The influence of root exudates of *Chloris gayana* and *Tagetes patula* on *Rotylenchulus reniformis*

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SUMMARY

Marigold *(Tagetes patula),* rhodes grass *(Chloris gayana),* and tomato *(Lycopersicon esculentum)* root exudates were collected from undisturbed rhizospheres. The influence of the root exudates on *Rotylenchulus reniformis* hatching, infection, development, and reproduction was assessed. Tomato hydrophobic root exudates and Hoagland's solution reduced the rate of hatch of *R. reniformis* as compared to a water control in *in vitro* testing. Marigold and rhodes grass hydrophobic root exudates did not reduce the rate of hatch. Applying marigold, rhodes grass and tomato root exudates to tomatoes growing in nematode infested soil significantly reduced numbers of *R. reniformis* in the soil, and also reduced root infection. Rhodes grass root exudates inhibited hatch and nematode orientation to host roots, while marigold root exudates seemed directly toxic to reniform nematode. Tomato root exudate activity in soil was ascribed to possible influence on hatch. Removing hydrophobic organic compounds from the root exudates did not eliminate the observed effects, suggesting that hydrophobic organic compounds were not responsible for the observed reduction of nematode numbers.

RÉSUMÉ

Influence des exsudats radiculaires de Chloris gayana *et* Tagetes patula *sur* Rotylenchulus reniformis

Des exsudats radiculaires sont collectés à partir de la rhizosphère non perturbée d'œillet d'Inde *(Tagetes patula),* de *Chloris gayana* et de tomate *(Lycopersicon esculentum).* L'influence de ces exsudats sur l'éclosion, le pouvoir infestant, le développement et la reproduction de *Rotylenchulus reniformis* a été testée. Les exsudats hydrophobes de tomate et la solution de Hoagland diminuent le taux d'éclosion de *R. reniformis* en comparaison d'un témoin-eau (essai *in vitro*). Les exsudats de *C. gayana* et d'œillet d'Inde ne diminuent pas le taux d'éclosion. L'application d'exsudats radiculaires hydrophobes d'œillet d'Inde, de *C. gayana* et de tomate à des tomates croissant dans un sol infesté réduit significativement le nombre des *R. reniformis* dans le sol, mais également le taux d'infestation. Les exsudats de *C. gayana* inhibent l'éclosion et l'orientation du nématode vers les racines de la plante-hôte, tandis que les exsudats d'œillet d'Inde sont directement toxiques envers le nématode. L'action des exsudats de tomate dans le sol est attribuée à une influence possible sur l'éclosion. Le retrait des composés organiques hydrophobes des exsudats radiculaires ne supprime pas les effets observés, ce qui laisse supposer que ces composés ne sont pas responsables de la diminution du nombre des nématodes observée.

Rotylenchulus reniformis Linford & Oliveira, 1941, the reniform nematode, is the most important pathogen of Hawaiian pineapple, and is controlled by nematicides and fallow periods (Caswell, Sarah & Apt, 1990). Rotation or cover crops are possible alternative nematode management strategies. The capacity of plants to influence terrestrial nematodes is well documented (Yeates, 1987), and marigold — *Tagetes* spp. — and rhodes grass — *Chloris gayana* Kunth — can reduce soil populations of several phytoparasitic nematode species (Daulton, 1963; Good, Minton & Jaworski, 1965; Hackney & Dickerson, 1975; Khan, Saxena & Mahmood, 1984; Gommers & Bakker, 1988).

There are conflicting reports on the host status of *Tagetes* spp. for *R. reniformis.* Our previous research has shown *T. patula* to be a very poor host for *R. reniformis,* and that growing *T. patula* reduces nematode numbers at least as well as fallow (Caswell *et al.,* 1989). *Chloris gayana* has been shown to reduce soil populations of root-knot nematode (Daulton, 1963). Roots of *C. gayana* are not penetrated by reniform nematode, and *C. gayana* reduces reniform nematode numbers as well as, or better than, fallow in greenhouse and field experiments (Caswell *et al.,* 1989).

