

# ATTRACTION OF *TYLENCHULUS SEMIPENETRANS* AND *MELOIDOGYNE JAVANICA* TO SALTS *IN VITRO*

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## ABSTRACT

Abou-Setta, M., and L. Duncan. 1998. Attraction of *Tylenchulus semipenetrans* and *Meloidogyne javanica* to salts *in vitro*. *Nematropica* 28:49-59.

A bioassay to measure the attractiveness of chemicals to plant parasitic nematodes was developed. Nematodes in the center of a petri dish of washed sand were permitted to migrate for 48 hrs, after which nematodes were extracted from six cores of sand removed from the edge of the dish. The cores encompassed alternating spots of 333  $\mu$ l test solution or distilled water, deposited on the sand at the time of nematode infestation. A preference index ( $I_p$ ) was calculated as the number of nematodes recovered from cores encompassing test solution as a proportion of the total nematodes recovered. Compared to water, sodium and potassium salts (0.3 M) of acetate, formate and chloride were preferred by *Tylenchulus semipenetrans* whereas water was preferred to those salts of citrate. Water was preferred to most of these salts by juveniles of *Meloidogyne javanica* with the exception of Na-acetate and Na-citrate which did not affect the migration of the nematode. Sodium carbonate and Na-bicarbonate were preferred by *M. javanica*, but water was preferred to Na-carbonate by *T. semipenetrans* and Na-bicarbonate did not affect *T. semipenetrans* migration. The  $I_p$  for *T. semipenetrans* and K-acetate was higher at 26-28°C than 18-21°C. Within a range of 4-20% soil moisture, assays with 6-10% moisture produced the highest  $I_p$ . Highest proportional recovery of *T. semipenetrans* from each bioassay unit occurred in spots with estimated 0.08-0.10 M Na-acetate. Above 0.2 M recovery was very low. Preference for Na-acetate by *T. semipenetrans* was pH-dependent. In the associated form, Na-acetate is preferred by the nematode whereas in the dissociated form (i.e. acetic acid at lower pH) it inhibits motility and eventually kills the nematode.

*Key words:* acetate, attraction, chemical recognition, *Meloidogyne javanica*, repulsion, *Tylenchulus semipenetrans*.

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## RESUMEN

Abou-Setta, M. y L. Duncan. 1998. Atracción de *Tylenchulus semipenetrans* y *Meloidogyne javanica* a sales, *in vitro*. *Nematropica* 28:49-59.

Se desarrolló un bioensayo, para medir la atracción de nematodos fitoparasíticos por compuestos químicos. Se colocaron nematodos en el centro de una placa petri con arena lavada y se les dejó migrar durante 48 horas, luego se extrajeron seis muestras de arena del extremo de la placa, y se realizaron extracciones de los mismos. Las muestras representaron 333  $\mu$ l de solución prueba o de agua destilada. Estas alícuotas fueron depositadas en la arena de forma alternada, al mismo tiempo en el que se realizó la infestación con nematodos. El índice de preferencia ( $I_p$ ) fue calculado como el número de nematodos recuperados en las muestras abarcadas por la solución prueba, expresado como una proporción del número total de nematodos recuperados en la solución prueba y en el agua. En comparación al agua, las sales de sodio o potasio (0.3M) en las formas acetato, formato o cloruro fueron preferidas por *Tylenchulus semipenetrans*, mientras que el agua se prefirió a la forma citrato de las referidas sales. Los juveniles de *Meloidogyne javanica*, mostraron más preferencia por el agua que por todas las otras sales, con la excepción del acetato y citrato de sodio, las que no afectaron la migración

de este nematodo. El carbonato y bicarbonato de sodio fueron las sales preferidas por *M. javanica*, pero *T. semipenetrans* prefirió el agua antes que el carbonato de sodio y el bicarbonato de sodio no afectó su migración. El  $I_p$  para *T. semipenetrans* y el acetato de potasio, fue mayor a 26-28°C que a 18-21°C. Dentro del rango del 4-20% de humedad del suelo, los ensayos con 6-10% de humedad, produjeron el mayor  $I_p$ . La mayor recuperación proporcional de *T. semipenetrans* en cada unidad de bioensayo, ocurrió en las manchas con una concentración estimada de 0.08-0.10 M de acetato de Na. La recuperación fue muy baja por encima de 0.2M. La preferencia de *T. semipenetrans* por el acetato de Na fue pH-dependiente. Los nematodos, prefieren al acetato de sodio, en su forma asociada, mientras que la forma disociada (ej: ácido acético a bajo pH) inhibe la motilidad y eventualmente causa la muerte de los mismos.

