Corn Rootworm

Bt Resistance and Management Recommendations for Illinois

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Western corn rootworm

Diabrotica virgifera virgifera

Northern corn rootworm

Diabrotica barberi

Current Rootworm Situation in Illinois

Populations (esp. NCR) have increased in continuous corn areas

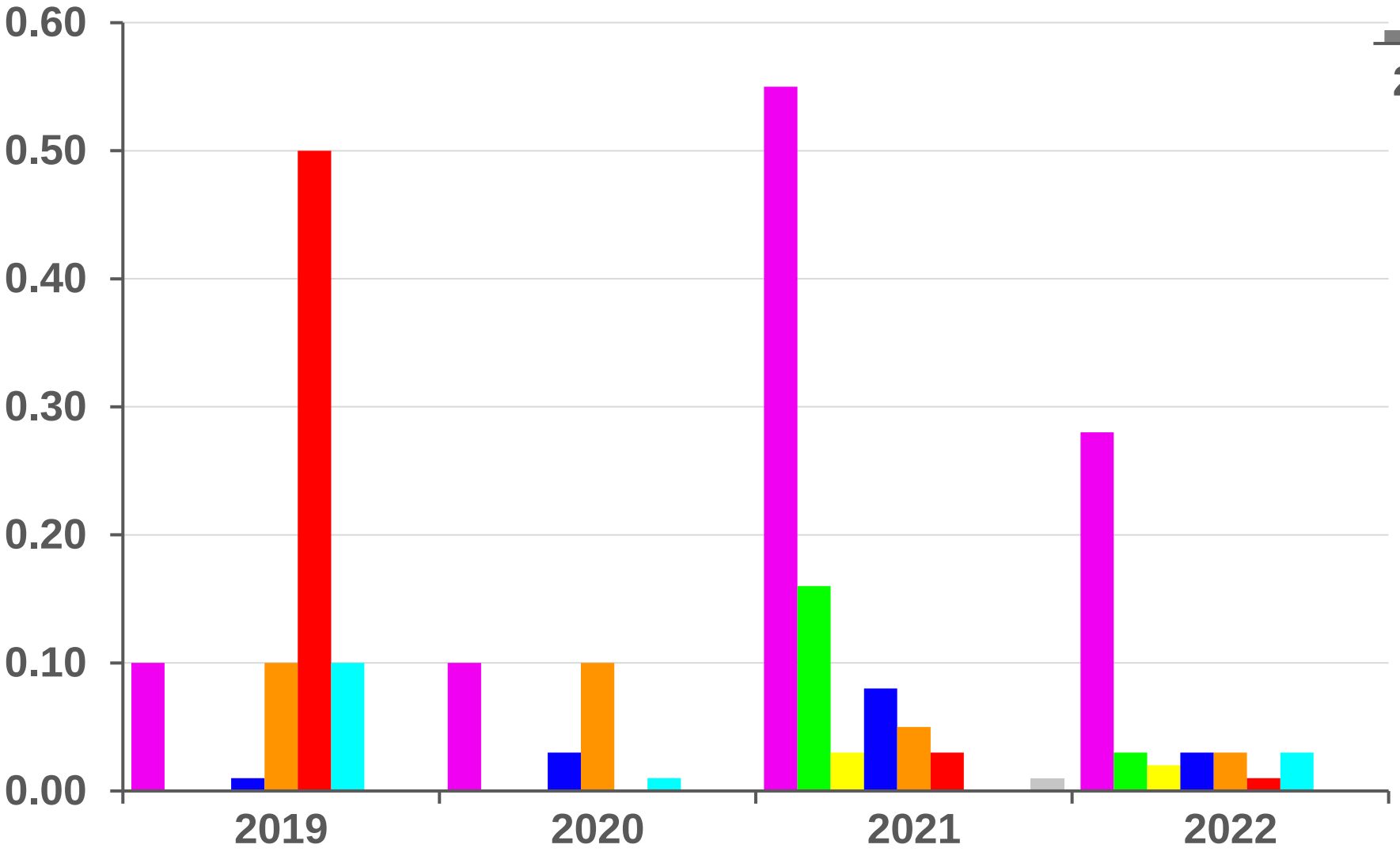
slightly less in 2022 compared with 2021

Rotation-resistant “variant” pressure is very low

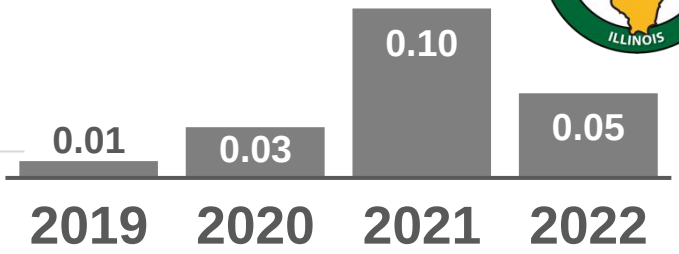
Populations of both western and northern corn rootworm have resistance to all available Bt traits in some areas