Root exudates and rhizosphere chemicals may stimulate nematode egg hatch and act as the stimulus for juvenile orientation to roots. For example, cucumber root extracts contain compounds that act as attractants and repellents to juveniles of *Meloidogyne incognita* (Castro *et al.,* 1990), and high concentrations of certain

salts, including Hoagland's solution salts, may be repellent to juveniles of *Meloidogyne javanica* (Prot, 1978). Depending on concentration, tomato root leachates may stimulate or suppress hatch of reniform nematode (Khan, 1985) and certain inorganic ions are attractive to reniform nematode (Riddle & Bird, 1985). Root exudates of *Tagetes minuta* L. have nematicidal activity against *R. reniformis* (Siddiqui & Alam, 1987). The influence of rhodes grass root exudates on reniform nematode has not been established.

This research was conducted to determine if marigold (*T. patula* cv. French Dwarf Double) or rhodes grass (*C. gayana* cv. Katambora) root exudates are capable of limiting infection, development, or reproduction of *R. reniformis*. Experiments were conducted in the laboratory and the greenhouse.

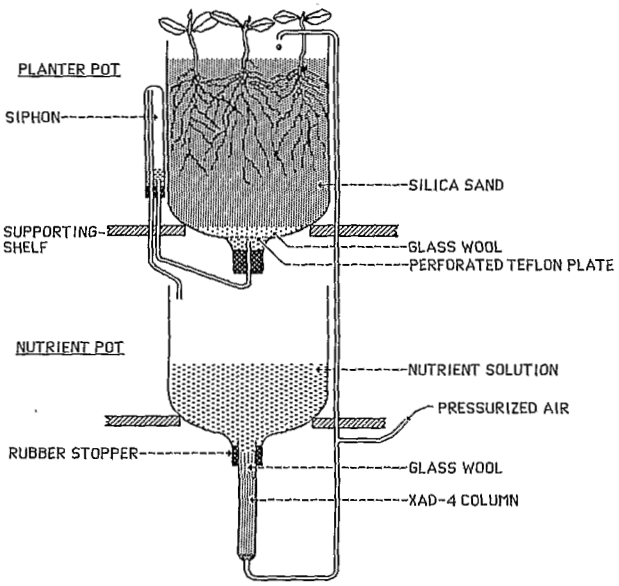

Fig. 1. Schematic diagram of the double-pot continuous root-exudate trapping system (DCRETS) used to collect root exudates. Hoagland's nutrient solution (1/20-strength) was recirculated through the system.

Materials and methods

Root exudate sources were marigold, rhodes grass, and tomato (*Lycopersicon esculentum* Mill. cv. Tropic). Seeds were germinated in moist 60-mesh silica sand contained in 10 cm-diam. Petri dishes. The seedlings were irrigated with 1/10-strength Hoagland's solution, and approximately 10 days after germination the seedlings were transplanted into double-pot continuous root-exudate trapping systems (DCRETS; Fig. 1; Tang, Komai & Huang, 1989) maintained in the greenhouse at temperatures of 22 to 28 °C. The DCRETS is a modification of the single-pot system (Tang & Young, 1982; Tang, 1986), by the addition of a reservoir pot beneath the planter pot. The DCRETS was designed for collecting root exudates from undisturbed rhizospheres.

In our experiments the planter pot contained 60-mesh silica sand, and the DCRETS pots and tubing were covered with aluminum foil to minimize exposing the root exudates to light (Fig. 2). DCRETS were irrigated by continuously circulating 1.5 l of 1/20-strength Hoagland's solution, which was replaced every three days. Once the root exudate donor plants were fully grown the root exudate solution was collected from DCRETS at three-day intervals and stored in brown glass bottles for a maximum of 3 days at 4 °C. An Amberlite XAD-4® resin column (Rohm & Hass Co.) of 12 ml bed volume was attached to the lower pot to remove hydrophobic organic root exudates from the recirculating solution. The XAD-4 columns were replaced at three-day intervals. These solutions were designated as root exudates minus hydrophobic organics (XAD-4-treated exudates).

