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. LC50 and LC90 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 LC50 values for *H. zea* ranged from 16.45 to 30.74 ng/ml, with a mean of 23.53 ng/ml. Chlorantraniliprole LC50 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 LC50 values and two populations had lower LC50 values compared to the laboratory colony. For chlorantraniliprole, three populations had greater LC50 values and three populations had lower LC50 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 sarcoplasmatic 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 phthalic acid diamide, and chlorantraniliprole (Prevathon, DuPont Crop Protection, Newark, DE), an anilinic 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 honmae (Yasuda), and tomato borer, Tuta absoluta (Meyrick) (Wang and Wu 2014, 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 S. 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 (Frechen-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 Stonafly 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

| Colony   | Species | Collection host | Month | Location         |
|----------|---------|----------------|-------|------------------|
| LAB      | H. zea  | Z. mays        | July  | 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 Stonely Heliothis Diet to yield 400 g of insecticide-treated diet for each concentration. Insecticide-treated diet 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, LC50, LC90, 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. LC50 and LC90 values were considered different when 95% confidence intervals did not overlap.

Results and Discussion
Significant differences in LC50 and LC90 values were observed among populations of *H. zea* for both chlorantraniliprole (Table 2) and flubendiamide (Table 3). Overall, mean LC50 and LC90 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 LC50 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 LC50 values for chlorantraniliprole ranged from 4.52 to 9.17 ng/ml (2.02 fold), with a mean of 5.68 ng/ml. The LC50 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 LC90 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 LC50 values that were significantly lower than the laboratory colony and three field populations had LC50 values that were significantly greater than the laboratory colony for chlorantraniliprole (Table 2). Similarly, two field populations had LC50

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°   | Slope (± SE)        | LC50 (95% CL)b (ng/ml) | LC90 (95% CL)b (ng/ml) | x² (df) | Pc |
|------------|---------|------|--------------------|------------------------|------------------------|--------|----|
| 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 (3.25–3.76)       | 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 (3.83–4.25)       | 5.61 (5.17–6.88)       | 5.25 (3) | 0.1541 |
| MSKL14     | *H. zea*| 634  | 3.82 (± 0.37)      | 4.21 (4.02–4.39)       | 5.86 (5.56–6.28)       | 8.57 (5) | 0.1275 |
| MSLE14     | *H. zea*| 448  | 1.84 (± 0.23)      | 3.79 (3.44–4.14)       | 7.61 (6.57–9.34)       | 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)       | 4.48 (3.54–5.26)       | 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 |
| NCI4       | *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 (4.85–5.49)       | 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).
values lower than the laboratory colony and five field populations had LC50 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. 2016). 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 LC50 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 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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### 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   | Slope (±SE) | LC50 (95% CL) | LC90 (95% CL) | χ² (df) | P   |
|------------|---------|-----|-------------|--------------|--------------|--------|-----|
| 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 (19.23–23.56) | 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 (27.58–30.30) | 35.33 (34.07–37.37) | 0.70 (2) | 0.7042 |
| MSLEL14    | H. zea  | 384 | 4.32 (±0.90) | 24.43 (21.82–25.90) | 32.87 (30.86–36.73) | 9.06 (5) | 0.1068 |
| MSNAT1     | 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 |
| MSYZAZ14   | H. zea  | 560 | 6.46 (±0.72) | 30.74 (29.99–31.52) | 37.49 (35.95–39.87) | 1.03 (3) | 0.7948 |
| NCI4       | 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) | 28.53 (26.74–31.49) | 4.34 (5) | 0.3009 |

*Total number of insects tested.

Confidence limits.

Goodness of Fit test (P > 0.10).
References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Adams, A. 2016. Baseline susceptibility of selected lepidopteran pests to diamides and use strategies in Mississippi soybean. Ph.D. Dissertation, Mississippi State University, Mississippi State, MS.

Brown, T. M., P. K. Bryson, D. S. Brickle, S. Pimprale, F. Arnette, M. E. Roof, J. T. Walker, and M. J. Sullivan. 1998. Pyrethroid-resistant Helicoverpa zea and transgenic cotton in South Carolina. Crop Prot. 17: 441–445.

