Evaluation of RNA Interference (RNAi) and Bt Traits for Management of Larval Corn Rootworm in Northeastern Iowa

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The purpose of this study was to evaluate the effectiveness of two Bt rootworm traits (Cry3Bb1 and Cry34/35Ab1) with and without an RNA interference trait (DvSnf7) for management of corn rootworm (CRW) larvae. The two CRW trait packages evaluated in this study were a pyramid Cry3Bb1 and Cry34/35Ab1 (SmartStax®), and a pyramid of Cry3Bb1, Cry34/35Ab1 and DvSnf7 (SmartStax PRO®). Both of the CRW trait packages were replicated twice, using two different hybrids with each trait package. Additionally, two non-CRW Bt hybrids were included in this study as untreated checks.

Materials and Methods

Study location. The study was conducted at the Iowa State University Northeast Research and Demonstration Farm (NERF). The field site had been planted the previous year with a trap crop, which is a mixed-maturity blend with a greater proportion of late-maturing varieties. This trap crop constitutes a favorable environment for adult female rootworm late in the season when other fields are maturing, and typically results in a high abundance of rootworm larvae the following year.

Field plot design. This study was split-plot design with four replications. Two separate hybrid families were used for each treatment. Treatments were four rows wide, and 35 ft. long. Plots were trimmed to 30 ft. in length after planting.

Planting. This study was planted May 16, using a four-row John Deere Max EmergeTM 7100 Integral Rigid Frame Planter with 30-in. row spacing. The study was planted at a depth of two in., with a spacing of six in. between seeds (35,600 seeds/acre).

Stand counts. On June 9, early season stand counts were measured in all treatments. These were measured by using a two in. PVC pipe cut to the length of 17.4 ft. (1/1,000 of an acre for 30 in. row spacing) that was placed between two rows of corn and the number of plants in both rows then counted. Late season stand counts were measured October 18, following the same procedure as early season stand counts. Measurements for both dates were averaged to provide a single value for stand counts (Table 2).

Root injury. After the majority of corn rootworm larvae had finished feeding on corn roots, roots were dug to assess feeding injury. Roots were dug July 21. Prior to leaving the field, all roots were labeled with study name and plot number using a permanent marker. On July 26, roots were cleaned at the Iowa State Johnson Farm's root washing station. Roots first were soaked in water for two to eight hours, then washed with a hose to remove any remaining soil. After being washed, roots were evaluated for rootworm feeding injury following the Iowa State Node Injury Scale (0-3) (Table 1).

Node Injury Scale (0-3)

0.00 - No feeding injury (lowest rating that can be given).
1.00 - One node (circle of roots), or the equivalent of an entire node, pruned to within 1.5 in. of the stalk or soil line
2.00 - Two nodes pruned
3.00 - Three or more nodes pruned. (Highest rating that can be given)

Injury in between complete nodes pruned was noted as the proportion of the node injured (e.g., 1.50 = one and a half nodes pruned and 0.25 = one quarter of one node pruned).

Product consistency. Percent product consistency was calculated as the percentage of times a treatment limited feeding injury to 0.25 nodes or less (greater injury may result in economic yield loss, especially when plants are moisture stressed).

Lodging counts. Lodging counts were collected on October 18 prior to harvest. A plant was considered lodged if it was leaning greater than 30 degrees from vertical. Lodging counts were taken at the same time as final stand counts. Percent lodging was calculated per plot as the number of lodged plants divided by the total stand, with this quotient then multiplied by 100 (Table 3).

Yields. This study was machine harvested October 18 with a modified John Deere 9450 plot combine owned by Iowa State University. Weight (pounds) and percent moisture were recorded using a Shivvers brand 5010 moisture meter and Avery-WeighTronix weigh system. These measurements were converted to bushels/acre of No. 2 shelled corn (56 lb./bushel at 15.5% moisture) in Microsoft Excel. (Table 3)

Data analysis. Data were analyzed with analysis of variance (ANOVA) in SAS Enterprise Guide 7.1. The treatment means were compared using LSMEAN procedure with an experimentwise error rate of $P < 0.05$.

Results and Discussion

There was high rootworm pressure at this location this year, with both of the untreated checks (non-CRW Bt corn) suffering over two nodes of root injury (Table 1). Both of the hybrids containing a pyramid of Cry3Bb1 and Cry34/35Ab1 suffered more than half a node of feeding injury (Table 1). The US Environmental Protection Agency considers more than half a node of rootworm feeding injury to pyramided corn to be greater than expected injury, and it is likely this injury arose because of the presence of resistance to both Cry3Bb1 and Cry34/35Ab1 by western corn rootworm in Iowa. Corn pyramided with Cry3Bb1, Cry34/35Ab1 and DvSnf7 suffered around a quarter of a node of injury, which would be considered an acceptable level of root protection (Table 1). Patterns of product consistency were similar to root injury (Table 1). Stand counts were somewhat reduced for the non-Bt hybrids and for one of the hybrids containing all three rootworm traits, but were similar among the other hybrids (Table 2). One of the non-CRW Bt hybrids suffered significantly higher lodging, but no other differences in lodging were observed (Table 3). Yields were reduced for the non-CRW Bt hybrids, which likely arose from the high level of rootworm feeding injury these hybrids suffered (Tables 1 and 4). Yields also were somewhat lower for one of the rootworm traited hybrids, but this was likely caused by the lower stand counts observed for that hybrid (Tables 2 and 4).

