SUSCEPTIBILITY OF TARNISHED PLANT BUG TO SELECT INSECTICIDES AND DEVELOPMENT OF DIAGNOSTIC DOSES

Moneen Jones
University of Missouri
Portageville, MO

Abstract

To determine baseline susceptibility of *Lygus lineolaris (Palisot de Beauvois)* to conventional and reduced-risk insecticides, serial dilutions of dicrotophos, dicrotophos+bifenthrin, bifenthrin, acetamiprid, acephate, zeta-cypermethrin, dimethoate, imidacloprid+bifenthrin and sulfoxaflor and others were each prepared from a 1000ppm stock solution. A petri dish was sprayed to 100% coverage with each representative solution using a Crown Spray-Tool system (Woodstock, Illinois) and allowed to dry. A green bean was dipped into solution with 0.25% Silwet L-77 surfactant, allowed to dry, and added to the petri dish. Five adult plant bugs were added to each petri dish, the dish was sealed with parafilm to prevent escape and held at 26.8±2ºC, 70% RH, 14:10(L:D). Mortality was assessed after 48h. Probit mortality lines for three diagnostic doses, LD_{50}, LD_{75}, and LD_{95}, for each chemical were estimated using POLO (LeOra Software, 2007).

Introduction

The 3 counties of southeast Missouri (Dunklin, New Madrid, and Pemiscot) contain about 98% of the State's cotton acreage, much of which consists of large-scale commercial fields. Many of the current management practices were developed and implemented in this region, and keen grower support for research and extension can be counted on. This is a high-yield industry which has seen tremendous increase in insecticide use over the past 5 years in response to drought and pests, made possible in part by strong market prices for cotton. However, these prices and practices may not be sustainable in the future. Furthermore, most cotton managers in the area were brought up with a strong commitment to integrated pest management (IPM), and there is great concern over the possible consequences of present insecticide use practices, including the likelihood of control failure due to insecticide resistance. Therefore there is keen interest in and support for this research in the cotton community.

Bollworms, tarnished plant bug (TPB), and cotton aphid are just a few of the key economic pests of cotton that have caused enormous losses to yields (Williams 2013). Insecticide resistance is confirmed in each of these species to organophosphates and to the second generation of pyrethroids (Martin et al. 1999; Leonard and Cook 2007; Snodgrass et al. 2008), specifically permethrin, cypermethrin and deltamethrin. Newer chemistries of pyrethroids (i.e. fenvalerate, lambda-cyhalothrin and beta-cyfluthrin) have developed over the decades that are more persistent in the environment.

Monitoring the presence and prevalence of resistant individuals in a population is a key step in insecticide resistance management (Roush and Miller 1986). These authors indicated that goals for resistance monitoring programs included detecting resistance before control failures, estimating the frequency of resistant individuals, making field-level choices of insecticides, and monitoring changes in resistance frequency. Because resistance is a genetically-based shift in population response, resistance monitoring relies on initial quantification in baseline responses of susceptible populations (Robertson et al. 2007). Thus it is necessary to determine baseline susceptibilities of TPB to key insecticides by estimation of statistical parameters of their concentration-response relationships in order to monitor resistance development and explain spray failures now and in the future (Robertson et al. 2007).

The goals of this research were to (1) determine baseline susceptibility of *Lygus lineolaris (Palisot de Beauvois)*, to select commercial and reduced-risk insecticides; and (2) develop a working diagnostic dose to monitor susceptibility of tarnished plant bug to key modes of action in field populations of these pests exposed to intensive versus modest insecticide use.

Methods

The main objectives of these bioassays were to develop concentration-response curves for key insecticides of tarnished plant bug.

Chemicals

Commercial formulations of acetamiprid (Intruder 70 WSP, DuPont Agricultural Products, Wilmington, DE), imidacloprid+bifenthrin (Brigadier, FMC Agricultural Chemicals, Philadelphia, PA), dimethoate (Dimethoate 4EC), zeta-cypermethrin (Mustang Maxx, FMC Agricultural Chemicals, Philadelphia, PA), sulfoxaflor (Transform WG, Dow Agrosciences, Indianapolis, IN), dicrotophos+bifenthrin (Bidrin XPII, Amvac Chemical Corp. Los Angeles, CA), dicrotophos (Bidrin 8, Amvac Chemical Corp. Los Angeles, CA), bifenthrin (Brigade 2EC, FMC Agricultural Chemicals, Philadelphia, PA), and acephate (Orthene 97, Amvac Chemical Corp. Los Angeles, CA) were diluted in de-ionized water for use as residual application.

