

GENERAL INFORMATION

DATA TITLE: Data for: Inheritance and fitness costs for western corn rootworm with laboratory-selected resistance to Gpp34/Tpp35Ab1 corn

DATA ABSTRACT

Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a serious pest of corn and is currently managed with corn hybrids that produce insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt). Bt corn kills rootworm larvae and reduces larval feeding injury to corn roots. The Bt protein Gpp34/Tpp35Ab1, previously named Cry34/35Ab1, has been widely used in transgenic Bt corn for management of western corn rootworm, and field-evolved resistance has been found in some populations. In the United States, the refuge strategy is used to manage Bt resistance, with refuges of non-Bt host plants serving as a source of Bt-susceptible individuals, which in turn reduce the frequency of homozygous resistant individuals within a population. As such, the dominance of resistance strongly influences resistance evolution, with faster evolution of resistance when resistance is not recessive. Additionally, selection for resistance by a Bt crop leads to the accumulation of resistance alleles within refuge populations, thereby reducing the capacity of refuges to delay resistance. However, fitness costs can remove resistance alleles from refuge populations and preserve the dynamic of refuges producing Bt-susceptible genotypes. Bt-susceptible and Gpp34/Tpp35Ab1-resistant western corn rootworm were used to quantify the inheritance and fitness costs of resistance. We found that Gpp34/Tpp35Ab1 resistance was not recessive and had the accompanying fitness costs of slower developmental rate to adulthood and lower egg viability. This research will help improve insect resistance management by providing a better understanding of the risk of western corn rootworm evolving resistance to transgenic corn that produces Gpp34/Tpp35Ab1. Dataset contains tabular data divided between seven .xlsx files.

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This dataset was used in the publication:

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FILE LIST

1. Inheritance.xlsx is the file containing the data for the inheritance assay. The headers of this file are: "WCR_strain," which refers to the strain of western corn rootworm used; "Corn_hybrid" refers to whether a Bt or non-Bt corn hybrid was used to rear the western corn rootworm; "Rep" is the replication number in the assay; "larvae added" is the number of larvae added to each replicate; "adults collected" is the total adults collected, or survival, from each replicate; "Prop. Survival" is the "adults collected" divided by the "larvae added" for each replicate; and "prop corrected survival" is the adults collected for each replicate on Bt corn divided by the adults collected on each corresponding non-Bt replicate for the same wcr-strain with empty cells indicating a blank value. These data are found in Table 1 and Figure 1 of the paper publication.
2. Head Capsule.xlsx is the file containing the data for the head capsule size from the fitness cost assay. The headers of this file are: "WCRstrain," which refers to the strain of western corn rootworm used; "larva added" is the number of larvae added to each replicate; "adults collected" is the total adults collected, or survival, from each replicate; "cage" is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; "Rep" is the replication

number for each strain in the assay; “sex” refers to the sex of each individual beetle determined by looking at basal-tarsal morphology; “Head capsule (um)” is the measurement on the adult head capsule (the outermost boundary between the compound eyes) in micrometers. These data are found in Table 2 and Figure 2 of the paper publication.

3. Longevity.xlsx is the file containing the data for the length of the adult beetles’ lives from the fitness cost assay. The headers of this file are “WCRstrain,” which refers to the strain of western corn rootworm used; “larvae added” is the number of larvae added to each replicate; “adults collected” is the total adults collected, or survival, from each replicate; “cage” is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; “sex” refers to the sex of each individual beetle determined by looking at basal-tarsal morphology; “Days2Dead” is the adult longevity, which was determined for all adults and was calculated by subtracting the average day of adult emergence by sex within a replicate from the day on which each individual died. These data are found in Table 2 and Figure 2 of the paper publication.
4. Survival.xlsx is the file containing the data for the overall survival from the fitness cost assay. The headers of this file are “WCR strain,” which refers to the strain of western corn rootworm used; “Rep” is the replication number for each strain in the assay; “cage” is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; “larva added” is the number of larvae added to each replicate; “females” is the number of adult females collected from each cage; “male” is the number of male adults collected from each cage; “Total adults collected” is the total adults collected, or survival, from each replicate; “sex” refers to the sex of each individual beetle determined by looking at basal-tarsal morphology; “Prop. Survival” is the “total adults collected” divided by the “larva added” for each cage. These data are found in Table 2 and Figure 2 of the paper publication.
5. Fertility.xlsx is the file containing the data for the viability of the eggs collected for each cage from the fitness cost assay. The headers of this file are “AveViability.” Egg viability was calculated as the number of larvae that eclosed divided by the number of eggs evaluated, and this was measured over an average of 3.7 ± 0.8 (mean \pm standard

