Journal of Chemical Ecology, Vol. 16, No. 4, 1990

STRONG REPELLENCY OF THE ROOT KNOT NEMATODE, *Meloidogyne incognita* BY SPECIFIC INORGANIC IONS

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(Received May 12, 1989; accepted June 27, 1989)

Abstract—Simple inorganic salts of the ions K^+, NH_4^+, Cs^+, NO_3^-, and Cl^- are strongly repellent to infective second-stage larvae of the root knot nematode, *Meloidogyne incognita*. Some of these salts are known to be beneficial to plant growth. The results suggest a new means of plant protection.

Key Words—Chemotaxis, repellents, *Meloidogyne incognita*, root knot nematode, inorganic ions, salts, gradients.

INTRODUCTION

Attraction of parasitic nematodes by plant roots may be the key to host recognition, specificity, and subsequent infection (Dusenbery, 1987; Steiner, 1925). On the other hand, environmentally tolerable repellents offer an alternative to pesticides in the protection of plants from these root parasites.

We have recently described a quantitative bioassay for the attraction and repulsion of plant parasitic nematodes by chemical substances (Castro et al., 1989). The method was illustrated with infective second-stage larvae of *Meloidogyne incognita* (MiJ_2) and chemotactic fractions isolated from cucumber roots. In the course of this work we have found that fertilized cucumber seedlings contained an additional repellent fraction. Examination of the fertilizer constituents prompted a scan of the response of MiJ_2 to a variety of inorganic salts. In this work we describe the surprisingly strong repellency of simple inorganic ions to this parasite. Our results suggest that salts beneficial to plant nutrition,

0098-0331/90/0400-1199$06.00/0

suitably applied, may be used to shield roots from nematode attack. They suggest a new means of plant protection.

METHODS AND MATERIALS

^{14}C-Labeled glycerine, glycine, and sodium acetate were commercial samples (New England Nuclear, Dupont) and were used without purification. Specific activities were UL labeled glycerine 9.1 mCi/mmol, [1-^{14}C]glycine 4.5 mCi/mmol, and [1-^{14}C]sodium acetate 1.8 mCi/mmole. HFeEDTA · $2H_2O$ was prepared by literature procedures (Garcia Basalotte et al., 1986) and recrystallized twice from water–acetone before use. Monosodium and potassium salts were obtained from it by reaction with stoichiometric amounts of the corresponding carbonates. The resulting monohydrate complexes were recrystallized from water–acetone. All other salts used in this work were purchased as analytical reagent-grade substances.

Bioassay. The essence of our bioassay is to effectively restrict nematode movement to one dimension. Narrow 0.8% agarose tracks are employed. Precise gradients of test substance can be established, and the population of nematodes along the track can be monitored with time. The test substance is placed at the end of the track at fixed concentration. After a suitable time (ΔT), the nematodes (100–150 larvae) are inoculated into the center of the track and the population distribution is allowed to "develop." Employing the average number of animals in each 0.5-cm section of track from replicate runs (N), and the distance of that section from the center (D), we have defined an attractant (A) repellent (R) ratio at time t as:

$$(A/R)_t = \frac{\sum (N)(D)_{\text{toward}}}{\sum (N)(D)_{\text{away}}}$$

The sum $(N)(D)$ is the distribution of the population weighted for the distance it has moved towards or away from a given substance in that time (t). The details of this procedure have been presented (Castro et al., 1989).

Gradients. The salts KNO_3, NH_4NO_3, and NaOAc and the water-soluble substances glycerine and glycine all establish the same gradient under the same conditions of time and concentration. For example, the 22-hr, 10^{-2} M gradient for KNO_3 shown in Figure 1 is identical for all of these substances. Consequently, we assume the gradients for the other salts in this report are the same in this matrix. The concentration of radiolabeled substances in each 0.5-cm section of track was determined by liquid scintillation counting as previously described (Castro et al., 1989). Nitrate ion was determined spectrophotometrically by reduction to nitrite, diazotization of sulfanilamide, and coupling with N-(1-naphthyl)ethylenediamine (Chow and Johnstone, 1962). The intensity of