The collected root exudates from each donor species were tested in the laboratory for their ability to affect hatching, and were also applied to nematode-infested soil in which bioassay tomatoes were growing. The bioassay tomatoes were maintained in the greenhouse at 22 to 28 °C, and each received approximately 100 ml of donor exudate per day.

The nematode infested soil for the bioassay was obtained from Dole Company field 4141 in Mililani, Oahu (soil type Lahina Silty Clay; Foote *et al.*, 1972). The soil was sieved through a 0.6-cm mesh screen to remove small pebbles and soil aggregates, and was mixed in a rotary cement mixer before placement into pots. Three tomato seeds were placed in each of the bioassay pots and the soil was saturated with tap water. Immediately after germination, each pot was thinned to one tomato plant. During the experiment the tomato bioassay plants were watered with root exudates or control solutions. Treatments were randomly assigned to pots as soil was added, and a 50 or 10 cm^3 soil sample was taken for determining initial population density *(Pi)*. The pots were arranged in a completely randomized design. Root balls were removed from pots at sampling and final nematode densities *(Pf)* were assessed from rhizosoil using modified centrifugal flotation (Jenkins, 1964). Roots were gently rinsed, weighed, and stained (Byrd, Kirkpatrick & Barker, 1983), and females in the roots enumerated. Nematode counts were recorded as numbers per 100 cm^3 soil or as numbers per gram of fresh root weight, and soil *Pi* and *Pf* values included all motile stages.

Data normality was assessed using the Shapiro-Wilk statistic (Schlotzhauer & Littell, 1987). Data on egg hatch were subject to linear regression analysis and comparison of regression slopes, while data on infection and development were subject to analysis of variance (using PC SAS ver. 6.03, SAS Institute Inc., Cary, NC).

