0.01 , regardless of soil source or heat treatment. To attain uniform bulk densities, the water content in experiments 2 and 3 was 9.0%.

To determine whether heat treatment changed soil pH, pH of non-heat-treated and heat-treated soil was measured before soil was packed into vials in trials 2 and 3. The pH of site 4 soil was 6.5 in trial 2 and 6.4 in trial 3, regardless of heat treatment. The pH of site 5 soil was 4.5 in trial 2 and 4.6 in trial 3, regardless of heat treatment.

In vials receiving no pellets, 0 and <1% of the assay nematodes had adhering conidia in site 4 and site 5 soil, respectively (data not shown). Thus, resident *Hirsutella rhossiliensis* was not present or was present in very small numbers.

Decreasing the pH of site 4 soil (experiment 2). Addition of acid reduced the pH of site 4 soil (Fig. 2A). The data were similar among trials, but the pH reduction was less in leached soil than in nonleached soil. Soil pH increased with greater leaching time (trial 5 > 4 > 3). Heat treatment did not affect the pH of the nonacidified soils that were leached (Fig. 2A).

Electrical conductivity and therefore salt concentration increased substantially with the addition of acid (Fig. 2B). Leaching removed much of the salt from soils treated with the highest acid rate and removed nearly all of the salt from soils treated with lower acid rates. Heat treatment did not affect the electrical conductivity of the nonacidified soils that were leached (Fig. 2B).

Fig. 1. Activity of pelletized *Hirsutella rhossiliensis* in two vineyard soils (experiment 1). Soil from two vineyards (sites 4 and 5) was heat-treated or not treated and packed into vials with one pellet of *Hirsutella rhossiliensis* per vial. After 2 weeks at 20°C, assay nematodes were added to each vial. Assay nematodes were recovered from soil 2 days later and examined for adhering *Hirsutella rhossiliensis* conidia. Experiment 1 was performed three times: twice with a water content of 11% (trials 1 and 2) and once with water contents of 9 and 11% (trial 3). Each value is the mean ($\pm$ SE) of 8 (trials 1 and 2) or 10 (trial 3) replicate vials.

In nonheated and nonacidified soil from site 4, the percentage of assay nematodes with adherent conidia was near zero in four of five trials (Fig. 2C). Heating of the nonacidified soil greatly increased the percentage of assay nematodes with conidia (Fig. 2C). Addition of intermediate levels of acid to non-heat-treated soil also increased the percentage of assay nematodes with conidia (Fig. 2C). The percentage of assay nematodes with conidia was less in soil receiving 2 ml of acid than in the heated controls, but the differences were relatively small and were not statistically significant (in an analysis across all three trials, $P = 0.16$).

In the leached site 4 soil, numbers of bacterivorous nematodes decreased as soil pH decreased (mean numbers recovered per vial

were 100, 82, 31, and 20 at pH 6.6, 5.3, 4.5, and 3.7, respectively); numbers of nematodes in other trophic groups were small (usually <10 per vial) regardless of treatment (data not shown). In nonleached site 4 soil, resident nematode numbers were small (usually <10 of any trophic group per vial) regardless of treatment (data not shown). Enchytraeids were rarely observed.

Increasing the pH of site 5 soil (experiment 3). Addition of $\text{Ca}(\text{OH})_2$ increased the pH of site 5 soil (Fig. 3A). The increase was moderated by leaching (Fig. 3A). Nontreated, nonleached soil from site 5 was saltier than nontreated, nonleached soil from site 4 (Fig. 3B versus Fig. 2B). Electrical conductivity increased slightly with addition of $\text{Ca}(\text{OH})_2$ and was greatly reduced by leaching (Fig. 3B).

Fig. 2. Effect of sulfuric acid on soil pH, soil electrical conductivity, and activity of pelletized *Hirsutiella rhossiliensis* in site 4 soil (experiment 2). Non-heat-treated soil from site 4 was treated with sulfuric acid and was either not leached (trials 1 and 2) or leached (trials 3 to 5). After leaching, half of the soil receiving no acid was heated to 60°C for 2 h; no other soil was heat-treated. **A**, Soil pH. Before the soils were assayed for fungus activity, subsamples were moistened to a saturated paste with 0.01 M CaCl_2 , and pH was determined. **B**, Soil solutes. Before the soils were assayed for fungus activity, the electrical conductivity (deciSiemens per centimeter) of a saturated extract was determined. **C**, Fungus activity. The soils were adjusted to 9% soil water content and packed into vials with one pellet of *Hirsutiella rhossiliensis* per vial. After 2 weeks at 20°C, assay nematodes were added to each vial. Assay nematodes were recovered from soil 2 days later and examined for adhering *Hirsutiella rhossiliensis* conidia. Each value for pH and electrical conductivity represents one determination. Each value for fungus activity is the mean ($\pm$ SE) of 10 replicate vials.