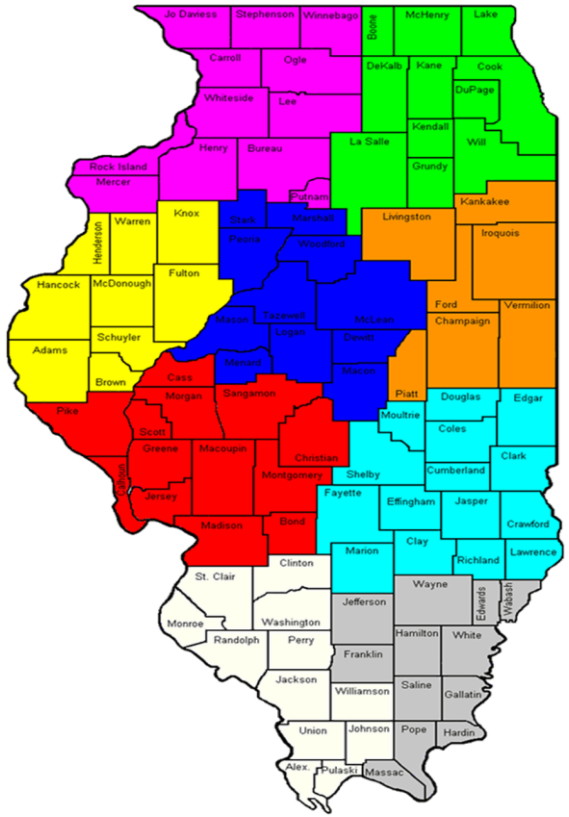
Western Corn Rootworm

Average number of beetles per plant



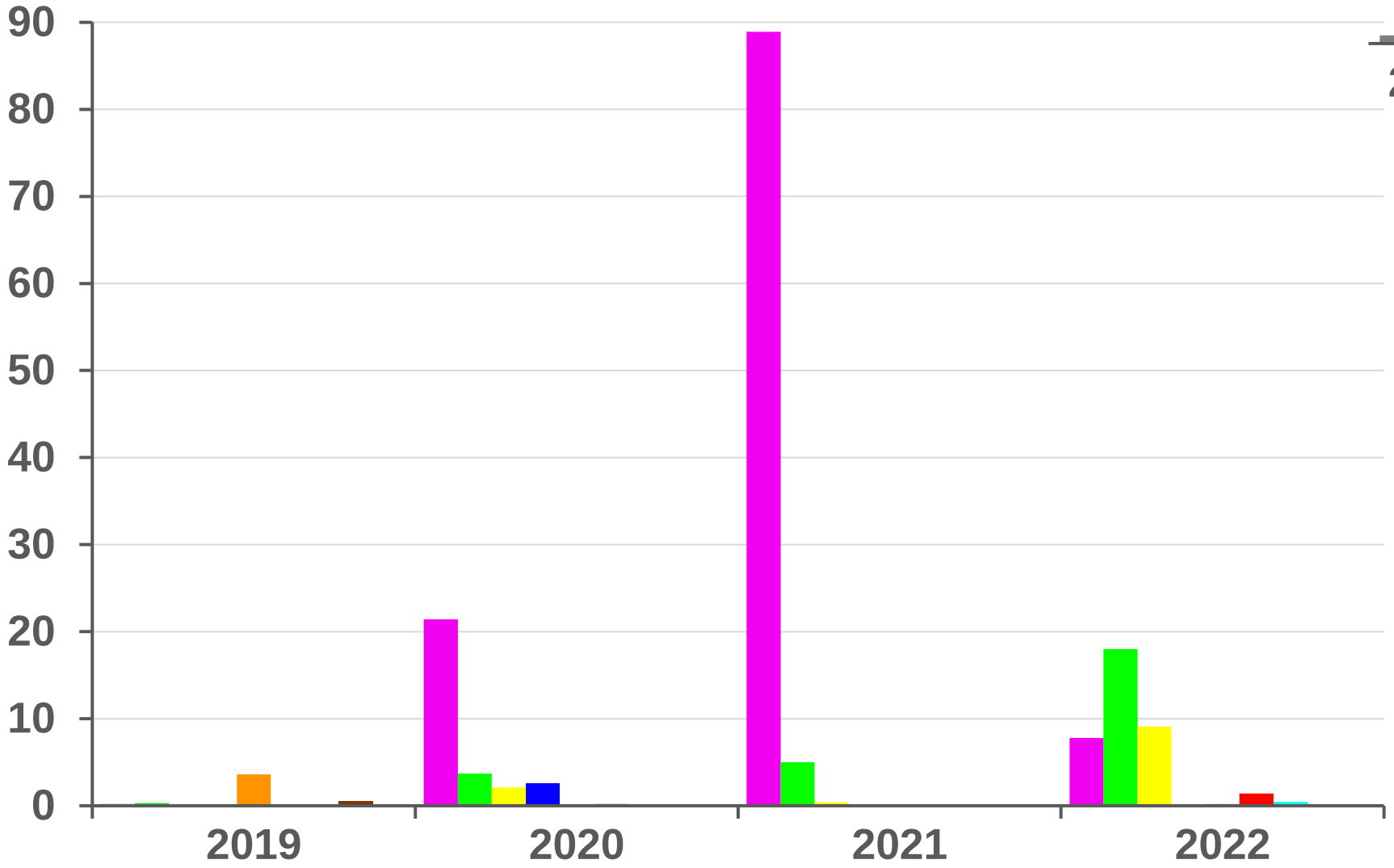
State Average



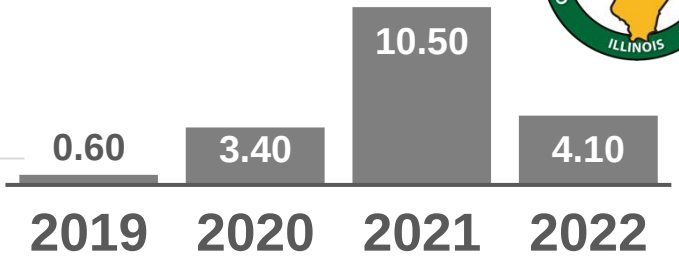


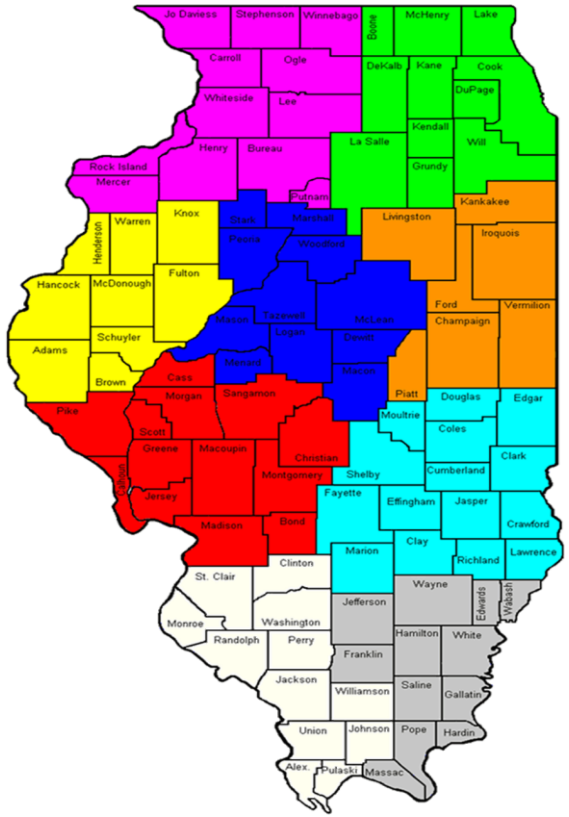
Northern Corn Rootworm

Average number of beetles per 100 sweeps



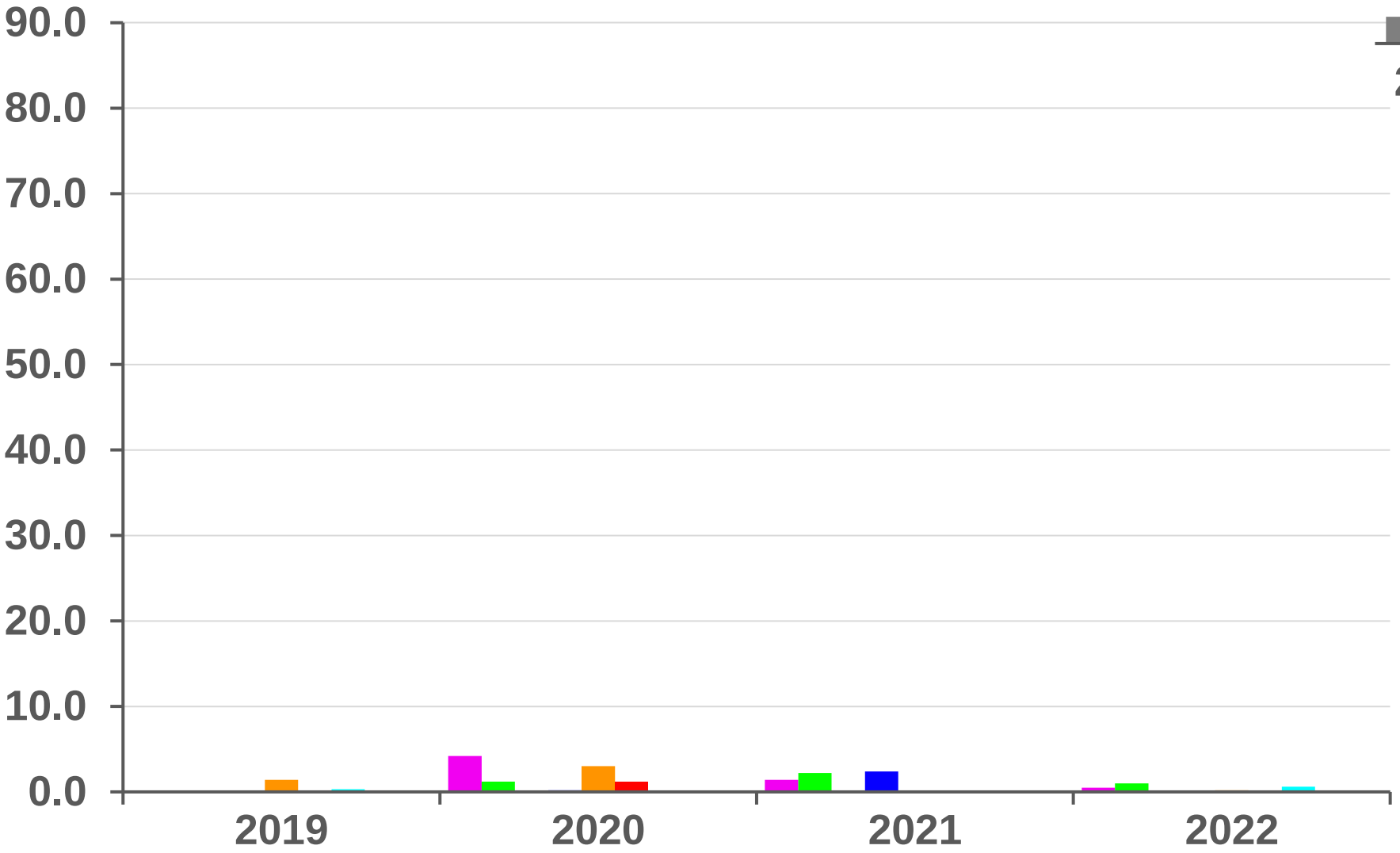
State Average



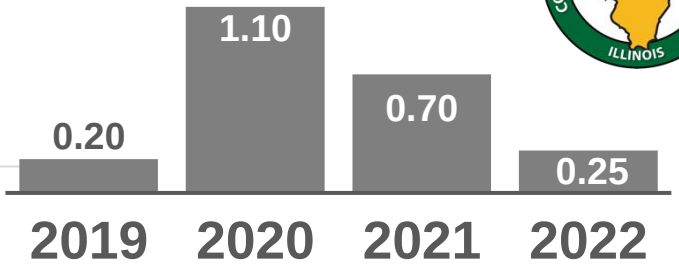


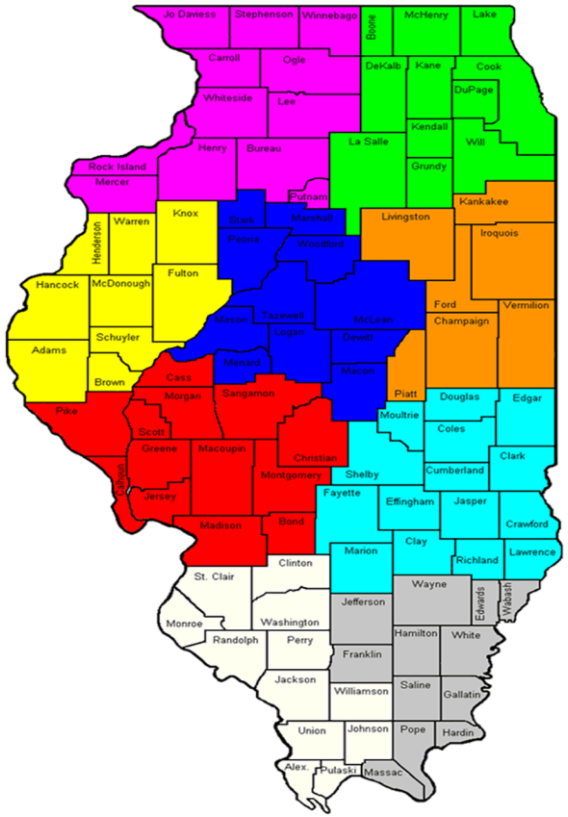
Western Corn Rootworm

Average number of beetles per 100 sweeps



State Average





Corn Rootworm State Average

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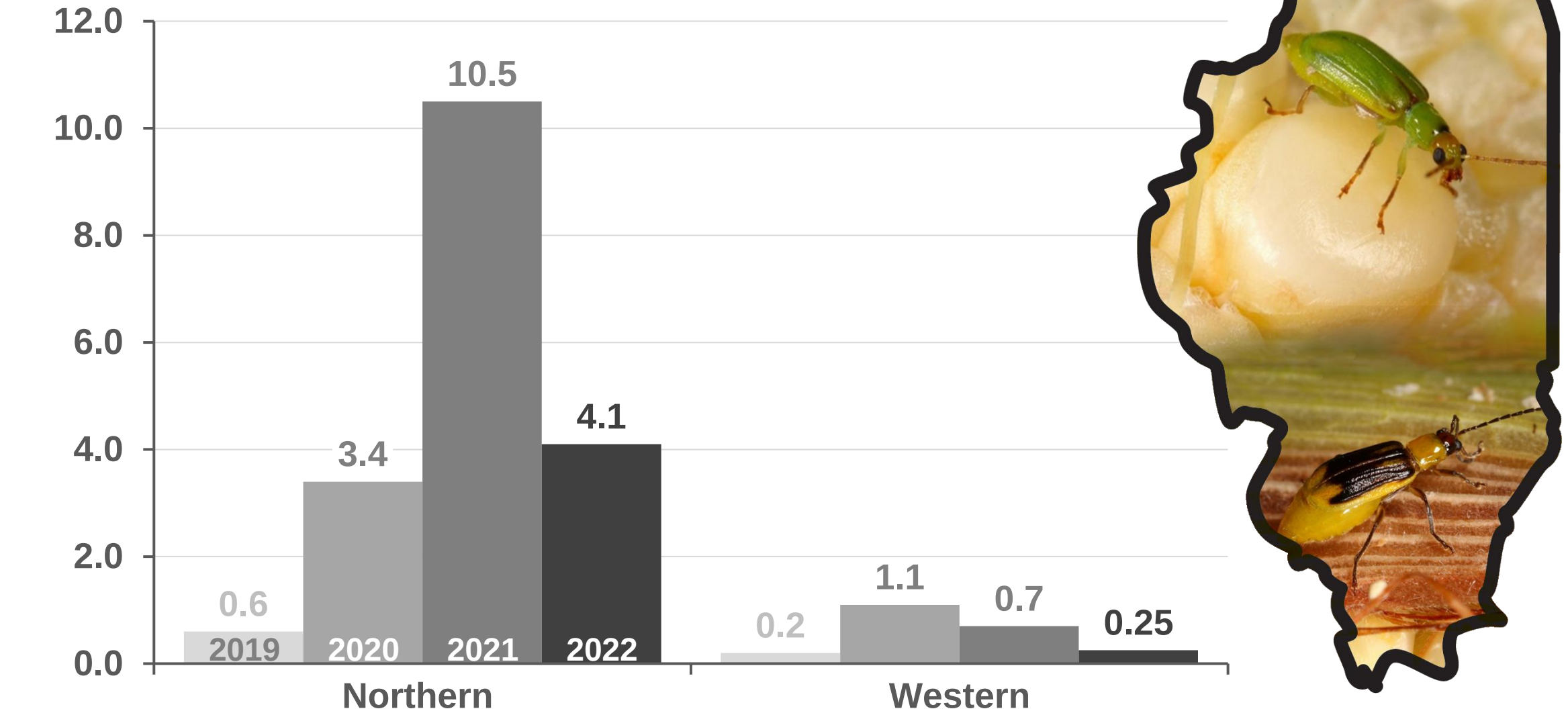
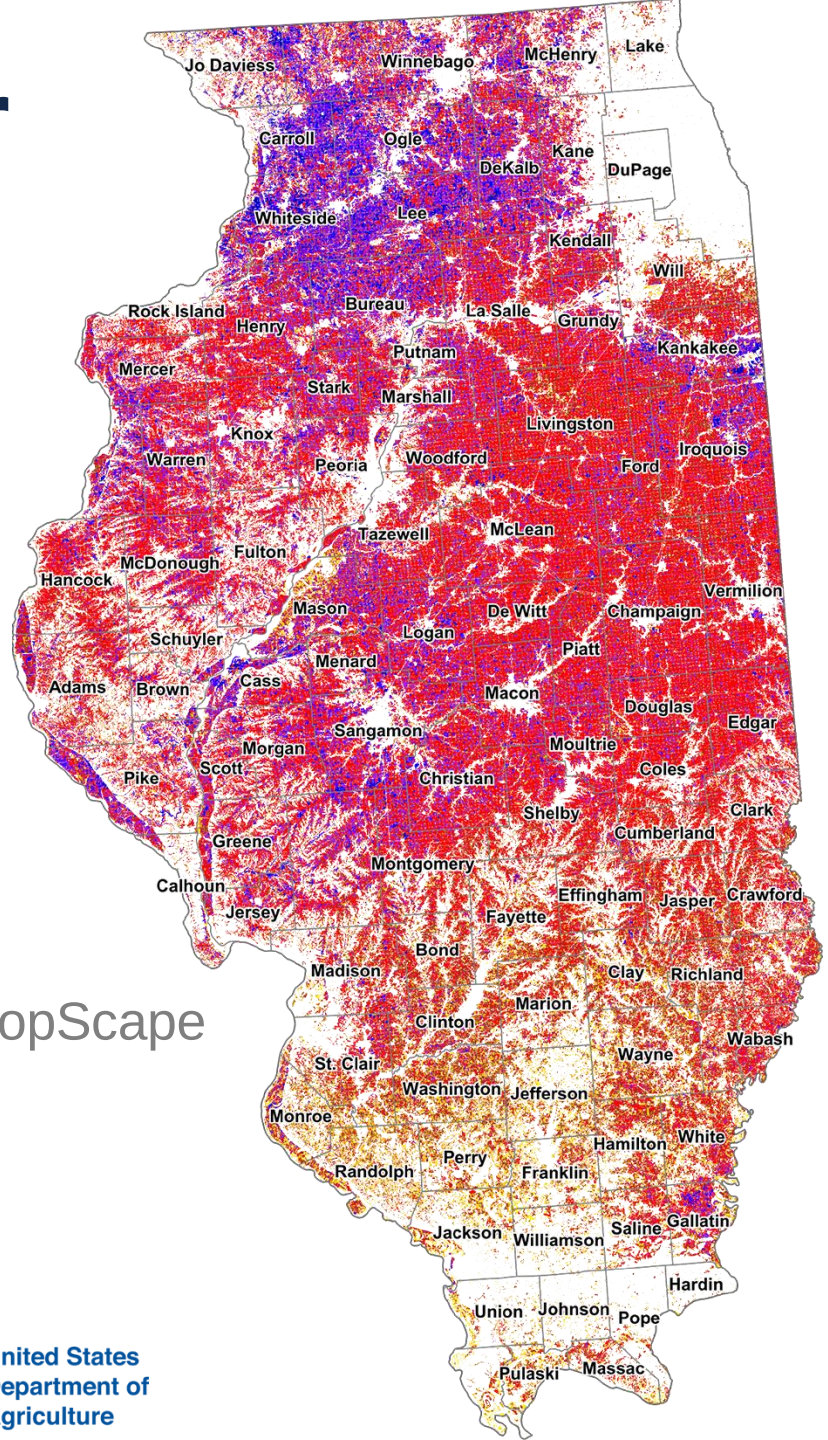


Corn Frequency Layer
2008 to 2021



Land Cover Categories

- 0 Year
- 1 Year
- 2 Years
- 3 Years
- 4 Years
- 5 Years
- 6 Years
- 7 Years
- 8 Years
- 9 Years
- 10 Years
- 11 Years
- 12 Years
- 13 Years
- 14 Years

Produced by CropScape
http://nassgeodata.gmu.edu/CropScape

USDA United States Department of Agriculture

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Four Bt proteins, “two” modes of action, several combinations

SmartStax = Cry3Bb1 + Cry34/35Ab1

Acremax Xtreme = mCry3A + Cry34/35Ab1

Qrome = mCry3A + Cry34/35Ab1

Agrisure 3122 = mCry3A + Cry34/35Ab1

Duracade = mCry3A + eCry3.1Ab

Similar mode of action(“Cry3” traits)

- Cry3Bb1
- mCry3A
- eCry3.1Ab

Distinct mode of action

- Cry34/35Ab1

Cross-resistance in WCR among Cry3Bb1, mCry3A, eCry3.1Ab
Fitness cost reported for Cry34/35Ab1 resistance, but not for Cry 3 resistance

https://www.texasinsects.org/bt-corn-trait-table.html

Rootworm Trait Packages with RNA-interference Mode of Action

SmartStax Pro

Bayer

Limited release: 2022

Commercial release: 2023

Above-ground:
Cry1A.105, Cry2Ab2, Cry1F

Below-ground:
Cry3Bb1, Cry34/35Ab1, DvSnf7 dsRNA

Herbicide:
glyphosate, glufosinate

Vorceed Enlist

Corteva

Limited release: 2023

Larger release in subsequent years

Above-ground:
Cry1A.105, Cry2Ab2, Cry1F

Below-ground:
Cry3Bb1, Cry34/35Ab1, DvSnf7 dsRNA

Herbicide: glyphosate, glufosinate, 2,4-D

VT4Pro

Bayer

Estimated commercial release in 2024

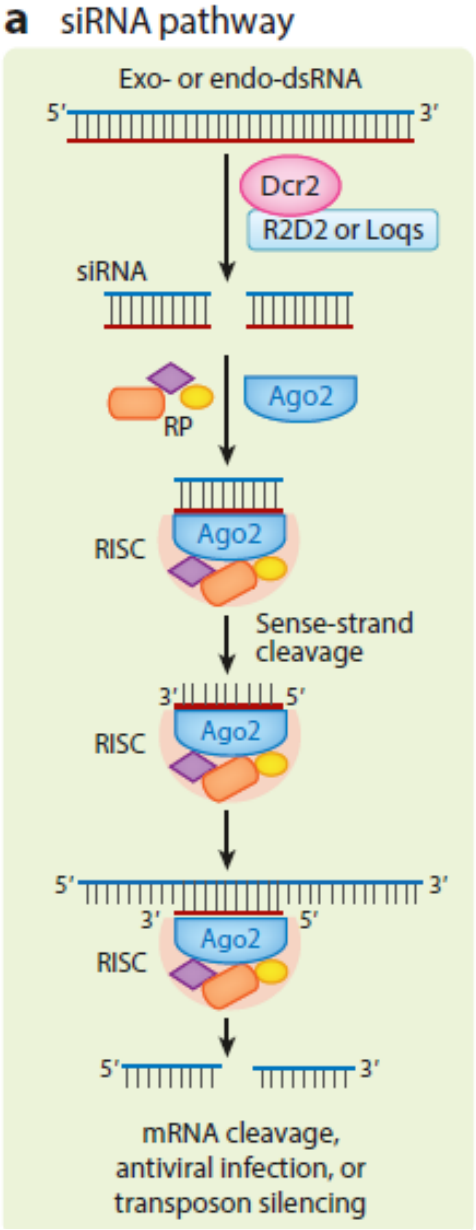
Above-ground:
Cry1A.105, Cry2Ab2, Vip3Aa20

Below-ground:
Cry3Bb1, DvSnf7 dsRNA

Herbicide:
glyphosate

How does RNAi work?

