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Author(s): J. R. Prasifka , R. L. Hellmich , D. V. Sumerford , and B. D. Siegfried

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Bacillus thuringiensis Resistance Influences European Corn Borer (Lepidoptera: Crambidae) Larval Behavior After Exposure to Cry1Ab

J. R. PRASIFKA,¹ R. L. HELLMICH,² D. V. SUMERFORD,² AND B. D. SIEGFRIED³

Energy Biosciences Institute, Institute for Genomic Biology, Room 1117, University of Illinois, Urbana, IL 61801

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ABSTRACT The behavior of pests targeted by *Bacillus thuringiensis* (Bt) crops has been recognized as an important factor to define resistance management plans. However, most data do not include the possible impact resistance may have on the behavior of pests. To examine whether resistance influences behavior of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), neonates after exposure to dietary Bt, the responses of Cry1Ab-resistant, -susceptible, and hybrid (F1) lines from two populations were compared in laboratory tests by using artificial diet mixed with 10–50% Cry1Ab or non-Bt isoline corn, *Zea mays* L., tissue. In no-choice tests, resistant (and usually hybrid) lines were less likely to be irritated (i.e., to move away after physical contact with diet containing Cry1Ab) than susceptible larvae after exposure to diets containing 10–50% Cry1Ab leaf tissue. Early in the no-choice tests (8 h), neonate *O. nubilalis* also were more likely to move off of diets that contained 10% non-Bt tissue compared with diets with 25 or 50% non-Bt tissue. In agreement with results from no-choice tests, choice tests with 10 or 25% tissue indicated that resistant (and sometimes hybrid) larvae were more likely than susceptible neonates to be found on diet with Cry1Ab. For choice tests, differences among lines seemed dependent on the amount of Cry1Ab tissue incorporated into diets. Results suggest differences in behavior are a result of reduced physiological susceptibility to Cry1Ab and are not an independent behavioral component to resistance.

KEY WORDS behavioral resistance, feeding, dispersal, insect resistance management, transgenic

To reduce the likelihood of pests becoming resistant to crops that express *Bacillus thuringiensis* (Bt)-derived toxins, the U.S. Environmental Protection Agency (USEPA) requires an approved insect resistance management strategy before commercial release of any crop that produces Bt toxins. All resistance management strategies in the United States require that target pests have a refuge from Bt toxins to maintain a source of susceptible alleles and decrease selection for resistance. A variety of refuge strategies have been considered, including planting of both Bt and non-Bt crops (in blocks, strips, or seed mixtures), limiting the expression of toxins to be time- or tissue-specific, or using noncrop hosts to produce Bt-susceptible pests (Bates et al. 2005).

However, the resistance management approach first used for target pests of Bt crops is the high dose/refuge (HDR) strategy, so called for its basic components: 1) crop varieties that express a Bt toxin at doses sufficient to kill susceptible homozygotes and most heterozygotes, and 2) areas planted to non-Bt crop

varieties used as a source of susceptible homozygotes (USEPA 2001a). The HDR strategy incorporates three basic assumptions. First, resistance to Bt is recessive and controlled by two alleles (*R*, resistant; *S*, susceptible) at one locus. Second, the initial frequency of *R* alleles in pest populations is low. Finally, random mating between resistant (*RR*) adults and susceptible (*SS*) moths (from refuges) keeps *R* alleles rare.

Within the framework provided by the HDR strategy, specific information about the behavior of target pests is important for appropriately defining the details of resistance management. For example, in the primary corn (*Zea mays* L.)-producing area of the United States (the Corn Belt), planting Bt corn hybrids toxic to the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), requires growing refuge corn without Cry1Ab or Cry1 F toxins over at least 20% of the total planted area; the refuge also must be located within 800 m of the Cry1Ab or Cry1 F corn and have a minimum width of four rows (Matten et al. 2004). Research on the premating dispersal behavior of *O. nubilalis* adults helped to define 800 m as a reasonable distance between Bt corn and required refuge (USEPA 2001a, Hunt et al. 2001, Showers et al. 2001) that encourages mating between Bt-resistant and -susceptible adults. Similarly, narrow (less than four rows wide) strips or in-row mixtures of

¹ Corresponding author, e-mail: prasifka@illinois.edu.

² USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory c/o Insectary, Iowa State University, Ames, IA 50011.

³ Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE 68583.

Bt and non-Bt seed were eliminated as options for refuge planting (i.e., only blocks or strips are currently permitted) because European corn borer larvae moving from plant to plant (see Ross and Ostlie 1990) may effectively receive a lower dose of Bt toxins, increasing the likelihood of heterozygote survival and potentially accelerating the development of resistance (Mallet and Porter 1992, USEPA 1998, NCR-46 2001).

Field exposure to toxins present in Bt corn seems to increase the likelihood that *O. nubilalis* larvae will move between plants (Davis and Onstad 2000), which may further hasten resistance evolution. Similar behavioral responses that include increased movement often have been observed for other lepidopteran larvae on Bt plants (Ramachandran et al. 1998 [*Plutella xylostella* (L.)], Parker and Luttrell 1999 [*Heliothis virescens* (F.)], Li et al. 2006 [*Trichoplusia ni* (Hübner)]) or exposed to Cry toxins in the laboratory (Berdegué et al. 1996 [*Spodoptera exigua* (Hübner)], Davis and Coleman 1997 [*O. nubilalis*], Harris et al. 1997 [*Epiphyas postvittana* (Walker)]). Fewer studies have examined the potential for resistance to interact with the behavioral responses to Bt toxins (but see Gould and Anderson 1991, Schwartz et al. 1991, Berdegué et al. 1996, Huang et al. 2001, and Harris et al. 2006), with only one study (Huang et al. 2001) including *O. nubilalis*, the primary lepidopteran target of Bt corn. Given the increasing availability of laboratory-selected pests resistant to Bt toxins (for *O. nubilalis*, see Siqueira et al. 2004, Siegfried et al. 2007), opportunities exist to contribute to Bt resistance management by understanding the possible effects of resistance on pest behavior.

Accordingly, a series of laboratory-based tests were conducted to investigate the possible effects of Cry1Ab resistance on the behavior of *O. nubilalis* larvae. Neonates from Cry1Ab-resistant, -susceptible, and hybrid (F1) lines derived from two populations were observed to determine whether Bt resistance influences responses to Cry1Ab. Separate experiments over a range of toxin concentrations were designed to determine 1) whether irritability of larvae exposed to dietary Cry1Ab is influenced by resistance in no-choice trials, and 2) whether resistance increases larval ability to reduce exposure to Cry1Ab when a choice (non-Bt diet) is available.

Materials and Methods

In an attempt to see patterns of how resistance influences behavior, lines derived from *O. nubilalis* populations in Minnesota and Iowa were used. Resistant and susceptible "Kandi" lines were established from larvae collected in non-Bt corn in Kandiyohi Co., MN, during 2001 (R-Kandi and S-Kandi in Siegfried et al. 2007). "Ames" lines were derived from moths collected in central Iowa in 1996. Cry1Ab resistance in both resistant lines is >1,000-fold compared with the susceptible controls (established concurrently from the same *O. nubilalis* populations). Details on the selection and resistance for lines from the Kandi and Ames populations can be found in Crespo et al. (2009)

and Sumerford et al. (2009). The Cry1Ab-resistant lines do not conform to the HDR assumption of recessive resistance based on a single locus. As a result, using the abbreviations *RR*, *SS*, and *RS* to refer to lines from each population would be misleading. Instead, lines of *O. nubilalis* are referred to as resistant, susceptible, and hybrid for both the selected lines and their F1 crosses (hybrids are not maintained as distinct lines but are produced from crosses to obtain F1 larvae as needed).

