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Influence of Urea, Hydroxyurea, and Thiourea on *Meloidogyne javanica* and Infected Excised Tomato Roots in Culture[1]

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Abstract: Urea (U), hydroxyurea (HU), and thiourea (TU), in various concentrations, were added to chemically defined plant tissue culture medium on which *Meloidogyne javanica* was reared on excised tomato roots. Concentrations as low as 3 ppm HU or 12 ppm TU inhibited nematode maturation by 70–90% 4 weeks after inoculation, and the coenocytes in the parasitized tissue were poorly developed. Gall weight was also inhibited by 50% in cultures treated with 3 and 6 ppm HU. However, exposing juveniles of *M. javanica* and *Tylenchulus semipenetrans* or juveniles and adults of *Pratylenchus thornei* to increasing concentrations of HU or TU, up to 100 ppm, was not lethal. These two urea derivatives still inhibited nematode maturation when the infected region of the root was not in direct contact with the chemicals. Therefore, we suggest that these urea derivatives inhibit nematode development by affecting the plant metabolism essential to coenocyte formation, an occurrence similar to the hypersensitive reaction in a naturally resistant plant.

Key words: inhibition, nematode maturation, coenocytes.

The root-knot nematode, *Meloidogyne* spp., depends on successful formation of feeding sites (coenocytes) in susceptible plants for its development and reproduction. These coenocytes usually fail to develop in roots of nonhosts and resistant cultivars (2,5,6).

Attempts to prevent coenocyte formation, and thus hamper nematode development, by using plant growth hormones (10,13) or plant growth retardants (4,7,12) have failed in most cases to inhibit coenocyte formation and if they did, they severely damaged the host plant (15). The idea of inducing the natural root-knot resistance reaction by artificial means has been examined in earlier reports. Overman and Woltz (16) proposed the use of amino acid analogs as antimetabolites for inhibiting development of *Meloidogyne* spp. in tomato roots. Rodriguez-Kabana and King (17) noted a reduction in galling of squash roots by adding urea to the soil. Kochba and Samish (10) indicated that thiourea inhibited development of the root-knot nematode *M. javanica* in peach roots. Orion et al. (14) found that high concentrations of ammonium nitrate in the medium inhibited coenocyte and gall formation in cultured tomato roots infected with *M. incognita.* Orum et al. (15) reduced *M. incognita* infection in tomato by applying herbicides each as oryzalin to the plants. Spiegel et al. (18) showed that the application of ammonium chloride reduced nematode populations in roots and induced a high percentage of males in the population.

The purpose of this study was to examine the effects of urea (U) and two of its derivatives, thiourea (TU) and hydroxyurea (HU), on the development of galls and the root-knot nematode in root cultures of susceptible tomato plants.

Materials and Methods

Roots of tomato, *Lycopersicon esculentum* cv. Hosen Eilon (susceptible to root-knot nematode), were grown in petri dishes on the chemically defined basal medium of Skoog, Tsui, and White (14) and inoculated with egg masses of *M. javanica,* using a procedure previously described (14). The following concentrations of urea and its derivatives were added to the medium to determine their effects on the root and on nematode development: U—9, 18, 36, 72, and 144 ppm; HU—3, 4, 5, 6, 12, and 46 ppm; and TU—6, 12, 18, 23, and 46 ppm. Each treatment was replicated 12 times.

Root growth and nematode development were examined 4 weeks following inoculation by weighing the fresh roots, both infected and noninfected, and by sampling 100 ± 5 nematode individuals per culture

