

Efficacy of Abamectin B1 for the control of *Meloidogyne arenaria*

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Summary – The avermectins (AVM) are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis*. They have potent, broad spectrum anthelmintic, insecticidal and miticidal activities. The present investigation was carried out to explore the nematocidal properties of one AVM, Abamectin B1, commercialized under the name Vertimec® against the root-knot nematode *Meloidogyne arenaria* on tomato. Preliminary tests showed that 1 mg/l AVM B1 solution inhibited the hatching of *M. arenaria* after 12 days incubation and that juveniles were paralysed after 24 h exposure when dipped in a low concentration (0.3 mg/l). Experiments on tomato seedlings, in greenhouse, to control nematode penetration in tomato roots by application of AVM B1 on foliage were not effective. In contrast, direct application of AVM B1 to the soil induced a significant reduction in nematode penetration. AVM B1 showed therefore limited downward movement when sprayed onto leaves (only 20 % of activity) and acted mainly by the soil (80 % of activity). However, AVM B1 may be only used as an effective nematocide in sandy or low organic soils because of its rapid decomposition in high organic soils. Some result given in the literature make analogues (AVM B2a-23-ketone and less lipophilic derivatives) promising nematocidal candidates in more soils.

Résumé – Efficacité de l'Abamectine B1 contre *Meloidogyne arenaria*. – Les avermectines (AVM) sont des lactones macrocycliques produites par l'actinomycète *Streptomyces avermitilis*. Elles possèdent des activités antihelminthiques (large spectre), insecticides et acaricides. Le présent travail a pour but l'étude des propriétés nématocides d'une AVM, l'Abamectine B1, commercialisée sous le nom de Vertimec®, contre le nématode à galles *Meloidogyne arenaria* sur tomates. Des tests préliminaires indiquent qu'une solution d'AVM B1 à 1 mg/l inhibe l'éclosion des œufs de *M. arenaria* après 12 jours d'incubation et que les juvéniles sont paralysés après 24 h d'immersion dans de faibles concentrations (de l'ordre de 0,3 mg/l). Des essais réalisés en serre sur plants de tomates en vue de réduire les pénétrations des juvéniles dans les racines montrent l'inefficacité des traitements par pulvérisation foliaire de l'AVM B1. Par contre, l'application directe de l'AVM B1 dans le sol entraîne une réduction significative des pénétrations. Ainsi l'AVM B1 agirait plutôt par traitement du sol (80 % d'efficacité) que par pulvérisation sur les feuilles (seulement 20 % d'efficacité). Cependant, du fait de sa rapide décomposition dans les sols très organiques, l'AVM B1 ne pourra être efficace que dans des sols sableux ou à faible teneur en matière organique. Des données bibliographiques feraient d'analogues, tels que l'AVM B2a-23-cétone ou de dérivés moins lipophiles, des nématocides plus prometteurs et efficaces dans plusieurs types de sols.

Key-words : Avermectins, *Streptomyces avermitilis*, *Meloidogyne arenaria*, *Lycopersicon esculentum*, chemical control.

The avermectins (AVM), first reported by Burg *et al.* (1979) and Miller *et al.* (1979) are a family of aleandrose disaccharide derivatives of pentacyclic lactones, isolated from fermentation broths of *Streptomyces avermitilis*, and developed by the scientists from Merck Sharp Dohme Research Laboratories.

Fermentation of the actinomycete *Streptomyces avermitilis* produces four homologous pairs of closely related compounds : A1 (a & b), A2 (a & b), B1 (a & b) and B2 (a & b) (Figure 1). Because the separation of the components is very tedious and impractical on a large scale, and because the biological activities of the two homologues are practically identical, the AVM components are often used as a mixture of these two homologues. In such cases they are just defined as A1, A2, B1 or B2, which means a mixture of not less than 80 % of components « a », and not more than 20 % of components « b ».

