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Baseline Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to Indoxacarb, Emamectin Benzoate, and Chlorantraniliprole in Australia

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ABSTRACT Baseline susceptibility of *Helicoverpa armigera* (Hübner) to emamectin benzoate, chlorantraniliprole, and indoxacarb was determined in feeding assays on insecticide-incorporated artificial diet in the laboratory. The intraspecific variation of *H. armigera* was established from field populations collected between September 2012 and March 2013, primarily from commercial farms across eastern Australia. Emamectin benzoate had the highest toxicity with a median lethal concentration (LC_{50}) of 0.01 µg/ml diet ($n = 20$ strains). The LC_{50} for chlorantraniliprole was 0.03 µg/ml diet ($n = 21$ strains), while indoxacarb had the lowest relative toxicity with an average LC_{50} of 0.3 µg/ml diet ($n = 22$ strains). Variation in susceptibility amongst field strains was 2.3-fold for emamectin benzoate and 2.9-fold for chlorantraniliprole and indoxacarb. Discriminating concentrations of 0.2, 1, and 12 µg of insecticide per milliliter of diet for emamectin benzoate, chlorantraniliprole, and indoxacarb, respectively, were calculated from toxicological data from field *H. armigera* strains as a first step in resistance management of these classes of insecticide in Australia. The low intraspecific tolerance, high slope values, and goodness-of-fit to a probit binomial model obtained in this study suggest that a feeding assay using diet incorporated insecticide is an effective laboratory method for measuring the dose–responses of these classes of insecticides in *H. armigera*.

KEY WORDS insecticide, *Helicoverpa armigera*, resistance monitoring, emamectin benzoate, chlorantraniliprole

Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner), is major pest of field crops in Australasia, Asia, Africa, and South America and has developed resistance to many conventional insecticides including synthetic pyrethroids (Forrester et al. 1993), organophosphates (Gunning et al. 1998), carbamates (Gunning et al. 1996a), and spinosad (Wang et al. 2009). This capacity to develop resistance is partly due to its distribution, which is one the widest for any insect pest (Fitt 1989). This species is also highly polyphagous, with 72 known host species distributed in 29 families in Australia (Zalucki 1986). In addition, *H. armigera* is a significant pest of cotton with ∼30% of pesticide applications made worldwide used to target this pest (Ahmad 2007).

Since the early 1980s, a resistance monitoring program has been in place for detecting changes in resistance frequencies in Australian field populations of *H. armigera* (Forrester et al. 1993; Gunning et al. 1996b, 1998). The resistance monitoring program is currently part of a broader preemptive insecticide resistance management strategy used primarily by the Australian cotton industry to formulate responses to emerging *H. armigera* resistance issues (Bird et al. 2013). Monitoring methods for the insecticide resistance management strategy were originally developed for broad-spectrum contact insecticides such as synthetic pyrethroids, organophosphates, and endosulfan. However, management of *Helicoverpa* spp., particularly *H. armigera*, is becoming increasingly reliant on newer selective insecticides such as emamectin benzoate, chlorantraniliprole, and indoxacarb because of their low toxicity to beneficial insects (Chukwudebe et al. 1997, Hewa-Kapuge et al. 2003, Bostanian et al. 2004, Gonzalez-Zamora et al. 2004, Dinter et al. 2009) and, therefore, high compatibility with integrated pest management programs.

Techniques for monitoring insecticide resistance in *Helicoverpa* spp. in Australia were originally developed for contact insecticides using topical application of a discriminating concentration of insecticide (Forrester et al. 1993). Resistance to selective chemistries was also monitored in Australia by topical application since they were first commercialized; emamectin benzoate in 2000, indoxacarb in 2001, and chlorantraniliprole in 2008 (Rossiter et al. 2008). However, emamectin benzoate, chlorantraniliprole, and indoxacarb intoxicate insects via both contact and ingestion, with the latter considered the primary route whereby insects accumulate a lethal dose of insecticide (Lasota and Dybas 1991, Wing et al. 2004, Temple et al. 2009).