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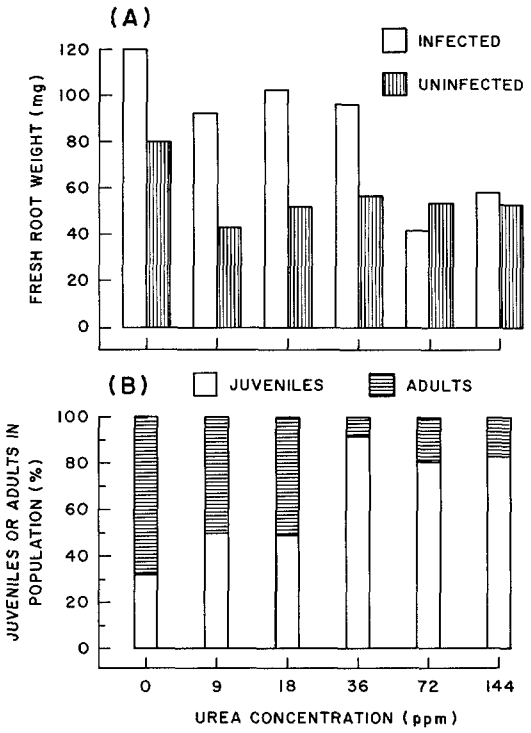

FIG. 1. Excised tomato root weight (A) and development of *Meloidogyne javanica* (B) on roots grown on medium containing different concentrations of urea.

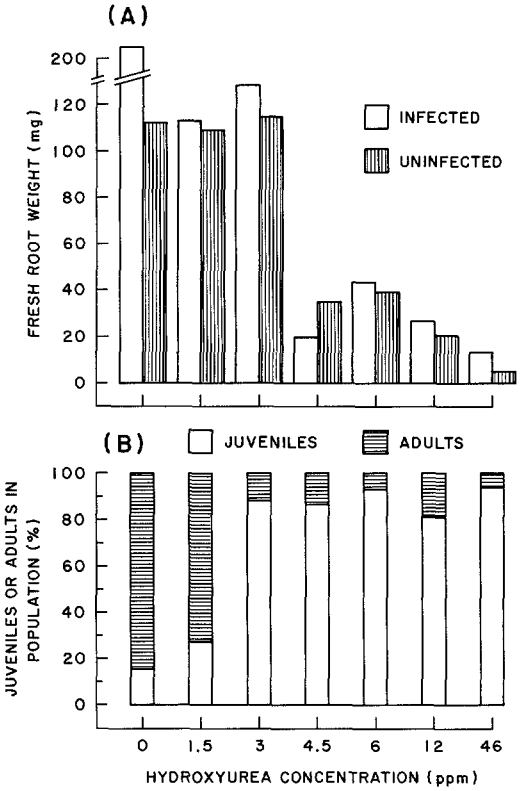

FIG. 2. Excised tomato root weight (A) and development of *Meloidogyne javanica* (B) on roots grown on medium containing different concentrations of hydroxyurea.

from galled roots stained for 5 minutes in boiling acid fuchsin–lactophenol and cleared for 24 hours in lactophenol. Root samples removed from cultures were prepared for observation with a scanning electron microscope (SEM) following the procedure of Wergin (20).

The nematicidal action of HU and TU were examined by two methods: (i) Second-stage juveniles of *M. javanica* or *Tylenchulus semipenetrans,* or juveniles and adults of *Pratylenchus thornei* were placed in 50-ml conical flasks containing 25-ml sterile solutions of HU and TU at concentrations of 0, 1, 10, and 100 ppm with six replicates of each treatment. The flasks were kept in the dark at 4 C for 96 hours. Every 24 hours the number of moving nematodes per 1-ml sample from each flask was counted. Excised tomato root cultures were inoculated with *M. javanica* juveniles previously exposed for 96 hours to HU or TU at the concentration of 100 ppm. Twenty-eight days after inoculation, the gall tissues and nematode development were examined as described above. (ii) A 3-cm petri dish containing basal medium was placed in a 9-cm dish containing basal medium enriched with 3 ppm of HU or 12 ppm of TU. The excised tomato roots were placed in the smaller dish and the lateral roots were allowed to grow in both dishes. Only roots in the small dish were inoculated to prevent direct nematode contact with the chemical being tested. Each treatment was replicated 10 times. Four weeks after inoculation, samples of 100 ± 5 nematodes per culture were taken for analysis of nematode development. The results were compared with those from control treatments, in which both petri dishes contained basal medium.