*Palabras claves:* Acetato, atracción, *Meloidogyne javanica*, reconocimiento químico, repulsión, *Tylenchulus semipenetrans*.

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## INTRODUCTION

Understanding mechanisms by which plant parasitic nematodes locate host roots could provide powerful opportunities to intervene in the nematode life cycle. Identification of nematode chemical receptors would permit attempts to block their function (Zuckerman, 1983). Chemicals that attract or repel nematodes could be used to disrupt normal host-finding behavior by disorienting nematodes or as baits to increase the efficacy of chemical or biological control (Castro *et al.*, 1989; Duncan and Abou-Setta, 1995; Jaffe *et al.*, 1989; Meyer *et al.*, 1997; Robinson and Jaffe, 1996). Attractive or repellent chemicals of host-plant origin could provide useful markers in programs to breed resistant plant cultivars (Kaplan, 1981).

Despite the high potential to utilize chemicals involved in nematode rhizotaxis, the host-finding process remains poorly understood. Nematode orientation in gradients created with carbon dioxide (Dusenbery, 1983), salts (Castro *et al.*, 1990; Diez and Dusenbery, 1989; Prot, 1978b, 1978c, 1979a, 1979b; Riddle and Bird, 1985) and various undefined root emanations (Castro *et al.*, 1989; Papademetriou and Bone, 1983; Prot, 1978a; Prot and VanGundy, 1981; Viglierchio, 1961) have been described. Vanillic acid has been identified as a sex phero-

mone for *Heterodera glycines* (Jaffe *et al.*, 1989). However, the primary chemical events involved in host-finding have not been elucidated for any nematode-host combination.

Some bioassays to measure nematode attraction to chemicals and root emanations have employed agar or agarose as the support matrix, which permits direct observation of the nematode responses. Citrus root leachates repelled *Tylenchulus semipenetrans*, but attracted *Radopholus similis* and *Pratylenchus coffeae* on water agar (unpublished data). Repellency of nematodes by root emanations in bioassays appears to be common (Castro *et al.*, 1989; Diez and Dusenbery, 1989) and may result from loss of attractive (particularly volatile) components (Castro *et al.*, 1989), from interactions between the agar matrix and chemicals in the leachate, and from oversimplification of the rhizosphere environment with a loss of important interactions between stimuli.

We developed a simple, rapid bioassay using a sand matrix to study chemotaxis in *T. semipenetrans* Cobb 1913. In this paper, we describe the bioassay and the attractiveness of salts of inorganic elements and organic acids to *T. semipenetrans* and *M. javanica* (Treub, 1885) Chitwood 1949. Elements of the salts are commonly found in soil solution, derived either from the soil, fertilizer or irrigation water, or as exudates from the roots of plants (Rovira, 1965).

## MATERIALS AND METHODS

*Bioassay:* *Tylenchulus semipenetrans* used in the assay were obtained by soaking infected field grown citrus roots in aerated tap water overnight. *Meloidogyne javanica* were obtained by incubating field-grown tomato roots in water. Juveniles collected over 24 hrs were placed on Baermann funnels and recovered the next day.

The bioassay unit consisted of moistened sand contained in either large (90 mm diam) or small (60 mm diam) plastic petri dish covers. Astatula fine sand (92% sand, 2% silt, 6% clay) was washed through a 40 mesh sieve (380  $\mu\text{m}$  opening) onto a 100 mesh sieve (140  $\mu\text{m}$  opening) from which it was recovered to provide a standard particle size range. Sand was washed with HCl followed by NaOCl and was rinsed with tap water, followed by deionized water. Sand was also reused in experiments after being washed with 2 M NaCl and rinsed repeatedly with deionized water. The sand was dried (130°C) overnight and then moistened to 6% (w/w) with distilled water unless otherwise indicated. Dishes were filled completely with equivalent weights of the moistened sand.

