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An Induced Resistance Effect of Hydroxyurea on Plants Infected by *Meloidogyne javanica*[1]

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Abstract: Aqueous solutions of hydroxyurea (HU) in various concentrations were applied as soil drenches to *Meloidogyne javanica*-infected plants. At a concentration of 15 ppm, the chemical hampered giant cell formation and the number of females on the roots was 20% of that of the control but the growth of the host plants was not affected. Additional HU applications after the one at infection did not add to the inhibitory effect. HU exerted its effect on *M. javanica*-infected tomato in five soil types. Soil temperature of 32 C neutralized the HU-induced resistance in much the same way that high temperature breaks the natural resistance in *M. incognita*-resistant tomato. This study provides further evidence of the role of HU as an induced resistance agent.

Key words: induced resistance, nematode development, plant growth.

Application of nontoxic chemicals to plants to control plant parasitic nematodes has been studied by several workers. Although promising results were obtained applying nitrogenous compounds such as urea (12), thiourea (7), amino acid analogs (10), the herbicide oryzalin (9), and ammonium chloride (13), their modes of action were not elucidated and their activity was attributed to nematicidal properties. High concentrations of ammonium nitrate hampered giant cell development in *Meloidogyne incognita* in tomato root cultures, causing poor nematode development (8). We recently reported that two urea derivatives, thiourea (TU), and hydroxyurea (HU), also hampered giant cell development in *M. javanica*-infected excised tomato root cultures and thus indirectly inhibited development of the nematode as well (4). A similar mechanism, the hypersensitive reaction, occurs in plants naturally resistant to root-knot nematodes (RKN), and therefore we proposed that TU and HU exert their influence by inducing resistance in susceptible tomato roots. Between the two chemicals, HU proved to have a stronger induced resistance effect and was active at concentrations as low as 3 mg/liter, a concentration which did not affect root growth. Encouraged by the findings obtained in vitro, we extended our study and examined the effect of HU on RKN-infected intact plants under greenhouse conditions.

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Materials and Methods

Tomato (*Lycopersicon esculentum* cv. Hosen Eilon) seedlings were grown in autoclaved silica sand in 750-ml plastic pots in a greenhouse at 22–28 C. The plants were fertilized with a commercial mineral nutrient solution once a week. When the plants were at the four-leaf stage they were inoculated with 1,800–2,000 *M. javanica* eggs obtained from monoxenic cultures (5). Approximately 50% of the root systems were covered with galls 5 weeks after inoculation. Aqueous solutions of HU were applied to the plants at 100 ml/pot as a soil drench. Each treatment in the various experiments was replicated 10 times. The effects of HU were evaluated 5 weeks after inoculation by weighing fresh root systems and shoots and by staining 1 gram of infected roots from each replicate in boiling acid fuchsin-lactophenol and counting the mature females. Root samples from various treatments were prepared for observation by scanning electron microscope (SEM) (14).

Five experiments were conducted:

A. HU concentrations of 0, 5, 15, 30, and 90 mg/liter were applied in water at 1-week intervals for 4 weeks to *M. javanica*-infected and noninfected tomato seedlings.
B. HU at 15 mg/liter was applied to *M. javanica*-infected tomato seedlings one, two, three, and four times at 1-week intervals starting 7 days prior to inoculation. A nontreated control was also included.
C. HU at 15 mg/liter was applied to *M. javanica*-infected tomato seedlings grown in five soil types obtained from

TABLE 1. Characterization of five soil types examined for influence of hydroxyurea, applied as soil drench, on *Meloidogyne javanica*-infected tomato plants.

Soil type	Location	Particle size properties (%)				pH	OM*
		Clay	Silt	Fine sand	Coarse sand		
Loess	Gilat	16.0	28.0	54.0	2.0	7.8	0.6
Sandy loess	Magen	5.6	6.2	48.8	39.4	8.5	0.3
Loam	Bet Dagan	14.8	38.4	37.7	9.1	7.6	0.8
Sandy loam	Nordia	14.4	30.4	34.8	20.4	6.5	0.1
Silica sand	Ramon				100	Inert medium	

* Organic matter, percent.

different locations in Israel, as indicated and characterized in Table 1. These soils were dry heat sterilized at 100 C for 24 hours prior to use.

D. HU at 15 mg/liter was applied to three *M. javanica*-infected plant species: cabbage (*Brassica oleracea* L. cv. Tasty), cucumber (*Cucumis sativus* L. cv. Dixie), and pea (*Pisum sativum* L. cv. Target).

E. HU at 15 mg/liter was applied to *M. javanica*-infected and noninoculated tomato seedlings growing in pots immersed in water temperature tanks at 18, 25, or 32 C.