- Traited corn expresses double stranded RNA that codes for an essential rootworm protein
- Cellular machinery of the rootworm essentially recognizes this dsRNA as “foreign”, prevents protein expression
- Because this protein is essential for rootworm growth, the insect dies in ~5 days
- Requires both a local and a systemic response (response spreads among cells)
  – both occur readily in beetles



From Zhu and Palli 2020, Annu. Rev. Entomol. 65: 293-311

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Herbicide:
glyphosate

Potential for resistance

Resistant western corn rootworm population was developed in lab from field-collected beetles

Reduced uptake of dsRNA

No cross-resistance to Bt traits

Cross-resistant to other dsRNAs

https://doi.org/10.1371/journal.pone.0197059

PLOS ONE

RESEARCH ARTICLE

Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte

Chitvan Khajuria*, Sergey Ivashuta, Elizabeth Wiggins, Lex Flagel, William Moar, Michael Pleau, Kaylee Miller, Yuanji Zhang, Parthasarathy Ramaseshadri, Changjian Jiang, Tracey Hodge, Peter Jensen, Mao Chen, Anilkumar Gowda, Brian McNulty, Cara Vazquez, Renata Bolognesi, Jeffrey Haas, Graham Head, Thomas Clark

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OPEN ACCESS

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Abstract

The use of dsRNA to control insect pests via the RNA interference (RNAi) pathway is being explored by researchers globally. However, with every new class of insect control compounds, the evolution of insect resistance needs to be considered, and understanding resistance mechanisms is essential in designing durable technologies and effective resistance management strategies. To gain insight into insect resistance to dsRNA, a field screen with subsequent laboratory selection was used to establish a population of DvSnf7 dsRNA-resistant western corn rootworm, *Diabrotica virgifera virgifera*, a major maize insect pest. WCR resistant to ingested DvSnf7 dsRNA had impaired luminal uptake and resistance was not DvSnf7 dsRNA-specific, as indicated by cross resistance to all other dsRNAs tested. No resistance to the *Bacillus thuringiensis* Cry3Bb1 protein was observed. DvSnf7 dsRNA resistance was inherited recessively, located on a single locus, and autosomal. Together these findings will provide insights for dsRNA deployment for insect pest control.

Introduction

Transgenic maize expressing *Bacillus thuringiensis* (Bt) proteins has been rapidly adopted on farms across the Midwestern U.S. Corn Belt to control WCR, *Diabrotica virgifera virgifera LeConte* and other corn rootworm (CRW) species[1, 2]. However, at least some resistance has been reported to all five Bt proteins currently used to control WCR[3–8], emphasizing the need for alternative management tools.

Since the first report of expressing insecticidal dsRNA in plants[9], the use of dsRNA to control insect pests via the RNA interference (RNAi) pathway has been explored by numerous researchers globally[10–15]. DvSnf7 dsRNA-expressing maize (*Zea mays* L.) targeting Western corn rootworm (WCR) was the first insecticidal dsRNA-expressing plant registered by US

NCR and WCR have a long history of overcoming management tactics.

Insecticides, Crop Rotation, Bt Corn Hybrids

Genetically-based decreases in tactic susceptibility, are called “resistance”.

Adaptation and Invasiveness of Western Corn Rootworm: Intensifying Research on a Worsening Pest*

Michael E. Gray,[1] Thomas W. Sappington,[2] Nicholas J. Miller,[2] Joachim Moeser,[3] and Martin O. Bohn[1]

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[2]USDA-ARS, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, Iowa 50011; email: Tom.Sappington@ars.usda.gov, nicholas.miller@ars.usda.gov

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Annu. Rev. Entomol. 2009. 54:303–21

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi: 10.1146/annurev.ento.54.110807.090434

Key Words

Diabrotica virgifera virgifera, maize rotation

Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is an established insect pest of maize (*Zea mays* L.) in North America. The rotation of maize with another crop, principally soybeans, *Glycine max*

insects

MDPI

Review

The Use of Insecticides to Manage the Western Corn Rootworm, *Diabrotica virgifera virgifera*, LeConte: History, Field-Evolved Resistance, and Associated Mechanisms

Lance J. Meinke [1,*], Dariane Souza [2] and Blair D. Siegfried [2]

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[2] Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, USA; dariane.souza@ufl.edu (D.S.); bsiegfried1@ufl.edu (B.D.S.)
* Correspondence: lmeinke1@unl.edu

Simple Summary: The structure of agricultural enterprises in the western United States Corn Belt (large irrigated monocultures, continuous planting of maize, strong aerial pesticide application and livestock industries) has led to a tradition of extensive insecticide use over time to manage the western

insects

MDPI

Review

Resistance to Bt Maize by Western Corn Rootworm: Effects of Pest Biology, the Pest–Crop Interaction and the Agricultural Landscape on Resistance

Aaron J. Gassmann

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Insects **2021**, *12*, 136. https://doi.org/10.3390/insects12020136 https://www.mdpi.com/journal/insects

Bt Traits for Corn Rootworm Management

Four Single Bt toxins for corn rootworm management have been commercialized:

- Cry3Bb1 (Yieldgard Rootworm)
- mCry3A (Agrisure RW)
- eCry3.1Ab (Agrisure Duracade)

} **"Cry3" toxins**

- Cry34/35Ab1 (Herculex CRW)

Bt Traits for Corn Rootworm Management

Pyramided rootworm Bt hybrids express ≥2 rootworm Bt toxins in a single plant.

- Multiple MOAs are more durable; larvae need to be resistant to ≥2 Bt toxins.
- To fully exploit the benefits, novel toxins should be pyramided.

Rootworm Bt trait/toxin commercialization dates:

2003: Cry3Bb1

2006: Cry34/35Ab1

2007: mCry3A

2010: Cry3Bb1 + 34/35Ab1

2012: mCry3A + 34/35Ab1

2014: eCry3.1Ab + mCry3A

2022: Cry3Bb1 + 34/35Ab1 + DvSnf7 (RNAi)

Rootworm Resistance to Bt Traits

2009

First evidence of field-evolved resistance to Cry3Bb1 in western corn rootworm: *6 years after commercialization*.

- Gassmann et al. 2011 PLOS One 6 (7)

Cross-resistance among Cry3Bb1, mCry3A, and eCry3.1Ab

- Gassmann et al. 2014. PNAS https://doi.org/10.1073/pnas.1317179111
- Zukoff et al. 2016. J. Econ. Ent. https://doi.org/10.1093/jee/tow073 PMID: 27106225

Rootworm Resistance to Bt Traits

2016 - 2019

Confirmed reports of field-evolved resistance to Cry34/35Ab1 in Iowa and Minnesota

- Gassmann et al. 2019. Pest Man. Sci. DOI: 10.1002/ps5510
- Gassmann et al. 2016. J. Econ. Ent. DOI: 10.1093/jee/tow110
- Ludwick et al. 2017. J. Appl Ent. https://doi.org/10.1111/jen.12377

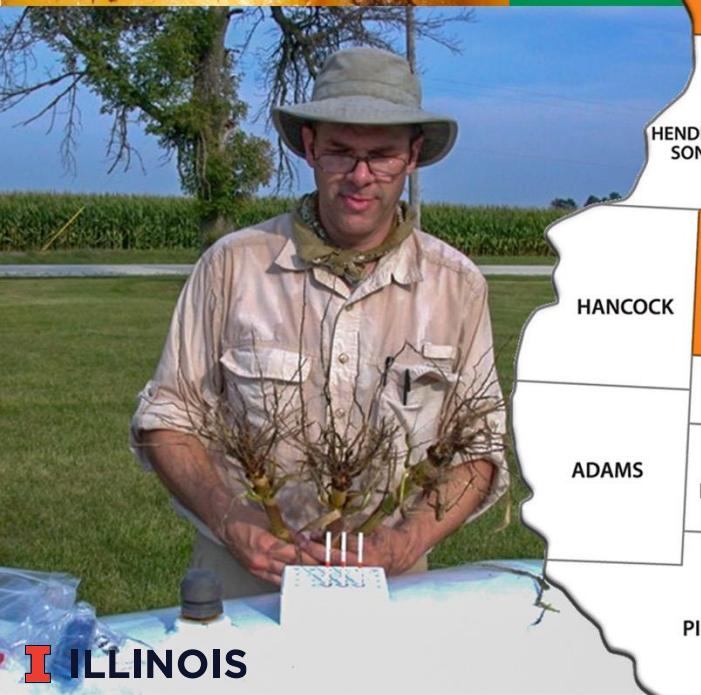
Rootworm Resistance to Bt Traits

2019

Confirmed reports of field-evolved resistance to Cry3Bb1 & Cry34/35Ab1 in North Dakota WCR & NCR!

- Calles-Torrez et al. 2019. J. of Econ. Ent. DOI: 10.1093/jee/toz111

2022 Illinois Bt Resistance Status
(~2012-2022)



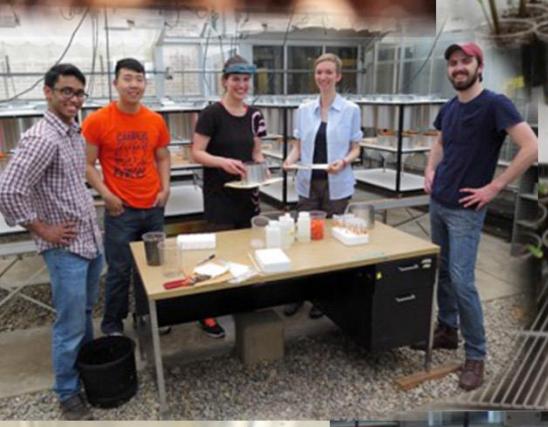
- **Counties with Bt resistance in *continuous* corn.**
- **Counties with Bt resistance in *rotated* corn.**
- **Injury due to Bt resistance possible in at least northern half of Illinois.**
- **Evidence of Cry3Bb1 resistance found wherever WCR were tested.**
- **Cry34/35Ab1 reduced susceptibility or resistance present in recent years.**

Single-plant Bt-resistance bioassay (Gassmann et al. 2011)

Single Bt trait hybrids (Cry3Bb1 + Cry34/35Ab1) + isolines

SmartStax® PRO family of hybrids

Suspected "R" populations vs. USDA Bt-susceptible WCR



→ **Measure: proportion surviving larvae & proportion 3rd instars among survivors**

Gassmann et al. 2011. *PLoS ONE* 6(7): e22629.

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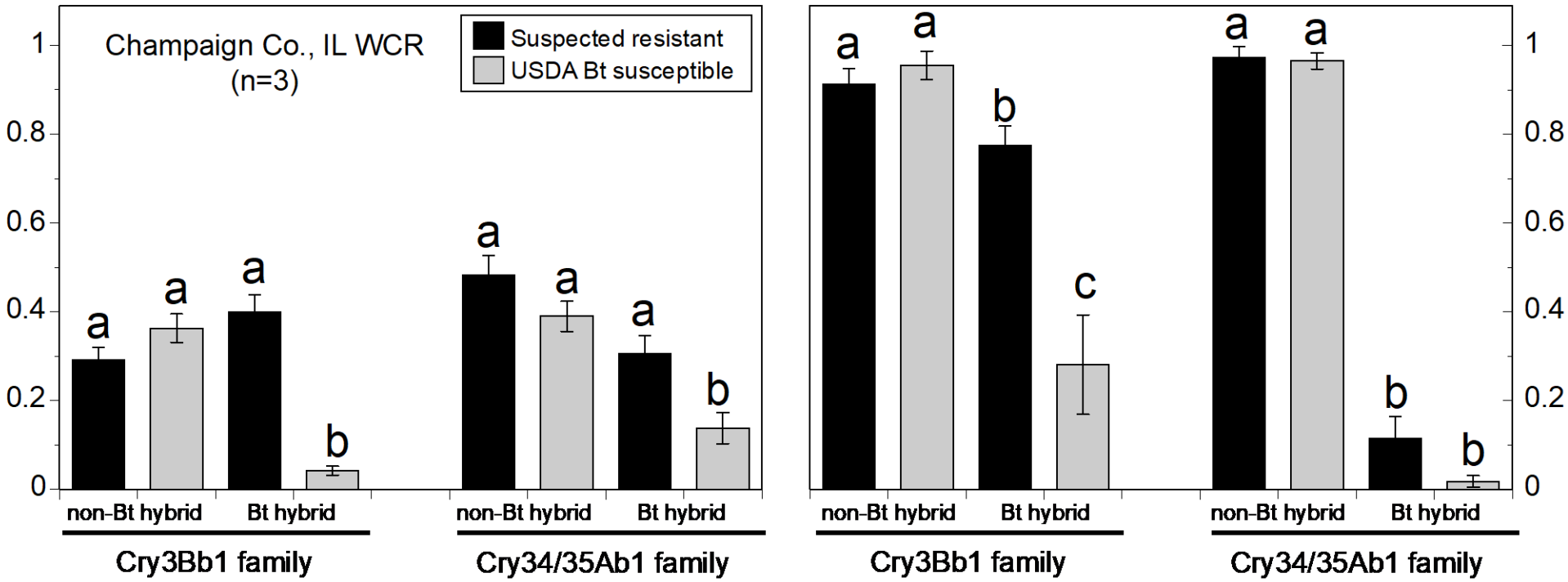
2022 IL
Single Trait Bt Bioassay

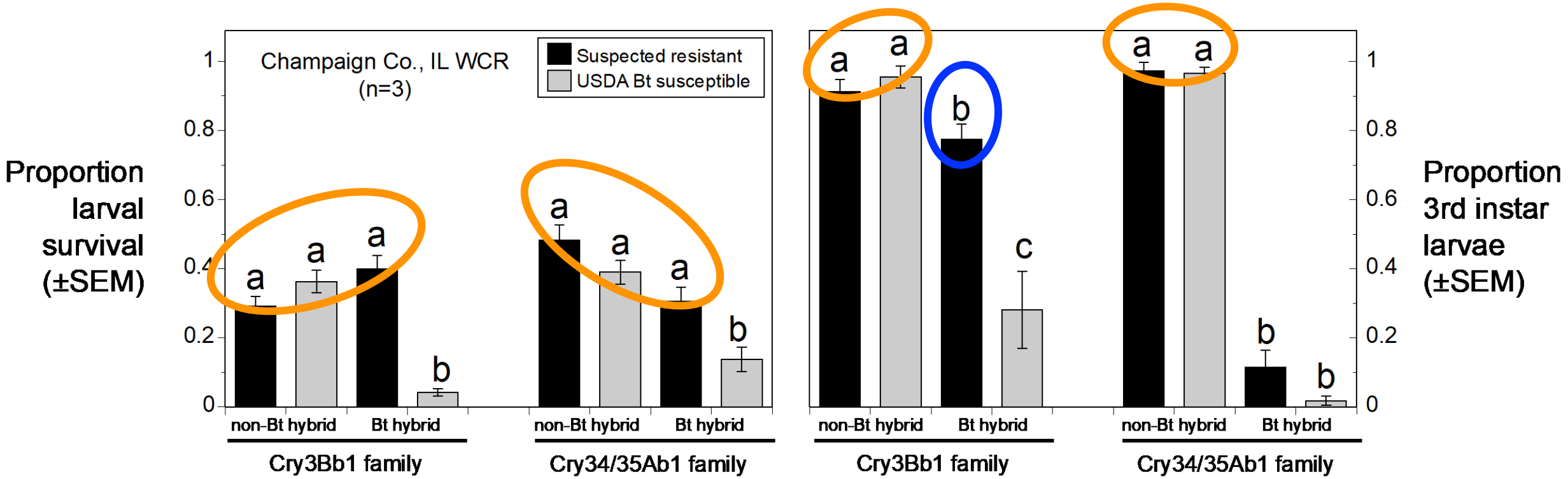
Proportion larval survival (±SEM)



Proportion 3rd instar larvae (±SEM)

