

Efficacy of abamectin seed treatment on *Pratylenchus zae*, *Meloidogyne incognita* and *Heterodera schachtii*

Wirkung einer Saatgutbehandlung mit Abamectin auf *Pratylenchus zae*, *Meloidogyne incognita* und *Heterodera schachtii*

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Abstract

The aim of this study was to test the effect of various concentrations of abamectin on the reduction of early root penetration of three nematode species and on plant growth, when applied as a seed treatment on maize, cotton and sugar beet. The study revealed that penetration of *Pratylenchus zae* was reduced more than 80% in maize at a dose of 1.0 mg a.i. seed⁻¹. The number of galls caused by *Meloidogyne incognita* race 3 in cotton was also reduced more than 80% with 0.1 mg a.i. seed⁻¹. Penetration of *Heterodera schachtii* in sugar beets was reduced over 60% when seeds were treated at a concentration of 0.3 mg a.i. seed⁻¹. Root length, as well as shoot and root weights in all plants treated with abamectin were not significantly different from controls. Our investigation determined that using abamectin as a seed treatment is an effective way of reducing early root infestation of the three different nematodes at low concentrations in a variety of crops and does not negatively affect plant growth.

Key words: avermectins, chemical control, cyst nematode, effectiveness, lesion nematode, root-knot nematode

Zusammenfassung

Ziel der vorliegenden Arbeit war es, den Einfluss verschiedener zur Saatgutbehandlung eingesetzter Abamectin-Konzentrationen auf Befall von Mais, Baumwolle und Zuckerrübe mit pflanzenparasitären Nematodenarten zu untersuchen sowie mögliche Nebenwirkung auf das Pflanzenwachstum zu ermitteln. Die Untersuchungen zeigten, dass bei einer Saatgutbehandlung mit Abamectin mit einer Konzentration von 1.0 mg a.i. seed⁻¹ die Eindringung von *Pratylenchus zae* in Mais über 80% reduziert wurde. Im Falle von Baumwollsaatgut, welches mit einer Abamectin-Konzentration von 0.1 mg a.i. seed⁻¹ behandelt wurde, betrug die Wirksamkeit gegenüber *Meloidogyne incognita* (Rasse 3) ebenfalls 80%, bei Zuckerrübensaatgut, behandelt mit einer Abamectin-Konzentration von 0.3 mg a.i. seed⁻¹, wurde eine Wirksamkeit gegenüber *Heterodera schachtii* von 60% festgestellt. Wurzellänge als auch die Spross- bzw. Wurzelgewichte von Pflanzen, deren Saatgut mit Abamectin behandelt wurde, unterschieden sich nicht signifikant von denen unbehandelter Pflanzen. Die Untersuchungen verdeutlichten, dass eine Saatgutbehandlung mit Abamectin für die praktische Anwendung geeignet ist. Eine hohe Wirksamkeit in verschiedenen Kulturen ohne negative Auswirkungen auf das Pflanzenwachstum wurde festgestellt.

Stichwörter: Avermectine, chemischer Pflanzenschutz, Effektivität, wandernde Wurzelneematoden, Wurzelgallenneematoden, Zystenneematoden

1 Introduction

There are a number of important plant parasitic nematodes limiting crop production in temperate, tropical and sub-tropical agriculture (EVANS et al. 1993; LUC et al. 2005). For example, *Pratylenchus zae*, a migratory endoparasitic nematode, is often encountered in maize cultivation throughout the world and causes significant yield losses (MCDONALD and NICOL 2005). *Heterodera schachtii* is a sedentary endoparasitic cyst nematode that causes significant levels of damage to sugar beet in most major growing areas (COOKE 1993). *Meloidogyne incognita* is a sedentary endoparasitic root-knot nematode that reduces yield in many crops world wide, for example in large and small scale cotton production (STARR et al. 2005). Despite the use of crop rotation, soil amendments, resistant/tolerant varieties, catch crops and biocontrol agents, the control of these plant parasitic nematodes still relies heavily on the use of chemical nematicides. However, large scale and repetitive application of these nematicides can cause accumulation in soil and water (HOMEYER 1971; FRANZMANN et al. 2000) or can lead to rapid degradation by soil microorganisms thereby losing their efficacy (STIRLING et al. 1992; OU et al. 1994; KARPOUZAS and GIANNAKOU 2002; McLEAN and LAWRENCE 2003).

