Effect of Cry1F maize on the behavior of susceptible and resistant *Spodoptera frugiperda* and *Ostrinia nubilalis*

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Abstract

Understanding the behavior of pests targeted with *Bacillus thuringiensis* Berliner (Bt) crops is important to define resistance management strategies. Particularly the study of larval movement between plants is important to determine the feasibility of refuge configurations. Exposure to Bt maize, *Zea mays* L. (Poaceae), has been suggested to increase larval movement in lepidopteran species but few studies have examined the potential for resistance to interact with behavioral responses to Bt toxins. Choice and no-choice experiments were conducted with *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) to determine whether Cry1F resistance influences neonate movement. Leaf discs of Cry1F maize and the corresponding isoline were used to characterize behavioral responses. In both experiments, the location (on or off of plant tissues) and mortality of susceptible and Cry1F resistant neonates was recorded for 5 days, but the analysis of larvae location was performed until 7 h. Our results indicated no strong difference between resistant and susceptible phenotypes in *S. frugiperda* and *O. nubilalis*, although a small percentage of susceptible neonates in both species abandoned maize tissue expressing Cry1F. However, significant behavioral differences were observed between species. *Ostrinia nubilalis* exhibited increased movement between leaf discs, whereas *S. frugiperda* selected plant tissue within the first 30 min and remained on the chosen plant regardless of the presence of Cry1F. *Spodoptera frugiperda* reduced larval movement may have implications to refuge configuration. This study represents the first step toward understanding the effects of Cry1F resistance on Lepidoptera larval behavior. Information regarding behavioral differences between species could aid in developing better and more flexible resistance management strategies.

Keywords: fall armyworm, European corn borer, transgenic maize, refuge configuration, Cry1F resistance, resistance management, *Bacillus thuringiensis*, Lepidoptera, Noctuidae, Crambidae

Introduction

The United States Environmental Protection Agency (USEPA) has mandated the high-dose/refuge strategy (HDR) to delay the evolution of insect resistance to *Bacillus thuringiensis* Berliner (Bt) crops (USEPA, 2001). The HDR strategy requires farmers to plant refuges of non-Bt crops to promote the survival of susceptible insects. Refuges allow susceptible insects to survive and to outnumber the few resistant individuals that could survive in Bt crop fields, thereby reducing the probability that resistant insects will mate with each other. If resistance is recessive, mating between susceptible and resistant adults will produce heterozygous offspring that cannot survive on Bt plants because of the high-dose expression. Consequently, the frequency of resistant individuals will be greatly reduced (Bates et al., 2005).

To facilitate mating between resistant and susceptible insects, several refuge strategies have been considered, including blocks, strips, or seed mixtures (Bates et al., 2005). Onstad et al. (2011) suggested that neither blocks nor mixtures are clearly superior. Seed mixtures generate greater adoption by farmers, but make monitoring more difficult and may increase the risk of resistance because of larval movement between expressing and non-expressing plants.

On the other hand, block refuges assist delaying resistance, but have the disadvantage of refuge compliance by farmers (Onstad et al., 2011). Current Lepidoptera insecticide resistance management (IRM) strategies mainly involve blocks and in-field strips for events expressing one toxin (USEPA, 2001; Onstad et al., 2011) and for some pyramided events expressing more than one toxin, the USEPA has approved the use of seed mixtures with activity against Lepidoptera (USEPA, 2010). However, IRM practices should not be expected to be suitable for all species and each species must be considered independently (USEPA, 1998; Onstad et al., 2011). Thus, specific information about the behavior of multiple pests is important to appropriately define IRM plans. Particularly, the study of adult and larval movement is important to better estimate the durability of various refuge configurations (Ross & Ostlie, 1990).

Current refuge strategies are based on studies indicating larval movement from plant to plant (Mallet & Porter, 1992). Behavioral studies of lepidopteran larvae have shown that exposure to Bt toxins present in maize, *Zea mays* L. (Poaceae), seems to increase the likelihood of larvae moving between plants (Ross & Ostlie, 1990; Razze & Mason, 2012). Movement may expose larvae to a lower dose of Bt toxins increasing the likelihood of heterozygote survival and potentially accelerating the evolution of resistance (Mallet & Porter, 1992; USEPA, 1998). This behavioral response has been reported in lepidopteran species feeding on Bt plants including European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) (Davis & Coleman, 1997; Davis & Onstad, 2000; Razze & Mason, 2012), diamondback moth, *Plutella xylostella* (L.) (Ramachandran et al., 1998), tobacco budworm, *Heliothis virescens* (Fabricius) (Parker & Luttrell, 1999), cabbage looper, *Trichoplusia ni* (Hübner) (Li et al., 2006), and fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Binning et al., 2014). Similar results were found in the beet armyworm, *Spodoptera exigua* (Hübner) (Berdegue et al., 1996), and the light brown apple moth, *Epiphyas postvittana* (Walker) (Harris et al., 1997) exposed to Cry toxins in the laboratory.

