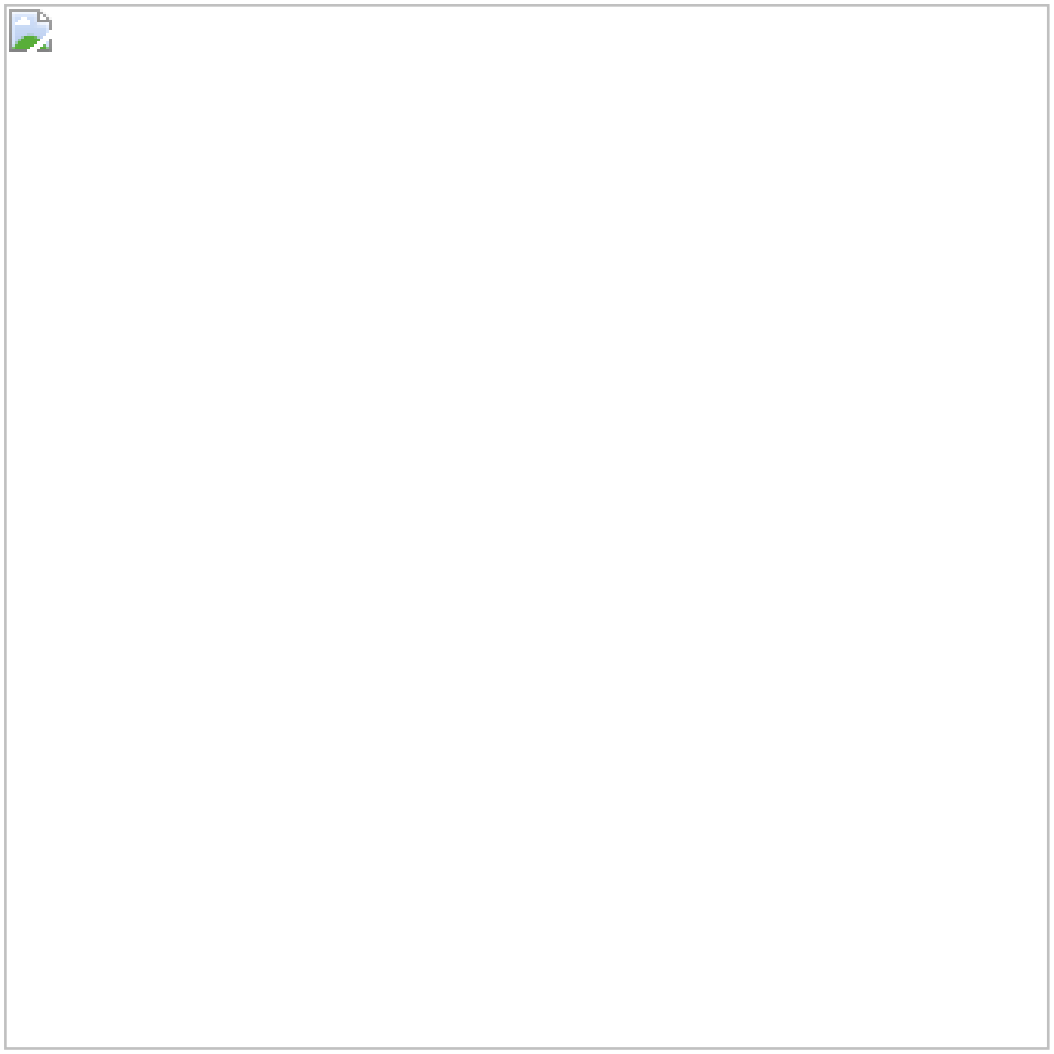

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Susceptibility of *Helicoverpa zea* (Lepidoptera: Noctuidae) Neonates to Diamide Insecticides in the Midsouthern and Southeastern United States

