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Michael A. Rausch
Iowa State University

Jeremy A. Kroemer
Iowa State University

Aaron J. Gassmann
Iowa State University, aaronjg@iastate.edu

Richard L. Hellmich
U.S. Department of Agriculture, rlhellmi@iastate.edu

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Abstract
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Keywords
Ostrinia nubilalis, plant abandonment, plant establishment, Onslmo, foraging

Disciplines
Agriculture | Behavior and Ethology | Entomology | Genetics | Population Biology

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On-Plant Selection and Genetic Analysis of European Corn Borer (Lepidoptera: Crambidae) Behavioral Traits: Plant Abandonment Versus Plant Establishment

MICHAEL A. RAUSCH,[1,2] JEREMY A. KROEMER,[1] AARON J. GASSMANN,[1] AND RICHARD L. HELLMICH[3]

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ABSTRACT Although some studies have investigated how insect behavior could influence resistance evolution to transgenic plants, none have determined if behavioral traits respond to selection pressure and how they may be inherited. We investigated plant establishment and abandonment traits for the European corn borer, *Ostrinia nubilalisi* (Hübner) (Lepidoptera: Crambidae), by conducting a laboratory selection experiment and quantifying patterns of gene expression. Egg masses with emerging larvae were placed on maize plants and silking individuals were collected every 15 min during a 4-h period to generate a plant abandonment (PA) colony. Plants were dissected 24–72 h later, and larvae were collected for a plant establishment colony. Selection of the PA colony showed an increased propensity to abandon the host plant by the third generation. The propensity for larvae to establish on the plants, however, did not show a significant response until the sixth generation. Quantitative real-time–polymerase chain reaction (qRT-PCR) was used to determine expression profiles for behavior associated genes (*foraging* and *Onslmo*). Egg samples from the two selected colonies and nonselected laboratory colony were collected at 0, 24, 48, 72, and 96 h after egg deposition, and first instars were sampled after exposure to maize tissue. Compared with the plant establishment and nonselected laboratory colonies at the 0-h time period, *foraging* and *Onslmo* showed higher expression in the PA colony. This is the first study that has specifically selected for these traits over several generations and analyzed behavior-associated genes to elucidate genetic changes.

KEY WORDS *Ostrinia nubilalis*, plant abandonment, plant establishment, *Onslmo*, *foraging*

Deployment of transgenic crops producing insecticidal crystal toxin (Cry) proteins derived from the bacterium *Bacillus thuringiensis* (Berliner) (Bt) has effectively managed insect pest populations while reducing use of chemical insecticide (Carrie're et al. 2003, Huang et al. 2005, Cattaneo et al. 2006, Wu et al. 2008, Hutchison et al. 2010, James 2011). Extensive planting of Bt crops and season-long exposure to toxins has caused concern that Bt resistance may develop in target pests (Tabashnik et al. 2008). This concern is supported by the development of Bt-resistant strains in laboratory settings (Tabashnik et al. 1990, Bolin et al. 1999, Huang et al. 1999, Pereira et al. 2008, Tabashnik et al. 2008, Gassmann et al. 2009, Crespo et al. 2009), and the discovery of Bt-resistant populations surviving in the field (van Rensburg 2007, Tabashnik et al. 2008, Storer et al. 2010, Dhurua and Gujar 2011, Gassmann et al. 2011, Zhang et al. 2011). Many insect resistance management (IRM) strategies have been considered, but the high-dose refuge (HDR) strategy has been adopted by regulatory, academic, and industry scientists as the standard for IRM (Gould 1998, Tabashnik et al. 2004). This strategy combines a high dose of toxin from Bt plants with a refuge of non-Bt plants that serves as a reservoir for susceptible insects. Certain aspects of insect behavior, in particular patterns of dispersal, have the potential to limit the effectiveness of HDR. Using maize plants in a laboratory setting, we conducted artificial selection on a laboratory colony of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), to test the response to selection for the behavioral trait of dispersal and then analyzed patterns of gene expression associated with this behavior.

Although resistance to Bt toxins may occur from a variety of mechanisms, Bt resistance research has focused primarily on physiological resistance, especially modifications of Bt targeted midgut receptors in larvae (Ferré and Van Rie 2002; Ma et al. 2005; Ferré et al. 2008). Relatively few studies, however, have considered the effects of behavior on the evolution of resistance to Bt crops. Hoy and Head (1995) found differences in behavior between Bt resistant and susceptible strains of Colorado potato beetle (*Leptinotarsa decemlineata* Say (Coleoptera: Chrysomeli-

[1] Department of Entomology, Iowa State University, Ames, IA 50011.
[2] Corresponding author, e-mail: mrausch@iastate.edu.
[3] USDA–ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State, University, Ames, IA 50011.

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dae)), with resistant strains more likely to disperse from leaf discs containing Bt than susceptible strains. In this case behavior response was correlated with physiological resistance, which suggests larval behavior could be an important factor for insect evolution of resistance to Bt crops.

O. nubilalis is a serious pest of maize in the United States and is managed through planting of Bt maize (Siegfried and Hellmich 2012). Neonates of this pest can disperse from their natal host plant with a strand of silk, either lowering themselves to a nearby plant directly or being carried aloft by the wind. Using this behavior, *O. nubilalis* neonates are capable of extensive interplant movement soon after hatching (Ross and Ostlie 1990). This dispersal has potential implications for IRM as movement of neonates could influence survival in a transgenic landscape (Mallet and Porter 1992, Gould 2000). Specifically, *O. nubilalis* neonates may ingest and recover from a sublethal dose of Bt toxin because they can detect the presence of Bt within 1 h of exposure (Davis and Coleman 1997). The presence of Bt insecticidal toxins influences movement of *O. nubilalis* neonates, with larvae abandoning Bt plants nearly two times more frequently than non-Bt plants (Goldstein et al. 2010). This detection and deterrence may have little effect on resistance evolution when Bt and non-Bt are in spatially segregated blocks, as the interface between transgenic and nontransgenic plants is quite small (limited to the border of the two blocks or completely absent in cases where they are planted in different fields). However, movement may become important when Bt and non-Bt plants are interspersed in a seed mixture (i.e., a blended refuge). In this scenario, movement of insects between transgenic and nontransgenic plants is likely because non-Bt refuge plants are surrounded by Bt plants. The ingestion and recovery of a sublethal dose of Bt toxin combined with the behavioral biology of *O. nubilalis* has the potential to cause resistance to evolve more rapidly in this scenario (Davis and Onstad 2000, Gould 2000).

