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Plant Protection with Inorganic Ions

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Abstract: Gradients of salts of the specific ion repellents for *Meloidogyne incognita*—NH_4^+, K^+, Cl^-, and NO_3^-—have been demonstrated to shield tomato roots from infestation in soil. The strategy of these greenhouse experiments was to interpose a salt barrier in a soil column between the plant roots and the nematodes. The relative effectiveness of the salts as a barrier to infective second-stage juveniles in a sandy loam was NH_4NO_3, NH_4Cl > KNO_3 > KCl. Some of these ions are beneficial to plant growth, and the results suggest that a new environmentally tolerable means of plant protection is possible.

Key words: ion, *Lycopersicon esculentum, Meloidogyne incognita,* nematode, protection, repellent.

Plant protection based upon the chemotactic responses of parasitic nematodes holds much promise (4). However, despite Steiner's early hypothesis (8) of chemoreception as a means of host detection, little work at the molecular level is in place. No chemical structures derived from plant roots have been forthcoming. Recent reports indicate, however, that attractant and repellent fractions for *Meloidogyne incognita* (Kofoid & White) Chitwood can be obtained from both cucumber (2) and tomato (3) roots. In addition, a swarming factor has been reported in the leaf of *Solanum elangifolium* for *Orrina phyllobia* (7), and a female generated pheromone from *Heterodera glycines* Ichinohe has recently been characterized as vanillic acid (3-methoxy-4-hydroxybenzoic acid) (5).

An impediment to the delineation of chemotactic agents has been the lack of a quantitative bioassay for actual parasite movement toward or away from a given substance. We have recently developed such an assay and demonstrated its effectiveness with infective second-stage juveniles (J2) of *M. incognita* (2). During our examination of plant root exudates, it became clear that fertilized cucumber seedlings contained an additional water soluble fraction repellent to J2. This led to an examination of the constituents of the fertilizer and a general examination of inorganic ions. To our surprise, some very simple specific ions were found to be highly repellent to J2 (1). Highly repellent cations and anions were $NH4^+$, K^+, Cs^+, Cl^-, and NO_3^-. Any salt composed of these ions exhibited a strong repellent effect at very low triggering concentrations (ca. 0.1 ppm).

Some of these ions are known to be beneficial to plant growth. The objective of this research was to determine whether salts composed of repellent ions could shield plant roots from nematode attack.

MATERIALS AND METHODS

Meloidogyne incognita J2 were isolated from the egg masses of sterilized females (2). The general strategy was to subject the roots of growing tomato (*Lycopersicon esculentum* Mill.) seedlings to infective juveniles with or without a salt barrier or gradient placed between them.

The specific regimen employed for these experiments was as follows: A 2-week-old tomato seedling was planted in a 178-ml styrofoam cup containing 285 g steam sterilized sandy loam soil wetted to field capacity. After 24 hours, a polyvinylchloride (PVC) tube (5 cm long, 2 cm i.d.) filled with the same moist soil, but fitted at the top with a 100 mesh nylon screen (150-μm-pore opening) and at the bottom with a PVC cap, was treated with a concentrated aqueous solution containing 6×10^{-4} moles (for NH_4NO_3, 0.048 g) of test salt. Analytical reagent grade test salts were ammonium nitrate, ammonium chloride, potassium nitrate, and potassium chloride. Each salt solution (0.2 ml) was applied evenly to the top of the tube through the screen. This corresponds to ca. 0.2 moles/liter in the top 1 cm of the soil water volume. The

