

DATA TITLE: Rootworm Bioassay Data and Root-Injury Scores: PNAS 2025

DATA ABSTRACT: Transgenic crops that produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) are grown worldwide to manage insect pests. Western corn rootworm is a serious pest of maize in the United States and is managed with Bt maize. However, some field populations of western corn rootworm have evolved resistance to all types of Bt maize that are currently used to manage this pest. This data set contains data on feeding injury to Bt maize by western corn rootworm in multiple fields in Iowa, USA. For several of these fields, adult western corn rootworm were collected and their larval progeny used in plant-based bioassays to measure resistance to Bt maize, and these data also are included in this data set. Finally, as part of this study, a meta-analysis was conducted using bioassay data spanning 12 years to test for changes over time in resistance to Bt maize by western corn rootworm, and the data used for this meta-analysis are provided.

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COLLECTION INFORMATION: Data on rootworm feeding injury to maize plants and rootworm survival in bioassays were collected between 2019 and 2022. Data used for meta-analysis were collected between 2009 and 2022. Rootworm bioassays were conducted at Iowa State University, and root injury data are from fields in Iowa, USA.

FILE LIST

1) “2020 Bioassay Survival.csv” File contains six columns of data, which are for plant-based bioassays conducted with western corn rootworm larvae during 2020. These bioassay data are described in Figure 2 and Table S2 of the Associated Publication (Gassmann et al. 2025). Information on individual field populations, described in column 2, “Population Code,” is provided in Figure 1 and Table S1 of the Associated Publication (Gassmann et al. 2025). The columns found in this file, with comma separated values (csv), are as follows:

1) “Population Type (1= Field; 2 = Control),” describes the type of rootworm population: 1 = field population and 2 = Bt-susceptible laboratory control population.

2) “Population Code,” provides the population code, with alphanumeric codes that begin with “B” corresponding to the various field populations that were studied. More information on individual field populations can be found in Figure 1 and Table S1 of the Associated Publication. In addition to these field populations, a Bt-susceptible control population also was tested and is labeled as “Control.” The control population was tested multiple times, and each separate testing event is labeled sequentially as Rep 1, Rep 2, Rep 3, etc. For example, the second replication of bioassays (testing event) run with the Bt-susceptible control population is labeled as “Control Rep 2.”

3) “Maize Type,” gives the maize hybrid that was used in bioassays. In cases where Bt maize hybrids were used, the types of rootworm active Bt toxins are given (i.e., Cry3Bb1, Gpp34/Tpp35, or the combination of these two, which is labeled as “Pyramid”). For each of these Bt hybrids, rootworm were also tested on a corresponding non-Bt hybrid, which was a genetic isolate to the Bt hybrid but lacked rootworm active Bt toxins. These are labeled with the prefix ISO. For example, the corresponding non-Bt hybrid to the Cry3Bb1 hybrid is labeled as “ISO Cry3Bb1.”

4) “Bioassay Replicate,” gives the replicate number for each combination of Population Code by Maize Type. A replicate is an individual maize plant onto which western corn rootworm larvae were placed. Each replicate represents a single plant-based bioassay.

5) “Larvae Placed on Plant,” provides the number of neonate western corn rootworm larvae used in an individual plant-based bioassay.

6) “Larvae Recovered from Plant,” is the number of live western corn rootworm larvae recovered at the end of the bioassay.

2) “2021 Bioassay Survival.csv” File contains six columns of data, which are for plant-based bioassays conducted with western corn rootworm larvae during 2021. These bioassay data are described in Figure 3 and Table S2 of the Associated Publication (Gassmann et al. 2025). Information on individual field populations, described in column 2, “Population Code,” is provided in Figure 1 and Table S1 of the Associated Publication (Gassmann et al. 2025). The columns found in this file, with comma separated values (csv), are the same as those found in, “2020 Bioassay Survival.csv.”

