Baseline susceptibility and resistance monitoring of *Striacosta albicosta* (Lepidoptera: Noctuidae) Vip3A, a *Bacillus thuringiensis* protein, and foliar insecticides

by

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Baseline susceptibility and resistance monitoring of *Striacosta albicosta* (Lepidoptera: Noctuidae) Vip3A, a *Bacillus thuringiensis* protein, and foliar insecticides

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*Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) is an emerging pest of corn (*Zea mays* L.) in the Great Lakes region. In the past fifteen years, *S. albicosta* has experienced a rapid range expansion, moving from Nebraska to the Maritime Provinces of Canada. Larvae of *S. albicosta* feed on corn causing economic damage and the potential for mycotoxin contamination. Transgenic corn expressing the *Bacillus thuringiensis* (Bt) vegetative insecticidal protein Vip3A and foliar insecticides are recommended for the management of *S. albicosta*; however, with the development of *S. albicosta* resistance to Cry1F Bt protein, resistance management is of concern for many researchers, corn producers, and product developers. The objective of this research was to determine the susceptibility and degree of exposure of *S. albicosta* larvae to these technologies. First instar *S. albicosta* were very susceptible to Vip3A protein and foliar insecticides. However, the susceptibility of older instars of *S. albicosta* to these technologies was lower than 1st instars. The estimated exposure of *S. albicosta* instars to Vip3A and foliar insecticides varied greatly depending on corn tissues assessed. First instar *S. albicosta* are likely to be exposed to high doses of Vip3A and foliar insecticides under field conditions; however, older instars may not be exposed to high doses of Vip3A and foliar insecticides under the same conditions. Specific to Vip3A, corn tissue assays showed higher survival of later instars of *S. albicosta* on various corn tissues, and developmental measures such as larval weights, body length, and head capsule width, of larvae that were exposed to Vip3A-expressing tissues did not
differ from those exposed to the control. Specific to foliar insecticides, the persistence of each insecticide in the field after application varied greatly. The results of this research suggest management of 1\textsuperscript{st} instar \textit{S. albicosta} with Vip3A and foliar insecticides is achievable; however, using these technologies, control may not be sufficient to prevent damage by older \textit{S. albicosta} instars that can cause potential for mold infection.
PREFACE

The presented dissertation is organized as a series of manuscripts accepted for publication or to be submitted for publication in peer-reviewed scientific journals. Each manuscript was written by Yasmine Farhan, and the references for the accepted publication and under review for publication included here as Chapter 2 and Chapter 3, respectively, are listed below:


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1 INTRODUCTION

1.1 DESCRIPTION

*Striacaosta albicosta* was first described in 1887 from a collection of Arizona moths (Smith 1887). It was first placed in the genus *Agrotis* (Lepidoptera: Noctuidae), and then moved to a new genus, *Loxagrotis* (McDunnough 1928). The entire genus *Loxagrotis* was then placed into the preexisting genus *Richia* (Poole 1989). Lafontaine (2004) moved *R. albicosta* to *Striacaosta*, a newly created genus.

*Striacaosta albicosta* is a univoltine noctuid with a wing expanse of 3.8 cm (Hoerner 1948, Michel et al. 2010). The wings are dark brown to black in colour with a near white stripe on the costal margin of the forewings, a white circle in the centre of the forewings, and a white and brown coloured crescent below the white circle (Hoerner 1948).

1.2 DISTRIBUTION AND HOST RANGE

*Striacaosta albicosta* is native to North and Central America (Douglass et al. 1957). The geographic distribution of *S. albicosta* was described by Crumb (1956) as ranging from eastern Mexico, through Texas, New Mexico, Arizona, Colorado, Kansas, Nebraska, Iowa, Utah, and Idaho, all the way north to southern Alberta, Canada. The Technical Bulletin report documented damage by *S. albicosta* to beans in Colorado and Idaho (Crumb 1956). Douglass et al. (1957) reported *S. albicosta* as a pest of dry beans (*Phaseolus* spp. L.) in Colorado in the 1940’s (Douglass et al. 1957). Prior to that, Hoerner (1948) documented reports of cutworm type injury to pinto beans in Colorado in 1915, 1925, and 1930, followed by sporadic injury to pinto beans in the 1940s. In 1945, injury to beans was reported in Idaho, with damage noticed in the previous
4 years (Hoerner 1948). The earliest record of *S. albicosta* on corn, *Zea mays* L., was in Colorado from an August 13, 1896 collection (Hoerner 1948). By 1969, *S. albicosta* was found throughout Nebraska and was considered a serious pest of corn (Keith et al. 1970). By 1980, the distribution of *S. albicosta* expanded to include Oklahoma, South Dakota, and Wyoming (Blickenstaff and Jolley 1982). Although Iowa was included in the 1956 description of the geographic range, *S. albicosta* was only sporadically reported and the first report of economic injury on corn did not occur until 2000, after which the eastward expansion accelerated (Rice 2000). *Striacosta albicosta* was reported for the first time on corn in Minnesota in 1999 (O’Rourke and Hutchison 2000), followed by Illinois, Missouri, and Wisconsin in 2004 (Dorhout and Rice 2004, Cullen 2007), Indiana, Michigan, and Ohio in 2006 (Dorhout and Rice 2008, Difonzo and Hammond 2008), Ontario, Canada, in 2008 (Baute 2009), and Pennsylvania, New York, and Quebec in 2009 (Tooker and Fleischer 2010, Baute and Bruggeman 2011). More recently, *S. albicosta* was reported as a dominant corn pest in Xochimilco and Coahuila, Mexico (Sánchez-Peña et al. 2016). In 2017, *S. albicosta* was confirmed in Prince Edward Island and Nova Scotia, Canada (Baute 2017).

The Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) began monitoring *S. albicosta* in Ontario in 2007 after reports of the insect in Michigan; no moths were captured that year (Smith et al. 2018b). The program continued, and in 2008, an average of 9.6 moths were captured per pheromone trap in Southwestern Ontario (Smith et al. 2018b). Trap counts continued to increase with an average of 3.7 moths per trap in 2009 to an average of 134.2 moths per trap in 2012 (Smith et al. 2018b). The average number of moths captured per trap in Ontario continues to increase with the exception of 2014 (Smith et al. 2018b). The first Ontario
report of economic injury by *S. albicosta* was on corn, in 2010 (Baute and Bruggeman 2011, Smith et al. 2017). Incidence and severity of injury to corn by *S. albicosta* continued to increase from 2010 to 2014 (Smith et al. 2018b). Furthermore, observations of successful overwintering by *S. albicosta* in Ontario and Michigan showed that it had become an established pest in the Great Lakes region (Smith et al. 2018b).

The mechanism(s) behind the geographic expansion of *S. albicosta* is (are) unknown; however, several factors that may have affected the expansion have been suggested. Miller et al. (2009) envisaged two general categories of environmental barriers that previously confined *S. albicosta* to the west of the Missouri River. The first category of barriers is a durable one, such as a physical landscape feature that might still be in existence. The second is a temporary barrier, subject to change, such as farming practices that make the environment unsuitable as a habitat. Miller et al. (2009) hypothesized that if the barrier was a durable one, at the beginning of the recent range expansion, a small number of colonizers overcame the barrier resulting in a population bottleneck indicative of a founder effect. The results of the Miller et al. (2009) study showed no differences in overall genetic diversity indicative of a bottleneck between the recently founded and the established populations. These results suggest that the barrier that previously confined *S. albicosta* to the west of the Missouri River was likely not a durable one, but more likely a temporal barrier that had recently changed allowing for the range expansion (Miller et al. 2009). Hutchison et al. (2011) suggested potential factors that may have influenced the range expansion of *S. albicosta*. One of these factors included the increased use of transgenic corn expressing proteins obtained from *Bacillus thuringiensis* (Bt). The wide spread use of Bt corn may have created a favourable situation for *S. albicosta* by reducing the use of insecticides and
competition with pests controlled by Bt corn (EPA 1999, Hutchison et al. 2011). Other factors that may have contributed to the eastward expansion by *S. albicosta* include climate change and cultural practices, such as conservation tillage, both of which may have facilitated improved overwintering survival (Hutchison et al. 2011). It is also likely that these factors interacted to enable the range expansion of *S. albicosta*.

1.3 LIFE HISTORY AND BIOLOGY

1.3.1 Reproductive Behaviour and Pheromone Responses

Moth flight begins in late June and continues until August, with peak flight occurring in mid- to late-July (Douglass et al. 1957, Smith et al. 2018b). Adult females of *S. albicosta* reach sexual maturation 4-6 days (d) after pupal eclosion (Konopka 2013) and their oviposition period can range from 2 to 13 d with an average of 8 d (Douglass et al. 1957). Females lay eggs in groups on the upper surface of corn leaves in the whorl or top of the plant (Blickenstaff 1983). On average, females lay over 300 eggs (Douglass et al. 1957, Blickenstaff and Jolley 1982) and egg masses vary in size from 20 to 200, but typically average around 50 eggs per mass (Douglass et al 1957, Hagen 1962). *Striacosta albicosta* eggs are dome-shaped, coarsely ribbed and about 0.08 cm in diameter (Hoerner 1948), resembling tiny white cantaloupes in shape. Eggs are typically white when first deposited, then become tan and purple as the head capsule of the larvae develops (Dyer et al. 2013). The total time of egg development from oviposition to larval hatch is 5 to 7 d (Douglass et al. 1957, Dyer et al. 2013). However, time of egg hatch and larval development are dependent on temperature and humidity.

Newly-hatched larvae incubated at 26.6°C, 70-80% RH, and a photoperiod of 16:8 (L:D) h, require about 3 days to reach the 2nd instar; 1st and 2nd instars are approximately 2.5 and 3.8
mm in length, respectively (Dyer et al. 2013). Larvae reach the 3rd instar stage 8 days after hatching, and are 7.7 mm in length on average. Larvae reach the 4th instar stage 13 days after hatching and are on average 15.3 mm in length. Sixteen days are needed after hatching for larvae to reach the 5th instar stage, where larvae are 19.3 mm in length on average. In the 6th, and typically the final larval stage, larvae reach 29.1 mm in length on average 21 days post hatch (Douglass et al. 1957, Dyer et al. 2013). As larvae mature through each of the six instar stages, they transform in colour from a dark grey to a pink brown colour (Hagen 1962, Dyer et al. 2013). Sixth instar larvae drop off the plant in early fall and burrow into the soil where they construct soil chambers using salivary gland secretions for overwintering (Douglass et al. 1957, Michel et al. 2010). Striacosta albicosta remain in a prepupal stage throughout the winter, then pupate and complete the cycle the following summer (Michel et al. 2010).

1.3.2 Larval Behaviour and Mobility

Larvae, upon hatching, eat the egg-shells (chorion) before dispersing (Douglass et al. 1957). Typically, in corn, newly hatched larvae move towards the tassel. After feeding on the anthers and pollen, larvae will then move down towards the developing silk and into the corn ear, feeding on the silk and ear tip. Larvae may also create entry holes in the corn husk to access the kernels (Hagen 1962, Paula-Moraes et al. 2012).

The distance that larvae move from plant to plant appears to be related to the host plant and density of its canopy (Blickenstaff 1983). The dispersal of larvae from a single egg mass in corn has been reported as over 3 m in diameter and larvae move more readily within rows than across rows (Douglass et al. 1957, Hagen 1962). Hagen (1962) noted that there was no correlation between the number of eggs in the mass and the size of the infested area. On beans,
Blickenstaff (1983) found that larval dispersal was almost twice the distance of that observed by Douglass (1957) and Hagen (1962) and suggested that the denser canopy of beans may facilitate greater larval movement. Blickenstaff (1983) also observed that at higher egg densities, dispersal across rows increased, but the majority of the damage occurred within an area 1.8 m wide by 3.7 m long. Larval dispersal commonly occurs by walking or ballooning, which is when larvae use silk to hang off of plant structures to come into contact with another structure or be taken by wind (Goldstein et al. 2010). As larvae grow, ballooning becomes impossible due to their increased weight, and dispersal for those larvae mostly occurs by walking (Zalucki et al. 2002).

1.4 *STRIACOSTA ALBICOSTA* AS A CORN PEST

1.4.1 Corn Injury

Larvae of *S. albicosta* feed on tassel, silk, and ears; however, most feeding is concentrated on the ear tip or side (Michel 2010). Appel et al. (1993) showed that an average infestation of one larva per plant caused yield loss of 0.25 T ha\(^{-1}\) or more, equivalent to over 3% yield loss relative to uninfested fields. Heavy infestation of several larvae per ear can result in 30 to 40% yield loss (Michel et al. 2010). Furthermore, ears with damaged tips and holes in the husk are prone to infestation by fungi, decreasing grain quality (Hagen 1962). Smith et al. (2018a) investigated the relationship between injury caused by *S. albicosta* feeding and deoxynivalenol, a mycotoxin produced by the mycotoxigenic fungi *Fusarium graminearum* (Schwabe). The study showed that the mere incidence of injury by *S. albicosta* increases the potential for the development of mycotoxins (Smith et al. 2018a). This relationship was facilitated by common environmental conditions in Ontario that tend to favour development of infection by
mycotoxigenic fungi (Smith et al. 2018a). Dietary exposure to mycotoxins can result in adverse effects to humans and livestock (Bowers et al. 2013).

1.4.2 Trapping Adults

Determining the presence of *S. albicosta* adults is crucial for timing when to scout for eggs and larvae (Michel et al. 2010). Pheromone traps and light traps are the easiest way to detect *S. albicosta* adults, however, pheromone traps are preferred due to their ease of use and species specificity. Pheromone trap height should be at least 1.2 m from the soil surface (Michel et al. 2010, Smith et al. 2018b). In Ontario, pheromone traps were set up to monitor *S. albicosta* adults in 2007 after the insect was first reported in Michigan (Smith et al. 2018b). Although no adults were captured that year, they were the following year, and annual trap catch numbers generally increased since then (Smith et al. 2018b). In Ontario, the earliest adult capture occurred in the first week of June, and latest capture occurred in the second week of September, while peak adult flight occurred mid- to late-July (Smith et al. 2018b). Trapping *S. albicosta* in corn and dry bean fields should follow the same technique, with traps placed along the edge of fields in areas that encourage consistent monitoring, pheromone lures changed every three weeks, and traps emptied at least once a week (Michel et al. 2010, Smith et al. 2018b).

1.4.3 Scouting

Scouting for *S. albicosta* eggs plays a crucial role in management of this pest. Corn plants should be inspected for eggs and larvae after *S. albicosta* moths are captured, and in Michigan and Ontario, scouting should occur over a minimum of 5 weeks starting mid-July (Smith et al. 2018b). Black light and/or pheromone traps can be used to monitor adult flight, population size, and to time egg scouting (OMAFRA 2017). Repeated scouting of the same fields may be
necessary to make appropriate management decisions based on current action thresholds (Smith et al. 2018b). Scouting for *S. albicosta* eggs entails the examination of 20 plants in five areas of the field, focusing on the top 3 to 4 upper leaves of the plant, with preference given to pre-tassel corn fields (OMAFRA 2017). OMAFRA (2017) recommends foliar insecticide application if 5% of the plants, cumulative over several scouting events, have *S. albicosta* eggs or small larvae.

### 1.5 MANAGEMENT

#### 1.5.1 Cultural and Biological Control

Management of *S. albicosta* using cultural practices such as plowing and tillage have been suggested by Seymour et al. (2010); however, these methods may not provide reliable control, have not been adequately tested, and may contradict other benefits sought from reduced tillage. Furthermore, Douglass et al. (1957) stated that adjusting planting dates of corn might not be effective to avoid injury by *S. albicosta*, as the oviposition period is nearly two to three weeks long and early planting might leave farmers susceptible to frost damage.

Biological control of *S. albicosta* has been observed in the field. Lady beetles (Coccinellidae), pirate bugs (*Orius* spp.), lacewing (Chrysopidae) larvae, and other predaceous beetles consume eggs and larvae of *S. albicosta* (Michel et al. 2010, OMAFRA 2017). Later instars and prepupae are vulnerable to predation by birds and ground-dwelling vertebrates (Michel et al. 2010). The microsporidian pathogen *Nosema* sp. was found to infect the midgut of *S. albicosta* (Helms and Wedberg 1976). *Striacosta albicosta* infected with *Nosema* sp. can experience chronic and debilitating effects such as reduced fecundity, reduced egg hatch, delayed larval development, reduced adult lifespan, and increased overwintering mortality (Lewis et al. 2009).
1.5.2 Transgenic Corn

*Striaco*sta albicosta* may be managed by using transgenic corn expressing proteins from *Bacillus thuringiensis* (Bt), a gram-positive bacterium. During sporulation, Bt strains produce crystalline inclusions, which contain delta-endotoxin crystalline (Cry) proteins that are toxic to some insects (Lee et al. 2006). The Cry protein mode of action begins with the ingestion of Bt crystal or tissue containing the inactive Cry protein. The Cry endotoxin is activated in the alkaline gut of the insect, where the toxin then binds to receptors in the gut epithelium, causing perforation of the gut membrane leading to starvation or septicemia (Lee et al. 2006). The use of Cry proteins, such as Cry1A, Cry1Ab, and Cry2Ab did not show control of *S. albicosta* (Catangui and Berg 2006, Eichenseer et al. 2008, Bowers et al. 2014). However, Catangui and Berg (2006) reported no infestation by *S. albicosta* in ears of transgenic corn event TC1507 (co-developed by Dow AgroSciences and Pioneer Hi-Bred International, Johnston, IA) expressing Cry1F protein in 2003, suggesting that Cry1F protein may offer protection against *S. albicosta*. Furthermore, Eichenseer et al. (2008) demonstrated that corn expressing Cry1F protein sustained less damage by *S. albicosta* than non-Bt hybrids in 2002-2006, however, Cry1F plants were not immune to damage and 17% of larvae developed to 6th instar. Studies in the early 2000s suggested that *S. albicosta* susceptibility to purified Cry1F protein was highly variable among field-collected populations (Ostrem et al. 2016). Because of the status of *S. albicosta* as a secondary pest (EPA 1999), continuous monitoring of susceptibility was not required and therefore not conducted. Subsequent testing revealed reduced Cry1F susceptibility among *S. albicosta* in Colorado, Nebraska, New Mexico, and Texas, and a recent study in Ontario found
no control of *S. albicosta* with Cry1F-expressing corn, supporting the conclusion that resistance to Cry1F has occurred (Dyer et al. 2013, Ostrem et al. 2016, Smith et al. 2017).

Event MIR162 (Syngenta Seeds LLC, Research Triangle Park, NC), expressing the vegetative insecticidal protein (Vip) Vip3A, was approved in the United States and Canada in 2010 (USDA/APHIS 2010, CFIA 2010). Unlike Cry proteins, Vip proteins are produced during the vegetative growth phase of Bt (Lee et al. 2006). Similar to Cry proteins, Vip3A is activated in the alkaline gut of insects; however, Vip3A and Cry proteins bind to different receptors in the midgut membrane (Lee et al. 2003, 2006; Sena et al. 2009; Liu et al. 2011). The Vip3A event has activity against Noctuidae (Lepidoptera) such as *Spodoptera frugiperda*, *Agrotis ipsilon* (Hufnagel), *Sp. exigua* (Hübner), *Sp. littoralis* (Boisduval), *Sp. albula* (Walker), *Sp.sinmoioides* (Walker), *Sp.eridania* (Cramer), *Heliothis virescens*, and *S. albicosta* (Estruch et al. 1996, Kurtz et al. 2007, Bergamasco et al. 2013, Bowers et al. 2013, 2014). In artificially infested field experiments, Vip3A provided 100% control of *S. albicosta* (Bowers et al. 2013, 2014). However, the toxicity of purified Vip3A to *S. albicosta* has not been determined.

### 1.5.3 Foliar Insecticides

Insecticide application is recommended to be timed with 95% tassel emergence in Nebraska and western US states (Appel et al. 1993, Michel et al. 2010). However, in Ontario, it is recommended that corn producers time insecticide application with silk emergence so that insecticides can be tank-mixed with a fungicide to manage ear-mold infection (Smith et al. 2018a). Hagen (1962) compared the efficacy of multiple foliar insecticides in controlling *S. albicosta* in corn in the southwest USA, and found organochlorine and carbamate insecticides to be the most effective, resulting in 12-34% decrease in damage per ear relative to the untreated
control. Since then, synthetic pyrethroids have become widely used in that region and concerns about resistance development have arisen (Archibald et al. 2017). The selection of pyrethroids over other control options is based on price (Klein 2017, Archibald et al. 2017) and a relaxed level of control needed, as growers in Nebraska may not face the same risk of mycotoxin contamination that is present in the Great Lakes region (Smith et al. 2018a, b). In Ontario, insecticide options for the control of *S. albicosta* are currently limited to one anthranilic diamide active ingredient (a.i.), one spinosyn, and two synthetic pyrethroids (OMAFRA 2017).

Chlorantraniliprole was the first insecticide produced from the anthranilic diamide class (Lahm et al. 2009). Chlorantraniliprole binds to the ryanodine receptors in muscle cells activating the uncontrolled release of calcium stores, resulting in feeding cessation, lethargy, contractile paralysis, and death (Lahm et al. 2009, IRAC 2017). Spinetoram, a spinosyn, is a nicotinic acetylcholine receptor allosteric activator that affects nerve action causing hyper-excitation of the nervous system (IRAC 2017). The pyrethroids, lambda-cyhalothrin and deltamethrin, are sodium channel modulators that affect nerve action by keeping sodium channels open, causing hyper-excitation and nerve blockage (IRAC 2017). Additionally, methoxyfenozide, from the class diacylhydrazine, reduced *S. albicosta* pod damage on dry beans and sweet corn (Trueman 2013; Goudis et al. 2015). Methoxyfenozide acts on the ecdysone receptor resulting in premature moulting (IRAC 2017). The susceptibility of *S. albicosta* populations in Ontario to the insecticides listed above has not been determined but is required for monitoring and managing resistance.
1.6 MONITORING FOR RESISTANCE

Resistance monitoring is a vital part of insect resistance management (IRM). Determining the baseline susceptibility of a pest population to a pesticide before commercialization or widespread used is an important first step in monitoring and managing resistance (Jutsum et al. 1998) in order to track the evolution of resistance. These data provide insight into the natural variability among target pest populations and can be used to assess shifts in susceptibility to the active ingredient after frequent use (Jutsum et al. 1998, Priesnitz et al. 2016). Furthermore, baseline susceptibility data can be used to define discrimination concentrations to distinguish susceptible and resistant populations (Roush and Miller 1986). Additionally, when compared with plant tissue expression levels, baseline data help determine dose status, vital to the high-dose/refuge IRM model. The high-dose/refuge model entails: 1) the resistance trait be a rare and recessive allele within the population; 2) the expression of a high toxin dose that will cause mortality at 25 to 50 times the dose that kills 99% of the population (LD$_{99}$); and 3) the planting of a refuge of non-Bt host plants along with Bt crops to promote survival of susceptible individuals (Hutchison et al. 1997, EPA 1999, Caprio and Sumerford 2000). This strategy was developed to delay the development of resistance in *Ostrinia nubilalis* (Hübner) to Cry proteins by providing numerous homozygous dominant susceptible alleles from refuge plants to mate with rare heterozygous recessive resistance alleles from traited plants (Siegfried et al. 2014).