Single Bt trait expressed in plants

2022 IL Single Trait Bt Bioassay



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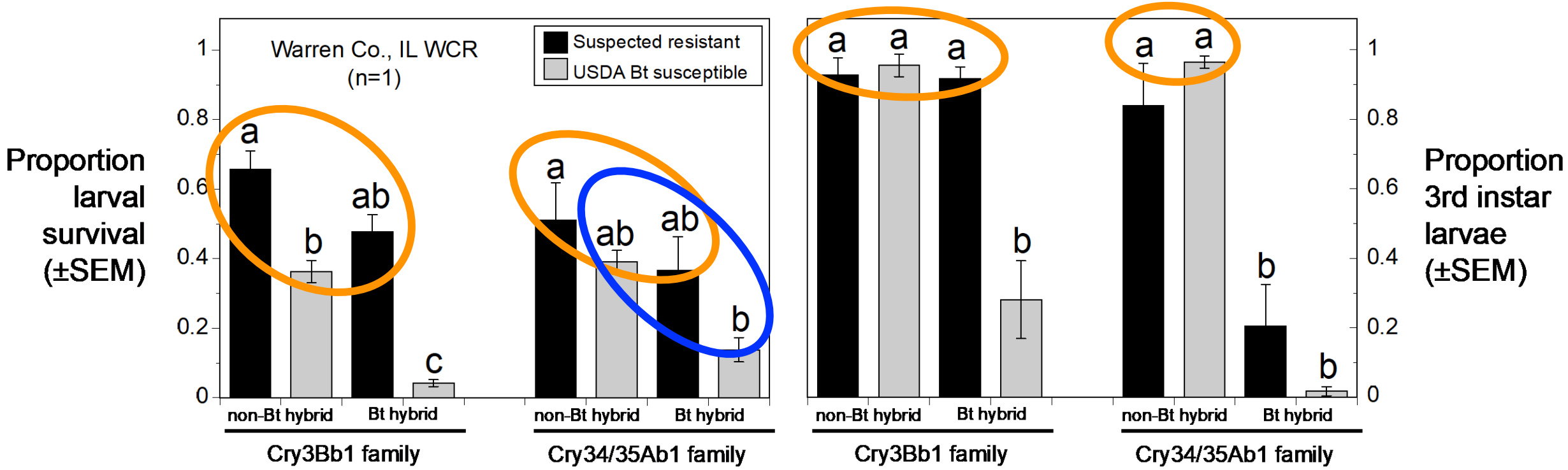
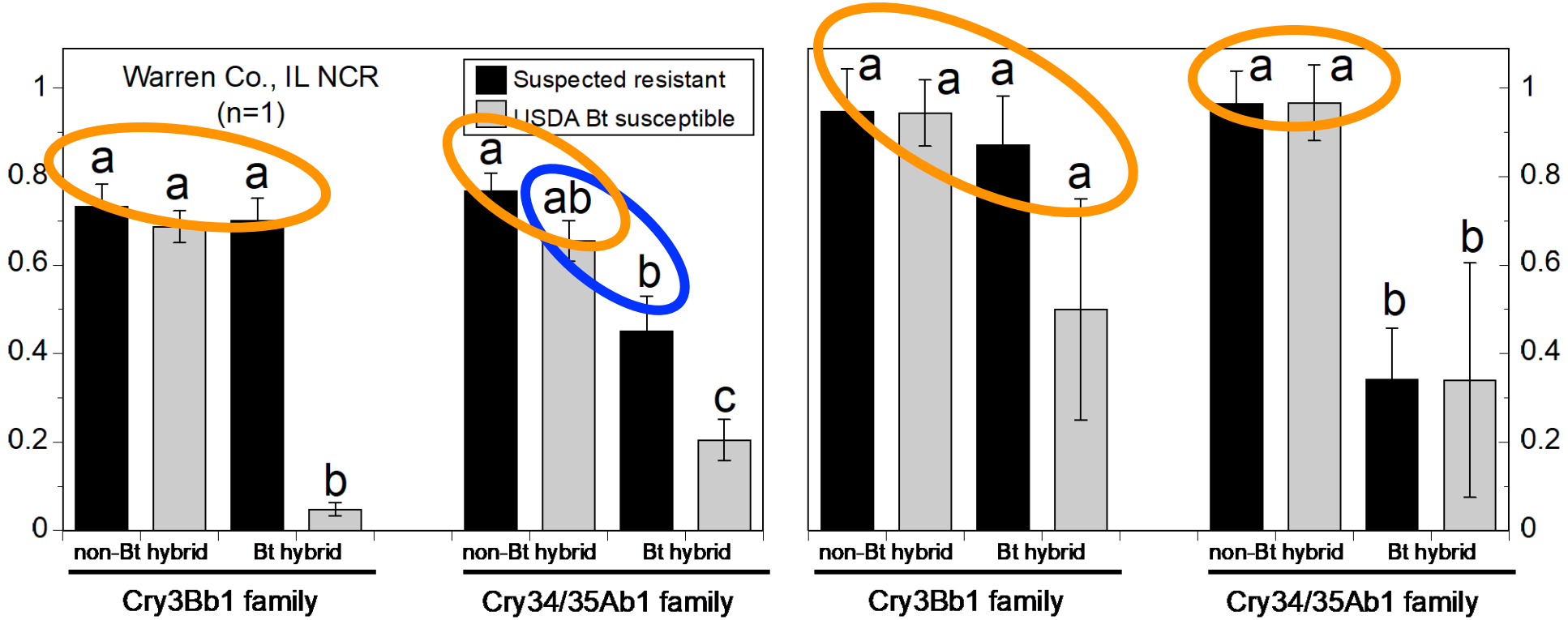
Single Trait Bt Bioassay



Single Bt trait expressed in plants

2022 IL
Single Trait Bt Bioassay

Proportion larval survival (±SEM)



Proportion 3rd instar larvae (±SEM)

Single Bt trait expressed in plants

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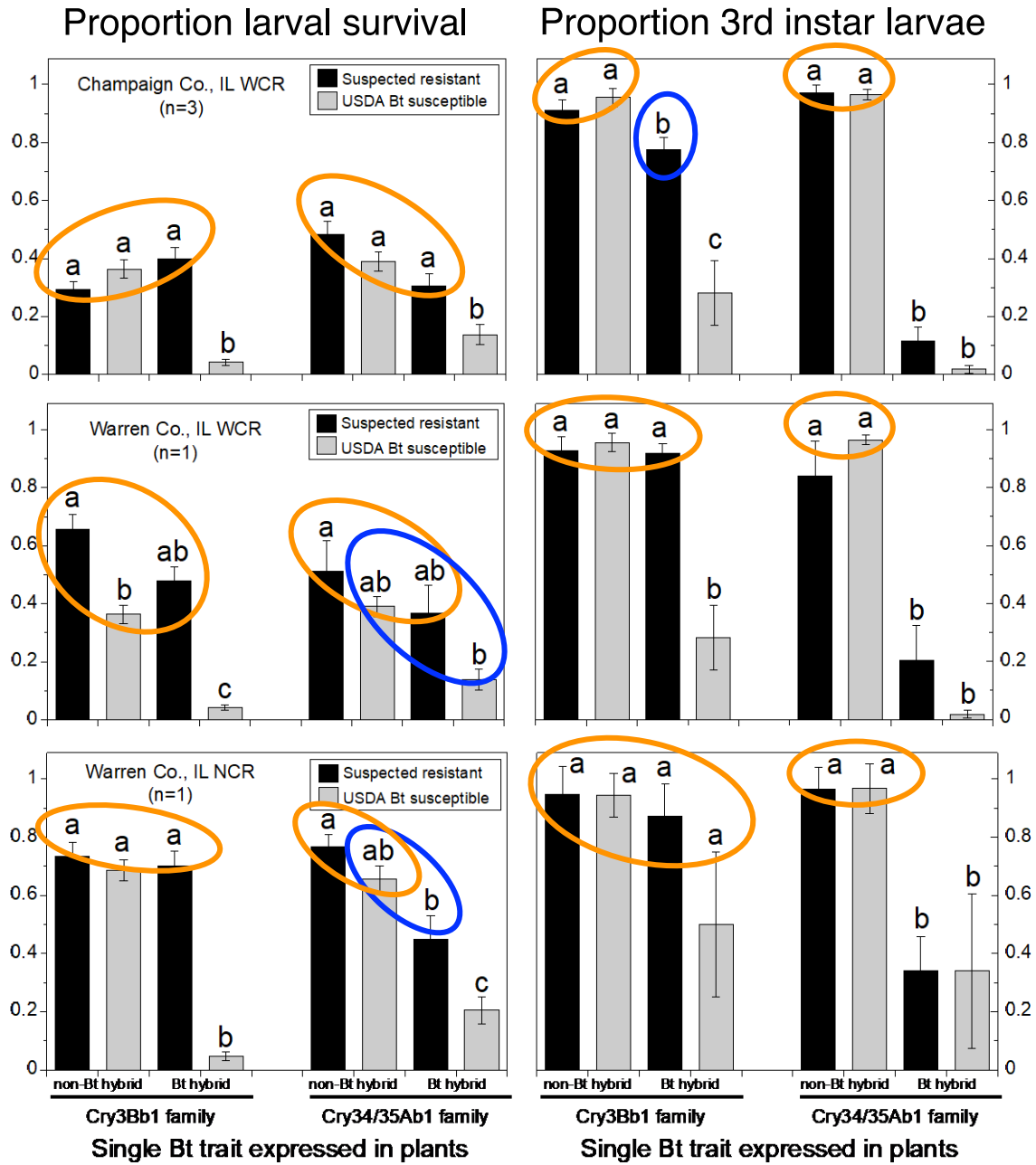
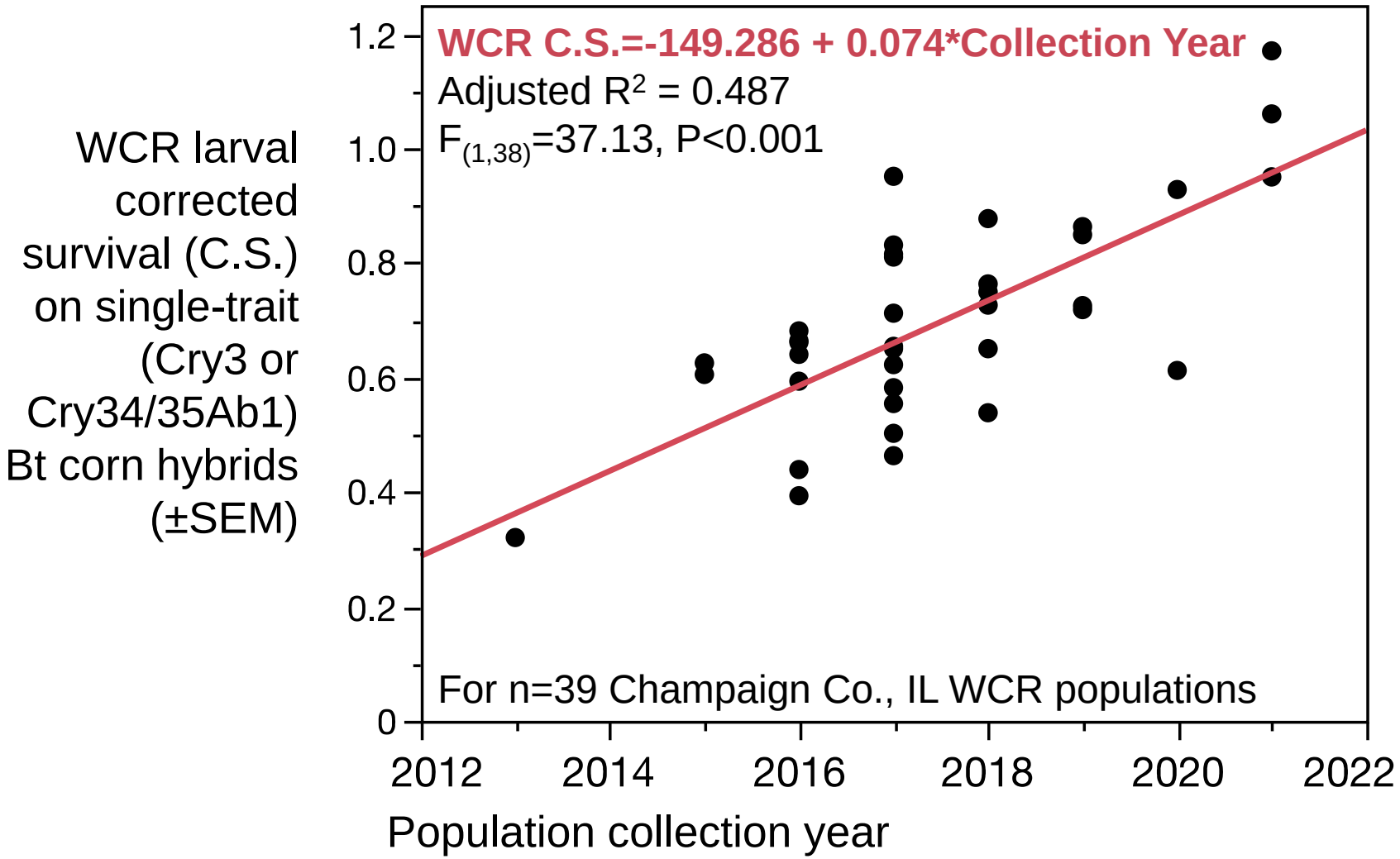


- NCR & WCR survive equally well on Cry3Bb1 and non-Bt hybrid in bioassays.
- CRW survival on Cry34/35Ab1 hybrid is almost equal to survival on non-Bt hybrid.
- Cry3Bb1 survivors may have minor developmental delays; Cry34/35Ab1 survivors have significant delays.

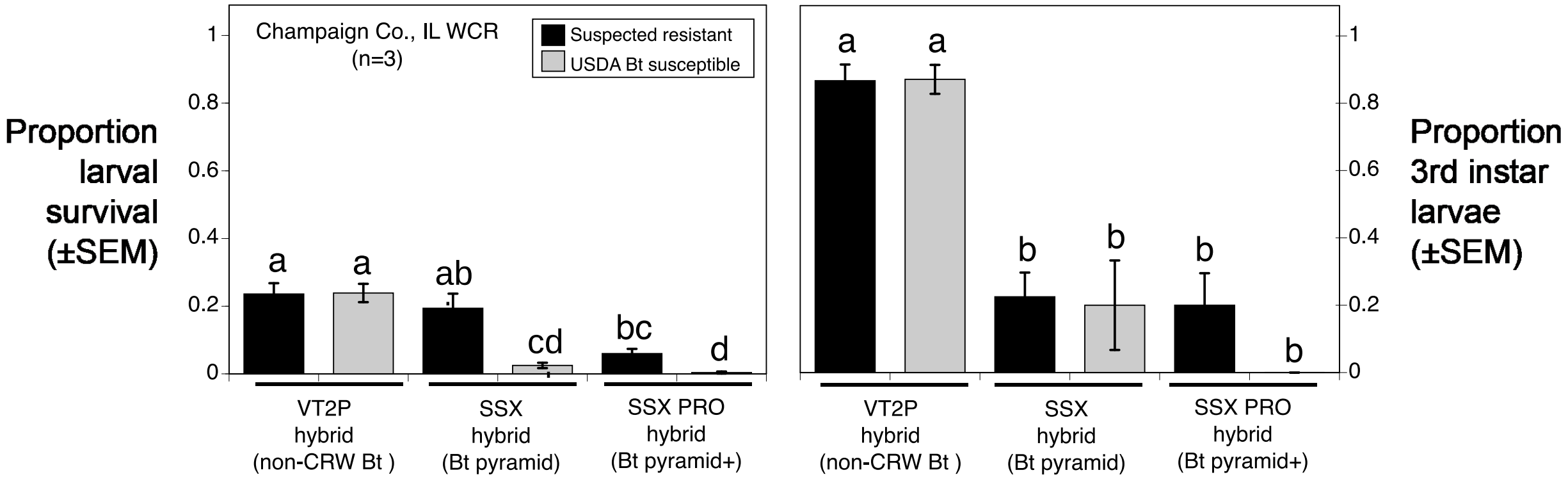
WCR Resistance to Bt traits is increasing in Illinois



- Corrected survival (C.S.) is the quotient of larval survival on a Bt maize hybrid divided by larval survival on the non-Bt hybrid.
- Lower values indicate greater efficacy.