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Abstract

Corn earworm, *Helicoverpa zea* (Boddie), is a significant pest of agroecosystems in the midsouthern and southeastern regions of the United States. These insects have developed resistance to, or inconsistent control has occurred with, most insecticide classes. With their unique mode of action, insecticides in the diamide class have become a key component in management of agriculturally important lepidopteran pests. In this study, field populations of *H. zea* were collected in the southern United States and compared to susceptible laboratory colonies to generate baseline concentration–mortality data. LC_{50} and LC_{90} values were generated for flubendiamide and chlorantraniliprole using neonates. To achieve equivalent levels of mortality, a higher concentration of flubendiamide was required compared to chlorantraniliprole. Flubendiamide LC_{50} values for *H. zea* ranged from 16.45 to 30.74 ng/ml, with a mean of 23.53 ng/ml. Chlorantraniliprole LC_{50} values for *H. zea* ranged from 2.94 to 4.22 ng/ml, with a mean of 3.66 ng/ml. Significant differences were observed for some field populations relative to the laboratory colony. For flubendiamide, five populations had greater LC_{50} values and two populations had lower LC_{50} values compared to the laboratory colony. For chlorantraniliprole, three populations had greater LC_{50} values and three populations had lower LC_{50} values compared to the laboratory colony. The response of these populations most likely represents natural variability among populations and does not indicate a significant shift in susceptibility of this species.

Key words: corn earworm, chlorantraniliprole, flubendiamide

Lepidoptera is the most damaging insect order in soybean, *Glycine max* (L.) Merr., production in the southern United States (Fitt 1989, Musser et al. 2015). Corn earworm, *Helicoverpa zea* (Boddie); soybean looper, *Chrysodeixis includens* (Walker); beet armyworm, *Spodoptera exigua* (Hübner); and fall armyworm, *Spodoptera frugiperda* (Smith) are widely distributed polyphagous pests of numerous cultivated crops throughout the midsouthern and southeastern United States. In 2014, these insects resulted in a combined US$138,874,796 economic loss across Alabama, Arkansas, Louisiana, Mississippi, North Carolina, Tennessee, and Virginia in soybean (Musser et al. 2015). Foliar applications of synthetic insecticides are instrumental in the management of lepidopteran pests in the southern United States. The widespread use of synthetic insecticides has led to resistance and/or inconsistent control with most insecticide classes, including chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and benzoylphenylureas (Sparks 1981, Brown et al. 1998, Temple et al. 2006, Jacobson et al. 2009, Lai and Su 2011).

The diamide class of insecticides was introduced in 2008 and is the newest major class of insecticides (EPA 2008). This class has a novel mode of action and is classified as ryanodine receptor modulators (MoA Group 28) (IRAC 2014). Ryanodine receptors (RyR) are intracellular calcium channels located in the sarcoplasmic reticulum that specialize in the rapid and massive release of calcium from intracellular stores, which is necessary for excitation contraction coupling in striated muscle (Ebbinghaus-Kintscher et al. 2006). Calcium serves as the primary physiological regulator of insect ryanodine receptors (Xu et al. 2000, Scott-Ward et al. 2001). Diamide insecticides bind to the ryanodine receptor complex, prompting the prolonged release of intracellular calcium stores, resulting in cessation of feeding and uncoordinated muscle contraction of intoxicated insects, eventually causing mortality (Ebbinghaus-Kintscher et al. 2006, Nauen et al. 2007, Hannig et al. 2009, Roditakis et al. 2015). The diamide insecticides are characterized by their low mammalian toxicity and are effective against a large number of lepidopteran

species (Sattelle et al. 2008, Tohnishi et al. 2005, Lahm et al. 2009, Teixeira and Andaloro 2013, Qi et al. 2014). Two representatives from this class of insecticides are flubendiamide (Belt, Bayer CropScience, Raleigh, NC), a pthalic acid diamide, and chlorantraniliprole (Prevathon, DuPont Crop Protection, Newark, DE), an anthranilic diamide (Lahm et al. 2009). Although they are structurally independent, these insecticides share the same target site (Lahm et al. 2009, Teixeira and Andaloro 2013). Eight years after their introduction to the global market, these two active ingredients comprise 7% of global insecticide use (Sparks 2013). Large global market shares result from the favorable biological, ecological, and toxicological attributes of this insecticide class (Teixeira and Andaloro 2013). It is perceived that the use of this insecticide class will continue to increase globally on a wide variety of crops (Teixeira and Andaloro 2013, Roditakis et al. 2015).