Growers that plant Bt crops are required to plant a specific proportion of their crop with a non-Bt hybrid. Blocks or strips of non-Bt refuge can be planted near blocks or strips of Bt hybrids or non-Bt seed can be mixed with the Bt seed and planted in as an integrated refuge. The integrated refuge system was marketed to simplify the grower implementation of refuge and to
increase compliance with IRM requirements (Siegfried and Hellmich 2012, IRAC 2013). Selecting between structured and integrated refuge systems is situation- and pest-dependent. Before implementing an integrated refuge system, the insect’s life history and mating behaviour, movement between plants, feeding preferences, stage-specific mortalities, and possible gene dominance should be considered to maximize the delay in the development of resistance (Mallet and Porter 1992, Siegfried and Hellmich 2012).

Plant to plant larval movement is an important behaviour influencing the effectiveness of the integrated refuge approach because young larvae may feed on a Bt plant, become sick, and move to a non-Bt plant. In such scenarios, a larva that has a copy of the resistance gene would have greater fitness than susceptible insects (Siegfried and Hellmich 2012). However, in a strip or block refuge, most larvae that move will encounter the same type of plant (Bt or refuge), providing less opportunity for larval survival on Bt plants (Siegfried and Hellmich 2012). Use of an integrated refuge presents additional resistance management challenges with respect to late instar insect dispersal (Mallet and Porter 1992, Yang et al. 2014). Insects can develop on non-Bt refuge plants as young larvae and move on to adjacent Bt plants when they are larger and better able to metabolize the toxin. *Striacosta albicosta* larvae are quite mobile and have been documented to move between plants along and across rows up to 3 m away or more from their egg mass site (Douglass et al. 1957, Pannuti et al. 2016). Although, susceptibility of older *S. albicosta* larvae has not been determined, the decrease in susceptibility as larvae grow has been documented in several other lepidopteran pests such *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner), *Spodoptera frugiperda, Heliothis zea* (Boddie), *Trichoplusia ni* (Hübner), and *O. nubilalis* (McGaughey 1978, Hornby and Gardner 1987, Huang et al. 1999).
Another problem arising from the deployment of integrated refuge is cross-pollination where pollen from Bt and non-Bt plants can fertilize individual kernels in each other’s ears resulting in a mosaic of Bt protein expression. Cross-pollination can result in reduced expression in kernels on Bt corn ears and the expression of Bt in kernels of non-Bt refuge ears (Mallet and Porter 1992, Chilcutt and Tabashnik 2004, Yang et al. 2014). Cross-pollination can compromise the refuge strategy by exposing susceptible insects to Bt toxins and may also expose insects feeding on Bt corn to sub-lethal levels of the Bt toxin (Yang et al. 2014). During the range expansion of *S. albicosta* into the Corn Belt and southern Canada, Cry1F-expressing corn was mainly deployed with a 5% integrated refuge strategy. This refuge strategy may have played a role in the development of resistance by exposing larvae on refuge plants to Cry1F through cross-pollination. The cross-pollination problem of integrated refuges and the larval movement of *S. albicosta* suggest that a structured refuge system would delay resistance better than an integrated refuge, however, in Ontario, Vip3A with integrated refuge is currently available for sale.

Adult migration also plays a pivotal role in the evolution of resistance by allowing for gene flow between populations (Miller and Sappington 2017). Movement of insecticide susceptible individuals into an area with high insecticide resistance will generally dilute resistance in the population by mating with the resistant individuals (Miller and Sappington 2017). This mixing of susceptible and resistant individuals is the same basis for the use of refuge to maintain a susceptible pest population. Conversely, it is also possible for resistance genes to be introduced into a population by individuals migrating from an area where resistance is established or widespread. In the case of resistance management for *S. albicosta*, it is important
to monitor the rate of adult migration. This migration is especially important with the knowledge that reduced efficacy of some foliar insecticides against *S. albicosta* has been reported in Nebraska (Archibald et al. 2017). A survey distributed to growers, crop consultants, and other agricultural professionals to obtain information on the efficacy of management tools for *S. albicosta* had yielded reports of reduced efficacy of three pyrethroid active ingredients, bifenthrin, permethrin, and zeta-cypermethrin (Archibald et al. 2017). Reduced efficacy of pyrethroids may be the first signs of development of pyrethroid resistance in *S. albicosta*. If pyrethroid-resistance in *S. albicosta* arises in Nebraska, it may become an Ontario problem if adults migrate into the Great Lakes region. Resistance to pyrethroids will make *S. albicosta* management in Ontario more difficult by further limiting available options for control. Additionally, the reduced efficacy of pyrethroids may be a greater management issue for Ontario than Nebraska, as the risk of infection by mycotoxigenic fungi is frequently high in this area, and the mere incidence of *S. albicosta* kernel damage increases the chances of mycotoxin contamination (Smith et al. 2018a).

*Striacosta albicosta* is a corn pest of primary concern in the Great Lakes region (Smith et al. 2018b); however, during the deployment of Cry1F-expressing corn, *S. albicosta* was considered a secondary pest (EPA 1999, Smith et al. 2017), and consequently susceptibility to Cry1F was not monitored. Within a few years of Eichenseer et al. (2008) reporting some control of *S. albicosta* by Cry1F-expressing corn, Smith et al. (2017) provided evidence for field-evolved resistance of *S. albicosta* to Cry1F-expressing corn. The development of resistance by *S. albicosta* to Cry1F protein and the reported reduced pyrethroid efficacy in Nebraska further emphasize the need for baseline susceptibility data and continuous monitoring. Currently, Vip3A
is the only available transgenic trait effective against *S. albicosta* (Bowers et al. 2013, 2014, Smith et al. 2018a), and wide deployment in Ontario is expected in the coming years (Smith et al. 2018b). Deploying Vip3A as single trait event without an IRM plan might lead to resistance development, as was seen with Cry1F. Additionally, deploying Vip3A in an integrated refuge strategy may further increase the risk of resistance development because: Vip3a is a single-trait, cross-pollination between Bt and non-Bt corn can occur, and *S. albicosta* exhibits larval movement between plants. With *S. albicosta* as a corn pest of primary concern in the Great Lakes region (Smith et al. 2018b), it is crucial that baseline susceptibility of *S. albicosta* to available control methods be determined before widespread use of these control methods. Also, monitoring *S. albicosta* populations for variability in susceptibility will allow for interventions before the development of resistance.

### 1.7 CONCLUSIONS AND RESEARCH OBJECTIVES

Since the first report of *S. albicosta* in the Great Lakes region in 2006, trap catches have generally increased annually in Ontario. The increase in trap catch numbers has indicated the establishment of a resident *S. albicosta* population in the region. As *S. albicosta* populations established in its new territory, increases in yield loss and reduction of corn quality, through the introduction of mycotoxogenic fungi, have also been documented. To manage *S. albicosta*, Cry1F-expressing corn was recommended, as early studies showed some levels of control over *S. albicosta*. However, Cry1F control of *S. albicosta* was short lived with reports of significant injury to Cry1F hybrids. The susceptibilities of *S. albicosta* populations to Cry1F showed a great deal of variability; however, susceptibilities of *S. albicosta* populations were determined after
widespread commercial deployment of Cry1F, and continuous monitoring of susceptibility of *S. albicosta* to Cry1F was not conducted throughout the use of the Bt product.

Current recommendations for the management of *S. albicosta* in Ontario include the use of Vip3A and foliar insecticides. Although field studies show that management of *S. albicosta* using Vip3A is promising, the key to sustaining this insect’s susceptibility to Vip3A is to determine baseline susceptibility of 1st and older instars prior to wide commercial deployment, follow appropriate refuge practices, and monitor for changes in susceptibility among field populations. Additionally, *S. albicosta* susceptibility to the recommended foliar insecticides has not been assessed and a monitoring program for the development of resistance has not been initiated. Thus, the objectives of this research were: 1) to determine the baseline susceptibility of Ontario field populations of *S. albicosta* at different larval instars to Vip3A and to determine if Vip3A-expression in plant tissues meets the high dose criteria relative to susceptibility of these instars; 2) to determine the efficacy of Vip3A-expressing tissue on later instars of *S. albicosta* compared with 1st instars; and 3) determine the susceptibility of different *S. albicosta* instars to insecticides currently recommended for their management and to compare acute and residual insecticide concentrations with relevant susceptibility parameters following typical field application scenarios. Relative to my objectives, I hypothesize:

1) First instar *S. albicosta* will be highly susceptible to Vip3A protein and 1st instars will be more susceptible to Vip3A than older instars; however, Vip3A-expression in plant tissues will be higher than the dose required to kill older *S. albicosta* instars;
2) Vip3A-expressing tissues will cause high insect mortality at all instar stages, with 1st instars having the highest mortality relative to older instars; and

3) Striacosta albicosta instars will be susceptible to the recommended foliar insecticides, with 1st instars being more susceptible than 3rd instars, however, the field application rates will still be higher than that required to kill 3rd instars.
2 BASELINE SUSCEPTIBILITY OF STRIACOSTA ALBICOSTA (LEPIDOPTERA: NOCTUIDAE) IN ONTARIO, CANADA TO VIP3A BACILLUS THURINGIENSIS PROTEIN

2.1 ABSTRACT

Striacosta albicosta (Smith) (Lepidoptera, Noctuidae) is a pest of corn (Zea mays L.), which has recently expanded its range into Ontario, Canada. Genetically modified corn expressing Vip3A insecticidal protein from Bacillus thuringiensis is a biotechnological option for the control of S. albicosta. To support an insect resistance management program, we conducted a study of baseline susceptibility of 10 field-collected S. albicosta populations in Ontario, Canada to Vip3A before widespread commercial adoption. First instars were exposed to artificial diet overlaid with Vip3A. The LC$_{50}$ ranged from 22.7 to 53.5 ng Vip3A cm$^{-2}$. The EC$_{50}$ ranged from 11.4 to 30.2 ng Vip3A cm$^{-2}$. There was low inter-population variation in susceptibility to Vip3A, which we believe represents natural geographical variation in response and not variation caused by previous exposure to selection pressure of the Vip3A protein.
2.2 INTRODUCTION

Native to southwestern North America, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), a pest of corn, *Zea mays* L. and dry edible beans, *Phaseolus* spp. L., was first documented in Arizona in 1887 (Smith 1887) and was largely confined to Colorado, Kansas, Nebraska, and Idaho as an occasional pest of dry edible beans before 1950 (Hoerner 1948). From the 1950s onwards, *S. albicosta* became increasingly more destructive on beans in western Nebraska (Hagen 1963, 1976) and was found attacking corn crops in southern Idaho (Douglass et al. 1957) and western Nebraska (Hagen 1962). By the early 2000s, *S. albicosta* expanded its geographic range eastward into Iowa, Wisconsin, Illinois, Missouri, Indiana, Michigan, and Ohio, predominantly attacking corn crops (Rice 2000, Dorhout and Rice 2004, Cullen 2007, Dorhout and Rice 2008, DiFonzo and Hammond 2008). By 2008, *S. albicosta* reached the province of Ontario, Canada and in 2010, economic injury by this insect was first reported in the region (Baute 2009).

Several life history characteristics are relevant to *S. albicosta* management in corn. Females lay egg masses on the upper side of corn leaves, in the upper portion of the canopy (Siegfried and Hellmich 2012). After hatching, the larvae move to the whorl to feed on tassel tissue and then move into the ear via the silk, or chew through the husk. Larvae are also able to move between plants along and across rows up to 1.5 m away from their egg mass (Appel et al. 1993). The resulting damage to corn ears impacts the quality of grain and increases crop susceptibility to ear mold fungi that may produce mycotoxins harmful to humans and livestock (Eichenseer et al. 2008, Parker et al. 2017). Infestation of one larvae per plant can result in yield loss of 0.25 T ha\(^{-1}\) or more (Appel et al. 1993).
To manage *S. albicosta*, foliar insecticides, primarily from the diamide and pyrethroid classes, are used in Ontario when the percentage of plants with egg masses within a scouted area of a field exceeds the recommended threshold of 5% (OMAFRA 2017). These insecticides kill young larvae, but are ineffective after larvae enter ear husks (Michel et al. 2010). Furthermore, diamides and pyrethroids exhibit harmful effects on many non-target arthropods that transgenic crops expressing *Bacillus thuringiensis* (Bt) proteins appear to have little effect on (Frisvold and Reeves 2014). However, continuous exposure to both foliar insecticides and Bt crops can result in resistance development (Frisvold and Reeves 2014).

The use of Bt Cry proteins, such as Cry1Ab, that effectively control *Ostrinia nubilalis* (Hübner) and other lepidopteran pests such as *Spodoptera frugiperda* (J. E. Smith) and *Helicoverpa zea* (Boddie), showed limited control of *S. albicosta* (Catangui and Berg 2006, Eichenseer et al. 2008, Bowers et al. 2014) and may have assisted in its range expansion through pest replacement and reduced insecticide use (Catangui and Berg 2006, Hutchison et al. 2011). Eichenseer et al. (2008) demonstrated that transgenic corn event TC1507 (co-developed by Dow AgroSciences and Pioneer Hi-Bred International, Johnston, IA) expressing Cry1F protein sustained less damage by *S. albicosta* than non-Bt hybrids, although Cry1F plants were not immune to damage. Studies in the early 2000s suggested that *S. albicosta* susceptibility to purified Cry1F protein was highly variable among field-collected populations (Ostrem et al. 2016). However, subsequent testing showed reduced susceptibility to Cry1F among *S. albicosta* in Nebraska, Texas, New Mexico, and Colorado over time, and recent studies in Ontario found no control of *S. albicosta* with Cry1F-expressing corn hybrids, supporting the conclusion that resistance to Cry1F has occurred (Dyer et al. 2013, Ostrem et al. 2016, Smith et al. 2017).
Event MIR162 (Syngenta Seeds LLC, Research Triangle Park, NC), which expresses the Vip3Aa protein, was approved in the U.S. and Canada in 2010 (CFIA 2010). This event expresses vegetative insecticidal protein from Bt and has activity against Noctuidae (Lepidoptera) such as *Sp. frugiperda*, *Agrotis ipsilon* (Hufnagel), *Sp. exigua* (Hübner), *Sp. littoralis* (Boisdouval), *Sp. albula* (Walker), *Sp. cosmioideis* (Walker), *Sp. eridania* (Cramer), *Heliothis virescens* (Estruch et al. 1996, Kurtz et al. 2007, Bergamasco et al. 2013), and *S. albicosta* (Bowers et al. 2013, 2014). The Vip3A insecticidal protein kills larvae of susceptible insects by a series of steps that resemble those caused by Cry proteins in their mode of action: however, Vip3A and Cry proteins bind to different receptors in the midgut membrane (Lee et al. 2003, 2006, Sena et al. 2009, Liu et al. 2011).

The insect resistance management (IRM) strategy for *O. nubilalis* and Bt-corn has guided IRM practices for other insect pests (Ostlie et al. 1997). Current efforts to manage resistance to Bt crops center on three theoretically complementary principles: 1) the resistance traits are rare and genetically recessive; 2) the provision of a non-Bt refuge that provides a source of susceptible insects to mate with potentially resistant insects; and 3) expression of a high toxin dose that will cause mortality at 25 to 50 times the dose that kills 99% of the population (LD99) (Hutchison et al. 1997, EPA 1999, Caprio and Sumerford 2000). The Canadian Food Inspection Agency (CFIA 2002) recommended the planting of a structured refuge arranged in strips or blocks within or near the insect-resistant corn field as a component of IRM. However, with the development of pyramided Bt-corn products that express multiple insecticidal proteins targeting the same pest, integrated refuge is now widely adopted whereby non-Bt and Bt seeds are blended before planting to distribute the refuge randomly throughout the field (Yang et al. 2014). It is
expected that the IRM plan for Vip3A will follow this trend. A major concern with integrated refuge is cross-pollination between Bt and non-Bt corn causing Bt proteins to be expressed in refuge corn plants, effectively compromising the refuge due to low survival rates of susceptible insects or increased survival of resistant heterozygotes due to sub-lethal exposure (Yang et al. 2014).

To monitor and identify changes in susceptibility to Bt proteins, the baseline susceptibility of the target organism should be determined before widespread commercial adoption occurs in the field (Wu 2014, Priesnitz et al. 2016). The baseline susceptibility of *S. albicosta* to Vip3A has not been reported and it is not known whether this protein meets the high-dose assumption of the current IRM strategy. The objective of this study was to determine the baseline susceptibility of field-collections of *S. albicosta* in Ontario to Vip3A using laboratory-based, concentration-response diet-overlay bioassays.

### 2.3 MATERIALS AND METHODS

#### 2.3.1 Striacosta albicosta Collections

Assays were conducted directly on 1st instars from field-collected egg masses or egg masses from field-collected adults. Collections were made in 2016 from 10 commercial corn fields. Collection sites were numbered from the western to the eastern regions of southwestern Ontario where economic infestations of *S. albicosta* have been reported in recent years. Economic infestations of *S. albicosta* have been observed annually near Bothwell, ON (West; fields 1-5) since 2010; however, economic infestations near Norwich, ON (East; fields 6-10) have been observed since approximately 2013 (OMAFRA 2017) (Fig. 2.1, Table 2.1).
Field-collected egg masses were cut from corn leaves, placed into plastic sandwich bags in coolers containing ice, and transferred to the laboratory. Based on information derived from growers or field signs, collection sites were planted with hybrids that expressed non-Vip3A Bt events; however, trait expression was not confirmed immunologically. In the laboratory, egg masses were placed on moistened filter paper inside petri plates, held at 26°C, 60-70% RH, with a photoperiod of 16:8 hr (L:D), and monitored daily for hatching. Adults were collected from black light traps using a method described by Madsen and Sanborn (1962), modified with a cage that traps but does not kill insects, placed on the outer field edge. Adults collected from black light traps were transferred to the laboratory and placed into rearing cages (60 × 60 × 60 cm) containing late vegetative stage pinto bean plants (*Phaseolus vulgaris*) in professional growing mix (Sun Gro Horticulture, Agawam, MA) for oviposition and 5% sucrose-agar water solution *ad libitum* for drinking. Egg masses laid on bean plants were collected daily, placed on moistened filter paper and monitored daily for hatch. All stages were kept at the conditions mentioned above.

### 2.3.2 Bioassays

Diet was prepared following the method described by Dyer et al. (2013), and was modified by the addition of 16.6 mL of propionic phosphoric acid solution to inhibit fungal and bacterial growth. Diet was dispensed into 128-well assay trays (Bio-16, CD International, Pitman, NJ), using a repeater pipette at a rate of 1 mL per well. Trays were allowed to cool to room temperature and then immediately used or covered with adhesive ventilated covers and stored at 4°C for up to 2 weeks before use.
Lyophilized, purified Vip3Aa insecticidal protein (86.5% active ingredient (a.i.)) derived from an *Escherichia coli* expression system was provided by Syngenta Seeds, LLC (Research Triangle Park, NC) and stored at -80°C. The purified protein was solubilized in 0.1% Triton X-100 in water (Sigma Life Science, Oakville, ON) through gentle agitation until completely dissolved. Dilutions were prepared to obtain the desired concentrations prior to bioassay. Following preliminary range-finding bioassays to determine a range of concentrations encompassing 0-100% mortality, the concentrations tested were 0.00, 12.50, 25.00, 50.00, 100.00, and 200.00 ng a.i. cm$^{-2}$. Thirty-six wells of each concentration were treated with 30-µL of the prepared solution. Control wells were treated with 30-µL of Triton buffer solution without Bt protein. After treatment application, trays were tilted horizontally to ensure uniform coverage of the diet surface. Trays were placed under a fume hood at room temperature until the liquid component of the solution had evaporated from the diet surface.

Each well was infested with a single 1$^{st}$ instar (unfed and <24 h after eclosion) using a fine-tipped soft paintbrush. Adhesive covers were replaced following larval introduction into wells, and trays were held under rearing conditions described earlier. Trays were covered with lightweight cardboard paper to block overhead light, which helped to keep larvae on the diet and prevent condensation buildup. Mortality and larval weight were recorded 7 d after introduction and larvae that were unresponsive to gentle prodding with a fine brush or weighed less than 10 mg were considered dead or moribund for the mortality analysis. All larval weights were retained in the growth inhibition data set. Bioassays were replicated three times for each collection.
Figure 2.1: Collection locations of *Striacosta albicosta* in Ontario, Canada, 2016.
(Images captured from Google Earth® 2017).
Table 2.1: Collection details for *Striacosta albicosta* in Ontario, Canada used in Vip3A susceptibility bioassays.

<table>
<thead>
<tr>
<th>Fields</th>
<th>County</th>
<th>Nearest town</th>
<th>Collection date</th>
<th>Stage collected</th>
<th>Source</th>
<th>No. collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chatham-Kent</td>
<td>Ridgetown</td>
<td>19-Jul-16</td>
<td>Adult</td>
<td>Black light trap</td>
<td>350</td>
</tr>
<tr>
<td>2</td>
<td>Chatham-Kent</td>
<td>Duart</td>
<td>19-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Chatham-Kent</td>
<td>Thamesville</td>
<td>19-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Chatham-Kent</td>
<td>Thamesville</td>
<td>19-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
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</tr>
<tr>
<td>5</td>
<td>Chatham-Kent</td>
<td>Bothwell</td>
<td>19-Jul-16</td>
<td>Adult</td>
<td>Black light trap</td>
<td>200</td>
</tr>
<tr>
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<td>Courtland</td>
<td>18-Jul-16</td>
<td>Egg mass</td>
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<td>78</td>
</tr>
<tr>
<td>7</td>
<td>Norfolk</td>
<td>Courtland</td>
<td>18-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>Norfolk</td>
<td>Tillsonburg</td>
<td>18-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>Norfolk</td>
<td>Otterville</td>
<td>18-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>54</td>
</tr>
<tr>
<td>10</td>
<td>Norfolk</td>
<td>Delhi</td>
<td>18-Jul-16</td>
<td>Egg mass</td>
<td>Non-Vip3A field corn</td>
<td>45</td>
</tr>
</tbody>
</table>

2.3.3 Data Analysis

Mortality data were analyzed to estimate the lethal concentrations, LC$_{50}$, LC$_{95}$, and LC$_{99}$ with 95% fiducial limits and the slope of the concentration-response curve, using PROC PROBIT in SAS 9.4 (SAS Institute, Cary, NC). The OPTC option was used to correct for larval
mortality in the control. The Pearson chi-square goodness-of-fit test was used ($p < 0.10$) to test if the observed values followed the predicted regression model. Replicates having control mortality >25% were considered as failed tests and were not used in the analyses.

For each concentration, larval weights were pooled and divided by the initial number of larvae to calculate the average larval weight (Siegfried et al. 2005). The weights were then transformed to percent growth inhibition relative to the control and analyzed by nonlinear regression (PROC NLIN, SAS 9.4) fitted to a probit model (SAS Institute 2008) as described by Marcon et al. (1999) to determine the effective concentration, $EC_{50}$ (concentration of protein at which larval growth was inhibited by 50%). To determine if the $LC_{50}$ and $EC_{50}$ for each collection were different, pairwise comparisons were conducted and deemed significant if the 95% confidence intervals did not overlap.

### 2.4 RESULTS

Susceptibility of Ontario field-collections of *S. albicosta* expressed as $LC_{50}$ values differed between fields based on non-overlapping confidence intervals and ranged from 22.70 (field 4) to 53.51 ng cm$^{-2}$ (field 1) (Table 2.2). $LC_{95}$ values ranged from 72.02 (field 2) to 207.87 ng cm$^{-2}$ (field 10) (Table 2.2). The $LC_{99}$ values ranged from 98.70 (field 2) to 370.98 (field 9) (Table 2.2). The Chi-squared value for field 9 was significant, indicating a poor fit of the probit model (Table 2.2). Based on $LC_{50}$ values, field 1, 9, and 10 were 1.8, 2.0, and 2.4-fold less susceptible to Vip3A than the most susceptible field 4 (Table 2.2). Based on $LC_{95}$ and $LC_{99}$ values, field 2 was more susceptible than all other collections, except field 4 (Table 2.2).
Table 2.2: Susceptibility of *Striacosta albicosta* 1st instars collected from corn fields in Ontario, Canada in 2016 to Vip3A insecticidal protein in diet-overlay bioassays expressed as lethal concentration (LC$_{50}$, LC$_{95}$, LC$_{99}$) and growth inhibition (EC$_{50}$) (ng ai cm$^{-2}$).