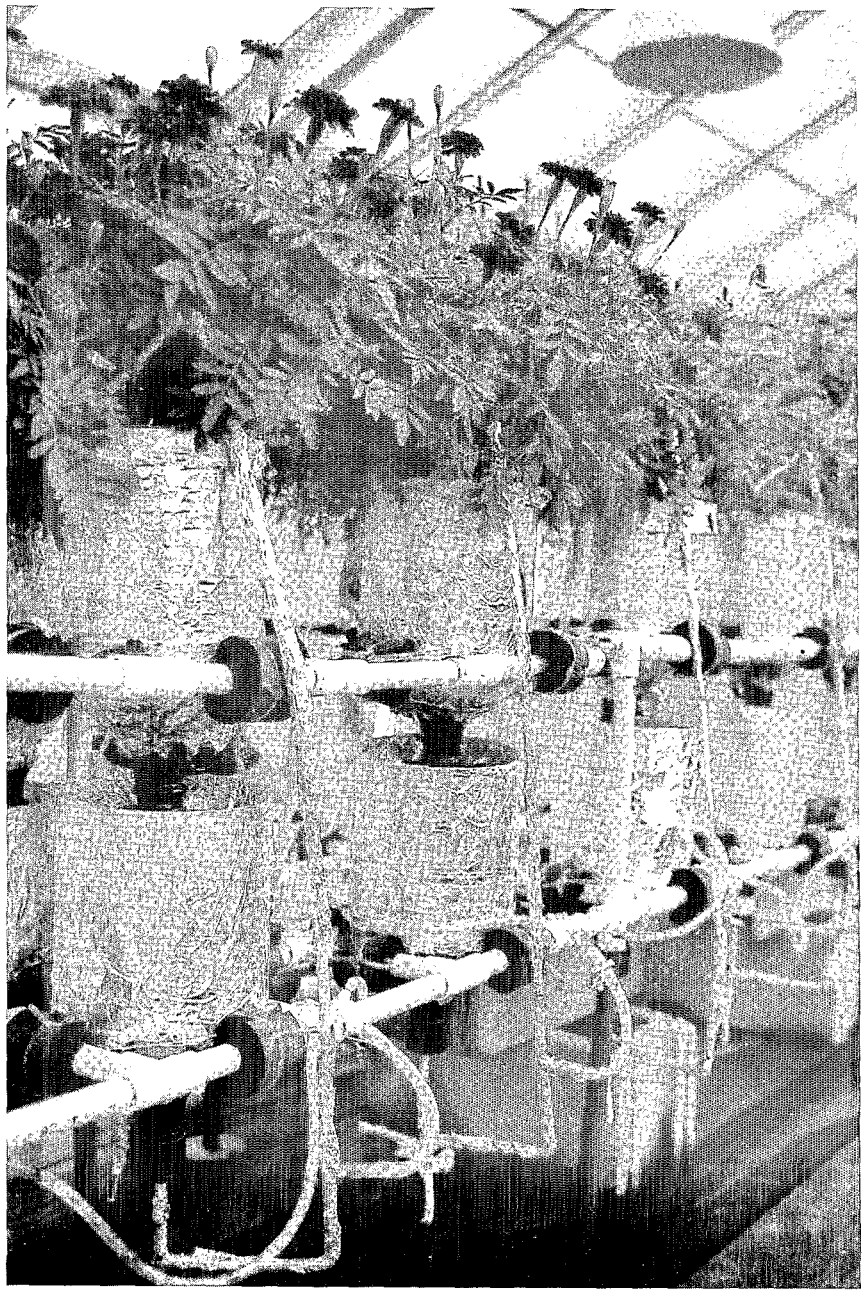

Fig. 2. The double-pot continuous root-exudate trapping system (DCRETS) used to collect root exudates from donor marigolds.

The Waller-Duncan Bayesian k-ratio *t*-test (k-ratio = 100), or LSD *t*-test (p = 0.05) multiple comparison procedures were used as appropriate (Chew, 1978).

Experiment 1

The influence of the hydrophobic root-exudate fraction of rhodes grass, marigold, and tomato on reniform nematode egg hatch was investigated in the laboratory. XAD-4 columns were collected from the DCRETS and rinsed with distilled water. Hydrophobic organics were eluted from the columns with 95 % EtOH and the eluants combined. The EtOH was removed using a rotary evaporator under reduced pressure. The remaining aqueous concentrate (10 ml) was used for the bioassay on reniform nematode egg hatch. Five treatments, including rhodes grass, marigold, and tomato hydrophobic root exudate concentrates, and a nutrient solution control collected from DCRETS without plants, were assessed at a 2 µl/ml water ratio. A water control was also included.

The bioassay eggs were recovered from reniform nematode cultures maintained on tomato-root explants growing on modified White's medium (White, 1934). Eggs were recovered by placing the culture contents in a blender, homogenizing the cultures for 30-45 seconds, and collecting the eggs on a 635-mesh sieve. The eggs were rinsed with sterile, oxygenated water and placed into Petri dishes (35 × 10 mm) containing 3 ml of the treatment solution.

The Petri dishes were kept in darkness in humid chambers at 25 to 28 °C in the laboratory. Numbers of eggs and juvenile stages in the dishes were counted each day for 7 days, and again after 16 days. The mean proportion of juveniles was subject to regression analysis with time (days) as the independent variable. The regression coefficients were compared using GT-2 95 % comparison intervals (Sokal & Rohlf, 1981).

Experiment 2

Treatments were : (a) water control, (b) XAD-4-treated rhodes grass exudates, and (c) complete rhodes grass exudates. Six replicates were destructively sampled 22 and 35 days after germination of the tomato bioassay plant. Roots were rinsed, weighed, and stained and female reniform nematodes enumerated on a per gram of root (fresh weight) basis. The ratio of the soil population density to the numbers of females per gram of root (soil/root ratio) was calculated to determine treatment influence on the proportion of nematodes in the root *vs* the soil.