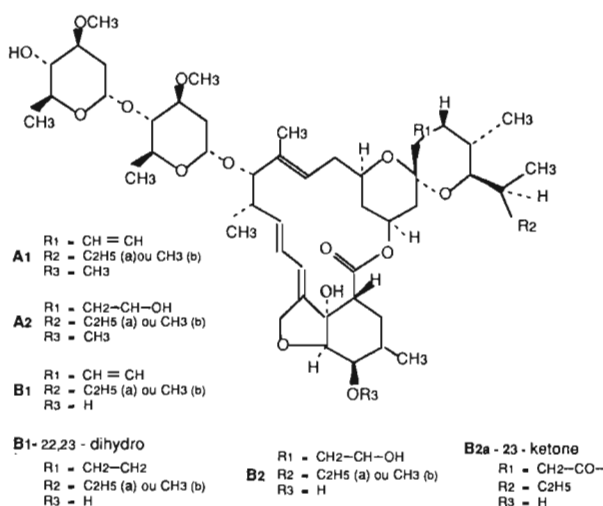


Fig. 1. Chemical structures of Avermectins.

AVM B1 is the most important because it has high potency against a broad spectrum of endo- and ectoparasites of farm animals and many agricultural mite and insect pests. It also serves as starting material for the semi synthetic 22, 23-dihydro analogue, which is used almost exclusively under the generic name Ivermectin for both prevention and cure of parasitic infections of animals (Campbell, 1981), especially for the control of Onchocerciasis (river blindness).

Abamectin (AVM B1) was introduced as an agricultural pesticide on a number of horticultural and agronomic crops in 1985 and its applications are continuing to expand; it is commercialized by MSD Agvet France under the names Vertimec®, Avid®, Avomec®, Affirm®, Agrimek® depending on the formulations.

The present investigation was carried out to explore the nematicidal properties of Vertimec® (AVM B1) against the root-knot nematode *Meloidogyne arenaria* on tomato.

In a preliminary test, different concentrations of AVM B1 were studied *in vitro* for their direct nematicidal effect on dipped *M. arenaria* eggs and juveniles. Furthermore, applications on tomatoes were studied in greenhouse (roots, foliage and soil treatments).

Materials and methods

MATERIALS

Eggs and juveniles of *Meloidogyne arenaria* used in these experiments were derived from a clone established from a single eggmass maintained on tomato plants (*Lycopersicon esculentum* cv. Saint Pierre) in the greenhouse (25 °C). Eggs at the two-blastomere-stage were selected and juveniles were extracted from galled roots using a mist chamber (Seinhorst, 1950) and only individuals collected within 24 h period were used.

Vertimec (AVM B1) is usually used in foliar spray applications and is formulated as a 1.8 % w/v emulsifiable concentrate (commercial formulation). A stock solution of 100 mg ai/l of Vertimec was prepared in distilled water and further diluted to requested experimental dilutions.

Five-week-old seedlings of tomato (*Lycopersicon esculentum* cv. Saint Pierre) raised in autoclaved sandy soil were used in all experiments. The seedlings were transplanted singly in 50 ml plastic tubes containing autoclaved sandy soil. The tubes were randomized on the greenhouse bench in metallic baskets.

METHODS

In vitro tests with AVM B1 on dipped M. arenaria eggs

The stock solution of 100 mg/l of AVM B1 was 10 and 100 times diluted in distilled water.

Ten two-blastomere-eggs were incubated in 5 ml of each solution in 5 ml Petri dishes. Each treatment was replicated four times. Eggs in distilled water served as controls. Fresh solutions (water and AVM B1) were

substituted each 48 h to avoid contamination. After 12 days, the number of juveniles were counted.

To estimate the hatching reactivation, the eggs whose development was stopped were collected and dipped in distilled water for 8 days. Then the number of juveniles were counted.

In vitro tests with AVM B1 on M. arenaria juveniles

The stock solution of AVM B1 was diluted in distilled water to give the following concentrations (in mg/l) : 100 - 90 - 70 - 60 - 50 - 40 - 30 - 20 - 10 - 1 - 0.9 - 0.8 - 0.7 - 0.6 - 0.5 - 0.4 - 0.3 - 0.2 - 0.1. The tests were done in 24 well Nunclon plates; each well received 1 ml of the test concentration to which 100 *M. arenaria* juveniles were added in 20 µl of water. Each treatment was replicated four times. Toxicity was estimated according to the mean percentage of paralysed nematodes after 2, 4, and 24 h of exposure. Juveniles in distilled water served as controls.