Repeated field applications of the diamide insecticides has resulted in numerous reports of resistance development for several lepidopteran species (Roditakis et al. 2015). To date, cross resistance between chlorantraniliprole and flubendiamide has been documented for diamondback moth, *Plutella xylostella* (L), smaller tea tortrix, *Adoxophyes honmai* (Yasuda), and tomato borer, *Tuta absoluta* (Meyrick) (Wang and Wu 2012, Uchiyama and Ozawa 2014, Roditakis et al. 2015). Also, resistance to chlorantraniliprole has been documented for rice stem borer, *Chilo suppressalis* (Walker), cutworm, *Spodoptera litura* (F), and *S. exigua* (Su et al. 2012, Che et al. 2013, Gao et al. 2013). Furthermore, resistance to flubendiamide has been documented in *C. suppressalis* (Wu et al. 2014). Because resistance has occurred among a number of pest species globally, monitoring susceptibility levels of target pest species is important for pest management and resistance management efforts. Furthermore, development of successful insecticide resistance management strategies requires the establishment of baseline susceptibility levels of target pest species while resistant allele frequencies are low (Ffrench-Constant and Roush 1990, Cook et al. 2005). Baseline responses of laboratory and field strains of target pest populations to novel modes of action act as a historical reference, and are necessary to mitigate resistance development, prolonging the effectiveness of novel modes of action. Although the diamide class has been used commercially since 2008, no field control failures have been documented for *H. zea* in the southern United States. The primary objective of the present study was to generate dose-mortality responses of *H. zea* to flubendiamide and chlorantraniliprole after several years of commercial use for future resistance monitoring efforts, development of resistance management strategies, and development of a more rapid assay method.

Materials and Methods

Insects

The *H. zea* susceptible colony was a laboratory colony maintained at the Mississippi State University Department of Entomology, Mississippi State, MS insect rearing facility. This colony originated from non-Bt corn in 2006 and wild individuals collected from non-Bt corn were incorporated into the colony on a yearly basis. Prior to the initiation of this experiment the susceptible colony was not known to be exposed to insecticides. Field-derived populations for this study were composed of 15 *H. zea* colonies collected during 2013 and 2014 (Table 1). Each collection consisted of at least 300 third instars. Larvae were placed in 36-ml Solo cups (Bio-Serv, Frenchtown, NJ) containing Stonefly Heliothis Diet (Product No. 38-0600, Ward's Natural Science, Rochester, NY) with matching lids. At pupation, ~50 pupae were placed in 3.79-liter cardboard containers with matching lids with the corresponding colony and generation information labeled on the outside of each bucket. Adults were fed a 10% sugar–water solution that was changed daily. For the purpose of egg collection for bioassays, the cardboard containers were lined with Reynolds Cut-Rite Wax Paper (Reynolds Consumer Products, Lake Forest, IL). The center of each lid was removed so that only the rim remained. Cotton cloth was placed over each bucket and kept in place by the lid to serve as an oviposition substrate. Eggs were collected daily and new cloths and wax paper were applied to every bucket. Collected egg sheets and wax paper from each colony were kept in 3.79-liter Ziploc (S.C. Johnson & Johnson, Inc., Racine, WI) bags until larvae hatched for use in bioassays. The laboratory susceptible colonies and field derived