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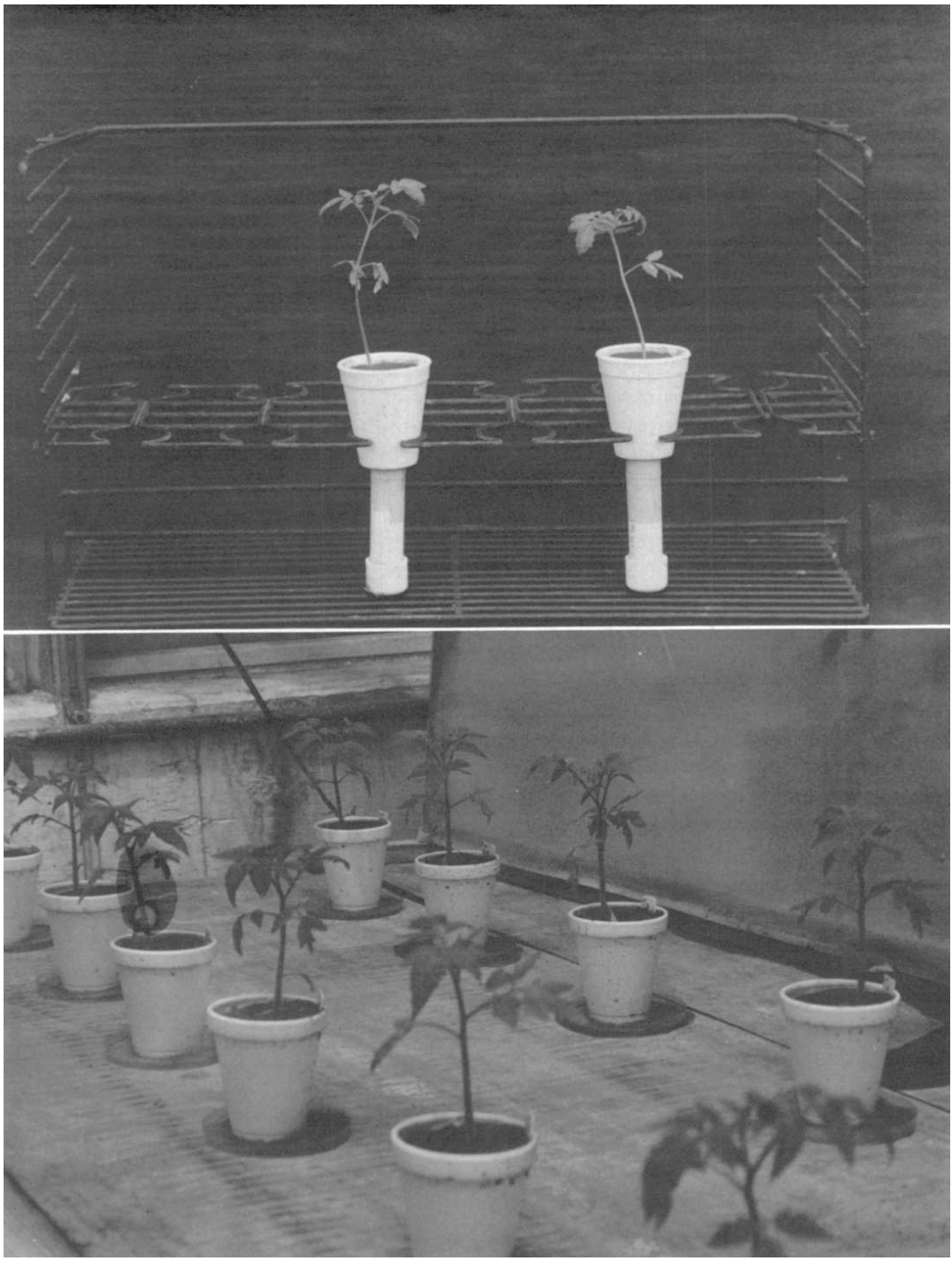

FIG. 1. Cup–tube experimental arrangement.

tube was then inserted into a hole in the bottom of the cup containing the plant (Fig. 1), with the top of the tube 1 cm above the bottom of the cup and no air space at the tube–cup interface.

For control treatments, the same procedure was followed except that 0.2 ml glass-distilled water replaced the salt solution. After another 24 hours, the PVC cap was removed from the bottom of the

tube and 1.5×10^4 J2 in 1 ml water were inoculated into the soil in the bottom of the cap which was then refitted to the tube. Usually four or five replicates of the nematode inoculated, salt treated and untreated soil tube–plant experiments were allowed to stand for 48 hours at 24 C under screen shaded conditions. Plants were watered daily to field capacity beginning the second day and throughout the holding period. They were not fertilized. After 2 days the tubes were disconnected from the plant cups, and the plants were held for 21 days at a soil temperature of 27 C. The roots were then washed and stained with Erioglaucine (6), and egg masses were counted.

One of the more promising ion combinations (NH_4^+, NO_3^-) (1) was subjected to further examination in order to determine the threshold or dose rate at which no response was observed. Ion gradients were established with NH_4NO_3: 6×10^{-4} moles, 3×10^{-4} moles, 2×10^{-4} moles, 1×10^{-4} moles, 0.5×10^{-4} moles.

To be sure ion gradients were established in these soil tubes under our conditions, radiolabelled sodium acetate of high specific activity was employed as a marker. In place of the test salts, 0.2 ml of a 0.1 M solution of sodium 1-[^{14}C]acetate (specific activity 1.9 mCi/mmol, 1.4×10^6 dps) was added to the top of the soil column. After 24 hours, the column was extruded and sliced into 0.5-cm sections. These were counted for ^{14}C in a Tricarb Model 3255 liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) equipped with background subtraction and automatic external standard. Soil samples were suspended in Packard "Instagel" counting cocktail, and all counts were quench corrected and had background subtracted from them. A nonradiolabelled sample of soil was used to establish the background counting rate. Counting times were adjusted so that the disintegrations per minute above background were at least at the 95% confidence level. The 24-hour gradient was determined (Fig. 2).

