Biologically derived insecticides for use against beet armyworm

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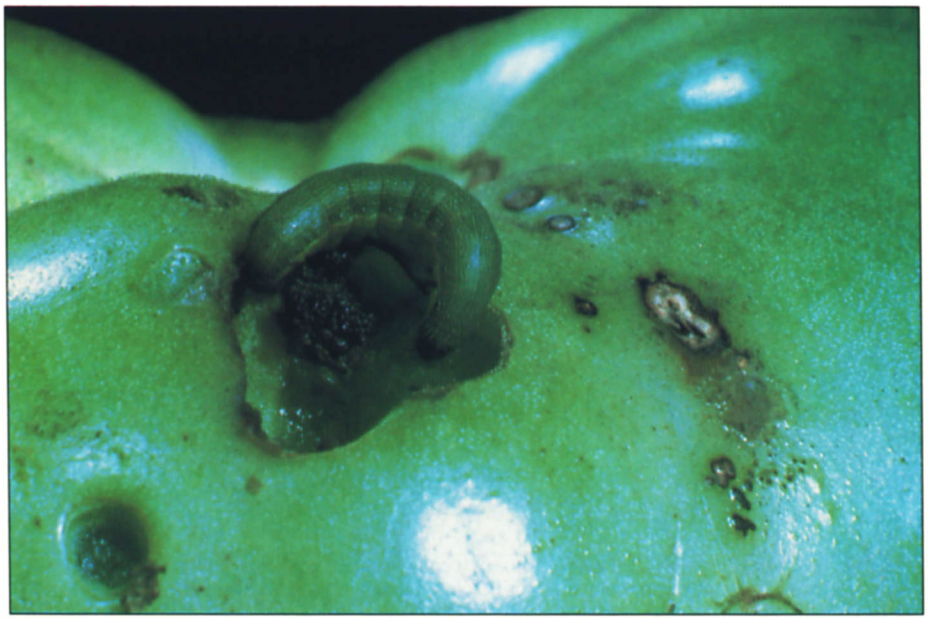
New microbial insecticides are good prospects for use in tomato and celery IPM programs.

Max Badgley



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Only a small amount of beet armyworm damage is commercially tolerated in celery (above) and tomato (below). The pest rapidly develops resistance to chemical controls.

The beet armyworm is a key insect pest of celery and fresh market tomatoes, and the economic threshold is quite low in both crops; usually no more than 3 to 5 percent crop damage is tolerated. Frequent insecticide applications are therefore needed for control. The beet armyworm, *Spodoptera exigua* (Hübner), however, has been shown to be resistant to many insecticides, including some chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids. Recently, high tolerances to the carbamate methomyl have been documented, a finding of particular concern, since methomyl is the most widely used pesticide in California for suppressing beet armyworm populations.

Also, methomyl and most other pesticides effective against beet armyworm do not control another important insect pest of celery and tomatoes, the leafminer *Liriomyza trifolii* (Burgess). Our research has shown that use of these compounds may actually increase leafminer populations by killing its parasites. Because the leafminer has demonstrated the potential for development of resistance to all chemicals currently registered in California, conservation of leafminer parasites is essential to long-term sustainable control.

Our first objective in the research reported here was to examine insecticides with novel modes of action against insects for potential incorporation into integrated pest management (IPM) programs. We evaluated several recently developed insecticides, as well as combinations of these with microbial insecticides that have been available for many years.

In our earlier field tests, commercially available microbial insecticides based on the HD-1 isolate of *Bacillus thuringiensis* var. *kurstaki* (BTK) did not reduce beet armyworm populations below the economic thresholds. The lack of control was due, in part, to the rapid breakdown of BTK in the field and to tolerance by the pest. This failure is unfortunate, because these microbial insecticides, which have a narrow host range, are relatively nontoxic to the environment, and their novel mode of action could be useful in a pesticide rotation schedule to help combat resistance. A new isolate of BTK, designated NRD-12, has recently been found to have greater activity towards beet armyworm. A second goal of our study was to evaluate the toxicity of both the new and the old isolates of BTK to newly hatched beet armyworm.

