EMAMECTIN BENZOATE: A NOVEL AVERMECTIN DERIVATIVE FOR CONTROL OF LEPIDOPTEROUS PESTS IN COTTON

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Abstract

Emamectin benzoate (MK-0244) is a novel semi-synthetic derivative of the natural product abamectin in the avermectin family of 16-membered macrocylic lactones. This epi-methyl amino derivative has unprecedented potency against a broad spectrum of lepidopterous pests with LC_{90} values ranging between 0.001-0.02 *u*g/ml in ingestion-based foliar spray assays. Emamectin benzoate is ca. 1,500-fold more potent against certain armyworm species than abamectin. It is more potent against tobacco budworm, *Heliothis virescens* (F.) and beet armyworm, *Spodoptera exigua* (Hübner), than other new insecticides, such as fipronil, chlorfenapyr, and tebufenozide. In the field, the compound is very effective at controlling all lepidopterous pests on various crops at low use rates (8.4-16.8 g ai/ha). The mode of action is similar to abamectin (GABA- and glutamate-gated chloride channel agonist) and is not cross resistant with any other compound currently used commercially. The first registrations for the compound in the U.S. and Japan are anticipated for 1997. Registration on cotton is expected prior to the 1999 use season. An overview of its potential for control of lepidopterous pests on cotton is provided.

Introduction

Avermectins are a family of 16-membered macrocyclic lactone natural product homologues produced by the soil microorganism, *Streptomyces avermitilis* MA-4680 (NRRL 8165) and were isolated at Merck Research Laboratories from a soil sample collected in Japan by researchers at the Kitasato Institute (see Campbell, 1989 and references therein). Isolation of the crude fermentation product of *S. avermitilis* yielded a complex of eight closely related avermectin homologues ($A1_a$, $A1_b$, $A2_a$, $A2_b$, $B1_a$, $B1_b$, $B2_a$, and $B2_b$), of which avermectins $B1$ (a and b) were the major components. Abamectin, the non proprietary name assigned to avermectin $B1$, is a mixture of $B1_a$ ($\geq$ 80%) and $B1_b$ ($\leq$ 20%). This mixture was very potent against mites and certain insect species (Dybas *et al.*, 1989). Abamectin was developed for crop protection and is currently sold commercially for control of mites and certain insect pests on several ornamental and agronomic crops, including cotton, in over 50 countries. Ivermectin, a 22,23 dihydro semi-synthetic derivative of abamectin, was developed widely for control of ecto- and endoparasites of food and companion animals as well as for control of the causative agent of river blindness, *Onchocerca volvulus*, in man (see Campbell, 1989; Lariviere *et al.*, 1985).

Although abamectin was potent against mites and a select number of insects, it was considerably less potent against most Lepidoptera. This spectrum deficiency prompted a focused, medicinal chemistry and biological testing program that resulted in the discovery of 4"-epi-methylamino-4"-deoxyavermectin $B1$ (emamectin) in 1984 (Figure 1). MK-0243 (the hydrochloride salt of emamectin), which was derived from abamectin via a five-step synthesis (Cvetovich *et al.*, 1994), was discovered after screening several hundred avermectin derivatives in an *in vivo* screen using tobacco budworm, *Heliothis virescens* (F.), and southern armyworm, *Spodoptera eridania* (Cramer) (Dybas and Babu, 1988; Dybas *et al.*, 1989; Mrozik, 1994; Mrozik *et al.*, 1989). The benzoate salt of emamectin (coded MK-0244) had improved thermal stability and greater water solubility compared with the hydrochloride salt. MK-0244 was assigned the nonproprietary name emamectin benzoate and is currently being developed as a crop protection insecticide; first registrations in the U.S. and Japan are anticipated for 1997. The present paper presents an overview of the potential of emamectin benzoate for control of lepidopterous pests on cotton.

Mode of Action

The mode of action of the avermectins has been reviewed by several authors (Arena, 1994; Fisher and Mrozik, 1984; Rohrer and Arena, 1995; Turner and Schaeffer, 1989). All studies suggested that there are few qualitative differences in the mode of action of the avermectin compounds studied, including emamectin benzoate; thus, it is believed that most, if not all, avermectins have a similar mode of action.