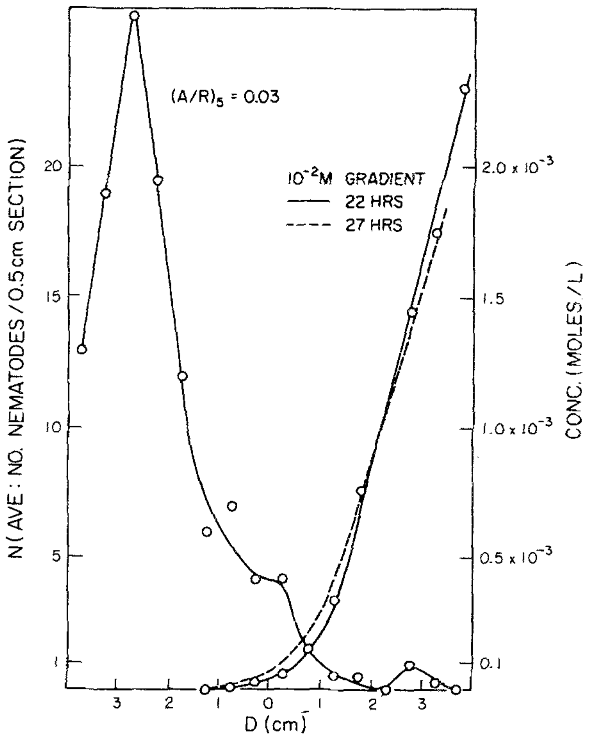

FIG. 1. Population distribution of MiJ_2 at 5 hr (left ordinate) in response to a 22-hr 10^{-2} M KNO_3 gradient (solid line, right ordinate) and the 27-hr gradient (dashed line, points not shown).

the band at 543 nm was determined with a Cary 118C spectrophotometer. In this work we have employed as standard an initial gradient of 10^{-2} M and a ΔT of 22 hr, that is, the end 0.5-cm section of track was made 10^{-2} M and the gradient was allowed to establish for 22 hr before inoculating the center of the track with larvae.

RESULTS AND DISCUSSION

Figure 1 shows the gradient (right ordinate) established with 10^{-2} M potassium nitrate at 22 hr (ΔT), and the population density of MiJ_2 5 hr after inoculation (t) (left ordinate). The gradient at 27 hr is also sketched in (dashed line). The N values plotted are an average of four replicate determinations. The concentration at the center that triggered this response was 4×10^{-5} M. It will be noted that the gradient does not change appreciably during the 5 hr allotted for the population distribution of the nematode to develop. Clearly the larvae are strongly repelled by this salt.

The $(A/R)_5$ values for some representative salts of similar charge type are shown in Table 1. All initial gradients were 10^{-2} M. All salts containing K^+, NH_4^+, Cl^-, and NO_3^- are consistently repellent. The variation of $(A/R)_5$ with initial gradient concentrations of these salts is plotted in Figure 2. While the response to the individual salts is measurably different, we have chosen to sketch one line through all of the points to illustrate the general character of the response. Potassium nitrate is the most repellent and is represented by the lower points at each concentration. The concentration of the test substance at the center of each track, the triggering concentrations, at $\Delta T = 22$ hr for 5×10^{-3} M and 5×10^{-4} M gradients are 1×10^{-5} and 1×10^{-6} M respectively. For potassium nitrate, the triggering concentration of 10^{-6} M corresponds to 0.1 ppm. The results suggest these salts may be used to shield plant roots from nematode attack.

A clearer picture of the specificity of the response of these larvae to certain salts of the group IA alkali metals can be discerned from the data in Table 2. There are some trends in these responses. Cations showing little or no effect at these concentrations are Li^+, Na^+, and Rb^+. Repellent cations are K^+, CS^+, and NH_4^+. Employing the data of both Table 1 and Table 2, the following anions show little or no effect: F^-, HO^-, OAc^-, $Fe^{III}edta^-$, SO_4^{2-}, and PO_4^{3-}. Except for fluoride, the other halide ions are repellent, as is nitrate ion. A rough ordering of repellency from Table 2 for repellent anions would be: $NO_3^- > Cl^- > Br^-, I^-$. The repellent cations exhibit about the same potency, with potassium showing a slightly greater effect: $K^+ > CS^+, NH_4^+$. The chloride and nitrate salts of these ions are all strong repellents $(A/R)_5 < 0.1$.

TABLE 1. RESPONSE OF SECOND STAGE LARVAE OF *Meloidogyne incognita* TO VARIOUS SALTS: 10^{-2} M GRADIENTS, ΔT 22 hr

Salt	$(A/R)_5$
None	1.0 ± 0.2[a]
NaOH	1.2
NaOAc	0.9
$NaFe^{III}edta$	1.1
$KFe^{III}edta$	0.5
NaCl	0.1
KNO_3	0.02
KCl	0.1
NH_4Cl	0.03
NH_4NO_3	0.06

[a] The ideal control response is 1.0. Thus, the first three salts exhibit no effect.