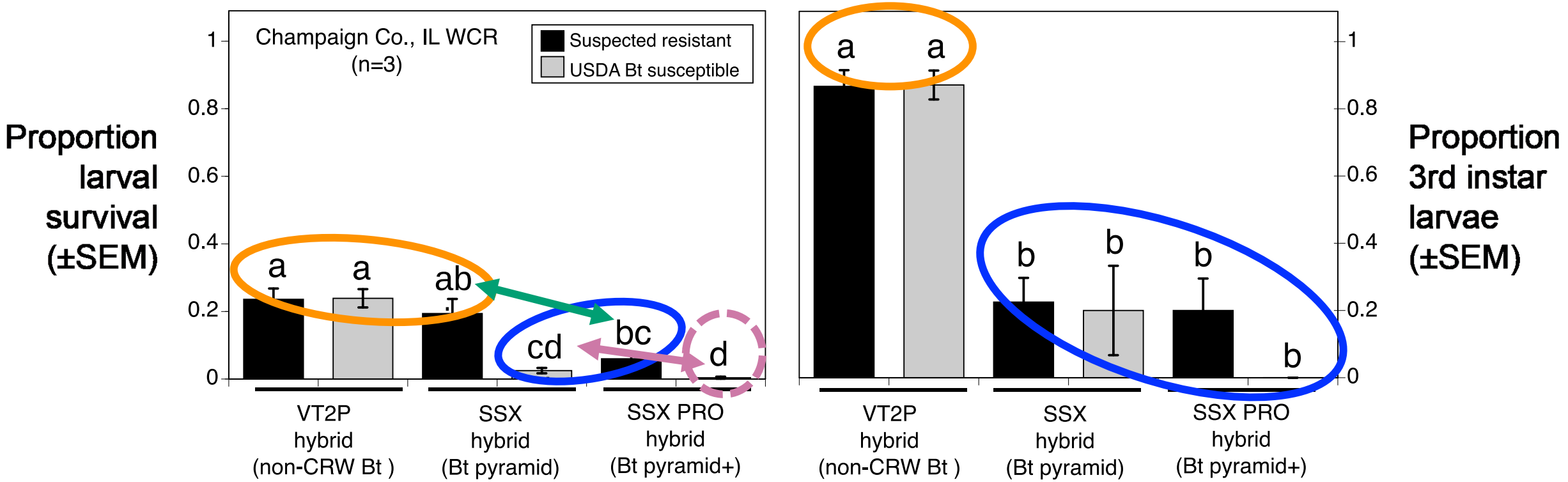
2013-2021 larval survival on single trait Bt hybrids increased at 7.4%/year

2022 IL SmartStax® Pro Bioassay



SmartStax® PRO family of corn hybrids
(Cry3Bb1 + Cry34/35 + ds RNA)

2022 IL SmartStax® Pro Bioassay



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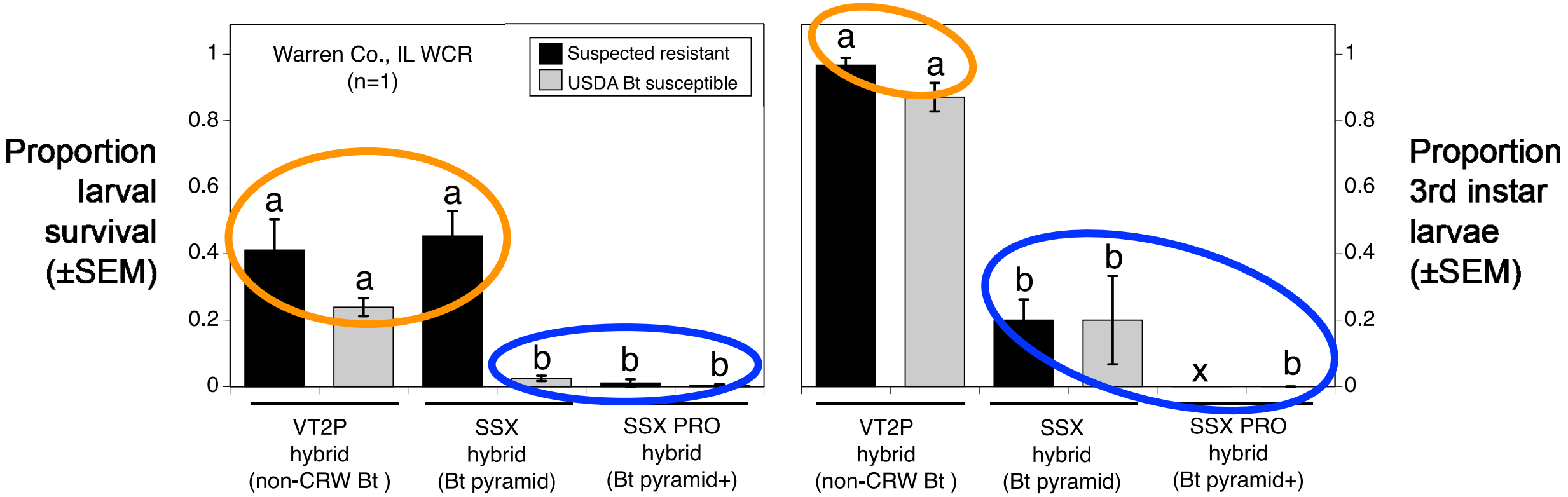

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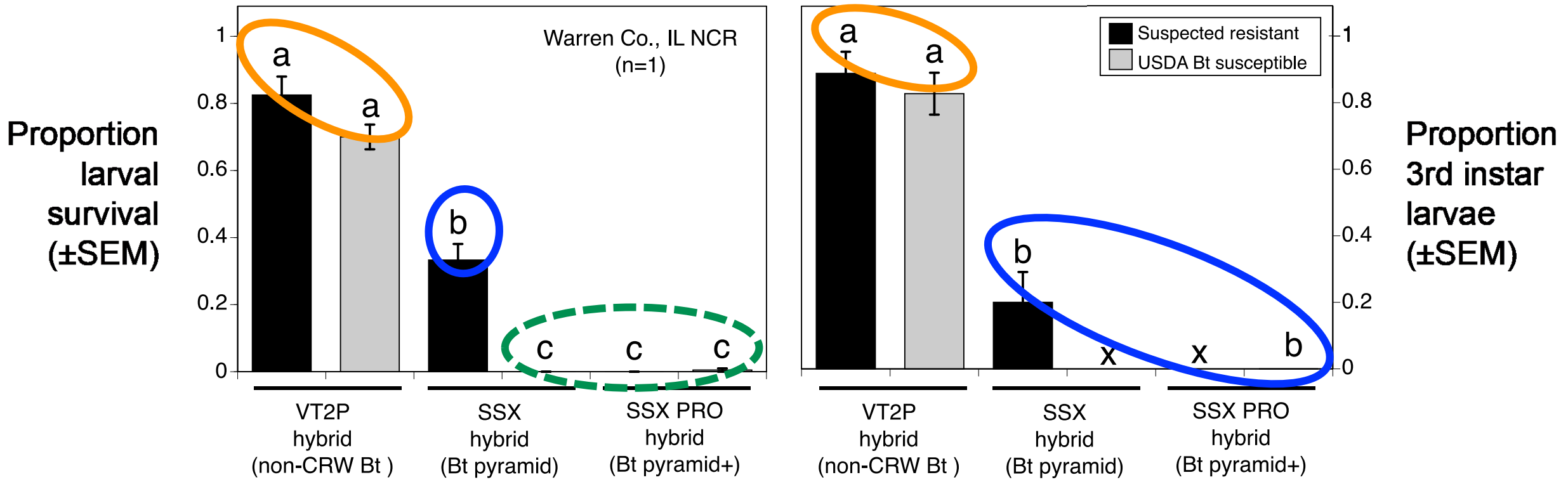

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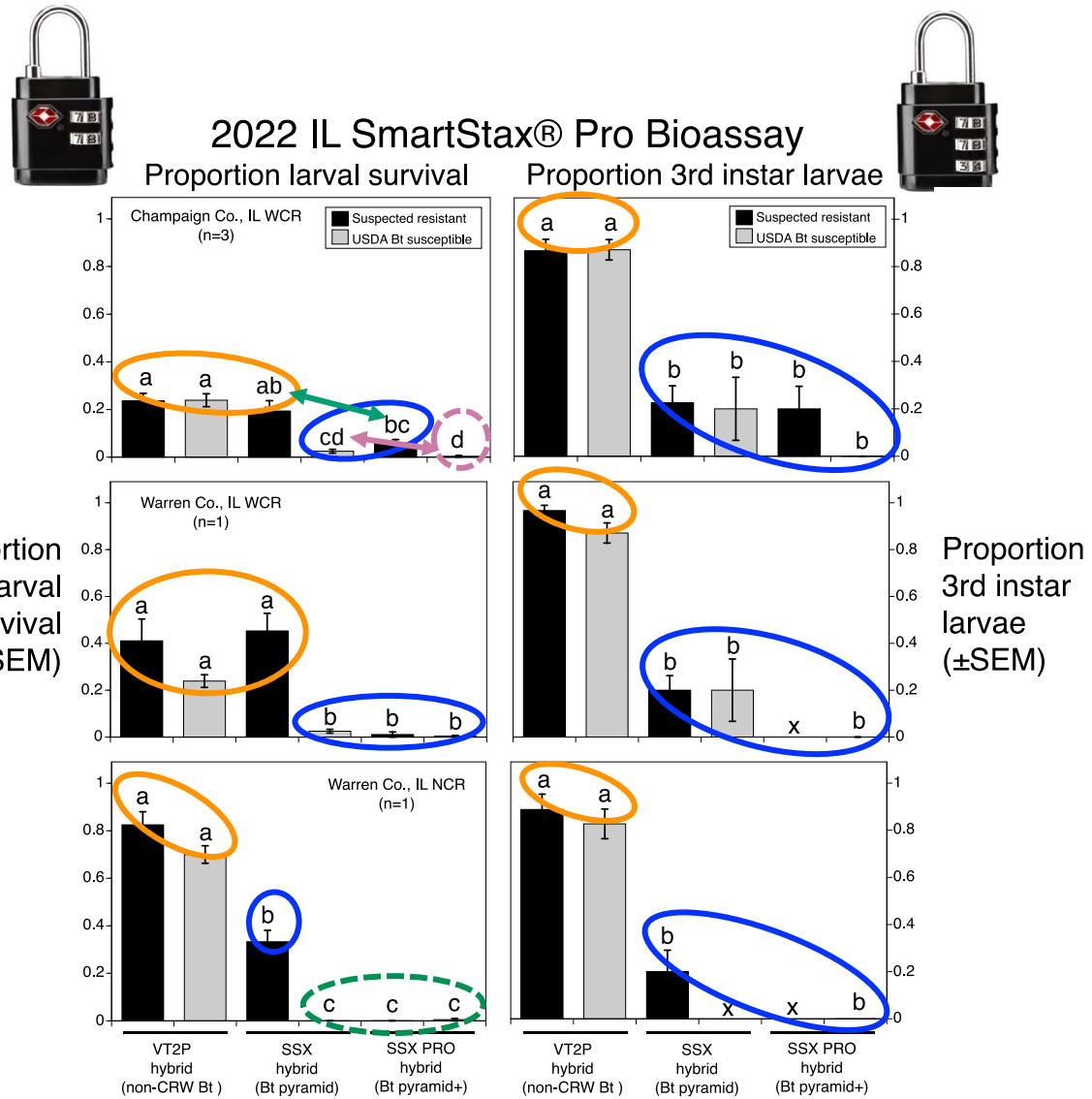

- CRW survival on the SSX Bt pyramid is nearly equivalent to survival on non-Bt VT2P in bioassays
- CRW susceptibility to SSX PRO Bt+RNAi pyramid is good; Champaign Co. WCR were slightly (but significantly) less susceptible to SSX PRO.
- Surviving larvae from SSX and SSX PRO hybrids have significantly slowed development in bioassays.

Delayed larval development delays adult emergence & may disadvantage the surviving beetles by:

Shortening adult span
vs. earlier emerging adults

Shortening period for access to the most nutritious foods
Slowing egg development

Shortening period for laying eggs

Reducing total reproduction relative to beetles on non-Bt plants

Bioassay and resistance summary:

- Bt resistance is increasing
  - Resistance is inevitable, we can slow it down.
  - There is survival & development evidence
- RNAi pyramids performed well on NCR & WCR
  - Potential for resistance to RNAi is present
- Use monitoring to put the right hybrid on the right acres and limit unnecessary selection
  - Non-economic populations don’t need to be treated
  - Rotation is the best tactic against high pressure

https://go.Illinois.edu/PestManagementResearchReport

Rootworm Control Evaluations 2018 to 2022

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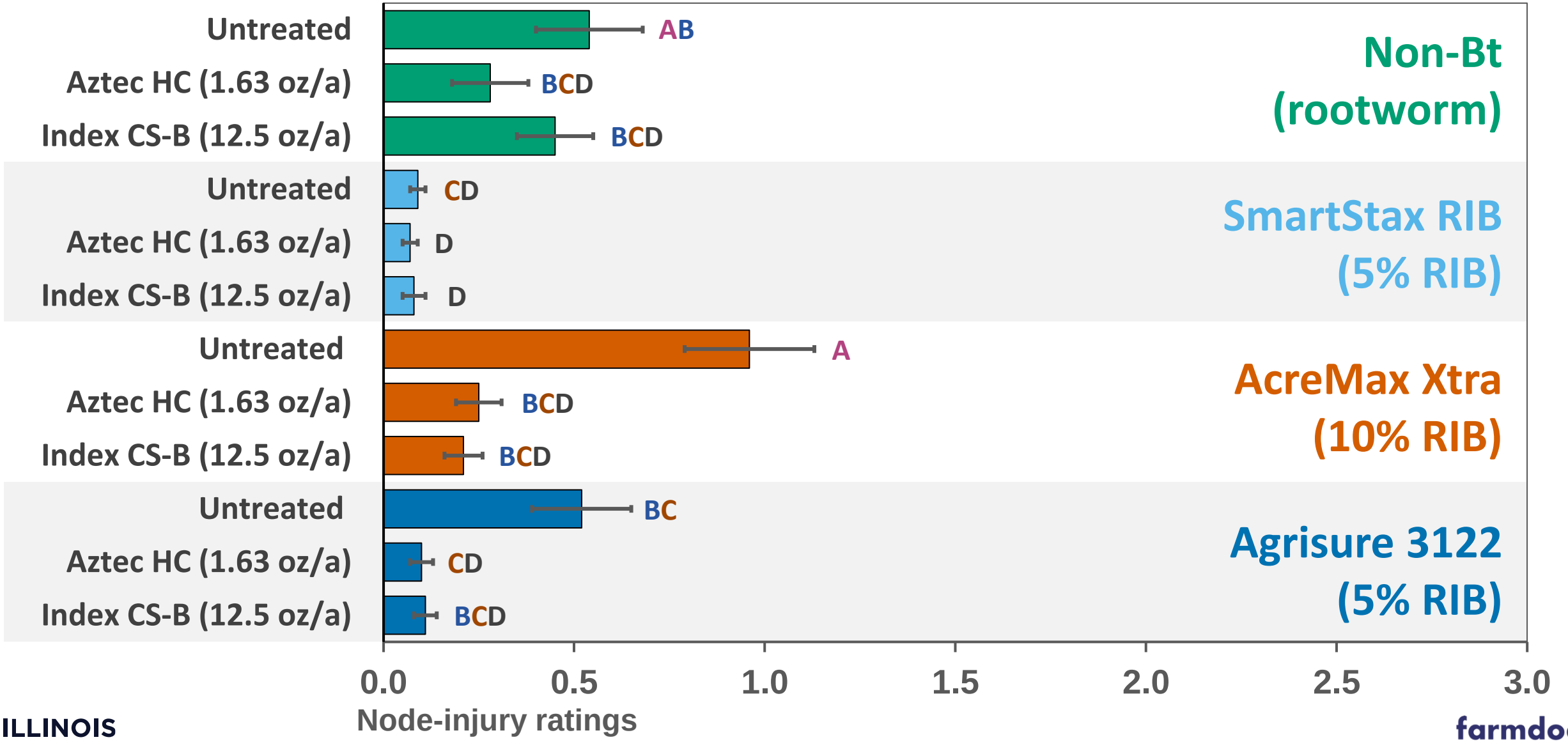
0 to 3 Node-injury rating