The anthelmintic properties of the avermectins are due predominantly to potentiation and/or direct opening of glutamate-gated chloride channels, whereas in insects, it is likely that avermectins bind to multiple sites (including glutamate and GABA) in insect chloride channels. In general, the chloride ion flux produced by the opening of the channel into neuronal cells results in loss of cell function and disruption of nerve impulses. Consequently, invertebrates are paralyzed irreversibly and stop feeding. Maximum mortality of arthropods is achieved within 4 days. Although the avermectins do not exhibit rapid knock down activity against insects, paralysis is rapid, and feeding damage to crops is minimal because insects cease feeding shortly after ingestion. Avermectins intoxicate arthropods via contact and ingestion, although ingestion is considered to be the primary route whereby arthropods accumulate a lethal dose. The wide margin of safety for avermectin compounds to mammals is attributed to (1) the lack of

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glutamate-gated chloride channels in mammals; (2) the low affinity of avermectins for other mammalian ligand-gated chloride channels; and (3) their inability to readily cross the blood-brain barrier (Arena *et al.*, 1995).

Potency and Spectrum of Activity of Emamectin Benzoate

Emamectin benzoate is highly potent to a broad spectrum of lepidopterous pests. LC_{90} values for emamectin benzoate against a variety of lepidopterous pests range between 0.002-0.89 *u*g/ml (Dybas, 1989; Cox *et al.*, 1995b; Jansson and Dybas, 1996) (Table 1). Emamectin hydrochloride was up to 1,500-fold more potent against armyworm species, e.g., beet armyworm, *S. exigua* (Hübner), than abamectin (Dybas *et al.*, 1989; Mrozik *et al.*, 1989; Trumble *et al.*, 1987). Emamectin hydrochloride was also 1,720-, 884-, and 268-fold more potent to *S. eridania* than methomyl, thiodicarb, and fenvalerate, respectively, and 105- and 43-fold more toxic to cotton bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm larvae than abamectin (Dybas and Babu, 1988). Recent studies showed that emamectin benzoate was 875- to 2,975-fold and 250- to 1,300- fold more potent than tebufenozide to tobacco budworm and beet armyworm, respectively. Emamectin benzoate was also 12.5- to 20-fold and 250- to 500-fold more potent than lambda cyhalothrin and 175- to 400-fold and 2,033 to 8,600-fold more potent than fenvalerate to these two Lepidoptera, respectively (Jansson *et al.*, 1997). In addtion, recent studies showed that emamection benzoate was 2.0 to 4.8 orders of magnatude more potent to lepidopterous pests of cotton (eg. tobacco budworm and beet armyworm) than other new insecticides including chlorfenapyr, fipronil and tebufenozide (figure 2.)

Emamectin benzoate is markedly less toxic to most non-lepidopterous arthropods (Table 1). It is about 8- to 15-fold less toxic to the serpentine leafminer, *Liriomyza trifolii* (Burgess) and the twospotted spider mite, *Tetranychus urticae* (Koch), respectively, than abamectin (Cox *et al.*, 1995a; Dybas *et al.* 1989). Emamectin benzoate and abamectin are comparable in their potency against Mexican bean beetle, *Epilachna varivestis* Mulsant, and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Dybas, 1989). Emamectin benzoate is markedly less toxic to black bean aphid, *Aphis fabae* Scopoli, than abamectin (Table 1).

Like abamectin, emamectin benzoate is less toxic to most beneficial arthropods (e.g., honey bees, parasitoids, predators), especially when exposure occurs beyond one day after application (Lasota and Dybas, 1991 and references therein; Cox *et al.*, unpublished). Foliar residues of emamectin benzoate were only slightly toxic (< 20% mortality) to most beneficial insects, including honey bees, *Apis mellifera*, and several predators and parasitoids, within one day after application and often within a few hours after application (Cox *et al.*, unpublished). The low toxicity was related to the short half-life of emamectin benzoate on foliage. On celery, the half-life of foliar dislodgeable residues was estimated to be approximately 0.66 days (Dunbar *et al.*, unpublished). Kok *et al.* (1996) showed that emamectin hydrochloride (MK-0243) displayed minimal adverse effects against two hymenopterous parasitoids (*Pteromalus puparum* and *Cotesia orobenae*). Like abamectin, emamectin benzoate provides ecological selectivity (and in some cases physiological selectivity) to a wide range of beneficial arthropods. For this reason, it is compatible with integrated pest management (IPM) programs.