The reversible effect of the treatments was estimated according to the following method : the larvae whose mobility was inhibited after various immersion periods in the different concentrations were collected and dipped in distilled water. The proportion of truly dead nematodes was estimated after 24 h in water. Nematodes were considered truly dead if they did not move when probed with a fine needle.

Root-dip treatment with AVM B1

Preliminary tests showed that root-dip treatment of tomato seedlings with high AVM B1 concentrations induced a very damaging phytotoxic effect. The following low concentrations, tolerated by tomato seedlings, were consequently selected : 1 - 0.4 - 0.3 - 0.2 - 0.1 mg/l. Tomato seedlings were carefully uprooted and their roots washed with distilled water. Roots of the test seedlings were separately dipped for 2, 6 and 24 h in the different AVM B1 concentrations. Tomato seedlings dipped in distilled water served as controls. Each treatment was replicated five times. Then seedlings were transplanted singly in plastic tubes. Each seedling was inoculated with 200 *M. arenaria* juveniles in 1 ml water droplet. The plants were uprooted 8 days after inoculation and their roots were washed free of soil and stained with cotton blue lactophenol (de Guiran, 1967). Juveniles in the roots were counted with the aid of a dissecting microscope.

Application of AVM B1 on foliage

The objective of these experiments was to observe the nematicidal properties of AVM B1 against *M. arenaria* when applied on tomato foliage. AVM B1 was then tested according to two methods : as aerial spraying and as foliage-dip treatment on upside-down plants.

Foliage spraying : Tomato seedlings were transplanted singly in plastic tubes and each seedling was inoculated with 200 *M. arenaria* juveniles in a 1 ml water droplet. AVM B1 was sprayed on the foliage with a household

sprayer. Each seedling was treated singly with 40 µl of solution.

The influence of three parameters was considered : spraying timing, AVM B1 concentrations and nematodes inoculation timing.

Spraying timing : Tomato seedlings were inoculated 8 days after transplanting. An aqueous solution of AVM B1 (100 mg/l) was sprayed on the foliage either before or after the inoculation of nematodes : two days before inoculation (I - 2d.), one day before inoculation (I - 1d.), one day after inoculation (I + 1d.), two days after inoculation (I + 2d.). Each treatment was replicated five times.

Juveniles in the roots were counted 8 days after inoculation by staining roots according to the above-mentioned method.

AVM B1 concentrations : Tomato seedlings were inoculated 8 days after transplanting. The following AVM B1 concentrations (in mg/l) were sprayed on the foliage two days before nematode inoculation : 10 - 20 - 30 - 40 - 50 - 60 - 70 - 80 - 90 - 100. Tomato seedlings sprayed with distilled water served as controls. Each treatment was replicated five times.

Juveniles in the roots were counted 8 days after inoculation by staining roots according to the previously described method.

Nematodes inoculation timing : An aqueous solution of AVM B1 (100 mg/l) was sprayed on tomato seedlings 8 days after transplanting. Tomato seedlings sprayed with distilled water at the same time served as controls. Each seedling was inoculated with 200 *M. arenaria* juveniles at the following delayed periods after the spraying (S) : S + 2d., S + 4d., S + 9d., S + 11d. Each treatment was replicated five times.

Juveniles in the roots were counted as previously indicated.

Foliage-dip treatment of upside-down tomato seedlings : Eight days after transplanting, tomato seedlings were turned upside down and the foliage was dipped for 5 s in an aqueous solution of AVM B1 (100 mg/l). Tomato seedlings dipped in distilled water in a similar manner served as controls.

Immediately after the treatment, seedlings were kept for 1 h in a horizontal position to ensure complete evaporation of the solution and avoid contamination of the soil. Then the culture tubes were randomized in standard upright position on the greenhouse bench and each seedling was inoculated with 200 *M. arenaria* juveniles at the following periods of time after dipping (D) : D + 2d., D + 4d., D + 7d., D + 9d., D + 11d. Each treatment was replicated five times.

Juveniles in the roots were counted according to the standard described method.