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Emamectin benzoate is a semisynthetic insecticide derived from the avermectin family of compounds and belongs to the Insecticide Resistance Action Committee (IRAC) mode of action class 6 (IRAC 2012). Avermectins are naturally occurring macrocyclic lactones isolated from fermentation products of the soil microorganism *Streptomyces avermitilis*. Emamectin benzoate acts as an agonist for gamma-aminobutyric acid-gated chloride channels (Kass et al. 1980). The resulting irreversible activation of chloride channels causes disruption of nerve impulses and rapid paralysis in a range of lepidopteran species (Jansson et al. 1997, Argentine et al. 2002, Ishaaya et al. 2002).

Chlorantraniliprole is an anthranilic diamide belonging to the IRAC mode of action class 28 (IRAC 2012), providing a broad range of activity across a range of lepidopteran species (Temple et al. 2009). As a selective agonist for ryanodine receptors in the muscle cells of insects, it stimulates the release and depletion of calcium from the internal stores in the sarcoplasmic reticulum, causing impaired regulation of muscle contraction leading to feeding cessation (Cordova et al. 2006).

Indoxacarb is a pyrazoline-type sodium channel blocker with activity against a range of lepidopteran, coleopteran, and sucking insect pests (Wing et al. 1998). It belongs to the IRAC mode of action class 22 (IRAC 2012). Metabolism studies in Lepidoptera have shown that indoxacarb is bioactivated to the active *N*-decarbomethoxyllated metabolite, which blocks voltage-dependent sodium channels, preventing the influx of sodium into neurons (Wing et al. 2004).

The objectives of this study were to 1) develop a feeding bioassay method to determine toxicity of the selective insecticides to *H. armigera*, and evaluate the use of this method as an appropriate alternative to the traditional topical method of bioassay; 2) accumulate baseline susceptibility data to determine the full range of intraspecific tolerance in field populations of *H. armigera*; and 3) utilize the baseline data generated to determine discriminating concentrations for emamectin benzoate, chlorantraniliprole, and indoxacarb for use in resistance monitoring programs.

Materials and Methods

Insect Strains. A laboratory-susceptible strain was used to check for consistency of bioassay at 4- to 6-wk intervals throughout the duration of the study. The laboratory strain New GR was established from a cohort of a general laboratory strain GR. Progenitor strains of New GR were sourced during the mid-1980s from a series of collections from cotton fields in the Namoi Valley, northern New South Wales (NSW).

Field strains were primarily sourced from major cropping areas located across NSW and in southern and central Queensland (QLD) in Australia. Populations were established from insects ranging from egg to pupa collected between September 2012 and March 2013 from cotton, sorghum, pigeon pea, maize, pulses, and a scrophulariaceous weed host, *Verbascum virgatum* Stokes. All strains were tested within three generations of establishment in the laboratory. A minimum of 50 field-collected individuals constituted any one geographically distinct strain.

Insect Rearing. Rearing methods used to maintain both field and laboratory strains were the procedures described by Teakle and Jensen (1985) except that formalin was omitted and soybean flour was heat-treated in a microwave for 10 min to remove enzyme inhibitors. Rearing trays (Tacca Plastics, Sydney, Australia) were covered and heat-sealed with perforated lids (Oliver Products, Grand Rapids, MI). Moths were provided with a 4% honey/sugar solution fed through a cotton wick and housed in containers open at the top and covered with cloth liners secured around the lip of the containers.

In the larval stage, insect strains were maintained under a laboratory environment of 25 ± 2°C, a photoperiod of 14:10 (L: D) h, and 45–55% relative humidity. Adults were maintained in a separate facility under the same conditions of light and temperature with relative humidity maintained at 70–80% for the duration of the dark cycle using a steam humidifier.

Insect Bioassays. The dose–responses of laboratory and field strains were measured by performing bioassays on artificial diet into which formulated insecticide was incorporated. The ratio of diet to toxin determined the concentration calculated as micrograms of insecticide per milliliter of diet. Formulated insecticides were diluted in distilled water producing twofold serial insecticide dilutions spanning six or seven insecticide concentrations that were expected to induce from 1 to 99% mortality. Serial dilutions were added to 200 ml of diet and incorporated by vigorous shaking by hand for 30 s to produce a homogenous mixture. Insecticide-incorporated diet was then dispensed into 45-well bioassay trays (Tacca Plastics, Sydney, Australia), each with approximately 1.5 ml of diet per well.