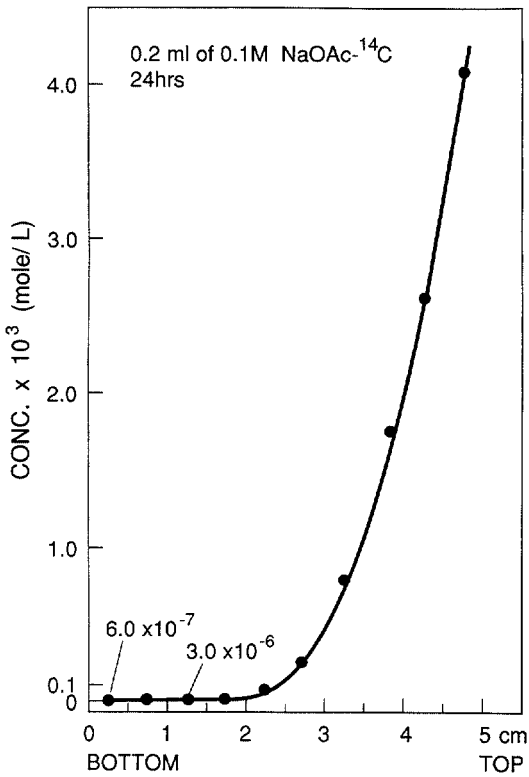

FIG. 2. Sodium acetate gradient in the soil tube at 24 hours.

RESULTS

In this report of initial greenhouse experiments, we confirm the repellency observed in the bioassay (1) in soil. Moreover, it is established that tomato seedlings can be shielded from infection by J2 with simple salts. Treated plants were larger and in many cases nearly free of *M. incognita* infection, relative to the controls (Fig. 3).

A more accurate assessment of the effectiveness of the various ion combinations to shield plant roots from infection by J2 was obtained by determining the number of egg masses found on each root following the 21-day incubation period. The root protection percentage was calculated from these numbers with the expression: 100 (control–treatment)/control, wherein control and treatment represent the number of egg masses per root system of each pair of untreated and salt protected plants. All data were obtained for a 2-day expo-