Relatively few studies have evaluated differences between susceptible and resistant insects feeding on Bt toxins or Bt-expressing plant tissue. The results of these studies differ depending on the species and Bt toxin tested. For example, a study with a susceptible and a toxin-adapted strain of *H. virescens* indicated that both strains avoided moderate and high concentrations of diet treated with Dipel 2X (Gould & Anderson, 1991). Moreover, a study with a susceptible and Dipel-resistant strain of *O. nubilalis* showed that the resistant strain appeared to avoid Dipel-treated diet more than the susceptible strain (Huang et al., 2001). Berdegue et al. (1996) reported that resistant *S. exigua* larvae feeding on Cry1C-treated and untreated diet were more active than susceptible larvae. In contrast, experiments with *O. nubilalis* indicated that Cry1Ab-resistant larvae were more likely than susceptible larvae to be found on Cry1Ab expressing maize leaf tissue (Prasifka et al., 2009). Video-tracking of *O. nubilalis* neonates indicated reduced movement and increased local searching with Cry1Ab-resistant neonates when exposed to Cry1Ab maize tissue compared to susceptible larvae (Prasifka et al., 2009). Based on these results, it was predicted that resistant larvae are more likely to disperse onto adjacent non-Bt plants, although such behavior may reflect greater survival after toxin exposure for resistant larvae rather than increased activity (Prasifka et al., 2010).

The objectives of this study were to determine whether resistance influences larval movement on Cry1F maize and compare the behavior of two Lepidoptera species that exhibit Cry1F resistance. *Spodoptera frugiperda* is an important pest of maize in the Tropics and throughout the USA as a late-season pest in late-planted crops (Buntin, 1986; Mitchell et al., 1991). *Spodoptera frugiperda* has been controlled with maize hybrids containing Cry1F since 2003. However, in 2006 field resistance to Cry1F maize was reported in Puerto Rico (Matten et al., 2008; Tabashnik et al., 2009). Moreover, Cry1F resistance in *O. nubilalis* has not been reported in the field but a laboratory-selected strain has been previously generated and characterized (Pereira et al., 2008a). Cry1F resistance in both species is similarly inherited and has been identified as recessive, autosomal, and conferred by a single locus (Pereira et al., 2008b; Storer et al., 2010; Velez et al., 2013). The availability of *S. frugiperda* and *O. nubilalis* Cry1F resistant strains allowed to perform choice and no-choice tests to investigate the possible effect of Cry1F resistance on the behavior of larvae. Results of this study will help promote improved IRM strategies based on increased understanding of larval movement.

Materials and methods

Insect strains and plant material

Dupont Pioneer (Johnston, IA, USA) generated the Cry1F-selected *S. frugiperda* strain from several hundred field-collected fall armyworm egg masses from Puerto Rico maize fields during October 2008 and January 2009. Neonates were exposed to Cry1F maize leaf discs and larvae that survived 4-day exposure were used to establish the fall armyworm Cry1F-selected strain (Velez et al., 2013). *Spodoptera frugiperda* susceptible strain was purchased from BioServ (Frenchtown, NJ, USA). Both strains were maintained using standard rearing techniques (Perkins, 1979) with slight modifications. The resistant *O. nubilalis* strain originated from insects collected throughout

the central US Corn Belt in 1996 and has been maintained in the laboratory with repeated exposure to a diagnostic concentration of Cry1F that allows only resistant homozygous individuals to survive (Pereira et al., 2008a). The susceptible *O. nubilalis* bivoltine E strain was generated in 1985 from field-collected insects near Geneva, NY, USA, and was augmented with additional field collections in 1996. *Ostrinia nubilalis* strains were maintained using standard rearing techniques (Lewis & Lynch, 1968) with slight modifications (Siqueira et al., 2004).

