SELECTED BIOLOGICAL INHIBITORS FOR *HETERODERA SCHACHTII* CONTROL

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Accepted:
24.IV.1989
Aceptado:

ABSTRACT

Viglierchio, D. R., and F. F. Wu. 1989. Selected biological inhibitors for *Heterodera schachtii* control. Nematrópica 19:75–79.

The potential of traditional nematicides to effect environmental and health hazards has led to a proliferation of wide ranging alternative schemes for nematode control. Even so, it is generally accepted that chemical agents will likely constitute major means for the foreseeable future. Unfortunately, the reservoir of environmentally safe chemical agents remains virtually unexplored. The intent herein is to illustrate the potential of selected biochemical inhibitors for control of *Heterodera schachtii*. Hydroxyurea and aminooxyacetic acid, inhibitors of ethylene biosynthesis and *d*-limonene, an insect nerve transmission inhibitor, exhibit a capacity to retard nematode population increases comparable to traditional nematicides.

Key words: aminooxyacetic acid, *Beta vulgaris*, cadusafos, control, *d*-limonene, fenamiphos, *Heterodera schachtii*, hydroxyurea, inhibitors, nematicides, nematodes.

RESUMEN

Viglierchio, D. R., y F. F. Wu. 1989. Algunos inhibidores biológicos para el combate de *Heterodera schachtii*. Nematrópica 19:75–79.

El peligro que potencialmente pueden causar los nematicidas tradicionales al ambiente y a la salud, ha hecho que se desarrolle un rango amplio de alternativas para el control de los nematodos. No obstante, es posible que los nematicidas van a ser el control principal en el futuro. Desafortunadamente, la mayoría de los agentes químicos no se han evaluado como nematicidas. El propósito de este trabajo es mostrar el potencial que tienen algunos inhibidores biológicos en al control de *Heterodera schachtii*. Hydroxyurea y ácido aminooxyacetico, inhiben la biosintesis de etileno y *d*-limonene, un inibidor de transmicion en los nervios de los insectos. Hydroxyurea y ácido aminooxyacetico retardan la población de los nematodos así como los nematicidas tradicionales.

Palabras claves: ácido aminooxyacetico, *Beta vulgaris*, cadusafos, control, *d*-limonene, fenamiphos, *Heterodera schachtii*, hydroxyurea, inhibidores, nematicidas, nematodos.

INTRODUCTION

Despite the myriad of nematicideless schemes proposed for nematode control, chemical means will remain a major component for the foreseeable future (2). While the overuse of the currently available

nematicides and their limited modes of action (fumigation for membrane disruption and acetylcholinesterase inhibition) may foster their demise, there are a wealth of other possibilities if nature is taken into consideration. Candidates for a third generation of agents for chemical control must be selected, targeting a susceptible characteristic of the nematode whether biological, biochemical, or behavioral. Recent research (4,5,9,11–13) has begun to focus on this promising aspect for nematode control. It was of interest therefore, to further this approach by trials with other substances and nematodes. In this paper we report the efficacy of aminoacetic acid, *d*-limonene, and hydroxyurea for control of *Heterodera schachtii* Schmidt and mechanisms by which these compounds may effect nematode control.

MATERIALS AND METHODS

Sugar beet seedlings (*Beta vulgaris* L.) cv. SSY1 were transplanted into 15-cm-diam pots (1 seedling/pot) containing a sand mixture (1 part white quartz sand: 2 parts coarse river sand) and watered daily with half-strength Hoagland's nutrient solution and maintained under greenhouse conditions. When the sugar beets leaves attained a height of approximately 20 cm, each plant was inoculated with approximately 2 300 freshly-hatched *H. schachtii* second-stage juveniles (J2). One week after inoculation, lots consisting of four-pot replicates were drenched to excess with different concentrations (ppm a.i.) of solutions of aminoacetic acid, cadusafos, *d*-limonene, hydroxyurea, or fenamiphos (Table 1). After 3 months, the experiment was terminated and the sand in each pot was wet sieved (3) through an 850-μm-pore sieve onto a 246-μm-pore sieve, and was submitted to sugar flotation (8). White females and cysts were collected, washed, and then air dried for subsequent counting.

RESULTS

From the data (Table 1) it is evident that in terms of population increase, fenamiphos (8 ppm) drenches were no different from the control; whereas another organophosphate, cadusafos (8 ppm), reduced the population by 80%. Hydroxyurea which was reported to reduce population levels of *Meloidogyne javanica* (Treub) Chitwood (5) was ineffective with *H. schachtii* in the same range of concentrations. Aminooxyacetic acid (25 ppm) reduced populations to 37% of the control, but at 50 ppm reduced the population to 17% of the control. At 100 ppm *d*-limonene reduced the *H. schachtii* population to less than 3% of the control. In all cases whether or not nematode populations were reduced, root weights of plants grown in treated sand were not different from the control, excluding the high concentrations of *d*-limonene

Table 1. Drench treatment effects on root growth and *Heterodera schachtii* population development.

Treatment	Rate (ppm a.i.)	Root wt. (g)	White females: cysts	White females + cysts
Cadusafos	8	212 a	0.89 b	1 574 cd
Fenamiphos	4	202 a	0.19 b	7 006 abc
Hydroxyurea	40	199 a	0.25 b	8 827 ab
Hydroxyurea	80	194 a	0.38 b	6 679 abc
Cadusafos	4	191 ab	0.29 b	7 604 ab
Control		186 ab	0.22 b	9 697 a
Aminooxyacetic acid	25	186 ab	0.76 b	3 628 bcd
Hydroxyurea	20	182 ab	0.36 b	7 866 ab
Aminooxyacetic acid	50	180 ab	3.00 a	1 657 cd
Fenamiphos	8	168 ab	0.30 a	8 079 ab
d-limonene	100	118 b	2.35 a	232 d
d-limonene	200	24 c	—	—
d-limonene	400	14 c	—	—

Data are means of four replications. Means with same letter are not different ($P < 0.05$) according to Duncan's multiple-range test.

which were highly phytotoxic to sugar beets. The higher ratios of white females/cysts (Table 1) suggest that, in these treatments, there was a delay in population increase within the time frame of the trial.