RESULTS

Urea inhibited root growth and nematode development at all concentrations tested (Fig. 1). Neither HU at less than 3

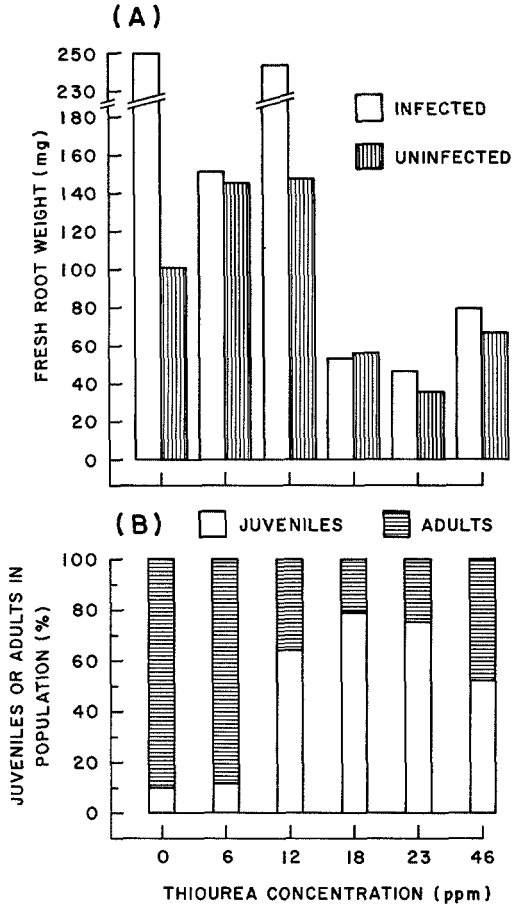

FIG. 3. Excised tomato root weight (A) and development of *Meloidogyne javanica* (B) on roots grown on medium containing different concentrations of thiourea.

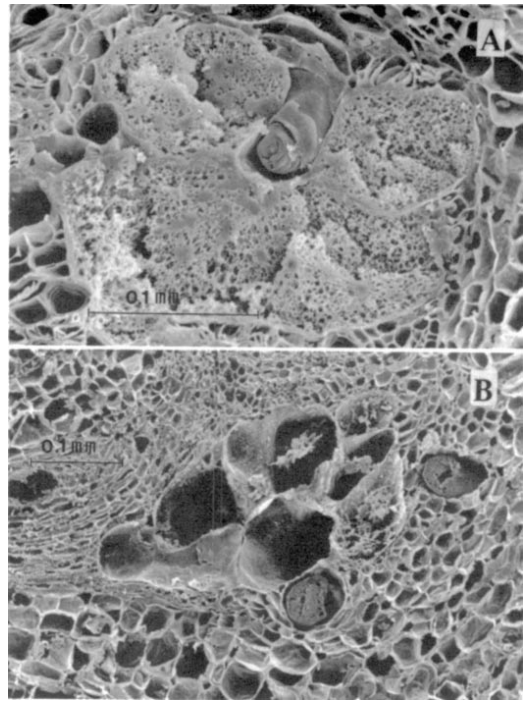

FIG. 4. Portion of fractured tomato root illustrating coenocytes in nontreated roots with dense cytoplasm induced by *Meloidogyne javanica* (A) and in roots exposed to 3 ppm hydroxyurea (B).

ppm nor TU at less than 12 ppm affected root growth (Figs. 2A, 3A). Both HU and TU at all concentrations above 1.5 and 6 ppm, respectively, inhibited nematode development (Figs. 2B, 3B). Only 12% of the nematodes developed into mature adults (males and females) at 3 ppm of HU and only 36% matured at 12 ppm of TU, compared with 90% in the nontreated control.

SEM examination of fractured gall tissue from nontreated cultures revealed that the coenocytes were well developed and full of cytoplasm (Fig. 4A). On the other hand, galled tissue from cultures treated with 3 ppm HU or 12 ppm TU had poorly developed coenocytes which were usually devoid of cytoplasm (Fig. 4B).