Table 1. Description of *H. zea* field-derived populations and laboratory colonies by identification code, collection host, month collected, and collection location

Colony	Species	Collection host[a]	Month	Location
LAB	*H. zea*	*Z. mays*		MSSTATE, MS
AR14	*H. zea*	*Z. mays*	July	Lonoke County, AR
GA14	*H. zea*	*Z. mays*	July	Tift County, GA
LA14	*H. zea*	*Z. mays*	July	Franklin Parish, LA
MSKIL14	*H. zea*	*T. incarnatum*	May	Montgomery County, MS
MSLEL14	*H. zea*	*T. incarnatum*	May	Washington County, MS
MSNAT14	*H. zea*	*T. incarnatum*	May	Adams County, MS
MSSTARK13-1	*H. zea*	*T. incarnatum*	May	Oktibbeha County, MS
MSSTARK14-1	*H. zea*	*Z. mays*	July	Oktibbeha County, MS
MSSTONE13-1	*H. zea*	*Z. mays*	June	Washington County, MS
MSSTONE13-2	*H. zea*	*S. bicolor*	July	Washington County, MS
MSSTONE13-3	*H. zea*	*C. arietinum*	August	Washington County, MS
MSVIC14	*H. zea*	*T. incarnatum*	May	Warren County, MS
MSYAZ14	*H. zea*	*T. incarnatum*	May	Yazoo County, MS
NC14	*H. zea*	*Z. mays*	July	Washington County, NC
SC14	*H. zea*	*Z. mays*	July	Barnwell County, SC
TN14-1	*H. zea*	*Z. mays*	June	Madison County, TN
TN14-2	*H. zea*	*Z. mays*	July	Madison County, TN

[a] All collections from *Z. mays* were from non-Bt.

populations of each species were reared at the Mississippi State University insect rearing facility under the following conditions: 25 °C, 80% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. All assays were conducted on first- and/or second-generation progeny of field-collected colonies.

Bioassays

Concentration–mortality bioassays were conducted to determine the susceptibility of *H. zea* to commercial formulations of flubendiamide (Belt; Bayer CropScience, Raleigh, NC) and chlorantraniliprole (Prevathon; DuPont Crop Protection, Newark, DE). Preparation of insecticide-treated diet was similar to Temple et al. (2009). Dilutions of flubendiamide and chlorantraniliprole in distilled water were made from a stock solution with a concentration of 1,000 ng/ml and 500 ng/ml, respectively, to yield eight concentrations ranging from 0 to 35 ng/ml for flubendiamide and 0 to 6.8 ng/ml for chlorantraniliprole. Aliquots from these solutions were combined with Stonefly Heliothis Diet to yield 400 g of insecticide-treated diet for each concentration. Insecticide treated diet was stored in 0.95-liter Ziploc bags and refrigerated. All diet was used or disposed of within 7 d of preparation. Insecticide-treated diet for each concentration was dispensed into 16 wells of a 128-well bioassay tray (Product No. BAW128, Frontier Agricultural Sciences, Newark, DE) in 0.5-ml aliquots. Each well was infested with one neonate (<24 h after hatching). Cells were covered with perforated, clear 16-well lids (P.E. film, Bio-Serv, Frenchtown, NJ). Infested assay trays were labeled and placed in a rearing chamber maintained at 25 °C, 80% RH, and a photoperiod of 16:8 (L:D) h. All bioassays were replicated at least four times over different days based on date of oviposition. Insect mortality was measured 7 d later. Ingestion of the diamides results in feeding cessation (Nauen et al. 2007, Hannig et al. 2009). Typically the ability of larvae to right themselves after being flipped onto their dorsal surface is considered an appropriate criterion for determining mortality with intoxicated larvae (Temple et al. 2009). Based on preliminary data of four *H. zea* colonies (data not presented), it was observed that intoxicated larvae, though severely stunted, could still right themselves when flipped onto their dorsal surface. Given the mobility of intoxicated larvae, severe growth inhibition was a more reliable indication of mortality. Therefore, the criteria for mortality was defined as larvae that had not molted to the second instar and weighed <10 mg after 7 d (Siegfried et al. 2000).

