Temporary protection of egg-plant from *Meloidogyne incognita* by minute quantities of isazophos and aldicarb applied at seedling stage

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SUMMARY

Egg plants growing in sterilized soil were treated with ultra low quantities of aldicarb or isazophos three weeks after sowing. Two weeks after treatment plants were transferred to sterile soil and inoculated with juveniles of *Meloidogyne incognita*. Both nematicides were tested at dosages of 0, 0.9, 3.5, 14 and 56 mg per plant. While strongly reducing nematode infection, the dosages of 14 mg proved to be non-toxic to the plants. At this dosage both nematicides reduced nematode development. At 14 mg per plant, there was a highly significant reduction in juvenile penetration and the number of eggs in egg-masses was also significantly lower. For a period of seven weeks, egg-masses were counted at weekly intervals as a measure for the development of nematode populations. Significant differences were observed between each treatment and controls, this demonstrates a positive effect of nematicides over that period. The possibility to introduce low dosage treatment of nurseries as an economic method to control *Meloidogyne* during the early development of transplants is suggested.

RÉSUMÉ

Protection temporaire de l'aubergine contre Meloidogyne incognita
par application de micro-doses d'isazophos et d'aldicarbe aux plantules

Des aubergines poussant dans du sol stérilisé ont été traitées avec des micro-doses d'aldicarbe et d'isazophos trois semaines après le semis. Deux semaines après le traitement, les plants ont été transférés dans du sol également stérilisé et inoculés avec des juvéniles de *Meloidogyne incognita*. Les deux nématicides ont été utilisés aux doses de 0, 0.9, 3.5, 14 et 56 mg (m.a.) par plant. Pour les deux produits la dose de 14 mg n'est pas phytotoxique et réduit de façon hautement significative la pénétration des juvéniles ainsi que la production de masses d'œufs. Pendant sept semaines les masses d'œufs ont été comptées hebdomadairement pour mesurer la croissance de la population du nématode. Des différences significatives ont été observées entre chaque traitement et avec le témoin, prouvant une action positive des nématicides pendant cette période. La possibilité d'introduire en pépinière les traitements nématicides à très faible dose pour lutter économiquement contre les *Meloidogyne* pendant le début de végétation des plantules est envisagée.

Frequently, high populations of root-knot nematodes (*Meloidogyne* spp.) develop when susceptible crops are grown on apparently uninfested land. This phenomenon may be explained in several ways; non-hatched eggs considered to be in diapause (de Guiran, 1979, 1980) may constitute an inoculum which cannot be evaluated as appropriate techniques for measuring this source of inoculum have not yet been developed. Furthermore, nematode density may be so low that juveniles are not included in the soil samples analyzed. They may, however, represent a rather large inoculum as many plants attract juveniles of *Meloidogyne* to their roots from distances of over 40 cm (Prot & Netscher, 1978). However, explosive developments of *Meloidogyne* populations are mainly caused by the high rate of reproduction of the nematodes, due to their short life cycle, their high fecundity and their parthenogenetic mode of reproduction which enables every juvenile inside a root to develop into an egg laying female.

Short season crops like vegetables only sustain a limited number of generations of *Meloidogyne*. Protecting the plants from infection during early development reduces the total number of generations. Thus the point of massive invasion is delayed to a later stage when the plant is more vigorous, resulting in better yields.

Nonvolatile nematicides protect crops from early nematode infection. Hough and Thomason (1975) reported that hatching of eggs of *Heterodera schachtii* and *Meloidogyne javanica* is inhibited by sublethal concentrations of aldicarb. Root-knot symptoms of tomatoes grown on soil infested with *M. javanica* were reduced by a 10 ppm soil application of carbofuran (di Sanzo, 1973).

Unforfunately, the remanence of these extremely toxic systemic nematicides in plants prevents them from being used in many crops. However, the practice of transplanting from nurseries to the field opens the possibility of treating various vegetables at seedling stage

with nonvolatile nematicides. Thus, minute quantities of nematicides could be applied to transplants wich, while protecting them from nematode attack during early stages, will be either metabolized or diluted in later stages of plant development, thus reducing nematicide residues to acceptable levels.