Insects for use in bioassays were reared on untreated diet to the late second or third larval instar and then introduced to trays containing bioassay diet (one larva per well) and covered with heat-sealed, perforated lids. The use of late second- or third-instar larvae ensured that only strains with sufficient fitness were utilized as bioassay subjects. Each bioassay was performed in triplicate with individual treatments (insecticide concentrations) in replicates consisting of a minimum of 20 individuals; untreated diet was used as the control.

Bioassays were performed on a minimum of four nonsynchronous cohorts of New GR between November 2012 and May 2013. The results were pooled in the final analysis because there were no significant differences between cohorts for any of the insecticides tested. The average LC_{50} generated from these bioassays was used as the estimate of baseline susceptibility for the New GR strain.

Bioassays were maintained for 7 d under the same conditions described above for larval rearing. Larvae were considered dead if one or more of the following criteria were demonstrated: larvae unable to perform coordinated movement when prodded; paralysis of prolegs; larvae very slow to right themselves (time exceeding 3 s).

Insecticides. Commercial insecticide formulations were used in all bioassays: indoxacarb (Steward EC [15% active ingredient], Du Pont Australia Ltd., Macquarie Park, Australia), emamectin benzoate (Affirm [1.9% active ingredient], Syngenta Crop Protection), and chlorantraniliprole (Altacor [35% active ingredient], Du Pont Australia Ltd., Macquarie Park, Australia).

Data Analysis. The dose–responses of larvae to insecticides were corrected for control mortality using the formula of Abbott (1925). Slope, LC_{50} and $LC_{99.9}$ estimates, and associated 95% fiducial limits (FLs) were calculated by probit analysis using the POLO-PC software (LeOra Software, Berkeley, CA). The toxicity ratio of emamectin benzoate, indoxacarb, and chlorantraniliprole was calculated by dividing the LC_{50} value of each population by the LC_{50} value (average of four nonsynchronous cohorts) of the laboratory strain.

Results

Toxicity of Insecticides to Laboratory Strain. The relative toxicity of insecticides was highest for the susceptible strain. Emamectin benzoate was the most toxic of the three insecticides tested ($LC_{50} = 0.007$ μg/ml; Table 1). Chlorantraniliprole was twofold less toxic to the laboratory strain ($LC_{50} = 0.014$ μg/ml; Table 2), with indoxacarb the least toxic insecticide tested ($LC_{50} = 0.147$ μg/ml; Table 3).

Toxicity of Insecticides to Field Strains. The response of field populations of *H. armigera* to emamectin benzoate is shown in Table 1. There was a narrow range of variation between field strains with LC_{50} values ranging from 0.007 to 0.017 μg/ml. The dose–responses were accompanied by high slopes (3.5 from the pooled data of 20 field strains). High susceptibility was demonstrated by low toxicity ratios ranging between 1.0 and 2.3. A population collected from chickpea in St. George southern QLD had the highest tolerance, with an $LC_{99.9}$ value of 0.154 μg/ml.

The response of field populations of *H. armigera* to chlorantraniliprole is shown in Table 2. The range of intraspecific variation between field strains to chlorantraniliprole was narrow, with LC_{50} values ranging from 0.016 to 0.048 μg/ml. The pooled slope value from chlorantraniliprole bioassays of 21 strains was 2.5. Low toxicity ratios ranging between 1.1 and 3.4 indicate that susceptibility of field populations was relatively high. A population originating from a soya bean field at Wee Waa in northern NSW had the highest tolerance with an $LC_{99.9}$ value of 1.238 μg/ml.

The response of field populations of *H. armigera* to indoxacarb is shown in Table 3. High susceptibility of field *H. armigera* to indoxacarb was demonstrated by low toxicity ratios ranging between 1.2 and 3.5. There was a narrow range of intraspecific variation in indoxacarb toxicity between field strains, with LC_{50} values ranging from 0.175 to 0.518 μg/ml. The slope value from pooled indoxacarb bioassays of 22 strains was 2.3. The most tolerant strains had $LC_{99.9}$ values >11 μg/ml. In one case (North Star maize and sorghum) the $LC_{99.9}$ exceeded 14 μg/ml. However, this result was accompanied by a low slope value of 1.9.