Application of AVM B1 into the soil

Tomato seedlings were transplanted singly in plastic tubes filled with 50 g autoclaved low organic sandy soil (1.6 % o.m.). Eight days after transplanting, the soil was drenched with different quantities of an aqueous solution of AVM B1 (100 mg/l).

The basic smallest quantity (85 µl per tube) was correlated with the mean trickling volume usually recovered in the soil after an aerial spraying. The other tested quantities were : 100, 200 and 300 µl per tube. Tubes drenched with water served as controls.

Each seedling was infested with 200 *M. arenaria* juveniles, 2 days after the treatment. Each treatment was replicated five times.

Juveniles in the roots were counted 8 days after inoculation by staining roots according to the above-mentioned method.

Results

EFFECT OF AVM B1 ON *M. ARENARIA* HATCHING

Table 1 gives the percentage of hatching in AVM B1 solutions compared with water control. 1 mg/l concentration was sufficient to stop the two-blastomere eggs development and there was no hatching reactivation.

Table 1. Effect of AVM B1 concentrations on *Meloidogyne arenaria* hatching.

| AVM B1 concentration (mg/l) | Percentage of hatching after 12 days in AVM B1 solutions |
|---|--|
| 100 | 0 a |
| 10 | 0 a |
| 1 | 20 b |
| Control (distilled water) | 90 c |
| <hr/> | |
| Hatching reactivation after 12 days in 1 mg/l AVM B1 then 8 days in distilled water | 0 a |

Means (n = 4) followed by the same letter in the columns are not significantly different at the 95 % confidence level according to the Student's test.

DIRECT EFFECT OF AVM B1 ON DIPPED *M. ARENARIA* JUVENILES

Even at low concentration (0.3 mg/l) larval paralysis reached 80 % after 4 h exposure (Table 2). On and after the 0.6 mg/l concentration, paralysis was always 100 % after 2 h exposure.

Table 2. Effect of AVM B1 concentrations on mortality of dipped *Meloidogyne arenaria* larvae.

| Exposure time (h) | Percentage of paralysis (relative to controls) in different AVM B1 concentrations (mg/l) | | | | | | | | | | |
|--|--|------|-------|-------|-------|-------|-------|-------|-------|-------|-----------|
| | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 | 10 to 100 |
| 2 | 0 a | 0 a | 0 a | 0 a | 0 a | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| 4 | 0 a | 0 a | 80 b | 95 b | 95 b | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| 24 | 10 b | 90 b | 98 c | 100 c | 100 c | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |
| Post exposure mortality (after 24h in distilled water) | 50 c | 95 b | 100 c | 100 c | 100 c | 100 a | 100 a | 100 a | 100 a | 100 a | 100 a |

Means (n = 4) followed by the same letter in the columns are not significantly different at the 95 % confidence level according to the Student's test.

pH and salinity values were regularly measured in order to be sure that they were never responsible for the observed paralysis.

The proportions of dead larvae after 24 h in distilled water following immersion in different AVM B1 concentrations are presented in Table 2. All larvae totally paralysed after 24 h exposure in AVM B1 (from 0.4 to 100 mg/l concentrations) were unable to recover the mobility in distilled water.

When paralysis was not complete after 24 h exposure (from 0.1 to 0.3 ppm concentrations), percentage mortality increased in a water bath showing that a poisonous dose was firstly assimilated during the treatment and there was no recovery in distilled water. The toxic properties of AVM B1 on immersed *M. arenaria* juveniles was clearly demonstrated.

EFFECT OF ROOT-DIP TREATMENT WITH AVM B1

A bare-root dip in AVM B1 significantly reduced the percentage of penetration in tomato roots by *M. arenaria* juveniles. The reduction in nematode penetration gradually increased with the increase in the concentration of AVM B1 and the duration of the dip treatment (Table 3). Results at 2 h exposure were not sufficient as percentage penetration was about the same as in controls whatever the tested concentration. A significant reduction in nematode penetration was observed at highest concentrations (0.4 and 1.0 mg/l) after 6 h. The reduction in percentage penetration exceeded 90 % with the highest AVM B1 concentration (1.0 mg/l) after 24 hours. The reduction in nematode penetration is probably correlated with an impregnation of roots with AVM B1.

