were three treatments with twelve replications : soil infested with 500 *H. oryzae* or *H. spinicaudata*, and control without nematodes. *H. oryzae* and *H. spinicaudata* were raised on rice cv. Moroberekan in flooded clay pots in the greenhouse. The seedlings were maintained under artificial illumination with a light intensity of 20 000 lux and a 14 h photoperiod. After eighteen days, the nitrogen fixing activity was estimated on six replicates by the acetylene reduction method (Raimbault *et al.*, 1977) and the nitrogen content of the plants determined by the Kjeldahl method on the others.

Both nematodes significantly lowered the rhizospheric acetylene reduction activity (ARA) and the plant dry weight (Fig. 1). Since the rhizospheric ARA of seedlings is known to depend mainly upon root exudation which is linked to plant photosynthesis (Balandreau *et al.*, 1976), the nematodes were assumed to reduce the rate of exudation.

The nitrogen nutrition of the plant seemed to be less affected by the nematodes : its nitrogen content was significantly reduced only by *H. oryzae* (Fig. 1). This result may be explained by the fact that : (i) sufficient nitrogen was available in the seed and in the soil to allow for an adequate nitrogen nutrition of the plant for three weeks, and (ii) the nitrogen originating from N_2 fixation should be mineralized before being absorbed by the plant.

Thus, the nematodes *H. oryzae* and *H. spinicaudata*, appear to be partly responsible for the limitation of non symbiotic N_2 fixation in submerged rice soils.

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MELOIDOGYNE INCOGNITA DEVELOPMENT ON SOYBEAN TREATED WITH SELECTED AMINO ACIDS BY ALTERNATE METHODS

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Preliminary studies on the effect of amino acids against plant parasitic nematodes have indicated some promise for selected amino acids as a component in a pest management program.

Amino acid antimetabolites applied to the soil, have been reported to decrease the numbers of *Paratrichodorus minor (Trichodorus christiei)* and *Meloidogyne incognita acrita* on tomato (Overman & Woltz, 1962). DL-amino acid antimetabolites decreased the population of *Aphelenchoides ritzemabosi* on lucerne, while DL-alanine significantly decreased the number of *Heterodera* without injuring different host plants (Prasad & Webster, 1967). Selected amino acids applied as foliar sprays to tomato plants infected with *M. incognita* were reported to affect the development and reproduction of the nematode (Krishna & Setty, 1974). In subsequent reports (Parvatha Reddy, Govindu & Setty, 1975*a*) certain amino acids were observed toxic to tomatoes as soil drenches, while others reduced galling caused by *M. incognita* without plant injury. Subsequently in axenic cultures (1975*b*) these authors reported that DL-methionine in sterile culture reduced root galling, egg mass production and fecundity of the root-knot nematode, *M. incognita*, and in the process delayed the completion of nematode life cycle by about eight to nine days. The amino acid studies have included four nematodes and three host plants, too few to establish a generality. This

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Table 1

Responses of *Meloidogyne incognita* infected soybeans to certain amino acids applied as foliar sprays or soil drenches

Treatments	*Dose/plant (mg)*	*No. of galls*	*No. of nodules*	*Shoot Length (cm)*	*Foliage Weight (g)*	*No. of pods*	*No. of leaves*
L-phenylalanine							
spray	2.5	183	51	27.0	18.6	4.7	4.7
	5.0	136	0	22.0	16.2	3.7	4.3
	10.0	212	0	25.3	17.9	5.3	6.0
drench	100	95	16	26.7	18.3	5.3	4.3
	200	166	24	24.0	16.7	4.3	5.0
	400	243	6	25.6	17.0	5.0	4.7
L-valine							
spray	2.5	285	0	24.7	17.9	4.7	5.0
	5.0	276	0	26.7	18.3	6.0	5.7
	10.0	247	7	26.3	18.3	4.0	5.7
drench	100	229	0	20.7	15.5	1.3	13.0
	200	218	0	20.5	14.3	0.0	20.3
	400	173	0	19.7	13.2	0.7	7.3
L-cysteine							
spray	2.5	310	0	26.7	17.5	5.0	5.3
	5.0	213	0	27.7	18.6	4.7	5.7
	10.0	282	0	25.3	15.8	5.0	5.3
drench	100	187	0	28.0	19.2	5.3	4.3
	200	283	0	25.3	18.7	5.3	5.0
	400	229	1	33.3	20.7	5.0	5.3
L-methionine							
spray	2.5	207	0	26.3	17.0	4.0	4.7
	5.0	224	10	28.0	20.6	5.7	5.7
	10.0	187	0	31.3	17.3	5.0	8.0
drench	100	243	1	23.3	14.3	3.0	12.7
	200	179	22	22.3	13.2	0.0	8.0
	400	101	26	16.7	13.2	0.0	7.3
Control	—	323	14	20.0	14.6	3.3	4.0
LSD 5%							
spray		16.3		NS	2.4	NS	NS
drench		17.6		6.6	2.4	1.5	4.5
LSD 1%							
spray		22.0		NS	3.2	NS	NS
drench		23.7		8.9	3.2	2.1	6.1

NS = Not significant

research extended the study, comparing different amino acid concentrations as foliar sprays and soil drenches, and seed soak, on the development of *M. incognita* infecting soybeans under greenhouse conditions.