Data Analysis

Data were corrected for control mortality using Abbott's formula (Abbott 1925). Corrected data were analyzed with probit analysis to calculate slope, LC_{50}, LC_{90}, and confidence intervals (PROC PROBIT, SAS Institute 2012). Goodness of fit tests ($P > 0.10$) were evaluated to ensure the trend line fit the model. LC_{50} and LC_{90} values were considered different when 95 percent confidence intervals did not overlap.

Results and Discussion

Significant differences in LC_{50} and LC_{90} values were observed among populations of *H. zea* for both chlorantraniliprole (Table 2) and flubendiamide (Table 3). Overall, mean LC_{50} and LC_{90} data suggest that *H. zea* were ~6.4-fold (23.43 vs. 3.65 ng/ml) to 5.4-fold (30.51 vs. 5.65 ng/ml) more tolerant to flubendiamide than chlorantraniliprole, respectively. A similar study suggested that *S. frugiperda* was 13.67-fold more tolerant to flubendiamide compared with chlorantraniliprole (Hardke et al. 2011). Based on these results and those reported previously, lepidopteran larvae appear to be more sensitive to chlorantraniliprole than flubendiamide at lower concentrations.

The LC_{50} values for chlorantraniliprole ranged from 2.94 to 4.22 ng/ml (1.44-fold), with a mean of 3.66 ng/ml (Table 2). The LC_{90} values for chlorantraniliprole ranged from 4.52 to 9.17 ng/ml (2.02 fold), with a mean of 5.68 ng/ml. The LC_{50} values for flubendiamide ranged from 16.45 to 30.74 ng/ml (1.86 fold), with a mean of 23.53 ng/ml (Table 3). The LC_{90} values ranged from 21.22 to 35.33 ng/ml (1.66 fold), with a mean of 30.59 ng/ml. Overall, three field populations had LC_{50} values that were significantly lower than the laboratory colony and three field populations had LC_{50} values that were significantly greater than the laboratory colony for chlorantraniliprole (Table 2). Similarly, two field populations had LC_{50}

Table 2. Comparative susceptibility of *H. zea* neonates to chlorantraniliprole in dose–mortality curves generated with concentration–mortality bioassays with insecticide-treated diet