<table>
<thead>
<tr>
<th>Fields$^1$</th>
<th>n$^2$</th>
<th>Slope ± SE</th>
<th>LC$_{50}$$^3$ (95% CI)</th>
<th>LC$_{95}$$^3$ (95% CI)</th>
<th>LC$_{99}$$^3$ (95% CI)</th>
<th>$X^2$$^4$</th>
<th>EC$_{50}$$^3$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>648</td>
<td>3.17 ± 0.382</td>
<td>41.19 (32.80-49.35)ab$^5$</td>
<td>136.13 (107.03-196.21)a</td>
<td>223.38 (191.77-375.42)a</td>
<td>20.01</td>
<td>22.38 (12.89-34.20)ab</td>
</tr>
<tr>
<td>2</td>
<td>648</td>
<td>4.98 ± 0.866</td>
<td>33.66 (27.56-38.04)bc</td>
<td>72.02 (61.78-95.23)b</td>
<td>98.70 (79.07-152.04)b</td>
<td>14.21</td>
<td>19.62 (11.54-30.17)ab</td>
</tr>
<tr>
<td>3</td>
<td>648</td>
<td>2.31 ± 0.223</td>
<td>34.92 (29.11-40.95)b</td>
<td>179.47 (140.02-251.78)a</td>
<td>353.61 (252.01-569.20)a</td>
<td>15.48</td>
<td>12.13 (8.34-15.42)b</td>
</tr>
<tr>
<td>4</td>
<td>648</td>
<td>2.48 ± 0.288</td>
<td>22.70 (17.31-28.05)c</td>
<td>104.81 (78.68-161.83)ab</td>
<td>197.55 (133.47-369.35)ab</td>
<td>20.47</td>
<td>11.41 (10.60-12.15)b</td>
</tr>
<tr>
<td>5</td>
<td>648</td>
<td>2.43 ± 0.268</td>
<td>35.53 (28.33-42.85)b</td>
<td>169.00 (128.88-250.03)a</td>
<td>322.49 (222.88-562.41)a</td>
<td>8.64</td>
<td>30.19 (22.92-39.02)a</td>
</tr>
<tr>
<td>6</td>
<td>648</td>
<td>2.67 ± 0.392</td>
<td>38.51 (26.75-49.01)abc</td>
<td>159.11 (119.20-258.81)a</td>
<td>259.71 (193.25-397.74)a</td>
<td>20.11</td>
<td>15.38 (7.57-22.68)ab</td>
</tr>
<tr>
<td>7</td>
<td>648</td>
<td>2.62 ± 0.253</td>
<td>37.28 (31.13-43.46)b</td>
<td>157.93 (127.46-210.84)a</td>
<td>287.23 (214.55-432.12)ab</td>
<td>19.71</td>
<td>14.44 (9.89-18.44)b</td>
</tr>
<tr>
<td>8</td>
<td>648</td>
<td>2.35 ± 0.224</td>
<td>38.13 (31.63-44.75)b</td>
<td>190.50 (150.03-263.62)a</td>
<td>223.12 (155.94-640.21)a</td>
<td>13.56</td>
<td>26.10 (9.73-53.43)ab</td>
</tr>
<tr>
<td>9</td>
<td>648</td>
<td>2.60 ± 0.419</td>
<td>45.00 (31.71-58.74)ab</td>
<td>193.03 (132.08-388.88)a</td>
<td>370.98 (267.46-587.62)a</td>
<td>40.18*</td>
<td>25.05 (17.39-34.03)a</td>
</tr>
<tr>
<td>10</td>
<td>648</td>
<td>2.79 ± 0.224</td>
<td>53.51 (47.40-60.19)a$^5$</td>
<td>207.87 (169.79-270.67)ab</td>
<td>369.16 (256.32-642.70)a</td>
<td>18.66</td>
<td>19.93 (12.83-27.16)ab</td>
</tr>
</tbody>
</table>

$^1$ Collections were tested at F$_0$

$^2$ n=total number of larvae in the bioassay.

$^3$ Concentrations tested: 0.00, 12.50, 50.00, 100.00, and 200.00 ng a.i. cm$^{-2}$.
4 Value of the Pearson Chi-square goodness-of-fit test; * $p<0.10$.

5 Means within columns followed by the same letter are not significantly different as determined by non-overlapping 95% confidence intervals.
Growth inhibition of *S. albicosta* larvae treated with Vip3A expressed as EC$_{50}$ values ranged from 11.41 (field 4) to 30.19 ng cm$^{-2}$ (field 5). The range of variation in susceptibility indicated by larval growth inhibition was 2.5-fold which is similar to that indicated by mortality data (Table 2.2). The EC$_{50}$ value for fields 3, 4, and 7 were significantly lower than those of fields 5 and 9 (Table 2.2).

### 2.5 DISCUSSION

Establishment of baseline susceptibility of a target pest should be completed prior to wide commercial deployment of a Bt event (such as >5% of market share), and these data are necessary for defining susceptibility changes relating to exposure to Bt crops (Wu 2014). In the present study, baseline susceptibility of Vip3A was established for 10 field-collections of *S. albicosta* in Ontario, Canada. These baseline data will serve as a comparative reference for early detection of field-evolved resistance after deployment of Vip3A is expanded in Canada. Based on the current study, we calculated a LC$_{99}$ value of 371.0 (267.46-587.62) ng cm$^{-2}$ (Table 2.2) for the least susceptible collection of *S. albicosta* in Ontario. Using this LC$_{99}$ value, tissue expression levels of 9,274-18,549 ng cm$^{-2}$ would be required to meet the definition of high-dose against *S. albicosta* (25 to 50 times higher than the LC$_{99}$) as put forward by Environmental Protection Agency Scientific Advisory Panel (EPA-SAP 1999) and Caprio and Sumerford (2000). However, to determine if a plant incorporated protection protein, such as Vip3A, is expressed in corn hybrids at concentrations that meet the high-dose assumption, the EPA-SAP (1999) recommended that high-dose be verified by at least two of five given approaches. Ideally, plant expression levels of Bt proteins would be determined alongside baseline susceptibility
studies; however, technology providers typically do not allow trait expression studies by outside parties.

In general, Vip3A proteins have shown high toxicity against noctuid species. Vip3A presented higher insecticidal activity against *Spodoptera* species such as *Sp. albula*, *Sp. cosmioides*, and *Sp. eridania* than against *S. albicosta*, with LC$_{50}$ values of 3.90, 2.78, and 3.44 ng cm$^{-2}$ respectively (Bergamasco et al. 2013). Susceptibility to Vip3A by *Sp. littoralis* and *H. virescens*, two other noctuid species, with LC$_{50}$ values of 21 and 40 ng cm$^{-2}$ respectively, is similar to that of *S. albicosta* (Sattar et al. 2008, Ali and Luttrell 2011). However, not all noctuids are highly susceptible to Vip3A, some, such as *H. zea*, *H. armigera* (Hübner), and *Sp. frugiperda* with LC$_{50}$ values of 113, 111, and 612 ng cm$^{-2}$ respectively, require higher Vip3A concentrations to achieve similar mortality levels to *S. albicosta* (Lee et al. 2003, Bernardi, Sorgatto, et al. 2014, Wei et al. 2017). Furthermore, for other Lepidoptera, such as *O. nubilalis*, *Bombyx mori* (L.), and *Danaus plexippus* (L.) there was no detectable toxicity to Vip3A proteins (Estruch et al. 1996, Lee et al. 2003, Fang et al. 2007, Ruiz de Escudero et al. 2014).

*Striacosta albicosta*, *A. ipsilon*, and *Pseudaletia unipuncta* (Haworth), are the most common noctuid pest species of corn in Ontario (Showers et al. 1983, Schaafsma et al. 2007, Goudis et al. 2015). The impacts of exposure to corn hybrids expressing Vip3A should be considered for all of these species although *S. albicosta* is the primary target pest in this region. *Agrotis ipsilon* and *P. unipuncta* are sporadic insects that are insensitive to Cry1F and Cry1Ab (Showers et al. 1983, Estruch et al. 1996, Schaafsma et al. 2007, Pérez-Hedo et al. 2012). Studies show laboratory reared *A. ipsilon* susceptibility to Vip3A, with LC$_{50}$ in one study 17 and 62 ng cm$^{-2}$ in another (Estruch et al. 1996, Lee et al. 2003). Currently, no studies have addressed *P.*
unipuncta susceptibility to Vip3A. For both P. unipuncta and A. ipsilon, further tests are required to determine and monitor susceptibility to Vip3A, as it may be an effective management tool for these noctuids.

The 20-year success of O. nubilalis management in North America using transgenic Bt crops without resistance development is due to the application of the high-dose/refuge strategy (Siegfried and Hellmich 2012). Striacosta albicosta resistance to Cry1F was not unexpected given that a high-dose was not achieved with this protein. Cry1F showed moderate control of S. albicosta in the field (Eichenseer et al. 2008), but laboratory studies were unable to obtain reliable LC$_{50}$ values, as 100% mortality was never achieved (Dyer et al. 2013, Ostrem et al. 2016, Smith et al 2017). Additionally, pyramided Cry1F is sold mainly in an integrated refuge strategy, which is not ideal for the highly mobile S. albicosta larvae (Appel et al. 1993). Furthermore, S. albicosta exhibited a great deal of variability in susceptibility to Cry1F indicating that alleles conferring resistance were not rare in the population (Eichenseer et al. 2008). In the case of Vip3A susceptibility, variability in susceptibility was low among Ontario collections, with a two-fold difference in LC$_{50}$ values, indicating that alleles conferring resistance were rare in the Ontario populations. Therefore, the likelihood of resistance development to Vip3A is lower than for Cry1F. However, use of an integrated refuge for Vip3A will likely increase the risk of resistance development among S. albicosta.

Field studies have shown that Vip3A hybrids significantly reduce feeding injury by S. albicosta compared to non-Bt and Cry1F hybrids (Bowers et al 2013, Smith et al, unpublished). It is also important to test the susceptibility of different instars of S. albicosta to Vip3A, as susceptibility may differ among instars. Variability in susceptibility between instars may require
high-dose to be defined in terms of least susceptible larval stage in light of *S. albicosta* larval movement in an integrated refuge system (Huang et al. 1999, Michel et al. 2010). Diet-based bioassays with *O. nubilalis*, showed that the LC$_{50}$ and LC$_{95}$ were 98- and 168-fold higher, respectively, for 5$^{th}$ instars compared to 1$^{st}$ instars (Huang et al. 1999). These findings should be considered during the development of an IRM strategy for *S. albicosta* and Vip3A, as larvae may grow and develop on non-Bt corn before attacking Bt-corn.

With the recent report of field-evolved resistance to Cry1F among *S. albicosta* in Ontario (Smith et al. 2017), the adoption of Vip3A hybrids is expected to increase in this region. The current study measured the baseline susceptibility of 10 field-collections of *S. albicosta* to Vip3A in Ontario before widespread market adoption of this Bt event. The LC$_{99}$ value of 371 ng cm$^{-2}$ can be used as a diagnostic concentration for further monitoring of *S. albicosta* 1$^{st}$ instar susceptibility to Vip3A. The use of a diagnostic concentration can be an efficient susceptibility monitoring tool (Marcon et al. 1999, Ali and Luttrell 2011, Bernardi, Amado, et al. 2014). Resistance monitoring programs should aim to detect shifts in Vip3A susceptibility by regularly targeting areas with frequent exposure of *S. albicosta* to Vip3A corn hybrids.
3 SUSCEPTIBILITY OF DIFFERENT INSTARS OF STRIACOSTA ALBICOSTA (LEPIDOPTERA: NOCTUIDAE) TO VIP3A, A BACILLUS THURINGIENSES PROTEIN

3.1 ABSTRACT

Striacosta albicosta (Smith) is an important pest of corn, Zea mays L. in the Great Lakes region, which can be controlled by transgenic corn expressing Vip3A protein from Bacillus thuringiensis. To inform insect resistance management strategies, the susceptibility, survival, and development of 1st, 3rd, and 5th instar S. albicosta to Vip3A were determined using protein-overlay and corn tissue bioassays. Tissue bioassays were also used to determine the consumption of corn tissues with and without Vip3A-expression by various instars. In diet bioassays, greater tolerance to Vip3A was observed in 3rd and 5th instars compared to 1st instars; however, no difference in susceptibility was observed between older instars. In tissue bioassays, survival of larvae fed tissues expressing Vip3A ranged from 0-21% which was significantly lower than survival of larvae fed relative to other Cry’s tested; however, developmental measures of larvae fed Vip3A tissues did not differ from those fed artificial diet or tissues of other Bt events. Consumption of Vip3A × Cry1Ab tissues did not differ from that of Cry1Ab for any instar. Estimated Vip3A exposure of 1st instars ranged from 3-57 times higher than the concentration required for 99% mortality (LC99), based on the product of the reported Vip3A expression in transgenic corn tissues and the consumption observed in tissue bioassays; however, the estimated

1 Chapter 3 is based on joint work with Dr. Jocelyn L. Smith from the University of Guelph, Ridgetown Campus. Dr. Smith collected the tissue bioassay data from 2011-2013, however I conducted the remaining work including the diet and tissue bioassays. I also conducted the statistical analysis and writing of this chapter.
exposure of 3rd and 5th instars to Vip3A was lower than their respective LC99 values. These findings suggest that 1st instar S. albicosta are exposed to a high dose of Vip3A under field conditions; however, Vip3A expression in corn may not be high enough to control older instars, increasing the risk of resistance development.

### 3.2 INTRODUCTION

Striacosta albicosta (Smith) (Lepidoptera: Noctuidae) is an emerging pest of corn, Zea mays L., in the Great Lakes region of North America. It is native to the high plains of the United States (U.S.) where it was first documented as a pest of dry edible beans, Phaseolus spp. L., prior to feeding on corn (Hoerner 1948). Striacosta albicosta recently expanded its geographic range into the northeastern U.S and Canada. It was documented in Minnesota in 1999 (O’Rourke and Hutchison 2000), in Illinois, Missouri, and Wisconsin in 2004 (Dorhout and Rice 2004, Cullen 2007), and in Indiana, Michigan, and Ohio in 2006 (Dorhout and Rice 2008, Difonzo and Hammond 2008). Striacosta albicosta expanded its range into Ontario, Canada in 2008, and by 2010, economic injury to corn was reported (Baute 2009, Smith et al. 2018b). Some now consider S. albicosta a primary pest of corn in the Great Lakes region (Smith et al. 2018b).

Transgenic corn event MIR162 (Syngenta Seeds LLC, Research Triangle Park, NC) expresses the Bacillus thuringiensis (Berliner) (Bt) vegetative insecticidal protein, Vip3A, which was approved for release in the U.S. (USDA/APHIS 2010) and Canada (CFIA 2010) in 2010. This protein is active against several Noctuidae, including S. albicosta (Bowers et al. 2013, 2014, Bernardi et al. 2014, Farhan et al. 2017). The Vip3A protein kills susceptible larvae via ingestion in a series of steps similar to those for Cry proteins; however, Vip3A and Cry proteins bind to different midgut membrane receptors (Lee et al. 2003, 2006, Sena et al. 2009, Liu et al. 2011).
The current adoption of Vip3A-expressing corn hybrids in the Ontario market is low (M. Rice, 2018 personal communication); however, market adoption is expected to increase with greater availability of Vip3A expressing corn hybrids and documented resistance to the Cry1F protein (Smith et al. 2017).

To delay resistance development to Bt events, the high-dose/refuge model was developed for the original target pests, *Ostrinia nubilalis* (Hübner), *Leptinotarsa decemlineata* (Say), *Pectinophora gossypiella* (Saunders), and *Heliothis virescens* (Fabricius) (EPA 1998). The criteria of the high-dose/refuge model include: 1) that resistance alleles are rare and recessive within the population; 2) the expression of a toxin dose that is 25 times higher than the dose that kills 99% of the population (LD$_{99}$); and 2) planting refuge of non-Bt plants along with Bt crops to promote survival of susceptible individuals that will mate with resistant individuals resulting in susceptible heterozygotes (EPA 1998, Caprio and Sumerford 2000). It is unknown whether Vip3A corn provides a high dose against *S. albicosta*, and whether refuge configurations that are currently preferred in the market place, i.e., integrated refuge, where a small proportion of the total seeds (e.g. 5%) that do not express Bt protein are blended with Bt-expressing seed and occur throughout the field, will be effective for resistance management of Vip3A for *S. albicosta*.

Transgenic corn event TC1507 (co-developed by Dow AgroSciences and Pioneer Hi-Bred International, Johnston, IA) expressing Cry1F protein was shown to reduce feeding injury by *S. albicosta* early in its range expansion into the Corn Belt (Eichenseer et al. 2008); however, there is evidence that Cry1F did not meet the high-dose criteria for *S. albicosta* and resistance developed in a relatively short time. Laboratory studies in the U.S. and Canada were unable to
estimate reliably the concentration of Cry1F that caused mortality of 50% of the population (LC$_{50}$) using 1$^{st}$ instars and purified protein (Dyer et al. 2013, Ostrem et al. 2016, Smith et al. 2017) and declining control of S. albicosta injury was observed in field trials of Cry1F corn shortly after the findings by Eichenseer et al. (2008) (Smith et al. 2017, Smith et al. 2018).

Integrated refuge may not be an effective insect resistance management (IRM) strategy for S. albicosta given the factors of larval movement among corn tissues and neighboring plants with varying protein expression levels (Mallett and Porter, 1992, Davis and Onstad 2000, Pannuti et al. 2016). In an integrated refuge scenario, larvae that undergo early development on a non-Bt plant and move to a Bt plant as a late instar may be able to survive to adulthood (Mallet and Porter 1992, Huang et al. 1999). Additionally, cross-pollination can occur in an integrated refuge, resulting in a mosaic of Bt expression throughout the corn ear, reducing expression levels of Bt corn plants, and contaminating non-Bt refuge plants (Chilcutt and Tabashnik 2004, Yang et al. 2014). First instar S. albicosta typically move to the upper part of the corn plant soon after hatching to feed on tassel and pollen tissue, then move down to the ear as 3$^{rd}$ instars to feed on silk and developing kernels for the remainder of their larval development (Douglass et al. 1957, Michel et al. 2010, Paula-Moraes et al. 2012). Paula-Moraes et al. (2012) documented that tassel tissue was the best food source for early instars, satisfying their nutritional requirements prior to moving to developing kernels. Older instars often move from plant to plant, leading to potential exposure to lower and variable doses, increasing the risk for the development of resistance (Huang et al. 1999). Striacosta albicosta larvae may migrate from their natal plant to corn plants up to 3 m or more away (Douglass et al. 1957, Pannuti et al. 2016). The survival and
development of older instar *S. albicosta* on different corn tissues has not been assessed and the effects of varying Bt protein expression in corn tissues remain unknown.

First instar *S. albicosta* were found to be highly susceptible to Vip3A protein (Farhan et al. 2017); however, the susceptibility of older instars has not been determined. Older instars of *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner), *Spodoptera frugiperda* (J. E. Smith), *Heliothis zea* (Boddie), *Trichoplusia ni* (Hübner), and *O. nubilalis* were less susceptible to Bt proteins than younger instars (McGaughey 1978, Hornby and Gardner 1987, Huang et al. 1999, Bernardi et al. 2015, Miraldo et al. 2016).

In this study, the relative susceptibilities of 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} instar *S. albicosta* to Vip3A protein were compared using a diet-overlay bioassay method. Additionally, the effects of different corn tissues (leaf, tassel, silk) expressing various single and pyramided Bt proteins on the survival, development, and tissue consumption of *S. albicosta* larvae were investigated. In 2011-2013, tissue bioassays were conducted with 1\textsuperscript{st} and 3\textsuperscript{rd} instar *S. albicosta* using single (Cry1Ab, Cry1F) and pyramided (Cry1F × Cry1A.105 + Cry2Ab2, Cry1Ab × Vip3A) Bt proteins. In 2018, tissue bioassays were conducted with 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} instar *S. albicosta* using Cry1Ab and Cry1Ab × Vip3A tissues. Understanding changes in Vip3A susceptibility and feeding preferences as larvae develop are critical aspects for developing an effective IRM strategy for *S. albicosta* and Vip3A.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Insect Collection

In 2011-2013, *S. albicosta* larvae were sourced from laboratory colonies originating from field collections in ON in 2010. In 2016 and 2017, larvae were sourced from egg masses from
corn fields near Melbourne (Middlesex County) and Delhi (Norfolk County), ON respectively. In 2018, larvae were sourced from field-collected egg masses from Courtland, ON or egg masses from adults collected live in a black light trap (Madsen and Sanborn 1962) at Ridgetown, ON. Field-collected egg masses were placed on moistened filter paper inside petri plates in the laboratory, held at 26 ± 1°C, 60–70% RH, with a photoperiod of 16:8 (L:D) h, and monitored daily for hatching. Adults collected from black light traps were transferred to the laboratory and placed into rearing cages (60 × 60 × 60 cm) containing late vegetative stage pinto bean plants (*Phaseolus vulgaris* L.) in professional growing mix (Sun Gro Horticulture, Agawam, MA) for oviposition and 5% sucrose-agar water solution was provided *ad libitum* for drinking. Egg masses laid on bean plants were collected daily, placed on moistened filter paper and monitored daily for hatch.

Larvae were maintained in mass rearing tubes with artificial diet containing ~50 larvae until the desired instar stage was reached following the methods of Dyer et al. (2013). Instars were determined using body length and head capsule measurements as described by Dyer et al. (2013). In 2011-2013, larvae were reared on artificial diet as described by Dyer et al. (2013). In 2016, 2017, and 2018, larvae were reared on the Shorey and Hale (1967) diet with the addition of 28 g Fumagillin-B (Medivet Pharmaceuticals Ltd., High River, Alberta) and 4 mL of linseed oil (Sigma-Aldrich, St. Louis, Missouri) as described in Supplemental information.

3.3.2 Vip3A Protein Bioassays

Diet for bioassay trays was prepared as described in the diet validation experiment (supplemental Table S2) using the Shorey and Hale (1965) diet. Diet was dispensed using a repeater pipette at a rate of 1 mL per well into 128-well assay trays (Bio-16, CD International,
Pitman, NJ) for 1\textsuperscript{st} and 3\textsuperscript{rd} instars, and 15 mL per well into 32-well assay trays (Bio-16, CD International, Pitman, NJ) for 5\textsuperscript{th} instars. Trays were cooled to room temperature, covered with an adhesive ventilated cover, and stored at 4°C for up to 2 wk before use.