Determination of Discriminating Concentrations of Insecticide. Discriminating concentrations for use in monitoring resistance to emamectin benzoate, chlorantraniliprole, and indoxacarb in *H. armigera* were based on the $LC_{99.9}$ response of the most tolerant

Table 1. Bioassay on 20 field strains and 1 laboratory-susceptible strain of *H. armigera* tested as early third instars on diet-incorporated emamectin benzoate (19 g/liter) and assessed for mortality at 7 d

Collection data			LC_{50} (μg/ml diet; 95% FL)	$LC_{99.9}$ (μg/ml diet)	Slope ± SE	Toxicity ratio[b]	% Mortality at 0.095 μg/ml diet[c]
Origin of field strains (G[a])	Host crop	Collection date					
Pampas QLD (F_1)	Pigeon pea	Jan. 2013	0.007 (0.006–0.009)	0.047	3.9 ± 0.54	1.0	100 (60)
Nandi QLD (F_1)	Cotton/pigeon pea	Jan. 2013	0.008 (0.006–0.009)	0.027	5.5 ± 1.10	1.0	100 (60)
Wee Waa NSW (F_2)	Sorghum	Dec. 2012	0.008 (0.006–0.009)	0.067	3.3 ± 0.45	1.0	100 (60)
Narromine NSW (F_2)	Maize	Nov. 2012	0.008 (0.005–0.011)	0.046	4.0 ± 0.97	1.0	100 (56)
Wee Waa NSW (F_1)	Soya bean	Feb. 2013	0.009 (0.006–0.013)	0.058	3.8 ± 0.90	1.2	100 (60)
Emerald QLD (F_1)	Chickpea	Sept. 2012	0.009 (0.008–0.010)	0.052	4.1 ± 0.41	1.2	100 (60)
Breeza NSW (F_1)	Pigeon pea	Feb. 2013	0.009 (0.007–0.012)	0.040	4.9 ± 1.09	1.3	100 (60)
Griffith NSW (F_2)	Cotton/pigeon pea	Mar. 2013	0.009 (0.007–0.012)	0.031	5.9 ± 1.40	1.3	100 (59)
Wee Waa NSW (F_2)	Maize	Dec. 2012	0.010 (0.007–0.012)	0.040	4.9 ± 1.08	1.3	100 (56)
Mullaley NSW (F_2)	Maize	Dec. 2012	0.010 (0.009–0.011)	0.060	4.0 ± 0.39	1.4	100 (60)
North Star NSW (F_1)	Maize/sorghum	Mar. 2013	0.010 (0.008–0.013)	0.084	3.4 ± 0.56	1.4	100 (60)
Cecil Plains QLD (F_2)	Cotton	Jan. 2013	0.010 (0.009–0.012)	0.045	4.9 ± 0.52	1.4	100 (60)
Wee Waa NSW (F_1)	Pigeon pea	Jan. 2013	0.011 (0.008–0.014)	0.055	4.3 ± 0.88	1.4	100 (60)
St George QLD (F_1)	Pigeon pea	Jan. 2013	0.011 (0.009–0.014)	0.073	3.8 ± 0.58	1.5	100 (60)
Wee Waa NSW (F_1)	Mung bean	Feb. 2013	0.011 (0.008–0.015)	0.100	3.2 ± 0.61	1.5	100 (60)
St George QLD (F_1)	Pigeon pea	Feb. 2013	0.012 (0.009–0.015)	0.085	3.6 ± 0.53	1.6	100 (60)
ACRI NSW (F_1)	Sorghum	Feb. 2013	0.012 (0.011–0.014)	0.127	3.0 ± 0.28	1.6	98.3 (60)
Felton QLD (F_2)	Canola	Oct. 2012	0.012 (0.010–0.015)	0.146	2.9 ± 0.33	1.7	100 (100)
Loxton QLD (F_1)	Cotton	Jan. 2013	0.013 (0.010–0.017)	0.092	3.7 ± 0.61	1.8	100 (60)
St George QLD (F_2)	Chickpea	Sept. 2012	0.017 (0.013–0.022)	0.155	3.2 ± 0.52	2.3	98.8 (80)
Pooled			0.010 (0.009–0.011)	0.079	3.5 ± 0.19		
Laboratory strain (New GR)			0.007 (0.006–0.009)	0.030	5.1 ± 1.00		100 (60)

[a] Generation tested.
[b] Toxicity ratio = LC_{50} of population/LC_{50} of New GR strain (average of four nonsynchronous New GR cohorts).
[c] Mortality at highest concentration tested.