Several microbial by-products also show promise for beet armyworm control. Thuringiensin, a beta-exotoxin produced by some varieties of *Bacillus thuringiensis* (BT), disrupts protein synthesis and inhibits metamorphosis. Avermectin

B_1, a by-product of the actinomycete *Streptomyces avermitilis*, disrupts signal transmission between nerves and muscles in insects, inhibiting feeding and causing paralysis. Avermectin B_1 has already been shown to control leafminer while not damaging its parasites. Another natural product is neem, an extract from the seed kernel of the neem tree, *Azadirachta indica*. It disrupts insect growth as well as inhibiting feeding.

Our third goal was to determine the toxicity of thuringiensin, avermectin B_1, a related avermectin analogue, and neem against newly hatched beet armyworm. We tested paired combinations of most of these compounds for joint effects. The time needed to cause mortality also was evaluated for compounds singly and in combination. To examine these compounds under more realistic field conditions, we assessed avermectin B_1 and a related analogue against the third larval stage (instar) of beet armyworm for contact and residual activity on celery leaves, and recorded the feeding damage.

Toxicity study

Materials tested for toxicity to larvae were: Dipel 2X wettable powder containing the HD-1 isolate with 32,000 international units (IU) per milligram (mg); Javelin wettable powder containing the NRD-12 isolate with 16,000 *Spodoptera* units per mg; liquid thuringiensin (1.5 percent active ingredient); avermectin B_1 (18 grams active ingredient per liter); avermectin analogue (L656,748, 6 grams active ingredient per liter); and neem seed extract (from Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland).

Seven to eight concentrations plus a control were bioassayed for individual toxicants. Suspensions containing the toxicants were added to an artificial diet medium in containers placed in a laboratory growth chamber. Thirty insects were evaluated per concentration, with each concentration replicated six times. Larval mortality was assessed at day 7 in all tests.

Javelin was three to four times as active as Dipel 2X; the LC_{50}, or concentration at which 50 percent of the larvae died, was 81.2 vs. 299 micrograms per milliliter (µg/ml) diet. Concurrent field tests using Javelin at recommended field rates, however, did not result in satisfactory control.

The 37.5 µg/ml (active ingredient) diet of thuringiensin killed 50 percent of the beet armyworm population after seven days, but no insect at any treatment concentration molted into the second instar. Our future research will look into ways to exploit this attribute of thuringiensin while minimizing residues, and will investigate how this compound affects the population dynamics of beet armyworm and leafminer natural enemies.

The avermectin analogue was about 1500 times as active as avermectin B_1 (LC_{50}'s were 0.48 vs. 814 nanograms (ng)/ml active ingredient). This analogue has not been field-tested in California, so potential problems with stability or residues may still develop. However, if the avermectin analogue proves to have as little impact on parasites of the leafminer as does avermectin B_1, this will be an extremely useful compound in IPM programs. The LC_{50} for neem was 0.12 µl/ml diet.

Joint action

Each toxicant (except avermectin analogue) individually and in pairs was bioassayed in a growth chamber as before. For combinations, we estimated the amounts of each toxicant needed to cause 25 percent mortality and mixed them into the diets. Toxicant combinations were replicated six times, with 30 larvae per replicate. Mortality was recorded at 24-hour intervals for seven days.

Combination of Dipel 2X with thuringiensin dramatically increased mortality above the expected levels (fig. 1). Javelin in combination with thuringiensin produced nearly identical results. This response is important, because control of beet armyworm with low concentrations of biologically derived pesticides could reduce the potential for environmental contamination from broad-spectrum insecticides, human health problems, and the pesticide pressure responsible for resistance development. Additional studies are under way to determine the minimum amount of these pesticides that will provide an acceptable level of control.

When the biological pesticide neem was combined with Dipel 2X or avermectin B_1, mortality was much less than expected (fig. 1). We suspect that the feeding-deterrent action of neem caused less of the other compounds to be consumed and reduced the mortality. This result suggests that, when using only biological pesticides, it might be advisable to avoid combinations of BTK and neem and, in a rotational program, to apply BTK first or only after neem residues had dissipated.