Abamectin is a macrocyclic lactone derived from the soil bacterium *Streptomyces avermitilis* that has been shown to have nematicidal properties (PUTTER et al. 1981) and a different mode of action than the other currently available nematicides (TURNER and SCHAEFFER 1989). *Radopholus similis*, *Ditylenchus dipsaci*, *Rotylenchulus reniformis* and some root-knot nematodes have been controlled by general soil treatment with either granular or liquid formulations of abamectin (SASSER et al. 1982; GARABEDIAN and VAN GUNDY 1983; ROBERTS and MATTHEWS 1995; JANSSON and RABATIN 1997; 1998). The use of seed treatments, however, is an attractive alternative for nematode control since it requires less chemical inputs than large scale field nematicide applications, thereby reducing environmental impact and lowering investment costs. Chemical seed treatment is only active in the rhizosphere of soil surrounding the root system of young plants and therefore reduces the risk of undesired accumulation. In addition, the amount of active ingredient needed to treat one seed is lower than when applying liquid or granular formulations as a drench or incorporated to the soil. Furthermore, seed treatment is safer to handle than liquid or granular formulations, especially in areas where nematicides are incorporated into the soil and where labour is unskilled. Treating seeds directly reduces the high cost associated with all other application forms.

Abamectin is a suitable compound for seed treatment since it can be stored for several months while maintaining its nematicidal properties. It can be applied to seeds at high concentrations, does not bio-accumulate and is not taken up by plants (DYBAS et al. 1989). To date, the activity of abamectin on *P. zae* and *H. schachtii* and its use as a seed treatment on maize or sugar beet is still untested. Abamectin as a seed

treatment in cotton has been studied by MONFORT et al. (2006) and FASKE and STARR (2007), but the optimal dose for nematode control in soil was not investigated. The effect of abamectin seed treatment on nematode control at the seedling stage has to be investigated to enhance nematode management by giving high levels of nematode control at low cost, and to determine the existence of phytotoxicity, since other non-fumigant nematicides have shown this adverse effect (SINCLAIR et al. 1992; KENNEDY 2002). Therefore, the objectives of this study were to determine the 50 and 80 effective concentrations (EC₅₀ and EC₈₀) of abamectin as a seed treatment against *P. zeae*, *M. incognita* and *H. schachtii* in maize, cotton and sugar beet and investigate its effect on plant growth.

2 Materials and methods

2.1 Nematode cultures

Pratylenchus zeae isolated from maize was maintained in a monoxenic culture on maize roots. Maize seeds cv. Liberal were surface sterilized in 1.5% NaOCl followed by four rinses in sterile water and placed onto Murashige agar (10 g murashige basal medium, 15 g agar l⁻¹ distilled water) in a 9 cm diameter Petri dish. Maize seeds were allowed to germinate by incubating the plates for 5–8 days in the dark at 24°C. Seeds and shoots were aseptically removed and the roots on the plates were inoculated with an agar piece of an older culture containing *P. zeae*. After 6 weeks, nematodes were extracted from agar medium using the modified Baermann method for 20 hours (HOOPER 1990). Active nematode juveniles were collected on a 20 µm aperture sieve.

M. incognita race 3 was obtained from a natural infested soil in Florida, USA, and maintained on tomato cv. Furor under permanent cultivation in a green house at 27°C ± 5. Nematode eggs were extracted from 8 weeks old tomato roots using 1.5% NaOCl following the method described by HUSSEY and BARKER (1973).

Soil infested with *H. schachtii* was obtained from a sugar beet field in Elsdorf, Germany. Cysts were extracted using the wet sieve decanting technique (AYOUB 1980). To obtain the second stage juvenile inoculum, cysts were placed in a zinc chloride solution (0.68 g l⁻¹) in an Oostenbrink dish. After 24 hours, free-living nematodes were discarded from the inoculum. Second stage juveniles were collected every two days and used in this study.

2.2 Seed treatment

Seeds of maize cv. Liberal, cotton cv. DP90 and sugar beet cv. Macarena were treated with a liquid formulation of abamectin (Syngenta Crop Protection AG, Basel, Switzerland) at 0.03, 0.1, 0.3, 0.6 or 1.0 mg a.i. seed⁻¹. Seeds were abamectin treated to a specific concentration in an Erlenmeyer flask shaken by hand for 90 s. Untreated abamectin seeds were used as controls throughout the study.