Laboratory Colony

A laboratory colony was acquired from Mississippi State University in spring of 2014. The colony has been maintained at MSU for ca. 7 years and is considered to be susceptible to insecticides.

Bioassays

The treatments were prepared in such a way that the representative chemical was combined with de-ionized water to equal a 1000ppm stock solution. A series of dilutions were prepared from the stock solution. Fifteen adult plant bugs were collected from the susceptible lab-reared colony by a hand held aspirator. A vented petri dish (60mm) was sprayed to 100% coverage using a Crown Spray-Tool system (Woodstock, Illinois) and allowed to dry. A green bean (ca. 2 cm) was dipped for 10 sec into each representative insecticide solution with which 0.25% Silwet L-77 surfactant was added to allow even distribution of the insecticide on the bean. The bean was then place on a paper towel to dry, and then added to each petri dish. Dishes were aerated with mesh screen and sealed with parafilm to prevent escape. Treated adults were held at 26.8±2ºC, 70% RH, and a photoperiod of 14:10(L:D), and mortality was assessed after 48h by prodding the plant bugs with a blunt probe and observing their movement. A plant bug was considered to be alive if it was able to fly, walk, or move its antennae, legs, or head when prodded. Plant bugs that exhibited no walking and only twitching of the abdomen or antennae were considered to be moribund and combined with dead insects (no movement) for analysis. Each bioassay had a minimum of 4 replicates and was done between February 2014 – October 2014. Diagnostic doses were estimated between 20 May and 27 Aug 2014.

Statistical Analyses

Concentration-response (mortality) relationships were estimated with PoloPlus (LeOra Software, Petaluma, CA) as described by Robertson et al. (2007). Data were analyzed assuming the probit model. Plots of standardized residuals were examined for outliers (Robertson et al. 2007). Only concentrations between the lowest concentration that caused 100% mortality and the highest concentration that caused 0% mortality were used in analyses. Slopes, LD50's, LD75's, and LD95's were estimated for each bioassay.

Results

Thus far, baseline susceptibilities for tarnished plant bug to acetamiprid, (19.39, 33.72, 74.74ppm), imidacloprid+bifenthrin (10.32, 16.78, 33.77 ppm), dimethoate (9.55, 23.79, 88.35 ppm), zeta-cypermethrin (1.29, 3.16, 11.48 ppm), sulfoxaflor (0.05, 0.62, 5.90 ppm), dicrotophos+bifenthrin (5.31, 7.47, 10.15 ppm), dicrotophos (3.96, 7.86, 14.56 ppm), bifenthrin (0.39, 4.22, 35.97 ppm), and acephate (0.62, 5.09, 33.86 ppm) are completed (Tables 1 and 2). Diagnostic doses (LD50, LD75, and LD95) have been determined and tested on the susceptible lab colony for acetamiprid, imidacloprid+bifenthrin, dimethoate and zeta-cypermethrin (Tables 3 and 4).

Table 1. Concentration-mortality relationships for lab susceptible colonies of tarnished plant bug with conventional insecticides (Probit analysis).

Insecticide	n	Slope ± SE	χ^2	LD_{50}	95% CL Lower-Upper	LD_{75}	95% CL Lower-Upper	LD_{95}	95% CL Lower-Upper
Bifenthrin									
	300	0.65±0.09	2.28	0.39	0.18-0.81	4.22	1.91-12.88	35.97	11.96-212.57
Acephate									
	325	0.72±0.1	2.52	0.62	0.34-1.13	5.09	2.60-13.37	33.86	12.98-154.99
Zeta-cypermethrin									
	350	1.73±0.35	1.73	1.29	0.67-1.923	3.16	2.12-5.12	11.48	6.62-35.20
Dicrotophos									
	450	2.26±0.31	3.40	3.96	3.00-4.92	7.86	6.35-10.14	14.56	11.13-21.83
Dimethoate									
	570	1.70±0.25	5.87	9.55	4.87-14.27	23.79	16.09-38.99	88.35	50.20-296.46
Acetamiprid									
	480	2.81±0.32	3.13	19.39	16.30-22.53	33.72	28.90-40.77	74.74	58.31-108.10

Table 2. Concentration-mortality relationships for lab susceptible colonies of tarnished plant bug with reduced-risk insecticides (Probit analysis).