Time of mortality

To document the time required for beet armyworm mortality to occur, we calculated the mean time of mortality. The number of larvae that died on a given day was divided by the total mortality after seven days. This value then was multiplied by the respective day that mortality occurred. Values for days one to seven were added to produce a weighted average mortality. We calculated the mean time of mortality for all concentrations of each pesticide. Three replicates (30 larvae per replicate) of Dipel 2X, avermectin B_1,

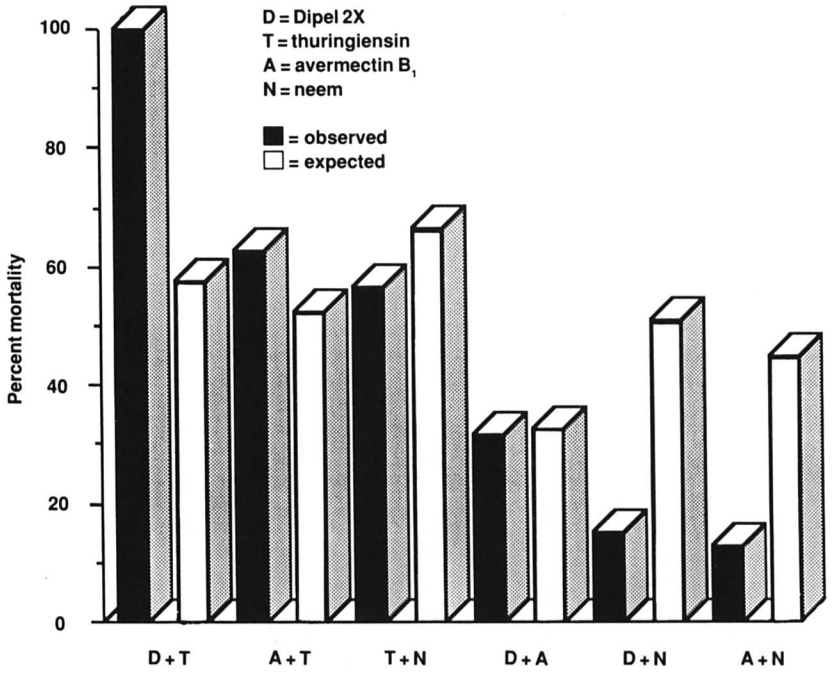

Fig. 1. The combination of Dipel 2X with thuringiensin increased mortality of first-stage beet armyworm larvae far above expected levels. Combinations of neem with Dipel 2X or avermectin B_1 gave lower than expected mortality.

neem, and thuringiensin were evaluated every 24 hours. The times for all paired combinations of pesticides were calculated from six replicates.

The results differed significantly for Dipel 2X, avermectin B_1, neem, and thuringiensin (table 1). Dipel 2X resulted in an increased rate of mortality when compared with the other compounds. Since all four compounds had different modes of action, there is a chance that the differences in times were due to the modes of action. Mean time of mortality could be helpful in determining when a certain insecticide should be applied to obtain the highest level of mortality, as well as when to evaluate treatment efficacy.

TABLE 1. Mean time of mortality for four toxicants tested individually and in combination against first-instar beet armyworm

Treatments*	Dipel 2X	Avermectin B_1	Neem	Thuringiensin
	days			
Dipel 2X	3.06	2.83	2.54	2.82
Avermectin B_1	—	4.13	2.87	5.13
Neem	—	—	5.42	6.06
Thuringiensin	—	—	—	6.23

* Mean time of mortality of treatments combined with themselves are individual compounds based on seven or eight concentrations used in probit analyses. All other combinations are mean times based on LC_{25}'s replicated six times.

If Dipel 2X was one of the paired compounds, the mean time of mortality was similar to that for Dipel 2X alone (table 1). This result implies that Dipel 2X determined the time necessary to induce mortality. When Dipel 2X was combined with thuringiensin, the tremendous increase in mortality occurred in the same amount of time as when Dipel 2X was used alone. This result suggests that Dipel 2X could be increasing the permeability of the larva's midgut, allowing the other compounds to reach their sites either quicker or in larger amounts.

Leaf-disc bioassay

In the contact-plus-residue and residue-only trials, methomyl (used as an industry standard), avermectin B_1, avermectin analogue, and a water control were applied with a backpack sprayer in 100 gallons per acre. All tests consisted of four replicates of 20 third-instar larvae per treatment.