Purified Vip3A insecticidal protein (86.5\% active ingredient [a.i.]) was provided by Syngenta Seeds, LLC (Research Triangle Park, NC) and stored at -80°C until use. Protein was diluted to a stock concentration of 2 mg Vip3A mL\textsuperscript{-1} in a 0.1\% Triton buffer (Triton X-100, Sigma Life Science, Oakville, ON) solution through gentle agitation until completely dissolved. Final overlay concentrations of 12.5, 25.0, 50.0, 100.0, and 200.0 ng a.i. cm\textsuperscript{-2} for 1\textsuperscript{st} instars and 1250, 2500, 5000, 10000, and 20000 ng a.i. cm\textsuperscript{-2} for 3\textsuperscript{rd} and 5\textsuperscript{th} instars, were prepared by serial dilution in the Triton buffer prior to bioassay. An additional dose of 15000 ng a.i. cm\textsuperscript{-2} was tested on the Middlesex County collection for 3\textsuperscript{rd} and 5\textsuperscript{th} instars. Twenty-four wells of each concentration were overlaid with 30 µl of the prepared concentration or control solution (0.1\% Triton buffer without Bt protein) for 1\textsuperscript{st} and 3\textsuperscript{rd} instars. Sixteen wells of each concentration were overlaid with 150 µl of the prepared concentration or control solution for 5\textsuperscript{th} instars. To ensure uniform coverage of the diet surface, trays were tilted in several directions, after which, trays were placed under a fume hood at room temperature until the liquid component of the solution had evaporated from the surface.

A single 1\textsuperscript{st}, 3\textsuperscript{rd}, or 5\textsuperscript{th} instar larva was introduced into each well from the 2016 (Middlesex County) and 2017 (Norfolk County) \textit{S. albicosta} collections using a fine-tipped brush or forceps. Diet trays were covered with an adhesive lid and held at 26 ± 1°C, 60–70\% RH, with a light-weight cardboard cover over top. Mortality and larval weights were recorded 7
d after larval introduction and larvae that were unresponsive to gentle prodding with a fine-tipped brush were considered dead. Bioassays were replicated three times for each collection.

3.3.3 Tissue Bioassays

In 2011, 2012, and 2013, replicated strips (4 rows × 10 m) of corn hybrids were planted on four dates, one week apart at Ridgetown, ON to provide tissue for use in laboratory bioassays. The hybrids 35F37 (non-Bt) and 35F40 (Cry1F) were near-isolines obtained from Pioneer Hi-Bred Production LP (Mississauga, ON). The near-isoline hybrids DKC50-47 (non-Bt), DKC50-44 (Cry1Ab), and DKC50-45 (SmartStax (SSX)) (Cry1A.105 x Cry2Ab2 x Cry1F) were obtained from Monsanto Canada (Winnipeg, MB). The near-isoline hybrids N53C-3000GT (Cry1Ab) and N53C-3111 (Vip3A × Cry1Ab) were obtained from Syngenta Seeds Canada (Arva, ON). In 2018, replicated strips (8 rows x 15 m) of the hybrids HZ3011A (Cry1Ab) and HZ4010 (Vip3A × Cry1Ab) (Horizon Seeds, Courtland, ON) were planted on three dates, one week apart at Ridgetown, ON.

Bioassay trays containing 32 wells (CD-32, CD International, Pitman, NJ) were lined with a piece of moistened filter paper (P5 grade, Fisherbrand™) in the bottom of each well and filled with leaf, tassel, or silk tissue. Expression of Bt events was confirmed in situ before tissue collection using EnviroLogix QuickStix (EnviroLogix Portland, ME). Corn tissues were collected from the field on the day of bioassay tray preparation. Leaf tissue was collected at the V6 stage (6 leaf collars), washed with distilled water, cut into squares from either side of the mid-rib and 1.0 g was placed into each well. Tassel tissue was collected from inside the whorl before tassel emergence and cut into 2.5 cm lengths. Fresh silk was obtained from newly developed ears. In 2011-2013, each well was filled with 2.0 or 3.0 g of tassel or silk tissue; in
2018, wells were filled with 1.0 or 2.0 g of tassel or silk tissue for 1st or 3rd instar larvae, respectively. In 2011-2013, control trays were filled with 20 mL of diet prepared in the same manner as that used for rearing (Dyer et al. 2013). In 2018, control trays were filled using a repeater pipette with 15 mL of the Shorey and Hale (1967) artificial diet (AD) prepared in the manner described above.

For kernel tissue bioassays, corn ears were removed from the field during the milk stage (R2) (Abendroth et al. 2011). Ears were cut to ~7 cm in length, weighed, and placed in 500 mL deli cups lined with coffee filter paper (Melitta, Toronto, ON) moistened with 1 mL of water. Deli cups with kernel tissues were also prepared without larvae to serve as a control for the change in moisture over time under experimental conditions.

For 1st and 3rd instar bioassays, one larva was transferred into each well using a fine-point paintbrush and covered with transparent, adhesive, ventilated covers (Frontier Agricultural Sciences, Newark, DE). Trays with larvae were placed into rearing conditions for the duration of the experiment (24 ± 1ºC, 60-70% RH, photoperiod of 16:8 (L:D) h). Larvae were transferred to new trays with fresh corn tissues prepared in the same manner every 2-3 d to replenish the supply and provide a clean environment; diet trays were not replenished. For each bioassay in 2011-2013, 32 larvae were treated per tissue replicate as well as the diet control. In 2018, the number of larvae available was limited when assay tissues were available. For 1st instar bioassays in 2018, 32 larvae were introduced to each AD, tassel, or silk tissues per replicate, with an exception of one replicate of non-Bt tassel, non-Bt silk, and Vip3A silk which had 22, 30, and 24 larvae, respectively. For 3rd instar bioassays in 2018, 24 larvae were treated per tassel, silk, and diet control, and 20 larvae were treated per kernel tissue and diet control. For 5th instar
bioassays 24 and 20 larvae per replicate were introduced onto silk and kernel tissues, respectively. Each bioassay was repeated 3 times, with the exception of the kernel tissue bioassays containing 3rd and 5th instars, which were replicated twice.

For 1st and 3rd instar bioassays, mortality and weight of surviving larvae were assessed at 7, 14, 21, and 28 days after introduction (DAI), and body length and head capsule width were measured using calipers. For 5th instar bioassays, mortality was assessed at 7 and 14 DAI. Larvae were deemed dead when unresponsive to gentle prodding with a fine brush. Surviving larvae were transferred to new trays with fresh corn tissues prepared in the same manner at each time point; artificial diet was replenished at 14 DAI. Consumption rates were calculated using standard gravimetric methods (Waldbauer 1968). Consumption values were determined by subtraction of the mean weight of the uneaten portion of the infested tissues from the mean weight of uninfested tissues.

To estimate the exposure of each instar to Vip3A protein under field conditions, the observed consumption of each tissue type in tissue bioassays was multiplied by the reported expression of Vip3A protein in fresh transgenic MIR162 corn tissues (USDA/APHIS 2007).

3.3.4 Data Analysis

Vip3A protein bioassays. To determine lethal concentrations (LC50, LC95, and LC99), with 95% fiducial limits, the slope of the concentration-response curve was fitted using PROC PROBIT in SAS 9.4. The OPTC option was used to correct for mortality of larvae in the control. The Pearson chi-square goodness-of-fit test was used (P < 0.10) to test if the observed values
followed the predicted model. Replicates with > 25% mortality in the control were not included in the analyses.

Larval weights were pooled and divided by the initial number of larvae infested for each concentration to calculate the average larval weight (Siegfried et al. 2005). Larval weights were transformed to percent growth inhibition relative to the control and analyzed by non-linear regression fitted to a probit model as described by Marcon et al. (1999) to determine the effective concentration, EC$_{50}$ (concentration of protein at which larval growth was inhibited by 50%). To determine if LC and EC values for each collection were different, pairwise comparisons were conducted and deemed significant if the 95% confidence intervals did not overlap. Tolerance ratios (TR$_{50}$, TR$_{95}$, and TR$_{99}$) were obtained by dividing LC$_{50}$, LC$_{95}$, and LC$_{99}$ values from 3$^{rd}$ and 5$^{th}$ instars with the LC$_{50}$, LC$_{95}$, and LC$_{99}$ values of the 1$^{st}$ instar for each collection (Huang et al. 1999).

_Tissue bioassays._ The effect of various Bt events on larval survival, weight, body length, head capsule width, and amount of tissue consumed for each instar was analyzed using a generalized linear mixed model (GLMM) in PROC GLIMMIX (SAS 9.4, SAS institute, Cary, NC). Fixed effects included tissue type, Bt event, DAI, and their interactions; replication was considered a random effect for all variables. Initial PROC GLIMMIX analyses determined that there was no difference in the survival and developmental measurements of larvae across years or among hybrids within Bt events; therefore, data from Cry1Ab hybrids (DKC50-44, N53C-3000GT, HZ 3011A), Cry1F hybrids (35F40, DKC50-45), Vip3A × Cry1Ab hybrids (N53C-3111, HZ 4010), and non-Bt hybrids (35F37, DKC50-47) were pooled for further analyses. Dependent variables measured over time for each instar were analyzed as repeated measures
fitted with a logit link and binomial distribution and the Laplace method was used for integral approximation (Bowley 2015). Larval survival was corrected for mortality on artificial diet (Abbott 1925). To fit a repeated measure covariance structure, an additional random statement was included defining the R-side covariance structure with the random subject term replication × tissue type. Compound symmetric (cs) and autoregressive order 1 (ar(1)) covariance structures were selected over other covariance structure models based on having the lowest AICC fit statistics for non-Gaussian larval survival and Gaussian larval weights, body length, head capsule width, and amount of tissue consumed, respectively. An F-test was used to determine the significance of the fixed effect (Bt-event) and least square means (LSMEANS) were compared pairwise with Tukey’s adjustment (Bowley 2015). The ilink option was used to convert means and standard error estimates into data scale (Bowley 2015). To confirm the assumptions of the analysis, scatterplots of studentized residuals against fixed effects and predicted values, and a Shapiro-Wilk test of normality were generated; no outliers were detected after studentized residual assessment (Bowley 2015).

3.4 RESULTS

3.4.1 Vip3A Protein Bioassays

The concentration/mortality relationship for the three instars of S. albicosta to Vip3A was satisfactorily described by probit analysis based on non-significant Chi square values (Bowley 2015) (Table 3.1). The LC$_{50}$, LC$_{95}$, and LC$_{99}$ values for 1$^{st}$ instars were lower than for 3$^{rd}$ and 5$^{th}$ instars for both S. albicosta collections (Table 3.1). There was no difference in lethal concentration estimates between 3$^{rd}$ and 5$^{th}$ instars for either collection. The susceptibility of the Middlesex and Norfolk County collections of S. albicosta expressed as LC$_{50}$ values ranged from
25.0 to 4058 ng a.i. cm\(^{-2}\) (Table 3.1) with tolerance ratios (the LC of older instars relative to the LC of 1\(^{st}\) instars) up to 162 for 5\(^{th}\) instars (Table 2). When expressed as LC\(_{95}\), values for both collections ranged from 101.1 to 21505 ng a.i. cm\(^{-2}\) (Table 3.1), with TR\(_{95}\) up to 213 (Table 3.2). The LC\(_{99}\) values ranged from 180.5 to 42915 ng a.i. cm\(^{-2}\) for both collections (Table 3.1), with TR\(_{99}\) up to 238 (Table 3.2).

Growth inhibition of \(S.\ albicosta\) larvae treated with Vip3A expressed as EC\(_{50}\) values is presented in Table 3.1. Values ranged from 20.5 ng a.i. cm\(^{-2}\) for 1\(^{st}\) instars to 3288 ng a.i. cm\(^{-2}\) for 5\(^{th}\) instars for the Middlesex County collection, and 30.3 ng a.i. cm\(^{-2}\) for 1\(^{st}\) instars to 2073. ng a.i. cm\(^{-2}\) for 5\(^{th}\) instars for the Norfolk County collection.

3.4.2 Tissue Bioassay

The variance analysis on corrected survival, weight, body length, head capsule width, and consumption amounts for 1\(^{st}\), 3\(^{rd}\), and 5\(^{th}\) instars of \(S.\ albicosta\) are presented in Table 3.3. Survival of the 1\(^{st}\) and 3\(^{rd}\) instars was <30% on leaf tissue across all Bt events, but was >55% on silk and tassel tissue on Cry1Ab, Cry1F and non-Bt, and <5% on all tissues of Vip3A x Cry1Ab (Fig. 3.1 a, b), producing a highly significant Bt event x tissue type interaction (\(P <0.0001\); Table 3.3). Survival of 1\(^{st}\) and 3\(^{rd}\) instars was <65% at 7 DAI, <50% at 14 DAI, and <40% at 21 and 28 DAI, producing a highly significant effect of DAI (\(P <0.0001\); Table 3.3). Survival of 5\(^{th}\) instars was >95% on kernel and tassel tissues expressing Cry1Ab at all DAI, and 21% on Vip3A x Cry1Ab kernels at 7 DAI, but was <1% on Vip3A x Cry1Ab kernels at 14 DAI and on Vip3A x Cry1Ab silk at all DAI (Fig. 3.1c), producing a highly significant Bt event by DAI by tissue type interaction (\(P <0.0001\); Table 3.3)
Table 3.1: Susceptibility of 1st, 3rd, and 5th instar Striacosta albicosta reared from egg masses collected in Ontario, Canada to Vip3A insecticidal protein in diet\(^4\)-overlay bioassays, expressed as lethal concentration (LC\(_{50}\), LC\(_{95}\), LC\(_{99}\)) and growth inhibition (EC\(_{50}\)) with 95% confidence intervals (ng a.i. cm\(^{-2}\)).

<table>
<thead>
<tr>
<th>Collection</th>
<th>Instar</th>
<th>n(^4)</th>
<th>Slope ± SE</th>
<th>LC(_{50}) (95% CI)</th>
<th>LC(_{95}) (95% CI)</th>
<th>LC(_{99}) (95% CI)</th>
<th>(\chi^2)</th>
<th>EC(_{50}) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middlesex 2016(^2)</td>
<td>1(^{st})</td>
<td>504</td>
<td>2.17 ± 0.291</td>
<td>25.0 (20.7-29.3)b(^5)</td>
<td>101.1 (79.6-142.0)b</td>
<td>180.5 (130.5-291.3)b</td>
<td>9.78</td>
<td>20.5 (17.3-23.9)a</td>
</tr>
<tr>
<td></td>
<td>3(^{rd})</td>
<td>480</td>
<td>2.47 ± 0.330</td>
<td>3433 (2251-4539)a</td>
<td>15888 (12630-21697)a</td>
<td>29977 (21910-48861)a</td>
<td>7.78</td>
<td>1346 (0.0-1798)a</td>
</tr>
<tr>
<td></td>
<td>5(^{th})</td>
<td>336</td>
<td>2.11 ± 0.255</td>
<td>4058 (2559-5496)a</td>
<td>21505 (16082-33070)a</td>
<td>42915 (28794-83197)a</td>
<td>20.27</td>
<td>3288 (0.0-4010)a</td>
</tr>
<tr>
<td>Norfolk 2017(^3)</td>
<td>1(^{st})</td>
<td>432</td>
<td>2.76 ± 0.359</td>
<td>46.3 (36.3-55.8)b</td>
<td>182.4 (141.4-269.3)b</td>
<td>322.1 (226.8-566.5)b</td>
<td>13.62</td>
<td>30.3 (21.6-42.1)a</td>
</tr>
<tr>
<td></td>
<td>3(^{rd})</td>
<td>432</td>
<td>2.38 ± 0.301</td>
<td>3583 (2957-4216)a</td>
<td>13988 (11050-19552)a</td>
<td>24597 (17889-39378)a</td>
<td>12.11</td>
<td>1823 (0.0-2297)a</td>
</tr>
<tr>
<td></td>
<td>5(^{th})</td>
<td>288</td>
<td>2.23 ± 0.322</td>
<td>2678 (1617-3812)a</td>
<td>14635 (10331-23941)a</td>
<td>29579 (18969-60206)a</td>
<td>12.25</td>
<td>2073 (0.0-2710)a</td>
</tr>
</tbody>
</table>

\(^1\) Shorey and Hale (1965).

\(^2\) Collection was tested at F\(_4\).

\(^3\) Collection was tested at F\(_2\).

\(^4\) n = total number of larvae used in the bioassay; 24 larvae per concentration for 1\(^{st}\) and 3\(^{rd}\) instars, 16 larvae per concentration for 5\(^{th}\) instars.

\(^5\) Concentrations tested: 0.0, 12.5, 50.0, 100.0, and 200.0 ng a.i. cm\(^{-2}\).

\(^6\) Concentrations tested: 0, 1250, 5000, 10000, and 20000 ng a.i. cm\(^{-2}\).

\(^7\) Concentrations tested: 0, 1250, 5000, 10000, and 20000 ng a.i. cm\(^{-2}\).

\(^8\) Value of the Pearson Chi-square goodness-of-fit test (\(P > 0.10\)).

\(^9\) Means within columns followed by the same letter are not significantly different as determined by non-overlapping 95% confidence intervals.
Table 3.2: Tolerance ratio (TR) of 3\textsuperscript{rd} and 5\textsuperscript{th} instar Striacosta albicosta compared to 1\textsuperscript{st} instars collected in Ontario, Canada to Vip3A insecticidal protein in diet-overlay bioassays.

<table>
<thead>
<tr>
<th>Collection</th>
<th>Instar</th>
<th>TR\textsubscript{50}</th>
<th>TR\textsubscript{95}</th>
<th>TR\textsubscript{99}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middlesex 2016\textsuperscript{1}</td>
<td>3\textsuperscript{rd}</td>
<td>137</td>
<td>157</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{th}</td>
<td>162</td>
<td>213</td>
<td>238</td>
</tr>
<tr>
<td>Norfolk 2017\textsuperscript{2}</td>
<td>3\textsuperscript{rd}</td>
<td>77</td>
<td>77</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>5\textsuperscript{th}</td>
<td>58</td>
<td>80</td>
<td>92</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Collection was tested at F\textsubscript{1}.
\textsuperscript{2} Collection was tested at F\textsubscript{2}.
\textsuperscript{3} Tolerance ratio = LC\textsubscript{50} of test instar/LC\textsubscript{50} of 1\textsuperscript{st} instar.
\textsuperscript{4} Tolerance ratio = LC\textsubscript{95} of test instar/LC\textsubscript{95} of 1\textsuperscript{st} instar.
\textsuperscript{5} Tolerance ratio = LC\textsubscript{99} of test instar/LC\textsubscript{99} of 1\textsuperscript{st} instar.

The weight, body length, and head capsule width of 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} instars increased with DAI for larvae fed AD or tissues expressing Bt events (Table 3.3). No differences between diet types were observed in these measures at earlier time points, but were evident at later time points (Table 3.4). Weight, body length, and head capsule width of 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} instars fed Vip3A × Cry1Ab expressing tissues did not differ from larvae fed AD or other Bt events, with the exception of 3\textsuperscript{rd} instar body length, which was over 0.4 mm shorter for larvae fed Vip3A × Cry1Ab expressing tissues relative to larvae fed AD and tissues of other Bt events (Table 3.4).
Table 3.3: Variance analysis for corrected survival, weight, body length, head capsule width, and amount consumed by 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} Striacosta albicosta instars.

<table>
<thead>
<tr>
<th>Instar\textsuperscript{1}</th>
<th>Corrected survival</th>
<th>Weight</th>
<th>Body length</th>
<th>Head capsule width</th>
<th>Amount consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>df\textsuperscript{2}</td>
<td>$P &gt; F$</td>
<td>df\textsuperscript{2}</td>
<td>$P &gt; F$</td>
<td>df\textsuperscript{2}</td>
</tr>
<tr>
<td>Bt event\textsuperscript{2}</td>
<td>3, 222</td>
<td>&lt;0.0001</td>
<td>4, 145</td>
<td>0.9138</td>
<td>0.4676</td>
</tr>
<tr>
<td>DAI\textsuperscript{3}</td>
<td>3, 222</td>
<td>&lt;0.0001</td>
<td>3, 145</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tissue\textsuperscript{4}</td>
<td>2, 222</td>
<td>&lt;0.0001</td>
<td>3, 145</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bt event × DAI</td>
<td>9, 222</td>
<td>0.3781</td>
<td>9, 145</td>
<td>&lt;0.0001</td>
<td>0.0402</td>
</tr>
<tr>
<td>Bt event × Tissue</td>
<td>6, 222</td>
<td>&lt;0.0001</td>
<td>6, 145</td>
<td>0.8496</td>
<td>0.4704</td>
</tr>
<tr>
<td>DAI × Tissue</td>
<td>6, 222</td>
<td>0.4158</td>
<td>6, 145</td>
<td>0.7973</td>
<td>0.3663</td>
</tr>
<tr>
<td>Bt event × DAI × Tissue</td>
<td>18, 222</td>
<td>0.9952</td>
<td>36, 145</td>
<td>0.9115</td>
<td>0.4222</td>
</tr>
<tr>
<td>Covariance parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication × Tissue</td>
<td></td>
<td></td>
<td></td>
<td>0.1494</td>
<td>0.1584</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instar\textsuperscript{1}</th>
<th>Corrected survival</th>
<th>Weight</th>
<th>Body length</th>
<th>Head capsule width</th>
<th>Amount consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>df\textsuperscript{2}</td>
<td>$P &gt; F$</td>
<td>df\textsuperscript{2}</td>
<td>$P &gt; F$</td>
<td>df\textsuperscript{2}</td>
</tr>
<tr>
<td>Bt event</td>
<td>3, 258</td>
<td>&lt;0.0001</td>
<td>4, 154</td>
<td>0.3186</td>
<td>0.0198</td>
</tr>
<tr>
<td>DAI</td>
<td>3, 258</td>
<td>&lt;0.0001</td>
<td>3, 154</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tissue</td>
<td>3, 258</td>
<td>0.0002</td>
<td>4, 154</td>
<td>0.0194</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bt event × DAI</td>
<td>9, 258</td>
<td>0.0485</td>
<td>12, 154</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bt event × Tissue</td>
<td>9, 258</td>
<td>&lt;0.0001</td>
<td>16, 154</td>
<td>0.9575</td>
<td>0.8320</td>
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<tr>
<td>Interaction</td>
<td>df</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
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<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>DAI × Tissue</td>
<td>9, 258</td>
<td>0.5292</td>
<td>12, 154</td>
<td>0.5589</td>
<td>0.0125</td>
</tr>
<tr>
<td>Bt event × DAI × Tissue</td>
<td>27, 258</td>
<td>0.9995</td>
<td>48, 154</td>
<td>0.9971</td>
<td>0.9993</td>
</tr>
<tr>
<td>Covariance parameters</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Replication × Tissue</td>
<td>0.3873</td>
<td>0.0698</td>
<td>0.0069</td>
<td>0.0011</td>
<td>1.0000</td>
</tr>
<tr>
<td>Fixed effects</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bt event</td>
<td>1, 18</td>
<td>&lt;0.0001</td>
<td>2, 16</td>
<td>0.0002</td>
<td>0.0081</td>
</tr>
<tr>
<td>DAI</td>
<td>1, 18</td>
<td>0.0011</td>
<td>1, 16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tissue</td>
<td>1, 18</td>
<td>0.0381</td>
<td>2, 16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bt event × DAI</td>
<td>1, 18</td>
<td>0.0324</td>
<td>2, 16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bt event × Tissue</td>
<td>1, 18</td>
<td>0.0095</td>
<td>4, 16</td>
<td>0.3805</td>
<td>0.4079</td>
</tr>
<tr>
<td>DAI × Tissue</td>
<td>1, 18</td>
<td>0.0048</td>
<td>2, 16</td>
<td>0.0003</td>
<td>0.1762</td>
</tr>
<tr>
<td>Bt event × DAI × Tissue</td>
<td>1, 18</td>
<td>0.0271</td>
<td>8, 16</td>
<td>0.0748</td>
<td>0.2729</td>
</tr>
<tr>
<td>Covariance parameters</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Replication × Tissue</td>
<td>0.4687</td>
<td>0.5583</td>
<td>0.5004</td>
<td>0.3159</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

1 Larval instar at initiation of the experiment.
2 Bt event – Artificial Diet (AD), Cry1Ab, Cry1F, Non-Bt, Vip3A × Cry1Ab.
3 DAI – Days after introduction (7, 14, 21, and 28).
4 Tissue – artificial diet (AD) (1st, 3rd, and 5th instars), kernel (3rd and 5th instars), leaf (1st and 3rd instars), silk (1st, 3rd, and 5th instars), and tassel (1st and 3rd instars) corn tissues.
5 df – numerator, denominator.
Figure 3.1: Corrected survival of a) 1st, b) 3rd, and c) 5th instar Striacosta albicosta larvae fed non-Bt or Bt corn tissues. Error bars indicate SE of the mean. Values followed by the same letter within instar are not significantly different according to Tukey ($P = 0.05$). Tissue types were kernel (3rd and 5th instars), leaf (1st and 3rd instars), silk (1st, 3rd, and 5th instars), and tassel (1st and 3rd instars) corn tissues. Bt events were Cry1Ab, Cry1F, Non-Bt, Vip3A × Cry1Ab.