For *O. nubilalis*, the larval behaviors of plant abandonment and establishment may be governed by a genetic component (Goldstein et al. 2010). Past research has found that 50% of neonates disperse before tasting maize tissue (Davis and Onstad 2000). Behaviors in many animals are linked to specific genes; for example, locomotory behavior in *Drosophila melanogaster* Meigen is affected by the genes *foraging* (*for*) (NCBI reference sequence NM_001169387.1), and *slowmo* (*slmo*) (NCBI reference sequence NM_001043629.1; Kalderon and Rubin 1989, Caldwell et al. 2003). An ortholog of *slowmo*, called *Onslmo* (NCBI reference sequence HQ116696.1), is found in *O. nubilalis*, and an ortholog of the *foraging* gene also occurs in *O. nubilalis* (Kroemer et al. 2011).

foraging encodes a cGMP-dependent protein kinase (PKG) in *D. melanogaster* (Kalderon and Rubin 1989). Kinases are enzymes that transfer phosphate groups to specific substrates (usually enzymes) modifying their activity, and PKG is known to affect food foraging behavior in larvae and adult *D. melanogaster* (Engel et al. 2000). Behavioral polymorphisms have been identified in this species that exist in natural populations at appreciable levels, which produce the behavioral phenotypes Rover and Sitter (Pereira and Sokolowski 1993). Rover phenotypes move a greater distance when feeding than Sitter phenotypes (Sokolowski 1980). Osborne et al. (1997) documented that these two behavioral phenotypes are influenced by the *foraging* gene. Enzyme assays on fly heads showed that behavior is correlated with PKG activity, with Rovers showing much higher enzyme activity than Sitters. To further test if PKG is causally related to activity, these authors overexpressed the *foraging* gene in Sitter larvae. The transgenic Sitter larvae then exhibited the Rover behavioral phenotype. It was also noted that these two phenotypes did not differ in their behavior in the absence of food (Pereira and Sokolowski 1993). The *foraging* gene could potentially influence *O. nubilalis* propensity to abandon the host plant, via silking, by influencing this food search behavior.

The *slowmo* gene encodes a mitochondrial protein of unknown function and has been shown to influence several aspects of behavior though peristaltic muscle contractions in insect larvae (Caldwell et al. 2003). A peristaltic muscle contraction propagates as a wave of contraction and relaxation along the body of the insect and is the chief mechanism of movement for many insect larvae. Genes influencing the ability of insects to create these muscle contractions could influence behaviors associated with movement. Carhan et al. (2004) found that *D. melanogaster* with mutations in the *slowmo* gene showed a significant reduction in the number of peristaltic muscle contractions, reduced movement, and reduced the ability to recover from being flipped onto their dorsal surface. The protein product of *slowmo* is associated with mitochondria, and it is possible that this gene influences muscle contractions through production of adenosine triphosphate or intracellular calcium levels, both of which are crucial for proper muscle contraction and coordination. Genes that affect movement, either through energy production or muscle physiology, could influence plant abandonment and establishment behavior in *O. nubilalis*.

In this study, we placed neonate larvae on non-Bt maize plants to determine if a response to selection could be elicited for plant abandonment (via silking within 4 h of placement on plants) and plant establishment (neonates present on plant after 24–72 h). In addition, we conducted quantitative real-time–polymerase chain reaction (qRT-PCR; Heid et al. 1996) to identify expression profiles and determine if differences in gene expression for *Onslmo* and *foraging* were associated with any response to selection. The results of this study expand the current knowledge on insect behavior and provide insights into the long-term effectiveness of non-Bt refuges to delay resistance by insects to Bt crops.

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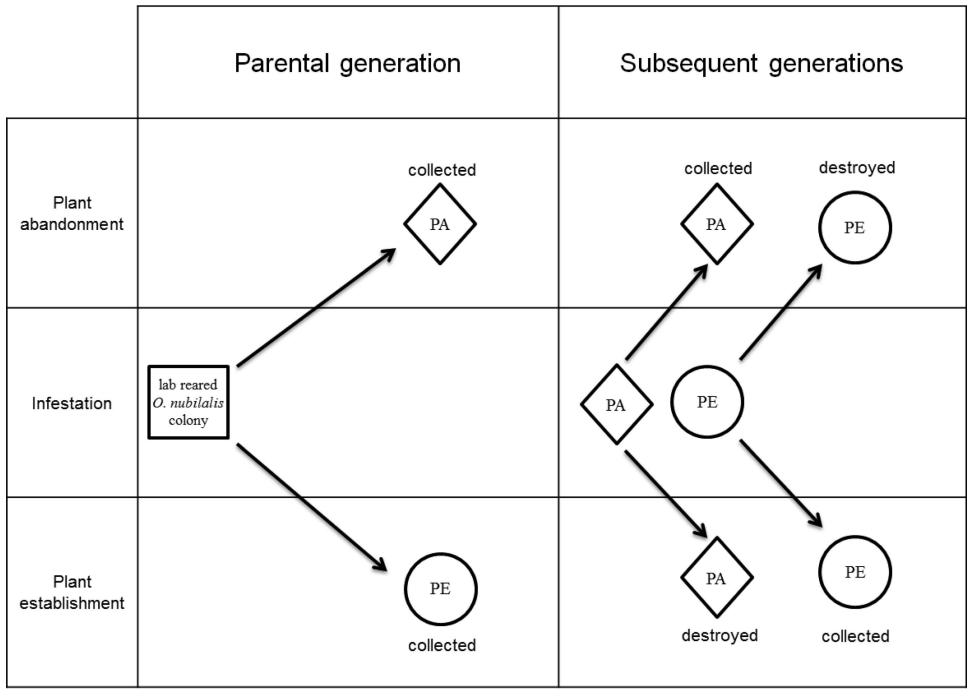

Fig. 1. Selection procedure: in the parental generation neonates exhibiting plant abandonment and plant establishment were collected and reared separately to found PA and PE colonies. In subsequent generations, neonates from the PA colony exhibiting plant abandonment behavior were tallied and collected; those exhibiting plant establishment behavior were tallied and destroyed. Neonates from the PE colony exhibiting plant abandonment behavior were tallied and destroyed and those exhibiting plant establishment were tallied and collected.