2.3 Effects of abamectin on nematode control and plant growth

An autoclaved field soil:sand mixture with 4% organic matter was used in this study. In tests with *P. zeae* and *M. incognita*, the soil:sand ratio was 1:1 (v:v). In studies with *H. schachtii*, the soil:sand ratio was 1:2 (v:v).

One seed was sown in every 10 cm diameter plastic pot filled with 250 ml of sand:soil mixture. Nematodes were inoculated by pipetting a nematode suspension into 3 cm deep holes made around the seed or seedling in each pot. *P. zeae* was inoculated at sowing of maize seeds, at a rate of 1000 juveniles per pot. *M. incognita* race 3 was inoculated at sowing of cotton seeds, at a rate of 3000 eggs per pot. *H. schachtii* was

inoculated two weeks after sowing sugar beets, at a rate of 1000 juveniles per pot. The control treatment was composed of untreated abamectin seeds inoculated with nematodes after sowing, while the absolute control consisted of abamectin untreated seeds in nematode-free pots.

Maize and sugar beet plants were harvested 14 days after nematode inoculation. Roots were gently washed with tap water and root and shoot fresh weight was assessed on a digital scale (Kern & Sohn GmbH, Balingen, Germany). Root length was measured using the WinRhizo program (Version 5.1; Regent Instruments Inc., Quebec, Canada) and a flatbed scanner (Agfa, Model SNAPSCAN TPO) as described by BOUMA et al. (2000). To assess nematode penetration, maize and sugar beet roots were stained in an acid fuchsin solution (875 ml lactic acid, 63 ml glycerol, 0.1 g acid fuchsin and 62 ml distilled water) and heated in a microwave for 2 min. Roots were drained and cut into approx. 1 cm long pieces and homogenized with tap water using an Ultra-Turrax (IKA-Werk, Staufen, Germany) at 11,000 rpm for 1.5 min. The homogenized roots were poured into a 150 ml graduated cylinder, which was filled up to 100 ml with tap water and shaken. The number of penetrated nematodes per gram of root was calculated after counting stained nematodes in two 10 ml sub-samples of homogenized root suspension.

Cotton plants were harvested 4 weeks after nematode inoculation and shoot fresh weight was taken. Roots were gently washed with tap water and root fresh weight was recorded. The number of galls per g of root was determined for each plant.

All experiments were arranged in a randomized complete block design with each treatment replicated six times and conducted under greenhouse conditions at 26 ± 5°C. Plants were watered as needed. Every experiment was conducted twice.

2.4 Statistical analysis

The data obtained after determining the number of penetrated nematodes or galls per g of root was transformed to percent of efficacy using ABBOTT'S (1925) formula. The efficacy (%) was determined separately for every experiment and its repetition, hereafter referred as experiment A and B, respectively. Combined data obtained from experiments A and B was subjected to non-linear regression analysis to determine the EC₅₀ and EC₈₀.

The effect of abamectin on plant growth was performed using combined data in two separated analysis. First, to determine if the plant growth effect was caused by the nematode inoculation and not by the seed treatment, the two controls namely nematode-control treatment (nematode inoculated untreated with abamectin) and absolute control (no nematode inoculation untreated with abamectin) were compared by T-Test ($P < 0.05$). A second analysis was performed only when the first analyses gave no significant differences. The second analysis consisted of an ANOVA test ($P < 0.05$) performed between the nematode-control treatment (nematode inoculated untreated with abamectin) and the rest of abamectin seed treatments.

The statistical program GenStat Discovery Edition 3 (VSN, International) was used to conduct the analysis as described by MOTULSKY and CHRISTOPOULUS (2003).

3 Results

3.1 Nematode control effectiveness of abamectin seed treatments

Abamectin seed treatment in maize against *P. zeae* caused a 80% reduction in early root penetration when 0.6 mg a.i. per seed was used in experiment A (Fig. 1). In experiment B a 50% reduction was achieved at the same concentration whereas at the highest dose tested 70% control was detected.

Cotton seeds treated with 0.1 mg a.i. of abamectin per seed reduced the galling of *M. incognita* over 90% in both experiments A and B (Fig. 2).