Table 2. Bioassay on 21 field strains and 1 laboratory-susceptible strain of *H. armigera* tested as early third instars on diet-incorporated chlorantraniliprole (350 g/kg) and assessed for mortality at 7 d

Collection data			LC_{50} (μg/ml diet; 95% FL)	$LC_{99.9}$ (μg/ml diet)	Slope ± SE	Toxicity ratio[b]	% Mortality at 0.5 μg/ml diet[c]
Origin of field strains (G[a])	Host crop	Collection date					
Nandi QLD (F_1)	Cotton/pigeon pea	Jan. 2013	0.016 (0.014-0.019)	0.161	3.1 ± 0.32	1.1	100 (60)
Emerald QLD (F_1)	Chickpea	Sept. 2012	0.018 (0.015-0.021)	0.223	2.8 ± 0.24	1.2	100 (60)
Loxton QLD (F_2)	Cotton	Jan. 2013	0.018 (0.012–0.024)	0.815	1.9 ± 0.28	1.3	100 (59)
ACRI NSW (F_1)	Sorghum	Feb. 2013	0.019 (0.014–0.025)	0.193	3.1 ± 0.54	1.3	100 (60)
Wee Waa NSW (F_1)	Mung bean	Feb. 2013	0.019 (0.016–0.023)	0.304	2.6 ± 0.26	1.4	100 (60)
Griffith NSW (F_2)	Cotton/pigeon pea	Mar. 2013	0.020 (0.015–0.025)	0.148	3.6 ± 0.55	1.4	100 (60)
Walgett NSW (F_2)	Chickpea	Sept. 2012	0.020 (0.015–0.027)	0.590	2.1 ± 0.29	1.4	100 (60)
Narromine NSW (F_2)	Sweet corn	Mar. 2013	0.023 (0.020–0.026)	0.192	3.3 ± 0.33	1.6	100 (60)
St. George QLD (F_1)	Pigeon pea	Feb. 2013	0.023 (0.020–0.026)	0.222	3.1 ± 0.32	1.6	100 (60)
Mullaley NSW (F_2)	Maize	Dec. 2012	0.023 (0.021–0.026)	0.139	4.0 ± 0.40	1.6	100 (60)
Wee Waa NSW (F_1)	Pigeon pea	Jan. 2013	0.024 (0.018–0.032)	0.137	4.1 ± 0.76	1.7	100 (60)
Wee Waa NSW (F_2)	Maize	Dec. 2012	0.025 (0.018–0.033)	0.305	2.8 ± 0.47	1.7	100 (60)
Felton QLD (F_2)	Canola	Oct. 2012	0.027 (0.023–0.032)	0.669	2.2 ± 0.20	1.9	98.3 (60)
St George QLD (F_2)	Chickpea	Sept. 2012	0.027 (0.024–0.031)	0.244	3.2 ± 0.27	1.9	100 (60)
Narromine NSW (F_2)	Maize	Nov. 2012	0.028 (0.022–0.036)	0.403	2.6 ± 0.35	1.9	100 (60)
Pampas QLD (F_2)	Pigeon pea	Jan. 2013	0.030 (0.025–0.036)	0.991	2.0 ± 0.18	2.1	100 (60)
Warren NSW (F_1)	Sunflower	Mar. 2013	0.030 (0.026–0.034)	0.237	3.4 ± 0.32	2.1	100 (60)
Wee Waa NSW (F_2)	Sorghum	Dec. 2012	0.031 (0.026–0.036)	0.505	2.5 ± 0.24	2.1	100 (60)
Breeza NSW (F_1)	Pigeon pea	Feb. 2013	0.035 (0.030–0.041)	0.585	2.5 ± 0.22	2.4	100 (58)
Cecil Plains QLD (F_1)	Cotton	Jan. 2013	0.041 (0.033–0.052)	0.698	2.5 ± 0.29	2.9	100 (80)
Wee Waa NSW (F_2)	Soya bean	Feb. 2013	0.048 (0.037–0.062)	1.238	2.2 ± 0.26	3.4	100 (58)
Pooled			0.025 (0.023–0.027)	0.454	2.5 ± 0.10		
Laboratory strain (New GR)			0.014 (0.013–0.016)	0.106	3.5 ± 0.30		

[a] Generation tested.
[b] Toxicity ratio = LC_{50} of population/LC_{50} of New GR strain (average of four nonsynchronous New GR cohorts).
[c] Mortality at highest concentration tested.