Materials and Methods

***O. nubilalis* Rearing and Selection Experiment.** The European corn borer colony used in this study was established from ≈350 adult females collected in the field using traps with an UV light source. This colony was maintained for 1 yr before this study was initiated at the U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS), Corn Insects and Crop Genetics Research Unit (Iowa State University, Ames, IA). Larvae were fed a meridic wheat germ diet that contained Fumigillin-B (Medivet Pharmaceuticals Ltd., High River, AB, Canada) to prevent microsporidial growth (Lewis and Lynch 1969), and larvae were reared in an environmental chamber (27°C, 50% relative humidity [RH], and a photoperiod of 24:0 [L:D] h). The constant lighting in the chamber ensured larvae did not enter diapause and allowed for the accurate prediction of egg emergence. Adults were allowed to emerge in wire mesh cages (Guthrie 1997) in an environmental chamber with a cyclic photoperiod (photoperiod of 16:8 [L:D] h) and controlled temperatures (27°C and 50% RH). Wax paper was provided as an oviposition substrate and changed daily.

We used non-Bt maize plants (33D31, Pioneer, Johnson, IA) that were transplanted from the field into 3.8-liter black pots (model Elite 300, ITML Horticultural Products Inc., Earth City, MO) with potting soil (Sta-Green all-purpose potting mix plus fertilizer, Lowes, Item no. 97889). Plants where then placed under fluorescent growth lights (6500K T5 bulbs, SunBlaze 44) in a controlled chamber (27°C, 59% RH, and a photoperiod of 16:8 [L:D] h). Water trays were placed under the plants to provide a water source for the plant and maintain humidity. After transplanting from the field, maize whorls were flushed with water from a garden hose and leaves were wiped down with a damp paper towel to remove possible insects on the plants.

For the parental generation, eggs (≥20 per egg mass) were collected from the USDA–ARS *O. nubilalis* colony. Two repetitions of initial infestation (dates: 2 and 9 July 2010) and selection were conducted (15 plants in each replicate, 30 total). Four to six egg masses were pinned on maize plants (V6 or V7) on the underside of the highest leaf with a complete leaf collar or the penultimate leaf (whichever was in a condition preferable for observations). Infested plants were inspected for neonates abandoning the plant via silking every 15–20 min for 4 h. Because of the small size of neonates, it was not possible to observe them feeding on the maize leaves at this time. Silking larvae were collected with a fine camel-hair brush and used to initiate the plant abandonment (PA) colony. After 24–72 h, the plants were dissected and remaining larvae were collected and these insects were used to initiate the plant establishment (PE) colony (Fig. 1).

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Eggs collected from the colonies were used in subsequent generations of infestation and selection as described above. However, neonates from the PE colony that were abandoning their host plant (observed every 15–20 m during a 4-h period) were tallied and destroyed; and neonates from the PA colony that were remaining on their host plant (dissected 24–72 h later) were tallied and destroyed (Fig. 1). After all on-plant data were collected during each generation, egg masses were collected from plants and the number of hatched eggs counted to determine the number of neonates per plant. From the number of emerged neonates, percentages of silking and established neonates were calculated for each phenotype. Throughout the experiment, the number of plants used for selection at each generation differed. This was because of 1) the number of maize plants available that met the conditions of the experiment and 2) the number of egg masses obtained were ≥20. However, the number of plants at each generation was never <11.

PA and PE neonates were reared to adulthood in separate plastic rearing cages (9 cm in height, 8 cm in diameter, and 0.44-liter capacity, Pioneer Plastics North Dixon, KY) containing artificial diet and placed in an environmental chamber (27 ± 0.2°C, 50% RH, and a photoperiod of 24:0 [L:D] h). For generations 1 through 3, individual pupae were removed and placed into plastic cups with plastic lids (2.5 cm in height, 4-cm in diameter, injection-molded 18.5-ml cups Anderson Tool and Die, Linden, NJ) and maintained at the same conditions. Adults of the same colony (PA or PE) were placed in screen breeding cages (Guthrie 1997), with ≈30 adults in each cage, and allowed to randomly mate. Water was provided though damp absorbent cotton placed at the bottom of the cage. For generations 4–7, adults of the same colony (PA or PE) were allowed to emerge in a screen cage, with a single cage used to house adults for each colony. The purpose of this change was to minimize the time required to maintain the colonies and provide a larger area for adults. Water was provided by lightly spraying the cages daily. For both these rearing methods, wax paper on the top of cage was provided as an oviposition site, and wax paper was changed daily.

At generation 4 (October 2010), at the end of the maize growing season, neonates were induced into diapause. Following selection, we took >300 larvae from generation 3 of the PA and PE colonies and reared them on artificial diet in a diapause-inducing chamber (23°C, 60% RH, and a photoperiod of 13:11 [L:D] h). When individuals reached the fifth instar, they were placed in a diapause maintaining chamber (8°C, 60% RH, and a photoperiod of 2:22 [L:D] h). The following spring (May 2011), insects were brought out of diapause and their progeny were used to continue selection for two more generations. This resulted in colonies that underwent selection for three generations during summer of 2010, did not undergo selection at generation 4, and underwent selection for two additional generations (F5 and F6) during summer 2011, for a total of five generations of selection.

Percentages of silking and established neonates were transformed by the arcsine square-root function. Statistics were performed in JMP, version 10 (SAS Institute Inc., Cary, NC, 1989–2012) using a multivariate analysis of variance repeated measure analysis of the silking and established phenotypes in both colonies over generations of selection. Factors in the analysis included colony as the main effect and generation as the repeated measure. Comparison of the colonies within a generation was accomplished using a Student's *t*-test.

Construction of Expression Profiles of *foraging* and *Onslmo*. Egg masses were collected from adults of the PA and PE colonies during the sixth generation and from a colony initiated in 2010 with field-collected adults, ≈350 adult females collected from field traps with an UV light source, 12 continuous generations laboratory reared. To ensure that egg masses were roughly the same age, fresh wax paper was provided in the evening, between 8–10 p.m. After 1 h (defined as hour 0 in presentation data), wax paper was collected, providing egg masses that were <1 h old.