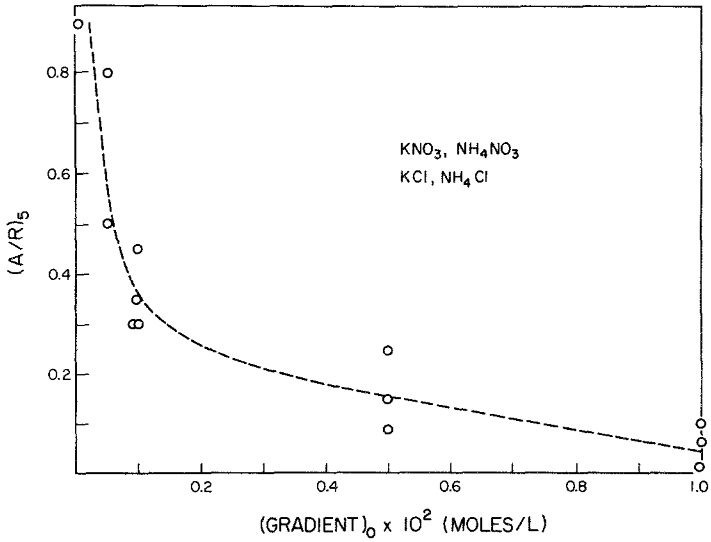

FIG. 2. Attractant/repellent ratios at 5 hr as a function of initial gradient concentration.

It is difficult to compare these results with previous related studies because the assay procedures employed were more qualitative in nature and they varied widely in design. Nevertheless, there are some interesting observations that should be noted. The bacteria-feeding nematode *Caenorhabditis elegans* was found to be attracted to a range of ions (Ward, 1973); these include Na^+, Li^+, K^+, Mg^{2+}, Cl^-, Br^-, I^-, and HO^-. Defined or calculated gradients were employed. The ions Na^+, Mg^{2+}, Cl^-, and OAc^- have also been reported to be attractive to the plant parasite *Rotylenchulus reniformis* (Riddle and Bird,

TABLE 2. $(A/R)_5$ VALUES FOR RESPONSE OF SECOND-STAGE LARVAE OF *Meloidogyne incognita* TO AMMONIUM AND OTHER ALKALI SALTS[a]

	Anions							
	F^-	Cl^-	Br^-	I^-	OAc^-	NO_3^-	SO_4^{-2}	PO_4^{-3}
Li^+	0.8	0.3	0.1	0.2	0.8	0.08		
Na^+	1.0	0.1	0.3	0.3	1.0	0.05	0.9	0.7
K^+	0.2	0.03	0.1	0.1	0.4	0.03		0.4
Rb^+	0.5	0.1	0.1	0.15_5	0.9	0.1		
CS^+	0.8	0.3	0.1	0.2	0.4	0.03		
NH_4^+	0.6	0.03	0.07	0.3	0.4	0.06	0.4	

[a]Reproducibility for $(A/R)_5 < 0.1$, ±30%, for $(A/R)_5 > 0.1$, ±20%.

1985). We find that none of these ions is attractive to *M. incognita*. Indeed K^+ and Cl^- are strongly repellent to this nematode. This opposite response to the same stimuli suggests a different set of molecular receptive sites are present in *M. incognita* or they reflect a different translation of the receptive event.

Differing responses of nematodes to metal ions has precedent in the work of Prot (1978a,b, 1979a,b). An examination of 12 "mineral salts" [NaCl, $NaNO_3$, NaH_2PO_4, KCl, KNO_3, KH_2PO_4, $CaCl_2$, $Ca(NO_3)_2$, $MgCl_2$, $MgSO_4$, $FeCl_2$, and $FeSO_4$] indicated all exhibited some degree of repellency toward *Meloidogyne javanica*, except perhaps $FeSO_4$. Moreover, Hoagland's solution [a solution containing all of the mineral salts noted above plus H_3BO_3, $MnSO_4$, $ZuSO_4$, $CuSO_4$ and $(NH_4)_6$ Mo_2O_{24}] was reported to be repellent to larvae of both *M. javanica* and *M. incognita*. Finally, differences in the response of juvenile *Heterodera oryzae* and *Scutellonema cavenessi* to salts that repelled *M. javanica* were noted.

Our results are limited to *M. incognita*, but, in the main, we believe they are in qualitative agreement with the work of Prot. Some of the ions we have delineated as repellent were present in the salts or salt mixtures he employed. As we have shown here, all salts do not elicit a chemotactic response with this nematode.

An explanation of these effects awaits further study, but it is clear that no simple correlation with size or charge of the ions is possible. For example, the approximate ionic radii (Cotton and Wilkinson, 1988) increase in the series NH_4^+, $K^+ < Rb^+ < CS^+$. The response of infective nematodes to these and other salts is under investigation. Efforts to demonstrate the effectiveness of simple salts to shield plant roots from infection are also underway.

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