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Effect of Seed Treatment With Delta-Endotoxins of *Bacillus* spp. on the Multiplication of *Heterodera glycines* on Soybean and Corn[1]

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SUMMARY

Sharma, R.D. & Gomes, A.C. Effect of seed treatment with delta-endotoxins of *Bacillus* spp. on the multiplication of *Heterodera glycines* on soybean and corn. **Nematol. Brasileira 20**(2): 21-29, 1996.

The effect of *Bacillus sphaericus* (Bs-2362), *B. thuringiensis* var. *israelensis* (Bti-H-14), and *B. thuringiensis* var. *kurstaki* (Btk-HD-1) delta-endotoxins applied to seeds of soybean and corn on the multiplication of *Heterodera glycines* was evaluated under greenhouse conditions. Fourty plastic pots were filled with 500 g of greenhouse soil infested with *H. glycines* (99 cysts + 3 juveniles + 3 eggs in 50 g of soil) of which half was used for soybean and the other one for corn. With soybeans, the treatments used were: 1) untreated control; 2) Bs-2362 1 g; 3) Btk-HD-1 10 g; and 4) Btk-HD-1 20 g per kg of soybean seed. In corn, the treatments used were: 1) untreated control; 2) Bti-H-14 1 g; 3) Bs-2362 10 g; and 4) Bs-2362 20 g per kg of corn seed. The experiments were evaluated after 26 days of soybean and corn planting. The percentage increase in the first generation cysts per plant over the control in soybean roots with treatments 2, 3, and 4 were 63.2, 138.8 and 145.9 respectively. The percentage decrease of cysts in soil in relation to the control with treatments 2, 3, and 4 were 56.4, 53.3 and 16.7 respectively. No cysts were found in the corn roots and the population in the soil were slightly reduced but remained equal regardless of the treatment. In general, seed treatment with toxins stimulated corn plant growth but a significant ($P < 0.05$) increase (42%) over the untreated control was observed only

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in treatment Bs 2362 20 g dose. The stimulatory effect of delta-endotoxins in increasing the female population in soybean roots can be used to increase the efficiency of soybeans as a trap crop in the control of *H. glycines*.

Key words: *Bacillus*, toxins, seed treatment, *Heterodera glycines*, control.

RESUMO

Sharma, R.D. & Gomes, A.C. Efeito do tratamento de sementes de soja e milho com delta-endotoxinas de *Bacillus* spp. na multiplicação de *Heterodera glycines*. **Nematol. Brasileira 20**(2):21-29, 1996.

Estudou-se o efeito de delta-endotoxinas de *Bacillus sphaericus* (Bs-2362), *B. thuringiensis* var. *israelensis* (Bti-H-14) e *B. thuringiensis* var. *kurstaki* (Btk-HD-1) no tratamento de sementes de soja e milho na multiplicação do *H. glycines*, em casa de vegetação. Foram usados 40 vasos plásticos contendo 500 g de solo, com população inicial do nematóide *H. glycines* formada por 99 cistos + 3 juvenis + 3 ovos por 50 g de solo. Para a cultura da soja, os tratamentos foram: (1) testemunha; (2) Bs-2362 1 g; (3) Btk-HD-1 10 g; e (4) Btk-HD-1 20 g/kg de semente da soja. Para a cultura do milho, os tratamentos foram: (1) testemunha; (2) Bti-H-14 1 g; (3) Bs-2362 10 g, e (4) Bs-2362 20 g/kg de semente de milho. A avaliação foi feita, para ambas as culturas, 26 dias após o plantio. Houve um aumento ($P < 0,05$) na população de cistos nas raízes da soja nos tratamentos Btk-HD-1 10 g e 20 g em relação à testemunha. Não foram encontrados cistos nas raízes de milho e as populações de cistos no solo foram iguais, independentemente do tratamento. Nas sementes de milho tratadas com delta-endotoxinas houve um estímulo ao crescimento das plantas em geral e um aumento significativo ($P < 0,05$) de 42% sobre a testemunha, observado somente no tratamento Bs-2362 20 g. O efeito estimulador de delta-endotoxinas no aumento dos cistos nas raízes de soja indica a possibilidade de seu uso para aumento da eficiência de culturas armadilhas no controle de *H. glycines*.

Palavras-chaves: *Bacillus*, toxinas, tratamento de sementes, *Heterodera glycines*, controle.

INTRODUCTION

Damage caused by plant-parasitic nematodes on host plant can be reduced by chemicals or antagonistic biological agents. Seed treatment provides sufficient initial protection to seedlings against nematodes, resulting

in better plant growth and yield (Rodríguez-Kábana & Weaver, 1987; Oostendorp & Sikora, 1989: Gogoi & Phukan,1990). However, Noel (1990) applied a commercial seed treatment with *Pseudomonas cepacia* but failed to control the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, 1952 on soybean (*Glycine max* (L.) Merr.). In a greenhouse trial, Zuckerman et al. (1993) incorporated an isolate of *Bacillus thuringiensis*, designated CR-371, into a methylcellulose seed coat and also applied as a drench and got good control of *Meloidogyne* on tomato.

The idea of treating the seeds with delta-endotoxins of *Bacillus* species in this work was to use trap crop by inducing egg hatching and infest the young seedlings of a susceptible host with high juvenile population and destroy the host before the nematode attains sexual maturity, i.e., 10 to 12 days after seed germination. The use of trap crop to control nematodes has been criticised by several workers in the past as an inefficient, costly, and risky method but no data on the subject were presented.

Hatching stimulants or hatching inhibitors can play a vital role as an important component of integrated management of cyst nematodes, and specially in SCN (Webber & Barker, 1968). Much time and effort (Shepherd & Clark, 1971; Clarke & Perry, 1977), has been spent in attempts to purify and characterize hatching stimulants since their first recognition in 1922 (Baunacke, 1922). However, success has been limited by the extremely small amounts of the active substance available. Masamune et al. (1982) isolated a natural hatching stimulus for the SCN from the roots of *Phaseolus vulgaris*, designated as glycinoeclipin A, which stimulates egg hatching *in vitro* at a concentration of 10^{-11} to 10^{-12} g.mL^{-1} in water at 25°C. Since this substance is not available in the market, we tested different doses of three delta-endotoxins of *Bacillus* species which are easily available in the market at an accessible cost. In a greenhouse trial, Sharma (1995) incorporated different doses of delta-endotoxins of *Bacillus* species in the soil infested with *H. glycines* on soybean observed both stimulatory and inhibitory effects on larval hatching and resulted in respective decrease and increase in soybean grain weights. In other greenhouse studies, Sharma & Gomes (1995) confirmed the stimulatory and inhibitory effects of these toxins on oviposition and juvenile hatching of SCN in the absence of soybean host. Due to lack of informations about the effect of delta-endotoxins, Bs 2362 and Bti-H-14 as seed treatment

of corn which is commonly grown in rotation with soybean to control *H. glycines* was included in these studies.The above mentioned delta-endotoxins and their respective doses were used for exploratory purposes to find out in future studies the optimal doses for seed treatment to inhibit or stimulate larval hatching as an component of the integrated SCN control. Therefore, an attempt was made to evaluate the effect of delta-endotoxins of *Bacillus* species applied as seed treatment on the multiplication of *H. glycines* and their effect on the initial growth of soybean cv. Cristalina (highly susceptible to *H. glycines*) and corn cv. Pioneer-3041 (nonhost to *H. glycines*).

MATERIALS AND METHODS

The effect of *Bacillus sphaericus* (Bs-2362), *B. thuringiensis* var. *israelensis* (Bti-H-14), and *B. thuringiensis* var. *kurstaki* (Btk-HD-1) delta-endotoxins applied to seeds of soybean and corn on the multiplication of *H. glycines* race 3 was evaluated under greenhouse conditions. Fourty plastic pots were filled with 500 g of soil infested with *H. glycines* half of which was used for soybean and the rest was for corn experiment. Before filling the plastic pots, the soil was thoroughly mixed and determined the initial population density of the nematode according to the methods of Fenwick (1940) and Coolen (1979). The initial population per 50 g of soil was 99 cysts, 3 juveniles and 3 eggs. The soil used in these experiments was a 50 % mixture (w:w) of Oxisol (Dark Red Latosol) and a coarse river sand after sieving (< 1 mm pore size). The above soil was limed with 1.72 g of limestone (PRNT 60 %) and fertilized with chemical fertilizers superphosphate 4.76 g; potassium chloride 0.41 g and micronutrients mixture (FTE BR-12) 0.25 g per kg of soil. Race 3 of *H. glycines* initially obtained from Chapadão do Céu, State of Goiás, Brazil was cultured on soybean cv. Cristalina for four months under greenhouse conditions.

For treating one kg of seed (soybean or corn) with different delta-endotoxins, 20 mL of 25 % sugar solution was used as an adherent. Seeds of control treatments were treated with 25 % sugar solution. The treated seeds were dried in shade for one hour before sowing. One seed per pot was sown of each crop. In experiment 1, the treatments used were: 1) untreated control; 2) Bs-2362 1g; 3) Btk-HD-1 10 g; and 4) Btk-HD-1 20 g per kg of soybean seed.

In experiment 2, the treatments used were: 1) untreated control; 2) Bti-H-14 1 g; 3) Bs-2362 10 g; and 4) Bs-2362 20 g per kg of corn seed.

Treatments were arranged in a completely randomized design on greenhouse benches. The greenhouse temperature varied between 25 and 30 °C during the experimental period. After 26 days of soybean and corn sowing, fresh top weight, fresh root weight and final population of cysts and juveniles in 50 g of soil and root systems were determined. All data were analyzed following standard statistical procedures (Snedecor & Cochran, 1969). Final nematode populations (Pf) constituted of cysts and juveniles due to the absence of eggs in the soil. The number of eggs/cyst was not determined in these studies. The Pf were log transformed before doing the analysis of variance.The means were compared using Tukey's test.

RESULTS AND DISCUSSION

No significant differences in fresh top weights and fresh root weights of soybean among treaments were observed; but a slight increase (3.2 %) in fresh top weights in treatments Bs-2362 1g and Btk-HD-1 10 g, and a decrease (11.7 %) in treatment Btk-HD-1 20 g per kg of seed in relation to the control were observed (Table 1). Fresh root weight of soybean in treatment Btk-HD-1 20 g dose was 10.2 % lower than the control because large number of nematodes present in the roots. The number of cysts in soil from control and in treatment Btk-HD-1 20 g dose per kg of soybean seed were higher ($P<0.05$) than Bs-2362 1 g and Btk-HD-1 10 g doses. The decrease in the cyst populations in 50 g soil in treatments Bs-2362 1 g, Btk-HD-1 10 g and Btk-HD-1 20 g in relation to the control were 56.4, 53.3 and 16.7 % respectively. The juvenile populations in 50 g soil were zero in treatment Btk-HD-1 10 g and significantly ($P<0.05$) low 1.0 in the control. An increase ($P<0.05$) in the cyst population in soybean roots was observed in treatments Btk-HD-1 10 g and Btk-HD-1 20 g doses over the control. The percentage increase in the female populations in treatments Bs-2362 1 g, Btk-HD-1 10 g and Btk-Hd-1 20 g over the control was 63.2, 133.8 and 145.9 respectively (Table 1).

Seed treatment with delta-endotoxins stimulated corn plant growth in general but a significant ($P<0.05$) increase in fresh top weight (41.9 %) over the control was observed in treatment Bs-2362 20 g dose per kg of corn seed

(Table 2). The increase in corn fresh top weights in treatments Bti-H-14 1 g, Bs-2362 10 g and Bs-2362 20 g over the control were 20.4, 13.8 and 41.9 % respectively. The increase in fresh root weight of corn over the control in treatments Bti-H-14 1g, Bs-2362 10 g and Bs-2362 20 g per kg of seed respectively were 10.9, 13.5 and 53.8 % but a significant ($P<0.05$) increase over the control was observed only in treatment Bs-2362 20 g. In corn roots, no cysts and juveniles were found and the female populations in soil were equal regardless of the treatment. The absence of cysts and juveniles in corn roots confirm that corn is a nonhost to *H. glycines* (Table 2).

A significantly ($P<0.05$) low population of the nematode was observed in the control treatment of corn than the control of soybean. The reduction of nematode population in corn was expected because corn being a nonhost might have inhibited juvenile hatching and their penetration in roots (Table 1 and 2).

The stimulatory effect of delta-endotoxins of Btk-HD-1 resulted in significant increase in hatching of juveniles of *H. glycines* and their consequent penetration in soybean roots. Significant stimulus in juvenile hatching of *H. glycines* induced by delta-endotoxins of Btk-HD-1 at 0.2 and 1 mg and Bs 2362 at 1 mg/g of soil in the absence of host plant was also observed by Sharma & Gomes (1995). Sharma (1995), observed that delta-endotoxins of *Bacillus* spp. induced initial stimulation or inhibition in larval hatching of *H. glycines* which resulted in respective decrease or increase in soybean grain weight. The data obtained in these experiments supports the earlier studies in part because of limited number of doses used. Further studies are needed where several doses of each toxin can be applied to determine the optimum stimulation and optimum inhibition levels on juvenile hatching to use in SCN control.

The corn roots were free of *H. glycines* regardless of the delta-endotoxins treatments and the nematode population in soil was significantly reduced too. The treatment of soybean (susceptible to SCN) seed with delta-endotoxin (Btk-HD-1 20 g/kg of seed) can be explored in trapping a large number of SCN juveniles in 10 to 12-day old seedlings followed by their destruction either by incorporating them in the soil or by systemic herbicides before the nematode reaches sexual maturity. Once the susceptible trap crop is destroyed, corn seeds treated with 20 g of delta-endotoxins of Btk-HD-1 can be planted to induce further juvenile hatching in order to starve them.

Table 1. Effect of seed treatment with delta-endotoxins of *Bacillus* spp. on soybean plant growth and number of first generation cysts and juveniles of *Heterodera glycines* in 50 g of soil and root systems under greenhouse conditions

Treatment (toxin)	Toxin dose (g/kg seed)	Fresh weight(g)		Number of cysts		Number of juveniles	
		top	root	soil	roots*	soil	roots
Control	0	1.86 a	1.56 a	156 a	55 b	1.0 a	78 a
		(100)	(100)	(100)	(100)**	(100)	(100)
Bs-2362	1	1.92 a	1.56 a	68 b	90 ab	0.4 ab	74 a
		(+3.2)	(0.0)	(-56.4)	(+63.2)	(-60.0)	(-5.1)
Btk-HD-1	10	1.92 a	1.58 a	73 b	128 a	0.0 b	68 a
		(+3.2)	(+1.3)	(-53.3)	(+133.8)	(-100)	(-12.8)
Btk-HD-1	20	1.66 a	1.40 a	130 a	136 a	0.4 ab	36 a
		(-11.7)	(-10.2)	(-16.7)	(+145.9)	(-60.0)	(-51.3)
CV (%)		19.40	28.73	5.49	13.80	112.14	26.60

*Values in each column followed by different letters differ statistically (P<0.05), according to Tukey's test; each value is the mean of five replications with one plant/pot/500 g of soil.

**Data presented in brackets are the percent increase (+) or decrease (-) over the control. Original nematode population data was log transformed, i.e., log (x+1).

Table 2. Effect of seed treatment with delta-endotoxins of *Bacillus* spp. on corn growth and number of cysts and juveniles of *Heterodera glycines* in 50 g soil under greenhouse conditions

Treatment (toxin)	Toxin dose (g/kg of seed)	Fresh weight* (g)		Cysts in soil	Juveniles in soil
		top	root		
Control	0	1.86 b	3.12 b	90 a	1.6 a
		(100)**	(100)		
Bti-H-14	1	2.24 ab	3.46 b	92 a	0.6 a
		(+20.4)	(+10.9)		
Bs-2362	10	2.12ab	3.54 b	89 a	1.6 a
		(+13.8)	(+13.5)		
Bs-2362	20	2.64 a	4.80 a	90 a	2.2 a
		(+41.9)	(+53.8)		
CV (%)		18.30	12.30	3.23	60.12

*Values in each column followed by different letters differ statistically P<0.05), according to Tukey's test; each value is the mean of five replications with one plant/pot/500 g of soil..

** Data presented in brackets are the percent increase (+) or decrease (–) over the control. Original nematode population data was log transformed, i.e., log (x+1).

The corn seed treatments with delta-endotoxins will have two beneficial effects: 1) starvation of the the juvenile population hatched after the destruction of the susceptible trap crop and 2) significant increase in plant growth resulting in higher yield. Three to four times more seed of the trap crop than normaly used for soybean grain production should be sown to cover maximum surface area. Planting of a trap crop to cover maximum surface area can be done either using broadcast method or by reducing 50% the spacing between the rows. In this way, both rotation time and money can be saved without increasing the cost of cultivation and without any residue problem for the environment and human beings.

CONCLUSIONS

1) The soybean cv. Cristalina seeds treated with delta-endotoxin, Btk-HD-1 resulted in increased hatching of *H. glycines* juveniles resulting in increased penetration in the roots. The stimulatory effect of this toxin can be used for trapping large populations of SCN in a susceptible host followed by its destruction resulting in a quick, cheap and simple method of population density suppression;

2) The corn seeds treated with delta-endotoxins of Bs-2362 20 g resulted in a significant increase (41.9 %) in fresh plant top weight and 53.8 % increase in fresh root weight over the control. The stimulatory effect of Bs 2362 on plant growth can be easily exploited to increase corn yield without any extra use of fertilizers besides controlling efficiently the soybean cyst nematode.

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