Fig. 3. Effect of calcium hydroxide on soil pH, soil electrical conductivity, and activity of pelletized *Hirsutiella rhossiliensis* in site 5 soil (experiment 3). Non-heat-treated soil from site 5 was treated with calcium hydroxide and either not leached (trials 1 and 2) or leached (trials 3 and 4). **A**, Soil pH. Before the soils were assayed for fungus activity, subsamples were moistened to a saturated paste with 0.01 M CaCl_2 , and pH was determined. **B**, Soil solutes. Before the soils were assayed for fungus activity, the electrical conductivity (deciSiemens per centimeter) of a saturated extract was determined. **C**, Fungus activity. The soils were adjusted to 9% soil water content and packed into vials with one pellet of *Hirsutiella rhossiliensis* per vial. After 2 weeks at 20°C, assay nematodes were added to each vial. Assay nematodes were recovered from soil 2 days later and examined for adhering *Hirsutiella rhossiliensis* conidia. Each value for pH and electrical conductivity represents one determination. Each value for fungus activity is the mean ($\pm$ SE) of 10 replicate vials.

Addition of $\text{Ca}(\text{OH})_2$ reduced the percentage of assay nematodes with conidia, but the reduction was exponential in non-leached soil whereas it was linear and less dramatic in leached soil (Fig. 3C). One lot of soil was used to start trials 1 and 3, and a second lot was used for trials 2 and 4; the percentage of assay nematodes with conidia was greater with the first lot of soil than with the second. Regardless of treatment, resident nematode numbers were small (usually <10 of any trophic group per vial) in site 5 soil (data not shown). Enchytraeids were rarely observed.

Relationship between fungus activity and soil pH or electrical activity. If data from all trials of experiments 2 and 3 were combined (excluding data from heat-treated controls), the percentage of assay nematodes with conidia was related to soil pH in site 4 soil (Fig. 4A). The percentage increased as pH decreased from 6.5 to approximately 4.2 and decreased at lower pH values. At pH values >4.2, the regression of percentage of nematodes with conidia on soil pH was significant ($P < 0.01$, $r^2 = 0.42$) in site 4 soil.

In site 5 soil, the percentage was greatest in nontreated soil (pH approximately 4.5) and decreased as the pH increased (Fig. 4B); the regression of percentage of nematodes with conidia on soil pH was significant ($P < 0.01$, $r^2 = 0.61$). Thus, the pattern was similar at both sites (activity was low when pH values were near 6.5 and activity was substantial when pH values were near 4.5).

In contrast, there was no clear relationship between electrical conductivity of the soil solution and fungus activity (Fig. 4C and D); regressions of fungus activity on electrical conductivity were insignificant at both sites.

DISCUSSION

As hypothesized, the activity of pelletized *Hirsutella rhossiliensis* substantially increased when a nearly neutral vineyard soil

was acidified. Similarly, the activity substantially decreased when an acidic vineyard soil was limed. In both soils, activity was greatest when the $\text{pH}_{\text{calcium chloride}}$ was approximately 4.5 (the normal pH for the acidic soil) and was minimal at pH 6.5 (the normal pH for the neutral soil). It follows that soil pH underlies much of the difference in how pelletized *Hirsutella rhossiliensis* performed in the two vineyards (12).

Although pH may directly affect the fungus, the current data point toward an indirect influence. Heating the neutral soil to 60°C for 2 h did not alter soil pH or soil electrical conductivity. It is also doubtful that such gentle heating greatly changed other chemical or physical properties. But heat treatment substantially suppresses soil organisms (19) and substantially increased pellet activity in the neutral soil. We infer that both heating and low pH suppress organisms that somehow interfere with *Hirsutella rhossiliensis* pellets. In at least three commercial tomato fields and in the neutral vineyard of this study, these unknown organisms are smaller than 20 μm ; exclusion of larger organisms did not improve pellet activity (12).

Our inference that heat treatment suppressed antagonists is consistent with the principles that soil is biologically buffered against introduced organisms (4,7,39) and that introducing nematophagous and other fungi is therefore easier if the soil community has been simplified (6,8,27,28,32). But the effect of heat treatment on pellet performance might be explained by other processes. Perhaps heating alleviated fungistasis by releasing nutrients required by the fungus or by suppressing organisms inducing dormancy (25,26). An explanation based on fungistasis, however, is inconsistent with the following observations: *Hirsutella rhossiliensis* pellets are nutrient-independent, i.e., hyphae grow from pellets and produce abundant conidia even when pellets are placed in plastic moisture chambers without exogenous nutrients (35); un-

Fig. 4. *Hirsutella rhossiliensis* activity as related to soil pH and soil solutes. Fungus activity data in experiments 2 (site 4) and 3 (site 5) are plotted on soil pH and on electrical conductivity (deciSiemens per centimeter). Data from heat-treated soil are excluded. Each value is the mean ($\pm$ SE) of 10 replicate vials.

like fungistasis-sensitive propagules, pelletized hyphae did not remain dormant in non-heat-treated soil (B. A. Jaffee and R. J. Zasoski, *unpublished data*); and most importantly, sensitivity to fungistasis should confer an ecological advantage (4), but *Hirsutella rhossiliensis* hyphae gain no advantage by remaining dormant in nonheated, nematode-infested soil.

