



2024 FINAL REPORT (project ongoing)

PROJECT TITLE: Monitoring and Risk Assessment of European Corn Borer (ECB) Resistance to Transgenic Bt Corn in Minnesota (Year 2)

PROJECT NUMBER: 6111-23DD

REPORTING PERIOD: 08/01/2023-02/28/2024

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1.) PROJECT ACTIVITIES COMPLETED DURING THE REPORTING PERIOD. (*Describe project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

Conduct F₂ screens of ECB populations collected from different locations in Minnesota to estimate resistance allele frequency against Cry1 and Cry2 Bt proteins: The objective of this study is to measure Bt resistance allele frequencies in ECB using F₂ screen techniques. Over 2023-2024, we have successfully established 62 F₂ families of ECB collected from different locations in Minnesota and Wisconsin. We have screened these populations against Cry1Ab, Cry1F, Cry1A.105, and Cry2Ab2 Bt proteins (Tables 1-4). All detailed data are summarized in Tables 1-4. In summary, the resistance allele frequencies for Cry1Ab, Cry1F, and Cry1A.105 in these ECB populations were found to be low in the surveyed fields in 2023 and 2024, suggesting the risks of resistance to these Cry1 proteins in ECB are low across the states (Tables 1-3). However, the resistance allele frequencies for Cry2Ab2 in these ECB populations were found to be high in the surveyed fields in 2023 and 2024, suggesting the risks of resistance to Cry2Ab2 protein in ECB are high. Especially, we found one population collected from Wisconsin and 16 out of 44 populations collected from Martin County, Minnesota survived well on the diagnostic concentration of Cry2Ab2 (Table 4), indicating the major resistance alleles are present in the states. Further analysis based on the survival of 3rd instar larvae on 270 ng/cm² Cry2Ab2 protein indicated that the Cry2Ab2 resistance in ECB populations ranged from recessive, incomplete recessive, to incomplete dominant, which is deviated from the assumption for high-dose refuge strategy that Bt resistance is completely recessive in target insect populations, highlighting the risk of Cry2Ab2 resistance in ECB (Table 5).

Document the resistance levels of Cry2Ab2 resistance: The objective of this study is to assess Cry2Ab2 resistance levels in multiple ECB populations derived from F₂ screening. Four populations were evaluated: one from Wisconsin (ECB-WI-WP#2-Cry2Ab2-RR), two from Minnesota (ECB-MN-EC#8-Cry2Ab2-RR and ECB-MN-EC#48-Cry2Ab2-RR), and a lab-

susceptible control population (ECB-SS). Bioassay results revealed that ECB-SS was highly susceptible to Cry2Ab2, whereas all three resistant populations exhibited strong resistance (Table 6, Figure 1). These findings suggest that the resistance alleles identified in the field are major contributors to Cry2Ab2 resistance in Bt corn.

2.) IDENTIFY ANY SIGNIFICANT FINDINGS AND RESULTS OF THE PROJECT TO DATE.