In experiment A, sugar beet seeds treated with 0.6 and 1.0 mg a.i. of abamectin per seed reduced *H. schachtii* infection to 50% (Fig. 3). In experiment B, 0.03 mg a.i. per seed caused a 50% reduction and 0.1, 0.3 and 0.6 mg a.i. per seed gave a 70% level of control. The highest concentration tested (1.0 mg a.i. seed⁻¹) was less effective than the three previous concentrations used.

3.2 Effective concentration

Using the combined data from experiments A and B the EC₅₀ and EC₈₀ of abamectin seed treatment in maize against *P. zaeae*

was established at 0.16 and 1.0 mg a.i. per seed, respectively (Table 1). The EC₅₀ and EC₈₀ in cotton against *M. incognita* was 0.016 and 0.28 mg a.i. per seed, respectively. Against *H. schachtii* in sugar beet, the EC₅₀ was 0.026 mg a.i. per seed and the EC₈₀ was not attained at the doses tested.

3.3 Root and shoot parameters

There were no significant differences in the T-Test ($P < 0.05$) between root and shoot weights of nematode control (nematode inoculation and no abamectin treatment) and absolute control (no nematode inoculation and no abamectin treatment), indicating that nematode inoculation alone did not affect root nor shoot growth (data not shown). The combined data of experiments A and B showed that abamectin has no

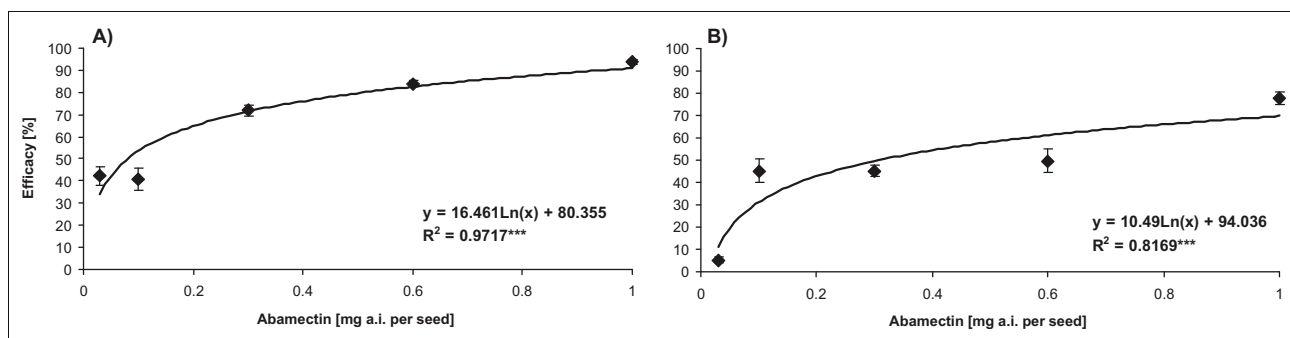


Fig. 1: Efficacy of abamectin seed treatment in controlling *Pratylenchus zaeae* on maize cv. Liberal, 2 weeks after nematode inoculation. A and B are the first and second experiments, respectively. Bars indicate the standard error of the means. N = 6. *** Significant at $P < 0.001$.

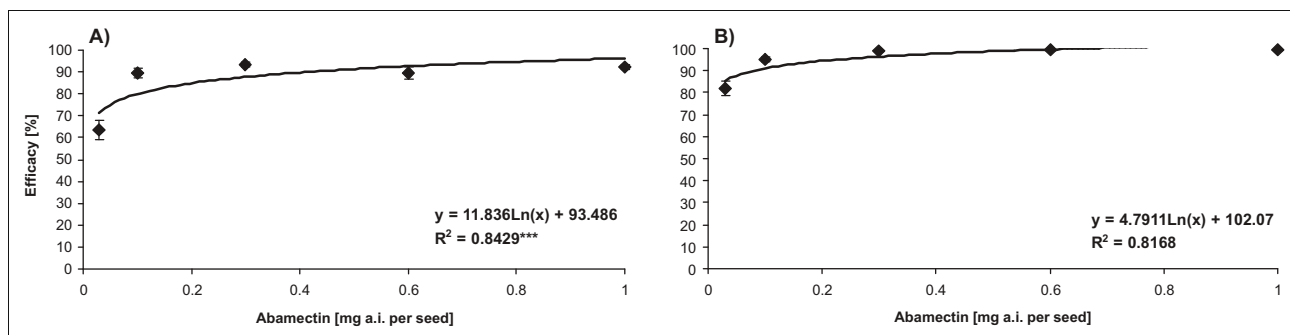


Fig. 2: Efficacy of abamectin seed treatment in controlling *Meloidogyne incognita* race 3 on cotton cv. DP90, 4 weeks after nematode inoculation. A and B are the first and second experiments, respectively. Bars indicate the standard error of the means. N = 6. *** Significant at $P < 0.001$.

