SCREENING OF BIOINSECTICIDES AGAINST THE COTTON BOLLWORM ON COTTON[1]

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ABSTRACT - Four bioinsecticides and a synthetic pyrethroid were evaluated against the cotton bollworm (*Heliothis zea* Boddie, 1850), (Lepidoptera: Noctuidae) in laboratory. Treatments were: cypermethrin, *Bacillus thuringiensis* var. *kurstaki* Berliner (BT), *Steinernema feltiae* Weiser, *Heliothis* Nuclear polyhedrosis Virus (HNPV), *Beauveria bassiana* (Bals) and the untreated check, applied over 2,8 cm^2 cotton leaf discs in three different concentrations. Mortality rate was recorded at one, two, seven and nine days after treatment. The efficiency of the bioinsecticides was also tested in a greenhouse. Treatments were the same but instead of HNPV an experimental formulation of BT (Mycogen®) was used. Each treatment consisted of four plants (Coker 310) sprayed with insecticide and infested with nine neonate larva. Results were evaluated seven days after treatment. In laboratory cypermethrin and BT showed a significant difference compared to control at day 1, despite concentrations. They did not differ statistically from each other. *S. feltiae* showed a significant mortality rate to control after day 2. HNPV was highly effective between two and seven days after treatment, killing all larvae. In greenhouse cypermethrin showed a significant difference compared to check.

Index terms: microbial control, pyrethroid, cotton pest management.

EFICIÊNCIA DE INSETICIDAS MICROBIANOS SOBRE A LAGARTA-DA-MAÇÃ EM ALGODÃO

RESUMO - Foi testada, em laboratório, a eficiência de quatro bioinseticidas e um piretróide contra a lagarta-da-maçã (*Heliothis zea* Boddie, 1850) (Lepidoptera: Noctuidae). Os tratamentos foram: cypermethrin; *Bacillus thuringiensis* var. *kurstaki* Berliner (BT); *Steinernema feltiae* Weiser; vírus da poliedrose nuclear de *H. zea* (HNPV); *Beauveria bassiana* (Bals), e o controle, em um delineamento completamente casualizado, com 20 repetições. Discos de folhas de algodão (Coker 310) com 2,8 cm^2 foram imersos nas soluções em três diferentes concentrações. Cada disco recebeu uma lagarta. A mortalidade foi avaliada após um, dois, sete e nove dias. Em casa de vegetação foram conduzidos quatro experimentos, com delineamento totalmente casualizado, com quatro repetições. Os produtos testados foram os mesmos, exceto HNPV, que foi substituído por BT (Mycogen®). Cada tratamento consistiu de quatro plantas de algodão (Coker 310) com botões, pulverizadas com os inseticidas e infestadas com dez lagartas de primeiro estádio. Dez botões do terço superior foram examinados sete dias após o tratamento. Em laboratório, cypermethrin e BT apresentaram diferenças significantes em relação ao controle no dia-1. *S. feltiae* mostrou índices de mortalidade que diferiram do controle no dia-2. HNPV mostrou 100% de mortalidade entre o dia-2 e o dia-7. Em casa de vegetação, cypermethrin mostrou diferença significante em relação ao controle nos quatro testes realizados.

Termos para indexação: controle microbiano, piretróide, manejo de pragas do algodão.

INTRODUCTION

The cotton bollworm, *Heliothis zea* (Boddie, 1850), is a worldwide important insect pest. It is especially noxious to cotton due to the damage of floral buds and because of the increasing difficulties in obtaining an acceptable control. Applications of different chemicals have triggered resistance

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mechanism to most classes of insecticides (Bull et al., 1979; Jackson, 1989). This fact has encouraged a search for alternative methods for management and suppression of the pest including bioinsecticides.