RESULTS

The influence of HU at various concentrations on the growth of tomato plants and on *M. javanica* development is presented in Figure 1. At 5 mg/liter, HU did not affect shoot or root fresh weight and the number of swollen females was 50% of the nontreated control. At 15 mg/liter, shoot weight was slightly lower than that of the control and root weight was not affected, but the number of females was only 20% of that of the control. SEM observations of fractured galls treated with 15 mg/liter HU showed small poorly developed giant cells. Treatments with 30 and 90 mg/liter inhibited plant growth. Varying the number of HU applications did not enhance the inhibitory effect over a single application at the combination level applied at inoculation (Fig. 2).

The influence of the various soil temperatures on HU activity is shown in Figure 2. At 18 C, growth of tomato plants and nematode development were rather slow and no females developed in any treatment. At 25 C, the optimal temperature for *M. javanica* development, shoot and root weights of infected plants treated with HU were 30% more than those of noninoculated controls and the *M. javanica* population in the control had 80% more females. At 32 C, shoot growth was not affected by the high soil temperature and root weight in all treatments was 20% less than at 25 C.

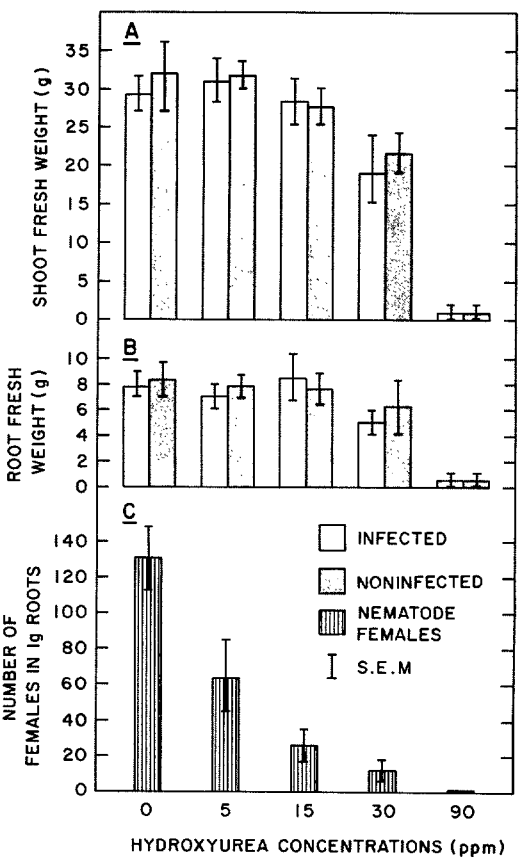

FIG. 1. Shoot weight (A), root weight (B), and number of *Meloidogyne javanica* females per gram of root (C) in tomato plants treated with various concentrations of hydroxyurea applied as soil drenches.

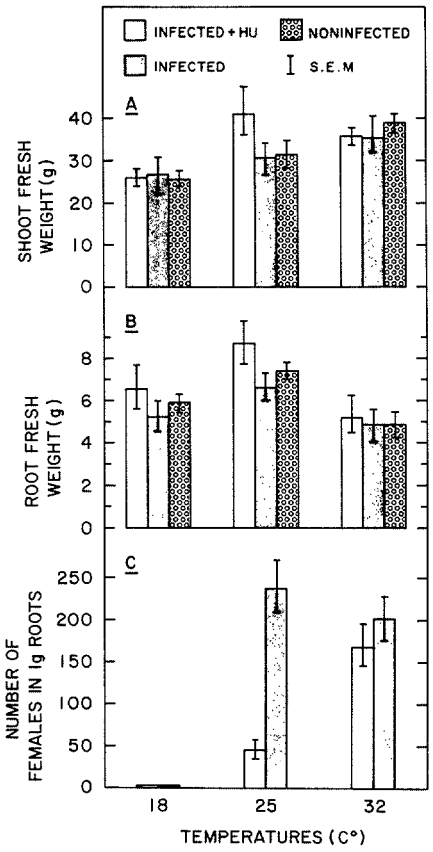

FIG. 2. Effect of hydroxyurea at 15 mg/liter applied as a soil drench on the growth of tomato plants and development of *Meloidogyne javanica* at three soil temperatures.