For all experiments, a standard *O. nubilalis* diet (Reed et al. 1972) was modified by replacing 10, 25, or 50% of the dry nutritive ingredients with an equivalent mass of tissue (see Wilson and Wissink 1986) from Cry1Ab (Pioneer 34N44) or nontransgenic (34N43) corn hybrids. Corn tissues were obtained by cutting plants (\approx V10 stage) at the collar of leaf 10, and removing leaves 10 and 11; the remaining tissue was freeze-dried, ground (<1-mm particle size), and stored at -80°C .

To avoid potential confusion surrounding terms related to behavioral resistance, basic distinctions outlined by Lockwood et al. (1984) are observed. In particular, behavioral resistance is taken to include only attributes resulting from selection in the presence of a toxin that enable a population to reduce mortality from or exposure to a toxin. A response to move away from a toxin after physical contact is referred to as irritability and is not equivalent to repellency, in which stimulation to move away takes place before (or without) physical contact.

No-Choice Bioassays. No-choice tests were used to establish any effects of Bt resistance on larval irritability by using diets with 10, 25, and 50% corn tissue (Cry1Ab or nontransgenic). Discs of diet (15 mm in diameter, 8 mm in depth) were produced by cutting a cross section from a cylinder of diet extracted with a no. 11 cork borer, after which discs were placed into individual wells of an 128-well bioassay tray (BIO-BA-128, C-D International, Pitman, NJ). Four neonates were placed into each cell and groups of 16 cells were covered with a transparent sheet (BIO-CV-16, C-D International). After 4, 8, 12, 24, 36, 48, and 72 h, the positions of all larvae were categorized as 1) on the top (cover) of the well, 2) on the sides, 3) visible on or in the diet, or 4) not visible (deep within or beneath the diet); larvae that seemed dead (inactive and desiccated) also were noted. For both Kandi and Ames populations, all combinations of line (resistant, susceptible, and hybrid), diet type (with or without Cry1Ab), and tissue content (10, 25, and 50%) were observed in eight groups (replicates) of four wells (=128 larvae).

Choice Tests. Preliminary trials with no-choice bioassays suggested that resistant larvae are less irritated by Cry1Ab than susceptible or hybrid larvae derived from the same population. Conversely, Huang et al. (2001) show *O. nubilalis* larvae resistant to a combination of Cry toxins were better able to reduce exposure to diet-incorporated Bt by moving onto a control diet in choice tests. Experiments similar to those of Huang et al. (2001) were conducted to test whether

resistant or hybrid larvae are able to reduce exposure to dietary Cry1Ab (relative to susceptible larvae) when provided a choice of diets with 10 or 25% corn tissue (with and without Cry1Ab). For choice tests, a no. 11 cork borer was used to produce discs (15 mm in diameter, 4 mm in depth) of corn tissue diet that were placed opposite each other in each small petri dish (50 mm in diameter; 351006, BD Biosciences Discovery Labware, Bedford, MA). In total, 25 neonates was placed on the bottom of each petri dish and allowed access to diets with the Cry1Ab and non-Bt corn tissue. After 4, 8, 12, 24, 36, 48 and 72 h, the positions of larvae in each dish were categorized as 1) on or in the Cry1Ab diet, 2) on or in the nontransgenic diet, 3) on neither diet, or 4) not visible (deep within the diet); larvae that seemed dead also were noted. For both Kandi and Ames populations, six line (resistant, susceptible, and hybrid) and tissue content (10, 25%) combinations were observed in eight (replicate) dishes (=200 larvae).