For the contact-plus-residue tests, larvae were placed on celery leaf discs in screen cages. The insects, leaf discs, and screen cages were treated, then cages were held in the laboratory at 80°F. This approach simulates field conditions while minimizing the environmental variability associated with field studies. In residue-only tests, larvae were placed in cages with leaf discs taken from previously treated celery. Leaf discs from each replicate in the residue studies were changed at 24-hour intervals for the 72 hours following each test. Samples were frozen. Leaf area eaten was determined with a Li-Cor Leaf Area Meter.

In the contact-plus-residue tests, the avermectin analogue proved to be as toxic as methomyl 24 hours after treatment (table 2). In residue studies, the avermectin analogue had significantly longer residual activity than avermectin B_1, and leaves treated with the analogue showed equal or less damage when compared with those treated with methomyl, even after 14 days posttreatment.

Although the avermectin analogue was much more active than avermectin B_1, additional field research will be needed to evaluate the analogue's effectiveness on beet armyworm and the leafminer, as well as any harmful effects on the leafminer parasite population.

TABLE 2. Mortality of third-instar beet armyworms and feeding damage in celery following methomyl, avermectin analogue, and avermectin B_1 application

CONTACT + RESIDUE

Treatment and rate	Cumulative percent mortality, posttreatment				
	3 hr	18 hr	24 hr	48 hr	72 hr
kg[AI]ha	percent				
methomyl, 1.008	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
avermectin analogue, 0.0112	81.3 b	86.3 b	100.0 a	100.0 a	100.0 a
avermectin B_1 0.0224	10.0 c	33.8 c	48.8 b	63.8 b	73.8 b
control	0.0 c	0.0 c	1.3 c	6.3 c	11.3 c

RESIDUE ONLY

Days after application, and treatment	Cumulative percent mortality posttreatment				Total surface area eaten†
	4 hr	24 hr	48 hr	72 hr	
	percent				*sq cm*
0					
methomyl	100.0 a	100.0 a	100.0 a	100.0 a	0.0 a
avermectin analogue	30.0 b	100.0 a	100.0 a	100.0 a	0.0 a
avermectin B_1	12.5 c	43.5 b	50.7 b	75.1 b	0.1 ab
control	2.5 c	2.5 c	2.5 c	2.5 c	0.1 b
7					
methomyl	38.8 a	70.0 a	89.7 a	96.3 a	0.0 a
avermectin analogue	0.0 b	29.0 a	34.0 a	57.7 a	0.0 a
avermectin B_1	1.3 b	6.9 b	10.6 b	27.4 b	0.3 b
control	1.3 c	1.3 b	2.5 c	2.5 c	0.2 b
14					
methomyl	0.0 a	16.6 a	40.7 a	55.9 a	0.2 c
avermectin analogue	0.0 a	0.0 b	11.6 b	30.6 b	0.0 a
avermectin B_1	0.0 a	0.0 a	0.0 c	4.0 c	0.3 d
control	0.0 a	0.0 b	0.0 c	0.0 c	0.1 b

*Means in columns within days posttreatment followed by the same letter are not significantly different at the $P \leq 0.05$ level, Duncan's new multiple range test.
† Average leaf area eaten per larva in 24 hours.

Conclusion

Several new insecticides and insecticide combinations show promise for beet armyworm control, including Javelin, combinations of thuringiensin with Dipel 2X or Javelin, and the avermectin analogue. The use of these products could help reduce the amount of broad-spectrum insecticides necessary for control. Because these compounds have novel modes of action, their use singly or in combination with other compounds could be valuable in a spray schedule designed to manage resistance.

Since we now know the time required by these microbial products to inflict mortality, we also have an idea as to how soon after application the efficacy of microbial pesticides should be examined. This information could lead to more efficient use, thereby reducing crop damage. Furthermore, as a cropping season draws nearer to harvest, knowledge of the time required can help in choosing an insecticide that will cause maximum mortality before harvest. Since many of these microbials are nontoxic to beneficial insects, any beet armyworms that escape pesticide treatment could be subject to attack by predators or parasites. This not only lowers the threat of resistance, but also reduces the problem of resurgence by the leafminer.

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