Colony	Species	N[a]	Slope (±SE)	LC_{50} (95% CL)[b] (ng/ml)	LC_{90} (95% CL)[b] (ng/ml)	χ^2 (df)	P[c]
LAB	*H. zea*	991	3.35 (±0.24)	3.58 (3.41–3.73)	5.25 (5.01–5.55)	6.13 (5)	0.2935
AR14	*H. zea*	256	2.83 (±0.48)	3.38 (2.85–3.76)	5.32 (4.79–6.23)	3.86 (5)	0.5700
GA14	*H. zea*	384	3.37 (±0.43)	3.09 (2.81–3.32)	4.52 (4.20–5.00)	6.27 (5)	0.2808
LA14	*H. zea*	288	3.38 (±0.94)	3.84 (2.85–4.25)	5.61 (5.17–6.88)	5.25 (3)	0.1541
MSKIL14	*H. zea*	634	3.82 (±0.37)	4.21 (4.02–4.39)	5.86 (5.56–6.28)	8.57 (5)	0.1275
MSLEL14	*H. zea*	448	1.84 (±0.23)	3.79 (3.44–4.14)	7.61 (6.57–9.54)	5.73 (4)	0.2202
MSSTARK13	*H. zea*	384	1.60 (±0.21)	4.11 (3.50–4.77)	9.17 (7.53–12.32)	3.89 (5)	0.5659
MSSTARK14	*H. zea*	512	4.02 (±0.64)	3.62 (3.42–3.84)	4.98 (4.54–5.86)	1.56 (5)	0.9059
MSSTONE13-1	*H. zea*	881	3.13 (±0.40)	2.94 (2.75–3.11)	4.43 (4.07–5.03)	4.93 (4)	0.2947
MSSTONE13-2	*H. zea*	1643	2.76 (±0.23)	3.21 (3.05–3.36)	5.10 (4.74–5.61)	4.44 (5)	0.4882
MSSTONE13-3	*H. zea*	1166	4.51 (±0.64)	3.52 (3.36–3.68)	4.68 (4.35–5.27)	6.09 (5)	0.2974
MSYAZ 14	*H. zea*	128	2.12 (±0.43)	4.09 (3.25–4.84)	7.47 (6.15–10.72)	8.43 (5)	0.1340
NC14	*H. zea*	256	5.54 (±1.01)	4.22 (3.86–4.49)	5.32 (4.98–5.91)	6.38 (5)	0.2702
SC14	*H. zea*	384	5.72 (±0.93)	4.05 (3.76–4.26)	5.06 (4.8–5.49)	9.07 (5)	0.1064
TN14-1	*H. zea*	256	5.21 (±0.96)	3.72 (3.37–3.98)	4.76 (4.44–5.32)	0.43 (5)	0.9946
TN 14-2	*H. zea*	256	2.49 (±0.61)	3.15 (2.19–3.67)	5.26 (4.65–6.53)	6.83 (5)	0.2336

[a] Total number of insects tested.
[b] Confidence limits.
[c] Goodness of Fit test ($P > 0.10$).

Table 3. Comparative susceptibility of *H. zea* neonates to flubendiamide in dose–mortality curves generated with concentration–mortality bioassays with insecticide-treated diet

Colony	Species	N[a]	Slope (±SE)	LC_{50} (95% CL)[b] (ng/ml)	LC_{90} (95% CL)[b] (ng/ml)	χ^2 (df)	P[c]
LAB	*H. zea*	447	4.54 (±0.53)	21.96 (20.36–23.21)	29.12 (27.71–30.95)	5.39 (4)	0.2497
AR14	*H. zea*	256	4.25 (±0.81)	21.88 (19.23–56.58)	29.59 (27.58–33.19)	6.29 (5)	0.2789
GA14	*H. zea*	256	5.66 (±1.06)	27.75 (26.23–28.99)	34.79 (32.67–39.24)	3.35 (5)	0.6459
LA14	*H. zea*	256	4.80 (±0.60)	23.34 (21.85–24.66)	30.47 (28.65–33.15)	7.27 (5)	0.2010
MSKIL14	*H. zea*	398	6.89 (±1.13)	29.34 (27.58–30.48)	35.33 (34.07–37.38)	0.70 (2)	0.7042
MSLEL14	*H. zea*	384	4.32 (±0.90)	24.43 (21.82–25.90)	32.87 (30.86–37.31)	9.06 (5)	0.1068
MSNAT 14	*H. zea*	192	5.22 (±1.16)	24.04 (19.94–26.28)	30.76 (28.57–33.74)	2.68 (3)	0.4439
MSSTARK13-1	*H. zea*	406	5.23 (±0.61)	17.02 (16.19–17.91)	21.75 (20.37–23.88)	1.25 (3)	0.5354
MSSTARK14-1	*H. zea*	256	5.04 (±1.03)	16.45 (15.25–17.77)	21.22 (19.29–25.73)	1.56 (5)	0.9060
MSSTONE13-1	*H. zea*	947	2.58 (±0.20)	19.72 (18.54–20.83)	32.38 (30.28–35.21)	3.28 (3)	0.1938
MSSTONE13-2	*H. zea*	672	2.75 (±0.76)	21.23 (15.30–23.73)	33.82 (30.75–43.90)	4.60 (3)	0.2035
MSVIC14	*H. zea*	320	8.04 (±1.43)	25.19 (22.51–26.86)	29.55 (27.91–31.13)	1.62 (2)	0.4443
MSYAZ14	*H. zea*	560	6.46 (±0.72)	30.74 (29.99–31.52)	37.49 (35.95–39.87)	1.03 (3)	0.7948
NC14	*H. zea*	288	9.07 (±1.46)	24.47 (23.22–25.36)	28.19 (27.23–29.60)	2.60 (3)	0.4572
SC14	*H. zea*	896	7.37 (±0.60)	25.70 (25.14–26.22)	30.58 (29.79–31.61)	9.23 (5)	0.1002
TN14-1	*H. zea*	256	3.72 (±0.63)	22.82 (20.37–24.64)	32.20 (29.66–36.70)	3.85 (5)	0.5718
TN14-2	*H. zea*	256	5.19 (±0.90)	22.30 (20.24–23.80)	28.55 (26.74–31.49)	4.34 (5)	0.5009