Conduct F₂ screens of ECB populations collected from different locations in Minnesota to estimate resistance allele frequency against Cry1 and Cry2 Bt proteins: All ECB insects collected in Minnesota and Wisconsin during 2023-2024 exhibited high susceptibility to the Cry1Ab, Cry1F, and Cry1A.105 Bt proteins, suggesting Cry1Ab, Cry1F, and Cry1A.105 appeared particularly effective in controlling ECB populations. The observed resistance allele frequencies for Cry1Ab, Cry1F, and Cry1A.105 in ECB populations were found to be low in the surveyed fields in 2023, indicating the low risks of ECB resistance to these Cry1 Bt proteins. However, we found a total of 17 populations collected from Wisconsin and Minnesota carried resistance alleles against the Cry2Ab2 protein, with 3rd instar larval survivorship of 1.6-32.8% in the F₂ screening bioassays. In particular, some populations displayed incomplete recessive and dominant resistance to the Cry2Ab2 protein, suggesting Cry2Ab2 is not very effective for control of ECB in these locations, leaving only Cry1 proteins effectively control ECB, which will accelerate the evolution of ECB resistance to Cry1 proteins if pyramiding technology is not fully functional. Further confirmation tests on higher Cry2Ab2 concentrations showed that ECB-WI-WP#2 from Wisconsin could survive well with the survivorship to 2nd instar of 86.7%, to 3rd instar of 76.6% on 360 ng/cm² Cry2Ab2, and to 2nd instar of 71.1%, to 3rd instar of 63.3% on 540 ng/cm² Cry2Ab2 after five generations of selection (Table 7). Similarly, the ECB-MN-ELMCRREK#8 from Martin County, Minnesota could survive well with the survivorship to 2nd instar of 75.5%, to 3rd instar of 64.7% on 360 ng/cm² Cry2Ab2, and to 2nd instar of 59.1%, to 3rd instar of 41.0% on 540 ng/cm² Cry2Ab2 after 3 generations of selection (Table 7). While the survivorship to 2nd and 3rd instar for ECB-BZ-SS was 0% on these two higher Cry2Ab2 concentrations (Table 7).

Document the resistance levels of Cry2Ab2 resistance: The ECB-SS insects are highly susceptible to the Cry2Ab2 protein, with an LC₅₀ value of 139.7 ng/cm² (Table 6). No SS insects survived Cry2Ab2 concentrations of 360 ng/cm² or higher (Figure 1). In contrast, the LC₅₀ values for the three resistant populations were 767,571 ng/cm² for ECB-MN-EC#8-Cry2Ab2-RR, 1,350 ng/cm² for ECB-MN-EC#48-Cry2Ab2-RR, and 189,329 ng/cm² for ECB-WI-WP#2-Cry2Ab2-RR, resulting in resistance ratios of 5,494.4-, 9.7-, and 1,355.3-fold compared to SS (Table 6). Concentration-mortality responses further support this resistance. ECB-WI-WP#2-Cry2Ab2-RR exhibited only 0.0–13.2% mortality across tested concentrations (90–5,400 ng/cm²), while ECB-MN-EC#8-Cry2Ab2-RR showed 1.6–4.9% mortality at 90–900 ng/cm². ECB-MN-EC#48-Cry2Ab2-RR displayed slightly higher mortality (13.9–38.2%) at 270–900 ng/cm², though still significantly lower than ECB-SS (Figure 1). These results indicate that all three resistant populations exhibit high levels of Cry2Ab2 resistance. The resistant alleles identified in the F₂ screening are major contributors to this resistance, confirming its prevalence in field populations.

3.) CHALLENGES ENCOUNTERED. *(Describe any challenges that you encountered related to project progress specific to goals, objectives, and deliverables identified in the project workplan.)*

No budget challenges were found during this period.

4.) FINANCIAL INFORMATION (*Describe any budget challenges and provide specific reasons for deviations from the projected project spending.*)

One postdoc working on this project left the position during the project year, and the projects will be continued by graduate students. We may need to do the re-budget and budget extension.

5.) EDUCATION AND OUTREACH ACTIVITIES. (*Describe any conferences, workshops, field days, etc attended, number of contacts at each event, and/or publications developed to disseminate project results.*)