Weight of *M. javanica* galls in the nontreated controls was twice that of galls treated with HU at 3 or 6 ppm (Fig. 5A). Nematode development was severely inhibited at both concentrations of HU (Fig. 5B); only 12–26% of the nematodes reached maturity by 35–56 days after inoculation, compared with 79–85% in the nontreated control. While males comprised less than 10% of the population of adults in the control cultures, they comprised more than 50% of the adults in the HU treated cultures.

Nematodes of *M. javanica, T. semipenetrans,* or *P. thornei* were not killed by incubation in aqueous solutions of HU or TU at concentrations of up to 100 ppm for 96 hours. The *M. javanica* individuals transferred from those solutions into petri dishes containing excised tomato root cultures developed normally. Eighty-seven percent of the nematode population were mature 27 days after inoculation.

Development of nematodes in the double petri dish test demonstrated that HU and TU moved systemically in tomato roots to inhibit development of *M. javanica* (Fig. 6). Only 30% and 15% of the nematodes

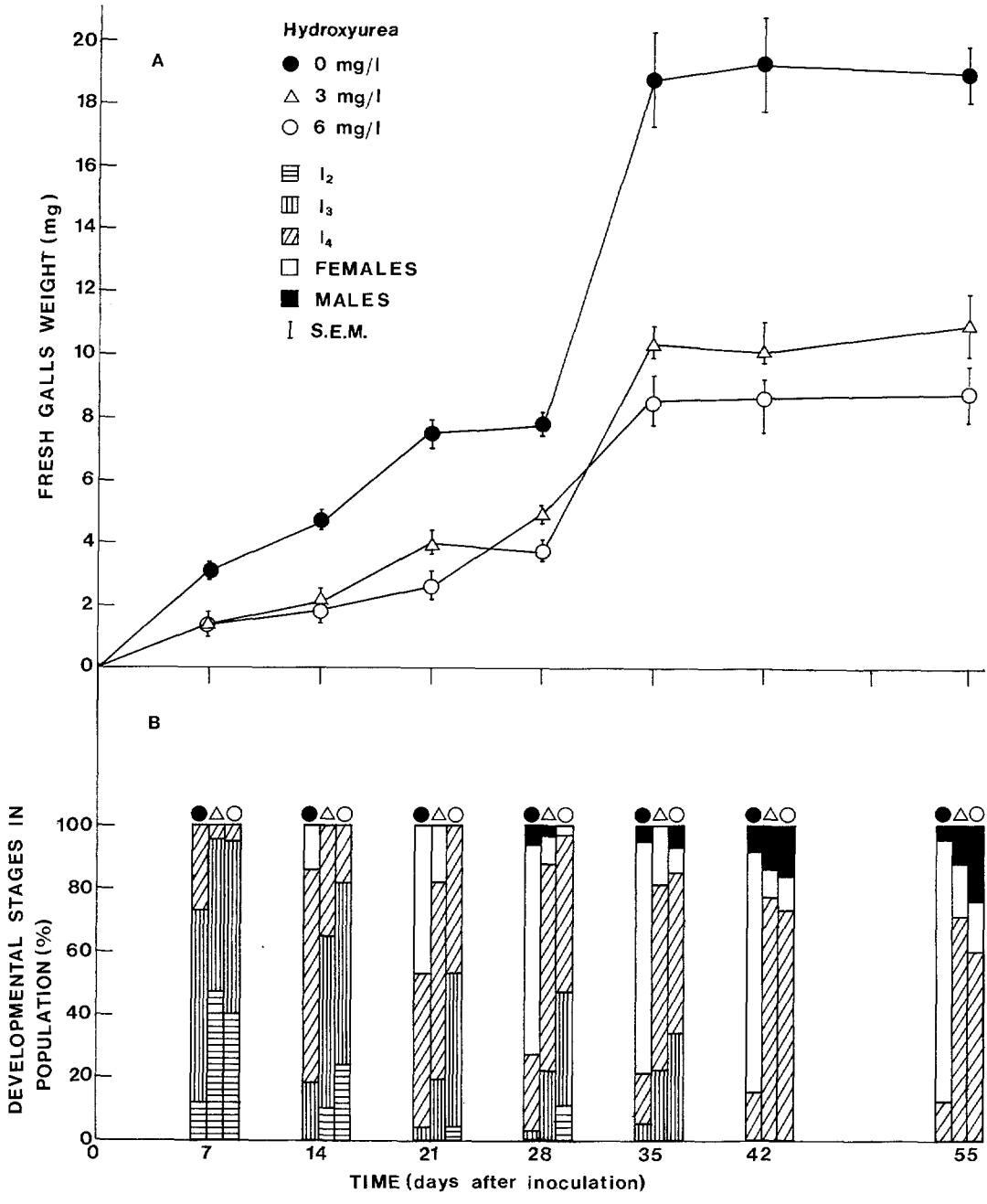

FIG. 5. Effect of hydroxyurea on gall weight (A) and nematode development (B) in *Meloidogyne javanica*-infected excised tomato root cultures over an 8-week period.

exposed to HU or TU, respectively, matured, compared with 90% maturity in the nontreated control cultures.

DISCUSSION

Both HU and TU inhibited development of *M. javanica* at low concentrations without affecting root development. In contrast, the highest concentrations of urea required to inhibit nematode development also inhibited root growth. Our results with tomatoes agree with those of Kochba and Samish (10), who reported that TU reduced the number of root-knot nematode

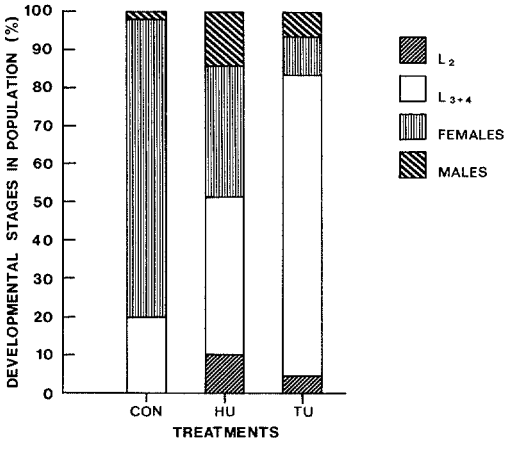

FIG. 6. Effect of 3 ppm hydroxyurea or 12 ppm thiourea on the development of *Meloidogyne javanica* 4 weeks following inoculation where nematode-infected roots were kept separate from roots exposed to hydroxyurea or thiourea.