Table 3. Effect of root-dip treatment of tomatoe seedlings with AVM B1 on the penetration of tomato roots by *Meloidogyne arenaria* larvae.

| AVM B1 concentrations (mg/l) | Percentage of penetration (relative to controls) in tomato roots after different exposure times of root-dip treatments (in hours) | | |
|------------------------------|---|----------|---------|
| | 2 h | 6 h | 24 h |
| 0.1 | 93.29 a | 87.95 a | 56.49 a |
| 0.2 | 90.16 ab | 74.76 b | 32.89 b |
| 0.3 | 89.27 b | 125.90 c | 35.60 b |
| 0.4 | 66.66 c | 26.11 d | 12.19 c |
| 1.0 | 89.61 b | 22.40 d | 8.30 c |

Means (n = 5) followed by the same letter in the columns are not significantly different at the 95 % confidence level according to the Student's test.

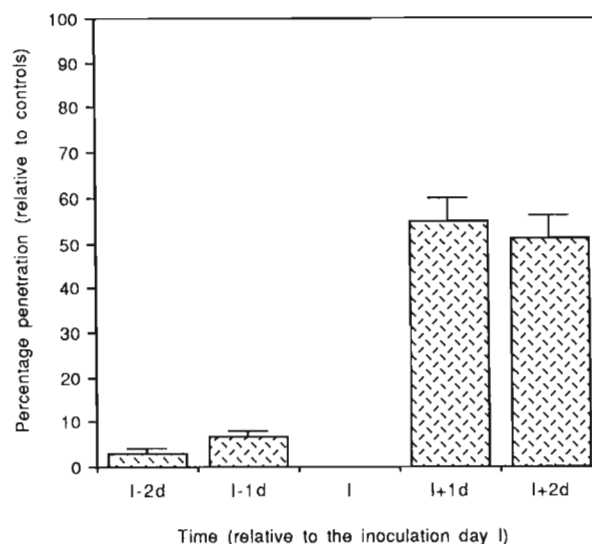


Fig. 2. Foliage spraying of tomato seedlings with AVM B1 (100 mg/l). Influence of spraying time on penetration of tomato roots by *Meloidogyne arenaria* larvae.

EFFECT OF AVM B1 APPLIED ON FOLIAGE

Effect of foliage spraying with AVM B1

Influence of spraying time : Figure 2 summarizes percentage of penetration recorded with the four different spraying periods in comparison with untreated controls (100 % penetration).

Inhibition of juvenile penetration increased when tomato seedlings were sprayed prior to inoculation. The best results were obtained with the earliest treatment (I - 2d.).

Post-inoculation treatments (I + 1d., I + 2d.) were unsatisfactory because most juveniles entered the roots immediately after inoculation.

Influence of AVM B1 concentrations : The reduction in nematode penetration gradually increased with the increase in the concentration of AVM B1, sprayed on the foliage 2 days before inoculation (Fig. 3).

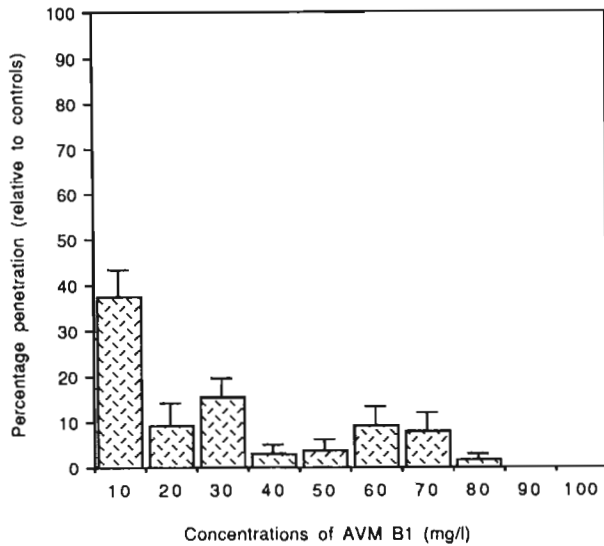


Fig. 3. Effect of foliage spraying of tomato seedlings with different concentrations of AVM B1 on the penetration of tomato roots by *Meloidogyne arenaria* larvae.