Catchot, A., J. Gore, and D. Cook. 2015. Bollworms showing up in soybean. Mississippi Crop Situation, July 17, 2015. (http://www.mississippi-crops.com/20150717/bollworms-showing-up-in-soybeans/ (accessed June 13, 2016).

Che, W., T. Shi, Y. Wu, and Y. Yang. 2013. Insecticide resistance status of field populations of Spodoptera exigua (Lepidoptera: Noctuidae) from China. J. Econ. Entomol. 106: 1855–1862.

Cook, D. R., B. R. Leonard, J. Gore, and J. H. Temple. 2005. Baseline responses of bollworm, Helicoverpa zea (Boddie), and Tobacco Budworm, Heliothis virescens (F.), to indoxacarb and pyridalyl. J. Agric. Urban Entomol. 22: 100–109.

Ebbinghaus-Kintscher, U., P. Luemmen, N. Lobitz, T. Schulte, C. Funke, R. Fischer, T. Masaki, N. Yasokawa, and M. Tohnishi. 2006. Phthalic acid diamides activate ryanodine-sensitive Ca2+ release channels in insects. Cell Calcium 39: 21–33.

EPA. 2008. Pesticide Fact Sheet, Chlorantraniliprole, (http://www3.epa.gov/pesticides/chem_search/registration/fs_PC-090100_01-Apr-08.pdf) (accessed January 2016).

French-Constant, R. H., and R. T. Roush. 1990. Resistance detection and documentation: the relative roles of pesticial and biochemical assays, pp 4–38. In R. T. Roush and B. E. Tabashnik [eds.], Pesticide Resistance in Arthropods. Chapman and Hall, New York, New York and London, United Kingdom.

Fitt, G. P. 1989. The ecology of Heliothis species in relation to agroecosystems. Ann. Rev. Entomol. 34: 17–32.

Gao, C., R. Yao, Z. Zhang, M. Wu, S. Zhang, and J. Su. 2013. Susceptibility baseline and chlorantraniliprole resistance monitoring in Chilo suppressalis (Lepidoptera: Pyralidae). J. Econ. Entomol. 106: 2190–2194.

Hannig, G. T., M. Ziegler, and P. G. Marcon. 2009. Feeding cessation effects of chlorantraniliprole on a new anthracnoid diameidicide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. Pest Manag. Sci. 65: 969–974.

Haridé, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011. Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 94: 272–278.

IRAC. 2007. Insecticide Resistance Action Committee, Resistance Management for Sustainable Agriculture and Improved Public Health. (http://www.irac-online.org/documents/irac-croplife-irm-booklet.pdf) (accessed January 2016).

IRAC. 2014. IRAC MoA Classification Scheme (Version 7.3.1). (http://www.irac-online.org) (accessed October 2015).

Jacobson, A., R. Foster, C. Krupke, W. Hutchinson, B. Pittendrigh, and R. Weinzierl. 2009. Resistance to pyrethroids insecticides in Helicoverpa zea (Lepidoptera: Noctuidae) in Indiana and Illinois. J. Econ. Entomol. 102: 2289–2295.

Lahm, G. P., D. Cordova, and J. D. Barry. 2009. New and selective cyanide receptor activators for insect control. Bioorganic Med. Chem. 17: 4127–4133.

Lai, T., and J. Su. 2011. Assessment of resistance risk in Spodoptera exigua (hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. Pest Manag. Sci. 67: 1468–1472.

Mussel, F. R., A. L. Catchot, Jr., J. A. Davis, D. A. Herbert, Jr., G. M. Lorenz, T. Reed, D. D. Reisig, and S. D. Stewart. 2015. 2014 Soybean Insect Losses in the Southern US. MidSouth Entomol. 8: 35–48.

Nauen, R., S. Konanz, T. Hirooka, T. Nishimatsu, and H. Kodama. 2007. Flubenamid: A unique tool in resistance management tactics for pest lepidoptera difficult to control. Pflanzenschutz-Nachrichten Bayer 60: 247–262.