Resistant and susceptible phenotypes were compared in each experiment. The susceptible *S. frugiperda* phenotype consisted of heterozygous individuals originated from the F1 progeny of the parental susceptible and resistant strains. *Ostrinia nubilalis* susceptible phenotype consisted of a mixture of homozygous susceptible with a low frequency of heterozygous individuals. Cry1F resistance in both species has been characterized as completely recessive, autosomal, and conferred by a single locus; therefore, heterozygous individuals are considered functionally susceptible (Pereira et al., 2008b; Velez et al., 2013). Heterozygous susceptible phenotypes were used because the homozygous susceptible *S. frugiperda* strain avoided leaf tissue within the first hours of the experiment, which was considered as an artifact of the continuous rearing on artificial diet. *Spodoptera frugiperda* heterozygous individuals were generated by mass crossing the homozygous susceptible and homozygous resistant strains; for this purpose, pupae from the resistant and susceptible strains were separated by sex based on morphological differences in genitalia and upon emergence (Heinrich, 1919; Capinera, 2000), virgin males and females were crossed with the opposite sex from each strain.

Bioassays on artificial diet were performed before each experiment to confirm susceptible and resistant phenotypes using the methodology described by Velez et al. (2013). Neonates of each phenotype were bioassayed with a Cry1F diagnostic concentration of 200 ng cm^{-2} for *S. frugiperda* and 60 ng cm^{-2} for *O. nubilalis*. Cry1F concentrations were confirmed by SDS-PAGE/densitometry (Crespo et al., 2008). After the phenotypes were confirmed, neonates within 12 h of eclosion were used for the choice and no-choice experiments.

Plant material

Leaf discs of Cry1F maize (event TC1507) and the respective near isoline were used to identify behavioral responses. Plants were grown in the greenhouse and the plant tissue used for the experiments ranged betweenV7 andV9. Cry1F expression was confirmed using Bt1F trait check lateral flow test (Strategic Diagnostic, Newark, DE, USA) prior from initiating the experiments. For both tests (choice and no choice)

fresh leaves were collected to assure freshness of the tissue, and leaf discs were cut using a number 13 cork borer that generates 1.7-cm-diameter leaf discs.

Choice and no-choice tests

Choice tests were performed to test whether resistant and susceptible phenotypes discriminated between Cry1F maize and isoline plants, and to determine whether behavior was affected by exposure to Cry1F-expressing plants. Experiments were performed in 16-well trays (5.1 × 3.8 × 2.9 cm) covered with Breathe-Easy sealing membrane (Sigma-Aldrich, St. Louis, MO, USA); one tray represented one phenotype. Three replications were performed at different times for a total of 48 larvae per phenotype for the choice test and 24 larvae for the no-choice test. Replications were represented by randomly selecting neonates' eclosing from eggs laid on 3 days to account for differences among cohorts (Robertson et al., 1995).

To prevent leaf tissue degradation and microbial contamination, 15 ml of a solution consisting of 10 g ml^{-1} of agar, 0.3 g ml^{-1} of sorbic acid, and 1.7 g ml^{-1} of methyl paraben was dispensed in each well. Using a small spatula, the agar was scored and the leaf discs were positioned vertically in the agar. Cry1F maize and isoline leaf discs were placed in each well for the choice experiment, and a single plant tissue type was placed in each well for the no-choice experiment. Leaf discs were placed facing each other in the choice experiment with a distance between leaf discs of ca. 1.5 cm. The position of leaf discs was randomized using Proc RANK (SAS Institute, 2011). One larva per well was transferred in the agar with a fine paintbrush. Experiments were held at room temperature at 22 ± 2 °C, 30 ± 20% r.h., and L14:D10 photoperiod.

The position of the larvae and mortality was recorded for 5 days. Behavior was categorized in the following way for the choice experiment: (1) on Cry1F maize, (2) on isoline, (3) off plant, (4) dead, and (5) missing; for the no choice experiment: (1) on plant tissue, (2) off tissue, (3) dead, and (4) missing. Data were collected every 30 min for the first 7 h, and after the first day, data were collected 39a day for 5 days (111 h).