To verify whether transplants can be protected with low concentrations of nonvolatile nematicides, the behaviour of *M. incognita* was studied on egg-plant seedlings grown in soil treated with isazophos or aldicarb and subsequently transplanted into soil infested with *M. incognita.*

Materials and methods

All experiments were carried out with eggplant *(Solanum melongena)* cv. Violette longue. Seedlings were grown in 700 cm^3 pots filled with sterile soil placed in a greenhouse. Mean air temperature and humidity were respectively 28° and 80 %. Three weeks after sowing isazophos and aldicarb were dissolved into water, each plant receiving 20 cm^3 of nematicide solution. Two weeks after treatment, plants were uprooted, washed free of soil, transplanted to 700 cm^3 pots filled with sterile soil and inoculated with juveniles of *M. incognita.* In all experiments each treatment was replicated ten times.

Experiment 1

Isazophos or aldicarb were applied at 0, 0.9, 3.5, 14 and 56 mg per pot. Four weeks after inoculation with 500 juveniles, plants were uprooted and their fresh weight determined. Egg-masses were removed with a powerful jet of water, collected on a 200 µm aperture sieve and counted.

Experiment 2

Two series of 20 plants each were treated with 14 mg of isazophos or aldicarb respectively. One week after inoculation with 500 juveniles, ten plants of each series were uprooted, roots washed free of soil and stained with cotton blue (de Guiran, 1966). Nematode counts of roots crushed between two glass plates were made using a dissecting microscope. Four weeks after inoculation the remaining plants were uprooted and egg-masses collected and counted, using the above mentioned method.

For hatching experiments egg-masses were placed in distilled water in the dark at 28°. As de Guiran (1979) had observed that hatching of egg-masses of *M. incognita* could last for a period of 80 days, the same hatching time was used in this experiment. After 80 days, egg-masses were dissociated in a 0.53 % solution of NaOCl (Hussey & Barker, 1973). After rinsing with water, eggs were allowed to hatch for another 20 days. Subsequently, they were stained with New Blue R (Shepherd, 1962) to distinguish dead eggs from living non-hatched ones.

Juveniles hatched from egg-masses which had been collected from plants treated with aldicarb, isazophos or control plants respectively, were inoculated to untreated egg-plant seedlings at the rate of 500 juveniles per plant. Four weeks after inoculation, plants were uprooted and egg-masses collected and counted.

Experiment 3

Starting four weeks after inoculation with 200 juveniles, three series of ten plants which were treated with 14 mg of isazophos, aldicarb or 20 cm^3 of water respectively, were uprooted at weekly intervals for seven weeks and the number of egg-masses per plant determined.

Results

Experiment 1

With increasing dosages of nematicides the number of egg-masses collected decreased to 0 at 56 mg per plant (Fig. 1 A). Although aldicarb did not suppress nematode development at 0.9 mg, higher dosages inhibited nematode development better than corresponding dosages of isazophos. Plant weight decreased when the nematicides were applied at 0.9 mg per plant, but with increasing dosages of both nematicides, it increased to reach a maximum at 14 mg (Fig. 1 B).

Experiment 2

Penetration of juveniles was significantly lower in treated plants. When nematodes developed, the number of egg-masses collected corresponded very closely to the number of juveniles which had penetrated : 25/26 (aldicarb); 45/46 (isazophos) and 331/332 (control), these figures being highly significant ($p = 0.001$).

Differences between the numbers of juveniles that hatched from egg-masses on treated plants and controls were significant ($P < 0.001$ according to the Mann Whitney U test). Dissociation of egg-masses did not have a pronounced effect on hatching of non-embryonated eggs considered to be in diapause (Fig. 2).

Hatching in all experiments was synchronous. Almost all eggs hatched within a period of 20 days. The proportions of hatched (h), living non-hatched (n) and dead (d) eggs at the end of the experiment were approximately equal for all treatments :

aldicarb : h = 84.4; n = 1.5; d = 14.1;
isazophos : h = 79.3; n = 0.8; d = 19.9;
control : h = 85.3; n = 0.9; d = 13.8.

No difference in egg-masses production was observed after inoculation with juveniles recovered from egg-masses extrated from treated or control plants : 309 (aldicarb), 318 (isazophos) and 314 (control).

