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EFFECTS OF AMINO ACIDS ON THE REPRODUCTION OF *HETERODERA ROSTOCHIENSIS*

BY

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Of several racemic mixtures of amino acids tested, the most toxic to *H. rostochiensis* and the least toxic to potato plants growing in pots was DL methionine. The D and L forms were equally toxic to the nematodes which became less sensitive as they aged. Methionine did not act directly by contact with the nematode: it had first to be taken up by the host plant and was presumably ingested in sap extracted from giant cells.

Although methionine affected populations of *H. rostochiensis* in field plots, even large amounts did not control it adequately.

As amino acids have chemotherapeutic effects on some diseased plants (Van Andel, 1966) and as they accumulate in the giant cells of nematode-infested plants (Owens & Novotny, 1960), Peacock (1966) suggested that they might be used as nematicides. Overman & Woltz (1962) had already found that some amino acids inhibit reproduction of *Trichodorus christiei* Allen, and *Helicotylenchus nannus* Steiner, and decrease galling of tomato roots by *Meloidogyne incognita* (Kofoid & White), and of eight amino acids tested by Prasad & Webster (1967) against four species of nematode, DL methionine and DL alanine decreased numbers of *Heterodera avenae* Woll. cysts on oats, DL ethionine and DL alanine the numbers of *Ditylenchus dipsaci* (Kühn) on oats, and DL amino butyric acid the numbers of *Aphelenchoides ritzemabosi* (Schwartz) on lucerne.

Of the several amino acids we tested against *Heterodera rostochiensis* Woll., infecting potato plants in pots, methionine was the most effective, and the one we tested in the field.

Materials and Methods

Potato plants (var. Arran Banner) were grown in 9 cm diam. pots of sterilised loam, each of which was inoculated with 2,000 or 5,000 freshly hatched larvae of *H. rostochiensis* (Pathotype A, British notation). The amino acids were applied as drenches in 15 ml of water. The males and females produced were collected and counted as described by Trudgill (1967). Cysts were extracted from dried soil using a Fenwick can and hatching tests were done on batches of 50 cysts in watch glasses containing a stock solution of root diffusate. The activity of larvae exposed to methionine solutions was assessed by their ability to pass through milk filters.

In the field, potatoes (var. King Edward) were planted in plots 3 m long and four rows wide, and the roots drenched with methionine solution. Eggs and cysts were counted in aliquots of representative soil samples taken before planting and after harvest. Plant heights and yields were measured.

Results

DL-α-amino-n-butyric acid, DL ethionine, DL methionine and hydroxy-L-proline, significantly decreased the numbers of males, females, cysts and eggs (Table I). DL ethionine and DL methionine killed almost all the nematodes when applied 3 days after inoculation, but whereas DL methionine did not affect plant growth adversely, all doses of DL ethionine, a synthetic product that does not occur naturally, did.

TABLE I

Effects of DL-amino acids on the numbers of adult males and females of H. rostochiensis *when applied 3 days after inoculation with larvae, and on the number of cysts and eggs per cyst when applied 28 days after inoculation. Percentages of untreated control*

Treatment	Dose (mg/plant)	3 days		28 days	
		Males	Females	Cysts	Eggs/Cyst
D-β-Phenyl-α-Alanine	150	61	36		
	300			74	90
DL-α-Amino-n-Butyric Acid	150	43	26*		
	300			96	52*
DL-Ethionine+	75	1**	1**		
	150			27*	34**
DL-Methionine	150			26**	45
	300	0**	0**	3**	21**
Hydroxy-D-proline	150			120	102
Hydroxy-L-proline	150	28*	65		
DL-Tyrosine	100			95	87
	300	80	51	118	107
DL-Valine	150			98	93
	300	41	36	122	102

+ Phytotoxic

Levels of significance 5% *
1% **

Table II shows the effect of a range of doses of DL methionine on the reproduction of *H. rostochiensis*, and the individual effects of the D- and L-stereoisomers. The LD_{50} and LD_{95} were 16 mg and 56 mg of DL methionine respectively, when applied 3 days after inoculation to infected plants growing in 200g of soil. The 28 day applications were less effective but a 300 mg dose decreased the numbers of cysts and the numbers of eggs they contained (P = 0.01). The D, L and DL forms were equally effective in preventing reproduction.

EDITORIAL

Throughout the 15-year existence of this journal, the cooperation between the Editors and the Publisher has always been excellent. Each has his own field of responsibility: the Editor the scientific aspects, the publisher the commercial aspects. Nevertheless the Editor needs money to pay for clerical work and the publisher has to keep prices under control, and it is there that there is a common field of concern which necessitates frequent contact.

In the course of the years, two trends have developed in the financial management of scientific periodicals:

1. The sole source of profit is the subscription price.
2. Authors help to pay part of the costs.

Nematologica has mainly followed the first trend, as "page charges" have not usually been asked for. There were, however, exceptions:

a. If papers were longer than 16 pages in print, payment was often asked for the excess pages.
b. If the number of Plates was greater than the journal could afford, payment was also asked.