Treatments were carried out in large dishes by spotting 333  $\mu\text{l}$  alternately of distilled water and test solution on the sand surface at each of six equidistant points 0.5 cm from the edge of the dish. Thus, a total of three gradients of test solution each separated by a gradient of distilled water were created. All measurement units were halved to prepare small dishes. Approximately three thousand nematodes (juvenile and male *T. semipenetrans* or second stage juveniles of *M. javanica*) in 0.5 ml water were spotted onto the sand at the exact center of the dish. The water added to dishes with test and control solutions and nematode inoculum raised the soil moisture content to 10.0% (-4.3 J/Kg).

Dishes were stacked inside of a plastic container and maintained for 48 hrs on a laboratory bench (24-25°C) unless otherwise indicated. Each dish constituted an experimental replicate.

At the end of the test, a 21 or 14 mm diameter cork borer was used to extract nematodes in the large or small petri dishes, respectively. Sand from each zone of the test gradient was removed in three single-cores taken 0.25 cm from the edge of the dish. The same procedure was used to remove sand from the three zones of the distilled water (control) gradient. All three cores of each treatment (test or control) were placed into a single 50 ml conical based centrifuge tube. Fifteen ml of tap water were added to the tubes which were shaken and allowed to settle for 5 sec before 5 ml of nematode suspension were removed for counting.

A preference index ( $I_p$ ) was calculated based on the nematode counts. The number of nematodes in the test-solution sectors was expressed as a proportion of the total number of nematodes recovered from the test-solution and control sectors. Thus, a mean proportion significantly greater than 50% defined preference for the test solution compared to water and a mean proportion significantly less than 50% defined non-preference.

*Preference by nematodes for salts:* The bioassay was used to determine an optimum dose for screening the attractiveness of salts to *T. semipenetrans*. Sodium acetate and K-acetate were tested at concentrations of 0.05, 0.1, 0.2, 0.3 and 0.5 M (five replicates each).

Based on the results of the previous experiment, 0.3 M of the following salts were screened in the bioassay using *T. semipenetrans* and *M. javanica*: Sodium chloride (NaCl), Potassium chloride (KCl), Calcium chloride ( $\text{CaCl}_2$ ), Na-acetate ( $\text{CH}_3\text{COONa}$ ), K-acetate ( $\text{CH}_3\text{COOK}$ ), Na-

formate (HCOONa), K-formate (HCOOK), Na-citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ ), K-citrate ( $\text{K}_3\text{C}_6\text{H}_5\text{O}_7$ ), Na-carbonate ( $\text{Na}_2\text{CO}_3$ ) and Na-bicarbonate ( $\text{NaHCO}_3$ ). Treatments were replicated four times and the experiment was conducted twice using 90 mm dishes and twice using 60 mm dishes for *T. semipenetrans*. The experiment using *M. javanica* was conducted once using 90 mm dishes. Data from all experiments involving *T. semipenetrans* were pooled ( $n = 16$ ) for analysis of variance.

*Gradient characterization:* Sodium acetate was used in the bioassay (90 mm dishes) with *T. semipenetrans* at concentrations of 0.05, 0.1, 0.2, 0.3 and 0.5 M. Four cores (7 mm diam) were taken in a line toward the center of the plate at distances of 0, 7, 14 and 21 mm, from the point of chemical introduction. In addition to enumerating the nematodes in the cores, the electrical conductivity of the nematode-sand suspension was measured. Conductivity values were used to estimate the Na-acetate gradients in dishes by comparison with a standard curve constructed by moistening sand with known concentrations of Na-acetate and processing cores of the soil as in the bioassay. Nematodes in each core were expressed as a proportion of all nematodes recovered in a dish and plotted against the estimated concentration of sodium acetate in the core to determine the concentration preference of the nematode. Each concentration was replicated three times. The experiment was repeated once with essentially the same results. Data from one experiment are presented.