A full inhibition of juveniles penetration was obtained at highest concentrations (90 and 100 mg/l) and a significant reduction in the penetration rate was observed at the lowest (10 mg/l).

Influence of inoculation time : The data presented in Table 4 (left column) show that penetration of juveniles was always very weak. It increased little as the inoculation time was delayed, denoting a gradual reduction of the treatment efficacy.

Effect of foliage-dip treatment of upside-down tomato seedlings

The data presented in Table 4 (right column) show that reduction of juveniles penetration after foliage-dip treatment appeared unsatisfactory in comparison with foliage spraying treatment.

Table 4. Effect of delaying inoculation after the treatment with AVM B1 (100 mg/l) on the penetration of tomato roots by *M. arenaria* larvae.

| Delayed inoculation, days after treatment (T +) | Percentage of penetration (relative to controls) in tomato roots after different foliage treatments with AVM B1 (100 mg/l) | |
|---|--|-----------------------|
| | Foliage spraying | Foliage-dip treatment |
| T + 2 | 3.09 a | 78.14 a |
| T + 4 | 4.23 a | 60.09 b |
| T + 7 | 5.31 a | 74.12 a |
| T + 9 | 9.91 b | 106.54 c |
| T + 11 | 9.11 b | 68.75 d |

Means (n = 5) followed by the same letter in the columns are not significantly different at the 95 % confidence level according to the Student's test.

Table 5. Effect of soil treatment with AVM B1 on the penetration of tomato roots by *Meloidogyne arenaria* larvae and the growth of entered larvae.

| | Quantity of AVM B1 (100 mg/l) per culture tube | | | |
|---|--|-------------|-------------|-------------|
| | 85 µl | 100 µl | 200 µl | 300 µl |
| Percentage penetration in tomato roots (relative to controls) | <u>1.54</u> | <u>0.23</u> | <u>0.59</u> | <u>0.35</u> |
| Percentage of developed larvae (relative to controls) | <u>100</u> | <u>100</u> | <u>75</u> | 0 |

Means (n = 5) underlined by the same line are not significantly different at the 95 % confidence level according to the Student's test.

EFFECT OF AVM B1 APPLIED INTO THE SOIL

The data presented in Table 5 show that percentage of penetration of juveniles after treatment was almost suppressed.

Furthermore, results show that development of larvae that entered into the roots was affected by the treatment denoting an influence of the treatment on the nematode growth.

Discussion

There is some other evidence to suggest that AVM may be extremely active against plant-parasitic nematodes.

OVICIDAL EFFECT ON *MELOIDOGYNE* SP. (*IN VITRO* TESTS)

We have observed that the exposure of two-blastomere eggs of *Meloidogyne arenaria* for 12 days in 100 to 1 mg/l AVM B1 aqueous solutions inhibited egg hatching without reactivation in water.

Nordmeyer and Dickson (1980) have also observed that exposure of egg masses of *Meloidogyne javanica*, *M. arenaria* and *M. incognita* to 1 mg/l (1.2 μ M) and 1/4, 1/16, 1/64 of AVM B2 aqueous solution, at 28 °C for 5 days, completely suppressed egg hatching. The inhibitory effect on all three species was more noted with AVM B2 than with phenamiphos > ethoprophos > aldicarb at 10 mg/l, oxamyl at 100 mg/l and carbofuran > carbosulfan at 150 mg/l. After transferring the treated eggmasses to water, they noted from 20 to 60 % of eggs hatched.

The inhibitory effect was more marked with AVM B2a-23-ketone, a metabolite derivative, which suppressed egg hatching within 24 h in 0.1 mg/l aqueous solution; but rinsed in water 4 days later, hatching resumed at a greater rate than in the controls (Wright *et al.*, 1984). This may indicate that embryogenesis proceeded normally and that hatching was halted by the immobilization of the juveniles by the chemical.

NEMATICIDAL EFFECT ON *MELOIDOGYNE* SP. JUVENILES (*IN VITRO* TESTS)

Our experiments showed that *M. arenaria* juveniles were hardly affected by dip-treatment with different AVM B1 concentrations (Table 2). These results correlate with other works.