The weight, body length, and head capsule width of surviving 1st instars were highest when fed AD, followed by silk and tassel, followed by leaf tissue (Table 3.5). Similarly, the weights of 3rd instars were highest when fed AD, followed by silk and tassel tissue, followed by kernel and leaf tissue (Table 3.5); the body length and head capsule width of 3rd instars were lower when fed leaf tissue relative to those fed AD and other corn tissues (Table 3.5). The weight, body length, and head capsule width of 5th instars were highest when fed kernel tissue, followed by AD, followed by silk tissue (Table 3.5).

There was a tissue type by Bt event by time interaction on the amount of tissues consumed by 1st, 3rd, and 5th instars (Table 3.3). There was no difference in the amount of Vip3A × Cry1Ab or Cry1Ab leaf or tassel tissue consumed at 7 DAI for any instar; however 3rd instars consumed 2.3 times more Cry1Ab than Vip3A × Cry1Ab kernels (Table 3.6). At 14 DAI, more Cry1Ab-expressing tissues was consumed than Vip3A × Cry1Ab tissues (Table 3.6). Consumption by 1st instars increased over time for tissues expressing Cry1Ab but not for tissues expressing Vip3A × Cry1Ab (Table 3.6). For 7, 14, and 21 DAI, there were differences in consumption between Cry1Ab-expressing tissue types, with 1st, 3rd, and 5th instars consuming over 3 times more silk tissue than other tissue types (Table 3.6). The estimated quantity of Vip3A protein in consumed tissues for each instar is presented in Table 3.6. Estimated exposure
depended on tissue type consumed ranging from 816-10241, 367-12445, and 992-48441 ng for 1st, 3rd, and 5th instars, respectively (Table 3.6).

3.5 DISCUSSION

We investigated the susceptibility, survival, and development of 1st, 3rd, and 5th instar S. albicosta exposed to Vip3A. Older instars were less susceptible and had higher survival on Vip3A-expressing tissues compared to 1st instars. The developmental measures showed no difference between larvae that fed on Vip3A-expressing tissues compared to those that fed on tissues expressing other Bt-events. The findings from this study suggest that Vip3A corn hybrids may provide a high dose against 1st instar larvae, but not against 3rd and 5th instar S. albicosta. The susceptibility of 1st instar S. albicosta reported in this study is within the baseline susceptibility range documented in Farhan et al. (2017); however, the difference in tolerance between early and later instars may increase the risk of resistance development if the amount of Vip3A produced by corn tissue is not high enough to kill later instars.

Measuring the amount of Vip3A protein that S. albicosta larvae are exposed to at different stages would inform pest management and IRM plans for S. albicosta; however, determination of Bt protein expression levels in plant tissue by an external party is not permitted by the technology providers. In order to estimate biologically relevant Vip3A exposure for S. albicosta, tissue-specific consumption rates of 1st, 3rd, and 5th instar larvae observed in this study were compared to publicly available protein expression data for Vip3A corn tissues (USDA/APHIS 2007). Estimated protein exposure demonstrates that the potential exposure to Vip3A for 1st instars is approximately 57- and 32- times higher on tassel tissue than the LC_{99} estimated for the Middlesex and Norfolk collections, respectively at 7 DAI. First instar S.
*albicosta* are most likely to feed on corn pollen and tassel in the field (Hagen 1962, Paula-Moraes et al. 2012). These tissues express the highest amount of Vip3A protein throughout the plant (USDA/APHIS 2007; Table 3.6). In contrast, 3rd and 5th instars primarily feed on silk and kernel tissues in the field (Douglass 1957, Hagen 1962), which express 3.4 and 1.4 times less Vip3A protein, respectively, than pollen (USDA/APHIS 2007; Table 3.6). The results of this study indicate that Vip3A corn hybrids do not meet the high-dose criteria for 3rd and 5th instar *S. albicosta*, as LC$_{99}$ values were higher than the estimated protein exposure; furthermore, up to 21% survival was observed on Vip3A-expressing corn tissues.

The survival of older *S. albicosta* instars on Vip3A-expressing tissues is especially concerning in an integrated refuge system when considering on-plant and plant-to-plant larval movement. An integrated refuge increases the possibility of cross-pollination between non-Bt and Bt corn leading to a mosaic of Bt expression in corn ears, the presence of Bt protein expression in refuge corn, and vice versa (Chilcutt and Tabashnik 2004). Integrated refuge also increases the chances that larvae produced on non-Bt refuge plants may migrate to Bt corn as later instars, when they are more tolerant to the Bt toxin, and therefore may survive exposure (Yang et al. 2014). The success of the integrated refuge strategy also depends on recessive resistance inheritance; however, the inheritance of Vip3A resistance by *S. albicosta* is currently unknown. Furthermore, the ability of 3rd and 5th instars to tolerate a higher dose of Vip3A may leave corn ears vulnerable to feeding by these larvae, potentially increasing mycotoxin contamination. Smith et al. (2018a) showed that the mere incidence of injury by *S. albicosta* increases the potential for the production of mycotoxins, which are harmful to humans and livestock when consumed (Eichenseer et al. 2008, Smith et al. 2018a).
Table 3.4: Mean weight, body length, and head capsule width of 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> instar *Striacosta albicosta* fed artificial diet (AD), non-Bt, or Bt corn tissues in tissue bioassays.

<table>
<thead>
<tr>
<th>Instar&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Bt event(s)</th>
<th>Weight (g)</th>
<th></th>
<th></th>
<th>Body length (mm)</th>
<th></th>
<th></th>
<th>Head capsule width (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAI 7</td>
<td>14</td>
<td>21</td>
<td>28</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>AD</td>
<td>0.007e</td>
<td>0.095e</td>
<td>0.385bc</td>
<td>0.663a</td>
<td>8.4e</td>
<td>16.9d</td>
<td>27.7b</td>
<td>32.3a</td>
</tr>
<tr>
<td></td>
<td>Cry1Ab</td>
<td>0.010e</td>
<td>0.069e</td>
<td>0.240d</td>
<td>0.418b</td>
<td>7.6e</td>
<td>14.5d</td>
<td>23.3c</td>
<td>28.1b</td>
</tr>
<tr>
<td></td>
<td>Cry1F</td>
<td>0.002e</td>
<td>0.069e</td>
<td>0.306bcd</td>
<td>0.377bc</td>
<td>6.1e</td>
<td>13.5d</td>
<td>22.7bc</td>
<td>22.6b</td>
</tr>
<tr>
<td></td>
<td>Non-Bt</td>
<td>0.007e</td>
<td>0.087e</td>
<td>0.292cd</td>
<td>0.384bc</td>
<td>7.3e</td>
<td>15.5d</td>
<td>23.6c</td>
<td>23.2bc</td>
</tr>
<tr>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.008e</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>5.7e</td>
<td>.</td>
<td>.</td>
<td>0.6cd</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>AD</td>
<td>0.160d</td>
<td>0.311bc</td>
<td>0.392abc</td>
<td>0.455ab</td>
<td>17.3d</td>
<td>24.2bc</td>
<td>28.5a</td>
<td>28.3a</td>
</tr>
<tr>
<td></td>
<td>Cry1Ab</td>
<td>0.088d</td>
<td>0.207c</td>
<td>0.272bc</td>
<td>0.368ab</td>
<td>15.5d</td>
<td>22.2c</td>
<td>26.9ab</td>
<td>29.5a</td>
</tr>
<tr>
<td></td>
<td>Cry1F</td>
<td>0.087d</td>
<td>0.216c</td>
<td>0.425ab</td>
<td>0.556a</td>
<td>16.4d</td>
<td>22.7c</td>
<td>28.5ab</td>
<td>31.0a</td>
</tr>
<tr>
<td></td>
<td>Non-Bt</td>
<td>0.116d</td>
<td>0.296bc</td>
<td>0.286ab</td>
<td>0.477ab</td>
<td>16.9d</td>
<td>22.8c</td>
<td>27.3ab</td>
<td>28.0ab</td>
</tr>
<tr>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.073d</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>8.7e</td>
<td>.</td>
<td>.</td>
<td>1.3d</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>AD</td>
<td>0.178b</td>
<td>0.369a</td>
<td>.</td>
<td>.</td>
<td>24.2bc</td>
<td>29.0a</td>
<td>.</td>
<td>2.4c</td>
</tr>
<tr>
<td></td>
<td>Cry1Ab</td>
<td>0.203b</td>
<td>0.349a</td>
<td>.</td>
<td>.</td>
<td>23.4c</td>
<td>27.1ab</td>
<td>.</td>
<td>2.6bc</td>
</tr>
<tr>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.136b</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>20.4c</td>
<td>.</td>
<td>.</td>
<td>2.8abc</td>
</tr>
</tbody>
</table>

<sup>2</sup> Larval instar at initiation of the experiment.

<sup>3</sup> Values followed by the same letter within instar and growth parameter are not significantly different according to Tukey ($P = 0.05$).

<sup>4</sup> No data were collected due to 100% mortality.
Table 3.5: Mean (SE) weight, body length, and head capsule width of 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} instar \textit{Striacosta albicosta} fed artificial diet (AD), and combined Bt and non-Bt corn leaf, silk, or tassel tissues in tissue bioassays at 28 DAI.

<table>
<thead>
<tr>
<th>Instar\textsuperscript{1}</th>
<th>Tissue</th>
<th>Weight (g)</th>
<th>Body length (mm)</th>
<th>Head capsule width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD</td>
<td>0.265 (0.0563)a\textsuperscript{2}</td>
<td>20.5 (1.74)a</td>
<td>2.7 (0.39)a</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.037 (0.0626)c</td>
<td>6.8 (2.58)c</td>
<td>0.9 (0.52)c</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>0.179 (0.0546)b</td>
<td>17.3 (1.68)b</td>
<td>1.9 (0.37)b</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>0.185 (0.0534)b</td>
<td>17.3 (1.61)b</td>
<td>2.0 (0.35)b</td>
</tr>
<tr>
<td>1\textsuperscript{st}</td>
<td>AD</td>
<td>0.314 (0.0428)a</td>
<td>24.1 (1.52)a</td>
<td>2.9 (0.23)a</td>
</tr>
<tr>
<td></td>
<td>Kernel</td>
<td>0.104 (0.0683)c</td>
<td>24.3 (2.47)a</td>
<td>2.9 (0.33)a</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.113 (0.0494)c</td>
<td>15.3 (1.65)b</td>
<td>2.1 (0.25)b</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>0.233 (0.0427)b</td>
<td>21.3 (1.37)a</td>
<td>2.6 (0.22)a</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>0.251 (0.0422)b</td>
<td>22.8 (1.35)a</td>
<td>2.9 (0.22)a</td>
</tr>
<tr>
<td>3\textsuperscript{rd}</td>
<td>AD</td>
<td>0.276 (0.0156)b</td>
<td>26.5 (1.30)a</td>
<td>2.7 (0.04)b</td>
</tr>
<tr>
<td></td>
<td>Kernel</td>
<td>0.386 (0.0399)a</td>
<td>25.8 (1.41)a</td>
<td>3.2 (0.10)a</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>0.188 (0.0380)c</td>
<td>22.1 (1.34)b</td>
<td>2.5 (0.09)b</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Larval instar at initiation of the experiment.

\textsuperscript{2} Values followed by the same letter within instar are not significantly different according to Tukey ($P = 0.05$).
Table 3.6: Mean consumption of 1st, 3rd, and 5th instar *Striacosta albicosta* fed corn tissues in bioassays and the estimated quantity of Vip3A protein in consumed tissue as determined at days after introduction (DAI) in Ontario, Canada in 2018.

<table>
<thead>
<tr>
<th>Instar¹</th>
<th>Tissue</th>
<th>Bt-event(s)</th>
<th>Tissue consumed (g)</th>
<th>Estimated quantity of Vip3A in consumed tissue (ng)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAI 7 14 21 28 7 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>Silk</td>
<td>Cry1Ab</td>
<td>0.089d³ 0.142c 0.245b 0.276b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.065de 0.002e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>Cry1Ab</td>
<td>0.251b 0.365a 0.384a 0.256b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.237b</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>Kernel</td>
<td>Cry1Ab</td>
<td>0.276ab 0.274ab 0.292a 0.252ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.123cde 0.016ef</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>Cry1Ab</td>
<td>0.091de 0.123d 0.151cd 0.185bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.073de</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>Cry1Ab</td>
<td>0.330a 0.295a 0.193bc 0.290a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.288a 0.023e</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>Kernel</td>
<td>Cry1Ab</td>
<td>1.957ab 2.834a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>1.625ab 1.345bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>Cry1Ab</td>
<td>0.201d 0.282cd</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vip3A × Cry1Ab</td>
<td>0.079d 0.094cd</td>
<td></td>
</tr>
</tbody>
</table>
1 Larval instar at initiation of the experiment.

2 Estimated Vip3A in consumed tissue was estimated as the product of the amount of tissue consumed per instar and Vip3A protein expression in MIR162 transgenic fresh corn tissue as reported in USDA/APHIS (2007).

3 Tissue consumption values followed by the same letter within instar are not significantly different according to Tukey ($P = 0.05$).

4 No data were collected due to 100% mortality.
Currently, Vip3A is the only commercially available Bt event that reduces injury by *S. albicosta* (Bowers et al. 2013, 2014, Farhan et al. 2017), since *S. albicosta* has developed resistance to Cry1F in the U.S. and Canada (Ostrem et al. 2016, Smith et al. 2017). In the current study, survival on Cry1F tissue was not different from survival on non-Bt and Cry1Ab but was significantly higher than on Vip3A tissues. The independent modes of action of Vip3A compared with Cry proteins suggest that cross-resistance is unlikely to occur between Cry1F and Vip3A in *S. albicosta*. Additionally, none of the 1<sup>st</sup> and 3<sup>rd</sup> instars fed Vip3A tissues developed to 5<sup>th</sup> instar, indicating that Vip3A controls early instars. However, when looking at growth parameters such as weight, body length, and head capsule width, there were no differences between larvae that survived exposure to Vip3A tissues and those feeding on artificial diet or non-Vip3A tissues up to 7 DAI. Exposure to Vip3A tissues may not have impacted larval growth within 7 days of feeding, however, larvae died with continued exposure to Vip3A. In an integrated refuge system, the duration of survival on Vip3A-expressing tissues may provide larvae enough time to move from Vip3A-expressing plants to non-Vip3A plants, which could accelerate the evolution of resistance to Vip3A protein.

The susceptibility, survival, development, and feeding habits of 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> instar *S. albicosta* presented here can inform IRM in transgenic corn. In this study, we found *S. albicosta* susceptibility decreased and survival on Vip3A tissue increased as larvae developed to older instars. Additionally, we found that the estimated Vip3A exposure to 3<sup>rd</sup> and 5<sup>th</sup> instars consuming Vip3A-expressing corn tissues may not be high dose. Future efforts looking into the survival and development of older *S. albicosta* in integrated refuge scenarios will provide more insight into the effect of larval movement and cross-pollination. Additionally, the mode of
inheritance of Vip3A-resistance by *S. albicosta* requires further investigation. The decrease in susceptibility as *S. albicosta* larvae develop confirms that the high-dose model should be evaluated in terms of larval stage and exposure.
4 SUSCEPTIBILITY AND FIELD EXPOSURE OF STRIACOSTA ALBICOSTA (LEPIDOPTERA: NOCTUIDAE) LARVAE IN ONTARIO, CANADA TO FOUR INSECTICIDES

4.1 ABSTRACT

_Striacosta albicosta_ (Smith) (Lepidoptera: Noctuidae) has become the primary pest of corn in Ontario, Canada. In Ontario, management of _S. albicosta_ relies on foliar insecticides such as chlorantraniliprole and lambda-cyhalothrin with little attention paid to resistance management. Susceptibility of _S. albicosta_ to these and other active ingredients (methoxyfenozide and spinetoram) and the field-realistic exposure of _S. albicosta_ after insecticide application is unknown. The objective of this study was to generate dose-mortality responses for 1st and 3rd instar _S. albicosta_ in laboratory bioassays and to determine the concentration of insecticide active ingredients on corn tissues up to 14 days after application (DAA) in the field. First instar _S. albicosta_ were more susceptible to insecticides than 3rd instars. Of the four insecticides, 1st and 3rd instars were most susceptible to chlorantraniliprole and lambda-cyhalothrin, respectively. Additionally, lambda-cyhalothrin caused the greatest in the shortest amount of time relative to chlorantraniliprole, methoxyfenozide, and spinetoram. After field application, the highest and lowest concentrations of active ingredients were measured on leaf tissue and silk, respectively. The concentration of chlorantraniliprole exceeded the 1st and 3rd instar LC<sub>99</sub> up to 14 DAA on all tissues tested. The concentration of methoxyfenozide and spinetoram was higher than the 1st instar LC<sub>99</sub> up to 7 DAA. The concentration of chlorantraniliprole, lambda-cyhalothrin, and methoxyfenozide exceeded the 3rd instar LC<sub>99</sub> up to 7 DAA. All four active ingredients were effective against _S. albicosta_; however, insecticide persistence varied by corn tissue. An
insecticide with longer persistence may affect later instars but may have implications for the development of resistance compared to insecticides with short persistence if concentrations are below lethal concentrations.

4.2 INTRODUCTION

*Striacosta albicosta* (Smith) is a univoltine noctuid moth that has become a major pest of corn, *Zea mays* L., in the Great Lakes region (Smith et al. 2018b). *Striacosta albicosta* is native to the high plains of the United States (US), and was initially documented as a pest of dry beans, *Phaseolus* spp. (L.) (Crumb 1956, Hoerner 1958). More recently, *S. albicosta* began an eastward range expansion into Minnesota (O’Rourke and Hutchinson 2000) and South Dakota (Catangui and Berg 2006) in 2000, Illinois and Missouri in 2004 (Dorhout and Rice 2004, Rice et al. 2004), Wisconsin in 2005 (Cullen and Jyuotika 2008), and Indiana, Michigan, and Ohio in 2006 (Dorhout and Rice 2008, DiFonzo and Hammond 2008). By 2008, *S. albicosta* was documented in Ontario, Canada, and economic injury to corn was reported by 2010 (Smith et al. 2018b). It continued to expand its range, reaching the maritime provinces of Canada by 2018 (Pohl et al. 2019).

Infestation by one *S. albicosta* larva per corn plant can result in yield loss of 0.25 T ha\(^{-1}\) or more (Appel et al. 1993). *Striacosta albicosta* feeding injury to corn ears increases contamination by ear mold fungi that can produce mycotoxins harmful to humans and livestock (Eichenseer et al. 2008, Bowers et al. 2014, Parker et al. 2017, Smith et al. 2018a). Smith et al. (2018a) found the incidence of feeding injury by *S. albicosta* to increase contamination of grain corn with deoxynivalenol in Ontario.
The event MIR162, expressing the Bt vegetative insecticidal protein Vip3A, was found to have activity against *S. albicosta* larvae (Bowers et al. 2013, Smith et al. 2018a); however, with the documented rapid development of resistance to Cry1F by *S. albicosta*, deploying Vip3A as a single trait may lead to a similar outcome (Roush 1998). The current Ontario market adoption of Vip3A corn hybrids is low (M. Rice, personal communication) because of limited commercial availability of suitable hybrids. Corn producers in Ontario currently rely on foliar insecticide applications to minimize injury by *S. albicosta* and to reduce the risk of mycotoxin accumulation in grain corn.

Foliar insecticides are recommended to manage *S. albicosta* in Ontario when the percentage of plants with egg masses within a scouted area of a field exceeds the threshold of 5%, accumulated over several scouting events (OMAFRA 2017); however, in some cases, insecticide applications may be scheduled without scouting. Additionally, it is difficult to ensure that larvae will encounter a lethal dose of insecticide due to larval movement and protected feeding behaviour (Michel et al. 2010). Furthermore, to properly time insecticide applications, monitoring for adults and scouting for egg masses should be conducted, because insecticide applications are ineffective after larvae enter the ear (Michel et al. 2010).

Corn producers are encouraged to rotate insecticides with different modes of action against *S. albicosta* to avoid the development of resistance to a single insecticide class (Roush 1993). There are two strategies for using multiple modes of action for insecticides. The first is to mix more than one mode of action and apply them at the same time (Roush 1993, 1998). Mixing multiple products with different modes of action against *S. albicosta* at the same time can delay resistance by requiring the unlikely development of resistance to both modes of action.
simultaneously (Attique et al. 2006, Ahmad et al. 2009). The second is to rotate to a different mode of action between applications (Roush 1993, 1998). Rotating products across generations will also slow the development of resistance by removing continuous selection pressure. In the case of *S. albicosta*, a univoltine pest, one insecticide application is usually conducted per year (OMAFRA 2017, 2018); thus, rotating annually with products containing different modes of action would decrease selection pressure from repeated use of the same mode of action. In either case, it is important to know what the baseline susceptibility of target field populations is to an insecticide before it is widely used, so that shifts in susceptibility can be detected (Cook et al. 2004, Hardke et al. 2011). Ultimately, the goal of monitoring susceptibility to insecticides is to allow for early intervention in resistance development.

In Ontario, the most commonly used insecticides for the control of *S. albicosta* are a diamide, chlorantraniliprole, and a synthetic pyrethroid, lambda-cyhalothrin (Goudis et al. 2015). In addition, the diacylhydrazine and spinosyn insecticides, methoxyfenozide and spinetoram, respectively, have shown potential for the control of *S. albicosta* in dry edible beans and sweet corn (Trueman 2013, Goudis et al. 2015). Spinetoram has recently been added to the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) insecticide recommendations for *S. albicosta* management in corn (OMAFRA 2018). Chlorantraniliprole targets ryanodine receptors in insect muscles and results in the uncontrolled release and depletion of internal calcium (IRAC 2017). Lambda-cyhalothrin acts as a sodium channel modulator affecting nerve action (IRAC 2017). Methoxyfenozide is an ecdysone receptor agonist acting on insect growth regulators (IRAC 2017). Spinetoram is a nicotinic acetylcholine receptor allosteric activator that affects
nerve action (IRAC 2017). These four insecticides have both contact and ingestion activity against insect pests (Bloomquist 1996, Pineda et al. 2006, EPA 2008).

The effectiveness of the four insecticides against *S. albicosta* larvae when exposed, either through topical application or by ingestion, has been documented by Trueman (2013) and Goudis et al. (2015) on sweet corn and dry beans, respectively, but the baseline susceptibility of *S. albicosta* larvae to these insecticides is unknown. Furthermore, the concentration of each insecticide that *S. albicosta* is exposed to after field application is unknown. Complicating things further, *S. albicosta* larvae undergo intra-plant movement that may result in varying levels of exposure for different larval instars, as insecticide concentrations may vary on different corn tissues or due to insecticide degradation. For the most effective management, understanding the field-relevant exposure of *S. albicosta* will also be vital for the development of application and for resistance management recommendations.