1. Calvin, W., Dwyer, T., Yang, F., 2024. European corn borer: Old pest, new problems. MN Crop News, <https://blog-crop-news.extension.umn.edu/2024/02/european-corn-borer-new-pest-old.html>
2. Dwyer, T., Calvin, W., **Yang, F.**, Status of Bt resistance in European corn borer (Lepidoptera: Crambidae) populations within Minnesota and Wisconsin. The Entomological Society of America Meeting, Phoenix, AZ, Nov 10-13, 2024.
3. Hanson, A., **Yang, F.**, Hutchison, B., Potter, B., Peltier, A., Miller, R., Stahl, L., 2024. Reducing Bt trait acres in 2024 Minnesota Corn Production? Implications for European corn borer. MN Crop News, <https://blog-crop-news.extension.umn.edu/2024/03/reducing-bt-trait-acres-in-2024.html>
4. Stahl, L., **Yang, F.**, 2024. Strategic Farming: Let's talk crops focused on corn insect pests. MN Crop News, <https://blog-crop-news.extension.umn.edu/2024/03/strategic-farming-lets-talk-crops-18.html>
5. **Yang, F.**, 2024. Field School for Ag Professionals. "Identification of pest & beneficial insects in field crops". July 30th, 2024.
6. **Yang, F.**, 2024. Management of corn insect pests: foliar & soil insecticides and seed treatment. 2024 CPM Short Course and MCPR Trade Show. Dec. 10-12th, Minneapolis, MN.
7. **Yang, F.**, 2025. Research Update for Management of Corn Rootworm and European Corn Borer. 2025 Research Update for Ag Professionals in Willmar, MN. Jan. 14th, Willmar, MN.
8. **Yang, F.**, 2025. Research Update for Management of Corn Rootworm and European Corn Borer. 2025 Research Update for Ag Professionals in Lamberton, MN. Jan. 15th, Lamberton, MN.
9. **Yang, F.**, 2025. Strategic Farming: Keeping track of changes in corn insect challenges. Feb. 26th, 2025, Webinar.
10. **Yang, F.**, Dwyer, T., Wangila, D., 2024. Scouting for European corn borer infestations. MN Crop News, <https://blog-crop-news.extension.umn.edu/2024/08/scouting-for-european-corn-borer>.
11. **Yang, F.**, Hunter N., and Dwyer T., Seed treatment for corn rootworm control and Bt resistance in European corn borer. 2025 MN AG Expo, Mankato, Jan 22-23, 2025.
12. **Yang, F.**, Potter, B. 2024. Scouting & decision making for corn rootworms & European corn borer. UMN Field School for Ag Professionals, July 31, 2024.

Table 1. ECB-F₂ families surviving a discriminatory concentration of 60 ng Cry1F/cm²

Year of collection	Collection site	No. F₂ families assayed	No. Surviving F₂ families	% of surviving F₂ families	Estimated No. major resistance alleles	Estimated major resistance allele frequency	Confidence interval (95%)
2023	Rosemount, MN	3	0	0	0	0.0253	(0, 0.0758)
	Crookston, MN	10	0	0	0		
	Waupaca County, WI	3	0	0	0		
	Wood County, WI	1	0	0	0		
	St. Croix, WI	1	0	0	0		
2024	Martin County, MN	44	0	0	0	0.0109	(0, 0.0327)
2023-2024	Overall	62	0	0	0	0.0078	(0, 0.0235)

Table 2. ECB-F₂ families surviving a discriminatory concentration of 11.2 ng Cry1Ab/cm²

Year of collection	Collection site	No. F₂ families assayed	No. Surviving F₂ families	% of surviving F₂ families	Estimated No. major resistance alleles	Estimated major resistance allele frequency	Confidence interval (95%)
2023	Rosemount, MN	3	0	0	0	0.0253	(0, 0.0758)
	Crookston, MN	10	0	0	0		
	Waupaca County, WI	3	0	0	0		
	Wood County, WI	1	0	0	0		
	St. Croix, WI	1	0	0	0		
2024	Martin County, MN	44	0	0	0	0.0109	(0, 0.0327)
2023-2024	Overall	62	0	0	0	0.0078	(0, 0.0235)

Table 3. ECB-F₂ families surviving a discriminatory concentration of 20 ng Cry1A.105/cm²