Table 3. Bioassay on 22 field strains and 1 laboratory-susceptible strain of *H. armigera* tested as early third instars on diet-incorporated indoxacarb (150 g/liter) and assessed for mortality at 7 d

Collection data			LC_{50} (μg/ml diet; 95% FL)	$LC_{99.9}$ (μg/ml diet)	Slope ± SE	Toxicity ratio[b]	% Mortality at 6 μg/ml diet[c]
Origin of field strains (G[a])	Host crop	Collection date					
Walgett NSW (F_2)	Chickpea	Sept. 2012	0.175 (0.132–0.229)	2.950	2.5 ± 0.35	1.2	100 (60)
Emerald QLD (F_1)	Chickpea	Sept. 2012	0.196 (0.154–0.248)	1.019	4.3 ± 0.78	1.3	100 (60)
Narromine NSW (F_2)	Sweet corn	Mar. 2013	0.215 (0.169–0.273)	3.881	2.5 ± 0.31	1.5	100 (60)
Griffith NSW (F_2)	Cotton/pigeon pea	Mar. 2013	0.217 (0.160–0.294)	3.843	2.5 ± 0.37	1.5	100 (60)
St George QLD (F_1)	Pigeon pea	Feb. 2013	0.229 (0.158–0.310)	5.285	2.3 ± 0.37	1.6	100 (60)
Wee Waa NSW (F_2)	Maize	Dec. 2012	0.231 (0.188–0.281)	1.665	3.6 ± 0.49	1.6	100 (60)
Warren NSW (F_1)	Sunflower	Mar. 2013	0.245 (0.188–0.312)	2.691	3.0 ± 0.44	1.7	100 (60)
Wee Waa NSW (F_2)	Sorghum	Dec. 2012	0.254 (0.222–0.290)	2.077	3.4 ± 0.33	1.7	100 (60)
ACRI NSW (F_1)	Sorghum	Feb. 2013	0.280 (0.213–0.357)	6.283	2.3 ± 0.29	1.9	100 (60)
Nandi QLD (F_1)	Cotton/pigeon pea	Jan. 2013	0.286 (0.247–0.329)	3.039	3.0 ± 0.28	1.9	100 (60)
St George QLD (F_2)	Chickpea	Sept. 2012	0.286 (0.240–0.342)	9.686	2.0 ± 0.18	1.9	100 (60)
Wee Waa NSW (F_1)	Soya bean	Feb. 2013	0.298 (0.243–0.358)	11.213	2.0 ± 0.19	2.0	100 (60)
Loxton QLD (F_1)	Cotton	Jan. 2013	0.305 (0.223–0.406)	6.731	2.3 ± 0.34	2.1	100 (60)
St George QLD (F_1)	Pigeon pea	Jan. 2013	0.315 (0.241–0.409)	3.702	2.9 ± 0.42	2.1	100 (60)
Pampas QLD (F_1)	Pigeon pea	Jan. 2013	0.327 (0.241–0.439)	4.898	2.6 ± 0.42	2.2	100 (60)
North Star NSW (F_1)	Maize/sorghum	Mar. 2013	0.346 (0.288–0.415)	14.803	1.9 ± 0.15	2.3	100 (60)
Breeza NSW (F_1)	Pigeon pea	Feb. 2013	0.348 (0.287–0.416)	12.372	2.0 ± 0.18	2.4	100 (60)
Mullaley NSW (F_2)	Maize	Dec. 2012	0.357 (0.287–0.441)	4.579	2.8 ± 0.33	2.4	100 (60)
Wee Waa NSW (F_2)	Pigeon pea	Jan. 2013	0.359 (0.303–0.422)	7.592	2.3 ± 0.21	2.4	100 (60)
Cecil Plains QLD (F_1)	Cotton	Jan. 2013	0.440 (0.339–0.579)	11.190	2.2 ± 0.30	3.0	100 (60)
Narromine NSW (F_2)	Maize	Nov. 2012	0.447 (0.350–0.563)	11.917	2.2 ± 0.23	3.0	100 (60)
Wee Waa NSW (F_1)	Mung bean	Feb. 2013	0.518 (0.405–0.659)	11.864	2.3 ± 0.25	3.5	98.3 (60)
Pooled			0.291 (0.270–0.314)	6.812	2.3 ± 0.10		
Laboratory strain (New GR)			0.147 (0.137–0.158)	1.172	3.4 ± 0.19		

