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SHORT COMMUNICATION

Susceptibility of northern corn rootworm *Diabrotica barberi* (Coleoptera: Chrysomelidae) to mCry3A and eCry3.1Ab *Bacillus thuringiensis* proteins

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Abstract The susceptibility of the northern corn rootworm *Diabrotica barberi* (Smith & Lawrence) to mCry3A and eCry3.1Ab proteins derived from *Bacillus thuringiensis* (Bt) was determined using a diet bioassay. Northern corn rootworm neonates were exposed to different concentrations of mCry3A and eCry3.1Ab, incorporated into artificial diet. Larval mortality was evaluated after 7 d. The mCry3A and eCry3.1Ab proteins were found to be toxic to the northern corn rootworm larvae. The LC₅₀ and LC₉₉ values for mCry3A were 5.13 and 2482.31 µg/mL, respectively. For eCry3.1Ab, the LC₅₀ and LC₉₉ values were 0.49 and 213.01 µg/mL. Based on the estimated lethal concentrations, eCry3.1Ab protein was more efficacious to northern corn rootworm larvae than mCry3A. These lethal concentration values will be used as diagnostic doses for routine annual monitoring for change in susceptibility of field collected northern corn rootworm to mCry3A, and eCry3.1Ab toxins.

Key words *Bacillus thuringiensis*; *Diabrotica barberi*; eCry3.1Ab; mCry3A; susceptibility

Introduction

The western *Diabrotica virgifera virgifera* LeConte and northern corn rootworms, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), are major pests of maize, *Zea mays* L., in certain regions of the U.S. Corn Belt, causing damage to maize by feeding directly on the roots (Chiang, 1973). The soil-inhabiting larval stage is essentially monophagous on corn roots, but also can survive on other grass species (Oyediran *et al.*, 2004, 2008). Because the damage by the corn rootworm complex has such a negative impact on corn production, several management tactics, such as soil-applied insecticides, crop

rotation, and foliar sprays (to reduce beetle populations) are used to control these pests (Krysan & Miller, 1986). However, extensive use of these tactics has resulted in the selection of insect resistance to several insecticides in the western corn rootworm (Ball & Weekman, 1962; Meinke *et al.*, 1998; Wright *et al.*, 2000). Crop rotation also has selected for northern corn rootworm individuals that have an extended egg diapause and can overwinter two or more years (Krysan *et al.*, 1984; Krysan & Miller, 1986). The occurrence of prolonged diapause in *D. barberi* eggs helps account for larval root damage observed in first-year cornfields (Bigger, 1932; Chiang, 1965, 1973; Krysan *et al.*, 1984, 1986; Levine *et al.*, 1992, 2002; Steffey *et al.*, 1992; Gray *et al.*, 1998; French *et al.*, 2014).

The failure of these primary control strategies for the northern and western corn rootworm has created a need that might be addressed by new technologies. Seed companies have developed maize hybrids containing genes from the soil bacterium *Bacillus thuringiensis*

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Berliner (Bt) that code for production of insecticidal proteins that have high levels of antibiosis to neonates of the rootworm complex. Currently, there are 4 Bt toxins registered in the United States that are active against *Diabrotica* species and are produced either singly (Cry3Bb1, Cry34/35Ab1, mCry3Aa, and eCry3.1Ab) or in pyramids (Cry3Bb1 + Cry34/35Ab1, mCry3Aa + Cry34/35Ab1, and mCry3Aa + eCry3.1Ab) (USEPA, 2005a, 2010, 2011 and 2014).

The widespread planting of crops genetically engineered to produce these insecticidal toxins places intense selective pressure on pest populations to evolve resistance. For example, in continuous maize fields the western corn rootworm is often managed through planting of Bt maize. And during 2009 and 2010, fields were identified in Iowa in which western corn rootworm imposed severe injury to maize producing Bt toxin Cry3Bb1. Subsequent bioassays revealed Cry3Bb1 resistance in these populations (Gassmann *et al.*, 2014).