deviation) cohorts of eggs for each replicate, and averaged to generate the values for “AveViability.” “Colony” refers to the western corn rootworm strain used; “cage” is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; “Rep” is the replication number for each strain in the assay. These data are found in Table 2 and Figure 2 of the paper publication.

6. Developmental Rate.xlsx is the file containing the data for the length of time between the larval to the adult stage from the fitness cost assay. The headers of this file are “WCRstrain,” which refers to the strain of western corn rootworm used; “cage” is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; “Rep” is the replication number for each strain in the assay; “larva number” is the number of larvae added to each replicate; “sex” refers to the sex of each individual beetle determined by looking at basal-tarsal morphology; “totaladults” is the total adults collected, or survival, from each cage; “days2adult” is the developmental rate, which was calculated per replicate as the average number of days between when larvae were added to seedling mats and the emergence of adults for each sex. These data are found in Table 2 and Figure 2 of the paper publication.
7. Fecundity.xlsx is the file containing the data for the number of eggs produced from the fitness cost assay. The headers of this file are “WCR strain,” which refers to the strain of western corn rootworm used; “Rep” is the replication number for each strain in the assay; “total eggs” is the total number of eggs collected from each cage over the entirety of the life span of the cage; “cage” is the cage number, which differs from rep, because this refers to total cages used in the entire experiment while rep is referring to each strain; “total females per cage” refers to the total number of adults collected through emergence for each cage; “Eggs per female” is “total eggs” divided by “total females per cage” which provides the number of eggs per female per cage. These data are found in Table 2 and Figure 2 of the publication.

METHODS AND MATERIALS

Insect Strains

The parental strain of western corn rootworm used in this research was generated by collecting adult rootworm from fields in Dodge City, KS, Caledonia, MN, and Janesville, WI, between 2002 and 2004. These field-collected strains were crossed with a nondiapausing laboratory strain at USDA-ARS Plant Genetic Research Laboratory in Colombia, MO, and subsequently pooled in 2009 to generate a parental strain ([Meihls et al. 2008](#), [Deitloff et al. 2016](#)). In 2010, this parental strain was sent to Iowa State University where it was divided into two strains: 1) Bt-susceptible strain and 2) Bt-resistant strain.

The Bt-susceptible strain was maintained on non-Bt corn and was never exposed to Bt corn. The Bt-resistant strain was selected for resistance on Gpp34/Tpp35Ab1 corn by rearing larvae from neonate to pupation on seedling mats of Gpp34/Tpp35Ab1 corn (event 59122-7; Corteva Agriscience, Indianapolis, IN) for 26 generations between the F1 and F47, and then rearing continuously on Gpp34/Tpp35Ab1 corn for eight generations (F48 to F55). Additionally, the Bt-resistant strain was back-crossed with the Bt-susceptible strain four times (F27, F36, F43, and F44) to increase the genetic similarity between the resistant and susceptible strains. Before initiating experiments, the Bt-resistant strain was reared on non-Bt corn for three generations (F56, F57, and F58) to avoid any sub-lethal maternal or paternal effects of Bt corn. Both strains were maintained at an average population size of between 2,000 and 2,500 adults per generation, although the population size of the Bt-resistant strain tended to be somewhat smaller than this average population size during generations of selection (~1,750 adults per generation). Population sizes were smaller during generations of selection because Bt corn killed larval insects.