*Effect of soil moisture and temperature on nematode response to K-acetate.* Potassium acetate (0.3 M) was used in the bioassay (60 mm dishes) with *T. semipenetrans*. Experiments were maintained in an incubator at temperatures of 18, 21, 25 and 28°C. A separate experiment was conducted for each temperature. The soil in four replicate

assay dishes was moistened to levels of 4, 6, 8, 10, 12, 16 and 20% (weight water:dry sand) in each experiment. Soil moisture corresponded to -6.2, -5.5, -4.8, -4.3, -3.8, -2.9 and -2.3 J/Kg, respectively, as determined gravimetrically. Preference indices from all experiments were pooled by temperature and by moisture for analysis.

*Effect of pH on nematode response to Na-acetate:* Sodium acetate (0.1 M) was adjusted to pH 3.0 and 6.0 with HCl. Acetic acid was adjusted with distilled water to 0.1 M (pH 3.1). *Tylenchulus semipenetrans* were placed in petri dishes containing these solutions or distilled water. After 4 hrs, the nematodes were placed on Baermann trays for 20 hrs, after which they were recovered and counted. The experiment was repeated once. In a separate observation, distilled water was adjusted to pH 3.0 with HCl and nematodes treated as above.

*Competition between citrus radicles and Na-acetate:* The inner and outer integuments were removed from Cleopatra mandarin seeds and seeds soaked between moistened filter paper for 5 days. Seeds with radicles were buried in bioassay dishes in the same locations used normally for test solutions. The bioassay was then used to test the preference by *T. semipenetrans* of: 1) buried seeds with radicles vs. distilled water, 2) Na-acetate 0.3 M vs. distilled water, 3) seeds with radicles vs. Na-acetate, 4) Na-acetate 0.3 M vs. distilled water with germinated seeds buried in the center of the dish and 5) Na-acetate 0.3 M vs distilled water with Na-acetate also added in the center of the bioassay unit.

## RESULTS

*Attraction of nematodes to salts:* *Tylenchulus semipenetrans* preferred K-acetate or Na-acetate to water when used in the assay at concentrations between 0.05 and 0.5 M (Fig. 1). Sodium acetate was preferred to

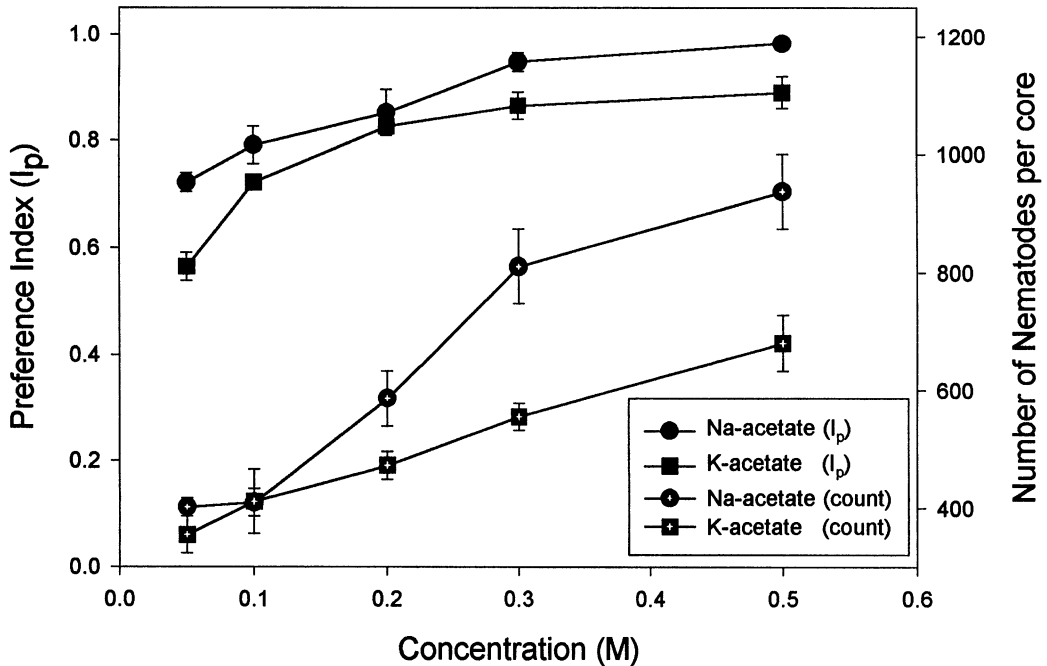


Fig. 1. Preference of *Tylenchulus semipenetrans* for K-acetate and Na-acetate compared to water. Preference index ( $I_p$ ) = number of nematodes recovered from salt sector cores/total number of nematodes recovered.