Egg masses were placed in an environmental chamber (27°C, 50% RH, and a photoperiod of 24:0 [L:D] h) and were allowed to develop. After 0 h (i.e., initial collect of eggs), samples were collected in 24-h increments as follows: 24, 48, 72, and 96 h. At these age periods, two egg masses were placed in RNase-free 1.5-ml microcentrifuge tubes (MAX-815, Phoenix Research Products, Phoenix, AZ), frozen in liquid nitrogen and ground to a fine powder using RNase-free pestles, with a separate pestle used for each sample. Immediately after grinding, 500 μl of Trizol (Invitrogen, Carlsbad, CA) was added and samples were immersed in liquid nitrogen and stored at −70°C. In addition, larvae were allowed to hatch from several egg masses (under same conditions as described above), and these neonate larvae were placed in petri dishes containing non-Bt maize leaf tissue for 4 h. Samples of 16 neonates from each colony and a nonselected laboratory colony (F12) also were processed following the same methods used with eggs. Four samples were taken at each time period in three independent trials.

RNA was isolated following the Trizol procedure provided by the manufacturer (Invitrogen, Carlsbad, CA). RNA was isolated from each trial on a different day to ensure that they were independent. Oligo-dT primer and Moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, WI) were used to perform cDNA synthesis reactions on all samples using 2 μg of total RNA.

Relative qRT-PCR was conducted according to Kroemer and Webb (2006) to determine differences in quantitative expression profiles in these genes. Reactions were performed on a Bio-Rad MyIQ real-time PCR thermalcycler. Gene specific primers were used for *foraging* (qFW GAG ACA CAC TAC CAG AAC GGC, qRV CTG CTA TCT CCC TCG TCC TTG), and *Onslmo* (qFW AAG CAG CGT GGA GGA AAT ATC CCA, qRV TCC AAT AAG TGC TTG GGC CCA TCT). One microliter of 1:10 diluted cDNA from each

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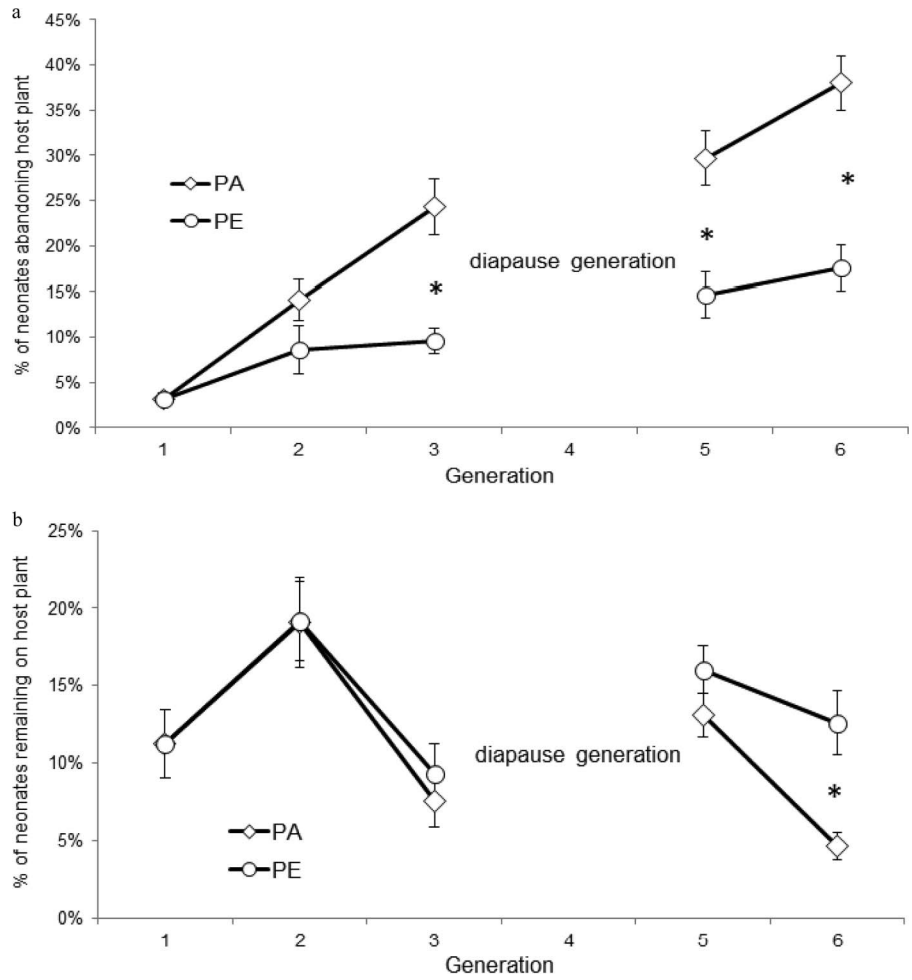

Fig. 2. Percentage of European corn borer neonates from the PA and PE colonies observed abandoning maize plants within the first 4 h of infestation (mean ± SE) over generations 1–3, 5, and 6. (b) Percentage of larvae from the same two colonies observed remaining on plants for at least 48 hours (mean ± SE) over generations 1–3, 5, and 6. An asterisk indicates a significant difference between the PA and PE colonies at a generation.

sample in a trial was subjected to 50 rounds of PCR in the presence of SYBR green dye (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. Standards of cDNA were prepared from serial dilutions (10-fold) of 0.001 μg from combined pools of each trial. Fluorescence intensity was measured after each round of PCR. Mean threshold PCR cycles of our samples were calculated from the three independent trials and normalized to the quantities of 18S rRNA (RPS 3; Li et al. 2005). Starting quantities for each sample were calculated by using the linear standard equation formulated from starting quantities and the mean log threshold fluorescence values obtained from standards. Each trial was done in triplicate for each candidate gene to confirm results; outliers were removed from the data. Data were combined to create expression profiles for the mean threshold PCR cycles of each candidate gene to compare RNA expression.

Statistical analyses of expression were conducted using JMP 10 to construct a restricted maximum likelihood mixed model with main effects colony, time, colony × time, and with trial treated as a random effect. Means were compared using a Tukey's honestly significant difference (HSD) test (Tukey 1953).