Corn containing three rootworm traits (Cry3Bb1, Cry34/35Ab1 and DvSnf7) suffered the lowest root injury in this study, and appears to offer farmers a valuable tool for managing larval corn rootworm. However, it is important to keep in mind western corn rootworm in Iowa has developed resistance to all available Bt traits for management of this pest. Additionally, laboratory research has found that western corn rootworm can develop resistance quickly to DvSnf7 and other RNAi traits. Consequently, there is a heightened risk of western corn rootworm evolving resistance to corn with Cry3Bb1, Cry34/35Ab1 and DvSnf7. One of the primary causes of Bt resistance in western corn rootworm is continuous corn cultivation coupled with continuous use of the same Bt traits. Diversified management, including crop rotation and use of non-rootworm traited corn with soil-applied insecticide, will be important to delay rootworm resistance to this new RNAi technology.

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Additional Information

Annual reports for the Iowa Evaluation of Insecticides and Plant-Incorporated Protectants are available online through the Department of Plant Pathology, Entomology and Microbiology at Iowa State University: ent.iastate.edu/dept/faculty/gassmann/rootworm

Table 1. Average root injury and product consistency. NERF, Floyd Co.[1]

Treatment[2]	Node- Injury[3,4,6]	Product Consistency[5,6]
Treatment 6: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	0.14a	70a
Treatment 5: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	0.29a	50ab
Treatment 3: Cry3Bb1 and Cry 34/35Ab1	0.68b	10c
Treatment 4: Cry3Bb1 and Cry 34/35Ab1	0.87b	20bc
Treatment 2: Non-CRW Bt	2.11c	0c
Treatment 1: Non-CRW Bt	2.11c	0c

[1]Planted May 16, 2022; evaluated July 26, 2022
[2]Non-CRW Bt = an absence of any Bt trait targeting corn rootworm
[3]Means based on 20 observations (5 roots/2 rows x 4 replications)
[4]Iowa State Node-Injury scale (0-3). Number of full or partial nodes completely eaten
[5]Product consistency = Percentage of times nodal injury was 0.25 (¼ node eaten) or less
[6]Significant difference between the treatment means for both Node-Injury and Product Consistency (ANOVA, P < 0.05)

Table 2. Average stand count. NERF, Floyd Co.[1]

Treatment[2]	Stand counts[3,4]
Treatment 3: Cry3Bb1 and Cry 34/35Ab1	27.4a
Treatment 4: Cry3Bb1 and Cry 34/35Ab1	27.2a
Treatment 6: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	26.3ab
Treatment 2: Non-CRW Bt	25.3abc
Treatment 1: Non-CRW Bt	24.4bc
Treatment 5: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	23.1c

[1]Planted May 16, 2022; evaluated June 9 and October 18, 2022
[2]Non-CRW Bt = an absence of any Bt trait targeting corn rootworm
[3]Data presented as plants per 1/1000 of an acre
[4]Significant differences between means (ANOVA, P < 0.05)

Table 3. Average yield. NERF, Floyd Co.[1]

Treatment[2]	Percent lodging[3,4]
Treatment 4: Cry3Bb1 and Cry 34/35Ab1	0.5a
Treatment 6: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	0.5a
Treatment 5: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	2.3a
Treatment 3: Cry3Bb1 and Cry 34/35Ab1	4.2a
Treatment 2: Non-CRW Bt	7.3a
Treatment 1: Non-CRW Bt	21.0b

[1]Planted May 16, 2022; evaluated October 18, 2022
[2]Non-CRW Bt = an absence of any Bt trait targeting corn rootworm
[3]Data presented as percentage of plants from the October 18, 2022 stand count that were lodged
[4]Significant differences between means (ANOVA, P < 0.05)

Table 4. Average yield. NERF, Floyd Co.[1]

Treatment[2]	Bushels/acres[3,4,5]
Treatment 6: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	219.5a
Treatment 3: Cry3Bb1 and Cry 34/35Ab1	207.8ab
Treatment 4: Cry3Bb1 and Cry 34/35Ab1	202.8ab
Treatment 5: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	191.7bc
Treatment 2: Non-CRW Bt	169.2cd
Treatment 1: Non-CRW Bt	165.0d

[1]Planted May 16, 2022; harvested October 18, 2022
[2]Non-RW Bt = an absence of any Bt trait targeting corn rootworm
[3]Means based on 4 observations (2-row treatment × 30 row-ft./treatment × 4 replications)
[4]Significant differences between means (ANOVA, P < 0.05)
[5]Yields converted to 15.5% moisture