Photostability and Translaminar Movement

Abamectin and emamectin benzoate are very susceptible to photodegradation. MacConnell *et al.* (1989) showed that the half-life of abamectin was < 10 h in simulated sunlight and that there were marked differences in the half-life of abamectin on Petri dishes and on leaves in light and dark environments. The half-life of emamectin benzoate on celery has been estimated to be 0.66 days; on cole crops, the half-life is expected to be even shorter. Numerous photodegradates of emamectin benzoate have been identified (Feely *et al.*, 1992).

Despite the short half-life for avermectin insecticides in sunlight, low levels of these compounds are taken up rapidly via translaminar movement into foliage. Translaminar movement of abamectin has been demonstrated in numerous studies (Dybas, 1989 and references therein; Wright *et al.*, 1985). Presence of abamectin and emamectin benzoate reservoirs in parenchyma tissue accounts for their long residual activity on certain crops under field conditions, and their ability to control several phytophagous pest species (Jansson & Dybas 1996).

Field Efficacy

Excellent efficacy of this compound at low use rates (8.4-16.8 g ai/ha) has been demonstrated against numerous lepidopterous pests in a variety of crops (Jansson and Lecrone, 1991; Jansson *et al.*, 1996; Leibee *et al.*, 1995; Merck, unpublished). Results from numerous field trials conducted in ***cotton*** were very consistent. Against beet armyworm, emamectin benzoate at 8.4 g ai/ha provided excellent efficacy equivalent to other new insecticides being developed for cotton; such as, chlorfenapyr and tebufenozide (figures 3 & 4.). Against cotton bollworm and tobacco budworm field performance with emamectin benzoate at 8.4 g ai/ha was comparable to spinosad, chlorfenapyr and RH-2485 (figure 5,6, & 7.). Collectively, these data demonstrate that emamection benzoate produces very high levels of efficacy against several key lepidopterous pests of cotton.

Cross Resistance and Resistance Management

Cross resistance between abamectin and emamectin benzoate and other classes of chemistry has not been documented widely, nor is it well understood. Recent

studies by Lasota *et al.* (1996) showed that there was no cross resistance between abamectin, emamectin benzoate, permethrin and methomyl in *P. xylostella* using an ingestion bioassay. Additional evidence from studies conducted at Merck Research Laboratories also suggested that there was no cross resistance between abamectin and emamectin benzoate. (Jansson *et al.*, unpublished).;

Pro-active resistance management programs were developed for abamectin. Similar programs are being formulated for emamectin benzoate. Part of this pro-active strategy includes the development of monitoring systems to detect resistance in high risk populations of arthropods. Monitoring programs for problematic Lepidoptera; such as, Spodoptera, Heloithis and Heliocoverpa , are planned.

To minimize the risk of resistance to emamectin benzoate in lepidopterous pests, most product labels will provide restrictions on the type, number, and sequencing of applications allowed per growing season. These and other strategies, such as advocation of rotation of emamectin benzoate with other chemical and biological insecticides with different modes of action, and advocation of IPM programs in all crops, should help to prolong the life of emamectin benzoate in the commercial sector.

Acknowledgments

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References

Arena, J.P. (1994). Expression of *Caenorhabditis elegans* mRNA in *Xenophus* oocytes: a model system to study the mechanism of action of avermectins. *Parasitology Today* 10: 35-37.