[a] Generation tested.
[b] Toxicity ratio = LC_{50} of population/LC_{50} of New GR strain (average of four nonsynchronous New GR cohorts).
[c] Mortality at highest concentration tested.

strains, and the empirical response to the highest concentration of the insecticide tested. In each case, a very low rate of survival was observed at the highest concentration of the serial dose response. To ensure that the discriminating concentration was rigorous enough to remove false positives, the concentration was set at double the dose at which this low rate of survival was observed.

Table 4. Comparison of toxicity of insecticides on a laboratory-susceptible strain using topical and feeding methods of bioassay

Insecticide	LD_{50} (μg/ml; 95% FL)	Slope ± SE	Reference
Emamectin benzoate	7.4 (5.9–9.0)	1.7 ± 0.10	Bird and Downes (2014)
Chlorantraniliprole	3.1 (2.4–3.9)	1.9 ± 0.12	Bird and Downes (2014)
Indoxacarb	25.5 (22.8–28.4)	4.0 ± 0.26	Bird and Downes (2014)

Empirical mortality observed at the highest concentration of the serial dose–response for emamectin benzoate (0.095 μg/ml), which produced between 98.3 and 100% mortality, is shown in Table 1. On the basis of the observed response to the highest concentration of emamectin benzoate tested, and taking into account the $LC_{99.9}$ value of the most tolerant strains, the discriminating concentration for emamectin benzoate is recommended as 0.2 μg/ml. Using observed data from the highest concentration of chlorantraniliprole tested (0.5 μg/ml; Table 2), and the $LC_{99.9}$ estimates of mortality in the most tolerant strain, a discriminating concentration for chlorantraniliprole of 1 μg/ml is recommended. To test the suitability of a discriminating concentration, strains were assessed for mortality at a dose of 6 μg/ml and mortality ranged from 98.3 to 100% (Table 3). Using these data and the $LC_{99.9}$ estimates of mortality in the most tolerant strains, the recommended discriminating concentrations for indoxacarb is 12 μg/ml.

Comparison of Toxicity of Insecticides Using Topical and Feeding Bioassay Methods. In the laboratory-susceptible stain, emamectin benzoate, chlorantraniliprole, and indoxacarb were more toxic when administered orally in a feeding bioassay as compared with contact activity when administered topically (Table 4). The slope values associated with the dose–response from the feeding bioassay were also higher than that for the topical bioassay (Table 4). In particular, the slopes were markedly higher in feeding bioassays for both emamectin benzoate and chlorantraniliprole with values of 5.1 and 3.5, respectively, as compared with slopes of 1.7 and 1.9 for emamectin benzoate and chlorantraniliprole, respectively, in topical tests.

Discussion

The use of a discriminating concentration technique in resistance monitoring programs is considered to be a highly efficient method for monitoring resistance in insect populations (Roush and Miller 1986). A discriminating concentration technique based on topical application of insecticide has been used in *Helicoverpa* spp. resistance monitoring in Australia since the mid-1980s. This technique was originally developed for broad-spectrum insecticides whereby toxicity is mediated primarily through contact mode of entry (Forrester et al. 1993). Although topical bioassays produce usable dose–response curves, they can be associated with low slope values.

More recently, selective chemistries have been introduced for which intoxication occurs primarily by ingestion (Lasota and Dybas 1991, Wing et al. 2004, Temple et al. 2009). Results from the present study demonstrate that emamectin benzoate, chlorantraniliprole, and indoxacarb are more toxic when administered by ingestion than orally in *H. armigera*. This study also demonstrates that high slope values are associated with the dose–response regressions from diet-incorporation bioassays. This suggests that delivery by ingestion using a diet-incorporation method of bioassay is highly effective for assessing the toxicity of these chemistries and would be an appropriate alternative method for use in resistance monitoring programs.

