Lannate and Larvin Resistance in Beet Armyworms from the Low Desert Regions of Arizona and California

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Abstract

Beet armyworm populations were collected in 1996-97 from spinach, melons, lettuce and alfalfa in Arizona and California, and tested for resistance to topical applications of Lannate. Resistance levels were found to be low to very high. The lowest level of resistance detected came from Blythe, CA, having no detectable resistance to Lannate, and from Parker, AZ, having a resistance level of approximately 24-fold. The highest level of resistance detected was a 685-fold increase, from a population collected from alfalfa in Imperial County, CA. In Yuma, larvae collected from alfalfa following an insecticide application that included Lannate, was 4.43-fold more resistant than the pre-application population. Only very low levels of resistance were found to Larvin, and no evidence of cross-resistance between Lannate and Larvin was found. Larvae resistant to topical applications of Lannate were found to be susceptible to Lannate given orally. Lannate resistance appears to be due to cuticular penetration and/or cuticular metabolism.

Introduction

The beet armyworm (BAW), *Spodoptera exigua* (Hübner), is a serious key pest of lettuce grown in the desert Southwest. Although this pest is most severe on lettuce planted in late August through September, it can present problems season long. BAWs will attack lettuce as soon as in emerges from the ground often resulting in severe stand loss. Later infestations will stunt the lettuce's growth, and damage and contaminate harvestable portions.

The BAW is a highly polyphagous pest feeding on many hosts including: alfalfa, cotton, lettuce, cole crops, corn, tomatoes and numerous weeds. Because BAWs feed of so many species of plants, they have an enormous capacity for detoxifying plant defense chemicals and insecticides. In lettuce, there are few insecticides that have efficacy to BAW. For over twenty years Lannate (methomyl) has been the product of choice for controlling BAW. More recently, Larvin (thiodicarb) has become a popular BAW material. Biorational pesticides such as *Bacillus thuringiensis* provide some control, but are not commercially acceptable when used alone. Almost all insecticide applications targeting BAW in lettuce contain either Lannate or Larvin. The potential for developing insecticide resistance in BAW to these materials is great. Lannate and Larvin are very similar insecticides with a potential for cross-resistance.

During the past five years many PCAs have been reporting that each year they have been having to use increasing amounts of Lannate to achieve commercially acceptable BAW control. During 1995, BAW populations were extremely active in the Yuma area. Many PCAs were reporting control failures with Lannate. BAW control failures have been attributed to both poor application and resistance. If resistance is at least partially to blame for control failures, alternative insecticides must be sought. Presently, there are no effective alternative insecticides for BAW commercially available. Fortunately, a Section 18 for Confirm (tebufenozide) has been granted in Arizona for use in lettuce, cole crops and leafy greens for BAW control.

BAWs from the Yuma area have been tested for resistance to Lannate on two previous occasions. In 1975, investigators concluded that there was no evidence of BAW resistance to Lannate (Meinke and Ware 1978). They found that a BAW strain collected from Yuma had an LD_{50} of 17.54 μg/g and did not differ from a Lannate susceptible strain. Because they detected no resistance, data from Meinke and Ware (1978) can serve as a historical baseline for comparing later populations. Another study conducted in during 1989, found that BAWs collected from the Yuma area had an LD_{50} of 52.57 μg/g and was 2.95-fold more resistant than a susceptible strain, and approximately 3-fold more resistant than the Yuma strain tested by Meinke and Ware (1978). A 3-fold level in resistance is not considered highly significant. The objective of this study was to assess the occurrence of Lannate and Larvin resistance in BAWs from the low desert agricultural regions of Arizona and California, and to determine if cross-resistance occurs between Lannate and Larvin.

Materials and Methods

From late February 1996 to June 1997, we collected BAW larvae and/or egg masses from spinach, melons, lettuce or alfalfa growing in the low desert agricultural regions of Arizona and California. These larvae were placed on artificial diet and reared to adults. The F1 generation obtained from these adults were tested for resistance to Lannate and Larvin.