Node Injury Scale	Description
0	No damage
1	One node or the equivalent of one node eaten within two inches of stalk
2	Two nodes eaten within two inches of stalk
3	Three nodes eaten within two inches of stalk

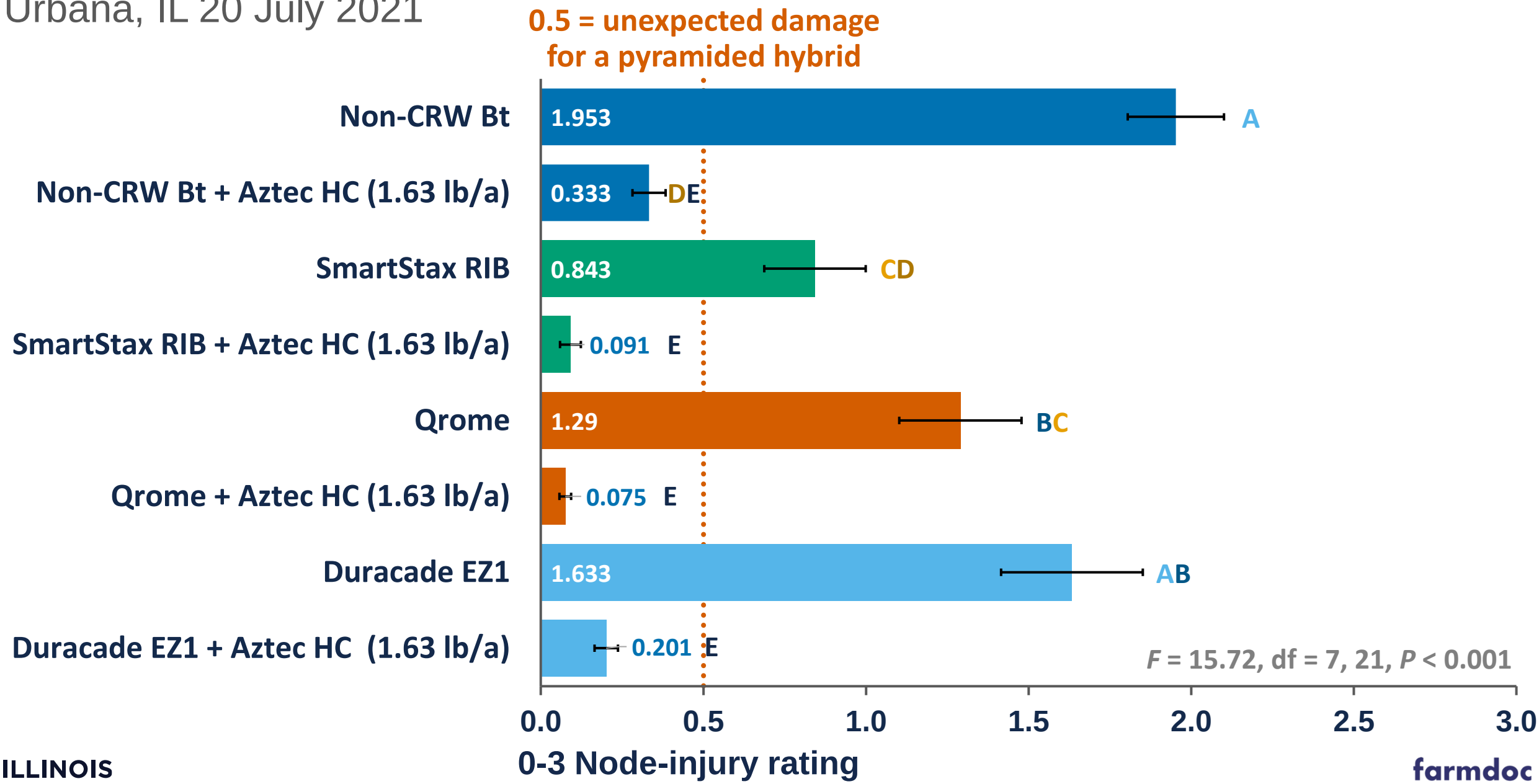
Evaluation of Traits in Combination with Insecticides

Urbana, IL 2019



Evaluation of Trait Packages with and without Aztec HC

Urbana, IL 20 July 2021



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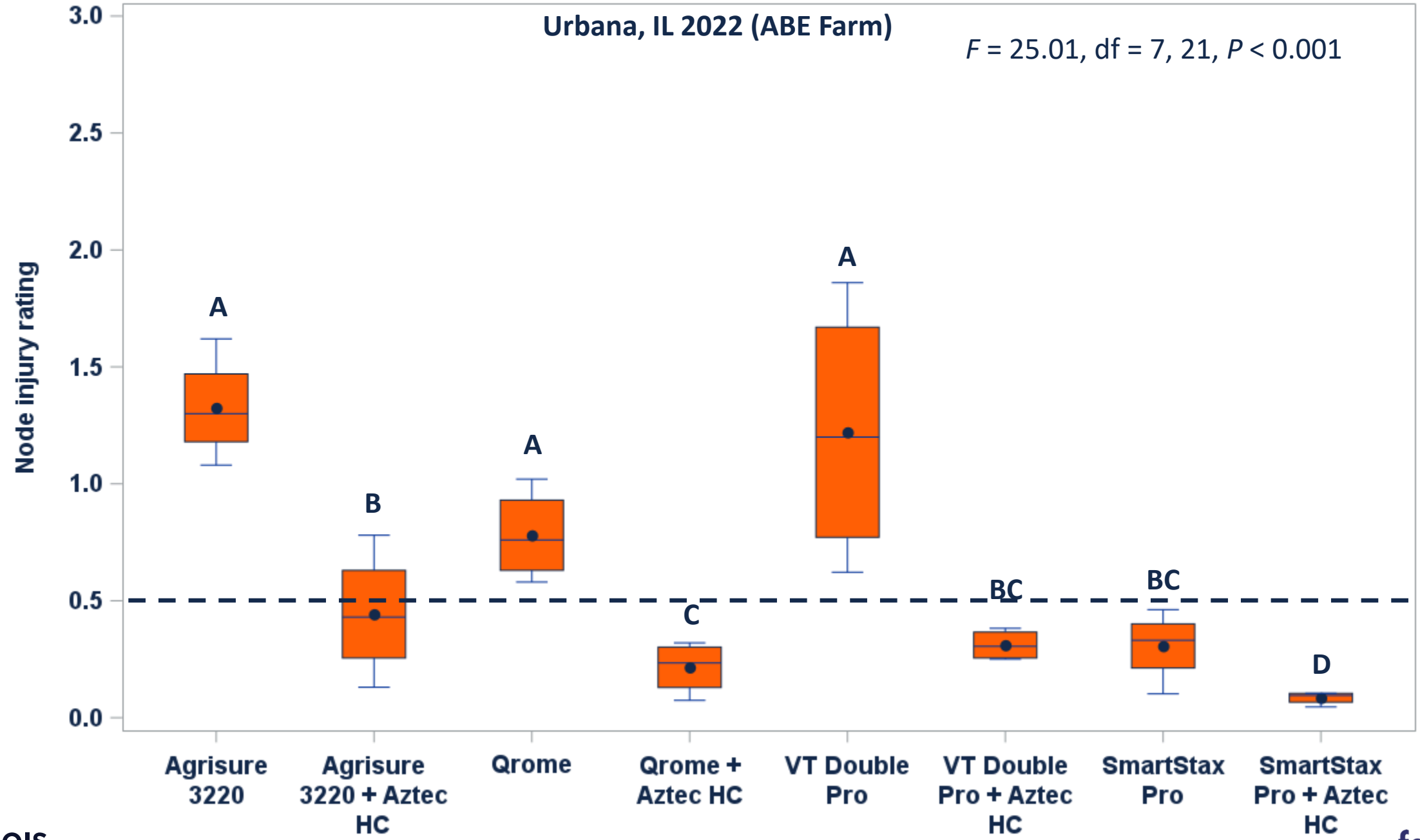
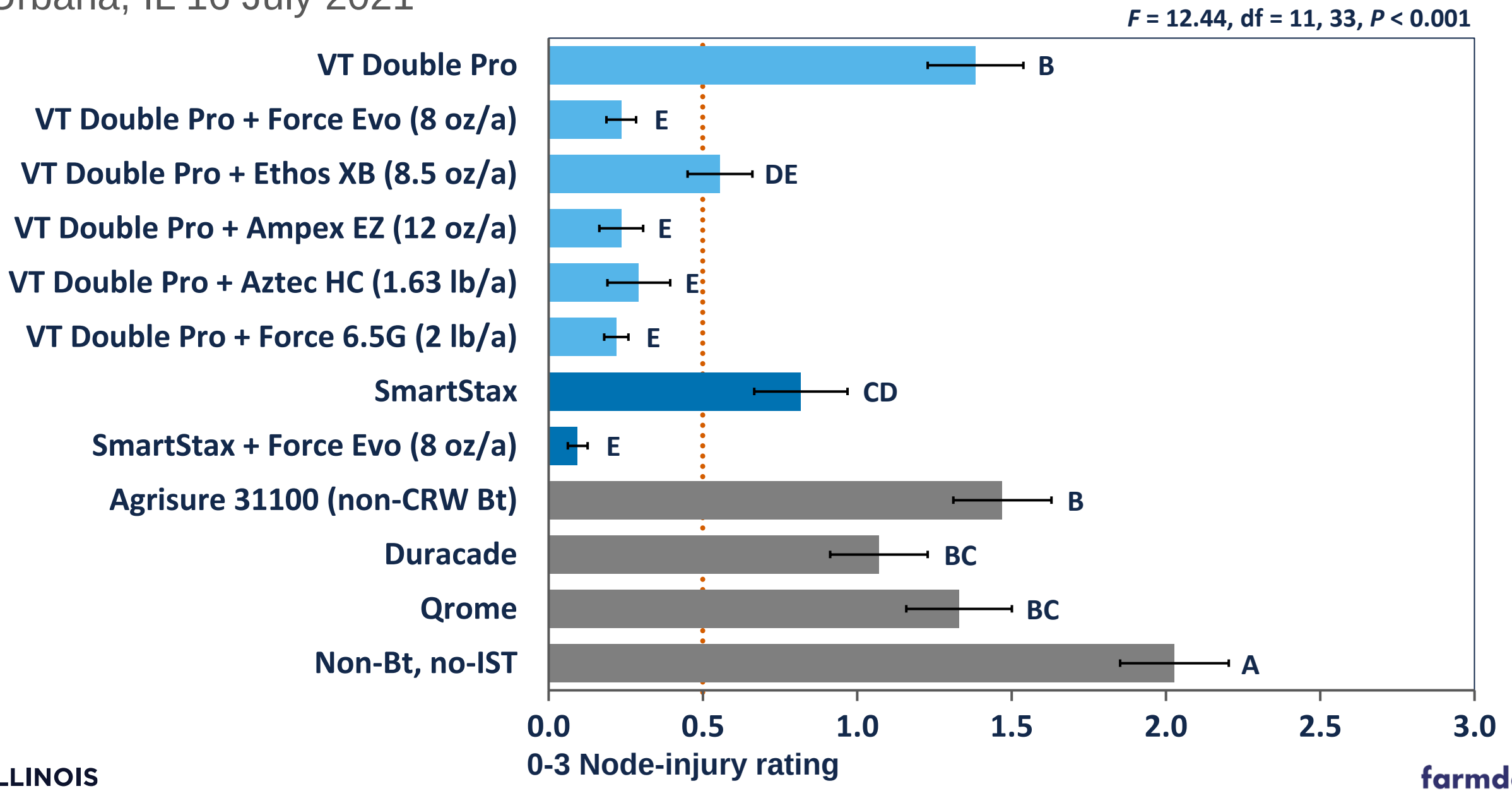
Corn Rootworm Evaluation - Traits plus Aztec HC (1.63 lb/a)