Both kinds of exception gave rise to difficulties. Sub. a): it is not the length but the contents of the paper that decide its scientific value. Sub. b): some authors were charged for plates that were essential to their papers. Moreover, in quite a number of cases, payment was out of the question, because of money regulations, lack of grants, etc. So a situation has developed in which some authors can furnish payment while others can not. Of course, payment never affected the scientific aspect: no priority or special attention was given to paid papers.

On the other hand, this unequal participation in the finances of the journal on the part of the authors is wrong, and it has therefore been decided to change our policy. In the future, the main source of income will be the subscription price. Of course, the consequence is that the subscription price will have to be much higher, and Vol. 18 (1972) will be priced at D. Fl. 175.— plus postage.

P. A. van der Laan
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TABLE II

Effects of methionine on reproduction when applied 3 or 28 days after inoculation with larvae

Treatment	Dose (mg/plant)	3 days (5,000 larvae/plant)		28 days (2,000 larvae/plant)	
		Males	Females	Cysts	Eggs/cyst
Control	—	320	847	325	412
DL-Methionine	12.5	275	450**		
,, ,,	25	148**	218**		
,, ,,	50	16**	38**		
,, ,,	75	9**	29**		
,, ,,	150	1**	0**	130	268
,, ,,	300	0**	0**	12**	126**
,, ,,	600	0**	1**		
D-Methionine	300			29**	70**
L-Methionine	300	0**	3**	32**	183**

LD_{50} Adults 16mg
LD_{95} Adults 56mg

Levels of significance 5% *
1% **

Applying 100, 200, or 400 mg of DL methionine, 7, 14, 21 or 28 days after inoculation and counting the cysts and eggs produced, showed that the earlier methionine is applied the more susceptible the nematodes are. Nevertheless the later applications significantly decreased the numbers of eggs (Table III). Weekly measurements of plant heights (Fig. 1) showed that a 100 mg dose barely affected plant growth, but 200 and 400 mg retarded growth immediately, and 400 mg retarded it for longest. However, growth was eventually resumed at the same rate as that of untreated plants, and the average weights of the tops 8 weeks after inoculation were 25.1, 27.7, 21.9 and 22.8 g after doses of 0, 100, 200 and 400 mg respectively.

TABLE III

Effect of time of treatment with DL-methionine on the numbers of cysts and of eggs per cyst. Means of four replicates

Dose (mg)	Time of treatment, days from inoculation			
	7	14	21	28
		cysts		
100	44*	17**	151	394
200	19**	80*	151	251
400	1**	1**	2**	215
Untreated control		399		
		eggs/cyst		
100	283	223	214	210
200	283	307	212	189*
400	314	144*	39**	103**
Untreated control		389		

Levels of significance 5% *
1% **

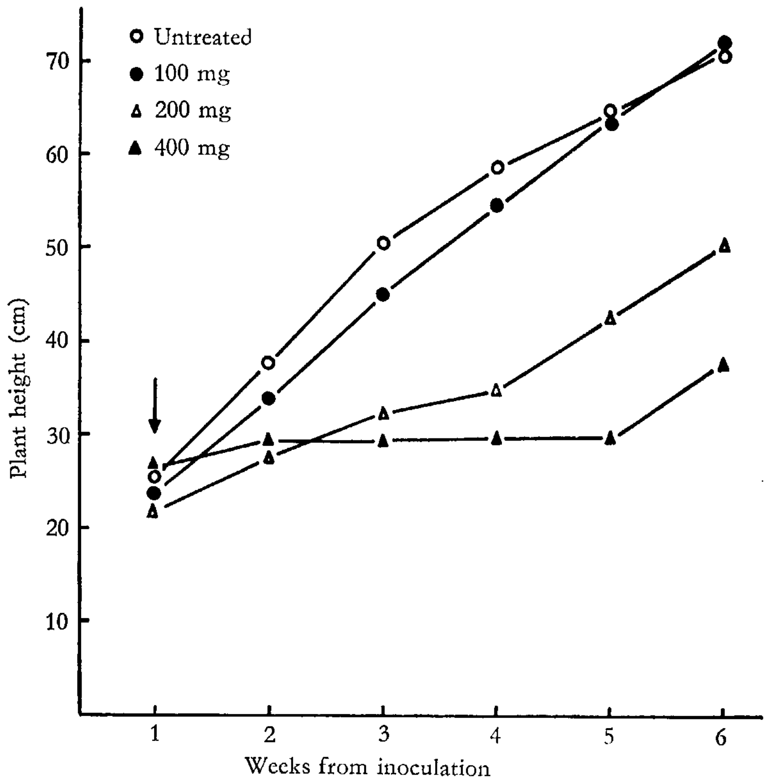

Fig. 1. Growth of potato plants receiving 0, 100, 200 or 400 mg of DL methionine in 15 ml of water. The arrow indicates the time it was applied.