Insecticide	n	Slope ± SE	χ^2	LD_{50}	95% CL Lower-Upper	LD75	95% CL Lower-Upper	LD_{95}	95% CL Lower-Upper
Sulfoxaflor	425	0.62±0.08	4.24	0.05	0.02-0.13	0.62	0.24-3.02	5.90	1.19-83.80
Dicrotophos+Bifenthrin	315	4.56±0.62	2.59	5.31	4.58-6.02	7.47	6.58-8.74	12.20	10.15-16.30
Bifenthrin+Imidacloprid	295	3.19±0.45	6.70	10.32	7.37-13.89	16.78	12.66-29.56	33.77	21.78-110.84

Table 3. Three estimated diagnostic doses for select chemicals on tarnished plant bug.

Chemical	**LD_{50}**	**LD_{75}**	**LD_{95}**
Dimethoate	9.55	23.79	88.35
Bifenthrin+Imidacloprid	10.32	16.78	33.78
Acetamiprid	19.39	33.72	74.74
Sulfoxaflor	0.05	0.62	5.9
Dicrotophos+Bifenthrin	5.31	7.47	12.2
Dicrotphos	3.96	7.86	14.56
Bifenthrin	0.39	4.22	35.97
Acephate	0.62	5.09	33.86

Table 4. Results of diagnostic doses on lab susceptible colony for verification. Mortality corrected using Abbott's formula (1925).

Lab Susceptible Colony		
Chemical	**n**	**% Mortality**
Dimethoate		
LD50	100	54
LD75	100	75
LD95	100	99
Zeta-Cypermethrin		
LD50	100	63
LD75	100	83
LD95	100	96
Bifenthrin+Imidacloprid		
LD50	100	59
LD75	100	73
LD95	100	97
Acetamiprid		
LD50	100	55
LD75	100	73
LD95	100	97

Discussion

Monitoring the presence and prevalence of resistant individuals in a population is a key step in insecticide resistance management (Roush and Miller 1986). Roush and Miller (1986) indicated that the goals for resistance monitoring programs include detecting resistance before control failures, estimating the frequency of resistant individuals, making field-level choices of insecticides, and monitoring changes in resistance frequency. Baseline data from susceptible populations are a prerequisite for understanding the development of resistance to insecticides in the field. Because resistance is a genetically-based shift in population response, resistance monitoring is aided by the initial quantification of responses to toxins by susceptible populations (Robertson et al. 2007). To determine the baseline susceptibility of *Lygus lineolaris* to these insecticides, we estimated the statistical parameters of concentration-response relationships observed in binary bioassays (Robertson et al. 2007). While we selected conventional and reduced-risk insecticides for evaluations, the two older classes of pyrethroid and organophosphate (i.e. Bifenthrin and acephate) continue to offer the highest toxicity to tarnished plant bug. Field populations of *Lygus* with varying degrees of insecticide exposure will be tested the spring and summer of 2015 with results to follow.

An alternative to using a full binary bioassay to detect resistance is the use of a single discriminating or diagnostic dose (Brown and Pal 1971, Roush and Miller 1986, Halliday and Burnham 1990, Usmani and Shearer 2001). Halliday and Burnham (1990) noted that the term "discriminating dose" is used when genetic and toxicological tests reveal differences in response by genotypes, whereas the term "diagnostic dose" is used to monitor changes in phenotypic response. When a diagnostic dose is administered to a sufficiently large sample of insects, survivorship that significantly exceeds the expected level (often 1 percent, but determined by the selection of the diagnostic dose) is considered possible evidence of resistance (WHO 1976). Even though the use of a diagnostic dose increases the efficiency of monitoring efforts designed to detect resistance at an early stage, thousands of insects must be tested to detect resistance at phenotypic frequencies of 0.001 or less (Roush and Miller 1986). To reduce the numbers of insects necessary to calculate effective diagnostic doses, this research is determining three diagnostic doses (i.e. LD_{50}, LD_{75}, and LD_{95}) for use in field monitoring. These doses will be tested on field populations of *Lygus* during spring and summer 2015.

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