Although heat treatment improved pellet performance in the neutral soil, it somewhat reduced activity in the acidic soil. If this reduction is real (the statistics were inconclusive), perhaps heating of the acidic soil solubilized some inhibitory component (23). This possibility is somewhat bothersome because it undermines our notion of "gentle heating".

The effect of pH might have been anticipated. First, nematode-endoparasitic and nematode-trapping fungi were detected more frequently in acidic than in neutral soils of Ireland (9); *Hirsutella rhossiliensis*, however, was not reported. Second, the optimum pH for *Hirsutella rhossiliensis* sporulation from parasitized nematodes in vitro was approximately 5.3 (21); these in vitro data show that a direct effect of pH cannot be completely discounted. Finally, among 10 peach and almond orchards surveyed in 1986, the one with the largest resident population of *Hirsutella rhossiliensis* (B. A. Jaffee, *unpublished data*) was very acidic; the pH of this soil, which was designated "soil M", was 4.4 (14,15). Soil M became the standard soil used by Jaffee and colleagues to study *Hirsutella rhossiliensis*.

The current data on pH help explain why *Hirsutella rhossiliensis* pellets performed much better in laboratory microcosms (24) than in campus microplots (16,18). Although loamy sands were used in both types of studies, soil pH was 4.4 in the laboratory (soil M was used) and 7.3 in the microplots.

The negative effect of pH in the microplots, as in the neutral vineyard, was largely indirect; gentle heating of microplot soil greatly improved pellet performance (16,18). The situation in the microplots, however, differed from that in the neutral vineyard and in three commercial tomato fields (12) because the microplots contained large numbers of enchytraeid worms (*Oligochaeta*) that are highly motile and consume *Hirsutella rhossiliensis* pellets (17). Consequently, pellet activity in the microplots was not greatly improved unless the soil was both heated and surrounded by mesh to prevent re-invasion by enchytraeids (19). Given the high soil pH and large numbers of enchytraeids, pelletized *Hirsutella rhossiliensis* had no chance to control plant-parasitic nematodes in the campus microplots.

Many others have found that soil pH affects biological control of soil pests (4). For example, giant vampyrellid amoebae perforated more fungal spores in neutral soils than in acidic soils (11). More recently, receptivity of soil to three root pathogens was correlated with soil pH (30); and although various soil chemical and physical factors were statistically significant, biological factors seemed most important because receptivity was greatly increased by sterilization of soil with gamma radiation.

We did not directly assess fungal growth and activity. Instead, we used a bioassay in which target nematodes were added to soil, recovered, and examined for attack by the control agent. Although the bioassay directly relates to biological control, it depends not only on conidial numbers but also on nematode movement through soil (35). If our treatments affected such movement, it would be incorrect to measure fungus activity by quantifying the percentage of assay nematodes with conidia. But most studies indicate a minimal direct effect of soil pH on nematodes (38). Moreover, heat treatment greatly increased the percentage of assay nematodes with conidia, and there is no basis for inferring that heat treatment increases nematode movement or susceptibility.

Specific ions, on the other hand, may attract or repel nematodes (1,2,33) and thereby affect the number of nematodes encountering conidia. Similarly, specific ions and osmotic potential may enhance or suppress *Hirsutella rhossiliensis* (5,20,21). Although osmotic or specific ion effects were indicated in the study (pellet

activity was greater in leached soil than in nonleached soil), these effects were clearly minor relative to those of pH.

Two other laboratories have reported greater success than ours in adding *Hirsutella rhossiliensis* to soil (3,31). It is unclear whether the success reflects differences in formulation quality, formulation quantity, soil, fungal isolate, or experimental procedure. Another formulation, consisting of vegetative hyphae of the same isolate used in this study, performed equally well in autoclaved or nonautoclaved organic soil; significantly, the soil pH was 4.7 (37).

We now better understand why pelletized *Hirsutella rhossiliensis* failed to control plant-parasitic nematodes in our campus microplots and why fungus activity was greater in one vineyard than in another. Applying this information, however, is not easy. We might suggest that the pellets be studied and tested only in acidic soils, but such soils are relatively rare in agriculture. Moreover, strongly acidic soils often are amended to raise pH, and the benefits from increased pH could outweigh those from nematode control. Instead, it may be useful to modify the formulation, thereby reducing antagonism by other organisms. A previous study using the neutral vineyard soil showed that inoculum consisting of *Hirsutella rhossiliensis*-parasitized nematodes rather than pellets performed well in bioassays whether the soil was heated or not (13). We again suggest that the pellet might perform better if it more accurately mimicked the fungus-parasitized nematode. A protective covering, like the nematode's cuticle, could be especially valuable.

ACKNOWLEDGMENTS

We thank S. Lange and J. Tecklenburg for allowing us to work in their vineyards, E. Caswell-Chen for suggestions, L. Epstein and T. Gordon for comments on a draft of the manuscript, and K. Johansen for technical help.

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