The literature on microbial control of the bollworm is abundant. Much attention has been given to the usage of *Bacillus thuringiensis* Berliner (BT) because of its safe mode of action producing a crystalline protein (∂-endotoxin) capable of paralyzing the larval gut, causing death. The use of the nematode *Steinernema feltiae* Weiser (=Neoaplectana carpocapsae) has increased tremendously because of its wide host range and ease mass propagation (Kaya & Burlando, 1989). This nematode is known by its mutualistic relationship with the bacteria *Xenorhabdus nematophilus* which is ultimately responsible for the death of the larvae. In the past 20 years fungi agents have played an important role in insect control programs. The advantages are both of being saprophitic and able to penetrate directly to the insect's cuticle although they are limited by the environmental conditions (Ferron, 1981). Interesting results with *Beauveria bassiana* (Bals) as larvicide for *Heliothis* spp. were reported by Gardner & Noblet (1978) and Pekrul & Grula (1979). Though more than 200 viral diseases are reported in insects of important commodities, only a few have been considered significant for applied control (Yearian & Youngs, 1982). In 1965 a feasible mass production of *H. zea* Nuclear Polyhedrosis Virus (HNPV) was reported by Ignoffo et al. (1965), and in 1976 it was registered under the name Elcar™.

Despite the advantages of using microbial control, chemical insecticides continue to be an indispensable means of controlling insect outbreaks because of their rapid, effective and flexible action in various crop situations. Pyrethroids, for instance, are active against all larval stages of *Heliothis* spp. The efficacy of pyrethroids used alone (Davis et al., 1975) or mixed with other insecticides (All et al., 1977) for control of bollworm is well established.

This study reports the results of a laboratory experiment treating cotton bollworm larva with four bioinsecticides in three different concentrations compared to a pyrethroid. A greenhouse bioassay was conducted to test the efficiency of four bioinsecticides based in the laboratory results.

MATERIALS AND METHODS

Laboratory test. This experiment was done at the laboratory facilities at the Entomology Department at University of Georgia. The solutions, except for the nematode and the pyrethroid, were tested at a standard dosage, one higher dosage (twice the standard) and one lower dosage (half of the standard). The treatments applied were: Cypermethrin (Cymbush™ 50 CE, ICI Corporation, Wilmington DE) at 7.19×10^{-5}, 0.71×10^{-5}, 71.9×10^{-5} g ia/ml; *B. thuringiensis* var. *kurstaki* (Dipel™ WP, with 2.5×10^{10} conidium/g, Abbott Laboratory, Chicago IL) at 1.2×10^{-2}, 2.4×10^{-2} and 0.6×10^{-2} g/ml; *S. feltiae* (cultures from *Galleria mellonella* (L.), University of Georgia) at 5.0×10^{3}, 1.0×10^{4} and 1.0×10^{3} infective juveniles/ml; *Heliothis* NPV (Elcar™ with 4×10^{9} PIB/g, Sandoz Corporation Protection Company, Desplaines IL) at 1.5×10^{-3}, 3.0×10^{-3} and 7.5×10^{-4} g/ml; *B. bassiana* [ABG-6178 (experimental formulation) Abbott Laboratory, Chicago IL] at 1.0×10^{-2}, 2.0×10^{-2} and 0.5×10^{-2} g/ml and the control. Each treatment consisted of twenty discs of 2.8 cm^2 of fresh cotton leaf (variety Coker 310) immersed in one of the insecticide solutions for 5 s. After dried out each leaf disc was placed into 1 oz--plastic cup plus one neonate larva. The cups were sealed and kept at 27 °C. After 24 hours all alive larvae were transferred to cups with Pinto Bean artificial diet. The percentage of larval mortality was recorded at one, two, seven and nine days. Differences between and within treatments means were compared by an Analysis of Variance and separated by Duncan's multiple range test (SAS, 1982). Based on the results of the laboratory test a greenhouse experiment was conducted using the same formulations.

Greenhouse test. The experiment was conducted in a greenhouse at the Southern Piedmont Extension Station in Watkinsville, Ga. The cotton plants (Coker 310) growing in plastic vases were set in stainless steel trays and watered by dripping irrigation.

Plants were sprayed in a chamber made of plastic walls having at the top a rotatory spray bar equipped with three hollow cone nozzles. A field situation was simulated with the equipment delivering 227 liters of insecticide solution per hectare at a pressure of 1.1 kg/cm^2 in a speed of 4.8 km/h. The treatments were: Cypermethrin at 0.054 kg a.i./ha; *B. thuringiensis* at 1.12 kg/ha; *B. thuringiensis* [(Mycogen™) (experimental formulation)] at 9.35 kg/ha; *S. feltiae* at 7.00×10^{8} infective juvenile/ha; *B. bassiana* (ABG-6178) at 4.2 kg/ha and a larval-infested check.