Stock cultures of *M. incognita* were maintained on tomato (*Lycopersicon esculentum* cv. VF 145) in the greenhouse. In the first trial seeds of soybean, cv. Williams, were sown in 10 cm pots containing a mixture of sterilized clay loan and sand (1 : 1). Twenty-day-old seedlings (1/pot) were inoculated with 1 000 freshly hatched larvae introduced into four holes around the base of the plant. Seven days after inoculation, solutions of four amino acids (L-phenylalanine, L-valine, L-cysteine, and L-methionine at three different concentrations) were uniformly sprayed over the foliage, or added to the soil as a drench. Soil was covered with aluminium foil to prevent contamination from the foliar sprays. The plants were harvested after 45 days.

In a second trial, the four amino acids were used at different concentrations to soak seeds for twelve or 24 hours. Control treatment seeds were soaked in distilled water for the same times. After treatment,

the seeds were planted in 10 cm pots containing the soil mixture previously described. Seven days after germination, all plants (1/pot) were inoculated with 1 000 newly hatched L_3 of *M. incognita*. After 45 days, the plants were harvested and root galls, nodules, and plant growth responses (shoot weights, heights, numbers of pods and numbers of leaves) were determined.

The results (Tab. 1) indicated that several amino acid foliar sprays significantly affected the *M. incognita* gall formation. Phenylalanine and methionine reduced nematode infection or reproduction, as measured by the reduction in the number of galls. Valine and cysteine were found to be less efficient in comparison to the other amino acids. Phenylalanine at 2.5 mg/plant increased three-fold the numbers of rhizobium nodules while valine at 10.0 mg/plant and methionine at 5.0 mg/plant reduced the numbers of nodules to less than that of the check treatment. All other treatments completely inhibited the development of nodules. Foliage weight of some treated plants were significantly different from untreated plants. All amino acids tested as soil drenches significantly decreased the number of galls (Tab. 1). Of all amino acids tested phenylalanine gave the best nematode control, and plant growth in terms of shoot length and foliage weight equaled controls or increased slightly. Valine decreased pod set but increased leaf number, at lower concentrations, while methionine decreased pod set at the higher concentrations, and increased leaf number at the low concentration.

Seed treatments at 2, 4 and 8 g/l with phenylalanine, methionine, valine and cysteine in 12 h soakings affected gall formation erratically, but all these tratments inhibited rhizobium nodule formation. In the 24 h seed treatments, cysteine reduced the numbers of galls significantly at all concentrations.

The potential of exogenous amino acids to reduce the number of *M. incognita* root galls formed has been demonstrated for tomato (Overman & Woltz, 1962 ; Krishna & Setty, 1974 ; Parvatha Reddy, Govindu & Setty, 1975*a*), sunflower (Osman & Viglierchio, 1981) and soybeans. Similar tests with other hosts would establish the generality of the effect which would in turn merit further studies on the mechanism of action. Speculation as to whether the mechanism is based upon stereoisomerism or other phenomenon appears to be premature and whether these observations can be developed into practical control measures remains to be seen.

The effect of amino acids on nodule formation is not understood. It would appear to be real since seeds, soil mix, nematode inoculum and nutrient all came from the same stock ; unfortunately a bacterial inoculum was not added to insure the presence of inducing organisms. The great differences in counts would support a suspicion that amino acids are able to influence nodule formation at the rates used.

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POTASSIUM PERMANGANATE AS A HATCHING AGENT FOR *HETERODERA SACCHARI*

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While attempting to distinguish between live and dead eggs of *Heterodera sacchari* using the potassium permanganate staining method of Jatala (1975), it was observed that 0.0625% potassium permanganate strongly stimulated the hatching rate of this species. This paper compares the hatching rate of crushed cysts of *H. sacchari* in water, in the presence of a rice seedling (host plant) and in 0.0625% potassium permanganate.

H. sacchari was reared on rice (*Oryza sativa* L.) cv. Moroberekan by inoculating 500 freshly hatched juveniles in 2.5 l plastic pots containing steam sterilized sandy soil, in which six rice seedlings were growing. Pots were then buried in sand at 28° in the green-

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