Data Analysis. All analyses were performed using SAS software (SAS Institute 1999) with specific procedures indicated in capital letters. Before analysis, data from the no-choice bioassays were converted to express the proportion of larvae in each replicate found off of the diet. Excluding any larvae that seemed dead, each four well (=16 larvae) replicate was scored with larvae on the top (cover) of the well or on the sides (with no part of the larva touching the diet) considered off the diet. Because the resulting data were proportions (often with a range of values >0.40), data were subsequently arcsine-square root-transformed. A preliminary repeated measures (RM) model (PROC MIXED) then examined how the proportion of larvae off of diets in the no-choice tests was influenced by source population (Kandi or Ames), line, diet type (Bt or non-Bt), tissue content (10, 25, or 50%), and time. Interactions (e.g., line \times tissue content, population \times tissue content) indicated a repeated measures analysis of variance (RM-ANOVA) for each source and tissue combination would be more appropriate. Subsequently, separate RM-ANOVA were conducted (PROC MIXED, heterogeneous compound symmetry [CSH] covariance structure and Kenward-Rogers [KR] adjustment to degrees of freedom) to determine how the proportion of larvae off of the diet was influenced by diet type, line, diet type \times line interaction and time. Interactions with time (e.g., time \times diet type, time \times line) were not considered because within a tissue type interactions were usually orderly (same direction but slight difference in slope between lines), and the experiments were intended to evaluate the entire 72-h interval. Pairwise differences between line and tissue type combinations were assessed using least-squared estimated means with the SLICE option and a Bonferroni adjustment to *P*-values.

Analyses of data for choice tests were performed according to the same approach used for no-choice bioassays, except that diet type was removed from the analysis (because both Bt and non-Bt diets were provided in choice tests). Data from choice tests were modified to reflect the proportion of larvae in each

Table 1. Repeated measures analyses on the proportion of neonate *O. nubilalis* from Kandi colonies found off of diets in no-choice tests

Tissue	Factor	df ^a	F	P
10%	Type	1, 44.5	544.7	<0.001
	Line	2, 44.5	24.2	<0.001
	Line \times type	2, 44.5	42.0	<0.001
	Time	1, 159	166.1	<0.001
25%	Type	1, 35	737.1	<0.001
	Line	2, 35	37.3	<0.001
	Line \times type	1, 35	52.2	<0.001
	Time	1, 135	98.5	<0.001
50%	Type	1, 42	686.0	<0.001
	Line	2, 42	12.66	<0.001
	Line \times type	2, 42	42.47	<0.001
	Time	1, 158	25.77	<0.001

^a Kenward-Rogers adjustment to degrees of freedom. Data are arcsine-square root-transformed for analysis.

dish that successfully reduced exposure to Cry1Ab at each time period (those found off of Bt diet). After excluding any larvae that seemed dead, the proportion of larvae off of Cry1Ab was simply one minus the proportion on Cry1Ab diet (combining larvae on non-Bt diet and off of both diets). For each combination of population and tissue content, separate RM-ANOVA (PROC MIXED, CSH covariance, and KR adjustment) were used to test the effect of line and time on the (arcsine-square root transformed) proportion of larvae off of the Bt diet. Pairwise comparisons between lines were made using *t*-tests on least-squares estimated means.

The repeated measures approach was useful to examine the lines over the entire 72 h tests. However, targeting specific time periods could also help answer questions about neonate response to diets. Previous observations indicated neonates on diet with 10% corn tissue often seemed irritated even in the absence of Cry1Ab (at ≤ 10 h; J.R.P., unpublished). Consequently, an ANOVA (PROC MIXED) was conducted to assess whether acceptance of non-Bt diet at 8 h improves as tissue content increases. The model tested the effects of source population, line, tissue content, and their possible two-way interactions on the (arcsine-square root-transformed) proportion of larvae off of the non-Bt diet. As with the choice tests, comparisons between lines were made using *t*-tests.