The sprayed plants were allowed to dry and then infested with ten neonate *H. zea* larvae distributed in the squares on the upper third canopy. A complete randomized

block design with six treatments and four repetitions was conducted. Plants were infested twice. The top ten damaged squares were tallied at seven days after infestation. The experiment was repeated four times at seven days interval. Data were analysed by an Analysis of Variance for a complete randomized block design and means were separated at P = 0.05 level by Duncan's multiple range test (SAS, 1982).

RESULTS AND DISCUSSION

Laboratory Assay. Statistic analysis is presented in Table 1. The first day showed the prompt effect of cypermethrin causing high mortality. All concentrations had significant differences comparing to check. Even the lowest concentration of cypermethrin was effective showing its characteristic "knock down" effect (Miller & Salgado, 1985). BT was also highly effective and did not differ from cypermethrin which is recommended against the first instar larva. This result coupled with those obtained for *H. zea* in soybean by Ignoffo et al. (1977). *S. feltiae* showed a highly significant difference in comparison with the control. Despite this, it should be considered as an intermediate control agent between the highly effective group (cypermethrin and BT) and the low effective group of insecticides (HNPV and *B. bassiana*). Bari & Kaya (1984) working with *Platyptilia carduidactyla* (Riley) also concluded that *S. feltiae* had a low effect against first instar larva. Bong (1986) obtained the best results against *H. zea* in corn using *S. feltiae* at 4 x 10^4 nematodes/ml after ten days. None of the treatments showed any significant difference related to the concentration of the insecticide solutions.

At the second day a significant percentage of mortality was observed in the treatment with *S. feltiae* compared to control. Since no further significant increase in the larvae mortality was recorded, an appreciation of the economic damage by the remaining population should be considered

TABLE 1. Mortality rate of *Heliothis zea* larvae on leaf discs of cotton (var. Coker 310) treated with four bioinsecticides and one pyrethroid in three different concentrations at one, two, seven and nine days after treatment.

Treatment	Rate		Control		Number of live larva at day			
					1	2	7	9
	71.90	x 10^{-5} g ia/ml	20a	00	Fb	00 Eb	00 Eb	00 Eb
Cypermethrin	7.19	x 10^{-5} g ia/ml	20a	00	Fb	00 Eb	00 Eb	00 Eb
	0.71	x 10^{-5} g ia/ml	20a	06	DEb	02 DEc	02 DEc	02 DEc
	2.40	x 10^{-2} g/ml	20a	04	EFb	00 Ec	00 Ec	00 Ec
B. thuringiensis	1.20	x 10^{-2} g/ml	20a	04	Ebc	01 Ec	00 Ec	00 Ec
	0.60	x 10^{-2} g/ml	20a	04	EFb	01 Ec	01 Ec	01 Ec
	1.00	x 10^{4} ij/ml	20a	10	CDb	06 CDc	03 CDEc	03 CDEc
S. feltiae	5.00	x 10^{3} ij/ml	20a	14	BCb	06 CDc	04 CDEc	04 CDEc
	1.00	x 10^{3} ij/ml	20a	12	Cb	07 Cc	05 CDc	05 CDc
	3.00	x 10^{-3} g/ml	20a	19	Aa	14 Bb	00 Ec	00 Ec
HNPV	1.50	x 10^{-3} g/ml	20a	19	Aa	17 ABa	00 Eb	00 Eb
	0.75	x 10^{-3} g/ml	20a	20	Aa	18 ABa	00 Eb	00 Eb
	2.00	x 10^{-2} g/ml	20a	18	ABa	16 ABa	07 Cb	07 Cb
B. bassiana	1.00	x 10^{-2} g/ml	20a	19	ABa	17 ABab	15 Bb	15 Bb
	0.50	x 10^{-2} g/ml	20a	20	Aa	19 Aa	13 Bb	13 Bb
Control			20A	20	A	19 A	19 A	19 A

Between treatment values in a column followed by the same capital letter are not significantly different (p = 0.05; Duncan's multiple range test). Within treatment values in a row followed by the same lower case letter are not significantly different (p = 0.05; Duncan's multiple range test).