Arena, J.P., Liu, K.K., Paress, P.S., Frazier, E.G., Cully, D.F., Mrozik, H. and Schaeffer, J. (1995). The mechanism of action of avermectin in *Caenorhabditis elegans:* correlation between activation of glutamate-sensitive chloride current, membrane binding and biological activity. *Journal of Parasitology* 81: 286-294.

Campbell, W.C. (ed.) (1989). Ivermectin and abamectin. Springer, New York, 363 pp.

Cox, D.L., Remick, D., Lasota, J.A. and Dybas, R.A. (1995a). Toxicity of avermectins to *Liriomyza trifolii* (Diptera: Agromyzidae) larvae and adults. *Journal of Economic Entomology* 88: 1415-1419.

Cox, D.L., Knight, A.L., Biddinger, D.G., Lasota, J.A., Pikounis, B., Hull, L.A. and Dybas, R.A. (1995b). Toxicity and field efficacy of avermectins against codling moth (Lepidoptera: Tortricidae) on apples. *Journal of Economic Entomology* 88: 708-715.

Cvetovich, R.J., Kelly, D.H., Di Michele, L.M., Shuman, R.F. and Grabowski, E.J.J. (1994). Synthesis of 4"-epi-amino-4"-deoxyavermectins B_1. *Journal of Organic Chemistry* 59: 7704-7708.

Dybas, R.A. (1989). Abamectin use in crop protection. In *Ivermectin and Abamectin* (ed. W.C. Campbell) pp. 287-310. Springer, New York.

Dybas, R.A. and Babu, J.R. (1988). 4"-deoxy-4"-methylamino-4"-epiavermectin B1 hydrochloride (MK-243): a novel avermectin insecticide for crop protection. In *British Crop Protection Conference. Pests and Diseases* pp. 57-64. British Crop Protection Council, Croydon, U.K.

Dybas, R.A. and A.S.J. Green. (1984). Avermectins: their chemistry and pesticidal activity. In *British Crop Protection Conference. Pests and Diseases* pp. 947-954. British Crop Protection Council, Croydon, U.K.

Dybas, R.A., Hilton, N.J., Babu, J.R., Preiser, F.A. and Dolce, G.J. (1989). Novel second-generation avermectin insecticides and miticides for crop protection. In *Novel Microbial Products for Medicine and Agriculture* (ed. A.L. Demain *et al.*) pp. 203-212. Society Industrial Microbiology, Annandale, VA.

Feely, W.F., Crouch, L.S., Arison, B.H., Vanden Heuvel, W.J.A., Colwell, L.F. and Wislocki, P.G. (1992) Photodegradation of 4"-(epimethylamino)-4"-deoxyavermectin B1a thin films on glass. *Journal of Agricultural and Food Chemistry* 40: 691-696.

Fisher, M.H. and Mrozik, H. (1984). The avermectin family of macrolide-like antibiotics. In *Macrolide Antibiotics* (ed. S. Omura) pp. 553-606. Academic Press, New York.

Jansson, R.K. and Dybas, R.A. (1996). Avermectins, biochemical mode of action, biological activity, and agricultural importance. In *Insecticides with Novel Modes of Action: Mechanism and Application* (ed. I. Ishaaya) Springer, New York (in press).

Jansson, R.K. and Lecrone, S.H. (1991). Efficacy of nonconventional insecticides for control of diamondback moth, *Plutella xylostella* (L.), in 1991. *Proceedings of the Florida State Horticultural Society* 104: 279-284.

Jansson, R.K., Halliday, W.R., Argentine, J.A. and Dybas, R.A. (1997). Comparison of miniature and high volume bioassays for screening insecticides. *Journal of Economic Entomology* (in review).

Jansson, R.K., Peterson, R.F., Mookerjee, P.K., Halliday, W.R. and Dybas, R.A. (1996). Efficacy of solid formulations of emamectin benzoate at controlling lepidopterous pests. *Florida Entomologist* 79: 434-439.

Kok, L.T., Lasota, J.A., McAvoy, T.J. and Dybas, R.A. (1996). Residual foliar toxicity of 4Ó-epimethylamino-4Ó-deoxyavermectin B1 hydrochloride (MK-243) and selected commercial insecticides to adult hymenopterous parasites, *Pteromalus puparum* (Hymenoptera: Pteromalidae) and *Cotesia orobenae* (Hymenoptera: Braconidae). *Journal of Economic Entomology* 89: 63-67.