Data analysis

All analyses were performed using SAS software v.9.3 (SAS Institute, 2011). Mortality started occurring after 7 h and by 24 h the mortality of susceptible larvae ranged between 13 and 41% in the choice and no-choice experiment. Therefore, the position of the larvae was analyzed only for the first 7 h to avoid the confounding effects of mortality. Missing larvae was less than 1% for all experiments. Dead and missing larvae were excluded from the analysis. The

position of the larvae for the choice and no-choice experiment were converted to proportions by generating two time points for each larvae: the first consisted of the proportion of larval location from 30 min to 3 h, and the second included the proportion of larval location from 4 to 7 h. Proportions of larvae in the different positions were transformed to arcsine $x^{1/2}$ (Martin & Bateson, 2007; Prasifka et al., 2009) prior to analysis. Both experiments were analyzed independently for each species using a repeated measures ANOVA with the Proc GLIMMIX procedure and the Kenward–Rogers adjustment for degrees of freedom(Prasifka et al., 2009). The main factors measured for the choice experiment were phenotype, time, and location, and for the no-choice experiment plant, phenotype, and time. Pairwise comparisons in both experiments were assessed using the least-square estimated means with a Bonferroni adjustment (Prasifka et al., 2009).

Results

Choice test

Phenotypes of resistant and susceptible neonates were confirmed based on the differences in mortality during the exposure period. After 111 h, 43% of susceptible *S. frugiperda* and 56% of susceptible *O. nubilalis* survived in the choice experiments (Figure 1). Surviving susceptible larvae were either severely stunted or had initiated feeding on isoline plants and were unaffected. Survival of the resistant phenotype for both species exceeded 90%.

In general, both resistant and susceptible *S. frugiperda* phenotypes exhibited less movement and more rapid choice of host, relative to either *O. nubilalis* phenotype, which spent more time moving between plant tissues. The *S. frugiperda* analysis indicated no significant differences between phenotypes or times, but the proportion of susceptible and resistant neonates differed among locations ($F_{1,551.4} = 88.14$, P<0.001) (Table 1). The proportion of larvae was higher on Cry1F maize tissue than on isoline (t = 3.4, d.f. = 551.6, P = 0.0022) for both susceptible and resistant phenotypes (Figure 2A and C). In addition, the proportion of larvae off plant tissue was lower than the proportion observed on Cry1F maize (t = 12.81, d.f. = 551.9, P<0.0001) and isoline (t = 9.41, d.f. = 550.8, P<0.001). Although the statistical analysis did not detect significant differences between phenotypes, 11% of the susceptible larvae left Cry1F maize tissue after 7 h (Figure 2A), whereas resistant larvae did not abandon Cry1F maize (Figure 2C). No significant interactions between factors were found in the repeated measures analysis for *S. frugiperda* (Table 1).

The *O. nubilalis* analysis revealed a similar trend as there were no significant differences between phenotypes ($F_{1,1.581} = 0.47$, P = 0.58) or times ($F_{1,513.6} = 0.07$, P = 0.79), but
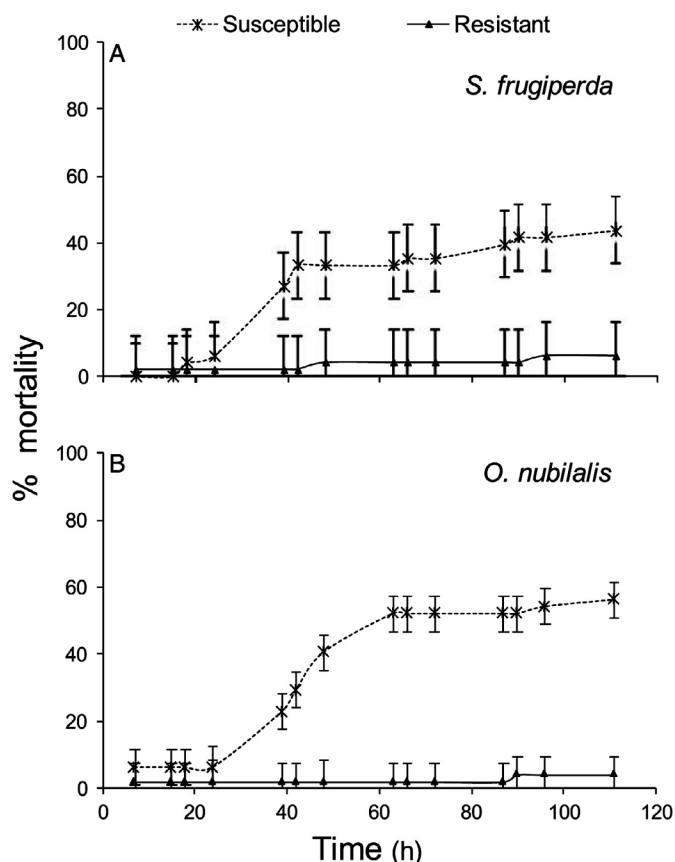