Lannate Topical Assays

Resistance to Lannate was evaluated using the same topical techniques as Meinke and Ware (1978). Insecticide dilutions were made by dissolving technical grade methomyl (Lannate) into acetone. Seven to thirteen dilutions were used in this study depending on the population tested. Dilutions were calculated based on average larval weights in μg-methomyl / g-larvae. Twenty-five to fifty 3rd instar larvae, which averaged 9-12 mg, were used for each dosage.. Topical applications were made with a 0.25-cc syringe and a 27 gauge needle using a hand driven Burkard microapplicator. Each larvae was assayed by placing a 0.5μl droplet of insecticide dilution on the dorsal thorax. The worms were then transferred to individual cups containing artificial diet and allowed to sit at ambient room temperature for 24 hr. After 24 hr, larvae that could not move about freely were considered dead. Dosage-mortality curves were estimated using Probit's analysis. Dosages required to kill 50% (LD_{50}) and 95% (LD_{95}) of the worm populations were estimated using the Probit regression, 95% fiducial limits were used to separate differences. Resistance ratios (RRs) of the LD_{50} of the field strain / LD_{50} of the 1976 historical standard was used to quantify resistance.

Larvin and Lannate Oral Assays

Since Larvin is primarily active as a stomach poison, resistance to Larvin was evaluated using a diet based technique. Insecticide dilutions were made by dissolving Larvin 3.2EC into water. These insecticide solutions were used to hydrate instant mix diet (Stonefly Industries, Bryan, TX), using 3.0 ml insecticide solution per 1.0 g diet. Dilutions were calculated based insecticide concentration in the artificial diet (mg-active ingredient / kg-diet). Insecticide-diet mixtures were transferred to 22.18 ml plastic cups with lids at 4.0 g per cup. Six to eight dilutions were used depending on the population tested. Thirty to seventy-five 2nd instar larvae were used for each dosage, one larvae was used per cup. The larvae were allowed to sit at ambient room temperature for 96 hr. After 96 hr, larvae that could not move about freely were considered dead. Dosage-mortality curves were estimated using Probit's analysis. Dosages required to kill 50% (LC_{50}) and 95% (LC_{95}) of the worm populations were estimated using the Probit regression, 95% fiducial limits were used to separate differences. Resistance ratios of the LC_{50} of each field strain / the LC_{50} of a susceptible laboratory strain obtained from the USDA-ARS, Stoneville, MS was used to quantify resistance. Additionally, to determine if the mechanism of resistance in a Lannate resistant population was related to cuticular penetration and/or cuticular metabolic processes, Lannate was evaluated using the same diet based assay technique. However, instead of calculating the RR based on the laboratory strain from Mississippi, a susceptible laboratory strain from the USDA-ARS in Phoenix, AZ was used.

BAW Populations

BAW populations were collected as egg masses and/or larvae from spinach, melons, lettuce and alfalfa during 1996-97. Populations were collected from Yuma and La Paz Counties in Arizona, and Imperial and Riverside Counties in California. Populations taken from the field were transported to the laboratory and reared on artificial diet. The F1 generation from these collections were used for the insecticide bioassays. Populations collected from Yuma County included: YAC96A, collected on 02/26/96 from spinach at the Yuma Valley Agricultural Center; DOM96, collected on 09/07/96 from lettuce in Dome Valley; SOM96, collected on 09/11/96 from lettuce near Somerton; TEX96, collected on 10/10/96 from lettuce near Texas Hill; GAD97, collected on 02/05/97 from lettuce near Gadsden; YMS97A, collected on 06/06/97 from alfalfa on the Yuma Mesa and YMS97B, collected on 06/09/97 from alfalfa on the Yuma Mesa. Populations collected from La Paz County included: SAL96, collected on 08/19/96 from watermelons near Salome and PAR97, collected on 06/14/97 from alfalfa near Parker. Populations from California included: ICA97, collected on 06/11/97 from alfalfa in Imperial County and BLY97, collected on 06/14/97 from alfalfa near Blythe.

The YMS97A population was collected one day prior to an insecticide application of Lannate at 0.67 lbs/A + Pounce at 6.0 oz/A, while the YMS97B was collected from the same field 2 days following the insecticide application. Control of BAW by the insecticides at this location appeared to be approximately 70%.