3) “Root Injury.csv” File contains six columns of data, which are for root injury to maize plants in agricultural fields that were sampled as part of this study. These fields are described in Figure 1 and Table S1 of the Associated Publication (Gassmann et al. 2025). The columns found in this file, with comma separated values (csv), are as follows:

- 1) “Year,” provides the year in which a field was sampled.
- 2) “Field,” gives an alphanumeric code for each field. Note that this is the same as the “Population Code” given in the files “2020 Bioassay Survival.csv” and “2021 Bioassay Survival.csv.” These alphanumeric codes also correspond to the names used for fields and populations in Figure 1, Table S1 and Table S2 of the Associated Publication.
- 3) “Transect,” lists the transect from which an individual root was sampled.
- 4) “Sample,” gives the position along the transect a root was sampled.
- 5) “Maize Type,” describes the type of rootworm active Bt toxins found in each maize plant. In most cases, plants contained two rootworm active Bt toxins (i.e., a pyramid of toxins). In some cases, maize plants did not contain any rootworm active Bt toxins and those plants are listed as “non-CRW Bt.” Additionally, in three cases, a maize plant was found to only contain one rootworm active Bt toxin, Cry3Bb1, and those plants are labeled as “Cry3Bb1 only.”
- 6) “Injury,” provides a measurement of rootworm feeding injury to each maize plant based on a 0 to 3 node injury scale.

4) “Bioassay Data for 2009 to 2012.csv” File contains seven columns of data, which provide a summary of bioassay data for rootworm populations sampled from fields with a high level of rootworm feeding injury to maize that produced Bt toxin Cry3Bb1 or mCry3A. These data correspond to Figure 4A in the Associated Publication (Gassmann et al. 2025). The columns found in this file, with comma separated values (csv), are as follows:

- 1) “Reference,” provides the reference (first author and year) for the corresponding publication in which the data were originally published, and these references are provided at the end of this section.
- 2) “Year,” refers to the year a rootworm population was sampled from the field, and also identifies the Bt-susceptible control populations that were tested alongside those field populations in plant-based bioassays.
- 3) “Time,” gives the coding for year in the regression analysis reported in Figure 4 of the Associated Publication (Gassmann et al. 2025), with the first year sampled coded as year 1 and subsequent years coded chronologically based on the number of years after the first year.
- 4) “Replicate for Bioassays,” gives the code for an individual population, or replicate of a population, tested in bioassays. Populations are coded as in their corresponding publication, and if not given a code in that publication, are described simply as Rep 1, Rep 2, Rep 3, etc.
- 5) “Corrected Survival,” gives the value of corrected survival for each population on a specific type of Bt maize. Corrected survival was calculated as the quotient of average proportion survival on a Bt hybrid (i.e. Cry3Bb1 maize) divided by average proportion survival on the corresponding non-Bt isolate.
- 6) “Maize Type,” is the Bt hybrid used in bioassays, either Cry3Bb1 maize or Gpp34/Tpp35Ab1 maize.

7) “Population Type,” describes the rootworm population tested in bioassays. Populations are labeled as either “Control,” which were Bt-susceptible control populations, or “Field,” which were populations collected from maize fields where maize plants suffered high levels of feeding injury from western corn rootworm larvae.

5) “Bioassay Data for 2013 to 2020.csv” File contains seven columns of data, which provide a summary of bioassay data for rootworm populations sampled from fields with high levels of rootworm feeding injury to maize that produced Bt toxin Gpp34/Tpp35Ab1, either alone or as a pyramid with either mCry3A or Cry3Bb1. These data correspond to Figure 4B in the Associated Publication (Gassmann et al. 2025). The columns found in this file, with comma separated values (csv), are the same as those found in, “Bioassay Data for 2009 to 2012.csv.”