Figure 1. Mean (± SE) mortality (%) of neonate (A) *Spodoptera frugiperda* and (B) *Ostrinia nubilalis* susceptible and Cry1F resistant phenotypes observed in the choice test (n = 48).

there were differences among locations ($F_{2,513.2} = 31.08$, P<0.0001) (Table 1). In contrast to *S. frugiperda*, the proportion of larvae on Cry1F maize tissue was lower than on isoline (t = –4.76, d.f. = 512.9, P<0.0001). In addition, the proportion of *O. nubilalis* larvae on Cry1F (t = –7.82, d.f. = 514.3, P<0.0001) and isoline (t = –3.07, d.f. = 512.5, P = 0.0067) was lower than the proportion of larvae that were not observed on either leaf disc (Figure 2B and C). The only significant interaction was time*location ($F_{2,512.6} = 28.98$, P<0.0001) (Table 1).

No-choice test

High mortality in the no-choice test was only observed in susceptible *S. frugiperda* and *O. nubilalis* feeding on Cry1F maize tissue (Figure 3). For both species, mortality of susceptible larvae was first observed after 7 h; *S. frugiperda* reached 75% mortality by the end of the experiment (111 h), whereas *O. nubilalis* reached 67%. Surviving susceptible larvae feeding on Cry1F maize were severely stunted. Survival of the resistant phenotype feeding on Cry1F maize exceeded 80%. Mortality data were used to confirm the phenotypes.
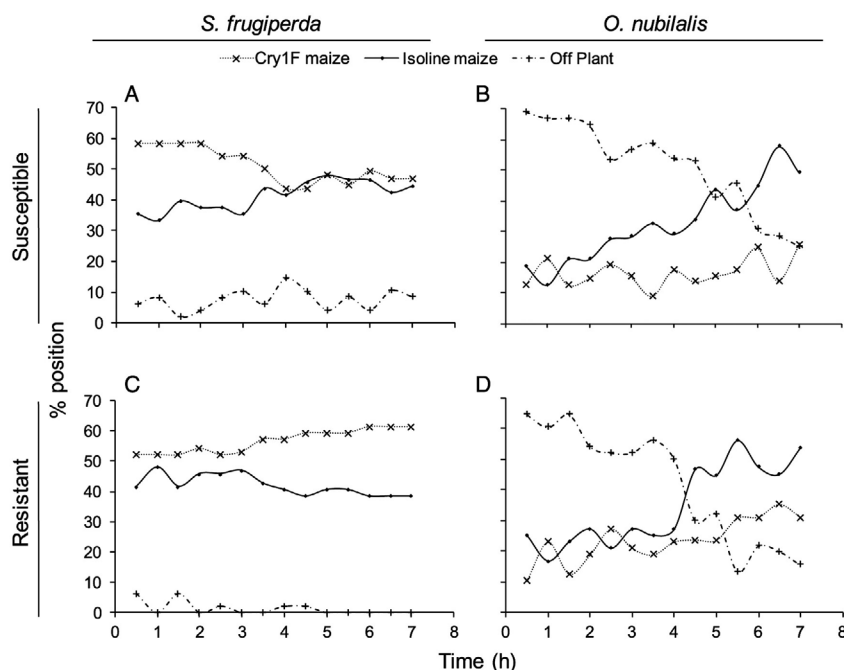
Figure 2. Choice test distribution (% position) of susceptible (A) *Spodoptera frugiperda* and (B) *Ostrinia nubilalis,* and Cry1F resistant (C) *S. frugiperda* and (D) *O. nubilalis* neonates (n = 42–48).

Table 1. Repeated measures analysis on the proportion of susceptible and resistant *Spodoptera frugiperda* and *Ostrinia nubilalis* neonates found on Cry1F maize, on isoline, and off plant in choice tests. Larval location was evaluated every 30 min over 7 h. Measurements were obtained from observations of 42–48 individuals per phenotype.