[a] Total number of insects tested.
[b] Confidence limits.
[c] Goodness of Fit test ($P > 0.10$).

values lower than the laboratory colony and five field populations had LC_{50} values that were greater than the laboratory colony for flubendiamide (Table 3). Statistical differences among populations were minimal and most likely represent natural variation among field populations. Although it cannot be ruled out because these insecticides have been used commercially since 2008, this does not appear to represent a major shift in *H.* zea susceptibility to this class of insecticides. Flubendiamide and chlorantraniliprole have been used extensively in soybean and grain sorghum to manage *H. zea* across the southern United States (Catchot et al. 2015) because of their high level of efficacy and long residual control (Tohnishi et al. 2005). As a result, *H. zea* populations in the southern United States have likely been exposed to some level of selection pressure to these active ingredients prior to being tested.

Concentration–mortality values of chlorantraniliprole for *H. zea* neonates in the current studies are considerably lower than those previously reported. Temple et al. (2009) reported mean LC_{50} values that were 15-fold greater than the results reported here (56 vs 3.6 ng/ml). These differences are most likely because Temple et al. (2009) tested third instars compared to neonates tested in the current study. The susceptibility of multiple lepidopteran species to several insecticides has been shown to increase at later instars (Yu 1983). Although direct comparisons cannot be made to previous research, the overall purpose of this study was to develop a more rapid method for testing *H. zea* susceptibility to diamide insecticides. In many cases, growers and consultants need to respond to a control failure and need an answer as quickly as possible to determine if resistance is the cause of poor control. Rearing larvae to the third instar increases the time needed to confirm resistance and will negatively impact the ability of growers to respond in a timely manner.

Baseline susceptibility to the diamide insecticide class was generated in Louisiana by Hardke et al. (2011) for *S. frugiperda* and Temple et al. (2009) for *H. zea*. However, data have not been produced for these species in Mississippi or other states included in this study. Furthermore, differences observed between this study and previous studies can be attributed to differences in growth stages of larvae tested. The long residual efficacy of the diamide insecticides may potentially expose multiple generations of the same species to the insecticide. Adams (2016) observed up to 90% and 60% control of *H. zea* 32 d after treatment in soybean field studies with both chlorantraniliprole and flubendiamide, respectively. This falls well within the generation time frame for *H. zea* during the warm summer months. Therefore, neonates were used in this study to account for subsequent populations that could potentially be exposed to the diamide insecticides as the growing season progresses and to develop a more rapid method for confirming resistance. Nevertheless, it is critical to document the variability in the response of field populations prior to the occurrence of control failures in the field. Resistance is defined as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (IRAC 2007). This study generated data that can serve as a reference point for future monitoring programs associated with *H. zea*, aiding in the detection of resistance alleles prior to field control failures. Future monitoring programs will aid resistance management efforts, allowing the diamide insecticide class to continue to play an important role in crop protection strategies.

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