Experiment 3

The same protocol as outlined for experiment 2 was used here. Treatments were : (a) complete tomato exudates, (b) XAD-4-treated tomato exudates, (c) complete marigold exudates, (d) XAD-4-treated marigold exudates, (e) complete rhodes grass exudates, (f) XAD-4-treated rhodes grass exudates, (g) and a DCRETS nutrient solution control. Soil nematode population densities were determined from six replicates at 0, 40, and 75 days after germination of the tomato bioassay plant. At 40 days roots were weighed, stained, and female reniform nematodes enumerated on a per gram of root basis and the soil/root ratio calculated. This experiment was conducted twice, with essentially similar results, and the data from the second trial are presented here.

Results

Experiment 1

The linear regressions of mean proportion of juveniles on time were highly significant ($p < 0.01$) for each treatment. The lowest coefficient of determination among all the regressions was 0.94. Tomato hydrophobic root exudates and the nutrient solution had significantly lower rates of hatch than did marigold hydrophobic exudates or the water control (Table 1). Rhodes grass hydrophobic exudates did not affect hatch as compared to all other treatments (Table 1).

Table 1

Influence of hydrophobic root exudates on egg hatch of *Rotylenchulus reniformis*. Regression of mean proportion of juveniles (n = 6) against time (days) using an initial mixed population of eggs and juveniles.

Root Exudate	*Regression Equation**	*Regression Coefficient***
Rhodes grass	Y = 23.14 + 2.19 X	2.19_{ab}
Marigold	Y = 21.95 + 2.35 X	2.35_{a}
Tomato	Y = 25.03 + 1.99 X	1.99_{b}
Nutrient control	Y = 27.75 + 1.86 X	1.86_{b}
Water	Y = 20.96 + 2.40 X	2.40_{a}

* For the regressions, $0.94 < R^2 < 0.99$, and all regressions were significant at $P < 0.01$.

** Regression coefficients followed by the same letters are not significantly different as judged by the GT-2 95 % comparison intervals.

Experiment 2

There were no differences in reniform nematode populations among treatments initially or after 22 days (Table 2). At 22 days there were no differences among treatments in soil nematode numbers, the numbers of females in the roots, or in the soil/root ratio (Table 2).

At 35 days rhodes grass root-exudate treatments had significantly fewer soil nematodes than did the water

control (Table 2), and fewer females per gram of root than did the water control. The rhodes grass XAD-4-treated treatment had significantly fewer females per gram of root than did the water control. The soil/root ratio for both rhodes grass treatments was greater than that of the water control (Table 2).

Table 2

Influence of plant root exudates on soil populations of *Rotylenchulus reniformis* (mean number/100 cm³ soil), and number of female reniform nematode per gram of root (fem/g) over time.

		Day 22			*Day 35*		
Root Exudate	*Initial*	*Pop*	*fem/g*	*Ratio**	*Pop*	*fem/g*	*Ratio*
Water control	154 $_a$	888 $_a$	345 $_a$	4.5 $_a$	668 $_a$	428 $_a$	0.5 $_b$
Rhodes grass (XAD-4 treated**)	144 $_a$	1 472 $_a$	293 $_a$	10.6 $_a$	606 $_{ab}$	48 $_b$	3.9 $_a$
Rhodes grass	128 $_a$	994 $_a$	171 $_a$	12.2 $_a$	484 $_b$	120 $_b$	2.4 $_a$

Means in the same column followed by the same letter are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (K ratio = 100), or the LSD *t*-test for multiple comparisons (p = 0.05).

* The ratio of soil nematode population density to numbers of female nematodes per gram of root.