Insect	Source	d.f.	F	P
S. frugiperda	Phenotype	1	0.69	0.46
	Time	1	0	0.99
	Location	1	88.14	<0.0001
	Phenotype*time	2	0	0.93
	Phenotype*location	2	1.58	0.11
	Time*location	2	0.16	0.86
	Phenotype*time*location	2	2.29	0.10
O. nubilalis	Phenotype	1	0.47	0.58
	Time	1	0.07	0.79
	Location	1	31.08	<0.0001
	Phenotype*time	2	0.02	0.89
	Phenotype*location	2	2.81	0.061
	Time*location	2	28.98	<0.0001
	Phenotype*time*location	2	0.45	0.64

Spodoptera frugiperda and *O. nubilalis* susceptible and resistant neonates exhibited similar behavior to that observed in the choice experiment: *S. frugiperda* moved less and chose more rapidly (Figure 4A and C), whereas *O. nubilalis* took longer to find the plant tissue and spent more time wandering in the arena (Figure 4B and D). In addition, susceptible larvae of both species tended to abandon Cry1F maize tissue, whereas the behavior of resistant larvae appeared to be unaffected by the presence of Cry1F, although differences between phenotypes were not significant (Table 2).

Spodoptera frugiperda repeated measures analysis only revealed significant differences over time ($F_{1,89}$ = 6.05, P = 0.016) and no interactions between factors. Although there was no significant interaction between plant and time ($F_{1,89}$ = 2.54, P = 0.12) there was a significant increase in larvae on isoline maize tissue over time (t = –2.88, d.f. = 121.1, P = 0.030). *Ostrinia nubilalis* analysis indicated no significant differences in any factor and/or interaction (Table 2).

Discussion

Results from both choice and no-choice experiments indicate differences in the behavior of neonate *S. frugiperda* and *O. nubilalis*. Most *S. frugiperda* larvae tend to select a
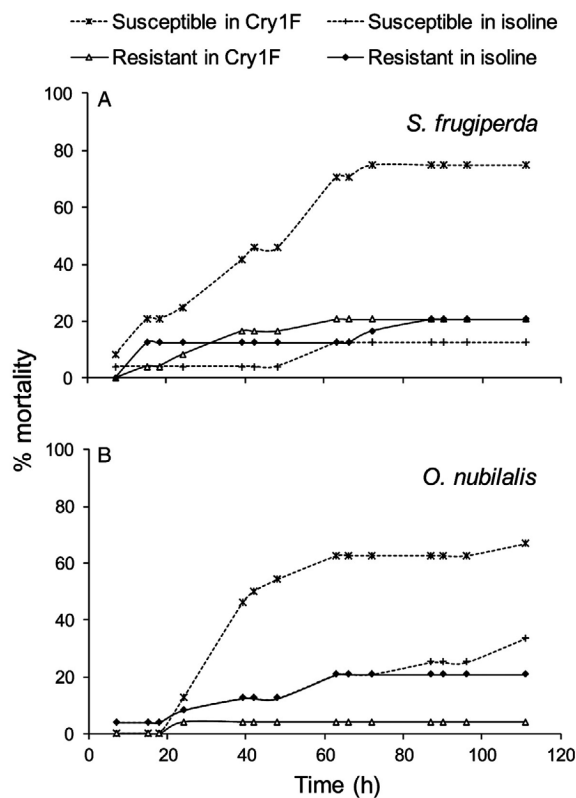
Figure 3. Mean (± SE) mortality (%) of neonate (A) *Spodoptera frugiperda* and (B) *Ostrinia nubilalis* susceptible and Cry1F resistant phenotypes observed in the no-choice test (n = 24).

Table 2. Repeated measures analysis on the proportion of susceptible and resistant *Spodoptera frugiperda* and *Ostrinia nubilalis* neonates on plant (Cry1F maize or isoline) in no-choice tests. Larval location was evaluated every 30 min over 7 h. Measurements were obtained from observations of 22–24 individuals per phenotype per type of plant tissue.