Results

No-Choice Bioassays. Mortality in the no-choice tests seemed low, with <1% of the 768 neonates ($n \leq 2$) in any colony \times tissue combination scored as dead after 48 h. Repeated measures ANOVA for the Kandi lines indicate that diet type, line, and time all consistently influenced the proportion of larvae found off of diets containing 10, 25, or 50% corn tissue (Table 1). Significant ($P < 0.05$) diet type \times line interactions also were detected, but this may primarily reflect differences among lines when confined on Bt diet; tests for an effect of line using only non-Bt diet indicated a difference for only one of the three tissue levels (50%

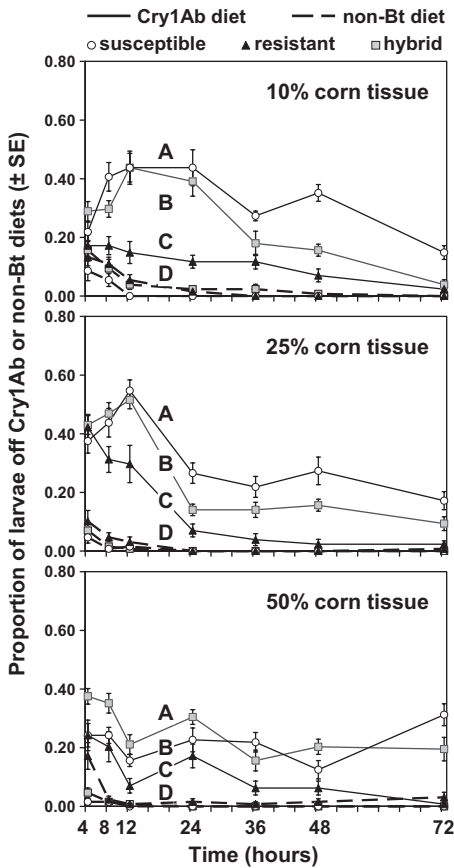


Fig. 1. Proportion of neonate *O. nubilalis* from Kandi populations found off of artificial diets containing Cry1Ab or non-Bt corn tissue. Significant differences in irritability among resistant, susceptible and hybrid lines on diets with Cry1Ab (solid lines) or non-Bt corn tissue (dashed lines) are indicated by different capital letters.

tissue; $F = 5.12$; $df = 2, 42$; $P = 0.010$). Pairwise comparisons among the six line and diet type combinations indicate the three lines differed when confined on Bt diet, but they were similar to each other when provided diet with non-Bt tissue (but different from all lines on Bt diet; Fig. 1).

For lines from the Ames population, RM-ANOVA also showed the proportion of larvae irritated by diets was related to diet type, line, and time at all tissue levels (Table 2). As with the Kandi lines, significant diet type \times line interactions were apparent in lines from the Ames population, but differences among lines on non-Bt diet only showed differences for one of the three tissue levels (10% tissue; $F = 3.44$; $df = 2, 35.9$; $P = 0.043$). In pairwise comparisons, resistant *O. nubilalis* consistently differed from susceptible and hybrid lines on Bt diet, whereas all three lines seemed similar when provided diet containing only non-Bt tissue (Fig. 2).

Results from analysis including both colonies on non-Bt diets after 8 h indicated early acceptance of non-Bt diet (at 8 h) depended on corn tissue content

Table 2. Repeated measures analyses on the proportion of neonate *O. nubilalis* from Ames colonies found off of diets in no-choice tests

Tissue	Factor	df ^a	F	P
10%	Type	1, 35.9	722.9	<0.001
	Line	2, 35.9	36.8	<0.001
	Line \times type	2, 35.9	75.6	<0.001
	Time	1, 145	165.7	<0.001
25%	Type	1, 33.8	1347.5	<0.001
	Line	2, 33.8	82.3	<0.001
	Line \times type	2, 33.8	74.8	<0.001
	Time	1, 132	11.7	0.008
50%	Type	1, 44.7	553.0	<0.001
	Line	2, 44.7	52.4	<0.001
	Line \times type	2, 44.7	43.8	<0.001
	Time	1, 128	20.7	<0.001

^a Kenward-Rogers adjustment to degrees of freedom. Data are arcsine-square root-transformed for analysis.