The objective of the current study was to determine the susceptibility of *S. albicosta* larvae in Ontario, Canada to four insecticides and to compare the susceptibility of different larval instars to acute and residual insecticide concentrations found on corn tissues after field application. I hypothesize that *S. albicosta* larvae will be highly susceptible to these insecticides, because these insecticides have only recently been approved for use against *S. albicosta* in Ontario. Additionally, I hypothesize that the insecticide concentrations found on tissues after field applications will be lethal to *S. albicosta* larvae. The baseline data will serve as a reference for future insecticide susceptibility surveys, to confirm field efficacy, and to support insect resistance management (IRM) recommendations to reduce the risk of resistance development to insecticides used against *S. albicosta*.
4.3 MATERIALS AND METHODS

Two experiments were conducted to investigate the susceptibility and field-relevant exposure of *S. albicosta* larvae to insecticides. The first experiment was designed to determine the baseline susceptibility of 1st and 3rd instar *S. albicosta* to four insecticides used against this pest using an insecticide diet-overlay bioassay in the laboratory. The second experiment was conducted to measure the concentration of these insecticides using liquid-chromatography mass-spectrometry (LC-MS) on corn tissues collected from the field following foliar application to estimate field-relevant exposure of *S. albicosta*.

*Insecticides.* Commercial insecticide formulations used in the laboratory bioassays and field application experiments included chlorantraniliprole at 200 g L\(^{-1}\) (DuPont\textsuperscript{TM} Coragen\textsuperscript{TM} Insecticide; E. I. Dupont Canada Company, Mississauga, ON), lambda-cyhalothrin at 120 g L\(^{-1}\) (Matador\textsuperscript{®} 120 EC; Syngenta Canada Inc., Guelph, ON), methoxyfenozide at 240 g L\(^{-1}\) (Intrepid\textsuperscript{™} SC; Dow AgroSciences, Calgary, AB), and spinetoram at 250 g L\(^{-1}\) (Delegate\textsuperscript{®} WG; Dow AgroSciences, Calgary, AB). Field application experiments also included commercial insecticide mixtures of chlorantraniliprole and lambda-cyhalothrin at 150 g a.i. L\(^{-1}\) (Voliam Xpress\textsuperscript{™} SC; Syngenta Canada Inc., Guelph, ON) and methoxyfenozide and spinetoram at 360 g a.i. L\(^{-1}\) (Intrepid Edge\textsuperscript{®} SC; Dow AgroSciences, Calgary, AB).

4.3.1 Laboratory Bioassay

*Insect rearing.* Collections of *S. albicosta* were made during the 2017 growing season from commercial corn fields in Ontario, Canada. Corn leaves with egg masses were collected in the field and placed in coolers until returned to the laboratory. Egg masses were cut from a portion of the corn leaf and placed on moistened filter paper inside petri plates that were held
under rearing conditions at 26°C, 60-70% RH, and a photoperiod of 16:8 (L:D) h. Larvae were mass reared with artificial diet containing ~50 larvae until the desired instar was reached following the method described by Dyer et al. (2013) and modified by Farhan et al. (2017). Third instars were determined using body length and head capsule measurements as described by Dyer et al. (2013).

**Bioassays.** Diet for bioassays was prepared following the method described by Dyer et al. (2013) and Farhan et al. (2017). Diet was dispensed into 128-well bioassay trays (Bio-16, CD International, Pitman, NJ) using a repeater pipette; 1 mL of diet, weighing 0.987 g on average, was dispensed into each well. The resulting diet surface area was 2.0 cm². After the diet had solidified in the wells, adhesive ventilated covers were placed over the wells. Trays were stored at 4°C until required for bioassay use, at which point the trays were removed from the refrigerator and warmed to room temperature before application of the insecticide solution.

Formulated products were dissolved in distilled water to create stock solutions of 100 µg mL⁻¹ and serial dilutions were made to encompass a range of 0-100% mortality (Table 4.1). Each well was administered 30 µL of insecticide solution; control wells were treated with 30 µL of distilled water without any insecticides. After insecticide solutions were applied, trays were tilted in all directions to cover the entire surface area of the diet with the insecticide solution and then left in a fume hood until the solvent components of the solution evaporated.

One 1st (<12 h old) or 3rd instar larva was transferred to each well of the bioassay tray using a fine-point paintbrush and tray covers were replaced following larval introduction into wells. Infested trays were placed into a rearing room at 24°C, 60-70% RH, and a photoperiod of
16:8 (L: D) h. They were covered with cardboard to block overhead light, which helped to keep the larvae on the diet and prevented condensation build-up. For each concentration in a bioassay replicate, 24 larvae were treated. Bioassays were replicated three times.

Table 4.1: Concentration ranges used for insecticide diet-overlay concentration-response bioassays with *Striacosta albicosta* larvae from Ontario, Canada, 2017.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Active ingredient</th>
<th>Larval stage</th>
<th>Concentrations tested (ng ai g⁻¹ diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coragen™</td>
<td>Chlorantraniliprole</td>
<td>1ˢᵗ instar²</td>
<td>0.00, 0.28, 0.56, 1.12, 2.24, 4.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3ᵗᵈ instar</td>
<td>0.00, 0.82, 2.46, 7.38, 22.15, 66.44</td>
</tr>
<tr>
<td>Matador®</td>
<td>Lambda-cyhalothrin</td>
<td>1ˢᵗ instar</td>
<td>0.00, 0.04, 0.08, 0.16, 0.31, 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3ᵗᵈ instar</td>
<td>0.00, 0.39, 0.78, 1.56, 3.11, 6.22</td>
</tr>
<tr>
<td>Intrepid™</td>
<td>Methoxyfenozide</td>
<td>1ˢᵗ instar</td>
<td>0.00, 5.31, 10.61, 21.23, 42.45, 84.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3ᵗᵈ instar</td>
<td>0.00, 31.44, 94.33, 282.99, 848.96, 2546.88</td>
</tr>
<tr>
<td>Delegate®</td>
<td>Spinetoram</td>
<td>1ˢᵗ instar</td>
<td>0.00, 3.06, 6.11, 12.23, 24.45, 48.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3ᵗᵈ instar</td>
<td>0.00, 33.20, 99.61, 298.83, 896.50, 2689.51</td>
</tr>
</tbody>
</table>

¹Coragen™ (chlorantraniliprole, 200 g a.i. L⁻¹, E. I. Dupont Canada Company, Mississauga, ON); Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L⁻¹, Syngenta Canada Inc., Guelph, ON); Intrepid™ SC (methoxyfenozide, 240 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB); Delegate® WG (spinetoram, 250 g a.i. L⁻¹, Dow AgroSciences, Calgary, AB).

²<12 h old.

Bioassays were assessed for mortality at 24 h intervals until 168 h after introduction. Final mortality was recorded at 168 h after introduction. Larvae that were unresponsive to gentle prodding with a fine brush or weighed less than 10 mg were considered dead. The concentration of each insecticide that resulted in 50% growth inhibition was also determined (EC₅₀).
4.3.2 Field Experiment

A field experiment was conducted at a commercial farm near Thamesville, ON. Plots were planted on 18 May 2018 using hybrid HZ 3011A (Horizon Seeds, Courtland, ON, 3000 CHU) expressing Cry1Ab, with three replicates at a row spacing of 0.76 m and seeding rate of 79,000 seeds ha\(^{-1}\). Insecticide treatments were arranged in a randomized complete block design. Plots consisted of eight 18-m long rows separated by a 1.0-m non-planted buffer between each plot and 12.2-m planted buffer between replicates. Fertilizers and herbicides were applied according to OMAFRA (2017) recommendations.

Insecticides were applied using a high clearance, commercial John Deere 4730 sprayer (John Deere and Co., Moline, IL) equipped with a custom modified 18-m spray boom divided into five, independent 3.6-m-wide sections for spraying multiple plots per pass. The plot layout included non-plot areas of corn for sprayer traffic. Each section had its own pressurized tank using on-board compressed air and was equipped with ABJ100.2 bubblejet yellow nozzle tips (ABJ Agri Products, Brandon, MB) spaced 50 cm apart. Tip extensions (C.L. Benninger Equipment, Chatham-Kent, ON), approximately 40 cm long, were attached to the boom with a swivel connection allowing the tips to point downwards towards the canopy. The boom was positioned to just clear the tassels of the corn canopy, at approximately 280 cm above the ground, depending on the height of the corn. Insecticides were delivered in 200 L ha\(^{-1}\) of water at 330 kPa at a ground speed of 5.3 km h\(^{-1}\). Treatments were applied at the early R1 stage (~100% tassel and silk emergence) on 26 July 2018. Untreated plots were not sprayed with anything.

To determine the concentration of each insecticide on leaf, tassel, and silk tissues, samples were collected from 3 plants per plot at 1-3 h after insecticide application (HAA), 1, 7,
9, and 14 d after insecticide application (DAA). Tissue samples were collected 2 m into plots, from plants within the two centre rows (rows 4 and 5) of each plot at each time point. The top leaf closest to the tassel, the full tassel, and all exposed silk were cut and placed separately into individual bags and placed in ice-chilled coolers prior to transferring to the laboratory for processing. Samples were quantified within 8 h of collection using high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS) to determine the concentration of active ingredient (see supplemental information for methods). A 2.5-g sample of leaf, tassel or silk was used in the washing procedure (see supplemental information 7.3 and 7.4). The extract from the tissue wash was filtered and then put through the HPLC-MS/MS immediately or stored at 4°C until the machine could be used. Specific parameters were optimized for each insecticide (see supplemental information 7.3 and 7.4).

4.3.3 Data Analysis

Bioassays. Only bioassays with ≥75% survival in the control were retained for statistical analysis. Mortality data for the collections tested were analyzed to determine LC\(_{50}\), LC\(_{95}\), and LC\(_{99}\) values (concentration of insecticide that resulted in mortality of 50, 95, and 99% of the individuals in a collection, respectively) with 95% confidence intervals and the slope of the concentration-response curve using PROC PROBIT in SAS 9.4 (SAS Institute, Cary, NC). The OPTC option was used to correct for larval mortality in the control (SAS Institute 2008). To compare the relative susceptibility of 1\(^{st}\) and 3\(^{rd}\) instars to insecticides, the tolerance ratio was calculated from the LC\(_x\) value of 3\(^{rd}\) instars divided by the LC\(_x\) value of 1\(^{st}\) instars (Haung et al. 1999).
Average larval weights for each concentration were calculated from pooled larval weights divided by the initial number of larvae infested (Siegfried et al. 2005). Weights were then transformed to percent growth inhibition relative to the control and analyzed by non-linear regression (PROC NLIN, SAS 9.4) fitted to a probit model (SAS Institute 2008) as described by Marcon et al. (1999) to determine the EC$_{50}$ (effective concentration of protein at which larval growth was inhibited by 50%).

The effect of each a.i. concentration over time on larval mortality was analyzed using PROC GLIMMIX (SAS 9.4, SAS institute, Cary, NC). Mortality was corrected for control (no insecticide) mortality (Abbott 1922). A response surface analysis was conducted with a quadratic polynomial model with a logit link and a binomial distribution with the Laplace method used for integral approximation (Bowley 2015). Since binomial distribution is restricted to the range 0 < y < 1, the values of 0 and 1 for mortality were replaced with the values 0.0001 and 0.9999, respectively. In order to fit a repeated measure covariance structure, an additional random statement was included defining the R-side covariance structure with the random subject term replication. Heterogeneous autoregressive order 1 (arh(1)) covariance structure was selected over other covariance structure models based on having the lowest AICC fit statistic for non-Gaussian larval survival (Bowley 2015). Solution, which requests that a solution for the fixed-effects parameters be produced, and noint options, which requests that no common intercept be included in the fixed-effects model, were specified (Bowley 2015). Contrast statements were used to determine significance between insecticide surface responses for each instar. Lack of fit was not significant and was removed from the model for both instars. Predicted values were calculated over the range of levels tested and the inverse logit, $y = \exp()/(1+\exp())$, was used to convert
predicted values to data scale. Surface response analysis was not conducted for lambda-cyhalothrin tested against 1st instars, as mortality was observed within 24 h at the lowest rates tested.

Field experiment. Generalized linear mixed model (GLMM) analysis was conducted for insecticide concentrations using a split-plot-in-time repeated measures model in PROC GLIMMIX (SAS 9.4, SAS institute, Cary, NC). Fixed effects included tissue type and DAA; replication was considered a random effect for all variables. To fit a repeated measure covariance structure, an additional random statement was included defining the R-side covariance structure. The random subject term replication was included in the random statement and modeled within the spatial power (sp(pow)) covariance structure (selected due to unequal time spacing) (Bowley 2015). A dummy variable for time, named DAA1, was created to fit spatial power model requirements. Solution and noint options were specified to generate coefficients (Bowley 2015). Contrast statements were used to determine significance between insecticide concentrations on corn tissues for each insecticide. Lack of fit was not significant and was removed from the model for all insecticides. Predicted values were calculated over the range of time points tested.

To confirm generalized linear mixed model assumptions, scatterplots of studentized residuals were analyzed against fixed factors and predicted values and a Shapiro-Wilk test of normality was generated for laboratory bioassay and field experiment data (Bowley 2015). Outliers were detected after assessment of the studentized residuals in the field experiments but were not removed, as they did not significantly change the outcome of the analyses (Bowley 2015).
4.4 RESULTS

4.4.1 Laboratory Bioassay

Lethal concentrations for chlorantraniliprole, methoxyfenozoide, and spinetoram on 1\textsuperscript{st} instars, showed them to be more susceptible to chlorantraniliprole than to methoxyfenozoide or spinetoram (Table 4.2). The LC\textsubscript{50} value for methoxyfenozoide for 1\textsuperscript{st} instars was higher than for spinetoram; however, there was no difference between these two insecticides in LC\textsubscript{95} and LC\textsubscript{99} values (Table 4.2). The EC\textsubscript{50} value of chlorantraniliprole on neonates was lower than that of methoxyfenozoide and spinetoram (Table 4.2). Methoxyfenozoide and spinetoram EC\textsubscript{50} values did not differ (Table 4.2). Lethal concentration values for lambda-cyhalothrin on 1\textsuperscript{st} instars could not be estimated because 100\% mortality was observed at all concentrations tested.

Third instars were most susceptible to lambda-cyhalothrin, followed by chlorantraniliprole, methoxyfenozoide, and spinetoram; however LC\textsubscript{99} values for methoxyfenozoide and spinetoram for 3\textsuperscript{rd} instars were similar (Table 4.3). The EC\textsubscript{50} value of chlorantraniliprole for 3\textsuperscript{rd} instars was lower than that of methoxyfenozoide and spinetoram (Table 4.3). The EC\textsubscript{50} value of lambda-cyhalothrin for 3\textsuperscript{rd} instars did not differ from chlorantraniliprole, methoxyfenozoide, or spinetoram; however, the upper limit of the confidence interval was higher than the concentrations tested (Table 4.3). Methoxyfenozoide and spinetoram EC\textsubscript{50} values on 3\textsuperscript{rd} instars did not differ (Table 4.3).
Table 4.2: Susceptibility of 1\textsuperscript{st} instar (<12 h old) \textit{Striacosta albicosta} larvae reared from field collections in 2017 in Ontario, Canada to insecticides in terms of lethal concentration (LC) with 95% confidence intervals (CI) in brackets.

<table>
<thead>
<tr>
<th>Insecticide\textsuperscript{1}</th>
<th>n\textsuperscript{2}</th>
<th>Slope ± SE</th>
<th>LC\textsubscript{50} \textsuperscript{(5% CI)}</th>
<th>LC\textsubscript{95} (95% CI)</th>
<th>LC\textsubscript{99} (95% CI)</th>
<th>(\chi^2)</th>
<th>EC\textsubscript{50} (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>432</td>
<td>3.58 ± 0.418</td>
<td>0.84 (0.71-0.98)a\textsuperscript{4}</td>
<td>2.43 (1.99-3.23)a</td>
<td>3.77 (2.89-5.62)a</td>
<td>7.28\textsuperscript{5}</td>
<td>0.64 (0.59-69)a</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>144</td>
<td>.\textsuperscript{6}</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>432</td>
<td>3.56 ± 0.417</td>
<td>11.22 (9.43-12.97)c</td>
<td>32.47 (26.61-43.31)b</td>
<td>50.23 (38.65-75.53)b</td>
<td>6.80</td>
<td>11.40 (5.94-20.06)b</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>432</td>
<td>2.92 ± 0.373</td>
<td>5.97 (4.66-7.21)b</td>
<td>21.85 (17.24-31.05)b</td>
<td>37.39 (27.08-62.22)b</td>
<td>7.76</td>
<td>6.86 (1.81-20.16)b</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Coragen\textsuperscript{TM} (chlorantraniliprole, 200 g a.i. L\textsuperscript{-1}, E. I. Dupont Canada Company, Mississauga, ON); Matador\textsuperscript{®} 120 EC (lambda-cyhalothrin, 120 g a.i. L\textsuperscript{-1}, Syngenta Canada Inc., Guelph, ON); Intrepid\textsuperscript{TM} SC (methoxyfenozide, 240 g a.i. L\textsuperscript{-1} SC, Dow AgroSciences, Calgary, AB); Delegate\textsuperscript{®} WG (spinetoram, 250 g a.i. L\textsuperscript{-1}, Dow AgroSciences, Calgary, AB).

\textsuperscript{2} n=total number of larvae infested in bioassay.

\textsuperscript{3} ng ai g\textsuperscript{-1} diet.

\textsuperscript{4} Values followed by the same letter within columns are not significantly different based on overlapping 95% confidence intervals.

\textsuperscript{5} Value of the Chi-square goodness-of-fit test; * \(p<0.10\).

\textsuperscript{6} “ . “ not estimable (>1000) because there was 100% mortality at all dosages tested.
Table 4.3: Susceptibility of 3rd instar *Striacosta albicosta* larvae reared from field collections in 2017 in Ontario, Canada to insecticides in terms of lethal concentration (LC) with 95% confidence intervals (CI) in brackets.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>n</th>
<th>Slope ± SE</th>
<th>LC$_{50}$ (95% CI)</th>
<th>LC$_{95}$ (95% CI)</th>
<th>LC$_{99}$ (95% CI)</th>
<th>$\chi^2$</th>
<th>EC$_{50}$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>432</td>
<td>2.22 ± 0.242</td>
<td>2.59 (1.99-3.24)b</td>
<td>14.23 (10.39-22.14)b</td>
<td>28.83 (19.04-53.11)b</td>
<td>12.71$^3$</td>
<td>0.71 (0.64-0.77)b</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>432</td>
<td>2.37 ± 0.267</td>
<td>0.84 (0.66-1.02)a</td>
<td>4.13 (3.15-6.11)a</td>
<td>8.01 (5.52-14.03)a</td>
<td>5.70</td>
<td>0.41 (0.26-0.54)a</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>432</td>
<td>1.85 ± 0.249</td>
<td>67.59 (43.93-93.09)c</td>
<td>524.84 (356.34-941.35)c</td>
<td>1227.00 (724.93-2873.00)c</td>
<td>3.96</td>
<td>25.19 (15.39-34.01)c</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>432</td>
<td>1.88 ± 0.217</td>
<td>219.34 (156.57-d</td>
<td>1642.00 (1139.00-d</td>
<td>3782.00 (2319.00-d</td>
<td>3.05</td>
<td>48.28 (35.71-61.33)d</td>
</tr>
</tbody>
</table>

$^1$ Coragen™ (chlorantraniliprole, 200 g a.i. L$^{-1}$, E. I. Dupont Canada Company, Mississauga, ON); Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L$^{-1}$).

Syngenta Canada Inc., Guelph, ON); Intrepid™ SC (methoxyfenozide, 240 g a.i. L$^{-1}$ SC, Dow AgroSciences, Calgary, AB); Delegate® WG (spinetoram, 250 g a.i. L$^{-1}$, Dow AgroSciences, Calgary, AB).

$^2$ n=total number of larvae infested in bioassay.

$^3$ ng ai cm$^{-2}$.

$^4$ Values followed by the same letter within columns are not significantly different based on overlapping 95% confidence intervals.

$^5$ Value of the Chi-square goodness-of-fit test; * $p$<0.10.
First instars were more susceptible to chlorantraniliprole, methoxyfenozide, and spinetoram than were 3rd instars (Table 4.4). The greatest tolerance ratio was observed with spinetoram, where 3rd instars were 36.4-, 75.7-, and 102-fold more tolerant than 1st instars based on LC₅₀, LC₉₅, and LC₉₉ values. The tolerance ratio for lambda-cyhalothrin could not be estimated, as the susceptibility of 1st instars could not be determined (Table 4.4).

The total regression model for each insecticide and larval instar was significant \( (P = 0.0001) \). Regression models were significantly different between insecticides for both instars \( (P < 0.0001) \); however, models were not significantly different between methoxyfenozide and spinetoram for either 1st \( (P = 0.8942) \) or 3rd instars \( (P = 0.1158) \). First and 3rd instar mortality increased with exposure time and insecticide concentration (Fig. 4.1 and 4.2). For both larval stages, greater mortality was observed with lambda-cyhalothrin in the shortest amount of time compared to the other insecticides. For 1st instars, lambda-cyhalothrin caused 100% mortality at all test concentrations after 24 h of exposure. For 3rd instars, lambda-cyhalothrin caused 100% mortality at the top concentration after 24 h (Fig. 4.2).

### 4.4.2 Field Experiment

The regression equations generated for each insecticide for the three tissue types are presented in Fig. 4.3 and 4.4. Comparisons of the response patterns revealed that tissue types have different concentrations of insecticides over time (Fig 4.3 and 4.4). The highest concentration of insecticides was observed immediately after application for all three tissue types when applied alone and in a mixture (Fig 4.3 and 4.4).
Table 4.4: Tolerance ratio (TR) of 3rd instar Striacosta albicosta larvae compared to 1st instars reared from field collections in 2017 in Ontario, Canada to insecticides in terms of lethal concentration with 95% confidence intervals (CI) in brackets.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>TR$_{50}$ (95% CI)</th>
<th>TR$_{95}$ (95% CI)</th>
<th>TR$_{99}$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>3.1 (2.8-3.3)</td>
<td>5.9 (5.2-6.9)</td>
<td>7.6 (6.6-9.5)</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>. ^3</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>6.0 (4.7-7.2)</td>
<td>16.2 (13.4-21.7)</td>
<td>24.4 (18.8-38.0)</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>36.7 (33.6-40.1)</td>
<td>75.1 (66.1-89.3)</td>
<td>101.2 (85.6-127.0)</td>
</tr>
</tbody>
</table>

^1 Coragen™ (chlorantraniliprole, 200 g a.i. L$^{-1}$, E. I. Dupont Canada Company, Mississauga, ON);
Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L$^{-1}$, Syngenta Canada Inc., Guelph, ON); Intrepid™ SC (methoxyfenozide, 240 g a.i. L$^{-1}$ SC, Dow AgroSciences, Calgary, AB); Delegate® WG (spinetoram, 250 g a.i. L$^{-1}$, Dow AgroSciences, Calgary, AB).

^2 Tolerance ratio = (LC$_x$ value of 3rd instar larvae/LC$_x$ value of 1st instars).

^3 “.” not estimable because there was 100% mortality at all concentrations tested on 1st instars.