Rearing of Strains

Adult insects were held in mesh cages (30 × 30 × 30 cm, MegaView Science Co. Ltd., Taichung, Taiwan) housed in a biological incubator (I41-LL Percival Scientific, Perry, IA; 25°C; 16:8 [L:D] hr). Adults were provided with a complete adult diet (Bio-Serv, Frenchtown, NJ), 1.5% agar solid as a source of water, and corn leaf tissue. Food and water resources were replaced three times per week. Eggs were collected in an oviposition dish, which was prepared by covering the bottom of a 10 cm Petri dish with a 5 mm layer of sieved field soil (<80 mesh

particle size). A piece of aluminum foil was then loosely placed over the Petri dish to generate a darkened environment to stimulate oviposition. After 6–10 d, eggs were washed from the soil in a 60-mesh sieve. Eggs were then added to seedling mats following [Ingber and Gassmann \(2015\)](#). Briefly, seedling mats contained 25 ml corn seeds, 80 ml water, and 500 ml of a 1:1 mixture of field soil and potting medium (Sunshine LC1 Professional Growing Mix, SunGro Horticulture, Vancouver, BC, Canada), and were held in a 1L clear plastic container with a lid (Dart C32DE, Dart Container Corporation, Mason, MI). Eggs were added to seedling mats at a density of 900 eggs per seedling mat during generations of selection with Bt corn, and a density of 600 eggs per seedling mat during generations on non-Bt corn. These smaller seedling mats were inverted, removed from their containers, and placed on top of larger seedling mats, which were held in 6 liter plastic boxes with lids (Sterlite 1642, Sterlite Corporation, Townsend, MA). Larger seedling mats contained 120 ml corn seeds, 500 ml water, and 3 liters of the soil mixture; and larvae completed development in these larger seedling mats. The larger seedling mats were at least 7 d old before the smaller seedling mats were transferred onto them, and larger seedling mats contained the same type of corn seed (i.e., non-Bt corn or Gpp34/Tpp35Ab1 corn) used in smaller seedling mats. A fabric cover under the lid was used to prevent larvae and adults from escaping. Upon emergence, adults were collected using an aspirator attached to a vacuum pump (1531-107B-6557x, Gast Manufacturing Inc., Benton Harbor, MI.) and adults were then placed inside a mesh cage.

DATA COLLECTION METHODS

This experiment was conducted between December 2019 and April 2020 and used the F58 of the Bt-resistant strain and the F60 of the Bt-susceptible strain. Two heterozygous crosses were made by reciprocally crossing unmated adults from the Bt-resistant and Bt-susceptible strains (i.e., resistant ♂ × susceptible ♀; susceptible ♂ × resistant ♀). Adults used for crosses were collected from non-Bt seedling mats within four hours of emergence to ensure they were unmated, following [Shrestha and Gassmann \(2020\)](#). Adults were held individually in small Petri dishes until sex could be determined by examining the basitarsi morphology following [Hammack and French \(2007\)](#) after which, adults were added in a 1-to-1 ratio to a small mesh cage (17.5 × 17.5 × 17.5 cm; 4S1515, BugDorm Store, Taiwan). The resistant ♂ × susceptible ♀ dorm received 710 total adults; the susceptible ♂ × resistant ♀ dorm received 822 adults. Simultaneously with the collection of eggs from the heterozygous crosses, eggs were collected from the Bt-resistant and Bt-susceptible strains. Eggs from each cross and the parental strains were held in a biological incubator (26°C, 40–60% RH, 16:8 [L:D] hr) until larval eclosion.

Each of the four genotypes (Bt-resistant, Bt-susceptible, resistant ♂ × susceptible ♀, and susceptible ♂ × resistant ♀) were added singly to seedling mats of two corn hybrids: Gpp34/Tpp35Ab1 corn (P8954XR) or the non-Bt isolate to Gpp34/Tpp35Ab1 corn (P8954R), with the presence and absence of Gpp34/Tpp35Ab1 confirmed, at the time each bag of seed was opened, with an ELISA based on a kit (Envirologix, Portland, ME). Sixteen replications were used for each combination of rootworm genotype by corn hybrid for a total of 128 seedling mats. Methods followed [Ingber and Gassmann \(2015\)](#) and used a two-stage, seedling-mat rearing method, similar to the methods applied for the rearing of strains, except that the seedling mats were smaller. Briefly, 18 neonates (<24 hr old) were placed on the exposed roots of a 5 d old small seedling mat (15 ml corn seeds, 50 ml water, and 200 ml soil mixture) placed in a cylindrical 500 ml container (DeliPRO TD40016, TRiPAK Industrial USA, LLC, White Plains, NY). Small seedling mats with larvae were then placed in a biological incubator (25°C; 60% RH; 16:8 [L:D] hr) for 10 d. After 10 d, each small seedling mat was placed on top of a 5 d old large seedling mat (25 ml corn seed, 80 ml water, and 500 ml soil mixture) placed in a 1 liter rectangular container (C32DE container, Dart Container Corp., Mason, MI), which was covered with white chiffon fabric and a lid with holes added for ventilation. These large seedling mats were checked every 2 to 3 d for adult emergence, with the number of adults emerging per day per seedling mat recorded.