Insect resistance management programs have been an integral part of delaying resistance in pest populations to maize plant incorporated protectants (PIPs) since their first U.S. commercial sales in 1996. Prior to the commercial release of a PIP containing maize hybrid, different resistance management tactics were evaluated and proposed. When transgenic corn for rootworm control was first registered in February 2003, an insect resistance management (IRM) plan, based on mandatory refuges, was required by the U.S. Environmental Protection Agency (USEPA, 2005b). The U.S. Environmental Protection Agency (USEPA, 2002) has required a resistance monitoring plan for all of the Bt insect protected products. The ability to effectively detect the evolution of insecticide resistance before a control failure is an integral component of resistance management strategies for transgenic plants that express Bt toxins and a regulatory requirement for registering Bt-expressing corn hybrids in the United States (USEPA, 1998, 2002; ILSI, 1998). The first step in implementing such programs include developing appropriate bioassay techniques and estimating baseline susceptibility to the Bt protein among insect populations across the geographic range of the target species.

Syngenta has received regulatory approvals to sell Agrisure RW (MIR604) and Agrisure Duracade (5307) containing mCry3A and eCry3.1Ab proteins derived from *B. thuringiensis* (Bt). As a condition of registration by U.S. Environmental Protection Agency, Syngenta is required to conduct annual monitoring of the northern corn rootworm to determine change in susceptibility of the insect to the 2 Bt toxins. The objective of this study was to determine the susceptibility of northern corn rootworm to mCry3A and eCry3.1Ab proteins.

Materials and methods

Insects

In this study, because of the difficulty in collecting field populations of northern corn rootworm, we used laboratory colony of northern corn rootworm to study the susceptibility of these insects to 2 proteins mCry3A and eCry3.1Ab. Northern corn rootworm eggs were obtained from a 1-year diapausing laboratory population at the USDA, ARS, North Central Agricultural Research Laboratory in Brookings, SD. The original beetles were collected near Brookings in 1996 and complete 1 generation each year. These beetles are reared similarly to *D. v. virgifera* LeConte (Jackson, 1986; Branson & Jackson, 1988; Branson *et al.*, 1988; Kim *et al.*, 2007; French & Hammack, 2010) except the egg dishes contain several small, ≈ 0.25 –1.0 cm soil clods in addition to the 80 mesh soil. Upon arrival, the eggs were incubated at 28 ± 2 °C and 60% RH for 20 d to initiate hatching. The neonate larvae that hatched from the eggs were used for the diet bioassay. Because egg hatching is not as synchronized, perhaps relating to quantitative genetic variation of the diapause trait (French *et al.*, 2014), in northern corn rootworm as it is with the western corn rootworm, the eggs did not hatch at the same time making it difficult to get enough larvae to run more replicates of the diet assay at the same time.

Due to the aforementioned difficulty of timing of egg hatch of this species, the laboratory colony was utilized instead of the field collected populations.

Source of protein

The Cry proteins, mCry3A and eCry3.1Ab were produced in recombinant *Escherichia coli* culture, and were provided by Syngenta Crop Product Safety (Research Triangle Park, NC, USA). The initial stock solution of mCry3A protein was prepared by solubilizing in purified water, 10% ethanol, 50 mmol/L Tris, 2 mmol/L EDTA, pH 10.5 buffer. The stock solution for eCry3.1Ab was prepared by solubilizing the protein in 10 mmol/L ammonium bicarbonate, purified water, 10% ethanol, 10 mmol/L Tris, 0.4 mmol/L EDTA, 0.1% Tween 20, pH 10 buffer. Dilutions were made in 50 mmol/L Tris, 2 mmol/L EDTA, pH 10.5 buffer and 10 mmol/L ammonium bicarbonate, pH 10 buffer for mCry3A and eCry3.1Ab. Lyophilized protein powder for mCry3A and eCry3.1Ab was added to the buffer, to make a stock solution of 2000 $\mu\text{g/mL}$ for mCry3A and 1000 $\mu\text{g/mL}$. The resulting stock solutions were used in making dilutions for the diet assays.