To develop a more homogeneous population resistant to Lannate for mechanism of resistance studies, a selection of BAWs surviving large Lannate dosages was made. Survivors from topical dosages exceeding 4000 μg-methomyl / g-larvae from the F1 generation of the YAC96A population were separated into a new population, YAC96B.

Results and Discussion

BAWs collected during 1996 and in 1997 have shown none to high levels of resistance to topical applications of Lannate relative to known susceptible laboratory and historical field populations (Table 1). The laboratory populations from USDA-ARS facilities in Mississippi and Arizona were highly susceptible to Lannate, having LD_{50}s of 19.92 and 21.78 respectively, and did not significantly differ from historically susceptible field populations.

We could detect no Lannate resistance in only one of the field populations tested, BLY97. The lowest detectable level of Lannate resistance came from the PAR97 population collected from alfalfa near Parker, AZ, having a RR of 24.4-fold. A 24.4-fold level of resistance appears to be a low level relative to levels found in other populations, and the laboratory and historically susceptible populations. Since Lannate use in the Blythe and Parker area is generally much lower than in the Yuma or in Imperial County, CA, low levels of resistance are not unexpected. The SAL96 population, also from La Paz County, AZ, was found to be high in resistance to Lannate with a RR of 137.74-fold. However, this population was exposed to 2 field applications of Lannate before it was collected which may have eliminated susceptible phenotypes.

Resistance levels of BAWs collected from Yuma ranged from moderately resistant, in the DOM96 and SOM96 populations, to highly resistant in the TEX96 and YMS97A populations, and very high resistance in the YAC96A, GAD97, and the YMS97B populations. The highest levels of resistance detected in Yuma occurred in October and February which follow the traditionally heavy Lannate use period in lettuce during September. YMS97A was collected 1 day before an application that included Lannate, and had a LD_{50} of 2,208 μg-methomyl / g-larvae and a probit regression slope of 1.00 ± 0.16. Two days after the insecticide application, YMS97B was collected and had a LD_{50} of 9,793 μg-methomyl / g-larvae, a 4.43-fold shift toward higher resistance. YMS97B had a probit regression slope of 0.52 ± 0.12, much flatter than that of the YMS97A population, suggesting the presence of greater proportions of SR and RR phenotypes, and possibly the involvement of major gene resistance.

The BAW population obtained from Imperial County, CA came from an area experiencing control failures with Lannate and Lorsban in alfalfa. Consequently, this population exhibited the highest level of Lannate resistance detected among all of the field populations tested, with a RR of 685.52-fold.

The YAC97A population collected from spinach on 02/26/96, had a very high level of resistance, with a RR of 313.80-fold. Larvae from this population that survived Lannate doses >4,000 μg-methomyl / g-larvae were selected to form a new population, YAC96B. When YAC97B was tested for resistance to Lannate it showed a significant shift towards a higher level of resistance, 754.11-fold, suggesting a more homogeneity for resistance. The YAC97B populations was created to perform tests to determine the mechanism of resistance to Lannate.

Susceptible laboratory strains, YAC96B and the F1 generations of moderate had highly Lannate resistant field populations were evaluated for resistance to Larvin. Larvin is chemically very similar to Lannate; it is essentially two Lannate molecules hooked together. In the insect, Larvin is cleaved to produce the primary toxicant, which is Lannate. Because of the chemical similarities between these insecticides, we expected that resistance to Lannate would confer cross-resistance to Larvin. However, resistance to Larvin was very low, never exceeding 6.3-fold (Table 2). The only difference in Lannate and Larvin is its method of delivery. Lannate is a contact poison, while Larvin must be ingested to induce its highest level of toxicity. Thus suggesting, that Lannate may not be able to penetrate the cuticle, or is being metabolized within the cuticle of Lannate resistant BAWs. To determine if the insects cuticle was responsible fcr Lannate resistance in BAWs, the cuticle was by-passed by using a oral ingestion technique. The YAC97B population was used as the standard, resistant to topical applications of Lannate, relative to the susceptible PHO96 population. When the YAC97B population was given Lannate orally, its RR dropped from 607.30-fold detected from the topical assay, to approximately 5.0-fold, differing only slightly from the RR of 3.43-fold detected for Larvin (Table 3). Therefore, Lannate resistance in BAWs collected from Yuma, AZ appears to involve cuticular penetration and/or cuticular metabolism.