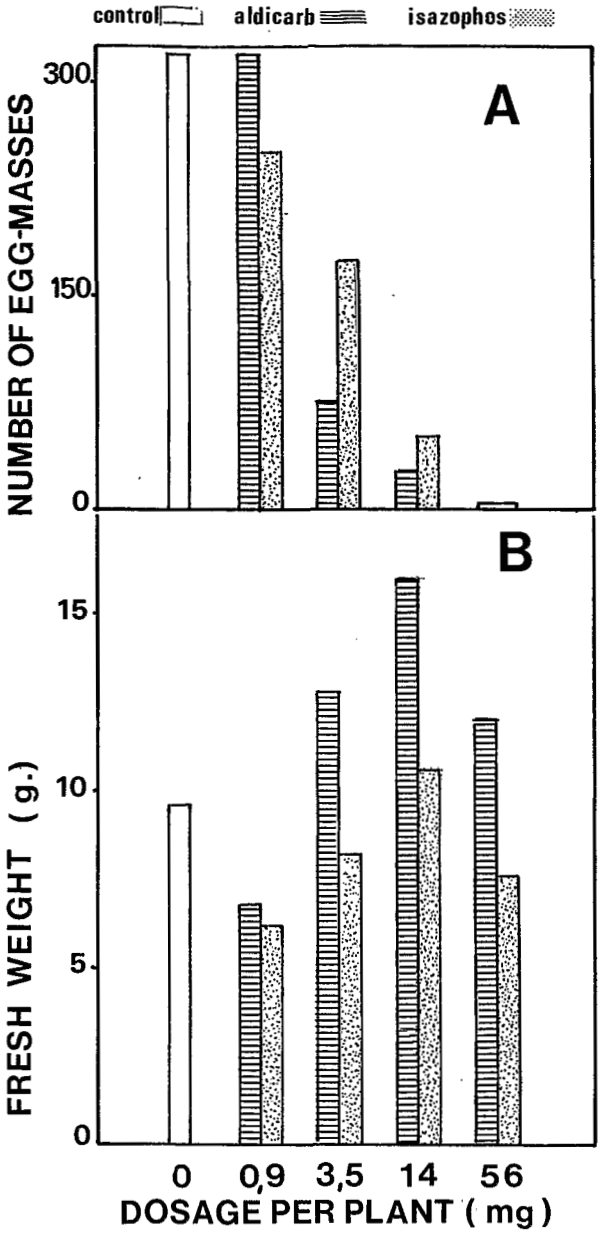

Fig. 1. Number of egg-masses on egg-plant treated with 0, 0.9, 3.5, 14 and 56 mg of aldicarb or isazophos (A) and fresh weight of these plants (B).

EXPERIMENT 3

Independently of the different treatments, the number of egg-masses collected per plant remained constant between three and six weeks after inoculation. Subsequently a significant increase was observed. A marked difference existed between the number of egg-masses collected on treated or control plants.

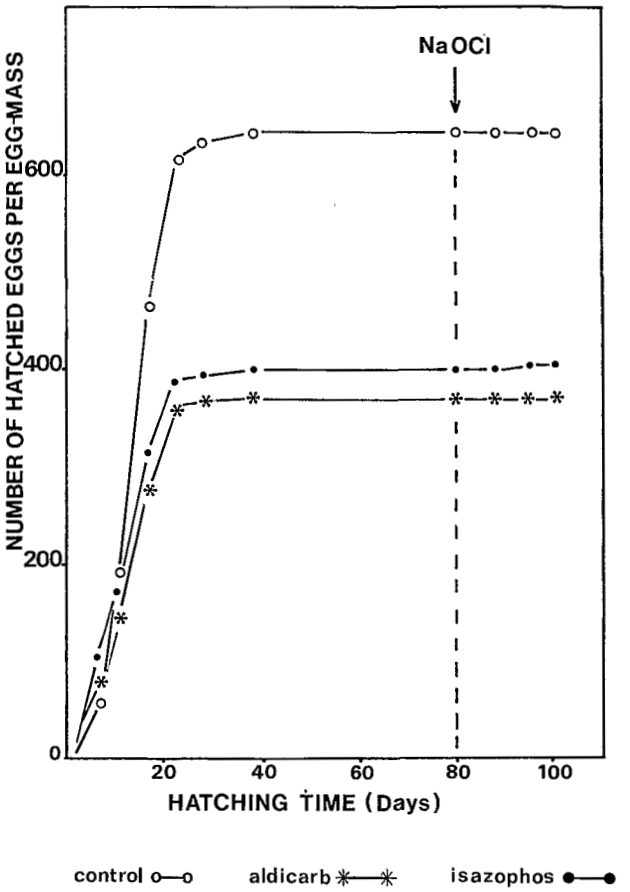

Fig. 2. Total number of hatched eggs per egg-mass incubated for 100 days.