Bioassay

The standard diet incorporation method was used to measure the susceptibility of northern corn rootworm to mCry3A and eCry3.1Ab toxins. A range of ten concentrations of lyophilized protein powder of mCry3A (0, 0.4, 1, 5, 10, 50, 100, 300, 600, and 1000 $\mu\text{g/mL}$), and 9 concentrations of eCry3.1Ab (0, 0.015, 0.03, 1, 5, 10, 25, 50, and 300 $\mu\text{g/mL}$) were incorporated into diet. The artificial diet used in all bioassays was prepared as described by Marrone *et al.* (1985), and antibiotics were added as described by Chen and Stacy (2006). For diet incorporation assays, the Cry proteins were individually suspended in buffer solutions. To obtain final concentrations for the 2 toxins, 500 μL of protein sample was mixed with an equal volume of $2\times$ molten diet and mixed to obtain a final concentration of 1000 $\mu\text{g/mL}$ as the highest dose for mCry3A and 300 $\mu\text{g/mL}$ for eCry3.1Ab, 1 mL amount of the different concentrations of mCry3A and eCry3.1Ab diets were dispensed into each 47-mm sterilized Petri dishes (Millipore, Billerica, MA, USA) representing all the concentrations tested and allowed to dry. Ten to 12 neonate northern corn rootworm larvae (<24-h old) were placed in each Petri dish. The bioassay dishes were held in an environmental chamber maintained at $28 \pm 2^\circ\text{C}$, 60% RH, and a photoperiod of 16 : 8 (L : D) h. Larval mortality for each dish recorded on the 7th day after inoculation.

Data analysis

Bioassays were conducted in duplicate on 3 different dates; mortality and the 50% lethal concentration (LC_{50}) and 99% LC_{99} were determined using the Environmental Protection Agency, Probit Analysis Software (version 1.5; Washington, DC, USA).

Results and discussion

The susceptibility of northern corn rootworm neonates exposed to proteins of mCry3A and eCry3.1Ab are presented in Table 1. The LC_{50} value for mCry3A was 5.13 $\mu\text{g/mL}$, and the LC_{99} value was 2482.31 $\mu\text{g/mL}$ (Table 1). The LC_{50} value for eCry3.1Ab was 0.49 $\mu\text{g/mL}$, and the LC_{99} value was 213.01 $\mu\text{g/mL}$ (Table 1).

The susceptibility of the northern corn rootworm to the mCry3A and eCry3.1Ab derived from *B. thuringiensis* (Bt) varied significantly. The eCry3.1Ab toxin was more toxic to northern corn rootworm larvae than mCry3A based on the estimated lethal concentrations from our diet incorporated assays. These lethal concentration

values will be used as diagnostic doses for routine monitoring of change in susceptibility of northern corn rootworm to mCry3A and eCry3.1Ab. The average percent mortality among northern corn rootworms treated with mCry3A ranged from 15% to 85%, and the mortality of larvae exposed to eCry3.1Ab ranged from 16% to 98%. The average percent mortality for the larvae in the control treatments was 16%.

For resistance management programs to be effective; monitoring, surveillance, and early detection of resistance are important prerequisites. Regular monitoring for resistance development helps to detect the emergence of resistant phenotypes in order to initiate timely remedial measures. Resistance monitoring also enables the evaluation of the effectiveness of resistance management strategies. Traditionally, log dose probit assays, and recently diagnostic dose assays have been routinely used to monitor development of insect resistance to insecticides (Forrester *et al.*, 1993; Kranthi *et al.*, 1997). The log dose probit assays are used to calculate resistance ratios (LC_{50} of the field strain \div LC_{50} of the susceptible reference strain), whereas the diagnostic dose assays help to discriminate between resistant and susceptible phenotypes. The LC_{50} values, and slope estimates can be used to distinguish resistant phenotypes only at a high frequency, and are not adequately sensitive enough to detect lower levels of resistance (Roush & Miller, 1986). Georgiou and Taylor (1976) emphasized the importance of diagnostic dose assays in detection of resistance to insecticides. After examining the genetic and logistical implications of resistance monitoring methods, Roush and Miller (1986) concluded that discriminating dose assays were more efficient than the log dose probit regression assays in monitoring for resistance. Sims *et al.* (1996) estimated diagnostic doses for *Heliothis virescens* (Fabricius) and *Helicoverpa zea* (Boddie). The determination of baseline susceptibility data is the first step in the development of a monitoring program designed to detect changes in susceptibility that may result from repeated and prolonged exposure to Bt toxins. These data may provide information that will allow development of diagnostic bioassays that would be more efficient in detection of resistant populations.