K-acetate at all tested concentrations, both in terms of  $I_p$  and total nematodes recovered. At 0.05 M,  $I_p$  was 0.73 and 0.54 for Na-acetate and K-acetate, respectively. The respective  $I_p$  at 0.5 M was 0.98 and 0.89.

Sodium acetate and K-acetate were the salts most preferred by *T. semipenetrans* of all compounds tested (Fig. 2A). Other salts preferred to water included  $\text{CaCl}_2$ , NaCl, KCl, K-formate and Na-formate. Water was preferred by the nematode to K-citrate, Na-citrate, and  $\text{Na}_2\text{CO}_3$ . Sodium bicarbonate had no effect on the distribution of *T. semipenetrans*. The preference index was not well correlated with the number of nematodes that migrated from the center of the dish to the sample zones. For example, formate salts were preferred to water by *T. semipenetrans*, but the numbers of nematodes recovered were similar to numbers for citrate salts which were non-pre-

ferred. However, acetate salts (including Ca-acetate-unpublished data) were the most preferred class of compounds and attracted the largest numbers of nematodes.

*Meloidogyne javanica* preferred Na-carbonate and Na-bicarbonate to water (Fig. 2B). Sodium-acetate, KCl, K-citrate and Na-citrate did not affect the distribution of *M. javanica*. The nematode preferred water to all of the other salts.

*Sodium acetate gradient characterization:* The different concentrations of Na-acetate produced well defined chemical gradients from the point-source to the center of the dish (Fig. 3). Electrical conductivity and Na-acetate concentrations for the standard curve were well-fitted by linear regression ( $R^2 = 0.998$ ). The average Na-acetate concentration per core was estimated to range from 0.002-0.396 M. Highest proportional