Wright *et al.* (1984) have shown that the response of juveniles of *Meloidogyne incognita* exposed to 0.1 mg/l aqueous solution of AVM B1 or B2a-23-ketone was triphasic: a rapid initial flaccid paralysis (within 10 min) while being responsive to touch, followed by a partial recovery within 30 min of exposure and then a more gradual decline in activity and irreversible loss of movement after 120 min (Birtle *et al.*, 1982; Wright *et al.*, 1984). In contrast, the acetylcholinesterase inhibitor, oxamyl, causes initial hyperactivity of juveniles followed by a progressive decline in movement.

It has been postulated by Fritz *et al.* (1979) that AVM have a nematicidal mode of action different from that of the organophosphates and carbamates. Those nematicides inhibit acetylcholine esterase (AChE), a neurotransmitter used by the excitatory motoneurons (ExMN); inhibitory motoneurons (InMN) do not use AChE for neurotransmission but γ -aminobutyric acid (GABA), found in both arthropods and nematodes (Gerschenfeld, 1973), and AVM should act at GABA-mediated synapses to open membrane chloride channels, either directly or by AVM-stimulated release of GABA from InMN. While this does not explain how

AVM may cause the triphasic response found in *M. incognita*, it does raise the possibility that this response could be due to the action of AVM on GABA-mediated InMN in different parts of the nematode nervous system.

Wright *et al.* (1984) have further investigated this mode of action with AVM B2a-23-ketone *in vivo* with infective juveniles (J2) of *M. incognita*. They have found that AVM B2a-23-ketone produced an initial hypoactive response in nematodes, as might be expected of a compound which is thought to act at inhibitory rather than excitatory synapses. Moreover, the GABA antagonists picrotoxin and bicuculline (Nistri & Constanti, 1979) antagonized the action of AVM on the locomotion of *M. incognita*; it was additional evidence that GABA-mediated transmission was the principal target for AVM in intact nematodes.

This work was confirmed in 1987 by Stretton *et al.* (1987) who have investigated the mode of action of AVM with two types of neuron as representative of ExMN and InMN; AVM had little or no effect on neuromuscular transmission when the ExMN was activated directly with electric current, but neuromuscular transmission from InMN was blocked by AVM. In addition to *M. incognita*, a triphasic locomotor response to AVM has been observed in the plant parasitic nematode *Heterodera schachtii* but was not found in the free-living species *Caenorhabditis elegans* (Wright *et al.*, 1984); it is not known whether these different responses are pharmacological or pharmacokinetic in nature.

It seems also that *Meloidogyne incognita* was less sensitive to AVM B1 than *M. arenaria* because we have only observed complete paralysis of *M. arenaria* juveniles at 0.6 mg/l concentration after 120 min exposure. Such differences in sensitivity were confirmed by Nordmeyer and Dickson (1985) with *M. javanica* which seems to be the least sensitive.

On agar plates, concentrations as low as 0.3 ng AVM B2a-23-ketone cm^{-3} significantly reduced the number of juveniles in cucumber roots and the proportion of juveniles which developed to a saccate stage (Wright *et al.*, 1984). The authors proposed in this context that AVM might affect the behavioural sequence preceding invasion, a mode of action also suggested for organophosphorus and carbamate nematicides (Wright, 1981). The nematode paralysis would probably prevent the nematode from migrating to its host plant, blocking chemoreceptor sites necessary for host finding.

According to Stretton *et al.* (1987), AVM B2 did not affect post-invasion development of *M. incognita* in tomato roots which were exposed to 1 mg/l solutions 48-72 h after invasion. We have observed in our own experiment that AVM B1 affects nematode physiology because after a soil treatment with the highest dose (300 μ l of aqueous solution 100 mg/l per 50 cm^3 of soil i.e. 0.6 mg/dm^3 of soil), the few *M. arenaria* which entered the roots were unable to develop and moult.