Cysts placed in solutions of DL methionine in potato root-diffusate for one week produced fewer larvae than cysts in root diffusate only, and the greater the concentration of methionine the fewer the larvae produced. After transferring the same cysts to root diffusate only for the second week, many more larvae hatched. Indeed, more larvae emerged from the cysts that had been in 0.1M methionine than from the others, presumably because they contained more unhatched eggs (Table IV).

TABLE IV

Effect of methionine on the number of larvae emerging from batches of 50 cysts. Means of four replicates

Root diffusate only	Root diffusate M/250 Methionine	Root diffusate M/50 Methionine	Root diffusate M/10 Methionine
	Week 1: Solutions of methionine in root diffusate		
4294	1600	1900	238
	Week 2: Same cysts transferred to root diffusate only		
4920	3645	3640	6740

TABLE V

Field potatoes treated with 10g of DL-methionine per plant in 2 litres water 7 weeks after planting in H. rostochiensis *infested soil. Means from two plots*

	Cysts/200g soil		Eggs/g of soil		Plant height (m)	Tuber yield (kg/plant)
	Pre-planting	Post-harvest	Pre-planting	Post-harvest		
Untreated control	82	319	124	319	47	0.57
DL-Methionine	78	111	131	121	45	0.68

To check whether methionine affects the larvae directly, batches were exposed to 0.004M, 0.02M and 0.1M solutions for 24 hours, and those that moved through a milk filter in 24 hours counted; the numbers were 3660, 3913 and 3905 respectively, compared with 4198 in the control without methionine, suggesting they were unaffected by exposure to methionine solutions.

Table V gives the results of the field experiment in which the soil around each plant was drenched with 10g of DL methionine in 2 litres of water, 7 weeks after planting. The cyst and egg populations of the soil increased in the untreated plots but, although the number of cysts increased slightly in the methionine-treated plots, the egg population decreased slightly. There was no obvious effect of methionine on top growth, but treated plants yielded slightly more than untreated.

Discussion

Antimetabolites are structurally similar to essential compounds and act by combining with enzymes for which the essential metabolite is the normal substrate. When the combination is reversible, the metabolite and its analogue compete for the enzyme, according to their relative concentrations and combining affinities. Antimetabolites that combine irreversibly are potent poisons.

Amino acids usually occur in plants as the L-isomers, so the D-isomers sometimes act as antimetabolites. *H. rostochiensis* females synthesise much protein to nourish their eggs, which begin to form about 25 days after plants are invaded, so amino acid anti-metabolites might be expected to hinder or prevent egg production. In fact methionine killed the young nematodes in the roots but doses that were lethal when applied 3 days after roots were invaded did not diminish egg production when applied later. Larger doses were required to interfere with egg development when treatment was 28 days after invasion (Table III) which might be expected because methionine forms less than 3% (proline >38%) by weight of the total amino acids in the egg shells (Clarke, Cox & Shepherd, 1967).

Davies (1966) showed that turnip storage tissue incubated with methionine accumulates S-adenosylmethionine, which is metabolically inactive and accumulates in the cell vacuoles. Removal of adenosine in this way slows oxidative phosphorylation and respiration in the cells, and might thereby affect the nematodes.

Alternatively the accumulation of amino acids in the giant cells of nematode-infected plants (Owens & Novotny, 1960) and the extra methionine ingested might inhibit oxygen metabolism within the nematode.

As a nematicide, methionine was more effective the earlier it was applied (Table III). It also greatly decreased hatching (Table IV), probably because the osmotic pressure of the solution was too great, for Wallace (1956) found that fewer *H. schachtii* Schm. larvae emerged in solutions of organic compounds stronger than about 0.01M and none in 0.65M solutions. In our experiments *H. rostochiensis* larvae exposed to methionine solutions up to 0.1M for 24 hours moved normally afterwards, suggesting that methionine is not a contact poison but a stomach poison for it must first be taken up by the host plant, where it is presumed to be ingested when larvae feed on giant cells.

The amounts applied in the field trial were equivalent to about 2½ cwts DL methionine per acre (260 kg/ha) and too costly to be economic. Attempts to use less by spraying the potato foliage with solutions distorted the leaves and killed few nematodes. However, our results do indicate that *H. rostochiensis* is sensitive to changes in the metabolism of the host, and that suitable antimetabolites might block reproduction. A search for such compounds might be more rewarding than the present empirical search for compounds that kill nematodes in soil.

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ZUSAMMENFASSUNG

Der Einfluß von Aminosäuren auf die Fortpflanzung von Heterodera rostochiensis

Bei der Prüfung razemischer Aminosäuregemische erwies sich DL Methionin als am giftigsten für *Heterodera rostochiensis* und am verträglichsten für eingetopfte Kartoffelpflanzen. Die D- und L-Formen waren gleichermaßen giftig für die Nematoden, deren Empfindlichkeit im Übrigen mit zunehmendem Alter geringer wurde. Methionin wirkte nicht direkt durch Kontakt. Es mußte erst von der Wirtspflanze aufgenommen werden und wurde dann von den Älchen wahrscheinlich mit dem Saft der Riesenzellen aufgenommen.

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