Chlorantraniliprole concentrations were lower on silk tissues relative to leaf tissues when applied alone ($F_{3,24} = 4.89, P = 0.0062$); however, concentrations of chlorantraniliprole when applied alone were not different between leaf and tassel tissue and silk ($F_{3,24} = 1.45, P = 0.2469$) and tassel tissues ($F_{3,24} = 1.52, P = 0.2278$) (Fig. 4.4). For Voliam Xpress™, concentrations of chlorantraniliprole were lower for silk relative to leaf ($F_{3,24} = 32.56, P <0.0001$) and tassel tissues ($F_{3,24} = 11.32, P <0.0001$) (Fig 4.3). Tassel tissue had lower concentrations of chlorantraniliprole relative to leaf tissue ($F_{3,24} = 7.44, P = 0.0006$; Fig. 4.4).
a) Chlorantraniliprole

b) Methoxyfenozide
c) Spinetoram

Figure 4.1: Surface response plots of the corrected mortality (%) of 1\textsuperscript{st} instar (<12 h old) Striacosta albicosta using diet-overlay bioassays at 24 h post-exposure intervals and concentrations (ng a.i. g\textsuperscript{-1} diet) of a) chlorantraniliprole (Coragen\textsuperscript{TM} (chlorantraniliprole, 200 g a.i. L\textsuperscript{-1}, E. I. Dupont Canada Company, Mississauga, ON)), b) methoxyfenozide (Intrepid\textsuperscript{TM} SC (methoxyfenozide, 240 g a.i. L\textsuperscript{-1} SC, Dow AgroSciences, Calgary, AB)), and c) spinetoram (Delegate\textsuperscript{®} WG (spinetoram, 250 g a.i. L\textsuperscript{-1}, Dow AgroSciences, Calgary, AB)).
a) Chlorantraniliprole

b) Lambda-cyhalothrin
c) Methoxyfenozide

![Graph showing corrected mortality over time and concentration for Methoxyfenozide](image)

- 75-100
- 50-75
- 25-50
- 0-25

Corrected mortality (%)

- Post-exposure (h)
- Concentration (ng a.i. g\(^{-1}\) diet)

---

d) Spinetoram

![Graph showing corrected mortality over time and concentration for Spinetoram](image)

- 75-100
- 50-75
- 25-50
- 0-25

Corrected mortality (%)

- Post-exposure intervals (h)
- Concentration (ng a.i. g\(^{-1}\) diet)
Figure 4.2: Surface response plots of the corrected mortality (%) of 3rd instar Striacosta albicosta using diet-overlay bioassays at 24 h post-exposure intervals and concentrations (ng a.i. g⁻¹ diet) of a) chlorantraniliprole (Coragen™ (chlorantraniliprole, 200 g a.i. L⁻¹, E. I. Dupont Canada Company, Mississauga, ON)), b) lambda-cyhalothrin (Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L⁻¹, Syngenta Canada Inc., Guelph, ON)) c) methoxyfenozide (Intrepid™ SC (methoxyfenozide, 240 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB)), and d) spinetoram (Delegate® WG (spinetoram, 250 g a.i. L⁻¹, Dow AgroSciences, Calgary, AB)).

Lambda-cyhalothrin concentrations were lower on silk ($F_{3,24} = 2.89, P = 0.0498$ alone; $F_{3,24} = 36.45, P < 0.0001$ in Voliam Xpress™) and tassel ($F_{3,24} = 5.34, P = 0.004$ alone; $F_{3,24} = 32.75, P < 0.0001$ in Voliam Xpress™) tissue relative to leaf tissues when applied alone and in a mixture (Fig. 4.3 and 4.4). Concentrations of lambda-cyhalothrin did not significantly differ between silk and tassel tissues when applied alone ($F_{3,24} = 0.38, P = 0.7713$) or in Voliam Xpress™ ($F_{3,24} = 0.12, P = 0.9448$).

Methoxyfenozide concentrations were lower on silk tissues relative to leaf tissues when applied alone ($F_{3,24} = 6.58, P = 0.0013$) or in Intrepid Edge® ($F_{3,24} = 10.98, P < 0.0001$). Concentrations of methoxyfenozide did not significantly differ between leaf and tassel when applied alone ($F_{3,24} = 1.32, P = 0.2835$) or in Intrepid Edge® ($F_{3,24} = 0.13, P = 0.9439$). Concentrations of methoxyfenozide were lower on silk relative to tassel tissue when applied in Intrepid Edge® ($F_{3,24} = 9.1, P = 0.0001$) but not when applied alone ($F_{3,24} = 2.09, P = 0.1202$).
a) Chlorantraniliprole

![Graph showing concentration over time for Chlorantraniliprole across different plant parts and stages.]

b) Lambda-cyhalothrin

![Graph showing concentration over time for Lambda-cyhalothrin across different plant parts and stages.]
c) Methoxyfenozide

[Graph showing the concentration of Methoxyfenozide over time for different parts of the plant: Leaf, Silk, Tassel, and 3rd instar LC99.]

---

d) Spinetoram

[Graph showing the concentration of Spinetoram over time for different parts of the plant: Leaf, Silk, Tassel, and 3rd instar LC99.]
Figure 4.3: Concentrations of a) chlorantraniliprole (Coragen™ (chlorantraniliprole, 200 g a.i. L\(^{-1}\), E. I. Dupont Canada Company, Mississauga, ON)) b) lambda-cyhalothrin (Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L\(^{-1}\), Syngenta Canada Inc., Guelph, ON)), c) methoxyfenozide (Intrepid™ SC (methoxyfenozide, 240 g a.i. L\(^{-1}\) SC, Dow AgroSciences, Calgary, AB)), and d) spinetoram (Delegate® WG (spinetoram, 250 g a.i. L\(^{-1}\), Dow AgroSciences, Calgary, AB)) insecticides measured on corn leaf, silk, and tassel tissues over time when applied alone using LCMS/MS following application in Thamesville, Ontario in 2018. Solid lines represent leaf tissue, dotted lines represent silk tissue, dashed lines represent tassel tissue, and dashed dotted lines represent 3\(^{rd}\) instar LC\(_{99}\) for each insecticide.

Spinetoram concentrations were lower on silk relative to leaf \((F_{3,24} = 5.4, P = 0.0038)\) alone; \(F_{3,24} = 7.61, P = 0.0005\) in Intrepid Edge®) and tassel \((F_{3,24} = 4.18, P = 0.0163\) alone; \(F_{3,24} = 6.54, P = 0.0013\) in Intrepid Edge®) tissue when applied alone or in Intrepid Edge® (Fig. 4.3 and 4.4). Concentrations of spinetoram did not significantly differ between leaf and tassel tissues when applied alone \((F_{3,24} = 0.66, P = 0.5796)\) or in Intrepid Edge® \((F_{3,24} = 0.04, P = 0.9882)\).

The concentration of chlorantraniliprole measured on the three tissues was higher than the LC\(_{99}\) values for 1\(^{st}\) and 3\(^{rd}\) instars at all time points measured when applied alone or in Voliam Xpress™ (Table 4.5). The highest concentration of chlorantraniliprole was measured on leaf tissue at 1-3 HAA and was over 1510 and 197 times higher than 1\(^{st}\) and 3\(^{rd}\) instar LC\(_{99}\) values, respectively. The lowest concentration of chlorantraniliprole was measured on tassel at 14 DAA and this was still over 8 times higher than 1\(^{st}\) instar LC\(_{99}\) and equal to 3\(^{rd}\) instar LC\(_{99}\) values. The concentration of chlorantraniliprole measured on the three tissue types at all time points exceeded the EC\(_{50}\) values by over 107 times for 1\(^{st}\) and 3\(^{rd}\) instar \(S.\ albicosta\).
a) Chlorantraniliprole

b) Lambda-cyhalothrin
c) Methoxyfenozide

![Graph of Methoxyfenozide concentration over time]

- Leaf
- Silk
- Tassel
- 3rd instar LC99

b) Spinetoram

![Graph of Spinetoram concentration over time]

Days after application
Figure 4.4: Concentrations of a) chlorantraniliprole and b) lambda-cyhalothrin (Voliam Xpress™ SC (chlorantraniliprole + lambda-cyhalothrin 150 g a.i. L⁻¹ Sus, Syngenta Canada Inc., Guelph, ON)), and c) methoxyfenozide and d) spinetoram (Intrepid Edge® SC (methoxyfenozide + spinetoram 360 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB)) measured on corn leaf, silk, and tassel tissues over time when applied in a mixture using LCMS/MS following application in Thamesville, Ontario in 2018. Solid lines represent leaf tissue, dotted lines represent silk tissue, dashed lines represent tassel tissue, and dashed dotted lines represent 3rd instar LC₉₉ for each insecticide.

A lethal concentration value could not be estimated for 1st instars with lambda-cyhalothrin because the lowest concentration tested caused 100% mortality (Table 4.5). The concentration of lambda-cyhalothrin measured on the three corn tissues exceeded the 3rd instar LC₉₉ value by over 6 times up to 7 DAA (Table 4.5). The concentration of lambda-cyhalothrin measured on the three tissues exceeded the EC₅₀ value for 3rd instars up to 14 DAA, except for silk tissues at 7 and 14 DAA when applied alone in the Matador® product and leaf and silk tissues at 14 DAA when applied in the pre-mixed Voliam Xpress™ product.

The concentrations of methoxyfenozide measured on the three tissues were over 12 times higher than the 1st instar LC₉₉ value at all measured time points (Table 4.5). The concentrations of methoxyfenozide measured on all three tissues were equal to the 3rd instar LC₉₉ value up to 14 DAA (Table 4.5). The concentrations of methoxyfenozide measured on the three tissues at all time points exceeded the EC₅₀ values by over 6 times for 1st and 3rd instars.
Table 4.5: Ratio of insecticide concentration to LC$_{99}$ of 1$^{st}$ and 3$^{rd}$ instar Striacosta albicosta on corn leaf, silk, and tassel tissues over three time points.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Tissue</th>
<th>1$^{st}$ instars</th>
<th>3$^{rd}$ instars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-3 HAA</td>
<td>7 DAA</td>
</tr>
<tr>
<td>Chlorantraniliprole$^3$</td>
<td>Leaf</td>
<td>937</td>
<td>493</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>211</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>650</td>
<td>282</td>
</tr>
<tr>
<td>Lambda-cyhalothrin$^3$</td>
<td>Leaf</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Methoxyfenozide$^3$</td>
<td>Leaf</td>
<td>191</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>121</td>
<td>38</td>
</tr>
<tr>
<td>Spinetoram$^3$</td>
<td>Leaf</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>Chlorantraniliprole$^4$</td>
<td>Leaf</td>
<td>1510</td>
<td>556</td>
</tr>
<tr>
<td></td>
<td>Silk</td>
<td>114</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>Tassel</td>
<td>954</td>
<td>359</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>Silk</td>
<td>Tassel</td>
</tr>
<tr>
<td>---------------</td>
<td>------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>.</td>
<td>.</td>
<td>.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>167</td>
<td>47</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinetoram</td>
<td>42</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Concentration of insecticide / LC₉₀ of 1st or 3rd instars

2 <12 h old.

3 Coragen™ (chlorantraniliprole, 200 g a.i. L⁻¹, E. I. Dupont Canada Company, Mississauga, ON); Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L⁻¹, Syngenta Canada Inc., Guelph, ON); Intrepid™ SC (methoxyfenozide, 240 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB); Delegate® WG (spinetoram, 250 g a.i. L⁻¹, Dow AgroSciences, Calgary, AB);

4 Voliam Xpress™ SC (chlorantraniliprole + lambda-cyhalothrin 150 g a.i. L⁻¹ Sus, Syngenta Canada Inc., Guelph, ON); Intrepid Edge® SC (methoxyfenozide + spinetoram 360 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB).

5 “.” not estimable (>1000) because there was 100% mortality at all dosages tested.
4.5 DISCUSSION

This study provides the basic tools by which the potential for development of resistance can be monitored. We determined the baseline susceptibility for the four main insecticides currently used to control *S. albicosta* in Ontario. For all insecticides, the recommended field application rates would prove lethal to 1\textsuperscript{st} instars as determined by lethal concentration estimates determined in the laboratory assays. However, there are some nuances when it comes to each insecticide regarding persistence and efficacy against later instars.

Differences in persistence were observed among the insecticides applied in field experiments. Factors that influence persistence will affect active ingredients differently (Edwards 1975). Factors that influence the persistence of insecticides include insecticide stability, volatility, solubility, and formulation (Edwards 1975). Additionally, environmental factors such as temperature, precipitation, humidity, and air movement can also affect the persistence of insecticides (Edwards 1975). Chlorantraniliprole persisted on corn tissue for 14 DAA at levels that exceeded the LC\textsubscript{99} of 3\textsuperscript{rd} instars, while lambda-cyhalothrin was below the level of detection by 8 DAA, if not earlier. ALL insecticide concentrations were reduced by 0-40\% at 1 DAA and by 50-99\% at 7 DAA. The variation in persistence of each insecticide on corn tissues has ramifications for both insect control and resistance management. Short insecticide persistence may be problematic for management of *S. albicosta* if the insecticide application is not synchronized with egg hatch and early larval movement. However, in the case of *S. albicosta*, synchronizing insecticide application with egg hatch is difficult as oviposition may occur from late June into early August (Douglass et al. 1957, Smith et al. 2018b). Additionally, persistence of insecticides may expose larvae to low doses of the insecticides, allowing for a greater chance
of resistance to develop (Tabashnik et al. 2004). Spinetoram concentrations at all time points and on all tissues were below the LC$_{99}$ of 3$^{rd}$ instars, while, lambda-cyhalothrin and methoxyfenozide concentrations past 7 DAA were below the LC$_{99}$ of 3$^{rd}$ instars.

Variability in insecticide concentrations among corn tissues may also influence insect control and resistance management. The highest concentrations of insecticides were measured on leaf tissue, which $S.$ $albicosta$ do not ingest and after hatching from the egg mass, larvae are infrequently found on corn leaves (Paula-Moraes et al. 2012). For all insecticides, the concentrations on leaf tissues were over 60 and 6% higher than on silk and tassel tissues, respectively. This intra-plant variation in concentration can expose larvae to lower concentrations of insecticides because $S.$ $albicosta$ larvae display intra-plant movement, with feeding preferences for silk and tassel over leaf tissues (Paula-Moraes et al. 2012). In addition to surviving larvae causing yield damage and mycotoxin contamination, this variation in concentration could expose larvae to sublethal doses of insecticides, and may allow for resistance to develop (Tabashnik et al. 2004). Perhaps the importance of the arena of exposure represented by silks has been underestimated in the management of $S.$ $albicosta$ and more attention to placement and persistence of insecticides in this arena needs to be considered.

Currently in Ontario, some corn producers tend to rely heavily on one insecticide product for their fields with little year-to-year product rotation. This continuous selection pressure can lead to insecticide resistance (McKenzie and Byford 1993, Zhao et al. 2010). Additionally, because of unclear commercial product naming, some producers may be unaware that they are applying continuous pressure on the insect population with the use of the same active ingredient. Some corn producers also apply insecticides without scouting for eggs in their field. This is
problematic as some producers may be applying insecticides when it is not necessary to do so (field is below threshold) or out of synchrony with egg hatching.

Among the insecticides evaluated in this study, we are unaware of any reports of lower than expected efficacy against *S. albicosta*. However, in a study surveying current management practices used for *S. albicosta* in Nebraska, one-third of respondents reported a decrease in efficacy against *S. albicosta* by pyrethroid insecticides (Archibald et al. 2017). Over 80% of survey respondents reported using pyrethroid insecticides with bifenthrin and/or zeta-cypermethrin as active ingredients. However, no evidence of a decrease in insecticidal efficacy has been documented in Nebraska (Montezano et al. 2016, 2017a,b). With survey reports of reduced insecticidal efficacy, there is a need to diversify insecticides and modes of action in Nebraska to preserve the efficacy of insecticides against *S. albicosta* (Archibald et al. 2017). Our results show that *S. albicosta* in Ontario are highly susceptible to the pyrethroid lambda-cyhalothrin at both 1\textsuperscript{st} and 3\textsuperscript{rd} instars. However, individuals from insecticide resistant populations that may be in Nebraska or other parts of the U.S could migrate into the Great Lakes region.

Insecticide mixtures, containing two active ingredients, can delay resistance if resistance genes to each compound are independently inherited and initially rare in the population (Roush 1993). For resistance to develop when using insecticide mixtures, insects would have to evolve resistance to both modes of action in a mixture simultaneously. Additionally, combining insecticides could have synergistic effects, where the effective concentration of each insecticide might be lower when combined than when applied alone (Attique et al. 2006). Insecticidal synergism between organophosphate and pyrethroid compounds has been investigated and utilized against agricultural pests (Bynum et al. 1997, Attique et al. 2006, Ahmed et al. 2009).
Synergistic effects between the four insecticides in this study should be investigated, as insecticide mixtures, such as Voliam Xpress™ containing chlorantraniliprole and lambda-cyhalothrin, are available and used to manage *S. albicosta*. With the approval of spinetoram for the management of *S. albicosta*, we expect the insecticide mixture Intrepid Edge®, containing methoxyfenozide and spinetoram, will also be approved. Combining insecticides with varying persistence, like chlorantraniliprole and lambda-cyhalothrin, may protect crops by initially providing two modes of actions and providing prolonged protection against later egg hatching. Additionally, if synergistic effects occur between the combined insecticides, the effective concentration of each insecticide may be lower, which may result in lethal concentrations that would otherwise be below LC values. However, Roush (1998) argues that as a result of incomplete coverage and concentration decay, the mortality of susceptible larvae is rarely consistently high enough for insecticide mixtures to be effective (Roush 1998). Additionally, Roush (1993) argues that mixing insecticides with varying persistence negates the intent of using insecticide mixtures because the insecticide with longer persistence acts as a single insecticide once the other compound has dissipated.

Rotating insecticides may delay resistance by removing the continuous selection pressure that may occur with the use of a single insecticide. In the rotation strategy, treatments are applied at different times so insects resistant to one insecticide are killed by the next insecticide in the rotation (Roush 1993). McKenzie and Byford (1993) found rotation of insecticides better suppressed pest populations and slowed resistance development relative to insecticide mixtures; however, both rotation and insecticide mixtures were better at delaying resistance than repeated use of the same insecticide. Additionally, rotating insecticide mixtures such as Voliam Xpress™
and Intrepid Edge® might be a viable option for resistance management in Ontario, as each has two unique active ingredients with activity, and potential synergistic effects, against *S. albicosta*. However, *S. albicosta* is more susceptible to the individual active ingredients in Voliam Xpress™ than to those in Intrepid Edge®, which may impact the amount of *S. albicosta* related injury that may occur when using one mixture over the other.

Information on *S. albicosta* susceptibility to the different insecticides presented in this study is important for development of successful IPM and IRM recommendations. Due to the potential of *S. albicosta* to develop resistance to insecticides, it is necessary to establish a baseline susceptibility database before or soon after the introduction of an insecticide. Although there is currently no insecticide resistance management strategy for *S. albicosta*, reducing the insecticidal pressure on *S. albicosta* populations is a commonly proposed strategy to delay evolution of resistance. Corn producers can reduce selection pressure by using insecticides only when necessary, using insecticide mixtures rather than single insecticides (Attique et al. 2006, Ahmed et al. 2009), and rotating between insecticide technologies including insecticide mixtures and Vip3A (McKenzie and Byford 1993, Zhao et al. 2010).
5 DISCUSSION AND CONCLUSIONS

*Striacosta albicosta* has become one of the most important pests of corn in the Great Lakes region due to yield loss and the relationship between *S. albicosta* injury and mycotoxin contamination (Smith et al. 2018b). Current recommendations for the management of *S. albicosta* in Ontario include the use of transgenic corn expressing the *Bacillus thuringiensis* (Bt) vegetative insecticidal protein Vip3A and foliar insecticides such as chlorantraniliprole, lambda-cyhalothrin, and spinetoram (OMAFRA 2018). Field studies show that management of *S. albicosta* using Vip3A and foliar insecticides are promising (Bowers et al. 2013, 2014, Bernardi et al. 2014, Goudis et al. 2015, Smith et al. 2017); however, the susceptibility of *S. albicosta* to these technologies has not been assessed, and as such, monitoring for the development of resistance by *S. albicosta* to these technologies has not been initiated.

Transgenic corn expressing the Bt protein Cry1F was reported to provide control of *S. albicosta* larvae (Eichenseer et al. 2008), however, by 2012, resistance was reported in Ontario, Canada (Smith et al. 2017). Resistance is likely to develop with low larval susceptibility to Bt or foliar insecticides, when a high frequency of resistance alleles exists in the pest population, with larval movement, and when an inappropriate refuge strategy is used (Georghiou and Tayler 1986, Caprio and Sumerford 2000, Pannuti et al. 2016). Additionally, exposure to low dosage through cross-pollination, low protein expression, and low persistence in the environment can also influence the development of resistance (Mallet and Porter 1992, Caprio and Sumerford 2000). Furthermore, resistance to Bt and foliar insecticides can arise with high selection pressure brought on by continuous (no rotation) use of the same technology.
Susceptibility of *S. albicosta* in Ontario, Canada to Vip3A and foliar insecticides decreased with larval development. First instar *S. albicosta* were very susceptible to Vip3A protein and foliar insecticides. However, the susceptibility of older instars of *S. albicosta* to these technologies was lower than 1st instars. The decrease in susceptibility as larvae developed could be related to the increase in larval size, which may dilute the dosage allowing for higher tolerance (Huang et al. 1999) or quicker detoxification of the toxin (Yu 1983). Increase in *S. albicosta* larval size may not be the sole reason for the increase in tolerance to Vip3A, as there was no difference in Vip3A tolerance between 3rd and 5th instars, although 5th instars are approximately 2.5-times larger than 3rd instars (Dyer et al. 2013). The variability of susceptibility between the Ontario populations tested was relatively low, suggesting that alleles conferring resistance are currently rare in the population. However, the alleles conferring resistance to Vip3A are currently unknown and the mode of inheritance has not been determined. The mode of inheritance (dominance, recessive, co-dominance, etc) is an important aspect of the high-dose/refuge model of resistance management (Caprio and Sumerford 2000). In the high-dose refuge model, it is essential that the alleles conferring resistance be recessive so that heterozygotes, produced by the mating of resistant and susceptible individuals, are still exposed to lethal concentrations (Caprio and Sumerford 2000).

The estimated exposure of each *S. albicosta* instar to Vip3A and foliar insecticides varied greatly. First instar *S. albicosta* are likely to be exposed to high doses of Vip3A and foliar insecticides under field conditions, however, Vip3A-expressing corn and foliar insecticides may not meet the ‘high dose’ definition against older instars. Specific to Vip3A, corn tissue assays showed higher survival of later instars of *S. albicosta* on various corn tissues. Additionally,
developmental measures such as weight, body length, and head capsule width, of larvae that were exposed to Vip3A-expressing tissues did not differ from those exposed to the control; suggesting limited sub-lethal impact of exposure to low doses of Vip3A. The tissue assay experiment was a single choice experiment and results may vary with the addition of non-Vip3A food choices. The results of a choice based experiment might be more field applicable and may offer insight into larval food aversion with the expression of Vip3A or other Bt products (Stapel et al. 1998). Food aversion may have an effect on the development of resistance, as food aversion may be a form of behavioural resistance (Stapel et al. 1998). Effective concentrations of Vip3A protein or insecticides at which larval growth was inhibited, presented in chapters 2, 3, and 4, may provide some evidence of food aversion by *S. albicosta* larvae; however, avoidance behavior was not evaluated in this study. Additional research is required to assess food aversion by *S. albicosta* larvae in the presence of insecticides or Vip3A-expressing tissues with the use of choice based experiments.