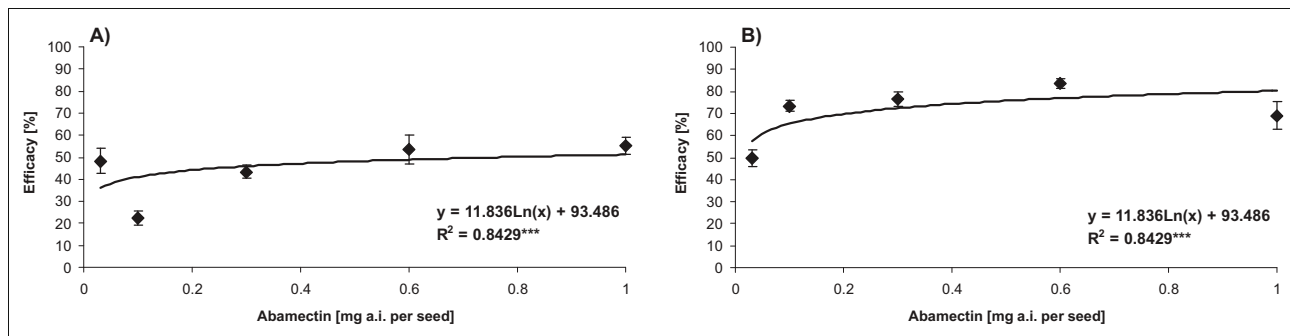


Fig. 3: Efficacy of abamectin seed treatment in controlling of *Heterodera schachtii* on sugar beet cv. Macarena, 2 weeks after nematode inoculation. A and B are the first and second experiments, respectively. Bars indicate the standard error of the means. N = 6. *** Significant at $P < 0.001$.

Table 1: Effective concentration 50 (EC₅₀) and 80 (EC₈₀) of abamectin seed treatment for controlling various plant parasitic nematodes in maize, cotton and sugar beet (combined data from experiments A and B)

Nematode	Host crop	Evaluation ^a	Abamectin (mg a.i. seed ⁻¹)		R ²
			EC ₅₀	EC ₈₀	
<i>Pratylenchus zeae</i>	Maize	# penetrated nematodes	0.160	1.00	0.97
<i>Meloidogyne incognita</i>	Cotton	# galls g root ⁻¹	0.016	0.28	0.81
<i>Heterodera schachtii</i>	Sugar beet	# penetrated nematodes	0.026	N.A.	0.84

^a Evaluation of penetrated nematodes was performed 14 days after nematode inoculation and galls per g of root were determined 4 weeks after inoculations. N.A.= not achieved at the concentrations tested. $P < 0.001$. N = 12.

Table 2: Root and shoot parameters of maize cv. Liberal, sugar beet cv. Macarena and cotton cv. DP90 in relation to different abamectin concentrations applied to seeds (combined data from experiments A and B)

Abamectin (mg a.i. seed ⁻¹)	Root length (m)		Shoot fresh weight (g)	Root fresh weight (g)
	Maize	Sugar beet	Cotton	Cotton
0.00	5.24 ± 1.61	13.50 ± 5.30	4.08 ± 0.78	1.94 ± 0.33
0.03	4.74 ± 1.32	13.25 ± 5.77	3.99 ± 0.67	1.86 ± 0.35
0.10	6.03 ± 2.04	13.83 ± 4.50	3.82 ± 0.59	1.82 ± 0.43
0.30	4.77 ± 1.61	15.07 ± 3.88	3.87 ± 1.01	1.91 ± 0.60
0.60	4.84 ± 1.52	15.68 ± 4.67	4.05 ± 0.85	1.96 ± 0.59
1.00	4.64 ± 1.48	12.87 ± 4.42	3.66 ± 0.73	1.69 ± 0.54
ANOVA ($p < 0.05$)	n.s.	n.s.	n.s.	n.s.