Results

Selection Experiment. Selection for plant abandonment and plant establishment were conducted for six generations (Fig. 1). When neonates from the PA colony were tested for plant abandonment (<4 h posteclosion) at each generation, the percentage that abandoned plants increased over generations (Fig. 2A). Percentage of neonates from the PE colony that abandoned the plants also increased over generations, but at a lower rate (Fig. 2A). A significant generation by phenotype interaction shows that the PA and PE phenotypes diverged across generations (F (4, 20) = 0.73; P = 0.0214), with the PA colony having a greater increase in propensity to abandon the host

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plant over generations. In addition, significant differences were present between the PA and PE strains in the proportion of insects abandoning plants at the third ($t(17) = 2.07$; $P = 0.0002$), fifth ($t(28) = 2.05$; $P = 0.0004$), and sixth generation ($t(28) = 2.05$; $P < 0.0001$), with the PA colony having a greater percentage of neonates abandoning the host plant.

When propensity to remain on the plant was analyzed, the generation by phenotype interaction was not significant ($F(4, 20) = 0.43$; $P = 0.1114$), but generation ($F(4, 20) = 9.40$; $P < 0.0001$) and phenotype ($F(1, 23) = 7.91$; $P = 0.0099$) were significantly different. There were no significant differences between larvae from the PA and PE colonies until the sixth generation ($t(26) = 2.05$; $P = 0.0008$) (Student's *t*-test) with the PE colony having a greater percentage of neonates remaining on the host plant (Fig. 2B).

qRT-PCR Analysis. The expression results for the *foraging* and *Onslmo* genes showed very high expression in the PA colony at the 0 h sampling time relative to the other age periods and the first instars exposed to maize tissue (Fig. 3). PA and PE colonies differed in the level of expression of these two genes (*foraging:* Colony $F(2, 142) = 4.82$; $P = 0.0095$. *Onslmo:* Colony $F(2, 142) = 30.28$; $P < 0.0001$), that the level of expression differed across age of the egg masses (*foraging:* Age $F(5, 142) = 6.05$, $P < 0.0001$. *Onslmo:* Age $F(5, 142) = 24.95$; $P < 0.0001$), and that the colonies differed in how expression changed across age of the egg masses (*foraging:* Age X Colony $F(10, 142) = 7.44$; $P < 0.0001$. *Onslmo:* Age X Colony $F(10, 142) = 42.72$; $P < 0.0001$). Comparison of means using a Tukey's test revealed that expression of *foraging* in the PA colony, at the 0 h sampling time, was significantly higher than all the other age periods and other colonies (PE and laboratory colony).

Discussion

There was a clear response to selection for plant abandonment in our PA colony, as the percentage of neonates silking from corn plants remained high and consistently increased over generations. In contrast, the response to selection for plant establishment seen in the PE colony was weak. Propensity of the PE colony to remain on the plant was only significantly greater than the PA colony at the sixth generation. The gene expression results from qRT-PCR analysis showed that among egg masses collected immediately after egg deposition, expression of *foraging* and *Onslmo* was significantly higher in the PA colony compared with the PE and laboratory colonies. Previous studies have found that these genes are associated with insect foraging and movement, which makes them likely candidates in the genetic determination of *O. nubilalis* plant abandonment behavioral phenotypes (Sokolowski 1980, Pereira and Sokolowski 1993, Osborne et al. 1997, Ben-Shahar et al. 2002, Carhan et al. 2004). Overall, these responses to selection, combined with our gene expression data, suggest that the behavioral trait for plant abandonment is likely to be under genetic control and that the genes *foraging* and *Onslmo* are involved. Although other studies have described *O. nubilalis* behavior on transgenic and nontransgenic plants (Ross and Ostlie 1990, Davis and Coleman 1997, Goldstein et al. 2010, Razze et al. 2011, Razze and Mason 2012), this is the first study that has specifically selected for these traits over several generations and analyzed behavior genes to elucidate genetic changes.

The *foraging* and *slowmo* genes influence behavior in *D. melanogaster* and have orthologs in *O. nubilalis.* Both of these genes had higher expression in the PA colony immediately after egg deposition than in the PE and laboratory colonies. Previous studies of the *foraging* gene have demonstrated that higher gene expression results in changes in behavior. *D. melanogaster* have Rover and Sitter phenotypes in natural populations, with the Rovers moving a greater distance while feeding and exhibiting higher expression of *foraging* (Sokolowski 1980, Pereira and Sokolowski 1993). The Sitter phenotype can be converted into the Rover phenotype by overexpressing the *foraging* gene (Osborne et al. 1997). In honey bees, *Apis mellifera* L., the transition from brood care to food gathering behavior in worker bees has been correlated with increased expression of the *foraging* gene (Ben-Shahar et al. 2002). These studies provide compelling evidence that the *foraging* gene influences behaviors associated with food searching in insects, with increased expression resulting in increased dispersal in the presence of a food source. Our PA colony has a higher propensity to leave the natal host plant and has much higher expression of the *foraging* gene than the PE colony. Because *foraging* influences food search behavior and abandoning the natal plant via silking is a form of food search behavior, it is likely that this gene may be involved with plant abandonment. The results of our selection experiment and genetic analysis on silking behavior in *O. nubilalis* appear to concur with other studies, suggesting that the *foraging* gene is involved with food-searching behaviors. Future research should focus on artificially influencing the expression of *foraging* in *O. nubilalis* neonates and determine any changes in dispersal behavior.

In *slowmo,* a mitochondrial-associated protein, artificial point mutations result in a reduction of peristaltic muscle contractions and reduced movement in *D. melanogaster* neonates. However, overexpression of this gene does not result in changes in locomotory behavior (Carhan et al. 2004). Currently, there are no known natural behavioral phenotypes associated with the *slowmo* gene, thus, it is not possible to draw comparisons with its influence on silking behavior in *O. nubilalis.* Although previous work has not found expression differences in *slowmo* to influence behavior, the involvement of this gene on silking behavior should not be discounted, and should be the focus of future research.