($F = 36.67$; $df = 2, 125$; $P < 0.001$), whereas source population, line, and any interactions were not significant components of the ANOVA. Paired comparisons

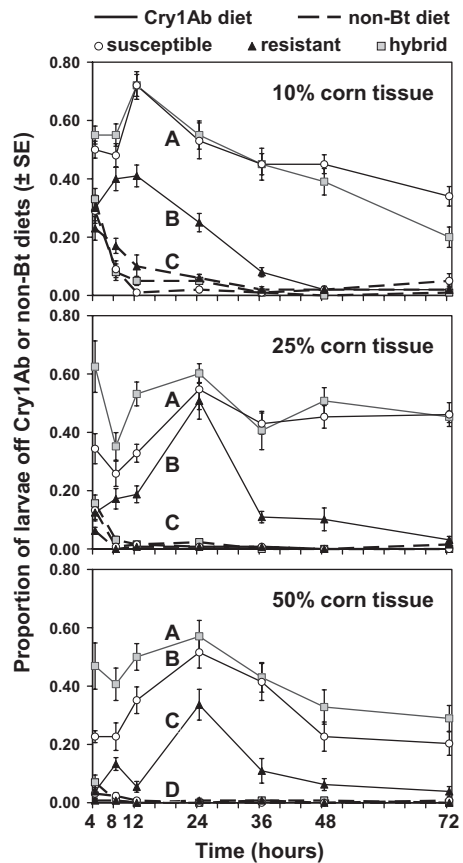


Fig. 2. Proportion of neonate *O. nubilalis* from Ames populations found off of artificial diets containing Cry1Ab or non-Bt corn tissue. Significant differences in irritability among resistant, susceptible and hybrid lines on diets with Cry1Ab (solid lines) or non-Bt corn tissue (dashed lines) are indicated by different capital letters.

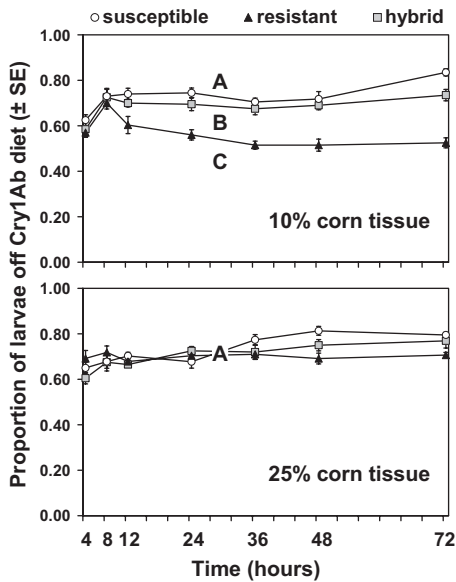


Fig. 3. Proportion of neonate *O. nubilalis* from Kandi populations found off of Cry1Ab diet in choice tests including Cry1Ab and non-Bt corn tissue. Significant differences among resistant, susceptible and hybrid lines are indicated by different capital letters.

suggested that larvae were more likely to move off of diets containing 10% tissue but found no differences between 25 and 50% corn tissue.

Choice Tests. In the choice tests, mortality after 72 h was <2% of the 600 neonates ($n \leq 10$) used in each colony \times tissue combination. Also, the percentage of larvae not accounted for in the choice tests was low; including all observations, an average of <3% of larvae ($n \leq 113$ of 4,200 larval observations [600 neonates \times 7 times]) were categorized as missing. For lines from the Kandi population, RM-ANOVA indicated significant effects of line ($F = 42.82$; $df = 2, 21.7$; $P < 0.001$) and time ($F = 8.55$; $df = 6, 59.9$; $P < 0.001$) on diets containing 10% corn tissue; with 25% Cry1Ab and non-Bt tissue, an effect of time was again detected ($F = 14.27$; $df = 6, 63.5$; $P < 0.001$), but no effect of line was apparent ($F = 1.05$; $df = 2, 23.9$; $P = 0.366$). The proportion of larvae off of Cry1Ab diets differed among all three lines on 10% tissue, but all three lines seemed similar when tissue content increased to 25% (Fig. 3).