Corresponding Citations for Column Entitled, “Reference”

Gassmann et al. 2011 corresponds to Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S. and Dunbar, M. W. 2011. Field-evolved resistance to Bt maize by western corn rootworm. *PLoS ONE* 6(7):e22629 doi:10.1371/journal.pone.0022629

Gassmann et al. 2012 corresponds to Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S. and Dunbar, M. W. 2012. Western corn rootworm and Bt maize: challenges of pest resistance in the field. *GM Crops and Food* 3:235-244

Gassmann et al. 2014 corresponds to Gassmann, A. J., Petzold-Maxwell, J. L., Clifton, E. H., Dunbar, M. W., Hoffmann, A. M., Ingber, D. A. and Keweshan, R. S. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. *Proceedings of the National Academy of Sciences USA* 111:5141-5146

Jakka et al. 2016 corresponds to Jakka, S. R. K., Shrestha, R. B. and Gassmann, A. J. 2016. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). *Scientific Reports* 6:27860 doi:10.1038/srep27860

Gassmann et al. 2016 corresponds to Gassmann, A. J., Shrestha, R. B., Jakka, S. R. K., Dunbar, M. W., Clifton, E. H., Paolino, A. R., Ingber, D. A., French, B. W., Masloski, K. E., Doudna, J. W. and St. Clair, C. R. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): root injury in the field and larval survival in plant-based bioassays. *Journal of Economic Entomology* 109:1872-1880

Gassmann et al. 2020 corresponds to Gassmann, A. J., Shrestha, R. B., Kropf, A. L., St. Clair, C. R. and Brenizer, B. D. 2020. Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other *Bacillus thuringiensis* traits in transgenic maize. *Pest Management Science* 76:268-276

Gassmann et al. 2025 corresponds to Gassmann, A. J., Brenizer, B. D., Kropf, A. L., McCulloch, J. B., Radosevich, D. L., Shrestha, R. B., Smith, E. M., St. Clair, C. R. 2025. Sequential evolution of resistance by western corn rootworm to multiple *Bacillus thuringiensis* traits in transgenic maize. *Proceedings of the National Academy of Sciences USA*

MATERIALS AND METHODS

Maize fields were visited during 2018, 2019 and 2020 in response to requests by crop consultants, farmers and regional agronomists who suspected high levels of feeding injury to Bt maize roots by corn rootworm larvae. For each field location, feeding injury was assessed for a total of 18 maize plants, which were sampled by excavating the entire rootstock, washing away soil from the roots, and scoring rootworm feeding injury based on the 0 to 3 node injury scale, with 0 indicating an absence of feeding injury (lowest possible rating) and 3 equaling three nodes of roots pruned by rootworm larvae (highest possible rating). Six plants were sampled at 2 m intervals along each of three transects. Transects were placed at least 15 m from the edge of the field, and were separated by at least 12 m. At each location, adult western corn rootworm were sampled to obtain eggs, which in turn were used to generate larvae for use in plant-based bioassays.

Adult western corn rootworm collected from each field were held separately in mesh cages, and eggs were collected in a Petri dish that contained sieved soil (< 165 µm particle size). Eggs were stored at 6°C for at least 5 months to break diapause, with resulting larvae used in plant-based bioassays during the following year (e.g., eggs collected in 2020 produced larvae that were used in bioassays in 2021). Plant-based bioassays followed Gassmann et al. (2020; Pest Management Science) and evaluated survival of larval corn rootworm on six maize hybrids: 1) Cry3Bb1 maize, 2) non-Bt genetic isolate to Cry3Bb1 maize, 3) Gpp34/Tpp35Ab1 maize, 4) non-Bt genetic isolate to Gpp34/Tpp35Ab1 maize, 5) maize containing a pyramid of Gpp34/Tpp35Ab1 and Cry3Bb1 and 6) non-Bt genetic isolate to maize containing a pyramid of Gpp34/Tpp35Ab1 and Cry3Bb1.

In all cases, the maize seed used in this study had been treated with an insecticidal seed treatment, and this seed treatment was removed following Gassmann et al. (2020; Pest Management Science) prior to growing maize for use in bioassays. Maize plants used in plant-based bioassays were grown in a greenhouse following Gassmann et al. (2020; Pest Management Science). Briefly, plants were grown individually in 1 L containers (Placon, Madison, WI, USA) filled with a potting medium [equal parts Metro Mix and Sunshine LC1 (Sun Gro Horticulture, Vancouver, British Columbia, Canada)], with supplemental light (16/8 L/D) provided by high-pressure sodium lamps. Plants were watered as needed and received supplemental fertilizer weekly. Plants grew in a greenhouse for 3 to 4 weeks, after which time they reached the four to six leaf stage (i.e., V4 to V6) and were used in bioassays.