Lasota, J.A. and Dybas, R.A. (1991). Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annual Review of Entomology* 36: 91-117.

Lasota, J.A., Shelton, A.M., Bolognese, J.A. and Dybas, R.A. (1996). Baseline toxicity of avermectins to diamondback moth (Lepidoptera: Plutellidae) populations: implications for susceptibility monitoring. *Journal of Economic Entomology* 89: 33-38.

Lariviere, M., Aziz, M., Weimann, D., Ginoux, J., Gaxotte, P., Vingtain, P., Beauvais, B., Derouin, F., Schulz-Key, H., Basset, D. and Sarfati, C. (1985). Double-blind study of ivermectin and diethylcarbamazine in African onchocerciasis patients with ocular involvement. *Lancet* 2: 174-177.

Leibee, G.L., Jansson, R.K., Nuessly, G. and Taylor, J. (1995). Efficacy of emamectin benzoate and *Bacillus thuringiensis* for control of diamondback moth on cabbage in Florida. *Florida Entomologist* 78: 82-96.

MacConnell, J.G., Demchak, R.J., Preiser, F.A. and Dybas, R.A. (1989). Relative stability, toxicity, and penetrability of abamectin and its 8,9-oxide. *Journal of Agricultural and Food Chemistry* 37: 1498-1501.

Mrozik, H. (1994). Advances in research and development of avermectins. In *Natural and Engineered Pest Management Agents, American Chemical Society Symposium Series No. 551.* (ed. P.A. Hedin *et al.*) pp. 54-73. American Chemical Society, Washington, D.C.

Mrozik, H., Eskola, P., Linn, B.O., Lusi, A., Shih, T.L., Tishler, M., Waksmunski, F.S., Wyvratt, M.J., Hilton, N.J., Andersen, T.E., Babu, J.R., Dybas, R.A., Preiser, F.A. and Fisher, M.H. (1989). Discovery of novel avermectins with unprecedented insecticidal activity. *Experientia* 45: 315-316.

Rohrer, S.P. and Arena, J.P. (1995). Ivermectin interactions with invertebrate ion channels. In *Molecular Action of Insecticides on Ion Channels. American Chemical Society Symposium series No. 591* (ed. J.M. Clark) pp. 264-283. American Chemical Society, Washington, D.C.

Royalty, R.N. and Perring, T.M. (1987). Comparative toxicity of acaricides to *Aculops lycopersici* and *Homeopronematus anconai* (Acari: Eriophydae, Tydeidae). *Journal of Economic Entomology* 80: 348-351.

Trumble, J.T., Moar, W.J., Babu, J.R. and Dybas, R.A. (1987). Laboratory bioassays of the acute and antifeedant effects of avermectin B1 and a related analogue on *Spodoptera exigua* (Hubner). *Journal of Agricultural Entomology* 4: 21-28.

Turner, M.J. and Schaeffer, J.M. (1989). Mode of action of ivermectin. In *Ivermectin and Abamectin* (ed. W.C. Campbell) pp 73-88. Springer, New York.

Wright, D.J., Loy, A., Green, A.S.J. and Dybas, R.A. (1985). The translaminar activity of abamectin (MK-936) against mites and aphids. *Med Fac Landbouww Rijksuniv Gent* 50(2b): 633-637.

Table 1. Comparative toxicity of abamectin and emamectin benzoate to different arthropod pests of agricultural importance.