Discussion

The results of the experiments reported here clearly demonstrate the systemic properties of isazophos and aldicarb. Exposure of egg-plant to soil treated with these nematicides resulted in low penetration and reduced egg production of the females developed in the roots.

The nematicides only seem to have had a quantitative effect on egg production and did not induce physiological changes. Hatching of egg-masses collected from treated plants and controls was completed in the same time (Fig. 2) and the proportions of hatched, non-hatched and dead eggs was equivalent. In fact, juveniles which succeeded in penetrating treated plants all developed into egg-laying females whose off-spring, as judged by their infectivity, were as viable as those of females developed on untreated plants (see above).

The number of egg-masses collected between four and six weeks after inoculation remained constant indicating that most juveniles penetrated shortly after inoculation (Fig. 3). The effect of the treatment was felt up to seven weeks after inoculation for both nematicides greatly reduced the level of secundary infection of treated plants (Fig. 3).

In a preliminary field experiment egg-plants were treated with either 14 mg of aldicarb or isazophos and transplanted to soil moderately infested by *M. incognita*. A phytotoxic effect was observed appearing as a slight inward rolling of the leaves. The seedlings were grown in plastic bags containing approximately half the quantity of soil used in the experiments reported here, therefore higher nematicide concentrations in the soil solutions may have been responsible for the phytotoxicity. In spite of this phytotoxicity, yields were definitly higher than controls, although no differences in the rate of galling could be observed. Probably both hatching and penetration of juveniles were reduced by the action of the nematicides, resulting in the protection of the plants during the early stages of development. Residue levels in fruits harvested from plants treated with aldicarb were well within the accepted tolerance level (0.01 ppm of aldicarb and its metabolites or less).

From a practical point of view, a dosage of 14 mg a.i. per plant corresponds to 200 gr a.i. per ha, making treatment at the seedling stage economically attractive. It is unlikely that farmers would be tempted to use higher dosages, because of the more pronounced phytotoxicity. This should safeguard the consumer from overdosage. Studies are under way to determine whether low dosage treatment of nurseries is feasible under farming conditions.

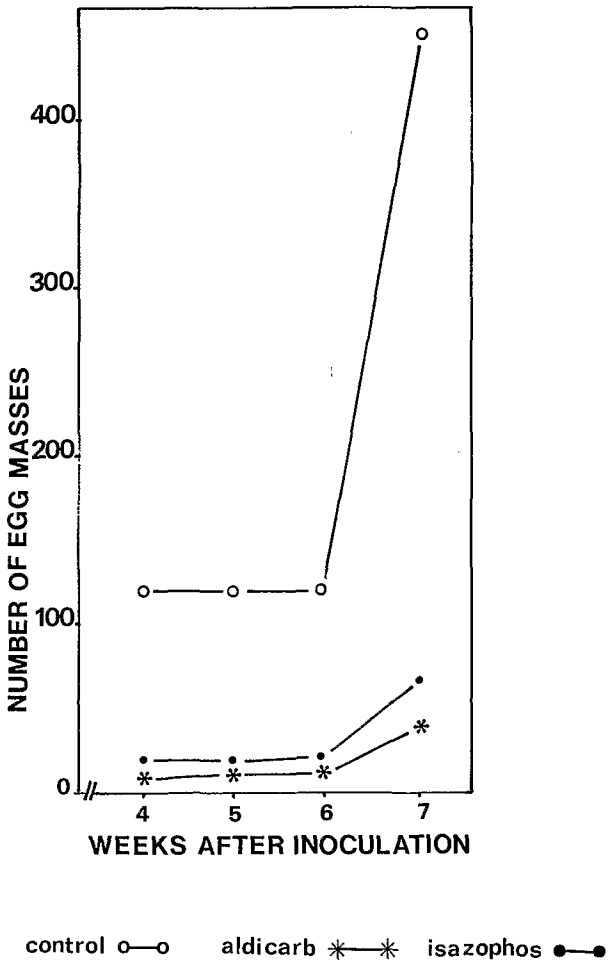

Fig. 3. Number of egg-masses recovered from egg-plant at weekly intervals, starting four weeks after inoculation.

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