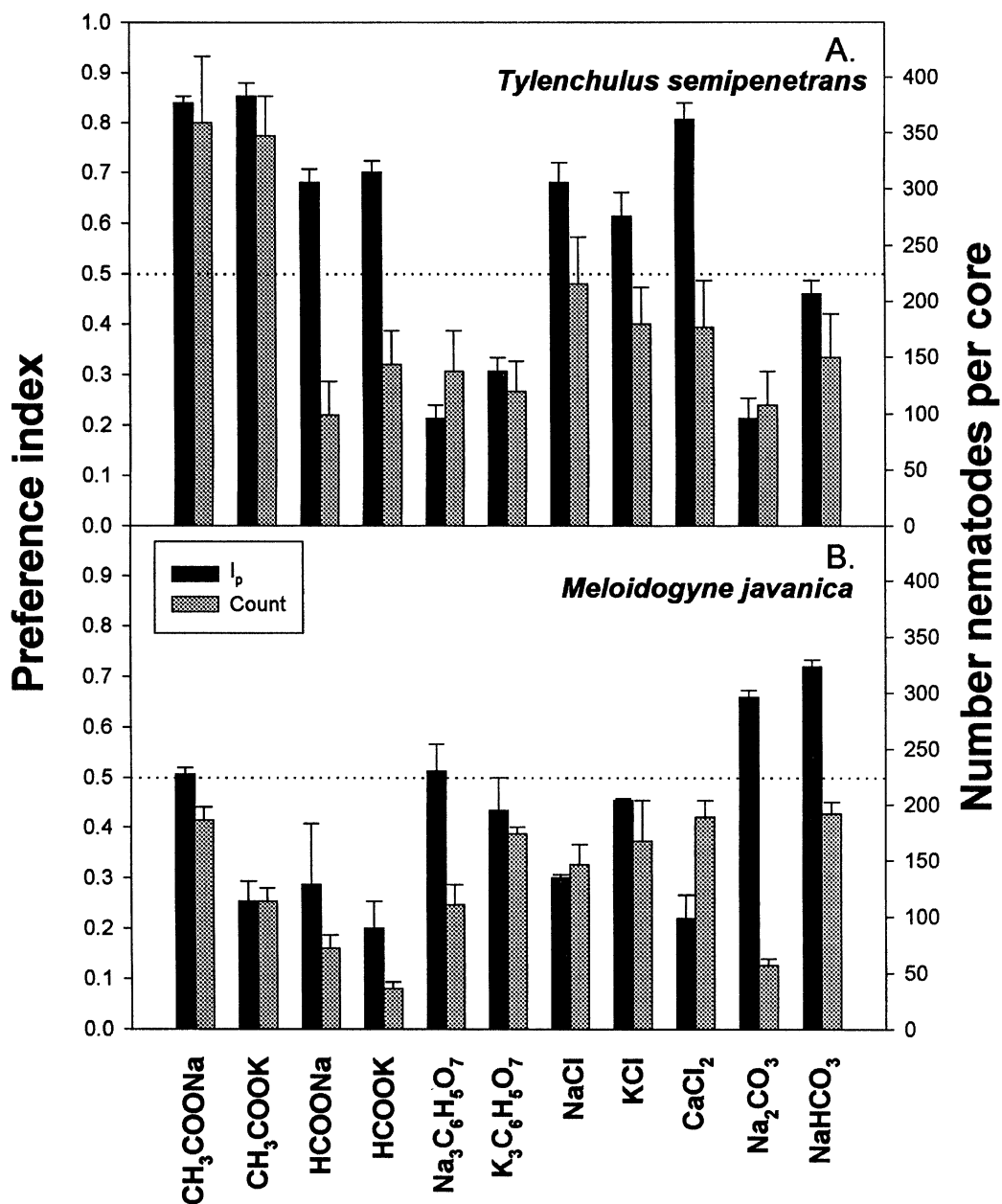


Fig. 2. Preference of *Tylenchulus semipenetrans* (A.) and *Meloidogyne javanica* (B.) for different salts (0.3 M) compared to water. Preference index ( $I_p$ ) = number of nematodes recovered from salt sector cores/total number of nematodes recovered.

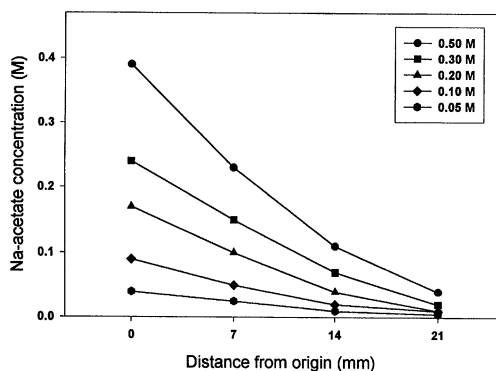


Fig. 3. Na-acetate gradient characterization in the bioassay unit using different concentrations of the salt.

recovery of *T. semipenetrans* from each bioassay unit occurred in sectors with approximately 0.08-0.10 M Na-acetate (Fig. 4). Few nematodes were recovered from sectors containing Na-acetate concentrations above 0.20 M.

*Effect of soil moisture and temperature on nematode response to K-acetate:* Across all temperatures, *T. semipenetrans* was most attracted to K-acetate at soil moisture between 6.0-10.0% (Fig. 4). The  $I_p$  across all soil moisture levels averaged 0.78-0.79 at temperatures of 25 and 28°C. The preference indices were not significantly differ-

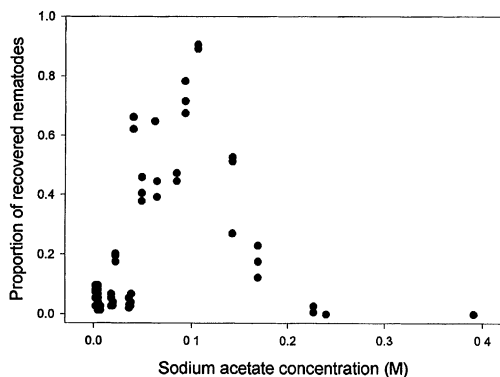


Fig. 4. Proportion of *Tylenchulus semipenetrans* recovered per core in relation to the estimated Na-acetate concentration.

ent from 0.50 at the lower temperatures of 18 and 21°C. The average numbers of nematodes recovered in the 18°C and 21°C experiments (230 and 63, respectively) were not greatly different than those at 25°C or 28°C (78 and 108, respectively).