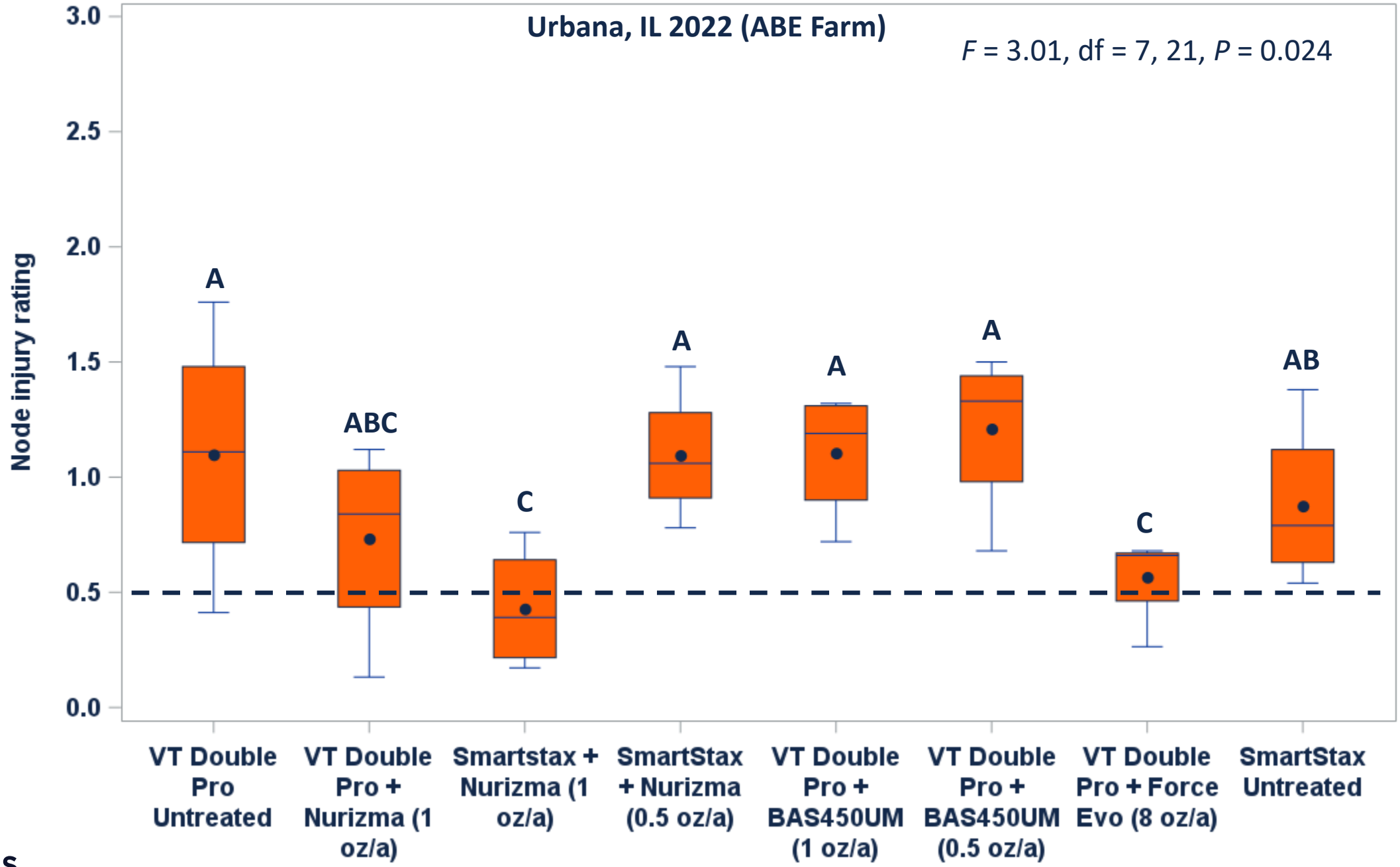


Standard Evaluation of Corn Rootworm Traits and Insecticides

Urbana, IL 16 July 2021

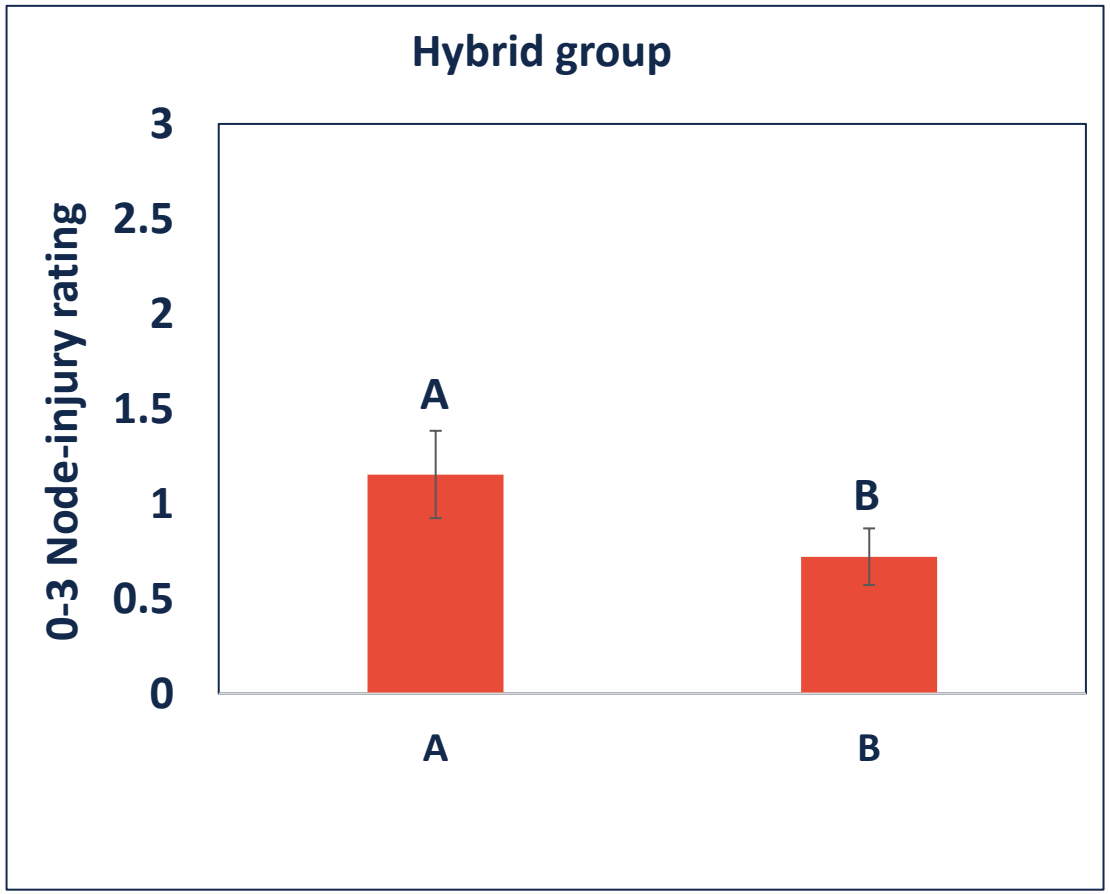


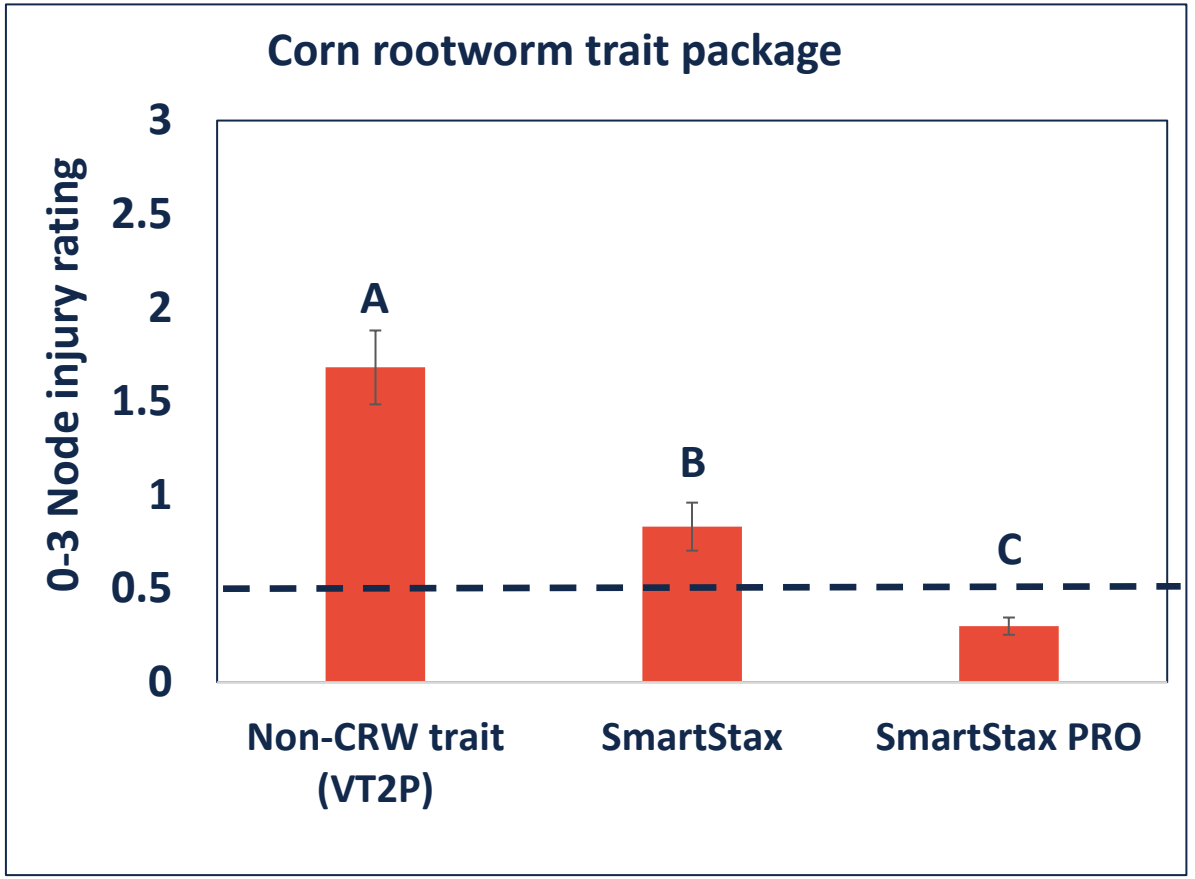
Corn Rootworm Evaluation - BASF-3 2022



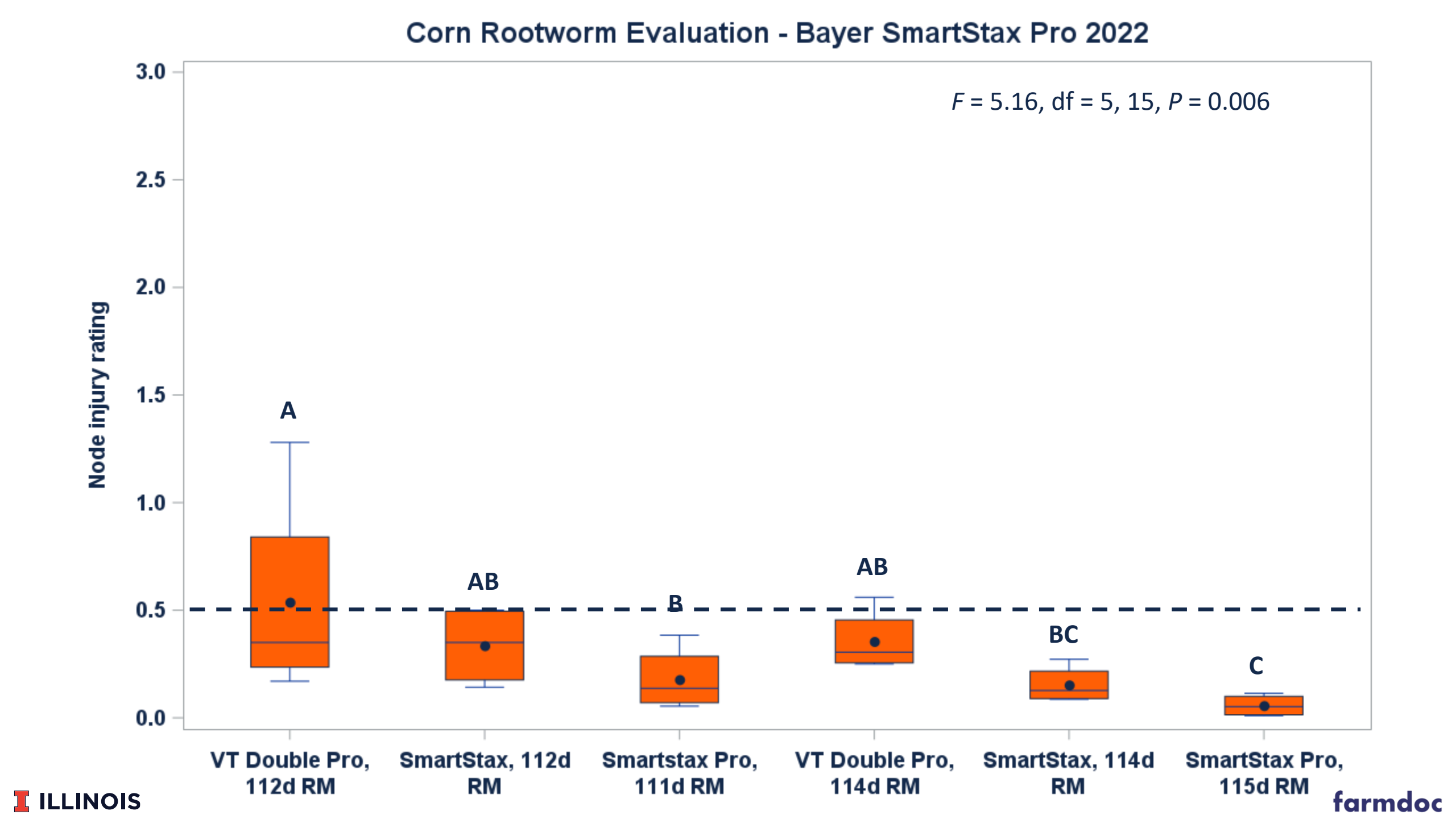
Evaluation of SmartStax PRO for western corn rootworm control

Urbana, IL 20 July 2021





Hybrid group: $F = 12.19$, df = 1, 15, $P = 0.003$
Trait package: $F = 42.18$, df = 2, 15, $P < 0.001$
Hybrid-trait interaction: $F = 2.14$, df = 2, 15, $P = 0.153$