Specific to foliar insecticides, the persistence of each insecticide in the field after application varied greatly. Variation in persistence may be problematic for management of *S. albicosta* if the insecticide application is not synchronized with egg hatch and early larval movement, as later instars are less susceptible to these technologies. However, synchronizing insecticide application with *S. albicosta* egg hatch is difficult as oviposition may occur from late June into early August (Douglass et al. 1957, Smith et al. 2018b). Additionally, the lack of insecticide persistence may expose larvae to low doses of the insecticides, allowing a greater chance for resistance to develop (Tabashnik et al. 2004). It is important to note that insecticide concentrations were measured on corn tissues, but the foliar insecticides applied, with the
exception of lambda-cyhalothrin, have some systemic activity (Carlson et al. 2001, Adams et al. 2016). The systemic activity of insecticides may have a greater effect on *S. albicosta* larvae when present in tissues consumed by larvae such as silk and tassel. The lethal concentrations tested in the laboratory measured the effect of both insecticide contact and consumption. Thus the tolerance ratios presented in chapter 4 may vary depending on larval consumption of corn tissues and extent of contact.

The integrated refuge strategy, where refuge seeds are combined in the same bag with traited corn, has been adopted for Vip3A-expressing corn. Integrated refuge can be problematic for resistance management of mobile insects such as *S. albicosta* (Mallet and Porter 1992, Pannuti et al. 2016). In an integrated refuge scenario, larvae developing on non-Bt corn may move to neighboring Bt corn when larvae are less susceptible to the Bt trait (Yang et al. 2014). *Striacosta albicosta* larvae display high degree of larval movement between plants and may move at later instars to Bt-corn when they are less susceptible to the Vip3A protein. Additionally, in an integrated refuge scenario, cross-polllination between the non-Bt and Bt trait is likely to occur (Mallet and Porter 1992). Cross-pollination can cause non-Bt plants to produce kernels that express Bt and vice versa (Mallet and Porter 1992). This is problematic, as larvae developing on non-Bt will then be exposed to Bt traits. Additionally, larvae on Bt corn may then be exposed to lower doses of the Bt protein. The larval movement of *S. albicosta*, the changes in susceptibility to Vip3A as larvae develop, and larval feeding on potentially cross-pollinated kernels suggest that structured rather than integrated refuge would be better for resistance management. Additional research is required to look into how cross-pollination in integrated
refuge with Vip3A-expressing corn affects the survival and movement of *S. albicosta* larvae in the field.

Corn producers in Ontario are aware of the need to control *S. albicosta* to reduce yield loss and mycotoxin contamination. Smith et al. (2018a) showed that the mere incidence of *S. albicosta* injury increases mycotoxin contamination; therefore it is important to corn producers to effectively reduce *S. albicosta* population size. This may require the adjustment of the current *S. albicosta* egg mass threshold for areas that are susceptible to ear mold infections. The tools studied here offer some control for 1st instar *S. albicosta*, but sufficient control may not be achieved with Vip3A and insecticides to prevent the minimal amount of damage by older instars of *S. albicosta* that can trigger mold infection. However, the effect of insecticide residues on larvae exposed to post-application tissues was not assessed in the current study. Such a study would provide greater insights into the efficacy of insecticides in reducing feeding damage.

Additionally, in field situations, it is difficult to determine whether larvae are able to move prior to accumulating a lethal exposure to insecticides and/or Vip, and whether there are other factors that may reduce or increase larval survival on insecticide-treated or Bt-expressing tissues. Unfortunately, due to limited oviposition in the field in 2018, it was not possible to determine field efficacy of the control methods discussed here. Field conditions, such as temperature, humidity, and other environmental factors may influence the effectiveness of control technologies (Iordanou and Watters 1969, Wang and Shen 2007, Athanassiou et al. 2008, De Silva et al. 2009). Additionally, determining the effect the control methods discussed here have on the relationship between *S. albicosta*, mycotoxin contamination, and yield loss would provide valuable information on which to base control technology recommendations. Limited
management options and divergence from appropriate resistance management strategies, such as the application of insecticides without scouting, leads to concerns over the development of resistance by *S. albicosta*. Resistance can be managed with the development of an appropriate insecticide resistance management strategy for *S. albicosta* that is actively utilized by corn producers.

The findings in my thesis provide baseline susceptibility data of *S. albicosta* to currently available management technologies. These data will assist monitoring efforts in determining shifts in *S. albicosta* susceptibilities. Monitoring of both insecticides and Vip3A will be crucial in the longevity of these technologies, as monitoring will allow for intervention when shifts in susceptibility become evident. In addition to monitoring for resistance, management strategies can be implemented by corn producers to reduce selection pressure on *S. albicosta*. Corn producers are encouraged to select hybrids that are less susceptible to ear mold; however, options for less ear mold susceptible hybrids are limited. When using Vip3A-expressing hybrids, structured rather than integrated refuge may be the best strategy for resistance management; however, corn producers are encouraged to follow bag tag instructions relating to refuge strategies. Corn producers are also encouraged to scout for *S. albicosta* egg masses and apply insecticides only when the threshold has been exceeded, apply insecticide mixtures with two insecticide active ingredients rather than single chemistries, and combine insecticides with fungicides to reduce risk of ear mold infection. Rotating technologies, including the rotation of Vip3A-expressing hybrids with insecticide mixtures, can contribute to the management of resistance. There are two currently available insecticide mixtures, with each having two insecticides with unique modes of action. Corn producers are encouraged to rotate between those
mixtures to reduce the chance of resistance developing. This strategy employs two resistance management techniques, technology mixtures and rotation, to delay resistance. An alternate approach is to rely on Vip3A as the foundational management tool for *S. albicosta*. Corn producers may then scout their fields for *S. albicosta* egg masses, and at a new and higher threshold, corn producers at an elevated risk for mycotoxin contamination may spray their field with an insecticide mixture.

Research into the synergistic effects of insecticide mixtures used in the management of *S. albicosta* is important for the development of insecticide recommendations. The four insecticides studied in this thesis come in two insecticide mixtures. The combinations in the insecticide mixtures may work additively or synergistically to reduce the lethal concentrations against *S. albicosta*. The lowered lethal concentrations may then expose larvae to lethal concentrations that would otherwise be below LC values; thus, insecticide mixtures may provide greater and potentially longer protection from *S. albicosta* larvae for corn producers. The findings in chapter 3 of this thesis have raised questions relating to the potential impact of decreased susceptibility to Vip3A protein as *S. albicosta* larvae develop in an integrated refuge system. Additional research into the survival and development of *S. albicosta* larvae in integrated refuge systems is important to determining the effect of larval movement and cross-pollination on the development of resistance. Additionally, the mode of inheritance of resistance by *S. albicosta* to Vip3A and foliar insecticides requires further investigation. A key element of the high dose/refuge strategy is that resistance is functionally recessive and the dose is high enough to kill the majority of heterozygotes (Caprio and Sumerford 2000). In scenarios where resistance is functionally dominant, the high dose would not kill heterozygotes. With a dominant resistance trait, random
mating between susceptible individuals that developed on refuge plants and resistant individuals produces heterozygotes that are functionally resistant to the Bt trait. Thus using a high dose/refuge strategy when resistance is functionally dominant may result in the development of resistance more quickly than when the trait is functionally recessive. Finally, monitoring susceptibility of *S. albicosta* to Vip3A and foliar insecticides is encouraged.
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7 SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION FOR CHAPTER 3

7.1 ARTIFICIAL DIET SELECTION FOR REARING

One artificial diet is described in the literature for rearing *S. albicosta* (Dyer et al. 2013). Two artificial diets have been reported for mass rearing of other Noctuidae: Shorey and Hale (1965) and McMorran (1965). The McMorran diet was developed for rearing spruce budworm, *Choristoneura fumiferana*, but has since been reported as a suitable diet for rearing 103 lepidopteran species (Hervet et al. 2016). The objective of this experiment was to compare the development of *S. albicosta* in the laboratory on these three artificial diets.

The diet in Dyer et al. (2013) is an amalgamation of two commercial formulations that are wheat germ, soy flour, and edible bean based. The McMorran (1965) diet is wheat germ based, and the Shorey and Hale (1965) diet is based on soaked edible beans. Diets were prepared as described in Dyer et al. (2013), McMorran (1965), and Shorey and Hale (1965) with slight modification. The McMorran (1965) and Shorey and Hale (1965) diets were modified by the addition of 28 g Fumagillin-B (Medivet Pharmaceuticals Ltd., High River, Alberta) (Dyer et al. 2013) and 4 mL of linseed oil (Sigma-Aldrich, St. Louis, Missouri) (Grisdale 1973), which are contained in the Dyer et al. (2013) formulation. Fifteen mL of each diet was poured into individual 30-mL translucent portion cups (Crawford Packaging, London, ON) using a repeater pipette. Diet-filled cups were cooled to room temperature and covered with a translucent plastic lid (Crawford Packaging, London, ON). A single 1st instar (<24 h old) was added to each cup for
a total of 30 individual larvae per diet. Three small pin-sized holes were added to each lid for ventilation. Infested cups were transferred to a rearing chamber held at 26±1°C, 60–70% RH. A light-weight cardboard cover was placed on top of the trays to block overhead light and prevent condensation buildup.

Daily observations of larval mortality were made. Prepupae were transferred to moistened vermiculite (Alltreat Farms, Arthur, ON) and monitored for pupation. Pupae were weighed, assessed for deformities, transferred back into moistened vermiculite, and monitored for adult eclosion. Analysis of variance was conducted on larval development time and pupal weight for the three diets using PROC ANOVA in SAS 9.4 (SAS Institute, Cary, NC). There was a significant difference in larval development time and pupal weight between diets, with larvae fed the Shorey and Hale (1965) diet having the shortest development time and heaviest pupae (Table S7.1). Survival and adult eclosion was highest on the Shorey and Hale (1965) diet, which also had the fewest pupal deformities. Therefore, the Shorey and Hale (1965) diet was selected for laboratory rearing of S. albicosta.
Table 7.1: The effect of artificial diet on larval survival, larval development time, pupal weight, pupal deformities, and adult eclosion of *Striacosta albicosta* under laboratory rearing conditions.

<table>
<thead>
<tr>
<th>Diet</th>
<th>n¹</th>
<th>Survival (%)²</th>
<th>Larval developmental time (d)³</th>
<th>Pupal weight (mg)</th>
<th>Pupal deformities (%)⁴</th>
<th>Adult eclosion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyer et al. (2013)</td>
<td>30</td>
<td>40</td>
<td>65.3 (1.93)b⁵</td>
<td>0.260 (0.0107)b</td>
<td>6.7</td>
<td>30</td>
</tr>
<tr>
<td>McMorran (1965)</td>
<td>30</td>
<td>53.3</td>
<td>61.9 (1.68)ab</td>
<td>0.284 (0.0093)ab</td>
<td>13.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Shorey &amp; Hale (1965)</td>
<td>30</td>
<td>100</td>
<td>59.1 (1.22)a</td>
<td>0.282 (0.0068)a</td>
<td>3.3</td>
<td>73.3</td>
</tr>
</tbody>
</table>

¹Larvae were reared from egg masses collected in corn fields in Middlesex County, Ontario in 2016 and tested at F₀.

²Survival = larvae that successfully reached pupation.

³Larval developmental time = time from experimental set-up to pupation.

⁴Pupal deformities – any irregularities in pupal form.

⁵Values followed by the same letter within columns are not significantly different according to Tukey (P = 0.05).
7.2 ARTIFICIAL DIET SELECTION FOR BIOASSAYS

An experiment was conducted to compare two artificial diets to select the optimal diet for use in diet-overlay bioassays to evaluate the susceptibility of different instars of *S. albidcosta* to Vip3A. The diets compared included that of Dyer et al. (2013) and Shorey and Hale (1965). The diet used in Dyer et al. (2013) was used previously to determine Cry1F and Vip3A susceptibility of 1st instar *S. albicosta* (Smith et al. 2017, Farhan et al. 2017), however, this diet is expensive to prepare (~500 CAD kg\(^{-1}\) or ~375 USD kg\(^{-1}\)). The Shorey and Hale (1965) diet was found to be suitable for rearing *S. albicosta* in a previous experiment and is a less expensive alternative to the Dyer diet. It was hypothesized that mortality and weight of *S. albicosta* larvae would not differ between the Dyer and Shorey and Hale diets in untreated bioassays.

Diet was dispensed into 128-well assay trays (Bio-16, CD International, Pitman, NJ) at a rate of 1 mL per well using a repeater pipette. Trays were allowed to cool to room temperature, covered with an adhesive ventilated cover (Bio-16, CD International, Pitman, NJ), and stored at 4°C for up to 2 wk before use. Twenty-four wells of each diet were overlaid with 30 µl of Triton buffer solution (0.1% Triton X-100 in water (Sigma Life Science, Oakville, ON)). To ensure uniform coverage of the diet surface, trays were tilted in several directions, after which, trays were placed under a fume hood at room temperature until the liquid component of the solution had evaporated from the surface.

Using a fine-tipped brush, each well was infested with one 1st instar (<24 h old) from the Middlesex collection. The adhesive covers were replaced following infesting, and trays were
held at 26±1°C, 60–70% RH. Trays were covered with light-weight cardboard paper to block overhead light, ensure larvae stayed on the diet surface, and prevent condensation buildup. Mortality and larval weights were recorded 7 d after larval introduction and larvae that were unresponsive to gentle prodding with a fine brush were considered dead. Bioassays were replicated three times. A t-test was conducted to compare the mortality and weight of larvae exposed to the two diets using PROC TTEST in SAS 9.4. There was no difference in larval survival or weight between diets (Table S7.2) and because the Shorey and Hale (1965) diet was less expensive, it was used for the diet-overlay bioassays in this study.

Table S7.2: The effect of artificial diet on mean mortality (%) and weight of 1st instar Striacosta albicosta1 (<24 h old) after seven days of feeding in diet bioassays.

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Mortality (%)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyer et al. (2013)</td>
<td>72</td>
<td>5.6 (3.67)a2</td>
<td>0.046 (0.0029)a</td>
</tr>
<tr>
<td>Shorey and Hale (1965)</td>
<td>72</td>
<td>5.6 (2.78)a</td>
<td>0.043 (0.0021)a</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.7273</td>
<td>0.6532</td>
</tr>
</tbody>
</table>

1 Larvae were reared from egg-masses collected in corn fields in Middlesex County, Ontario in 2016 and tested at F1.

2 Values followed by the same letter within columns are not significantly different according to t-test (P = 0.05).
SUPPLEMENTAL INFORMATION FOR CHAPTER 4

7.3 CHEMICAL REAGENTS AND EQUIPMENT

All reagents used in this study were HPLC grade or higher and, unless specified, obtained from Fisher Scientific (Oakville, ON, Canada). Acetonitrile (ACN) and methanol (MeOH) were obtained from J.T. Baker (Phillipsburg, NJ) and water was obtained from OmniSolv (Billerica, MA). Formic Acid was purchased from Fisher Scientific (Fairlawn, NJ). Methoxyfenozide and spinetoram technical grade standards were obtained from Dow AgroScience (Indianapolis, IN) while chlorantraniliprole and lambda-cyhalothrin were dissolved in ACN and purchased from Sigma Aldrich (Oakville, ON, Canada). Lambda-cyhalothrin D5 was dissolved in ACN and purchased from Sigma Aldrich (Oakville, ON, Canada). Two mL dSPE QuEChERS tubes containing 150 mg magnesium sulphate anhydrous, 50 mg primary secondary amine (PSA) and 50 mg C18 were purchased from Thermo Scientific (Waltham, MA). Magnesium sulphate anhydrous and crystalline sodium chloride was purchased from Fisher Scientific and sodium citrate tribasic and sodium hydrogen citrate sesquihydrate were both purchased from Sigma Aldrich.

Chemical analysis was performed using HPLC with ESI-MS/MS. An HTP Pal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 100 μL injection syringe linked to an 1100 Series HPLC unit with a G1316A thermostat column oven, G1312A binary pump, and G1322A degasser (Agilent Technologies, Santa Clara, CA) was used for HPLC separation. A PE SCIEX API 365 triple quadrupole mass spectrometer (AB SCIEX, Concord, ON, Canada) with an EP10 + detector modification (IONICS, Concord, ON, Canada) coupled
with a TurboIonSpray electrospray ion source (AB SCIEX) was used to perform mass spectrometry. A gas generator (>99% purity), using nitrogen, generated the curtain, drying and collision gases for the MS/MS analysis. Eluents A consisted of MeOH with 5 mM formic acid buffer while eluent B consisted of H$_2$O with 5 mM formic acid buffer.

Each 2.5 g tissue sample was placed into a centrifuge tube (Avantor, Allentown, PA) with 10 mL of Aquafina water (PepsiCo, Wichita, KS). Centrifuge tube lids were sealed with parafilm (Fisher Scientific, Ottawa, ON) and the sealed tubes were put on a shaker (Eberbach, Eberbach, Germany) for 90 min. After the shaker, 10 mL of acetyl-nitrate (ACN) and 2 mL of n-hexane were added to the tube and vortexed for 1 min. The tissue was then removed from the tube and a salt bag consisting of 4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic, and 0.5 g sodium hydrogen citrate sesquihydrate was added, and the tube was shaken immediately for 1 min. The tube was then centrifuged for 3 min at 3000 rpm in the Thermo IEC Centra CL2 centrifuge (Milford, MA). After centrifuging, 1 mL from the ACN layer (top layer) was taken and put into a QuEChERS tube (Sigma Aldrich, Oakville, ON) and vortexed for 1 min. The QuEChERS tube was centrifuged at 5000 rpm for 1 min in the Labnet Hermle Z180 centrifuge (Sigma Aldrich, Oakville, ON). After centrifuging, 400 µL of the extract from the QuEChERS tube were removed and put into a 5-mL test tube. Eight µL of lambda-cyhalothrin internal standard was added to the test tube. The solvent was dried in a Rapidvap Vertex Dry Evaporator (Labconco Corporation, Kansas City, MO) at 40°C with a gentle nitrogen stream. The dried extract was reconstituted in 400 µL of insecticide dilution solvent. The tube was sealed with parafilm and then vortexed for 2 min, after which, all the extract was removed from the tube and placed into a syringe with a filter tip. The extract was filtered through a 0.45-µm PTFE
syringe (Fisher Scientific, Ottawa, ON) into a 2-mL amber autosampler vial (Agilent, Mississauga, ON) with a 400-μL glass insert. The extract was put through the HPLC-MS/MS immediately or stored at 4°C until the machine could be used.

The HPLC used an injection volume of 50 μL to inject the sample aliquot into the system where it was eluted with a flow rate of 0.5 mL/min. A Gemini C18, 150 x 4.6 mm i.d., 5-μm column equipped with a 4 x 3-mm i.d. guard column of the same material (Phenomenex, Torrance, CA) was chosen for the elution. A 50:50 flow splitter diverted 25 μL of eluent to waste while the other 25 μL was received by the ESI-MS/MS. Elution began with 25% eluent A for 15 minutes were it was increased to 95% eluent A. After 19 min, the eluent composition was decreased back down to 25% eluent A for the remainder of the elution.

7.4 ANALYTICAL PERFORMANCE

Manual tuning for specific parameters for each insecticide were obtained by direct infusion of analytical standards (1 μg mL⁻¹ in PDS (50:50 v/v eluent A/B) solution) into the ESI-MS/MS at 10 μL min⁻¹ with a Fusion 100 infusion pump (Chemyx Inc., Stafford, TX) containing a 500 μL syringe (Gastight 1750, Hamilton, Reno, NV). Positive ion polarity mode was used to analyze each insecticide with a multiple reaction monitoring (MRM) procedure requiring one precursor ion (Q1) and two product ions (Q3). MRM parameters, declustering potential (DP), collision energy (CE) and cell exit potential (CXP), were optimized for each compound during injection (Table S7.1). The quantifier ion was identified as the product ion with the largest peak, while the qualifier ion was the other peak, which was used as confirmation. Instrument control, data collection, peak integration and parameter optimization were performed with Analyst 1.5
software (AB SCIEX, Concord, ON, Canada) and quantitation was done with MultiQuant 3.0.3 (AB SCIEX, Concord, ON, Canada). A matrix-matched calibration curve was used for each tissue including nine concentration levels between 31.3 and 1000 ng g\(^{-1}\) as well as a double blank (matrix extract) and a blank (matrix extract and internal standard). These curves were used to calculate the limit of detection (LOD) and the limit of quantification (LOQ) which were determined using the mean peak height detected using the mean height of the noise signal plus 3 and 10 times the standard deviation around the analyte retention time (Fig. S7.1).
Table S7.3: Optimized LC-ESI-MS/MS parameters, recovery (%), and limits of detection (LOD) and quantification (LOD) of insecticides measured on corn leaf, tassel, and silk tissues in Thamesville, Ontario in 2018.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Retention time (min)</th>
<th>Precursor ion (m/z)</th>
<th>DP²</th>
<th>Product ion (m/z)</th>
<th>CE⁴</th>
<th>CX⁵</th>
<th>Recovery (% ± s.d.)</th>
<th>LOD ng g⁻¹ (± s.d.)</th>
<th>LOD ng g⁻¹ (± s.d.)</th>
<th>LOD ng g⁻¹ (± s.d.)</th>
<th>LOD ng g⁻¹ (± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>15.5</td>
<td>484.1 [M - H]⁺</td>
<td>285.9/45</td>
<td>22/2</td>
<td>28/3</td>
<td>111.2</td>
<td>0.22</td>
<td>0.21</td>
<td>0.30</td>
<td>0.61</td>
<td>0.53</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>19.5</td>
<td>467.1 [M + NH₄]⁺</td>
<td>225.0/18</td>
<td>21/4</td>
<td>20/2</td>
<td>114.7</td>
<td>0.12</td>
<td>0.13</td>
<td>0.04</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>16.4</td>
<td>369.2 [M - H]⁺</td>
<td>149.0/13</td>
<td>22/3</td>
<td>13/1</td>
<td>75.4</td>
<td>1.02</td>
<td>1.13</td>
<td>1.78</td>
<td>2.98</td>
<td>3.18</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>12.9</td>
<td>748.5 [M - H]⁺</td>
<td>142.2/20</td>
<td>45/4</td>
<td>16/2</td>
<td>105.3</td>
<td>0.31</td>
<td>0.44</td>
<td>0.40</td>
<td>0.83</td>
<td>1.14</td>
</tr>
</tbody>
</table>

¹ Coragen™ (chlorantraniliprole, 200 g a.i. L⁻¹, E. I. Dupont Canada Company, Mississauga, ON); Matador® 120 EC (lambda-cyhalothrin, 120 g a.i. L⁻¹, Syngenta Canada Inc., Guelph, ON); Intrepid™ SC (methoxyfenozide, 240 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB); Delegate® WG (spinetoram, 250 g a.i. L⁻¹, Dow AgroSciences, Calgary, AB); Voliam Xpress™ SC (chlorantraniliprole + lambda-cyhalothrin 150 g a.i. L⁻¹ Sus, Syngenta Canada Inc., Guelph, ON); Intrepid Edge® SC (methoxyfenozide + spinetoram 360 g a.i. L⁻¹ SC, Dow AgroSciences, Calgary, AB).

² Declustering potential.
³ Quantifier/qualifier ions.
⁴ Collision energy.
⁵ Cell exit potential.
Figure S7.1: Intensity chromatograms of chlorantraniliprole (a), lambda-cyhalothrin (b), methoxyfenozide (c), and spinetoram in standard solution (1 μg mL$^{-1}$).