*Effect of pH on nematode response to Na-acetate:* Sodium acetate did not affect the number of motile *T. semipenetrans* at pH 7.6-6.0 (Table 1). Compared to controls, the motility of nematodes in water adjusted to pH 3.0 with HCl was not affected. No nematode motility was detected in acetic acid or in Na-acetate at pH 3.0. Nematodes were transferred to tap water during extraction; therefore, the treatment effects were nonreversible.

*Competition between citrus radicles and Na-acetate:* The preference index of Cleopatra mandarin radicles was 0.82, compared to 0.97 for Na-acetate (Table 2). Nematode migration was higher in the presence of Na-acetate than in that of radicles. The  $I_p$  for the radicles was reduced to 0.18 when nematodes were given a choice of moving toward either radicles or the salt. Germinated seeds or Na-acetate presence in the center of the bioassay unit with nematode inoculum resulted in reduction of nematode movement towards salt or water sectors, though the Na-acetate  $I_p$  was similar to treatment No. 2 (Table 2).

## DISCUSSION

As previously reported (Prot, 1979a; Riddle and Bird, 1985), preference for organic and inorganic salts varied among different species of plant parasitic nematodes. *Tylenchulus semipenetrans* migrated toward point sources of seven of 11 salts tested. The nematode preferred water to three of the four remaining salts. In general, the anionic portion of the salt appeared to determine the attractiveness

Table 1. Effect of 0.1 M Na-acetate on *T. semipenetrans* motility (n = 4) at different pH values (*in vitro*).

| Treatment             | pH  | Number of nematodes recovered | SE    |
|-----------------------|-----|-------------------------------|-------|
| Water                 | 7.0 | 530.6 a'                      | 77.08 |
| Na-acetate            | 7.6 | 471.8 a                       | 51.66 |
| Na-acetate (adjusted) | 6.0 | 470.2 a                       | 50.41 |
| Na-acetate (adjusted) | 3.0 | 21.0 b                        | 3.45  |
| Acetic acid (0.1 M)   | 3.1 | 15.0 b                        | 4.41  |

'Values in the same column not followed by the same letter are significantly different ( $P < 0.001$ ) using DMRT (SAS 1985).

to the nematode. Thus, Na and K salts of acetate, formate and chloride were preferred to water by *T. semipenetrans* whereas Na and K salts of citrate and carbonate repelled the nematode. Acetate has also been known to attract juveniles of *Rotylenchulus reniformis* (Riddle and Bird, 1985). The poor correlation of  $I_p$  and numbers of nematodes recovered may result from different gradients created by different salts or from different detection thresholds for different compounds.

The only salts preferred by *Meloidogyne incognita* were Na-carbonate and Na-bicarbonate. The nematode preferred water to six of the nine remaining salts. All

responses by this nematode were consistent with reports for this genus in other forms of assay (Castro *et al.*, 1989; Diez and Dusenbery, 1989). Nevertheless, further study of some compounds might result in different conclusions, because all salts were tested at a concentration (0.3 M) which was found to be optimum to demonstrate the attractiveness to *T. semipenetrans* of acetate salts.

The increased attractiveness of K-acetate at higher temperatures was not simply a function of greater mobility of the nematode at higher temperatures. It was not possible to directly compare counts of nematodes between different tempera-

Table 2. Attraction of *T. semipenetrans* to Cleopatra mandarin radicles and in competition with Na-acetate gradient (n = 3).

| Source of attraction                | $I_p$   | Number of nematodes recovered/core | SE    |
|-------------------------------------|---------|------------------------------------|-------|
| Radicles (vs. water)                | 0.81 b' | 489.7 b'                           | 41.70 |
| Radicles (vs. Na-acetate)           | 0.19 c  | 605.0 a                            | 24.39 |
| Na-acetate (vs. water)              | 0.97 a  | 677.7 a                            | 53.42 |
| Na-acetate (vs. water) <sup>y</sup> | 0.96 a  | 14.7 c                             | 1.45  |
| Na-acetate (vs. water) <sup>z</sup> | 0.90 a  | 30.0 c                             | 2.52  |

'Values in the same column not followed by the same letter are significantly different ( $P < 0.001$ ) using DMRT (SAS 1985).

<sup>y</sup>Germinated seeds were buried in center of the bioassay unit.