If Lannate resistance involves the insect's cuticle, then resistance may be effectively managed by encouraging BAWs to consume the Lannate rather than relying on contact poisoning. BAWs are known to be most active and feed primarily at night. Night-time or evening applications of Lannate may promote the ingestion of the material relative to day-time applications. Additionally, spray additives that stimulate BAW feeding may increase the possibility that the Lannate will be consumed.

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Table 1. Dose-mortality responses of BAW populations collected from Yuma to Lannate applied topically.

Population[a]	Date collected	Host	n	Slope ± SE	LD_{50} (95% fiducial limits)[b]	LD_{95}[c]	RR[d]
Historically susceptible populations							
1975	1975	cotton	229	1.08 ± 0.19	17.54 (10.12-26.04)	--[e]	1.0
1989	1989	cotton	600	1.72 ± 0.26	52.57 (41.11-61.66)	474.65	3.0
Laboratory susceptible populations							
MIS96	N/A	diet	350	1.90 ± 0.25	19.92 (15.49-24.18)	146.65	1.0
PHO96	N/A	diet	300	1.23 ± 0.17	21.78 (14.01-30.21)	472.13	1.0
Field collected populations (F1 generations)							
YAC96A	02/26/96	spinach	250	0.83 ± 0.28	5,504 (2,316-186,359)	555,709	313.80
SAL96	08/19/96	melons	600	1.98 ± 0.18	2,419 (2,114-2,745)	16,362	137.74
DOM96	09/07/96	lettuce	450	1.07 ± 0.10	934.58 (702.58-1,240)	32,308	53.28
SOM96	09/11/96	lettuce	600	0.79 ± 0.07	846.50 (609.21-1,165)	104,566	48.26
TEX96	10/10/96	lettuce	325	0.87 ± 0.14	4,976 (3,405-7,890)	380,635	283.69
GAD97	02/05/97	lettuce	330	1.45 ± 0.16	5,406 (4,174-6,936)	74,212	308.21
YMS97A	06/06/97	alfalfa	279	1.00 ± 0.16	2,208 (1,363-3,704)	97,102	125.88
YMS97B	06/09/97	alfalfa	280	0.52 ± 0.12	9,793 (3,838-64,918)	14,482,765	558.32
ICA97	06/11/97	alfalfa	300	0.83 ± 0.11	12,024 (7,098-25,694)	1,199,195	685.52
PAR97	06/14/97	alfalfa	300	0.67 ± 0.13	428.01 (159.63-850.31)	126,589	24.40
BLY97	06/14/97	alfalfa	279	0.56 ± 0.13	88.17 (8.44-250.12)	79,009	1.0
Laboratory selection for Lannate resistance (F2 generation)							
YAC96B[f]	N/A	diet	275	0.56 ± 0.13	13,227 (6,362-64,089)	12,165,829	754.11

[a]Populations. Historical standards: 1975 - collected from cotton (Meinke and Ware 1978); 1989 - collected from cotton (Aldosari 1990). Laboratory standards: MIS96 - USDA-ARS Stoneville, MS; PHO96 - USDA-ARS, Phoenix, AZ; YAC96A - Yuma Agricultural Center, Yuma, AZ; SAL96 - Salome, AZ; DOM96 - Dome Valley, AZ; SOM96 - Somerton, AZ; TEX96 - Texas Hill, AZ; GAD97 - Gadsden, AZ; YMS97A - Yuma Mesa, AZ; YMS97B - Yuma Mesa, AZ; ICA97 - Imperial County, CA; PAR97 - Parker, AZ and BLY97 - Blythe, CA; YAC96B - Yuma Agricultural Center, Yuma, AZ;.

[b] LD_{50} - Lethal dose (μg/g) required to kill 50% of the population tested.

[c] LD_{95} - Lethal dose (μg/g) required to kill 95% of the population tested.

[d]RR - Resistance ratio, or X-fold increase in resistance. Calculated by dividing the LD_{50} of the strain tested by the LD_{50} of the historically susceptible 1975 strain. A resistance ratio of 1.0, indicates that no resistance was detected based on over lapping 95% fiducial limits.