In conclusion, the lethal concentrations determined for mCry3A and eCry3.1Ab to detect change in Bt susceptibility will be used as diagnostic concentrations in monitoring northern corn rootworm resistance in corn growing regions. However, it should be noted that because the toxins are not high dose against the northern corn rootworm, using the LC_{99} as the diagnostic dose may be difficult to achieve because of the large amounts of purified proteins that would be required for testing large field populations.

Table 1 Susceptibility of northern corn rootworm neonates derived from laboratory population exposed to mCry3A and eCry3.1Ab toxins.

Toxin	<i>n</i>	Slope \pm SE	LC ₅₀ (95% CI) ^{†,‡}	LC ₉₉ (95% CI) [‡]	χ^2	df
mCry3A	460	0.86 \pm 0.12	5.13 (2.6–8.7)	2482.31 (702.6–22144.1)	9.49	9
eCry3.1Ab	444	0.88 \pm 0.11	0.49 (0.2–0.9)	213.01 (75.9–1155.1)	14.07	8

[†]Concentrations of mCry3A and eCry3.1Ab that produced 50% mortality by probit analysis.

[‡]mCry3A and eCry3.1Ab (in $\mu\text{g/mL}$) incorporated into the diet.

Chi-square significant ($P < 0.05$).

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Disclosure

The authors declare that they have no conflicts of interest.

References

- Ball, H.J. and Weekman, G.T. (1962) Insecticide resistance in the adult western corn rootworm in Nebraska. *Journal of Economic Entomology*, 55, 439–441.
- Bigger, J.H. (1932) Short rotation fails to prevent attack of *Diabrotica longicornis* Say. *Journal of Economic Entomology*, 25, 196–199.
- Branson, T.F. and Jackson, J.J. (1988) An improved diet for adult *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Journal of Kansas Entomological Society*, 61, 353–355.
- Branson, T.F., Jackson, J.J. and Sutter, G.R. (1988) Improved method for rearing *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, 81, 410–414.
- Chen, E. and Stacy, C. (2006) Modified Cry3A toxins and nucleic acid sequences coding. U.S. Patent 7030295.
- Chiang, H.C. (1965) Survival of northern corn rootworm eggs through one and two winters. *Journal of Economic Entomology*, 58, 470–472.
- Chiang, H.C. (1973) Bionomics of the northern and western corn rootworm. *Annual Review of Entomology*, 18, 47–72.
- Forrester, N.W., Cahill, M., Bird, L.J. and Layland, J.K. (1993) Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research-Supplement*, 1, 1–132.
- French, B.W. and Hammack, L. (2010) Reproductive traits of northern corn rootworm (Coleoptera: Chrysomelidae) in relation to female and male body size. *Annals of Entomological Society of America*, 103, 688–694.
- French, B.W., Coates, B.S. and Sappington, T.W. (2014) Inheritance of an extended diapause trait in the northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae). *Journal of Applied Entomology*, 138, 213–221.
- Gassmann, A.J., Petzold-Maxwell, J.L., Clifton, E.H., Dunbar, M.W. and Hoffmann, A.M. (2014) Field evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 5141–5146.
- Georghiou, G.P. and Taylor, C.E. (1976) Pesticide resistance as an evolutionary phenomenon. *Proceedings of the 15th International Congress of Entomology*, Washington DC, pp. 759–785.
- Gray, M.E., Levine, E. and Oloumi-Sadeghi, H. (1998) Adaptation to crop rotation: western and northern corn rootworms respond uniquely to a cultural practice. *Recent Research Developments in Entomology*, 2, 19–31.
- International Life Sciences Institute (1998) An evaluation of insect resistance management in Bt field corn: a science based framework for risk assessment and risk management. ILSI, Washington, DC.
- Jackson, J.J. (1986) Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi*. *Methods for the Study of Pest Diabrotica* (eds J.L. Krysan & T.A. Miller), pp. 25–47. Springer, New York.
- Kim, S.K., French, B.W., Sumerford, D.V. and Sappington, T.W. (2007) Genetic diversity in laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae), including a nondiapause colony. *Environmental Entomology*, 36, 637–645.
- Kranthi, K.R., Armes, N.J., Rao, N.G.V., Raj, S. and Sundaramurthy, V.T. (1997) Seasonal dynamics of metabolic

- mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in central India. *Pesticide Science*, 50, 91–98.
- Krysan, J.L., Jackson, J.J. and Lew, A.C. (1984) Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). *Environmental Entomology*, 13, 1237–1240.
- Krysan, K.J. and Miller, T.A. (1986) *Methods for the Study of Pest Diabrotica*. Springer Series in Experimental Entomology, New York.
- Levine, E.H., Oloumi-Sadeghi, H. and Fisher, J.R. (1992) Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. *Journal of Economic Entomology*, 85, 262–267.
- Levine, E., Spencer, J.L., Isard, S.A., Onstad, D.W. and Gray, M.E. (2002) Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. *American Entomologist*, 48, 94–107.
- Marrone, P.G., Ferri, F.D., Mosley, T.R. and Meinke, L.J. (1985) Improvements in laboratory rearing of the southern corn rootworm, *Diabrotica undecimpuncta howardi* Barber (Coleoptera: Chrysomelidae), on an artificial diet and corn. *Journal of Economic Entomology*, 78, 290–293.
- Meinke, L.J., Siegfried, B.D., Wright, L.R.J. and Chandler, L.D. (1998) Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. *Journal of Economic Entomology*, 91, 594–600.
- Oyediran, I.O., Hibbard, B.E., Clark, T.L. and French, B.W. (2004) Selected grassy weeds as alternate hosts of the northern corn rootworm (Coleoptera: Chrysomelidae). *Environmental Entomology*, 33, 1497–1504.
- Oyediran, I.O., French, B.W., Clark, T.L., Dashiell, K. and Hibbard, B.E. (2008) Prairie grasses as hosts of the northern corn rootworm (Coleoptera: Chrysomelidae). *Environmental Entomology*, 37, 247–254.
- Roush, R.T. and Miller, G.L. (1986) Considerations for the design of insecticide resistance monitoring programs. *Journal of Economic Entomology*, 79, 293–298.
- Sims, S.R., Berberich, S.A., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C. and Fuchs, R.L. (1996) Analysis of expressed proteins in fiber fraction from insect-protected and glyphosate-tolerant cotton varieties. *Crop Science*, 36, 1212–1216.
- Steffey, K.L., Gray M.E. and Kuhlman, D.E. (1992) Extent of corn rootworm (Coleoptera: Chrysomelidae) larval damage in corn after soybeans: search for the expression of the prolonged diapause trait in Illinois. *Journal of Economic Entomology*, 85, 268–275.
- USEPA (1998) *The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management*. Environmental Protection Agency, Washington, D.C., USA.
- USEPA (2002) Scientific Advisory Panel on Corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues, Part B: Insect Resistance Management Issues. <http://www.epa.gov/scipoly/sap/2002/august/august2002final.pdf>, Accessed 6 November 2002.
- USEPA (2005a) Environmental Protection Agency, *Bacillus thuringiensis* modified Cry3A protein (mCry3A) and the genetic material necessary for its production in corn; temporary exemption from the requirement of a tolerance. *Federal Registration*, 70, 17323–17327.
- USEPA (2005b) Environmental Protection Agency, Pesticide Fact Sheet. *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector zmir131) in Event MON863 Corn (006484). Publication No 730-F-03–01.
- USEPA (2010) Environmental Protection Agency, Biopesticides Registration Action Document: OptimumH AcreMax™ B.t. Corn Seed Blends. Washington, D.C.
- USEPA (2011) Environmental Protection Agency, Biopesticides Registration Action Document: MON 89034 x TC1507 x MON88017 x DAS-59122-7 (Smart-StaxH) B.t. Corn Seed Blend. Washington, D.C.
- Wright, R.J., Scharf, M.E., Meinke, L.J., Zhou, X., Siegfried, B.D. and Chandler, L.D. (2000) Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. *Journal of Economic Entomology*, 93, 7–13.

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