The genetic differences isolated in our colonies were found immediately after egg deposition, the 0-h time period. There are two possible explanations for

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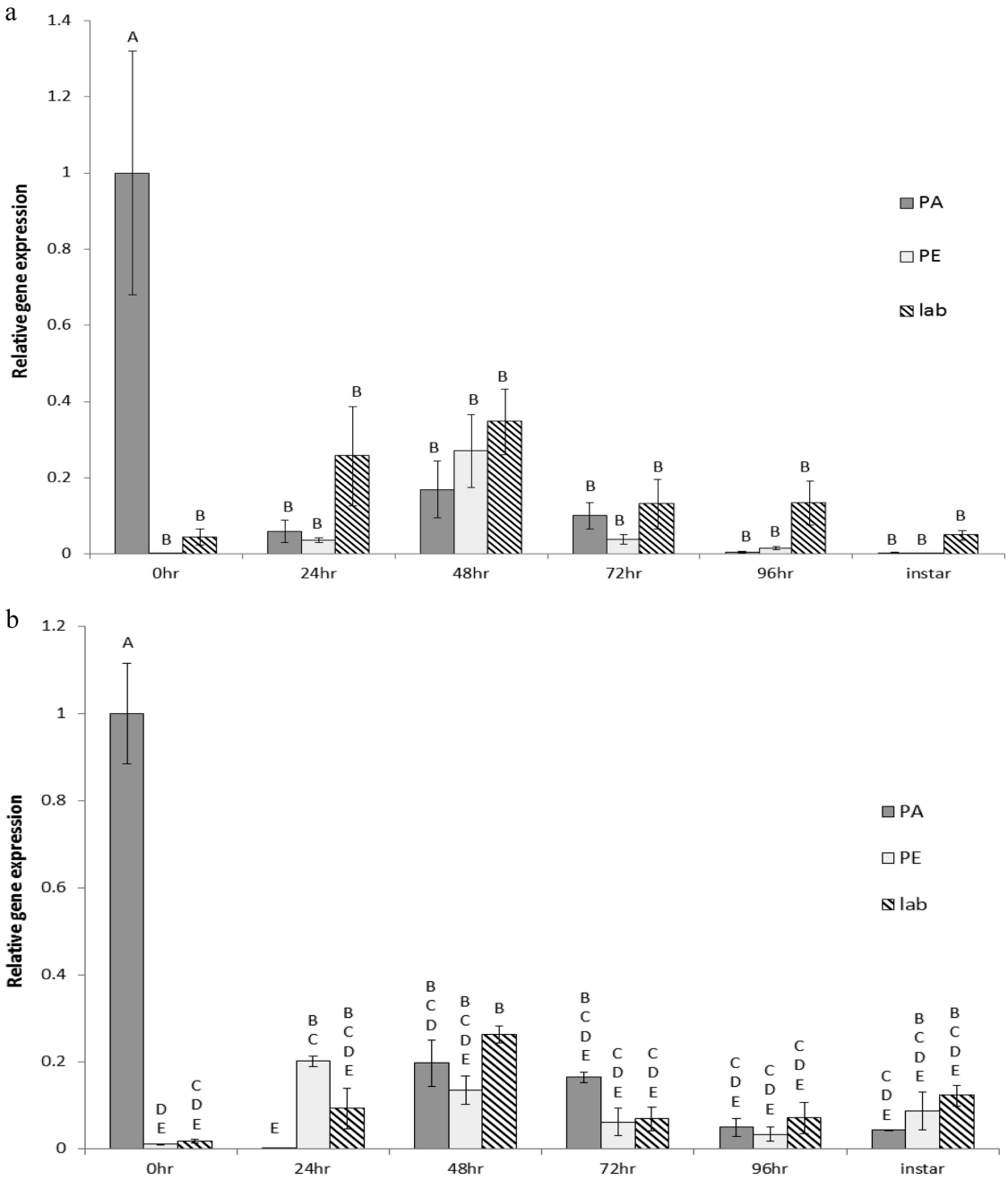

Fig. 3. qRT-PCR analysis of (a) *foraging* and (b) *Onslmo* gene expression on two egg masses collected at several time periods after putative 0 hr and 16 first-instar neonates exposed to maize tissue for 4 h (mean ± SE). The data for each time period are normalized relative to a ribosomal control gene. Means that do not share the same letter are significantly different.

the high expression seen at this time, 1) the developing embryo is exhibiting high levels of transcription of those genes, or 2) during egg development the female is imparting numerous mRNA to the embryo, which is detected as high gene expression. This imparting of mRNA by females, known as the maternal effect, could explain inheritance of the plant abandonment trait. In addition, females may be conferring a benefit to their offspring by donating mRNA. Extensive transcription of genes for the plant abandonment phenotype can be energetically costly, but through the maternal effect, developing embryos can avoid this cost.

The results of our selection experiment suggest that dispersal of *O. nubilalis* neonates could evolve in the field and in turn influence the evolution of resistance to Bt maize. It has been previously documented that

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O. nubilalis neonates can survive a sublethal dose of Bt toxin in transgenic maize (Davis and Coleman 1997) and this sublethal exposure results in a deterrent effect, with neonates abandoning Bt plants more often than non-Bt plants (Goldstein et al. 2010). Neonates abandoning Bt plants and successfully reaching non-Bt plants would escape the high dose of toxin, hastening resistance evolution by increased survival of resistant heterozygotes (Mallet and Porter 1992). These effects, however, will depend on how the refuge is distributed in a field. In a blocked refuge strategy there should be little effect as the proximity between Bt and non-Bt plants is limited to the border of the two blocks, or completely absent when the refuge is planted in another field. The probability of neonates abandoning Bt plants and successfully reaching refuge plants is quite low in this scenario. However, in a mixed seed refuge, where refuge plants are randomly distributed in a field, a refuge plant is surrounded by Bt plants, creating much higher proximity between the refuge and Bt plants. In this case, individuals abandoning natal Bt plants would have the potential to encounter a refuge plant and survive. Previous work by other authors also has suggested that seed mixtures may be less durable than blocks for managing insect species with high dispersal capabilities (Davis and Onstad 2000, Gould 2000). The results presented here provide a genetic mechanism through which dispersal may evolve in a blended refuge, and consequently, influence the evolution of resistance to Bt corn.