LC_{90}, µg/ml Arthropod species	Abamectin	Emamectin Benzoate	PR[a]
Acarina			
Foliar spray/contact assay			
Tetranychus urticae, adults	0.03 [c]	0.29 [d]	0.07
Insecta			
Coleoptera			
Leptinotarsa decemlineata, neonates, foliar spray	0.03 [c]	0.03 [d]	1.0
Epilachna varivestis, neonates, foliar spray	0.2 [c]	0.2 [d]	1.0
Diptera			
Liriomyza trifolii, first instar, plant dip	0.19 [f]	1.45 [f]	0.13
Homoptera			
Aphis fabae, foliar spray/contact	0.2-0.5 [g]	19.9 [d]	0.01-0.02
Lepidoptera			
Manducca sexta, neonates, foliar spray	0.02 [c]	0.003 [d]	7
Plutella xylostella, neonates, foliar spray	0.02 [g]	0.002 [g]	10
Heliothis virescens, neonates, foliar spray	0.13 [d]	0.003 [d]	43
Trichoplusia ni, neonates, foliar spray	1.0 [c]	0.014 [d]	71
Helicoverpa zea neonates, foliar spray	1.5 [c]	0.002 [d]	750
Spodoptera exigua, neonates, foliar spray	1.97 [g]	0.005 [d]	394
Spodoptera eridania, neonates, foliar spray	6.0 [c]	0.005 [d]	1,200
Spodoptera frugiperda, neonates, foliar spray	25.0 [e]	0.010 [d]	2,500
Pseudoplusia includens, neonates, foliar spray	-	0.019 [g]	-
Ostrinia nubilalis, neonates, diet assay	-	0.024 [g]	-
Agrotis ipsilon, neonates, diet assay		0.041 [g]	-
Argyrotaenia velutinana, neonates, foliar spray	-	0.009 [g]	-
Cydia pomonella, neonates, diet	135.0 [h]	0.89 [h]	152

[a] PR, potency ratio = LC_{90} abamectin/LC_{90} emamectin benzoate
[b] Royalty and Perring (1987)
[c] Dybas and Green (1984)
[d] Dybas *et al.* (1989)
[e] Dyas (1989)
[f] Cox *et al.* (1995a)
[g] Merck, unpublished data
[h] Cox *et al.* (1995b)

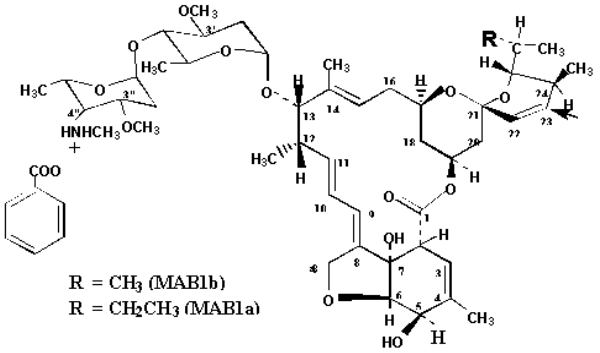

Figure 1. Structure of Emamectin benzoate.

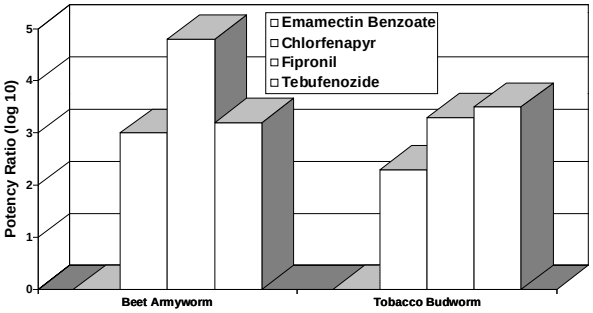

Figure 2. Log_{10} - Transformed ratio of the LC_{90} value of chlorfenapyr, fipronil, or tebufenozide divided by the LC_{90} value for emamectin benzoate against beet armyworm and tobacco budworm. LC values were generated using an agar-based artificial diet assay in which different concentrations of each compound were applied to the surface of the diet (Jansson et al., unpublished).

