

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<sub>3</sub> 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 treatments 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, 1975a), 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.

## REFERENCES

- KRISHNA, P.K.S. & SETTY, K.G.H. (1974). The effect of amino acids as foliar spray on root-knot nematode (*Meloidogyne incognita*) and tomato. *Indian J. Nematol.*, 4 : 88-90.
- OSMAN, A.A. & VIGLIERCHIO, D.R. (1981). Foliar spray effects of selected amino acids on sunflower infected with *Meloidogyne incognita*. *J. Nematol.* (submitted for publication).
- OVERMAN, A.J. & WOLTZ, S.S. (1962). Effect of amino acid antimetabolites upon nematodes and tomatoes. *Proc. Fla. St. hort. Soc.*, 75 : 166-170.
- PARVATHA REDDY, P., GOVINDU, H.C. & SETTY, K.G.H. (1975a). Studies on the effect of amino acids on the root-knot nematode *Meloidogyne incognita* infecting tomato. *Indian J. Nematol.*, 5 : 36-41.
- PARVATHA REDDY, P., GOVINDU, H.C. & SETTY, K.G.H. (1975b). Studies on the action of DL-methionine on *Meloidogyne incognita* infecting tomato. *Indian J. Nematol.*, 5 : 42-48.
- PRASAD, S.K. & WEBSTER, J.M. (1967). The effect of amino acid anti-metabolites on four nematode species and their host plants. *Nematologica*, 13 : 318-320.

Accepté pour publication le 13 novembre 1980.

POTASSIUM PERMANGANATE AS A HATCHING AGENT FOR *HETERODERA SACCHARI*

Georges Reversat \*

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-

\* Laboratoire de Nématologie ORSTOM, B.P. 1386, Dakar, Sénégal.

Table 1

Effect of different treatments on hatching of eggs of crushed cysts of *Heterodera sacchari*

Treatment number	Hatching medium	Exposure time	Number of hatched juveniles per batch of 50 crushed cysts mean $\pm$ S.D. *	Hatch as % of cyst content **
1	water	8 days	1,700 $\pm$ 330	9
2	water with a rice seedling	8 days	1,620 $\pm$ 325	9
3	0.0625% potassium permanganate	1 day	16,680 $\pm$ 1,800	93

\* Mean of 4 replicates and standard deviation.

\*\* An estimate of cyst content, made by dissecting 10 medium size cysts, gave an average of 360 eggs per cyst.

house and fourteen weeks after inoculation, brown cysts were recovered from roots by water spraying and from moist soil with the Fenwick can. Cysts were then stored in 0.3 M NaCl which inhibited hatching but allowed completion of the development of embryonated eggs to second-stage juveniles (Dropkin, Martin & Johnson, 1958; Reversat, 1975).

Medium sized cysts were picked individually and distributed at random into twelve dishes (diameter 40 mm) containing 0.5 ml of de-ionized water. When each sample contained 50 cysts, cysts were crushed with forceps and samples were subdivided into three groups for the experiment. Hatching media were then added to each dish: 8 ml of de-ionized water for treatment 1, 8 ml of de-ionized water and a five-day-old rice seedling for treatment 2 and 8 ml of 0.0625% potassium permanganate for treatment 3. Dishes for treatments 1 and 2 were maintained at 28° with a 12 hr / 12 hr artificial light for eight days and dishes for treatment 3 were kept at 28° in the dark for one day. At the end of the exposure time, the content of each dish was diluted and the number of hatched juveniles was counted in each of two samples of 5 ml.

Results (Tab. 1) showed that, under these conditions, hatching of *H. sacchari* was not stimulated by root diffusate of rice, a common natural host of this species. Working with cysts of *H. oryzae*, a related species, Merny (1972) reported that the stimulating effect of the rice root diffusate was appreciable only several weeks after application. Hanoomanjee (1977), cited by Clarke and Perry (1977), observed a stimulating effect of sugar cane root diffusate on hatching of *H. sacchari*.