Insect	Factor	d.f.	F	P
S. frugiperda	Phenotype	1	0	0.97
	Plant	1	1.09	0.30
	Time	1	6.05	0.016
	Phenotype*plant	1	1.37	0.25
	Phenotype*time	1	0.13	0.72
	Plant*time	1	2.54	0.12
	Phenotype*plant*time	1	0.38	0.54
O. nubilalis	Phenotype	1	3.31	0.14
	Plant	1	0.06	0.81
	Time	1	2.95	0.09
	Phenotype*plant	1	0.13	0.71
	Phenotype*time	1	0.48	0.49
	Plant*time	1	2.17	0.14
	Phenotype*plant*time	1	0	0.96

plant within the first 30 min and remain on the chosen tissue regardless of Cry1F presence. In contrast, *O. nubilalis* displayed inconsistent movement on and off plant tissue in both experiments. Increased movement in *O. nubilalis* might indicate that the pre-feeding movement phase in this species is longer compared to *S. frugiperda* (Zalucki et al., 2002). However, it could also be an artifact of the higher number of generations *O. nubilalis* strain have been reared in the laboratory resulting in less recognition of maize as a suitable host (Visser, 1986; Stuhl et al., 2008). An additional difference between species was in the initial preference of maize tissue in the choice test. *Spodoptera frugiperda* preferred Cry1F maize tissue, whereas *O. nubilalis* more frequently selected isoline, although it took longer for *O. nubilalis* larvae to make a choice. In general, *S. frugiperda* did not discriminate between plant types and for the initial choice preferred Cry1F maize tissue. The preference of susceptible *S. frugiperda* larvae for Cry1F maize was unexpected considering that no preference in diets with or without Bt toxins have been previously described in other Lepidoptera species (Stapel et al., 1998; Prasifka et al., 2009). These results may be related to the lack of response to the presence of Bt, to subtle differences in plant

quality between Cry1F and isoline tissues that could have affected initial plant choice (Goverde & Erhardt, 2003), and/ or to other innate behavioral factors not associated with the nutritional quality of the host (Thompson, 1988; Berdegue et al., 1996).

Although the differences between species were readily apparent, comparisons between susceptible and resistant phenotypes within each species were more difficult to assess. No significant differences among phenotypes were observed in the choice and no-choice experiments for either species. However, in both species a small percentage of susceptible larvae abandoned maize tissue expressing Cry1F in the choice test (Figure 3). After 7 h of feeding only 9% of susceptible *S. frugiperda* neonates were found off plant, whereas 25% of susceptible *O. nubilalis* larvae were found off maize tissue. In contrast, resistant larvae did not exhibit improved ability to reduce or avoid exposure, but seemed unaffected by the presence of Cry1F. In no-choice tests with *S. frugiperda* (Figure 4A and C), differences in larval position were detected over time. Susceptible larvae tended to move more with Cry1F maize as the sole choice compared to susceptible larvae on isoline. However, no differences were observed in the behavior of *S. frugiperda* resistant larvae feeding on Cry1F or isoline maize. *Ostrinia nubilalis* no-choice test analysis indicated no significant difference for any factor (Table 2, Figure 4B and D). However, a higher percentage of resistant compared to susceptible larvae was recorded on plant by the
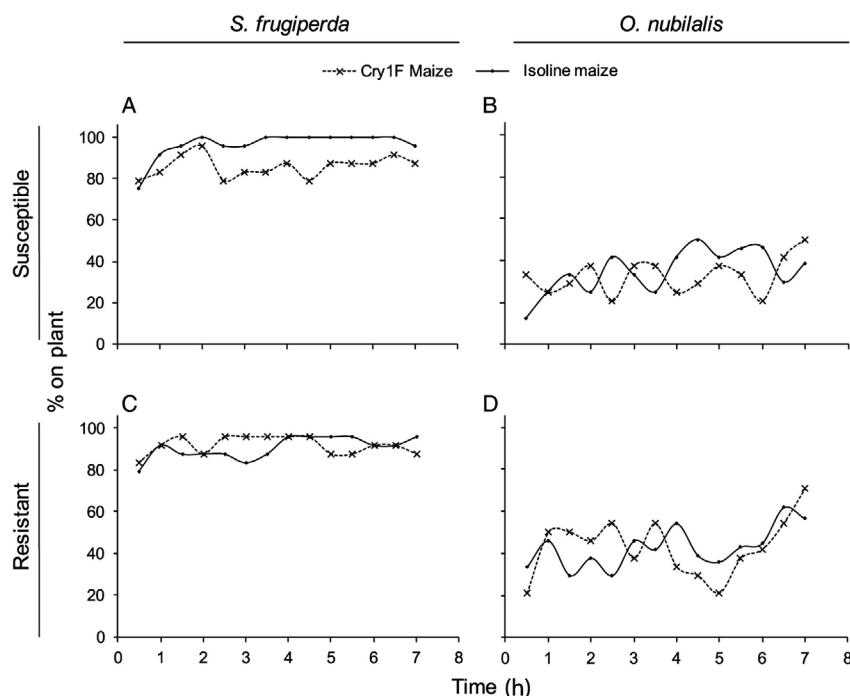
Figure 4. No-choice test percentage of susceptible (A) *Spodoptera frugiperda* and (B) *Ostrinia nubilalis,* and Cry1F resistant (C) *S. frugiperda* and (D) *O. nubilalis* neonates found on Cry1F maize and isoline (n = 22–24).