for *S. feltiae* profitable use. Since nematode concentrations did not differ significantly, using the lower concentration (1000 ij/ml) would be recommended. Similar result was achieved by Bari & Kaya (1984) working with *P. carduidactyla,* who concluded that the lowest effective concentration of *S. feltiae* was 1000 ij/ml. HNPV was ineffective in the first two observations except for the highe: concentration (3.0×10^{-2} g/ml) on the second da This was expected since viruses have relative slo action taking several days between vira consumption by the larva and its death (Stance et al., 1970). According to Alves (1986) the deatl of small larvae should be expected three days afte the ingestion. *B. bassiana* was also totally ineffectiv to the second day. Pekrul & Grula (1979) state i ideal conditions *B. bassiana* takes at least 18 hour to germinate and begin penetration in the larva' cuticle. Effects of the cuticle chemical compositio (Ferron, 1981) coupling with the appropriate enzymatic activity of the fungi's hypha (Smith et al 1981) may have a profound effect in the infectiou process.

At the seventh day HNPV presented a hig effective control achieving 100% mortality i treatments. The length of time needed for the viru to infect and kill the larvae was between three an seven days, confirming observations of Stancey et a (1970). The larvae died in the second instar havin their body characteristically disrupted, liquified an tarnished. This bioinsecticide may be used in IPM programs for control of the bollworm when time i not a crucial factor. *B. bassiana* reached significan results from the control with the highes concentration being most effective. The mummifiec bodies showed the caracteristic conidia-bearing mycelium covering completely the cadaver. This bioinsecticide had a low effect and little value as a microbial insecticide for control of bollworm in the conditions of this experiment. A similar conclusion was achieved by Tanada & Reiner (1962). No other different results were observed at a nine-day period.

Greenhouse Test. The percentage of damaged squares for each of the four tests is shown in Fig. 1. The first test shows a large numerical, but not significant, difference in the percentage of squares damaged between cypermethrin (0%), *S. feltiae* (0%), Mycogen (4.6%) and the control (20.4%). Cypermethrin showed its quick-killing action with a full protection effect. BT and *B. bassiana* showed intermediate results.

In test two, the pyrethroid treatment showed again no damaged squares, being significantly different

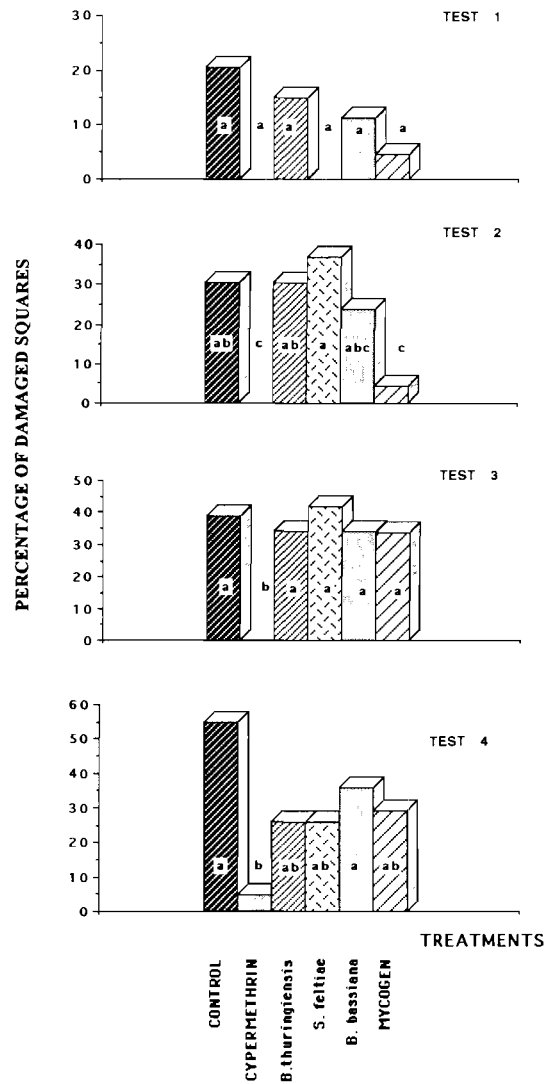

FIG. 1. Percentage of damage squares by *Heliothis zea* in cotton plants treated with four insecticides in greenhouse. (Columns in a same graphic with the same letter are not significantly different at p = 0.05 using Duncan's New Multiple Range Test).