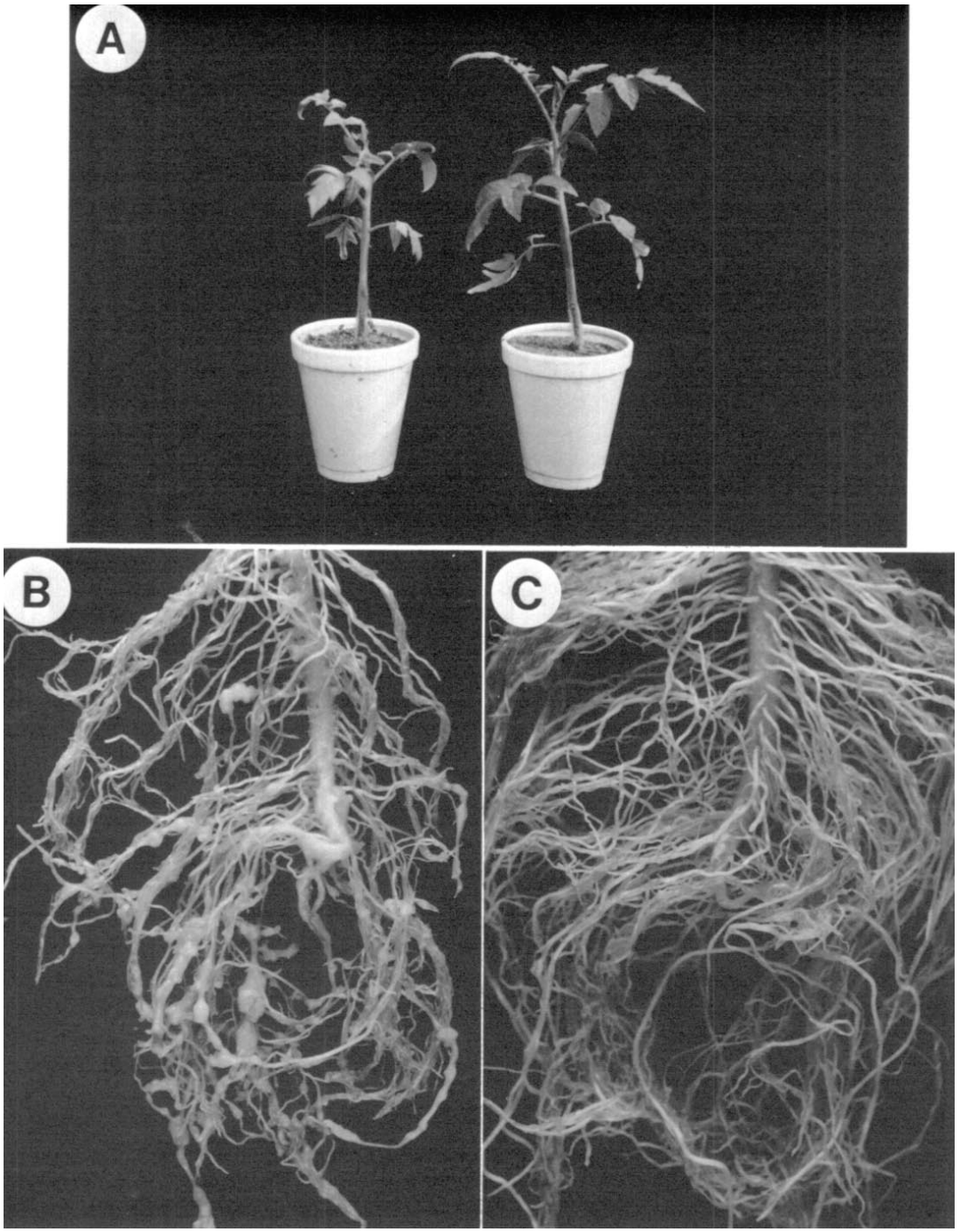

FIG. 3. Tomatoes, protected with NH_4^+, NO_3^- (6×10^{-4} mole) and exposed to 1.5×10^4 *Meloidogyne incognita* J2 for 2 days followed by a 21-day incubation period. A) Top growth control, left; treated, right. B) Control with galled roots. C) Treatment with little visible effects of nematodes.

sure to 1.5×10^4 J2 and an ion barrier established with 6×10^{-4} moles of salt.

Results are given in the sequence of salt, % protection ± standard deviation (σ), range, and the number of plant pairs in each experiment (replicates). They are as follows: NH_4NO_3, 99.2 ± 0.4, 98.7–100 (5); NH_4Cl, 93.2 ± 4.6, 92.7–100 (4);

KNO_3, 79.1 ± 6.7, 68.2–85.2 (4); KCl, 12.3 ± 9.8, −0.7–25.2 (4). The number of egg masses found in the controls usually ranged from 150 to 400. Whereas all combinations of the ions K^+, NH_4^+, Cl^-, and NO_3^- were strongly repellent in the bioassay, ammonium nitrate and ammonium chloride were the most effective in these soil experiments. Potassium chloride was only mildly repellent by comparison. With ammonium nitrate the number of egg masses was reduced by more than 98%. The more encompassing results with ammonium nitrate are summarized in the same format. The number of moles of NH_4NO_3 used to establish the gradient is the first number: 6.0×10^{-4}, 99.2 ± 0.4, 98.7–100 (5); 3.0×10^{-4}, 100 ± 0, 100–100 (5); 2.0×10^{-4}, 96.7 ± 2.9, 94.2–100 (3); 1.0×10^{-4}, 25.5 ± 39.3, −44–72 (7); 0.50×10^{-4}, 0 (5).These results show a sharp decline of effectiveness with gradients established with less than 2.0×10^{-4} moles of this salt. The results with 1.0×10^{-4} moles NH_4NO_3 indicate no statistically valid protection. In some of these replicates, the egg masses in the roots of treated plants exceeded those found in the controls. Lower amounts of salts (0.5×10^{-4} mole) showed no effect. The lowest effective dose for NH_4NO_3 in these experiments (2.0×10^{-4} moles) corresponds to 16 mg of salt.

Discussion

These experiments were designed to allow an interpretation of the data after only 2 days exposure. We have observed good protection for 3–4 days in some cases, but we opted for a 2-day standard as the basis for comparison. Thus, the time period associated with these results should be taken as a minimum. These data are encouraging, but it should be emphasized that these experiments were designed solely to establish whether repellent ions could shield plant roots from invasion by *M. incognita*. Clearly they can, but the best method of field application for maximum benefit over time remains to be determined. More important, we feel, is the fact that environmentally tolerable substances can be employed to shield roots. The potential for other substances to accomplish this remains large. The results also show the difficulty of making an extrapolation from bioassay to the greenhouse soil–tube experiments. The relatively mild effect of potassium chloride in the soil experiments suggests this salt may not readily establish a gradient in this soil. The sorption–desorption characteristics of a given soil can be a dominant factor in the efficiency of these or other chemotactic agents.

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