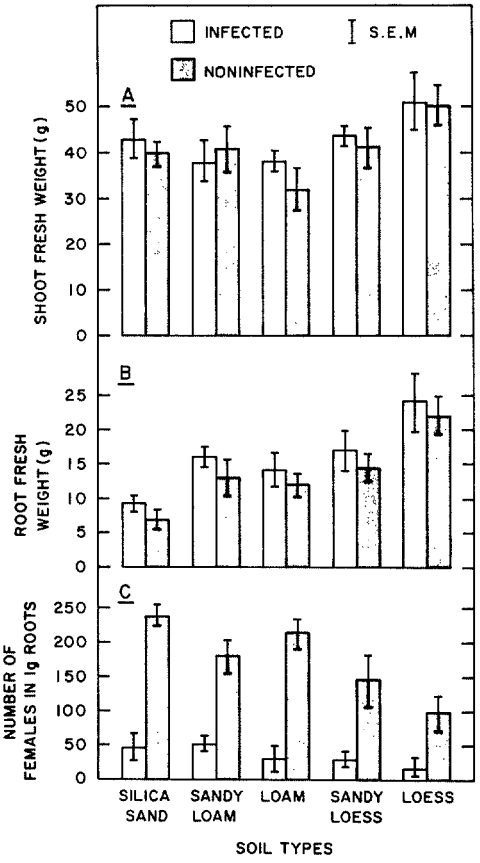

FIG. 3. Effect of hydroxyurea at 15 mg/liter applied as a soil drench on the growth of tomato and development of *Meloidogyne javanica* in different soil types.

In all the soil types tested, HU at 15 mg/liter inhibited female development by 70–80% compared with the nontreated controls (Fig. 3). Shoot and root weights varied with soil type; the number of females also varied, with a general tendency for higher numbers of females in roots growing in the lighter soil types.

Application of HU to four different *M. javanica*-infected host plants resulted in only 10–30% of the number of females compared with the infected nontreated control plants. Growth of plants of any species was not affected by the chemical treatment.

DISCUSSION

Recently, we have shown that under in vitro conditions urea derivatives, especially HU, alter the host compatability of RKN-susceptible excised tomato roots in a phenomenon similar to the hypersensitive reaction in naturally resistant plants (4). We proposed, therefore, that HU had an induced resistance property which complied also with Giebel's recent definition (3) that "induced resistance could be considered when the chemicals used for this purpose do not interfere with host-plant metabolism to such a degree as to be phytotoxic." The present study provided further information of HU performance as an agent to induce resistance when applied to the soil in a greenhouse under various environmental conditions. HU inhibited giant cell formation and consequently decreased by 70–90% the population of mature females in intact host plants belonging to four botanical families and in five soil types. High temperature breaking of resistance in RKN-resistant tomatoes (1) and the activity of HU as a giant cell formation inhibitor

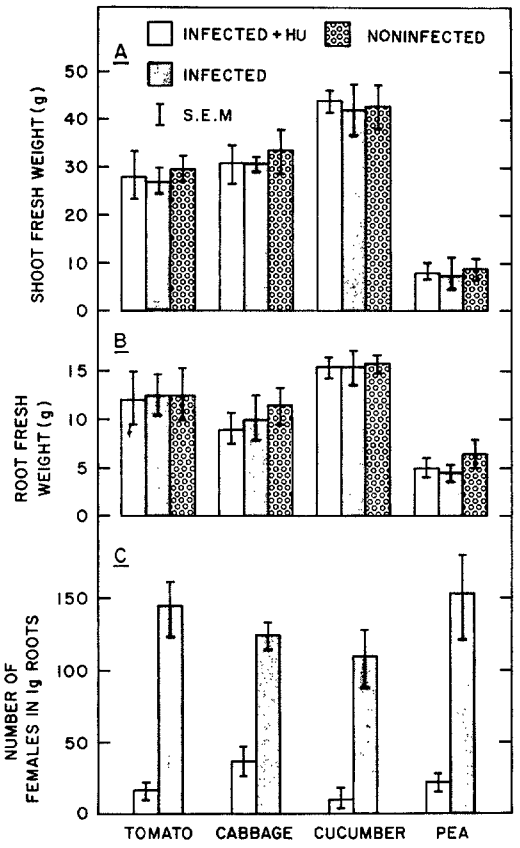

FIG. 4. Effect of hydroxyurea at 15 mg/liter on shoot and root weights and on development of *Meloidogyne javanica* on four host plants.

suggest that the natural and the induced resistance phenomena are similar. Hydroxyurea is an antimitotic compound specifically inhibiting DNA synthesis (2,6,11). The biochemical and cytological effects of HU depend on the concentration used, the duration of exposure, and the sensitivity of cell systems (2,11). Applying higher HU concentrations (30–90 mg/liter) to tomato plants arrested growth and caused other phytotoxic symptoms in the plants. Giant cells induced by RKN may be more vulnerable to HU activity at the 15 mg/liter concentration applied in this study than are other cell systems in the host plants such as apical meristems. This differential susceptibility is the reason for the specific impact of HU in inducing resistance.

Using these results, we are studying further the mode of action of HU, eventually aiming at practical development of induced resistance agents in hosts of RKN. Such studies may lead to a better understanding of natural resistance to root-knot nematodes.

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