** Root exudates passed through XAD-4 resin column before application to soil of tomato bioassay plant.

Experiment 3

There were no initial differences in reniform nematode numbers among treatments. At 40 days the complete marigold exudate treatment had significantly fewer soil nematodes than did the XAD-4-treated marigold exudate treatment, the XAD-4-treated rhodes grass exudate treatment, or the nutrient solution control (Table 3). The nutrient solution control had significantly higher densities of females per gram of root than did all other treatments with the exception of the marigold exudates (Table 3). The soil/root ratio of rhodes grass XAD-4-treated exudate was significantly higher than the ratio for marigold exudates and the nutrient blank.

At 75 days the rhodes grass root-exudate treatment had a significantly lower nematode numbers than either marigold treatment or the nutrient solution control (Table 3). At 75 days all of the exudate treatments had lower soil population densities than did the nutrient solution control (Table 3).

Discussion

Rotylenchulus reniformis is an obligate parasite with a unique life cycle. Host-plant root leachates, including tomato (Khan, 1985), may stimulate hatch. The second, third, fourth, and immature adult stages survive in the soil without feeding (Bird, 1984). Immature adult females penetrate the root, establish a feeding site, and develop into swollen, egg-producing adults (Bird, 1984). Males do not feed and are required for reproduction in Hawaiian populations.

Table 3

Influence of plant root exudates on soil populations of *Rotylenchulus reniformis* (mean number/100 cm³ soil), and number of female reniform nematode per gram of root (fem/g) over time.

				Day 40	
Root Exudate	*Initial*	*Day 40*	*Day 75*	*fem/g root*	*Ratio**
Tomato	2 025 $_a$	500 $_{ab}$	3 033 $_{cd}$	11.1 $_b$	69 $_{ab}$
Tomato (XAD-4-treated**)	2 064 $_a$	598 $_{ab}$	5 033 $_{bcd}$	10.9 $_b$	64 $_{ab}$
Marigold	*1 904* $_a$	392 $_b$	5 280 $_{bc}$	18.9 $_{ab}$	56 $_b$
Marigold (XAD-4-treated)	1 712 $_a$	757 $_a$	5 410 $_b$	14.1 $_b$	129 $_{ab}$
Rhodes grass	*1 932* $_a$	598 $_{ab}$	2 983 $_d$	6.2 $_b$	107 $_{ab}$
Rhodes grass (XAD-4-treated)	1 866 $_a$	747 $_a$	4 327 $_{bcd}$	7.4 $_b$	386 $_a$
Nutrient control	*1 621* $_a$	785 $_a$	8 690 $_a$	34.5 $_a$	75 $_b$

Means in the same column followed by the same letter are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (K ratio = 100), or the LSD *t*-test for multiple comparisons (p = 0.05).

* The ratio of soil nematode population density to numbers of female nematodes per gram of root.

** Root exudates passed through XAD-4 resin column before application to soil of tomato bioassay plant.

Mechanisms whereby root exudates may influence *R. reniformis* populations include : toxic allelochemic activity (nematicidal), disruption of female taxis to roots, disruption of male taxis to females, and through the production of anoxic rhizospheres. If root exudates exert direct toxic action they would kill a proportion of the soil nematode population. If the toxin did not affect taxis to roots, then the proportion of survivors entering roots would be the same as the control. This would result in lower soil and root numbers, but no change in the soil/root ratio as compared to the control.

If female taxis to roots was disrupted, soil nematode numbers would have increased relative to the controls, while the number in the roots would decrease, resulting in an increased soil/root ratio. If male taxis to females was disrupted, the numbers in soil and roots would be the same as in the controls during the first 20 to 30 days of the experiment. Subsequently, soil nematode num-

bers would decrease over time while the numbers of females in the roots would stay approximately the same as the controls yielding a soil/root ratio that would be the same as the controls for 20 to 25 days. The soil/root ratio would gradually increase as the soil population density decreased and adult female numbers stayed the same.