DISCUSSION

Organophosphates used as nematicides are presumed to target synaptic function to disrupt nerve conduction in nematodes. Cadusafos appears somewhat more effective than fenamiphos in blocking the particular form of acetylcholinesterase found in *H. schachtii* (14).

Ethylene production has been reported (6) for tomato plants infected by *Meloidogyne* spp. Moreover, inhibitors and stimulators of ethylene production affect gall development in *M. javanica*-infected tomato roots (7). According to Yang (15), the biosynthesis of ethylene proceeds according to the following pathway: methionine ⏵ S-adenosylmethionine (SAM) ⏵ 1-aminocyclopropane-1-carboxylic acid (ACC) ⏵ ethylene (Fig. 1). The rate-limiting step appears to be the conversion of SAM to ACC by means of the pyridoxalenzyme, ACC synthase. Certain natural elements e.g., germination, ripening, abscission, and other external factors such as wounding, disease, auxins, other chemicals, and various forms of stress enhance ethylene production largely by augmenting de novo synthesis of ACC synthase. Protein inhibitors therefore, inhibit ACC synthase synthesis to reduce or inhibit ethylene production. Hydroxyurea applied to tomato (*Lycopersicon esculentum* Mill.) infected with *M. javanica* was reported to inhibit gall development and giant cell formation, thereby reducing *M. javanica* reproduction by inhibiting DNA replication (5). Healthy tomato roots normally generate

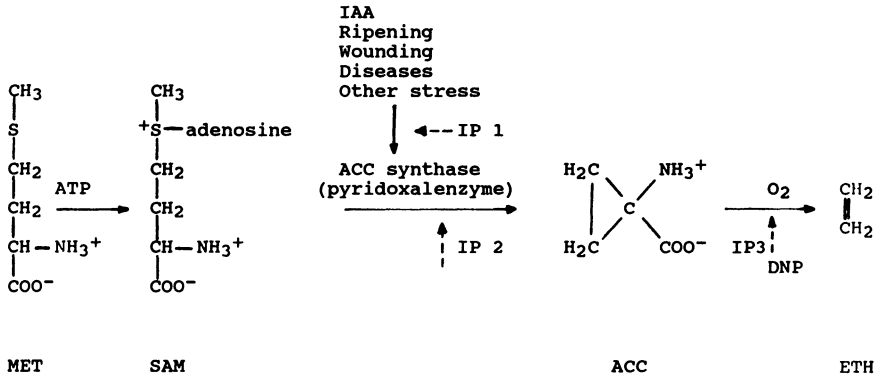

Fig. 1. Ethylene biosynthesis pathway. IAA = Indole-3-acetic acid; ATP = Adenosine triphosphate; MET = methionine; SAM = S-adenosylmethionine; ACC = 1-aminocyclopropane-1-carboxylic acid; ETH = Ethylene; DNP = dinitrophenol; and ◗ = inhibition points (IP).

very little ethylene; whereas *Meloidogyne*-infected roots with vigorously developing galls produce elevated levels of ethylene. In view of the inhibitory properties of hydroxyurea, its mechanism of action may be manifested in effecting an inhibition of protein synthesis, thereby preventing de novo ACC synthase at inhibition point 1 (Fig. 1) necessary for augmented levels of ethylene. *Heterodera schachtii* infections generate giant cells but no galls, have no need for de novo synthesis of ACC synthase and are therefore, unaffected by hydroxyurea treatments. Radish roots (used as a model to generate physiological data for sugar beets) produce ethylene in amounts large enough to be physiologically important (10). In sugarbeet roots, it appears that the normal ACC synthase level is adequate to convert SAM to ACC at a rate sufficient to generate the ethylene required for giant cell formation. ACC synthase is competitively inhibited by various agents e.g., aminoethoxyvinylglycine (AVG) (1) and aminooxyacetic acid (AOA) (16). These inhibitors block the conversion of SAM to ACC at inhibition point 2 (Fig. 1), thereby preventing the generation of ethylene by the indigenous synthase normally present in the root tissue. In either the case of a *Meloidogyne* infection of tomato treated with hydroxyurea or *H. schachtii* infection of sugar beet treated with AOA, the expected lack of ethylene production to support the development of giant cells on which the nematodes feed would be reflected in a delay of the population buildup. The lag in population increase was evident in the population levels after 3 months and supported by the significantly higher ratio of white females to cysts when compared to the control.

The mode of action of *d*-limonene on nematodes is unknown; however with insects, it appears to act on the nervous system by effecting leg paralysis followed by convulsions and death. Like conventional

fumigant nematicides, *d*-limonene exhibits properties of modest water solubility and fumigant action but is amenable to application around living plants. Phytotoxic levels, however, vary according to plant sensitivity; turf, for example, can tolerate much higher levels of *d*-limonene than can sugar beets or geraniol which could control *Meloidogyne* on tomato, both of which killed sugar beets at the same concentrations.

In our view, environmentally safe chemical agents exhibiting different modes of action not only offer an incredible potential for nematode control but also rather than defy, can be used to function in concert with nature.

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Received for publication:
9.IX.1988
Recibido para publicar: