CROP PROTECTION

Effect of Thiamethoxam on Entomopathogenic Microorganisms

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Neotropical Entomology 30(3): 437-447 (2001)

Efeito do Tiametoxam Sobre Microrganismos Entomopatogênicos

RESUMO - A compatibilidade de microrganismos entomopatogênicos com tiametoxam e outros inseticidas foi estudada através de ensaios *in vitro* e em campo. Os microrganismos testados foram: *Aschersonia aleyrodis*, *Bacillus thuringiensis*, *Baculovirus anticarsia* (VPNAg), *Beauveria bassiana*, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces farinosus*, *Sporothrix insectorum* e *Verticillium lecanii*. Em laboratório, foram testadas duas concentrações de cada produto, o que envolveu as recomendações máxima e mínima para uso em condições de aplicação no campo. Os produtos foram adicionados em meio de cultura específico para crescimento dos entomopatógenos. Foram avaliados os crescimentos reprodutivo e vegetativo para fungos e unidades formadoras de colônias (UFC) para bactérias. Para os testes conduzidos em campo foram consideradas as UFC para fungos e bactéria e a mortalidade de lagartas para o NPV de *Anticarsia gemmatalis* (Hueb.). Os resultados mostraram que: (1) a ação dos produtos fitossanitários sobre o crescimento vegetativo e a esporulação dos microrganismos variou em função da natureza química dos produtos, da concentração e da espécie do agente microbiano; (2) thiametoxam foi compatível com todos os microrganismos estudados; (3) endosulfam, monocrotofós e deltametrina foram os inseticidas que mais afetaram *B. thuringiensis*, *B. bassiana*, *M. anisopliae* e *S. insectorum*; (4) tiametoxam não interferiu no potencial de inóculo de *B. thuringiensis*, *B. bassiana* e *M. anisopliae* quando aplicado em lavoura de feijão (*Phaseolus vulgaris*); e (5) tiametoxam não afetou a eficiência do vírus de poliedrose nuclear de *A. gemmatalis*.

PALAVRAS-CHAVE: Insecta, inseticida, controle microbiano, compatibilidade.

ABSTRACT - The compatibility of entomopathogenic microorganisms with thiamethoxam and other insecticides was studied *in vitro* and under field conditions. The microorganisms tested were: *Aschersonia aleyrodis*, *Bacillus thuringiensis*, *Baculovirus anticarsia* (NPVAg), *Beauveria bassiana*, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces farinosus*, *Sporothrix insectorum* and *Verticillium lecanii*. Two concentrations of each product were tested in the laboratory, based on the maximum and minimum recommended rates for application in the field. The products were added to specific culture medium for growth of the entomopathogens. Reproductive and vegetative growth were evaluated for fungi, and colony forming units (CFU) were evaluated for bacteria. For the field test, CFUs were considered for both fungi and bacteria and caterpillar mortality for the NPV of *Anticarsia gemmatalis* (Hueb.). Results showed that: (1) the action of the pesticides on the vegetative growth and sporulation of the microorganisms varied as a function of the chemical nature of the products, its concentration and the microbial species; (2) thiamethoxam was compatible with all microorganisms studied; (3) endosulfan, monocrotophos and deltamethrin were the insecticides that most affected *B. thuringiensis*, *B. bassiana*, *M. anisopliae* and *S. insectorum*; (4) thiamethoxam did not affect the inoculum potential of *B. thuringiensis*, *B. bassiana* or *M. anisopliae* when applied to bean crop (*Phaseolus vulgaris*); and (5) thiamethoxam did not affect the efficiency of the nuclear polyhedral virus of *A. gemmatalis*.

KEY WORDS: Insecta, insecticide, microbial control, compatibility.

The conservation of biological control agents within agroecosystems is one of the strategies adopted for the exploitation of entomopathogens. Equaly important are the techniques of inoculative, inundative and incremental introductions. In all cases, either to preserve the entomopathogen or to use it in combination with chemical pesticides, it is necessary to know the action of these products on the microorganism and then determine their compatibility. This interaction should be considered before recommending a given chemical agent and represents an important tool in programs of integrated pest management.

Several studies have contributed information for the choice of pesticides with more selective action on the entomopathogens, and most of them were conducted under laboratory conditions and concerned entomopathogenic fungi (Ramaraje *et al.* 1967, Alves 1986, Calderon *et al.* 1991, Silva *et al.* 1993).

In Brazil, the fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Sporothrix insectorum,* as well as the bacterium *Bacillus thuringiensis* and the nuclear polyhedral virus of *Anticarsia gemmatalis* (NPVAg), are particularly outstanding for the number of studies carried out on them or for their practical use in extensive areas. These are the programs involving sugar cane and pasture spittlebugs, banana plant borers, rubber tree lace bugs, and velvetbean caterpillars, among others (Alves 1998). In addition to these, other entomopathogens occurring naturally in the agroecosystem also deserve attention, as is the case for *Nomuraea rileyi*, an important fungus that colonizes pest lepidopterans in various crops. With respect to this pathogen, Ignoffo *et al.* (1975) studied *in vitro* several chemical products employed in soybean crop and observed that almost all fungicides and some insecticides and herbicides tested inhibited fungal growth and affected fungal virulence. The fungi *Aschersonia aleyrodis*, *Hirsutella thompsonii*, *Paecilomyces farinosus* and *Verticillium lecanii* are pathogens of great importance for the natural control of different insects and mites and occurrs in various agroecosystems.

Pesticides can also act in a positive manner in combination with entomopathogens. At sublethal doses they interact with the latter causing or activating infectious diseases by stress, or turning the insects more susceptible to the action of microbial toxins. In this respect, Chen *et al.* (1974) observed that *B. thuringiensis,* in combination with carbaryl, had a synergistic effect on the control of *Heliothis virescens* (Fabr.) larvae. A similar effect was observed by McGaughey (1975).

The interaction between *B. bassiana* and mineral oil was evaluated by Batista Filho *et al*. (1995) in order to control the banana plant borer, *Cosmopolites sordidus* (Germ.). These investigators observed an additive effect of the combination, which caused 98% adult insect mortality compared to 70% caused by the fungus alone and 33% by mineral oil alone. Alves *et al.* (1998) concluded that triadimenol/disulfoton + *B. bassiana* could be an alternative for use in the integrated management of coffee pests and diseases.

Roberts & Campbell (1977) and Osborne & Boucias (1985) published an extensive review on the influence of pesticides on entomopathogenic fungi. In Brazil, Alves (1998), based on the work of several authors, published several compatibility tables between chemical products and entomopathogenic fungi of greater importance in the microbial control of insects, to be used for practical purposes. The author also presented a table showing that most combinations of insecticides with entomopathogenic fungi had an additive effect.

Moscardi & Sosa-Gómez (1992) reported no reduction in activity of NPVAg submitted to mixtures with various insecticides. Similarly, Crébio & Melhorança (1999) concluded that post-emergent herbicides tested in the field do not interfere with the efficacy of NPVAg in the control of velvetbean caterpillars.

Thiamethoxam is a neonicotinoid insecticide and belongs to a new insecticides group. It has a similar mode of action of nicotine, linking in the sinapses of the nervous connections to the acetylcholin receptors (Abbink 1991).

The objective of the present study was to investigate the toxic action of the insecticide thiamethoxam on the major entomopathogens used or naturally found in agroecosystems.

Material and Methods

The study was carried out at the Biological Control Laboratory and in research areas of the Centro Experimental do Instituto Biológico, located in Campinas, SP, and was divided into four experiments. The origin of each microorganism used is presented in Table 1.

Table 1. Microorganisms utilized in the experiments.

Species	Shain	Host	Origin
Aschersonia aleyrodis	ESALQ 1216	*Bemisia tabaci*	USP/ESALQ
Bacillus thuringiensis	DIPEL® PM	-	Abbott Lab. Brazil
Baculovirus anticarsia	CNPSo - 84	*Anticarsia gemmatalis*	EMBRAPA
Beauveria bassiana	CB 66	*Hypothenemus hampei*	Inst. Biológico
Hirsutella thompsonii	CEPLAC H6	*Leptopharsa heveae*	CEPLAC
Metarhizium anisopliae	SPL 358	*Anthonomus grandis*	USP/ESALQ
Sporothrix insectorum	CB - 79	*Leptopharsa heveae*	Inst. Biológico
Nomuraea rileyi	CB – 178	*Spodoptera frugiperda*	Inst. Biológico
Paecilomyces farinosus	CB – 144	Solo	Inst. Biológico
Verticillium lecanii	JAB - 02	*Coccus viridis*	UNESP/Jabot.

Entomopathogens x Insecticides in Laboratory. The culture medium potato-dextrose-agar (PDA) was autoclaved at 1 atm for 20 min. and the pesticides were added before solidification at a temperature of approximately 45°C. The mixture was then poured into petri dishes measuring 9 cm in diameter, three dishes per each insecticide concentration. After medium solidification, the fungi *B. bassiana*, *M. anisopliae* and *S. insectorum* were transferred to the medium containing the insecticides at the minimum and maximum doses recommended by the manufacturer (Table 2). Each fungus was inoculated at three points on the dish using a platinum loop. The control consisted of the culture media without insecticide. Thus, each treatment consisted of nine replicate colonies from three petri dishes which gave origin to nine colonies. The dishes were maintained in germination chambers (B.O.D.) at 26°C under a 14h photoperiod. Incubation period was 14 days for *B. bassiana* and *M. anisopliae* and eight days for *S. insectorum*.

For *B. thuringiensis* a similar method was used except that the medium was nutrient agar (NA), the incubation period was 48h and the dishes were fully inoculated.

For evaluating the compatibility with to the fungi, the colony size of each microorganism (vegetative growth) and the number of conidia (reproductive growth or sporulation) were considered. The vegetative growth of the colonies was measured with a common ruler by measuring in two directions and calculating the mean for the two measurements. For counting the number of conidia the colonies were cut out together with the culture medium and transferred to glass tubes (8.5 cm high x 2.5 cm in diameter) containing 10 ml sterile distilled water plus an adhesive spreader (Tween 80). Diluted microorganism suspensions were counted in a Neubauer hemocytometer. Colony forming unit (CFU) number was counted for *B. thuringiensis*.

A fully randomized experimental design with 20 treatments and nine replicates was used. Mean colony size and mean number of conidia in each treatment were submitted to analysis of variance followed by the F test and Tukey test at the 5% level of significance. The data were also standardized by the classification of Alves *et al*. (1998) based on the mean values as percent sporulation and vegetative growth of the fungal colonies, using the formula:

Table 2. Pesticides utilized in the experiments.

Technical name	Commercial name	Recommended concentrations (a.i./ha)
Acephate	Orthene® 750 BR	50 to 1500 g
Carbosulfan	Marshal® 200 SC	50 ml
Deltametrhin	Decis® 25 CE	30 to 400 ml
Diafentiuron	Polo® 500 PM	500 to 800 g
Endosulfan	Thiodan® CE	0.5 to 2.5 litros
Fipronil	Regent® 800 WG	15 to 250 g
Imidacloprid	Confidor® 700 GrDA	70 to 400 g
Monocrotophos	Azodrin® CE	300 to 2250 ml
Thiamethoxam	Actara® 250 WG	100 to 800 g

$$T = \frac{20[VG] + 80[SP]}{100}$$

where T is the corrected value of vegetative and reproductive growth for product classification, VG is percent vegetative growth compared to control, and SP is percent sporulation compared to control. The T values for the classification of the effect of chemical products on the fungi are as follow: 0 to 30 (very toxic), 31 to 45 (toxic), 46 to 60 (moderately toxic) and >60 (compatible).

Major Species of Entomopathogenic Fungi and Bacteria Used in Brazil X Thiamethoxam Under Field Conditions. Bean plots (*Phaseolus vulgaris*) of 1 m^2 were sprayed with *B. bassiana*, *M. anisopliae* and *B. thuringiensis* at concentrations of 0.1 g pure conidia for the fungi and 0.05 g commercial product (Dipel) for the bacterium. The insecticide thiamethoxam was then applied at the maximum recommended dose per hectare (800 g) to the plots treated with the entomopathogens. One hundred liters of water per hectare were used for the application of both the biological and the chemical products. The experimental design was random blocks with three replicates and eight treatments consisting of the plots treated with the microorganisms and the insecticide, the plots treated with the microorganisms alone, the plots treated with the insecticide alone, and the control plots receiving no treatment.

At 24, 48 and 72h after application, four leaflets were collected at random from each plot and one sample (1.6 cm in diameter) was obtained from each leaflet with the aid of a metal punch. This portion was washed in a tube containing 10 ml distilled water plus an adhesive spreader and 1 ml of the suspension thus obtained was applied with a Drigalsky loop to the surface of petri dishes containing fungus-specific culture medium (Dodine - acetato de n-dodecilguanidina 550 mg/l) and NA (Nutrient Agar) medium for bacteria. Twelve dishes (replicates) per treatment were prepared. The time and conditions of incubation were the same as described in the previous assay. CFU numbers were determined and the data were analyzed statistically by the Tukey test at the 5% level of significance.

Naturally Occurring Microorganisms X Thiamethoxam in Laboratory

In this experiment we studied the compatibility of the thiamethoxam with the major fungi naturally occurring in agroecosystems, some of them commercially exploited. The experimental setting, design, evaluation and statistical analysis were the same as described in experiment nº 1, except that the culture medium used was SDAY and the time of incubation was 10 days. Another modification specifically used for *Nomuraea rileyi* was the form of inoculation (full dish) because this fungus is yeast like at the beginning of growth. The experiment consisted of 15 treatments formed by the fungi *A. aleyrodis*, *H. thompsonii*, *N. rileyi*, *P. farinosus* and *Verticillium lecanii* inoculated into culture medium containing the insecticide at the minimum and maximum

doses and the control treatment. Each treatment consisted of nine replicates consisting of three petri dishes in which the fungi were inoculated at three points, giving origin to nine colonies.

Baculovirus anticarsia (NPVAg) X Thiamethoxam Under Field Conditions. The experiment consisted of the following treatments: a) NPVAg, b) NPVAg + thiamethoxam 800 g/ha, c) centrifuged NPVAg, d) thiamethoxam 800 g/ha, and control (destilated water). The centrifuged NPVAg treatment was obtained from a mixture of the concentrated virus suspension with the insecticide. The mixture was left at room temperature for 12h, after which the viral polyhedra were separated from the insecticide by successive differential centrifugations.

A random block design with five treatments and three replicates was adopted, with the plots consisting of 1 m^2 soy crop (*Glycine max*). Three border rows were left between plots and a 4-meter separation strip was left between blocks. A backpack hand-held sprayer was used to apply the mixtures. The concentration of the pathogen and the volume of fluid used were 1.0 x 10^{11} polyhedric inclusion bodies (PIB)/ha and 100 liters of water/ha, respectively. Thirty min. after the beginning of spraying, 20 leaflets located in the upper third of the plants were collected from each plot and sent to the laboratory. The material was placed in individual glass tubes (2.5 cm in diameter x 8.5 cm high), each containing one *A. gemmatalis* caterpillar at the beginning of 4th instar. Sixty individuals were used for each treatment, divided into three replicates of 20 insects each maintained in a room kept at 25^{o}C, RH = 70% and a 14h photoperiod. After 24h the caterpillars were transferred to glass tubes containing an artificial diet (Greene *et al.* 1976) and kept under the same climatic conditions.

The mortality rate obtained with each virus and insecticide was calculated and the data were submitted to analysis of variance, with the means of each treatment being compared by the Tukey test at the 5% level of probability.

Results and Discussion

Entomopathogens X Insecticides in Laboratory. The insecticides thiamethoxam 250 WG, carbosulfan 200 SC, imidacloprid 700 GrDA, acephate 750 BR, and fipronil 800 WG did not affect conidial production regardless of the concentration used (Table 3). The behavior of the fungus under this group of insecticides was similar in terms of vegetative growth, except for fipronil which at the minimum concentration reduced the diameter of fungal colonies (Table 4). When the data concerning reproductive and vegetative growth were submitted to the formula for the determination of T (Table 5), endosulfan, diafentiuron e deltamethrin were classified as incompatible with *S. insectorum* (Table 6). Imidacloprid, acephate and fipronil have been previously reported to be compatible with other species of entomopathogenic microorganisms (Morris 1977, Alves 1998).

On the other hand, endosulfan and monocrotophos used at maximum concentrations reduced the production of conidia and vegetative growth, whereas at minimum concentrations they had no effect on the reproductive growth of the fungus (Table 3 and 4). With respect to vegetative growth, at minimum concentrations, endosulfan and fipronil significantly reduced the diameter of *S. insectorum* colonies (Table 4). These results agree with those obtained by various authors who classified the insecticides endosulfan and monocrotophos as harmful to the development of some

Table 3. Number of conidia of *S. insectorum*, *M. anisopliae* and *B. bassiana* in culture medium containing insecticides at the maximum and minimum concentrations recommended in the field (Temperature 26^{o}C and photoperiod of 14h).

Inseticticides (n=5)	*S. insectorum* (x10^7conidia/ml)		*M. anisopliae* (x10^7conidia/ml)		*B. bassiana* (x10^7conidia/ml)	
	Maximum conc.	Minimum conc.	Maximum conc.	Minimum conc.	Maximum conc.	Minimum conc.
Control	1.8 ± 0.17 a	1.8 ± 0.17 ab	35.1 ± 4.10 a	35.1 ± 4.09 a	9.8 ± 4.21 ab	9.8± 4.21 abc
Thiamethoxam	1.5 ± 0.28 a	1.0 ± 0.26 ab	21.6 ± 5.08 abc	15.9 ± 0.99 ab	7.9 ± 2.03 ab	9.3± 0.92 abc
Carbosulfan[1]	1.8 ± 0.66 a	[1]	7.3 ± 1.71 bcd	[1]	2.2 ± 0.21 bcd	[2]
Endosulfan	0.2 ± 0.10 bc	0.3 ± 0.14 ab	0.2 ± 0.05 d	0.6 ± 0.06 c	0.3 ± 0.20 cd	0.7 ± 0.16 d
Diafentiuron	0.0 ± 0.00 c	0.1 ± 0.10 b	5.7 ± 3.73 cd	14.9 ± 6.83 b	14.1 ± 0.76 a	21.9 ± 3.05 a
Imidacloprid	0.9 ± 0.36 abc	1.2 ± 0.35 ab	11.7 ± 0.81 abcd	1.7 ± 0.31 c	4.5 ± 1.58 abcd	5.2 ± 0.14 bcd
Acephate	1.9 ± 0.21 a	2.3 ± 0.35 a	11.5 ± 8.43 abcd	7.7 ± 1.62 bc	7.7 ± 2.13 abc	16.1 ± 7.49 ab
Monocrotophos	0.1 ± 0.03 bc	0.5 ± 0.73 ab	0.6 ± 0.75 d	4.0 ± 0.77 bc	0.0 ± 0.00 d	0.7 ± 0.49 d
Deltamethrin	0.1 ± 0.14 bc	0.0 ± 0.00 b	0.4 ± 3.21 d	15.5 ± 0.34 ab	1.2 ± 1.43 bcd	0.7 ± 0.39 d
Fipronil	0.9 ± 0.11 ab	0.9 ± 0.58 ab	0.4 ± 0.51 d	12.8 ± 3.99 b	4.8 ± 3.58 abcd	3.5 ± 0.51 cd
Variation Coef. (%)	31	23	31	22	31	26

Means and ME followed by the same letter in the columns did not differ by the Tukey test at the 5% level of significance. Data transformed to $\sqrt{x+0.5}$.

[1]Single concentration.

Table 4. Diameter of colonies of *S. insectorum*, *M. anisopliae* and *B. bassiana* in culture medium containing insecticides at the maximum and minimum concentrations recommended for the field (Temperature 26ºC and photoperiod of 14h).

Insecticides	*S. insectorum* (cm)		*M. anisopliae* (cm)		*B. bassiana* (cm)	
	Maximum conc.	Minimum conc.	Maximum conc.	Minimum conc.	Maximum conc.	Minimum conc.
Control	2.8 ± 0.03 abc	2.8 ± 0.03 a	3.5 ± 0.06 a	3.5 ± 0.05 a	4.3 ± 0.52 a	4.3 ± 0.52 a
Thiamethoxam	2.5 ± 0.14 bc	2.4 ± 0.03 a	3.5 ± 0.58 a	3.0 ± 1.00 a	4.2 ± 0.26 a	4.2 ± 0.05 a
Carbosulfan[1]	2.9 ± 0.21 ab	[1]	3.7 ± 0.31 a	[1]	3.6 ± 0.20 a	[1]
Endosulfan	1.0 ± 0.11 de	1.5 ± 0.25 b	0.4 ± 0.15 b	1.1 ± 0.08 b	0.5 ± 0.03 b	1.0 ± 0.08 c
Diafentiuron	2.3 ± 0.05 bc	2.3 ± 0.06 a	3.9 ± 0.20 a	3.2 ± 0.40 ab	4.2 ± 0.03 a	4.4 ± 0.0 a
Imidacloprid	2.7 ± 0.23 abc	2.8 ± 0.23 a	3.5 ± 0.35 a	3.6 ± 0.46 a	3.3 ± 0.55 a	4.1 ± 0.08 a
Acephate	2.4 ± 0.10 bc	2.5 ± 0.05 a	2.1 ± 0.39 ab	3.6 ± 0.49 a	4.4 ± 0.09 a	4.3 ± 0.03 a
Monocrotophos	0.8 ± 0.0 e	2.5 ± 0.03 a	0.3 ± 0.16 b	3.8 ± 0.43 a	0.0 ± 0.0 b	2.1 ± 1.11 bc
Deltamethrin	1.8 ± 0.21 cd	2.2 ± 0.03 ab	2.8 ± 0.55 a	3.4 ± 0.08 a	0.5 ± 0.40 b	0.3 ± 0.15 c
Fipronil	3.6 ± 0.43 a	1.5 ± 0.25 b	0.6 ± 0.51 b	3.1 ± 0.21 ab	2.5 ± 0.75 a	0.6 ± 0.08 c
Variation Coef. (%)	14	11	26	26	24	24

Means and ME followed by the same letter in the columns did not differ by the Tukey test at the 5% level of significance. Data transformed to $\sqrt{x+0,5}$.
[1]Single concentration.

Table 5. T values (corrected value of vegetative and reproductive growth for product classification) for the classification of insecticides in terms of toxicity towards entomopathogenic fungi.

Insecticide	*B. bassiana*		*M. anisopliae*		*S. insectorum*	
	Max. C.	Min. C	Max. C.	Min. C	Max. C.	Min. C
Thiamethoxam	82.2	90.5	82.2	90.5	84.5	62.2
Carbosulfan	33.4	-	33.3	-	105.5	-
Endosulfan	4.5	10.1	4.5	10.1	15.2	27.2
Diafentiuron	126	187.2	37.5	56.4	16.4	20.8
Imidacloprid	51.6	59.1	47.3	25.2	63.7	69.7
Acephate	77.5	153.2	47.1	38.5	79.8	88.9
Monocrotophos	0	16.1	11	31	63.5	50.3
Deltamethrin	3.6	7.7	81.9	53.4	19	15.7
Fipronil	56.7	29.8	4.4	47.8	69.3	68.2

Table 6. Classification of the compatibility among insecticides and entomopathogenic fungi.

Insecticide	*B. bassiana*		*M. anisopliae*		*S. insectorum*	
	Max. C.	Min. C.	Max. C.	Min. C.	Max. C.	Min. C.
Thiamethoxam	C	C	C	C	C	C
Carbosulfan	I	-	I	-	C	-
Endosulfan	I	I	I	I	I	I
Diafentiuron	C	C	I	MT	I	I
Imidacloprid	MT	MT	MT	I	C	C
Acephate	C	C	MT	I	C	C
Monocrotophos	I	I	I	I	C	I
Deltamethrin	I	I	C	MT	I	I
Fipronil	MT	I	I	MT	C	C

I - Incompatible; MT - Moderately toxic; C - Compatible. (The T values that classified the products as very toxic and toxic were considered to be incompatible).

entomopathogenic species of a fungi nature (Alves 1986, Barbosa *et al*. 1997). In contrast, Olmert & Kenneth (1974) studied the action of various pesticides on the mycelial growth of isolates of the fungus *Verticillium* spp. and observed that both endosulfan and copper oxichloride moderately affected the fungus at their highest concentrations. These differences in results may be related to the different types of formulations used in the experiments. Endosulfan and deltamethrin were classified as incompatible at any concentration, while azodrin was compatible at the maximum concentration and incompatible at the minimum concentration (Tables 5 and 6). The insecticide diafentiuron had no negative effect on reproductive or vegetative growth when used at the minimum concentration. However, at maximum concentration, the insecticide prevented any conidial production even though vegetative growth was normal. Thus, diafentiuron was classified as incompatible for *S. insectorum* (Tables 5 and 6).

Statistical analysis showed that the reproductive and vegetative growth of *M. anisopliae* was not affected by thiamethoxam, imidacloprid or acephate used at maximum concentrations. However, at minimum concentrations, imidacloprid and acephate significantly reduced conidial production even though they did not affect colony diameter, demonstrating the absence of correlation between these parameters (Table 3). These two insecticides were classified as moderately toxic for *M. anisopliae* at the maximum dose and incompatible at the minimum dose (Tables 5 and 6).

The remaining insecticides affected the reproductive growth of the fungus regardless of concentration, except for deltamethrin 25 CE which was statistically similar to the control at the minimum concentration (Table 3). With respect to vegetative growth, the product showed the opposite behavior, with no difference from the control at maximum concentration (Table 4). This product was classified as moderately toxic and compatible for *M. anisopliae* (Tables 5 and 6). These results partially disagree with those reported by Camargo (1983) who observed that *M. anisopliae* is inhibited by different concentrations of pyrethroid insecticides. Deltamethrin had the highest inhibitory action. In the current study, the *M. anisopliae* colonies that grew in the presence of deltamethrin differed considerably in morphological aspects from untreated colonies, presenting irregular borders, vertical growth of the central part, changes in conidial color, and circular regions of white cotton-like mycelium.

Carbosulfan and diafentiuron showed a similar effect, namely not affecting the vegetative growth of the fungus but reducing conidial production (Tables 3 and 4). Carbosulfan used at a single concentration that was classified as incompatible, while diafentiuron was incompatible at maximum concentration and moderately toxic at minimum concentration (Tables 5 and 6). The same classification was obtained for the insecticide fipronil.

Among the insecticides tested, monocrotophos CE and endosulfan CE were those that most inhibited the fungus (Tables 3 and 4), being classified as incompatible with *M. anisopliae* (Tables 5 and 6). These results are similar to those obtained by Alves (1998), who observed that monocrotophos and endosulfan were moderately compatible at the minimum dose recommended and incompatible at the medium and maximum concentrations. On the other hand, thiamethoxam was compatible with the entomopathogen at both concentrations.

Monocrotophos, endosulfan and deltamethrin were the most harmful insecticides to *B. bassiana* development (Tables 3 and 4). As also observed for *S. insectorum* and *M. anisopliae*, endosulfan was classified as incompatible at both concentrations (Tables 5 and 6). These results partially agree with those reported by Alves (1986) who, in a study of endosulfan compatibility for *B. bassiana* and *M. anisopliae* at three concentrations (minimum, medium and maximum recommended concentrations), concluded that endosulfan was incompatible at medium and maximum concentrations and moderately compatible at minimum concentration, possibly harming fungal growth and sporulation. Mixture at subdosage was recommended.

Thiamethoxam, diafentiuron and acephate were compatible with *B. bassiana*, with no effect on reproductive or vegetative growth. Carbosulfan was classified as incompatible, significantly affecting conidial production, and imidacloprid was moderately compatible at both concentrations (Tables 3, 4 and 6). Imidacloprid was considered compatible by Alves *et al*. (1998). According to Roberts & Campbell (1977), the susceptibility of entomopathogenic fungi to chemical products varies widely among pesticides and among fungal isolates. This fact may explain the different results obtained by different authors.

For *B. thuringiensis*, the formation of two distinct groups is observed when the maximum concentration is considered. The first group, represented by the insecticides that did not differ significantly from the control treatment, consisted of thiamethoxam, carbosulfan, diafentiuron, imidacloprid, acephate, and fipronil (Table 7). Within this group, acephate

Table 7. Mean number of colony forming units of *B. thuringiensis* in culture medium containing insecticides at the maximum and minimum concentrations recommended for the field (Temperature 30ºC and photoperiod of 14h).

Insetictides (n=3)	Colony forming units (CFU)	
	Maximum concentration	Minimum concentration
Control	15.8 ± 2.51 ab	15.8 ± 2.51 cd
Thiamethoxam	43.9 ± 26.05 a	23.7 ± 3.78 bcd
Carbosulfan[2]	14.5 ± 1.76 ab	14.5 ± 1.76 cd
Endosulfan	0.0 ± 0.00 c	0.5 ± 0.66 d
Diafentiuron	9.9 ± 0.57 bc	105.5 ± 46.40 a
Imidacloprid	44.4 ± 11.24 a	18.3 ± 6.43 cd
Acephate	10.6 ± 2.51 bc	15.9 ± 0.57 cd
Monocrotophos	0.0 ± 0.00 c	12.1 ± 4.25 cd
Deltamethrin	0.0 ± 0.00 c	0.0 ± 0.00 d
Fipronil	20.8 ± 2.88 ab	43.1 ± 11.34 abc
Variation Coef. (%)	32	34

Means and ME followed by the same letter in the columns did not differ by the Tukey test at the 5% level of significance. Data transformed to $\sqrt{x+0.5}$.
[1]Single concentration.

has been reported to be one of the most compatible with *B. thuringiensis*, being recommended for use in programs of integrated management with bacteria (Morris 1977). The author also reported that the insecticides belonging to the carbamate group did not affect the multiplication of *B. thuringiensis*.

The second group consisted of endosulfan, monocrotophos and deltamethrin, which fully inhibited colony formation. With respect to deltamethrin, Morris (1977) showed that pyrethrins are highly bacteriostatic for the entomopathogen.

When the behavior of the bacterium was analyzed in the presence of the minimum concentration of the insecticides, most of the products did not differ significantly from the control, although endosulfan and deltamethrin practically inhibited colony formation. However, the occurrence of bacterial multiplication in the medium to which diafentiuron and fipronil were added should be pointed out. In these cases CFU number was very high, with a significant difference from the control treatment. Future studies may clarify whether there is this synergistic effect, as extensively reviewed by Benz (1971).

On the basis of the results obtained, it is clear that the action of pesticides on the vegetative growth and sporulation of microorganisms varied as a function of the chemical nature of the products, and their concentration, and species of the biocontrol agent. The extreme situations are represented by thiamethoxam, which proved to be compatible with all microbial agents, and by endosulfan, which was classified as incompatible. According to Morris (1977), the presence of emulsifiers and other additives in concentrated emulsifiable formulations contributes to the problem of insecticide compatibility with entomopathogens, thus representing an additional important factor to be controlled in the elaboration of new commercial formulations. Thus, even though the lethal action of some insecticides has been observed, we cannot state that the commercial products and their respective formulations were responsible for this result rather than the active ingredients themselves.

It should be emphasized, however, that these results were obtained in *in vitro* tests with maximum entomopathogen exposure to the insecticides, a fact that guarantees selectivity under field conditions for the insecticides classified as compatible. According to Alves *et al*. (1998), the high toxicity of the product *in vitro* does not always suggest a high toxicity in the field, but rather indicates only the possibility of the occurrence of damage of this nature. The authors state that the major problem of this type of study is the lack of standardization of the test conditions, which in most cases does not permit effective comparison between products.

Major Species of Entomopathogenic Fungi and Bacteria Used in Brazil X Thiamethoxam Under Field Conditions. Considering the three evaluations, it can be seen that thiamethoxam did not interfere with mean UFC number regardless of the microorganism studied. Analysis of Table 8 demonstrates that there was no significant difference when the microorganism was applied to fields treated or not with the insecticide. No difference in CFU production was observed between microorganisms during the first 48h.

Evaluation of compatibility 72h after application of the chemical and biological products was limited to fungi due to the great concentration of contaminating bacteria in the treatments involving the insecticides and *B. thuringiensis*. This fact may be explained by the action of climatic and natural factors such as the wind and the dissemination by arthropods, in addition to the absence of antibactericidal agents in the NA medium. A similar situation was observed for the thiamethoxam treatment, in which the presence of contaminating bacteria and of *B. thuringiensis* was observed. With respect to fungi, *M. anisopliae* showed a large CFU number in both the treatments with and without an insecticide, higher than that observed for *B. bassiana*. The progressive increase in CFU number observed along time for the entomopathogens demonstrates the dispersal ability of the agents and their potential for causing primary foci of the disease.

Table 8. Mean number of colony forming units of *M. anisopliae*, *B. bassiana* and *B. thuringiensis* on bean leaves submitted to the maximum concentration of the thiamethoxam insecticide.

Treatments (n=12)	24h	48h	72h
Control	0.0 b	0.0 b	0.0 c
Thiamethoxam	0.0 b	0.0 b	12.6 ± 7.03 ab
B. thuringiensis (B.t.)	2.9 ± 2.24 ab	1.7 ± 0.48 ab	-[1]
B.t. + Thiamethoxam	4.8 ± 3.10 a	2.3 ± 1.65 a	-[1]
M. anisopliae (M.a.)	1.1 ± 0.30 ab	0.9 ± 0.27 ab	20.8 ± 5.67 a
M.a. + Thiamethoxam	1.2 ± 0.42 ab	1.1 ± 0.32 ab	21.9 ± 9.67 a
B. bassiana (B.b.)	3.2 ± 0.90 ab	0.9 ± 0.60 a	5.4 ± 5.06 bc
B.b. + Thiamethoxam	2.7 ± 0.71 ab	1.2 ± 0.40 ab	4.3 ± 2.75 bc
Variation Coef. (%)	9	6	19

Means and ME followed by the same letter in the columns did not differ by the Tukey test at the 5% level of significance. Data transformed to log x + 10.

[1]Data not analyzed after 72h.

The field results confirm those obtained *in vitro* when thiamethoxam was classified as compatible with these microorganisms. This study suggests that the application of thiamethoxam for the control of pests such as the whitefly in bean crops, for example, does not interfere with the inoculum potential of *B. thruringiensis*, *B. bassiana* and *M. anisopliae*, microorganisms that are present in agroecosystems and that are responsible for control through the natural occurrence of the entomopathogen or through the use of strategic application. We also emphasize the possibility of the combined or alternate use of the insecticide with microbial control to combat a given pest as long as more specific studies are conducted with this objective and that this process is established as an alternative for the integrated management of pests.

Most of the studies carried out to detect the effect of pesticides on entomopathogens have been carried out *in vitro*. There is a need for data about these interactions under field conditions and for standardization of the methods. In Brazil, Barbosa *et al*. (1997) and Alves *et al*. (1998) tested the effect of insecticides on velvetbean caterpillars and on coffee berry borers, respectively. In the first case the authors concluded that natural infection of *N. rileyi* was reduced by the insecticides metamidaphos, endosulfan, diflubenzuron, thiodicarb and methyl parathion, while trichlorfon and chlorpyriphos ethyl did not affect the fungus. In the second case, Alves *et al*. (1998) observed that where endosulfan was applied more than once there was a reduction in CFU number of the fungus *B. bassiana*. The authors concluded that triadimenol/disulfoton + *B. bassiana* (two applications) could be an alternative for the integrated management of coffee plant pests.

The combination of pesticides with *B. thuringiensis*, *B. bassiana* and *M. anisopliae* is a strategy that may make the use of these microorganisms viable, with more efficient and economic applications. Thus, Morris (1976), Leite *et al*. (1992) and Batista Filho *et al*. (1995) studied the combination of these microorganisms with pesticides and other compounds and observed a synergistic effect in some situations.

Naturally Occurring Microorganisms X Thiamethoxam in Laboratory. Fungal behavior varied in the presence of different insecticide concentrations. For *P. farinosus* and *N. rileyi*, no differences in conidial production were observed compared to control, regardless of the concentration used (Table 9), and the microorganisms were thus classified as compatible (Table 10). *In vitro* studies by Ignoffo *et al.* (1975) using different pesticides demonstrated that the growth of *N.*

Table 9. Classification of thiamethoxam in terms of toxicity for entomopathogenic fungi.

Fungo	VG(%)[1]		RG(%)[2]		T[3]		Classification[4]	
	Max. C	Min. C	Max. C	Min. C	Max. C	Min. C	Max. C	Min. C
P. farinosus	100.0	100.0	180.6	110.4	164.5	108.3	C	C
N. rileyi	100.0	100.0	128.5	98.5	122.8	98.8	C	C
H. thompsonii	95.1	75.4	86.0	52.8	87.8	57.3	C	MT
V. lecanii	117.2	114.1	159.0	175.4	150.6	163.1	C	C
A. aleyrodis	117.3	108.7	58.8	72.7	70.5	80.0	C	C

[1]VG – Vegetative growth; [2]RG – Reproductive growth
[3]Max. C – Maximum concentration; Min. C. – Minimum concentration
[4]C – Compatible; MT - Medium toxicity

Table 10. Mortality rate of *A. gemmatalis* induced by NPVAg and thiamethoxam, in combination or not, for the evaluation of compatibility between insecticide and virus (Campinas, SP, 2000).

Treatments (n=3)	Mortality (%)		
	Insecticide	Virus	Other causes
Control	0.0 b	0.0 c	14.6 ± 2.88 ab
Thiamethoxam	45.9 ± 6.01 a	0.0 c	28.1 ± 3.33 a
Thiamethoxam+NPVAg[1]	45.2 ± 8.81 a	45.9 ± 6.66 b	5.8 ± 3.33 bc
Thiam.+NPVAg. Centrif.[2]	0.0 b	98.3 ± 1.66 a	1.4 ± 1.66 c
NPVAg	0.0 b	98.3 ± 1.66 a	1.4 ± 1.66 c
Variation Coef. (%)	5%	2%	8%

Means and ME followed by the same letter did not differ by the Tukey test at the 5% level of significance. Data were transformed to log x + 10.
[1]Virus mixed with the insecticide and immediately applied in the field.
[2]Virus kept in suspension with the insecticide for 12h and then centrifuged for the separation of viral polyhedra that were applied in the field.

rileyi was inhibited by almost all fungicides, some insecticides and herbicides used for soy crops, even when the products were tested at lower concentrations than technically recommended. Among the most harmful insecticides were monocrotophos and methyl parathion. Diflubenzuron, corbofuran, methomyl, acephate and carbonil did not inhibit fungal growth. In other *in vitro* studies, methyl parathion, monocrotophos, profenophos, chlorpyriphos, trichlorfon and endosulfan inhibited mycelial development and/or sporulation of *N. rileyi*, while DDT, malathion, acephate, diflubenzuron and permethrin had no deleterious effect on the fungus (Ignoffo *et al*. 1975, Gardner *et al*. 1979, Barbosa *et al*. 1986, Silva *et al*. 1993).

For the fungus *H. thompsonii*, thiamethoxam reduced conidial production at the minimum concentration tested (100 g/ha) compared to the highest concentration tested (800 g/ha) and to the control. On the other hand, vegetative growth was not affected, being significantly higher than in the control and similar to that obtained with the maximum concentration (Table 11). Considering the two parameters applied to the formula for T determination, the product was classified as moderately toxic at the minimum concentration and compatible at the maximum concentration (Table 9). It should be pointed out that the fact that some products affect conidial production without impairing mycelial growth has also been observed by Alves *et al*. (1993), in a study of the fungitoxic effects of some commercial insecticide formulations on some fungi used for agricultural pest control. By comparing the results obtained in the analysis of the two variables (colony diameter and number of conidia produced), the authors showed that there was no constant correlation between these parameters.

With respect to the fungus *V. lecanii*, greater reproductive growth was observed when the microorganism was inoculated into the culture medium containing the insecticide thiamethoxam at minimum concentration compared to control (Table 11). With respect to vegetative growth, the diameters of the colonies were similar in all treatments. On the basis of these results, the product was classified as compatible with *V. lecanii* (Table 9). In a study of the effects of some chemical products on this same fungus and also on *H. thompsonii* and *Aschersonia aleyrodis*, Alves *et al*. (1993) concluded that ferbutatin, fenproprathrin and quinomethionate oxide were the most selective ones. In another study, Olmert & Kenneth (1974) tested the compatibility of nine fungicides and 14 insecticides with the fungus *V. lecanii* and observed that almost all products caused some growth inhibition.

With respect to the reproductive growth of the fungus *A. aleyrodis* submitted to the maximum concentration, a lower conidial production and a greater vegetative growth were observed as compared to control (Table 11), confirming the fact that the action of pesticides on the vegetative growth and sporulation of entomopathogenic fungi varies with product concentration and microorganism species, possibly affecting fungal reproduction without reducing vegetative growth. Regardless of the concentration tested, thiamethoxam was classified as compatible. According to Alves (1986), ethyl parathion at the doses of 0.05 and 0.2% a.i. and vamidothion at the dose of 0.06% a.i. did not impair the sporulation of *A. aleyrodis*. On the other hand, diazinon and monocrotophos at the dose of 0.2% a.i. inhibited conidial production by this microorganism.

An important aspect of studies conducted to test the effects of pesticides on entomopathogenic fungi is the methodology adopted. The method used by Ignoffo *et al.* (1975) in the study of the action of various products on *N. rileyi* differed completely from that used by Olmert & Kenneth (1974) in similar studies conducted on *V. lecanii*. In the first case, the authors evaluated this action by measuring the diameter of the inhibition halo formed around the disk containing the pesticide. In the technique used by Olmert & Kenneth (1974), the pesticides were added to the culture medium and the diameters of the fungal colonies were later measured for the evaluation of the toxic effect. In these situations, a comparison becomes difficult.

The results obtained in the laboratory permit us to conclude that thiamethoxam is compatible with the major fungi that occur naturally controlling insects and mites, considering the maximum exposure of the microorganism to the action of the insecticide when submitted to *in vitro* studies. Thus, the insecticide should not affect the inoculum potential of the pathogen, favoring strategies of microbial control of he conservation type.

Table 11. Reproductive and vegetative growth of entomopathogenic fungi in culture medium containing thiamethoxam at the maximum and minimum concentrations recommended for field use (Temperature of 26ºC and photoperiod of 14h).

Fungi (n=9)	Reproductive growth (x10^7 conidia/ml)				Vegetative growth (colony diameter - cm)			
	Maximum conc.	Minimum Conc.	Control	CV(%)	Maximum conc.	Minimum Conc.	Control	CV(%)
P. farinosus	6.1 ± 1.14 a	3.7 ± 0.75 a	3.3 ± 2.55 a	38	-[1]	-[1]	-[1]	
N. rileyi	1.7 ± 0.62 a	1.3 ± 0.12 a	1.3 ± 0.08 a	20	-[1]	-[1]	-[1]	
H. thompsonii	11.3 ± 1.00 ab	6.9 ± 1.55 b	13.2 ± 0.79 a	20	3.8 ± 0.06 a	4.1 ± 0.31 a	2.9 ± 0.09 b	7
V. lecanii	0.9 ± 0.10 ab	1.1 ± 0.13 a	0.6 ± 0.10 b	12	2.2 ± 0.07 a	2.2 ± 0.04 a	1.9 ± 0.14 a	6
A. aleyrodis	6.3 ± 0.66 b	7.8 ± 1.02 ab	10.8 ± 1.65 a	17	1.4 ± 0.03 a	1.3 ± 0.04 a	0.9 ± 0.13 b	8

Means and ME followed by the same letter on the line did not differ by the Tukey test at the 5% level of significance. Data were transformed to $\sqrt{x+0,5}$.

[1]Data not used for statistical analysis due to the use of a full dish.

Baculovirus anticarsia (NPVAg) x Thiamethoxam Under Field Conditions. Analysis of Table 10 clearly shows that the NPV of *A. gemmatalis* was not inhibited by thiamethoxam, not even in the centrifuged treatment when it remained exposed to the insecticide for 12h and showed an excellent performance. It caused infection of about 98% of the insect population, identical to that observed for the virus used as control. This behavior agrees with data reported by Jaques & Morris (1981), who stated that most chemical insecticides, when mixed with entomopathogenic viruses, do not affect the biological activity of the pathogen.

Interestingly, in the treatment in which NPVAg was mixed with thiamethoxam shortly before application there was equilibrium in the mortality rates caused by the disease and by the action of the insecticide, each contributing with about 45% efficiency, with 90% of the deaths occurring within the first 72h of supplying contaminated food in the case of the chemical product (Table 10). The highest concentration of caterpillers killed by the virus occurred between the 7th and 11th day, for a total 86% rate of insects infected with the disease. During this same period of time, for the treatment in which the virus was used as a standard the mortality rate was 88%. The compatibility of the combination of virus and insecticide was confirmed also in this case.

These results permit us to conclude that thiamethoxam applications can be made to control whiteflies in soy culture without any harmful effect on the action of the virus for the control of velvetbean caterpillars. Indeed, the possibility exists of a joint application of these control agents when both pests are present at the same time. The use of the virus in a mixture with reduced thiamethoxam doses for the control of velvetbean caterpillars under conditions of infestation above the limits established for its application alone needs further studies for the determination of its efficiency. In this respect, Moscardi & Sosa-Gómez (1992) observed that the NPV of the velvetbean caterpillar mixed with several insecticides at concentrations up to 1/6 of the recommended ones resulted in adequate control of pest populations when they occurred beyond the limits established for the use of the virus alone. Similar results were obtained by Silva (1995) for extremely elevated population densities. According to Moscardi (1998), this strategy may be useful for cases in which the pest population exceeds the limit for use of NPV alone, considering that reduced doses of insecticide would involve less of human intoxication risk, reduction of populations of natural enemies and environmental damage. From an economic viewpoint, Crébio & Melhorança (1999) evaluated the possibility of applying NPVAg mixed with post-emergent insecticides and concluded that this technique is viable for soy culture management.

Acknowledgments

To Dr. Luís Francisco Angeli Alves (Centro de Ciências Biológicas e da Saúde Universidade Estadual do Oeste do Paraná) and Dr. Donald W. Roberts (Utah State University) for reviewing the manuscript, and to Dr. Valmir Antonio Costa (Instituto Biológico) for photographic documentation.

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Received 14/VI/2000. Accepted 30/VIII/2001.