When presented with a choice of diets with non-Bt or Cry1Ab tissue, the proportion of larvae from the Ames population found off of Bt diet was influenced by line ($F = 79.33$; $df = 2, 23.2$; $P < 0.001$), but no impact of time ($F = 2.07$; $df = 6, 61$; $P = 0.070$) was detected on diets containing 10% corn tissue. With 25% Cry1Ab and isoline corn tissue, effects of line ($F = 42.11$; $df = 2, 15.8$; $P < 0.001$) and time ($F = 4.57$; $df = 6, 56.9$; $P < 0.001$) were detected. All three lines from the Ames population differed for the proportion off of diet containing 10% Cry1Ab tissue, whereas susceptible and hybrid lines no longer differed when tissue content increased to 25% (Fig. 4).

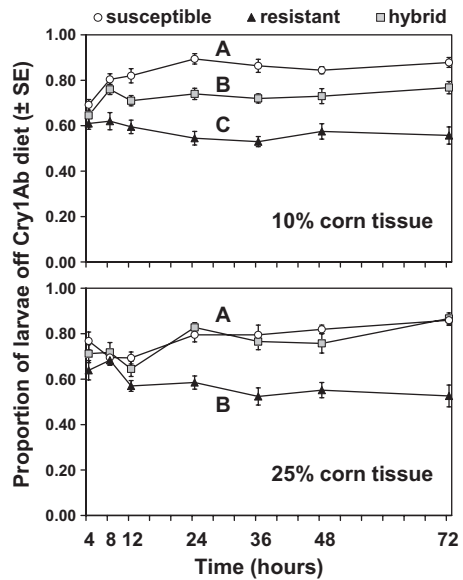


Fig. 4. Proportion of neonate *O. nubilalis* from Ames populations found off of Cry1Ab diet in choice tests including Cry1Ab and non-Bt corn tissue. Significant differences among resistant, susceptible and hybrid lines are indicated by different capital letters.

Discussion

The effects of resistance on *O. nubilalis* neonate behavior after exposure to dietary Cry1Ab were similar for two Cry1Ab-resistant lines independently derived from populations in Minnesota and Iowa. In no-choice tests, resistant (and usually hybrid) lines were less irritated than susceptible larvae over 72 h of exposure to diets containing ≈ 10 –50% of the Cry1Ab in Bt field corn (Tables 1 and 2; Figs. 1 and 2). Choice tests, which included 10 or 25% Cry1Ab and non-Bt corn tissue, indicated that resistance did not improve the ability of *O. nubilalis* larvae to reduce Cry1Ab exposure but instead increased exposure to the Bt toxin (Figs. 1 and 2). For choice tests, differences among lines seemed dependent on the amount of Cry1Ab tissue incorporated into diets, with lines becoming more similar as Bt corn tissue increased from 10 to 25%.

Interactions of resistance (i.e., line) with time and Cry1Ab tissue content for both no-choice and choice tests suggest extrapolation from laboratory-based tests to field behaviors may be difficult. However, the general effects of increasing levels Cry1Ab may be qualitatively predicted based on previous research. Tabashnik et al. (2004) show decreasing differences in survival between RS and SS larvae (“decreasing dominance”) as concentrations of Bt toxins increase. Similarly, for choice tests in which differences in *O. nubilalis* behavior were found between hybrid and susceptible lines (e.g., both Kandi and Ames populations at 10% tissue [Figs. 3 and 4]), no significant differences were found between the two lines at 25% Cry1Ab tissue.