Qi, S., P. Lummen, R. Nauen, and J. E. Casida. 2014. Diamide insecticide target site specificity in the Heliothis and Musca ryanodine receptors relative to toxicity. J. Agric. Food Chem. 62: 4077–4082.

Roditakis, E., E. Vasakis, M. Grispou, M. Stavrakaki, R. Nauen, M. Gravouil, and A. Bassi. 2015. First report of Tuta absoluta resistance to diame insecticides. J. Pest Sci. 88: 9–16.

SAS Institute. 2012. SAS 9.3 for windows. SAS Institute, Cary, NC.

Sattelle, D. B., D. Cordova, and T. R. Cheek. 2008. Insecticide resistance receptors: Molecular targets for novel pest control chemicals. Invert. Neurosci. 8: 107–119.

Scott-Ward, T. S., S. J. Dunbar, J. D. Windass, and A. J. Williams. 2001. Characterization of the ryanodine receptor-Ca2+ release channel from the thoracic muscles of the lepidopteran insect Heliothis virescens. J. Membr. Biol. 179: 127–141.

Siegfried, B. D., T. Spencer, and J. Nearmann. 2000. Baseline susceptibility of the corn earworm (Lepidoptera: Noctuidae) to the Cry1Ab toxin from Bacillus thuringiensis. J. Econ. Entomol. 93: 1265–1268.

Sparks, T. C. 1981. Development of insecticide resistance in Heliothis zea and Heliothis virescens in North America. Bull. Entomol. Soc. Am. 27: 186–192.

Sparks, T. C. 2013. Insecticide discovery: An evaluation and analysis. Pest. Biochem. Physiol. 107: 8–17.

Su, J., T. Lai, and J. Li. 2012. Susceptibility of field populations of Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. Crop Prot. 42: 217–222.

Teixeira, L. A., and J. T. Andaloro. 2013. Diamide insecticides: Global efforts to address insect resistance stewardship challenges. Pest. Biochem. Physiol. 106: 76–78.

Temple, J. H., D. R. Cook, P. L. Bommireddy, S. Micinski, W. Walmann, A. M. Stewart, B. Garber, and B. R. Leonard. 2006. Monitoring cypermethrin susceptibility in Louisiana bollworm 2004–2005, pp. 1236–1240. Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Council, Am., Memphis TN.

Temple, J. H., P. L. Bommireddy, D. R. Cook, P. Marcon, and B. R. Leonard. 2009. Susceptibility of selected lepidopteran pests to ryanyxyrr, a novel insecticides. J. Cotton Sci. 13: 23–31.

Tohnishi, M., H. T. Nakao, T. Furuya, A. Seo, H. Kodama, K. Tsubata, S. Fujioka, H. Kodama, T. Hirooka, and T. Nishimatsu. 2005. Flubenamid: a novel insecticide highly active against lepidopterous insect pest. J. Pestic. Sci. 30: 354–360.

Uchiyama, T., and A. Ozawa. 2014. Rapid development of resistance to diamide insecticides in the smaller tea tortrix, Adoxophyes honnaii (Lepidoptera: Tortricidae), in the tea fields of Shizuoka Prefecture, Japan. Appl. Entomol. Zool. 49: 1–6.

Wang, X., and Y. Wu. 2012. High levels of resistance to chlorantraniliprole evolved in field populations of Plutella xylostella. J. Econ. Entomol. 105: 1019–1023.

Wu, M., S. Zhang, R. Yao, S. Wu, J. Su, and C. Gao. 2014. Susceptibility of the rice stem borer, Chilo suppressalis (Lepidoptera: Crambidae), to Flubendiamide in China. J. Econ. Entomol. 107: 1250–1255.

Xu, X., M. B. Bhat, M. Nishi, H. Takeshima, and J. Ma. 2000. Molecular cloning of cDNA encoding a drosophila ryanodine receptor and functional studies of the carboxyl-terminal calcium release channel. J. Biophys. 78: 1270–1281.

Yu, S. J. 1983. Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larva. J. Econ. Entomol. 76: 219–222.