compared to the control. Pyrethroids are very effective against all larval stage of *Heliothis* spp. especially the first instar larva and are currently being employed for late season pest control in cotton (Elliott et al., 1978; Miller & Salgado, 1985). Mycogen (4.6%) had a significant performance compared to the pyrethroid (0%) and the control (30.6%). Also, the treatment with Mycogen (4.6%) showed a significant lower percentage of squares damaged than that with Dipel (30.3%). For BT (30.3%), *B. bassiana* (30.3%) and *S. feltiae* (36.9%) the percentage of damage squares was not significantly different as compared with the untreated check (30.6%) representing a whole lack of efficiency. Work by Bull et al. (1979) showed low efficiency of BT against bollworm leading to the conclusion that microbial pesticides may not work well in heavy infestations. This result is also corroborated by Ignoffo et al. (1977) and Bull et al. (1976). On the other hand, Ignoffo et al. (1977) observed a 69-96% reduction in the population larvae of *H. zea* in soybeans.

In tests 3 and 4 only cypermethrin (0% and 4.6%) gave significant level of protection relative to check (39% and 54.7%, respectively). An aspect to be considered is that the young larval stage is more susceptible to microbial infection than latter stages (Maddox, 1982).

In these experiments, the same plants were infested four times, the larvae surviving on the first insecticide application developed into further instars. This fact may have accounted for the lack of control observed in the treatments except for the cypermethrin which is active against all larval stages (Miller & Salgado, 1985). *S. feltiae* does not seem to be an appropriated pathogen for insect control above soil. Tanada & Reiner (1962) also concluded that the nematode DD-136 had low efficiency against *H. zea* when compared with HNPV and BT.

In the experiment the application was directed to the foliage without any protection. Probably, when the leaves dry out, the nematodes cannot move to search for host larvae becoming vulnerable to environment. Bong (1986) reported 70% mortality after second-day application and economic damage was not prevented. Mycogen should be considered for in other tests because it was effective against the cotton bollworm larvae in the first two tests.

CONCLUSIONS

1. Laboratory test at day-1 post applications showed that Cypermethrin and BT were very effective against *H. zea* larva in all the concentrations used. *S. feltiae* had a satisfactory result. At day-2 a high larval mortality was recorded for *S. feltiae*. HNPV was effective only for the highest concentration. At day-7 HNPV was highly effective in all concentrations used. *B. bassiana* also showed high larval mortality.

2. Test 1 in greenhouse showed no significant results in the percentage of damaged squares between treatments with Cypermethrin, *S. feltiae*, Mycogen and the control. In test 2 only Cypermethrin and Mycogen showed significant results. In tests 3 and 4 Cypermethrin was the only treatment with significant results compared to check.

REFERENCES

ALL, J.N.; ALLI M.; HORNYAK, E.P.; WEAVER, J.B. Joint action of two pyrethroids with methyl--parathion, methomil, and chlorpyrofos on *Heliothis zea* and *H. virescens* in the laboratory and in cotton and sweetcorn. **Journal of Economic Entomology,** v.70, p.813-817, 1977.

ALVES, S.B. Vírus entomopatogênicos. In: ALVES, S.B. (Coord.). **Controle microbiano de insetos.** São Paulo: Ed. Manole, 1986. p.171-187.

BARI, M.A.; KAYA, H.K. Evaluation of the entomogenous nematode *Neoaplectana carpocapsae* (=*Steinernema feltiae*) Weiser (Rhabditida: Steinernematidae) and the bacterium *Bacillus thuringiensis* Berliner var. *kurstaki* for suppression of the Artichoke Plume Moth (Lepidoptera: Pterophoridae). **Journal of Economic Entomology,** v.77, p.225-229, 1984.

BONG, C.F.J. Field control of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) using a parasitic nematode. **Insect Science Application,** v.7, p.23-25, 1986.