Rhodes grass hydrophobic root exudates did not reduce eclosion as compared to the water control (Table 1). However, experiment 1 is not definitive and does not eliminate the possibility that at different exudate concentrations different results would be obtained. Compounds known to stimulate nematode hatch often function at extremely low (ppb range) concentrations (Perry, 1987), and concentration may influence the inhibitory or stimulatory nature of root exudates (Khan, 1985).

Experiment 2 clearly demonstrated that rhodes grass root exudate could reduce nematode numbers in soil, and the numbers of females in roots. This experiment prompted us to further consider involvement of hydrophobic organic molecules in mediating the affect. We decided that dilute Hoagland's solution in DCRETS containing only sand, and changed every three days, would serve as our control in experiment 3.

In experiment 3 rhodes grass root exudates and XAD-4-treated root exudates reduced *R. reniformis* numbers in soil. Rhodes grass XAD-4-treated root exudates resulted in fewer females per gram of root, and a significantly increased soil/root ratio. The soil/root ratio together with reduced soil and root numbers indicates that the change in soil numbers was disproportionate to the change in root numbers, leading us to conclude that rhodes grass root exudates suppressed both hatching and the ability of females to find host roots. The XAD-4-treated rhodes grass exudate was apparently more effective in this regard. This is consistent with the results of experiments two and three, but not necessarily experiment 1. However, because the protocol in experiment 1 was limited to the hydrophobic-root exudate fraction, the results from experiment 1 did not contradict the conclusion that XAD-4-treated root exudates suppressed hatching.

Hydrophobic marigold root exudates did not alter the rate of reniform nematode hatch in experiment 1, but significantly reduced soil nematode numbers at days 40 and 75 in experiment 3. The soil/root ratio in experiment 3 revealed that the proportion of soil nematodes penetrating the roots was the same as observed for the control. We conclude that immature female orientation to roots was not affected by marigold exudates. Because the number of females in the root was not significantly reduced in experiment 3, and hatch was not inhibited in experiment 1, the root exudates probably exerted a direct toxic influence on juveniles and immature females in the soil. This is consistent with previous observations that *Tagetes minuta* exudates are toxic to *R. reniformis* (Siddiqui & Alam, 1987). Christie (1960) hypothesized that diffusates from *Tagetes* spp. might simply neutralize or mask host diffusates, making orientation to roots difficult. However, marigold roots are known to exude thiophenes (Tang, Wat, & Towers, 1986) that may be toxic to nematodes, although an exact mode of action has not been elucidated (Gommers & Bakker, 1988).

Tomato hydrophobic root exudates suppressed reniform nematode hatch in experiment 1, and suppression of hatch may have been involved in experiment 3. The soil/root ratio in experiment 3 showed that the same proportion of soil nematodes infected host roots as observed in the control, thus orientation of immature females to host roots was not affected. More probably, both the tomato root exudate and XAD-4-treated exudates suppressed hatch. This agrees with previous reports that tomato root leachates can stimulate or suppress reniform nematode hatch, and the suppressive or stimulatory affect depends on leachate age and plant density (Khan, 1985). However, the fact that tomato exudate and XAD-4-treated exudate were not significantly different suggests that the influence of hydrophobic organic compounds on hatch was not the primary determinant of our observed results.

The DCRETS effectively simulated natural soil conditions, and provided us with root exudates that were not confounded by physical disturbance of the root systems. In addition, the DCRETS system eliminated anoxia as a mode of action in our experiments because the circulating solutions were well oxygenated. These points are significant when comparing our results with other studies that have dealt with root extracts or root leachates.

Differences due to phenological variation will affect the quantity and quality of marigold and rhodes grass root exudates. Thus, we refrain from drawing broad conclusions here. However, marigold and rhodes grass seem to have potential use in reniform nematode management programs, as their root exudates can influence immature females seeking host roots. Specific practices using marigold and rhodes grass, such as cover cropping or companion plantings, for effective management of reniform nematode remain to be developed.

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