Year of collection	Collection site	No. F₂ families assayed	No. Surviving F₂ families	% of surviving F₂ families	Estimated No. major resistance alleles	Estimated major resistance allele frequency	Confidence interval (95%)
2023	Rosemount, MN	3	0	0	0	0.0253	(0, 0.0758)
	Crookston, MN	10	0	0	0		
	Waupaca County, WI	3	0	0	0		
	Wood County, WI	1	0	0	0		
	St. Croix, WI	1	0	0	0		
2024	Martin County, MN	44	0	0	0	0.0109	(0, 0.0327)
2023-2024	Overall	62	0	0	0	0.0078	(0, 0.0235)

Table 4. ECB-F₂ families surviving a discriminatory concentration of 270 ng Cry2Ab2/cm²

Year of collection	Collection site	No. F₂ families assayed	No. Surviving F₂ families	% of surviving F₂ families	Estimated No. major resistance alleles	Estimated major resistance allele frequency	Confidence interval (95%)
2023	Rosemount, MN	3	0	0	0	0.0513	(0.0065, 0.1399)
	Crookston, MN	10	0	0	0		
	Waupaca County, WI	3	1	33.3	1		
	Wood County, WI	1	0	0	0		
	St. Croix, WI	1	0	0	0		
2024	Martin County, MN	44	16	36.4	16	0.206	(0.1269, 0.3016)
2023-2024	Overall	62	17	27.4	17	0.1522	(0.0939, 0.2232)

Table 5. Survivorship for ≥ 3 rd instar of ECB F₂ family at discriminatory concentration of 270 ng Cry2Ab2/cm²

Year of collection	Collection site	F ₂ insect strain	% survival at 270 ng Cry2Ab2/cm ²	Possible dominance of resistance with 1 allele
2023	Waupaca County, WI	F2-ECB-WI-WP#2	15.8	Incomplete recessive
2024	Martin County, MN	F2-ECB-MN-ELMCREEK #8	20.4	Incomplete recessive
		F2-ECB-MN-ELMCREEK #26	32.8	Incomplete dominant
		F2-ECB-MN-ELMCREEK #30	11.7	Incomplete recessive
		F2-ECB-MN-ELMCREEK #48	13.1	Incomplete recessive
		F2-ECB-MN-ELMCREEK #50	23.1	Incomplete dominant
		F2-ECB-MN-ELMCREEK #51	19.7	Incomplete recessive
		F2-ECB-MN-ELMCREEK #53	9.8	Incomplete recessive
		F2-ECB-MN-ELMCREEK #57	22.9	Incomplete dominant
		F2-ECB-MN-ELMCREEK #88	1.6	Recessive
		F2-ECB-MN-ELMCREEK #95	24.6	Incomplete dominant
		F2-ECB-MN-ELMCREEK #97	11.5	Incomplete recessive
		F2-ECB-MN-ELMCREEK #108	26.2	Incomplete dominant
		F2-ECB-MN-ELMCREEK #109	9.8	Incomplete recessive
		F2-ECB-MN-ELMCREEK #111	6.6	Recessive
		F2-ECB-MN-ELMCREEK #120	18	Incomplete recessive
F2-ECB-MN-ELMCREEK #121	2.5	Recessive		

Table 6. LC₅₀ of different populations of ECB to Cry2Ab2 protein in diet-overlay bioassays

Insect genotype	N	LC₅₀ (95% FL) (ng/cm²)	Slope ± SE	X²	df	Resistance ratio
ECB-BZ-SS	512	139.7 (118.9, 162.1)	3.91 ± 0.43	43.3	26	1.0
ECB-MN-EC#8-Cry2Ab2-RR	437	767571	0.57 ± 0.76	13.3	18	5494.4
ECB-MN-EC#48-Cry2Ab2-RR	418	1350 (930.7, 4077)	1.70 ± 0.46	6.8	18	9.7
ECB-WI-WP#2-Cry2Ab2-RR	740	189329 (25014, 616283587)	0.68 ± 0.20	58.6	42	1355.3