<sup>z</sup>Na-acetate was also applied in the center of the bioassay unit.



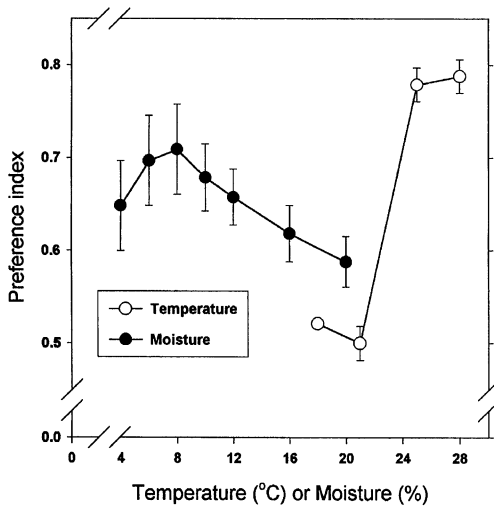


Fig. 5. Effect of soil moisture and temperature on the response of *Tylenchulus semipenetrans* to K-acetate (0.3 M) compared to water.

tures, because separate experiments were conducted for each. Nevertheless, mean numbers of nematodes recovered in each experiment were adequate to determine  $I_p$ . Thus, the nematode may have a lower response threshold for K-acetate at higher temperatures. This observation suggests that an inverse relationship between root infection and temperature below 25°C (O'Bannon *et al.*, 1966) for *T. semipenetrans* may be due in part to an inability of the nematode to orient to chemical cues at lower temperatures.

*Meloidogyne incognita* infection of tomato roots was inhibited by creating barriers of repellent salts in the soil (Castro *et al.*, 1991). The competitive ability of Na-acetate compared to citrus radicles shown in this study demonstrates the potential to reduce nematode parasitism with attractant chemicals that disrupt rhizotaxis. While the results are suggestive, the bioassay presented an arbitrary spatial dimension to the nematodes. The relative concentrations of attractants from roots are likely very low compared to that of the

salts which were selected to give maximum results in the assay. Accordingly, the stronger attraction of Na-acetate compared to radicles may not represent nematode preference, but rather the ability of the nematode to sense the salt at the distances imposed by the system. This is supported by the observation that relatively few nematodes moved toward Na-acetate when citrus radicles were placed in the center of the assay unit. The weak cation exchange capacity of sand will also result in a lower response threshold for most chemicals, because the concentration gradient is likely to be much more restricted in natural soils.

The relative availability of some of the chemicals with high  $I_p$  suggests the potential for their use as baits to enhance the efficacy of pesticides or sessile biocontrol agents (Duncan and Abou-Setta, 1995; Meyer *et al.*, 1997; Robinson and Jaffee, 1996). The efficacy of nematicides such as avermectin (which has low water solubility) and biocontrol agents such as *Pasturia* spp. are limited by the probability that a nematode will move into contact with the lethal agent. By increasing the probability of contact, attractants could affect the utility of control agents that are more target-specific and which pose less threat to other organisms.

An interesting characteristic of acetate is that it is an attractant to *T. semipenetrans* in the associated form but is lethal to the nematode in the dissociated form (at low pH). The pH-dependent response means that the attractiveness of the compound will vary with soil pH. *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* produce nematicidal concentrations of acetic acid in culture filtrates (Djian *et al.*, 1991). By formulating acetate with a slow release formulation of sulfate salt, it may be possible initially to attract *T. semipenetrans* and then kill the nematode as the pH is lowered by

the sulfate oxidation process. Provided that a small enough quantity of material was necessary, overall soil pH could be relatively unaffected.

The bioassay proved useful for the study of nematode chemotaxis. The typical coefficient of variation for a strong attractant such as Na-acetate was in the range of 5.0%, so that few replicates were required. It was simple to establish and requires only 2 days to complete an experiment. Nematode extraction from the sand was rapid. Compared with assays using agar or agarose, there was less opportunity for artifact due to experimental conditions and interaction of the substrate with chemicals and their gradients. Disadvantages of using sand compared to agar or agarose were the inability to visually observe nematode movement or to easily characterize their overall distribution in a dish. Similarly, the assay is inappropriate if fairly large numbers of nematodes are unavailable.

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