BULL, D.L.; HOUSE, V.S.; ABLES, J.R.; MORRINSON, R.K. Selective methods for managing insect pests of cotton. **Journal of Economic Entomology,** v.72, p.841-846, 1979.

BULL, D.L.; RIDGWAY, R.L.; HOUSE, V.S.; PRIOR, N.W. Improved formulations of the *Heliothis* nuclear polyhedrosis virus. **Journal of Economic Entomology,** v.69, p.731-736, 1976.

DAVIS, J.W.; HARDING, A.; WOLFENBARGER, D.A. Activity of a synthetic pyrethroid against cotton insects. **Journal of Economic Entomology,** v.68, p.373-374, 1975.

ELLIOTT, M.N.; JAMES, N.F.; POTTER, C. The future of pyrethroids in insect control. **Annual Review of Entomology,** v.23, p.443-469, 1978.

FERRON, P. Pest control by the fungi *Beauveria* and *Metarrhizium*. In: BURGES, H. D. (Ed.). **Microbial control of pests and plant diseases.** 1970-1980. London: Academic Press, 1981.

GARDNER, W.A.; NOBLET, R. Effects of host age, route of infection, and quality of inoculum on the susceptibility of *Heliothis virescens*, *Spodoptera eridania*, *S. frugiperda* to *Beauveria bassiana*. **Journal of Georgia Entomological Society,** v.13, n.3, p.214-218, 1978.

IGNOFFO, C.M.; CHAPMAN, A, J.; MARTIN, D.F. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virens* (Fabricius). III. Effectiveness of the virus against field populations of *Heliothis* on cotton, corn, and grain sorghum. **Journal of Invertebrate Pathology,** v.7, p.227-253, 1965.

IGNOFFO, C.M.; HOSTETTER, D.L.: BIEVER, K.D.; GARCIA, C.; THOMAS, G.D.; DICKERSON, W.A.; PINNEL, R. Evaluation of an entomopathogenic bacterium, fungus, and virus for control of *Heliothis zea* on soybeans. **Journal of Economic Entomology,** v.71, p.165-168, 1977.

JACKSON, G.J. Insect resistance - the challenge of the decade. In: GREEN, M.B.; LYON, D.J. de B. (Eds.). **Pest management in cotton.** West Sussex: Ellis Horwood, 1989.

KAYA, H.K.; BURLANDO, T, M. Development of *Steinernema feltiae* (Rhabditida: Steinernematidae) in diseased insect hosts. **Journal of Invertebrate Pathology,** v.53, p.164-168, 1989.

MADDOX, J.V. Use of insect pathogens in pest management. In: METCALF, R.L.; LUCKMANN W.H. (Eds.). **Introduction to insect pest management.** 2. ed. New York: John Wiley, 1982.

MILLER, T.A.; SALGADO, V.L. The mode of action of pyrethroids on insects. In: LEASLEY, J.P. (Ed.). **The pyrethroids insecticides.** London: Taylor & Francis, 1985.

PEKRUL, S.; GRULA, E. A. Mode of infection of the corn earworm (*Heliothis zea*) by *Beauveria bassiana* as revealed by scanning electron microscopy. **Journal of Invertebrate Pathology,** v.34, p.238--247, 1979.

SAS INSTITUTE. **SAS user's guide: statistics.** Cary: SAS Institute, 1982.

SMITH, R.J.; PEKRUL, S.; GRULA, E.A. Requirement for sequential enzimatic activities for penetration of the integument of the corn earworm (*Heliothis zea*). **Journal of Invertebrate Pathology,** v.38, p.335--344, 1981.

STANCEY, A.L.; YOUNG III, S.Y.; YEARIAN, W.C. Effect of larval age and mortality level on damage to cotton by *Heliothis zea* infected with *Baculovirus heliothis*. **Journal of Economic Entomology,** v.70, p.383-386, 1970.

TANADA, Y.; REINER C. The use of pathogens in the control of the corn earworm, *Helliothis zea* (Boddie). **Journal of Insect Pathology,** v.4, p.139-154, 1962.

YEARIAN, W.C.; YOUNGS, W. Control of insect pests of agricultural importance by viral insecticides. In: KURSTAK, E. (Ed.). **Microbial and viral pesticides.** New York: Marcel Dekker, 1982.