MICROPLOT, GREENHOUSE AND FIELD EXPERIMENTS

While AVM B1 and B2 have shown equally high activities in *in vitro* tests against *M. incognita*, on tobacco grown in microplots or in greenhouse experiments where granulated formulations of these entities were incorporated (at 0.2 to 1.5 kg ai/ha), AVM B2 was slightly more potent than B1 (Preiser *et al.*, 1981; Putter *et al.*, 1981) and was about 10-40 times more potent than several organophosphorus and carbamate nematicides (Putter *et al.*, 1981; Sasser *et al.*, 1982; Nordmeyer & Dickson, 1985). Putter *et al.* (1981) ascribed that AVM B2a was rapidly converted by soil microbes (half-life of 2-5 days) to B2a-23-ketone, having a soil half-life of about 30 days. The greater nematicidal potency of B2a seemed to be the result of its own nematicidal property combined with that of its metabolite, extended in greenhouse experiments for 2 months.

Other field studies investigated the efficacy of the AVM applied to soil as granules and liquid in furrows or by low pressure drip irrigation systems, at rates ranging from 0.09 to 0.34 kg ai/ha, as a single or three applications (each at 0.03 to 1.1 kg) on tomato plants infested with *M. incognita* (Garebedian & Van Gundy, 1983). AVM B1 and B2 were at least ten times more effective than aldicarb and oxamyl in reducing root-knot infections and galling in artificially irrigated situations; AVM protection of the roots remained constant throughout the first 5 weeks giving slightly longer protection than the other nematicides and with only 1/10 the volume of chemicals applied to the environment. However, when rates up to 0.6 kg ai/ha were applied by drip irrigation into thirteen equal weekly doses, AVM B1 did not significantly affect populations of *M. incognita* or yields of chrysanthemums (Overman & Price, 1984). The best field treatment occurred when AVM was applied at 0.04 kg ai/ha on each 3 consecutive days and then repeating the treatment three weeks later.

The water insolubility of the AVM (0.006 to 0.009 mg/l) did not appear to be a serious problem where the plants are frequently irrigated. Their rapid degradation in soil and poor leaching potential through many types of soil (Burg *et al.*, 1979; Bull, 1985) suggest that field applications would not result in persistent residues or contaminations of agricultural ground water.

AVM B1 and B2 showed limited downward movement when sprayed onto leaves : spraying of aerial parts of tomato plants with 1000 mg/l solutions resulted in only minor inhibition of root-galling by *M. incognita* (Stretton *et al.*, 1987).

All these results are confirmed in our own experiments with AVM B1. Comparison between foliage spraying and foliage-dip treatment of tomato seedlings (Table 4) shows that there is an important reduction of percentage of penetration with the spraying whereas the foliage-dip treatment is almost ineffective. The conclusion from such a marked difference is that foliage

spraying does not act through tomato leaves but by trickling of the sprayed solution onto the soil. That means that nematicidal properties of AVM B1 act mainly by way of the soil.

Such a hypothesis was clearly confirmed by direct application of AVM B1 in the soil which suppressed the juvenile penetration in tomato roots (Table 5).

Likewise, when AVM B1 was sprayed on the foliage at different periods, treatments preceding nematode inoculation were the best (Fig. 2) because they allow a good diffusion of the solution inside the soil.

In process of time, AVM B1 was diluted in the soil and lost its efficacy. Table 3 shows that the more nematode inoculation was delayed, the more percentage of penetration increased. After our experiments it appears that beside of the main soil effect of AVM B1, there is also a small effect through the plant tissues. Indeed, the reduction of juvenile penetration after foliage-dip treatment of upside down tomato seedlings was about 20%. Likewise, with the root-dip treatment, there was an impregnation of roots with AVM B1 which reduced nematode penetration.

In our experimental conditions, we come to the conclusion that AVM B1 acts according to two pathways : a main one (about 80 %) through the soil, and a secondary one (about 20 %) by impregnation of the plant tissues.

CONCLUSION

The novel mode of action of the AVM and their high nematicidal potency (only 40 mg AVM B1 in 40 ml water are necessary for the treatment of 1 m²), should make AVM promising nematicides. AVM B1 has major disadvantages : low solubility, failure of foliar applied treatments, rapid decomposition in high organic soils. Therefore AVM B1 should not be developed for use against plant parasitic nematodes in all soils unless further research on formulations leads to a better protection in high-organic soils. Further tests in sandy soils may be continued. Some more hydrophilic analogues or derivatives like B2a-23-ketone, which produces an active metabolite, should be more promising candidates for effective nematicides.

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