galls on peach roots. Abnormal coenocyte formation and inhibition of nematode development caused by HU and TU is similar to effects reported as caused by high concentrations of ammonium nitrate (14). This supports the concept that nematode maturation depends on the formation of coenocytes (2,5,6,14). The high proportion of males in the HU treatments indicates that the nematodes developed under stress conditions (3,11). The fact that HU and TU did not seem to directly affect three species of plant-parasitic nematodes, but limited maturation of *M. javanica* when applied indirectly to the infected root region, provides further indication that HU and TU act by altering host compatibility. One might conclude, therefore, that inhibition of nematode development by TU and HU was indirect due to inhibition of coenocyte development and is similar to the natural hypersensitive reaction in root-knot resistant cultivars (7,19) and thus could be considered as induced resistance.

In contrast to our study, in which the low concentrations of HU and TU did not suppress root growth while inhibiting both coenocyte formation and nematode development, in most other studies both coenocyte development and plant growth were suppressed (1,3,4,13). Giebel (7) recently suggested also 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."

Hydroxyurea is a known inhibitor of DNA synthesis (9), and TU has been reported to inhibit mitosis (8). Therefore, HU and TU may interfere with nematode-induced coenocyte formation by inhibiting plant DNA synthesis and/or mitosis. Urea failed to inhibit nematode development at low concentrations and may act as does ammonium nitrate (14) to increase plant resistance to root-knot nematode infection.

The precise mode of action of urea derivatives in suppressing coenocyte formation and development of *M. javanica* were not determined in this study, but further experiments along these lines seem warranted.

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