N.S. = numbers in the same column are not significantly different ($P < 0.05$). N = 12.

negative effects on root and shoot weights of maize, sugar beet and cotton seedlings at the concentrations tested (Table 2).

4 Discussion

In this study, abamectin proved to be very effective in reducing lesion and cyst nematode early root infection of maize and sugar beet roots and gall formation by root-knot nematodes in cotton. Abamectin at relatively low concentrations, ranging between 0.026 and 1.0 mg a.i. per seed, was able to diminish nematode infection to the three crops studied. The high efficacy can be related to previous observations which reported that abamectin inhibits nematode hatching and paralyzes juveniles (CAYROL et al. 1993). In addition, laboratory studies demonstrated that abamectin caused irreversible paralysis on root-knot nematodes and that increasing concentrations increased nematode mortality thus high nematode control effectiveness (FASKE and STARR 2006). A bioassay conducted by MONFORT et al. (2006) under greenhouse conditions showed that *M. incognita* galling rates decreased in relation to increasing concentrations of abamectin.

Current chemical control of root-knot nematodes in cotton is performed mainly by applying aldicarb (0.8 – 1.2 kg ha⁻¹) or the fumigant nematicide 1,3 dichloropropene (KOENNING et al. 2004). In sugar beet aldicarb (4.5 kg a.i.ha⁻¹), terbufos (9.0 kg a.i.ha⁻¹) and 1,3 dichloropropene are used towards the cyst nematode *Heterodera schachtii* (GRIFFIN 1988). In maize cultivation carbofuran (200 g a.i.100 m⁻¹), benfuracarb (300 g a.i.100 m⁻¹), terbufos (100 g a.i.100 m⁻¹), carbosulfan (100 g a.i.100 m⁻¹), aldicarb (100 g a.i.100 m⁻¹) and the fumigant ethylene dibromide (20 cm³ 100 m⁻¹) have been used (McDONALD et al. 1987). Our study revealed that chemical inputs can be reduced in the cultivation of these three crops by

the use of abamectin as a seed treatment. The EC₈₀ obtained with 0.28 and 1.0 mg a.i. of abamectin per seed in cotton and maize towards *M. incognita* and *P. zeae*, respectively, was attained with very low chemical input compared to the concentrations used with the nematicides listed above. In sugar beet the EC₅₀ was calculated to be 0.026 mg a.i. per seed demonstrating again a high level of efficacy with low chemical input.

The efficacy of abamectin against *H. schachtii* was lower than that on root-knot and lesion nematodes since the EC₈₀ was not achieved at the concentrations tested. Even at increasing abamectin dosages the cyst nematode reduction was not improved. In our tests with *H. schachtii* nematode inoculation was performed 2 weeks after seed sowing. Perhaps the efficacy was reduced due to the natural degradation of abamectin since its soil half-life has been reported to range between 2 weeks and 2 months (E.P.A. 1990). In addition, these results suggest that *H. schachtii* could be less sensitive to abamectin than the other two nematodes investigated. FASKE and STARR (2006) showed different sensibilities of nematodes to abamectin reporting that *M. incognita* was more sensitive than *Rotylenchulus reniformis*.

Root length of maize and sugar beet as well as shoot and root fresh weights of cotton were not affected by the abamectin seed treatment. In contrast to our results, cotton, MONFORT et al. (2006) obtained greater plant height in plants treated with 10 to 100 g of abamectin/100 kg seed than in control plants 45 days after planting. The reason why such differences were detected in their study but not in ours may be related to plant age and also nematode inoculum density. The nematode inoculum used by MONFORT et al. (2006) was higher than that used in the present study. In our study, nematodes did not significantly affect plant growth; this may be why no differences between growth parameters of the control and treated plants were observed.

In summary, our investigations demonstrated that using abamectin as a seed treatment is an effective way of reducing early root infection of the three economically important plant parasitic nematodes. It is highly effective in limiting nematode penetration in the roots of seedlings of a variety of crops and at low concentrations without negative effects on plant growth. Further studies using the concentrations determined by our study under field conditions using different plant cultivars and with yield measurements are required before abamectin, as a seed treatment, can be viably introduced into an integrated nematode management program.

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