Fitness Costs of Resistance

This experiment was conducted between February and July 2020 and used the F58 of the Bt-resistant strain and F61 of the Bt-susceptible strain. Fitness costs were measured for six life-history traits: 1) survival from the larval to the adult stage, 2) developmental rate, 3) longevity, 4) adult head capsule width, 5) fecundity, and 6) egg viability. A total of 20 replicates were established for both the Bt-resistant and Bt-susceptible strains. A replicate consisted of a single non-Bt seedling mat prepared following the methods described in [Ingber and Gassmann \(2015\)](#). A total of 18 neonate larvae were placed on each seedling mat, and adults were collected three times per week. Proportion survival to adulthood was calculated as the number of adults collected per seedling mat divided by the number of larvae added. Upon emergence, live adults were placed individually in Petri dishes and examined under a stereo-microscope (MZ6, Leica Microsystems, Wetzlar, Germany), with sex determined following [Hammack and French \(2007\)](#). Developmental rate was calculated per replicate as the average number of days between when larvae were added to seedling mats and the emergence of adults for each sex.

All adults that emerged from a single seedling mat were added to the same small mesh cage (17.5 × 17.5 × 17.5 cm; 4S1515, BugDorm Store, Taiwan), and cages were held in a biological incubator (25°C; 60% RH; 16:8 [L:D] hr). Adults were supplied with a complete diet, 1.5% agar solid, and corn leaf tissue, all of which were replaced every 3 to 4 d. When fresh food was provided, any dead adults were removed and placed in 85% ethanol. Adult longevity was determined for all adults and was calculated by subtracting the average day of adult emergence by sex within a replicate from the day on which each individual died. After death, head capsule width was measured as the outermost boundary between the compound eyes under a stereo-microscope using a digital camera (Motic North America, BC, Canada) with image analysis software (Motic Images Advanced V3.0 software, Motic North America, BC, Canada). For each specimen, sex was also determined following [Hammack and French \(2007\)](#).

Each cage received a 100 mm oviposition dish, which was replaced every 7 d. Each oviposition dish was washed following the procedures used for strain rearing. Fecundity per female per replicate was calculated as total eggs per cage divided by the total number of females in that cage. All but 25 eggs from each week's oviposition dish were stored in 70% ethanol. Stored eggs were quantified by photographing eggs, which were evenly distributed in a Petri dish, under a stereo-microscope and manually counting the photographed eggs within the ImageJ software ([Schneider et al. 2012](#)).

The 25 eggs that were set aside were used to determine egg viability. For each of the 40 small cages with adults, weekly cohorts of 25 eggs were assessed for egg viability. To quantify viability, eggs were placed on a 1.5% agar solid in a 50 mm Petri dish and examined three times per week under a stereo-microscope for newly eclosed larvae. All larvae were removed once counted. Petri dishes were checked for larvae until no new larvae eclosed for four consecutive observation periods. Egg viability was calculated as the number of larvae that eclosed divided by the number of eggs evaluated. During this process, eggs were held in a biological incubator (25°C, 60% RH, 0:24 [L:D] hr). On average, egg viability was measured for 3.7 ± 0.8 (mean \pm standard deviation) cohorts of eggs for each replicate. For one replicate with the Bt-resistant strain, we were unsuccessful in obtaining a measurement of egg viability because this replicated did not produce enough eggs to measure viability.

SOFTWARE

All data were analyzed with SAS 9.4 (SAS Institute 2015).

Name: SAS

Version: 9.4

URL: https://www.sas.com/en_us/home.html

Developer: SAS Institute

LICENSING

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