end of 7 h (57–71 vs. 39–50%). The lack of a behavioral response of resistant larvae to Cry1F maize in the choice and no-choice tests, might be explained by the ability of the larvae to overcome the toxin and by the absence of a fitness cost linked to Cry1F resistance in both species (Pereira et al., 2009; Velez et al., 2014).

The tendency of susceptible *O. nubilalis* larvae to stay off leaf material might be an indication of irritability generated by Cry1F ingestion as previously described for this species (Razze & Mason, 2012) and other lepidopterans exposed to Cry toxins (Berdegue et al., 1996; Stapel et al., 1998; Prasifka et al., 2009). Consequently, it is possible that susceptible first-instar *O. nubilalis* could move from a Cry1F-expressing plant to a non-Bt plant and recover from intoxication (Stapel et al., 1998; Li et al., 2006; Razze & Mason, 2012). Studies with *S. exigua* feeding on Bt-treated diets and *T. ni* feeding on Bt cotton indicate that larvae that fed on a mixture of non-Bt and Bt were able to survive (Stapel et al., 1998; Li et al., 2006), increasing the likelihood of heterozygote survival and potentially accelerating the evolution of resistance (Mallet & Porter, 1992; Davis & Onstad, 2000). In a study evaluating the movement and dispersal of neonate *O. nubilalis* on Cry1F-maize, a stacked pyramid expressing Cry1F and Cry1Ab, and a non-Bt sweet maize, Razze &Mason (2012) reported that neonate dispersal was significantly greater in Bt maize fields compared with non-Bt maize fields. Increased movement of *O. nubilalis* in response to Cry1F exposure indicates that current refuge configurations (i.e., blocks or strips) might be more suited for this insect (Ross & Ostlie, 1990).

In contrast to reports of behavior of other Lepidoptera species, the majority of susceptible *S. frugiperda* larvae (89%) remained on selected plant tissue regardless of toxin expression. Previous larval preference studies of *S. frugiperda* maize and rice strains with maize and stargrass, *Cynodon nlemfuensis* Vanderyst, indicated that neonates of both strains feed on the plant type that was encountered first and a substantial number of larvae remained on the selected plant tissue (Stuhl et al., 2008). These results suggest that the innate behavior of *S. frugiperda* neonates is to remain on the first plant tissue found. For *S. frugiperda*, more rapid host selection and reduced movement of early instars away from Bt maize may have important implications for refuge design. Binning et al. (2014) reported that susceptible third-instar *S. frugiperda* displayed an initial aversive response to Cry1F maize after ingestion, although the authors were unable to conclude whether host abandonment occurs after feeding. If the behavior of *S. frugiperda* neonates reported in this study is similar under field conditions and if the initial aversion of third instars reported by Binning et al. (2014) does not equate to host abandonment, the use of seed mixtures might be a suitable strategy for *S. frugiperda*.

This study represents the first step toward understanding the effects of Cry1F resistance in *S. frugiperda* and *O. nubilalis* larval behavior. Further greenhouse and field experiments are necessary to provide a more complete understanding of the effect of Cry1F on movement of susceptible and resistant larvae, and the differences between

S. frugiperda and *O. nubilalis*. Although laboratory behavior experiments are difficult to extrapolate to field behaviors (Prasifka et al., 2009), the apparent differences in the behavior of *O. nubilalis* and *S. frugiperda* exposed to Cry1F maize suggest that not all Lepidoptera species perform equally, and generalizations in behavior might not always be accurate. Understanding behavioral differences between species could help to develop better and more flexible resistance management strategies (USEPA, 1998; Onstad et al., 2011).

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