[e]Data not available.

[f]YAC96B is a population created from YAC96A larvae surviving >4,000 μg-Lannate / g-larvae.

Table 2. Dose-mortality responses of BAW populations collected from Yuma to Larvin applied orally.

Population[a]	Date collected	Host	*n*	Slope ± SE	LC_{50} (95% fiducial limits)[b]	LC_{95}[c]	RR[d]
Laboratory susceptible populations							
MIS96	N/A	diet	330	1.72 ± 0.23	498.18 (346.55-638.00)	4,509	1.0
PHO96	N/A	diet	240	3.09 ± 0.35	700.88 (605.86-811.73)	2,390	1.0
Field collected populations (F1 generations)							
SAL96	08/19/96	melons	300	3.35 ± 0.41	2,099 (1,876-2,393)	6,497	4.21
DOM96	09/07/96	lettuce	250	3.83 ± 0.48	2,569 (2,283-2,921)	6,907	5.16
SOM96	09/11/96	lettuce	240	4.21 ± 0.54	2,698 (2,415-3,042)	6,630	5.42
TEX96	10/10/96	lettuce	325	9.55 ± 1.09	3,117 (2,965-3,276)	4,643	6.26
Laboratory selection for Lannate resistance (F2 generation)							
YAC96B[e]	N/A	diet	300	9.94 ± 1.33	2,402 (2,273-2,524)	3,515	4.82

[a]Populations: Laboratory standards: MIS96 - USDA-ARS Stoneville, MS; PHO96 - USDA-ARS, Phoenix, AZ; SAL96 - Salome, AZ; DOM96 - Dome Valley, AZ; SOM96 - Somerton, AZ; TEX96 - Texas Hill, AZ and YAC96B - Yuma Agricultural Center, Yuma, AZ.
[b] LC_{50} - Lethal concentration (mg-ai / kg-diet) required to kill 50% of the population tested.
[c] LC_{95} - Lethal concentration (mg-ai / kg-diet) required to kill 95% of the population tested.
[d]RR - Resistance ratio, or X-fold increase in resistance. Calculated by dividing the LC_{50} of the strain tested by the LC_{50} of the MIS96 strain. A resistance ratio of 1.0, indicates that no resistance was detected based on over lapping 95% fiducial limits.
[e]YAC96B is a population created from YAC96A larvae surviving >4,000 μg-Lannate / g-larvae

Table 3. Dose-mortality responses of BAW populations collected from Yuma to Lannate applied orally.

	Lannate Oral			RRs		
Population[a]	*n*	Slope ± SE	LD_{50} (95% fiducial limits)[b]	Lannate oral[c]	Lannate topical[d]	Larvin oral[e]
PHO96	200	2.12 ± 0.26	329.33 (257.26-411.26)	1.0	1.0	1.0
YAC96B	245	7.00 ± 1.03	1,642 (1,459-1,814)	4.99	607.30	3.43

[a]Populations: PHO96 - Susceptible laboratory populations from USDA-ARS, Phoenix; YAC96B is a population created from YAC96A larvae surviving >4,000 μg-Lannate / g-larvae

[b] LC_{50} - Lethal concentration (mg-ai / kg-diet) required to kill 50% of the population tested.

[c]Lannate oral, RR - Resistance ratio, or X-fold increase in resistance. Calculated by dividing the LC_{50} of YAC96B by the LC_{50} of the PHO96 strain. A resistance ratio of 1.0, indicates that no resistance was detected based on over lapping 95% fiducial limits.

[d]Lannate topical, RR - Resistance ratio, or X-fold increase in resistance. Calculated by dividing the LD_{50} of YAC96B by the LD_{50} of the PHO96 strain. A resistance ratio of 1.0, indicates that no resistance was detected based on over lapping 95% fiducial limits. Data from Table 1.

[e]Larvin oral, RR - Resistance ratio, or X-fold increase in susceptibility. Calculated by dividing the LC_{50} of YAC96B by the LC_{50} of the PHO96 strain. A resistance ratio of 1.0, indicates that no resistance was detected based on over lapping 95% fiducial limits. Data from Table 2.