Even for comparisons between resistant and susceptible larvae, increasing the amount of Cry1Ab tis-

sue in diet seemed to reduce behavioral differences, because the proportion of resistant and susceptible larvae off of Cry1Ab diet for the lines from the Kandi population differed at 10% tissue but were statistically similar with 25% Bt corn tissue (Fig. 3).

One unexpected result was that early larval movement off of non-Bt diet was influenced by the amount of tissue incorporated into the artificial diet. This response could be described as apparent irritability, because both the artificial diet and the non-Bt leaf tissue were toxin-free. Including all lines from the Kandi and Ames populations, after 8 h more larvae were found off of the non-Bt diet in no-choice tests with 10% tissue compared with 25 or 50% corn tissue, but no differences between 25 and 50% tissue were detected (Figs. 1 and 2). These results suggest that although the standard *O. nubilalis* diet (Reed et al. 1972) is very effective for rearing *O. nubilalis*, it is less attractive than leaf tissue. However, after 12–24-h neonate *O. nubilalis* accepted the diet, suggesting that the amount of corn tissue had little effect on the larval response to Cry1Ab 24 h after exposure. Nonetheless, this potentially confounding effect of tissue and Cry1Ab content should be avoided in future tests. The simplest method would be to make all tissue-incorporated diets to a standard tissue concentration (e.g., 50%); increased amounts of Bt tissue would be offset by reductions in non-Bt tissue to achieve desired concentrations of Cry1Ab.

Both the no-choice and choice tests failed to provide evidence that resistant or hybrid larvae from either *O. nubilalis* source population have an enhanced ability to avoid dietary Cry1Ab (behavioral resistance sensu Lockwood et al. 1984); on the contrary, resistant (and sometimes hybrid) larvae were more likely to be found on diets with Cry1Ab than susceptible individuals from the same source population. However, other choice tests with Bt toxins have shown resistant larvae are more likely to be found on nontoxic diets compared with susceptible controls (e.g., Hoy and Head 1995 [*Leptinotarsa decemlineata* (Say)], Huang et al. 2001 [*O. nubilalis*]). Hoy and Head (1995) note two straightforward interpretations of finding a greater proportion of resistant individuals on nontoxic diet. First, more susceptible larvae may be found on Bt diet if the toxic effects of the diet render them unable to move away. Second, it is also possible that resistant larvae are inherently more likely to attempt to leave a diet containing Bt toxins. Both Hoy and Head (1995) and Huang et al. (2001) favored the second interpretation, that there was a behavioral component not explained by the physiological ability to withstand exposure to the toxin. Although lines from the Kandi and Ames populations differ in both their susceptibility to Cry1Ab and their behavioral response, the response of resistant larvae is contrary to that which would be expected with behavioral resistance (i.e., resistance actually increases toxin exposure and would most likely reduce survival). No direct comparison with the Dipel-resistant *O. nubilalis* used by Huang et al. (2001) is possible because the colony no longer exists, but based on the results from the resistant Kandi and Ames colonies, it seems that an

independent behavioral component to resistance for *O. nubilalis* may not be common.

The no-choice and choice laboratory tests were undertaken as a step toward using information on the behavior of resistant colonies to contribute to effective Bt resistance management. Given the variability of Cry toxin concentration in field-grown plants (USEPA 2001b [Table A2], Monsanto Company 2002 [Table 1]), the use of 50% tissue from Cy1Ab corn may be equivalent to the low end of field expression. However, the use of realistic toxin concentrations is essential and mixtures of artificial diet and corn tissue are increasingly difficult to handle beyond 50% tissue. Consequently, to best estimate how resistance impacts the likelihood of moving off of Bt corn and the frequency of survival (which influence the likelihood of resistance development, Onstad and Gould 1998), on-plant tests seem necessary. *O. nubilalis* colonies with resistance that more closely conforms to the high dose/refuge assumptions (i.e., based on one locus, recessive) also would be useful.

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