To conduct bioassays, plants were trimmed to a height of 20 cm and leaves were trimmed to a length of 10 cm. Potting medium at the base of the plant was moved aside to expose roots, and 12 neonate larvae (< 24 h old) were carefully transferred to roots using a small brush, with roots then gently covered with potting medium. To obtain larvae for bioassays, eggs were removed from cold storage and placed in a biological incubator (26°C; 0/24 L/D), with larvae eclosing from eggs 2 weeks thereafter. Maize plants with larvae were held in a biological incubator for 17 days (24°C; 16/8 L/D; 60% RH), after which the aboveground portion of the maize plant was excised, and the potting medium with maize roots and larvae was removed from the bioassay container and placed on a Berlese funnel, with live larvae subsequently collected in a vial containing 85% ethanol.

Eggs from a single field population were removed from cold storage every other week along with eggs from a Bt-susceptible control population. The Bt-susceptible control population

was a diapausing strain of western corn rootworm collected from Moody Co., South Dakota in 1986, and maintained in laboratory culture without exposure to Bt maize. During each of these biweekly sets of bioassays, up to 12 plants per hybrid received larvae from a field population and up to 6 plants per hybrid received larvae from the control population, depending on the availability of larvae each week. During 2020, bioassays were attempted for six field populations (originally sampled in 2019) with accompanying cohorts of the Bt-susceptible control population, and during 2021, bioassays were attempted for seven field populations (originally sampled in 2020) with accompanying cohorts of the Bt-susceptible control population.

Data are also provided from a meta-analysis spanning 12 years of bioassay data for western corn rootworm populations from Iowa, USA. The meta-analysis used bioassay data for western corn rootworm populations sampled from fields that experienced high levels of injury to maize that produced Gpp34/Tpp35Ab1 or Cry3 (i.e., Cry3Bb1 or mCry3A), either alone or together as a Bt pyramid. Fields with high levels of feeding injury were defined as those with either greater than one node of feeding injury, on average, to maize with a single Bt trait of either Gpp34/Tpp35Ab1 or Cry3; or greater than half a node of feeding injury, on average, to maize that produced a pyramid of Gpp34/Tpp35Ab1 and Cry3.

DATA PROCESSING METHODS

For data on root injury, a field was included in this study if it met the following three criteria: 1) was populated predominately by western corn rootworm ($\geq 95\%$), 2) contain Bt maize that produced a pyramid of Gpp34/Tpp35Ab1 and Cry3 (i.e., either Cry3Bb1 or mCry3A), and 3) the Bt maize plants suffered rootworm feeding injury that met the United States Environmental Protection Agency's threshold for greater than expected injury. For maize that produces two rootworm-active Bt traits, the threshold for greater than expected injury is reached when at least 50% of the Bt plants sampled suffer greater than half a node of feeding injury based on the 0 to 3 node injury scale.

For bioassays conducted as part of this study, bioassays were classified as successful and used in data analysis if at least eight replicates for each of the six hybrids were conducted, and the rootworm population was sampled from a field that experienced greater than expected injury to maize with a pyramid of Gpp34/Tpp35Ab1 and Cry3.

For bioassay data used in the meta-analysis, corrected survival was calculated as average survival on Bt maize divided by average survival on the non-Bt isoline, and this was done for both Cry3Bb1 maize and Gpp34/Tpp35Ab1 maize. In all cases, field populations were tested alongside one or more Bt-susceptible control populations, and corrected survival was calculated for replicates of the susceptible control populations for both Cry3Bb1 maize and Gpp34/Tpp35Ab1 maize following the same methods. Calculations of corrected survival followed Gassmann et al. (2020; Pest Management Science), with a value of 0 indicating no survival on Bt maize and a value of 1 indicating equal survival on Bt maize and non-Bt maize.

LICENSING

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