Numbers of hatched juveniles of *H. sacchari* were ten times as high in 0.0625% potassium permanganate after one day than in water after eight days (Tab. 1). This effect of potassium permanganate was reported by Nolte (1955) and Doliwa (1956) in *Globodera rostochiensis*, but, in further works, this result was confirmed neither with *H. schachtii* (Clarke & Shepherd,

1964) nor with *G. rostochiensis* (Clarke & Shepherd, 1968). Thus, in recent reviews (Shepherd & Clarke, 1971; Clarke & Perry, 1977), the only method suggested for hatching refractory species was chemical dissection by hypochlorite. The use of 0.0625% potassium permanganate with crushed cysts of *H. sacchari* permits rapid recovery of large numbers of freshly hatched juveniles for experimental purposes. Since the potassium permanganate solution was toxic for nematodes after several days of exposure (Jatala, 1975), juveniles must be rinsed by centrifugation after one day of exposure.

Considering the speed of hatching in potassium permanganate and the persistence of brown coloration of the empty egg-shell after treatment, it is suggested that juveniles are released passively by weakening of the egg-shell; oxidation caused by the permanganate may alter substances in the egg membrane (Doliwa, 1956), possibly in the lipid layer, which is important as a permeability barrier (Clarke & Perry, 1977).

## REFERENCES

- CLARKE, A.J. & SHEPHERD, A.M. (1964). Synthetic hatching agents for *Heterodera schachtii* Schm. and their mode of action. *Nematologica*, 10 : 431-453.
- CLARKE, A.J. & SHEPHERD, A.M. (1968). Hatching agents for the potato cyst-nematode *Heterodera rostochiensis* Woll. *Ann. appl. Biol.*, 61 : 139-149.
- CLARKE, A.J. & PERRY, R.N. (1977). Hatching of cyst-nematodes. *Nematologica*, 23 : 350-368.
- DOLIWA, U. (1956). Experimentelle Untersuchungen an Kartoffelnematoden (*Heterodera rostochiensis* Wollenweber). *Wiss. Z. Univ. Rostock*, 5 : 133-149.
- DROPKIN, V.H., MARTIN, G.C. & JOHNSON, R.W. (1958). Effect of osmotic concentration on hatching of some plant parasitic nematodes. *Nematologica*, 3 : 115-126.

- HANOOMANJEE, S. (1977). *The effect of temperature and hatching agents on the emergence of Heterodera sacchari larvae*, M. Sc. Thesis, Univ. of London, Imperial College.
- JATALA, P. (1975). Efficiency of potassium permanganate in differentiating between live and dead nematodes. *Ann. appl. Biol.*, 80 : 109-113.
- MERNY, G. (1972). Les nématodes phytoparasites des rizières inondées de Côte d'Ivoire III — Etudes sur la dynamique des populations de deux endoparasites : *Hirschmanniella spinicaudata* et *Heterodera oryzae*. *Cah. ORSTOM, Sér. Biol.* n° 16 : 31-87.
- NOLTE, H.W. (1955). Reizphysiologische Untersuchungen bei *Heterodera rostochiensis*. *Mitt. biol. Bund.Anst. Ld-u. Forstw.* n° 83 : 133-136.
- REVERSAT, G. (1975). Influence d'une inhibition préalable par la pression osmotique sur l'éclosion des masses d'œufs du nématode *Heterodera oryzae*. *Cah. ORSTOM, Sér. Biol.*, 10 : 189-206.
- SHEPHERD, A.M. & CLARKE, A.J. (1971). Molting and hatching stimuli. In : Zuckerman, B.M., Mai, W.F. & Rohde, R.A. (Eds) *Plant parasitic nematodes*, New York, Academic Press, Vol. 2 : 266-287.

*Accepté pour publication le 13 novembre 1980.*