Although the scenario described above would appear to favor selection for plant abandonment behavior, this would only influence resistance evolution if the behavior occurred directly in response to ingestion of Bt toxin. If emerging neonates abandoned Bt plants and non-Bt plants equally, this would result in a rerandomizing of neonates in the field. Selection for certain behavioral traits would only influence resistance evolution if it were linked with neonate response to Bt. It has been demonstrated that Bt-susceptible *O. nubilalis* larva can detect the presence of Bt toxins upon ingestion (Davis and Coleman 1997) and are two times more likely to abandon Bt plants (Goldstein et al. 2010), resulting in potential increased survival of resistant heterozygotes. Furthermore, physiological resistance may confer heightened behavioral response to Bt. Hoy and Head (1995) discovered that Bt resistant *L. decemlineata* larvae are more responsive to the presence of Bt toxin than susceptible beetles. In a graded-series of Bt toxin concentrations, resistant beetle larva were more likely to move from leaf discs containing high amounts of Bt to leaf discs containing zero amounts of Bt. This correlation with behavioral response and physiological resistance suggests that resistant beetle larva on transgenic plants are more likely to move to nontransgenic plants, resulting in indirect selection for physiological resistance (Hoy and Head 1995). It is currently unknown if physiologically resistant *O. nubilalis* larva exhibit an increased behavioral response to Bt.

The ability of Bt-susceptible and -resistant *O. nubilalis* larva to successfully disperse from Bt plants and establish on non-Bt plants may be compromised, limiting the effects on seed mixtures. Resistance to Bt may carry fitness costs, which could be in the form of reduced mobility. In addition, although susceptible *O. nubilalis* larva can survive a sublethal dose of Bt, they suffer reduced long-term survival, and this may limit the ability of larva abandoning transgenic plants to establish on nontransgenic plants. Future research should focus on dispersal capabilities of Bt-resistant larva and larva plant establishment following Bt ingestion.

Davis and Onstad (2000) also have speculated that extensive use of seed mixtures could influence dispersal capabilities in *O. nubilalis.* Genes influencing plant abandonment behavior, such as *foraging* and *slowmo*, would likely be involved in this process. Other studies have shown neonates disperse more from Bt versus non-Bt plants (Ross and Ostlie 1990, Davis and Coleman 1997, Goldstein et al. 2010, Razze et al. 2011, Razze and Mason 2012).

Our hypothesis that dispersal by neonate larvae of *O. nubilalis* is under at least partial genetic control is supported by the results of our selection experiment and gene expression profile data. The evolution of the propensity to disperse could in turn affect the evolution of resistance to transgenic plants in the field. Previous modeling studies have taken into account insect dispersal and how it could influence resistance evolution, but alterations in insect dispersal as a result of selection have not been considered (Davis and Onstad 2000). The data from our study could be used to construct more accurate models of field resistance evolution and hence be used to optimize strategies for preserving Bt crops.

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References Cited

Ben-Shahar, Y., A. Robichon, M. B. Sokolowski, G. E. Robinson. 2002. Influence of gene action across different time scales on behavior. Science 296: 741–744.

Bolin, P. C., W. D. Hutchison, and D. A. Andow. 1999. Long-term selection for resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in Minnesota population of European corn borer (Lepidoptera: Crambidae). J. Econ. Entomol. 92: 1021–1030.

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Caldwell, J. C., M. M. Miller, S. Wing, D. R. Soll, and D. F. Eberl. 2003. Dynamic analysis of larval locomotion in *Drosophila* chordotonal organ mutants. Proc. Natl. Acad. Sci. U.S.A. 100: 16053–16058.

Carhan, A., S. Reeve, C. T. Dee, and R. A. Baines. 2004. Mutation in *slowmo* causes defects in *Drosophila* larval locomotor behavior. Invertebr. Neurosci. 2: 65–75.

Carrière, Y., C. Ellers-Kirk, M. S. Sisterson, L. Antilla, M. Whitlow, T. J. Dennehy, and B. E. Tabashnik. 2003. Long-term regional suppression of pink bollworm by Bacillus thuringiensis cotton. Proc. Natl. Acad. Sci. U.S.A. 100: 1519–1523.

Cattaneo, M. G., C. Yafuso, C. Schmidt, C. -Y. Huang, M. Rahman, C. Olson, C. Ellers-Kirk, B. J. Orr, S. E. Marsh, L. Antilla, et al. 2006. Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. Proc. Natl. Acad. Sci. U.S.A. 103: 7571–7576.

Crespo, A. L., T. A. Spencer, A. P. Alves, R. L. Hellmich, E. E. Blankenship, L. C. Magalhaes, and B. D. Siegfried. 2009. On-plant survival and inheritance of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrinia nubilalis.* Pest Manage. Sci. 10: 1071–1081.

Davis, P. M., and S. B. Coleman. 1997. European corn borer (Lepidoptera: Pyralidae) feeding behavior and survival on transgenic maize containing cry1A(b) protein from *Bacillus thuringiensis.* J. Kans. Entomol. Soc. 70: 31–38.

Davis, P. M., and D. W. Onstad. 2000. Seed mixtures as a resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic maize expressing Cry1Ab protein. Entomol. Soc. Am. 93: 937–948.

Dhurua, S., and G. T. Gujar. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) from India. Pest Manage. Sci. 67: 898–903. doi: http://dx.doi.org/10.1002/ps. 2127.

Engel, J. E., X. J. Xie, and M. B. Sokolowski. 2000. A cGMP-dependent protein kinase gene, *foraging*, modifies habituation-like response decrement of the giant fiber escape response circuit in *Drosophila.* Learn Mem. 7: 342–352.

Ferré, J., and J. Van Rie. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 47: 501–533.

Ferré, J., J. Van Rie, S. C. MacIntosh. 2008. Insecticidal genetically modified crops and insect resistance management (IRM), pp. 41–85. *In* J. Romeis, A. M. Shelton, G. G. Kennedy (eds.), Integration of Insect Resistant Genetically Modified Crops Within IPM Programs. Springer, New York, NY.

Gassmann, A. J., Y. Carriere, and B. E. Tabashnik. 2009. Fitness costs of insect resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 54: 147–163.

Gassmann, A.J.J., L. Petzold-Maxwell, R. S. Keweshan, M. W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6: e22629.