Table 7. Confirmation of F₂ families surviving Cry2Ab2 protein

Insect Strain	Collection year	Location	No. survivor at 360 ng/cm ²			No. survivor at 540 ng/cm ²		
			No. Insect assayed	≥ 2nd instar	≥3rd instar	No. Insect assayed	≥ 2nd instar	≥3rd instar
ECB-BZ-SS	/	/	64	0 (0%)	0 (0%)	64	0 (0%)	0 (0%)
ECB-WP-WI#2-Cry2Ab-RR-F5	2023	Waupaca County, WI	128	111 (86.7%)	98 (76.6%)	128	91 (71.1%)	81 (63.3%)
ECB-MN-ELMCREEK#8-Cry2Ab-RR-F3	2024	Martin County, MN	184	139 (75.5%)	119 (64.7%)	61	36 (59.1%)	25 (41.0%)

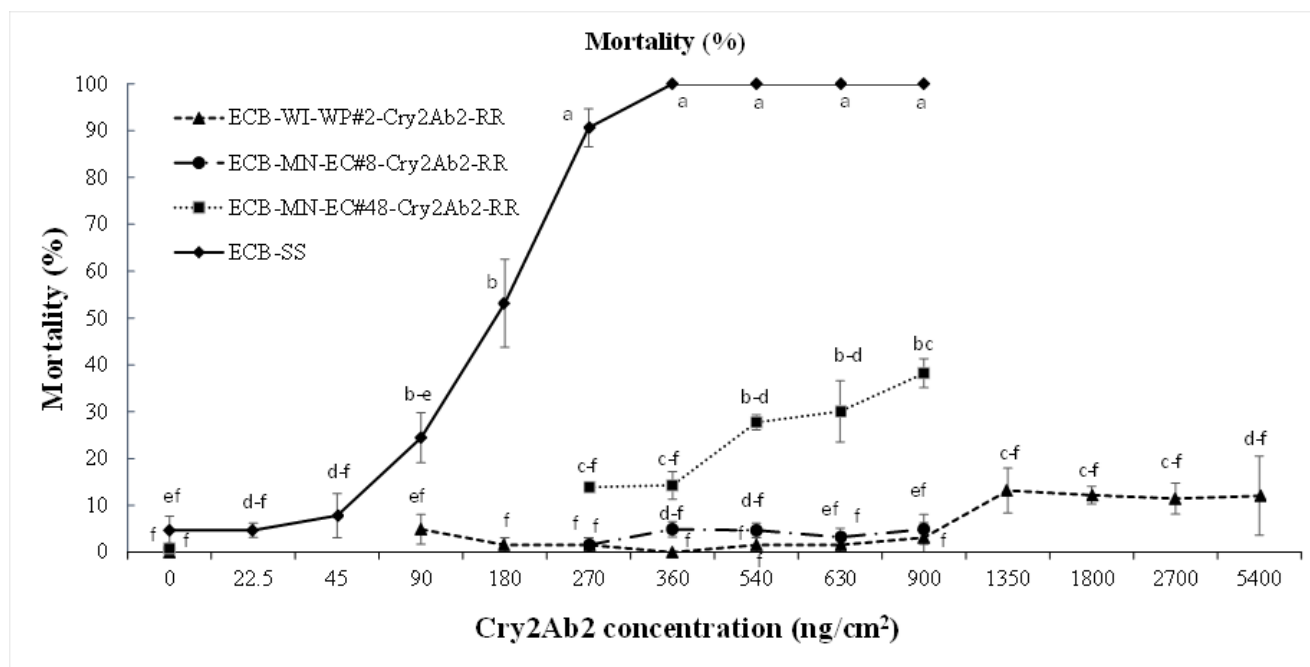


Figure 1. Concentration-larval mortality response of different ECB populations to Cry2Ab2 protein. The effect of insect genotype, protein concentration and the interaction between insect genotype and protein concentration were all significant ($F \geq 15.92$; $df = 3-16$, 99; $P < 0.0001$). Mean values followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD test).