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 1000 newly hatched L2 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 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.

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Table 1

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

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Hatching medium</th>
<th>Exposure time</th>
<th>Number of hatched juveniles per batch of 50 crushed cysts mean ± S.D. *</th>
<th>Hatch as % of cyst content **</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>water</td>
<td>8 days</td>
<td>1,700 ± 330</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>water with a rice seedling</td>
<td>8 days</td>
<td>1,620 ± 325</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>0.0625% potassium permanganate</td>
<td>1 day</td>
<td>16,680 ± 1,800</td>
<td>93</td>
</tr>
</tbody>
</table>

* 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°C with a 12 hr / 12 hr artificial light for eight days and dishes for treatment 3 were kept at 28°C 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, 1958). 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


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