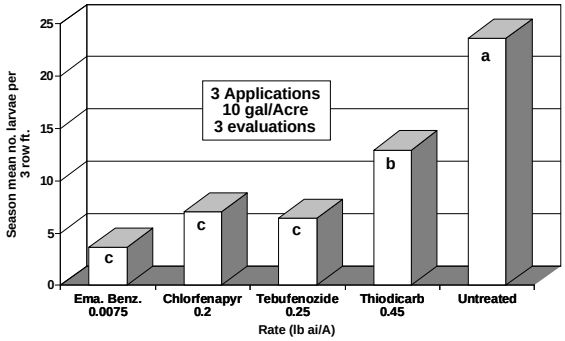

Figure 3. Beet armyworm control in Alabama. Research by Dr. R. Smith, Auburn University (1994). Means with the same letter are not significantly different (DMRT, p=0.05).

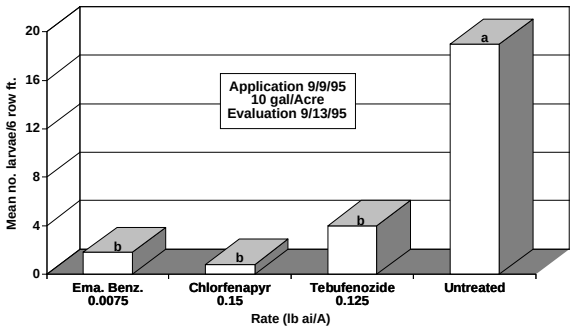

Figure 4. Beet armyworm control in Arkansas. Research conducted by Dr. D. Harlan, Mid-South Ag Research, Inc. (1995). Means with the same letter are not significantly different (DMRT, p-=0.05).

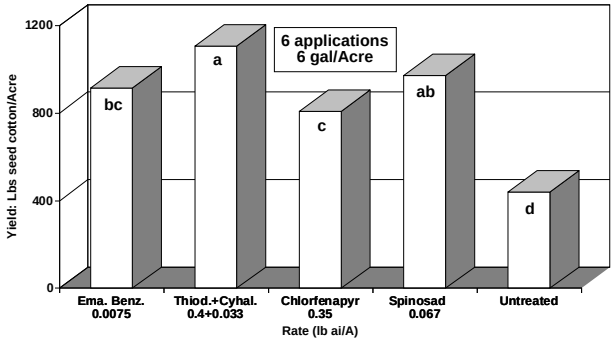

Figure 5. Control of cotton bollworm and tobacco budworm in Louisiana. Research conducted by Dr. R. Leonard, LSU Northeast Research Station. Means with the same letter are not significantly different (DMRT, p=0.05).

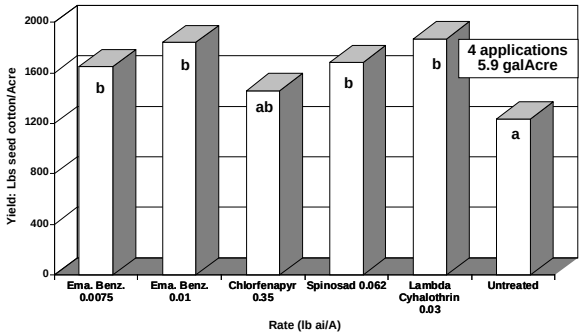

Figure 6. Control of cotton bollworm and tobacco budworm in Louisiana. Research conducted by Dr. Steve Micinski, LSU Red River Research Station (1996). Means with the same letter are not significantly different (DMRT,p=0.05).

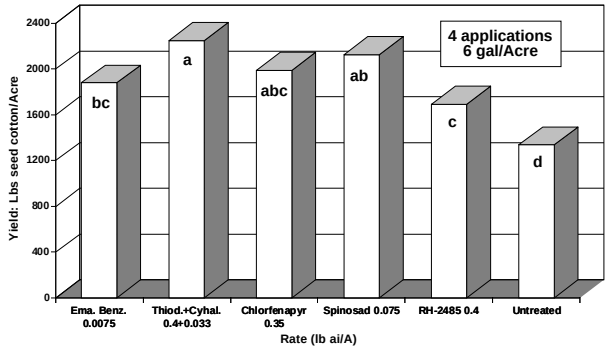

Figure 7. Control of cotton bollworm and tobacco budworm in Louisiana. Research conducted by Dr. R. Leonard, LSU Northeast Research Station (1996). Means with the same letter are not significantly different (DMRT, p=0.05).