Ideally, baseline susceptibility should be assessed before product commercialization, particularly for products with narrow spectra of activity, or where activity is concentrated against pests with historical problems of insecticide resistance (ffrench-Constant and Roush 1990, Jutsum et al. 1998). After more than a decade of commercial use of indoxacarb and emamectin benzoate, and several years of commercial use of chlorantraniliprole in Australia, increased resistance frequencies to these chemistries have yet to be reported from direct field screening of Australian populations of *H. armigera* using established topical bioassay methods (L.J.B. unpublished data). Results from the present study demonstrate high susceptibility in Australian populations of *H. armigera* to emamectin benzoate, chlorantraniliprole, and indoxacarb along with a narrow range (less than threefold) of intraspecific variation between geographically diverse populations. Notwithstanding the long-term registration of these products in Australia, it is unlikely that these small differences are the result of resistance selection. Rather they are likely to be the result of natural variability reflecting the true range of intraspecific tolerance inherent in this species to these chemistries.

Low intraspecific variation in baseline sensitivity to emamectin benzoate and indoxacarb has also been reported among populations of *H. armigera* from Pakistan (Ahmad et al. 2003), with low variation in baseline chlorantraniliprole toxicity reported for field populations of other noctuid species including *Spodoptera litura* (F.) (Su et al. 2012) and *Helicoverpa zea* (Boddie) (Temple et al. 2009). However, a narrow range of susceptibility among intraspecific populations may not preclude the potential to respond to selection pressure (Tabashnik 1994). For example, despite a low (less than fivefold) range of variation observed in baseline studies of chlorantraniliprole toxicity to *Plutella xylostella* (L.) (Wang et al. 2010), subsequent reports suggest rapid resistance development in this species (Wang and Wu 2012).

Increased detoxification enzyme activity in pest populations is considered one of the most important factors

for development of insecticide resistance (Denholm and Roland 1992, Li et al. 2007). Enhanced activities of mixed function oxidase and esterase enzymes are associated with pyrethroid resistance in Australian populations of *H. armigera* (Gunning et al. 1996b, Joußen et al. 2012, Teese et al. 2013). Increased metabolic capability has also been implicated as a mechanism for resistance in *H. armigera* to selective insecticidal chemistries and insecticidal toxins from the bacterium *Bacillus thuringiensis* (Gunning et al 2005, Alvi et al. 2012). High susceptibility to emamectin benzoate, chlorantraniliprole, and indoxacarb in Australian *H. armigera* with existing high frequencies of resistance to fenvalerate (L.J.B. unpublished data) suggest a lack of cross-resistance to pyrethroid. Further studies involving the use of enzyme inhibitors are required to confirm this. A lack of cross-resistance to indoxacarb was also found in pyrethroid-resistant populations of *H. armigera* from Pakistan (Ahmad et al. 2003) and Central Africa (Achaleke et al. 2009), and in pyrethroid-resistant strains of *P. xylostella* (Lasota et al. 1996, Yu and McCord 2007). On the other hand, pyrethroid-selected strains of *H. armigera* from India and Australia were shown to be negatively cross-resistant to indoxacarb (Gunning and Devonshire 2002, Ramasubramanian and Regupathy 2004).

Determination of a discriminating concentration of insecticide for resistance monitoring is an empirical compromise based, firstly, on the limits of tolerance, and secondly on a concentration of insecticide that kills most (99.9%) of the susceptible individuals. Various methods can be used to assign a discriminating concentration for use in resistance monitoring (Busvine 1971). But regardless of the method, it is important to identify a concentration that is sufficiently high so as to effectively discriminate between resistant and susceptible phenotypes without detecting false positives. On the other hand, the concentration should not be so high so as to mask resistance.

In the present study, determination of discriminating concentration is based on a theoretical estimate of the highest $LC_{99.9}$ value, while taking into account the empirical mortality observed at the upper limits of the dose–response curve (Robertson et al. 2007). Based on these criteria, we propose a discriminating concentration of 0.2, 1, and 12 μg/ml of diet for emamectin benzoate, chlorantraniliprole, and indoxacarb, respectively.

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