Goldstein, J. A., C. E. Mason, and J. Pesek. 2010. Dispersal and movement behavior of neonate European corn borer (Lepidoptera: Crambidae) on non-Bt and transgenic Bt maize. J Econ. Entomol. 103: 331–339.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Gould, F. 2000. Testing Bt refuge strategies in the field. Nat. Biotechnol. 18: 266–267.

Guthrie W. D. 1997. Advances in rearing the European corn borer on a meridic diet, pp. 46–59. *In* Toward Insect Resistant Maize for the Third World. Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico.

Heid, C. A., J. Stevens, K. J. Livak, and P. M. Williams. 1996. Real time quantitative PCR. Genome Res. 6: 986–994.

Hoy, C. W., and G. Head. 1995. Correlation between behavioral and physiological responses to transgenic potatoes containing *Bacillus thuringiensis* delta endotoxin in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 88: 480–486.

Huang, F. N., L. L. Buschman, R. A. Higgins, W. H. McGaughey. 1999. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. Science 284: 965–967.

Huang, J., H. Ruifa, S. Rozelle, and C. Pray. 2005. Insect-resistant GM rice in farmers' fields: assessing productivity and health effects in China. Science 308: 688–690.

Hutchison, W., E. C. Burkness, P. D. Mitchell, R. D. Moon, T. W. Leslie, S. J. Fleischer, M. Abrahamson, K. L. Hamilton, K. L. Steffey, M. E. Gray, et al. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330: 222–225.

James, C. 2011. Global Status of Commercialized Biotech/GM Crops: *ISAAA Brief* No. 43. ISAAA: Ithaca, NY.

Kalderon, D., and G. M. Rubin. 1989. cGMP-dependent Protein Kinase genes in *Drosophila.* J. Biol. Chem. 264: 10738–10748.

Kroemer, J. A., and B. A. Webb. 2006. Divergences in protein activity and cellular localization within the *Campoletis sonorensis* Ichnovirus Vankyrin family. Am. Soc. Mircrobiol. 80: 12219–12228.

Kroemer, J. A., T. Nusawardani, M. A. Rausch, S. E. Moser, and R. L. Hellmich. 2011. Transcript analysis and comparative evaluation of *shaker* and *slowmo* gene homologs for European corn borer, *Ostrinia nubilalis.* Insect Mol. Biol. 20: 493–506.

Lewis, L. C., and R. E. Lynch. 1969. Rearing the European corn borer, Ostrinia nubilalis (Hubner), on diets containing maize leaf and wheat germ. Iowa State J. Sci. 44: 9–14.

Li, H., B. Oppert, R. A. Higgins, F. Huang, L. L. Buschman, J. R. Gao, and K. Y. Zhu. 2005. Characterization of cDNAs encoding thee trypsin-like proteinases and mRNA quantitative analysis in Bt-resistant and –susceptible strain of *Ostrinia nubilalis.* Insect Biochem. Mol. Biol. 35: 847–860.

Ma, G., M. Sarjan, C. Preston, S. Asgari, and O. Schmidt. 2005. Mechanics of inducible resistance against *Bacillus thuringiensis* endotoxins in invertebrates. Insect Sci. 12: 319–330.

Mallet, J., and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Lond. B 250: 165–169.

Osborne, K. A., A. Robichon, E. Burgress, S. Butland, R. A. Shaw, A. Coulthard, H. S. Pereira, R. J. Greenspan, and M. B. Sokolowski. 1997. Natural behavioral polymorphism due to a cGMP-dependent protein kinase of *Drosophila.* Science 277: 834.

Pereira, H. S., and M. B. Sokolowski. 1993. Mutations in the larval foraging gene affect adult locomotory behavior after feeding in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. 90: 5044–5046.

Pereira, E. J., B. A. Lang, N. P. Storer, and B. D. Siegfried. 2008. Selection for Cry1F resistance in the European corn borer and cross-resistance to other Cry toxins. Entomol. Exp. Appl. 126: 115–121.

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Razze, J. M., and C. E. Mason. 2012. Dispersal Behavior of Neonate European Corn Borer (Lepidoptera: Crambidae) on Bt Corn. J. Econ. Entomol. 105: 1214–1223.

Razze, J. M., C. E. Mason, and T. D. Pizzolato. 2011. Feeding behavior of neonate Ostrinia nubilalis (Lepidoptera: Crambidae) on Cry1Ab Bt corn: Implications for resistance management. J. Econ. Entomol. 104: 806–813.

Ross, S. E., and K. R. Ostlie. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field maize. J. Econ. Entomol. 83: 831–836.

Siegfried, B. D., and R. L. Hellmich. 2012. Understanding successful resistance management: the European corn borer and Bt corn in the United States. GM Crops and Food 3: 184–193.

Sokolowski, M. B. 1980. Elucidating the behavioral-phenotype of *Drosophila melanogaster* larvae-correlations between larval foraging strategies and pupation heights. Behav. Genet. 10: 498.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031–1038.

Tabashnik, B. E., N. J. Cushing, N. Finson, and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671–1676.

Tabashnik, B. E., F. Gould, and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasingdominance and heritability. J. Evol. Biol. 17: 904–912.

Tabashnik, B. E., A. J. Gassmann, D. W. Crowder, and Y. Carrière. 2008. Insect resistance to Bt crops: evidence versus theory. Nat. Biotechnol. 26: 199–202.

Tukey, J. W. 1953. The problem of multiple comparisons. *In* H. I. Braun (ed.), The Collected Works of John W. Tukey VIII. Multiple Comparisons. 1948–1983: 1–300. Chapman and Hall, New York, NY.

van Rensburg, J.B.J. 2007. First report of field resistance by stem borer, Busseola fusca (Fuller) to Bt-transgenic maize. S. Afr. J. Plant Soil 24: 147–151.

Wu, K.–M., Y.-H. Lu, H.-Q. Feng, Y.-Y. Jiang, and J.-Z. Zhao. 2008. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321: 1676–1678.

Zhang, H., W. Yin, J. Zhao, L. Jin, Y. Yang, S. Wu, B. E. Tabashnik, and Y. Wu. 2011. Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. PLoS ONE 6: e22874. doi:22810.21371/journal.pone.0022874.

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