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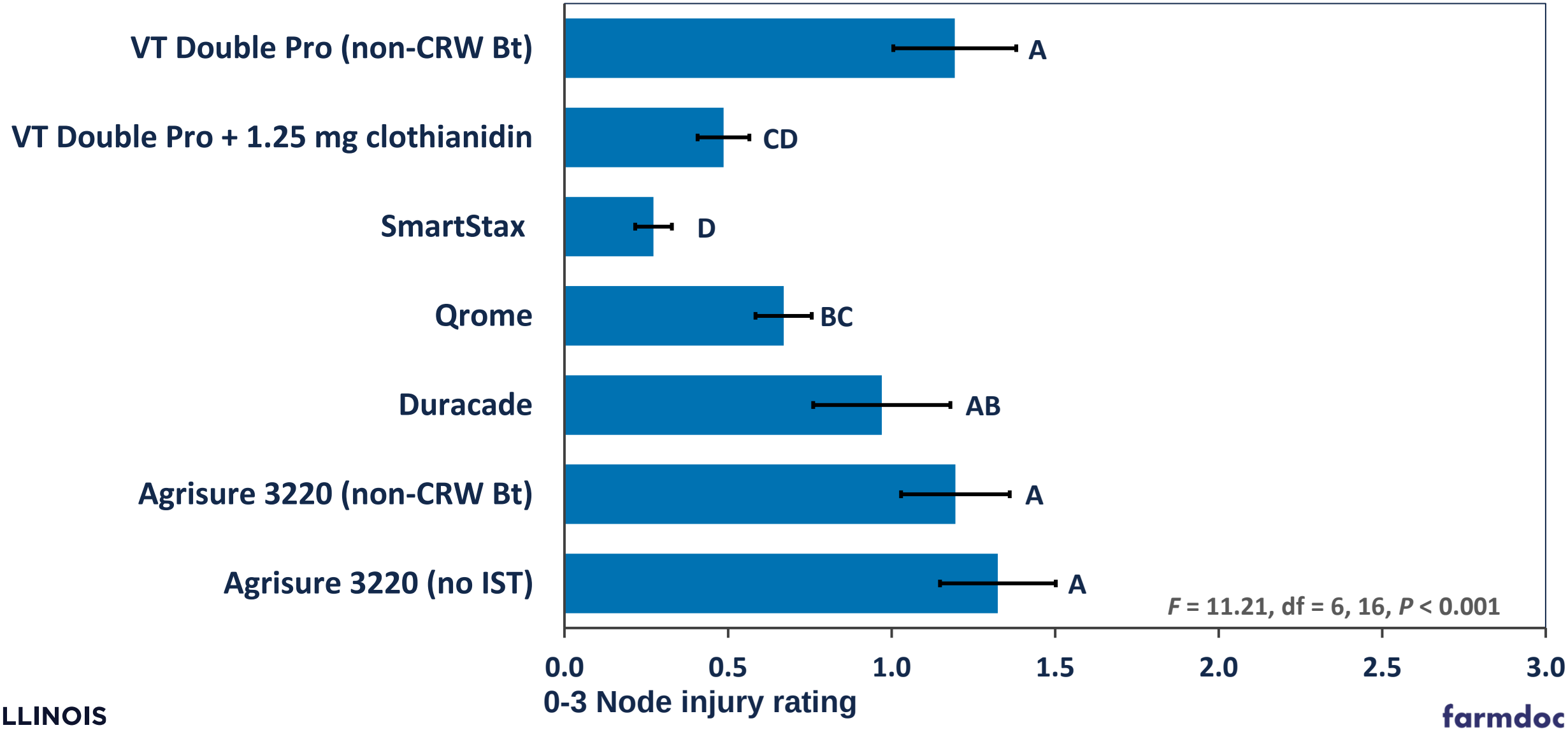
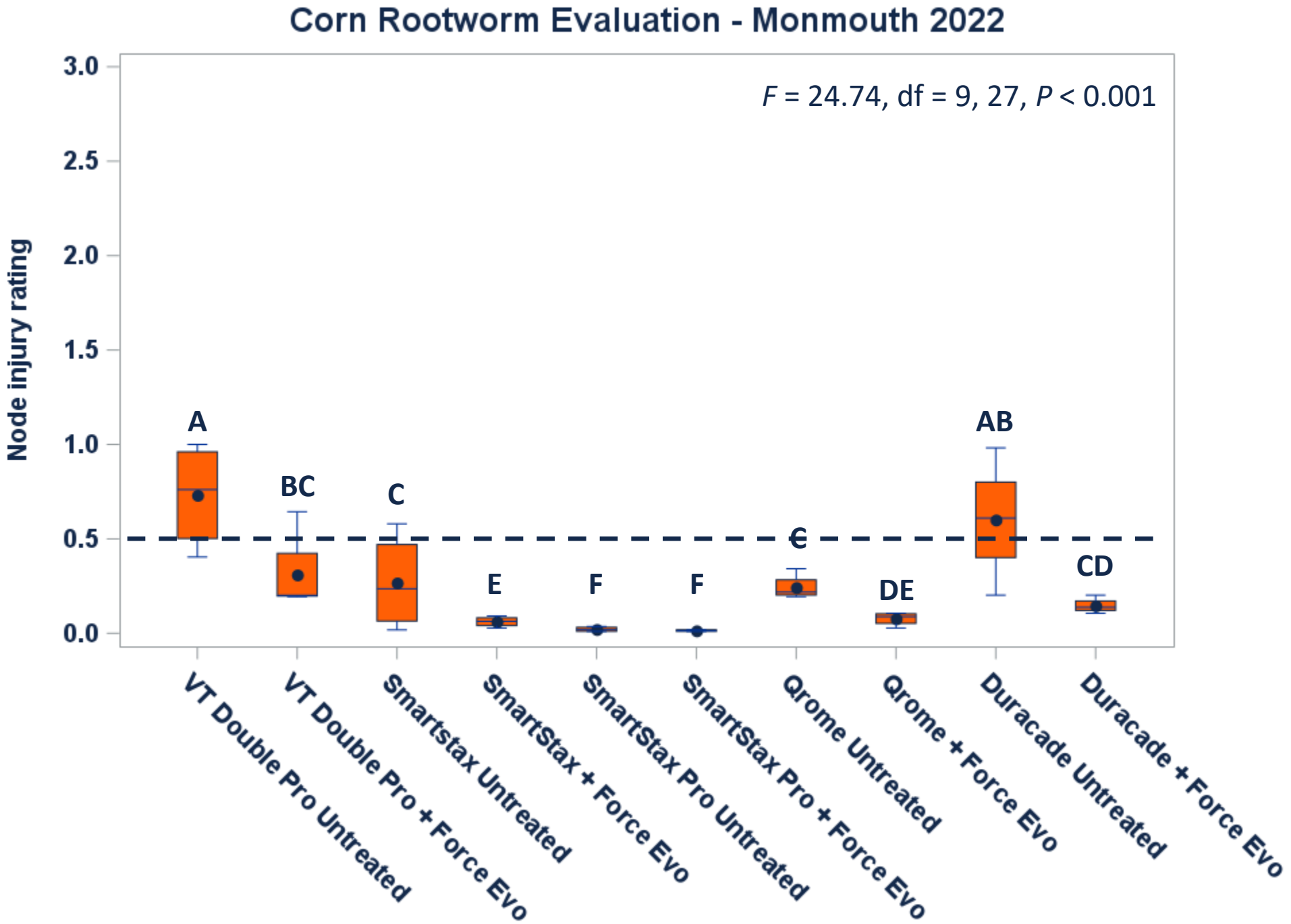
Evaluation of Corn Rootworm Trait Packages

Monmouth, IL 21 July 2021

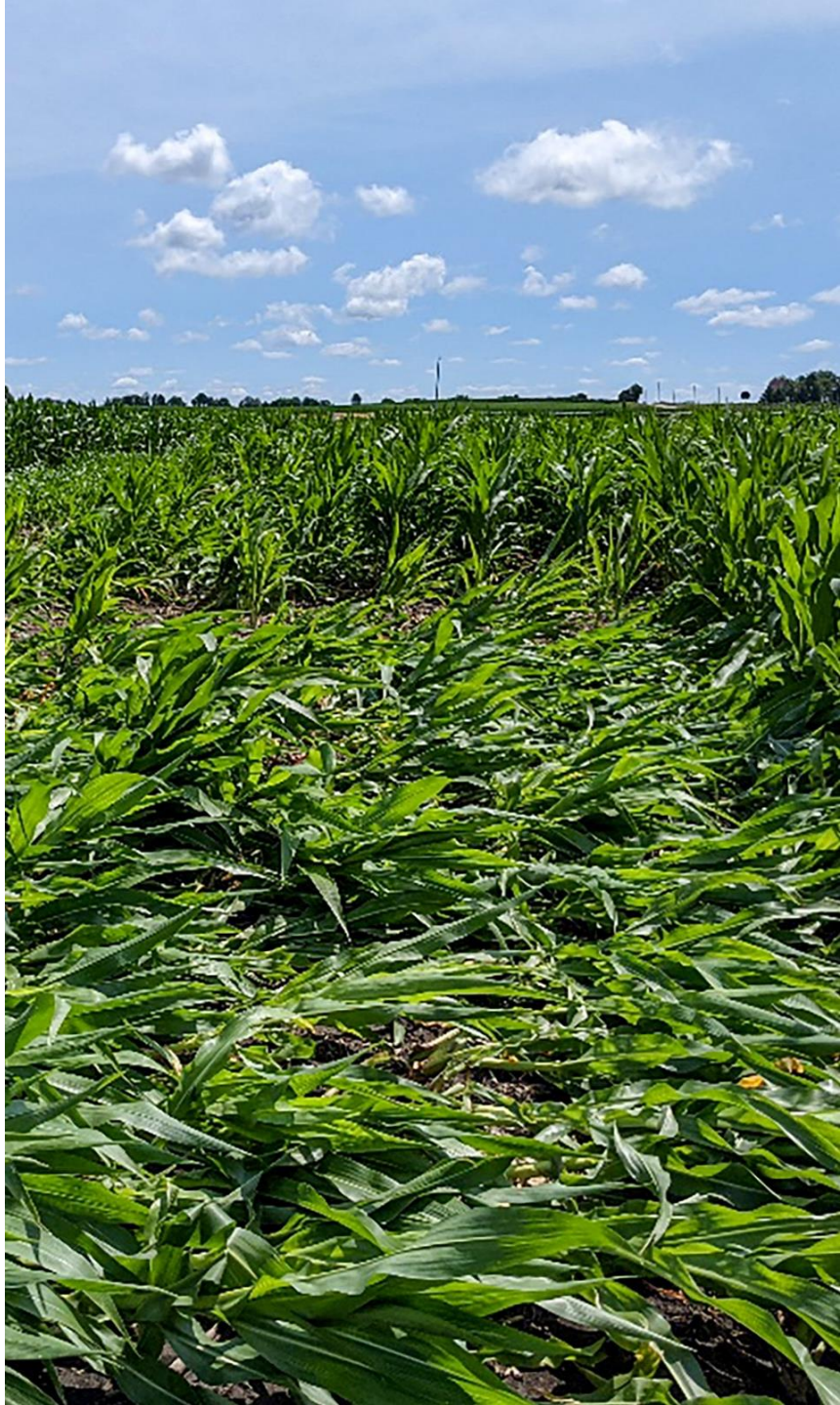



Corn Rootworm Evaluation - Monmouth 2022
F = 24.74, df = 9, 27, P < 0.001
Node injury rating
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
BC
C
E
F
F
E
DE
AB
CD
VT Double Pro Untreated
VT Double Pro + Force Evo
Smartstax Untreated
SmartStax + Force Evo
SmartStax Pro Untreated
SmartStax Pro + Force Evo
Qrome Untreated
Qrome + Force Evo
Duracade Untreated
Duracade + Force Evo

Corn Rootworm Summary
Illinois

Step back in corn rootworm pressure in 2022 after increasing for several years

Bioassays continue to show increasing resistance in both species to all traits



Corn Rootworm Summary
Illinois

Dramatic difference between continuous corn and heavy rotation regions within Illinois

- High rates of resistance, failures of pyramided traits, high northern corn rootworm populations north of I-80
- Low to no pressure in much of east central Illinois
- **Injury to first-year corn has been uncommon**

Corn Rootworm Summary
Illinois

Soil insecticides

have not lost efficacy in

Illinois field experiments

Current management recommendations

Where unexpected damage is observed and/or resistance is expected:

Best option: Rotate field to soybean (kills all WCR larvae in the soil at hatch)

Next best: Use a soil insecticide

Worst option: Continuous corn, same trait package

Local practices have a demonstrated impact on corn rootworm resistance development

Unexpected damage (EPA definition)

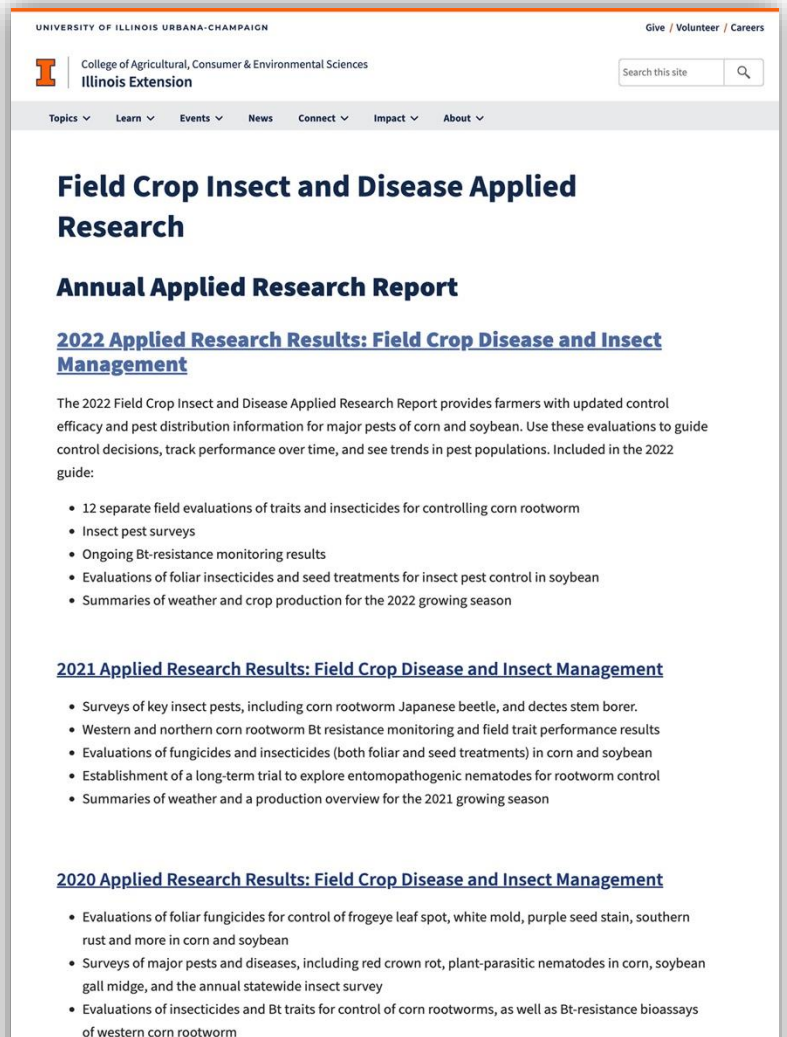
- ½ node pruned pyramided hybrid
- 1 node pruned single-trait hybrid

Resources

Applied Pest Management Research

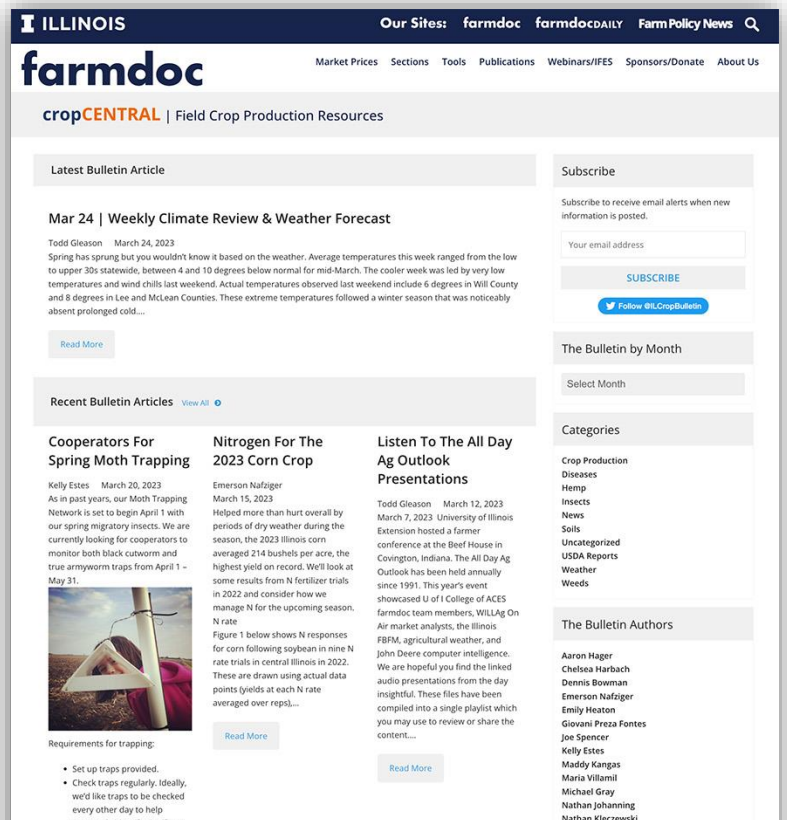
2018 to 2022 reports available

go.Illinois.edu/PestManagementResearchReport



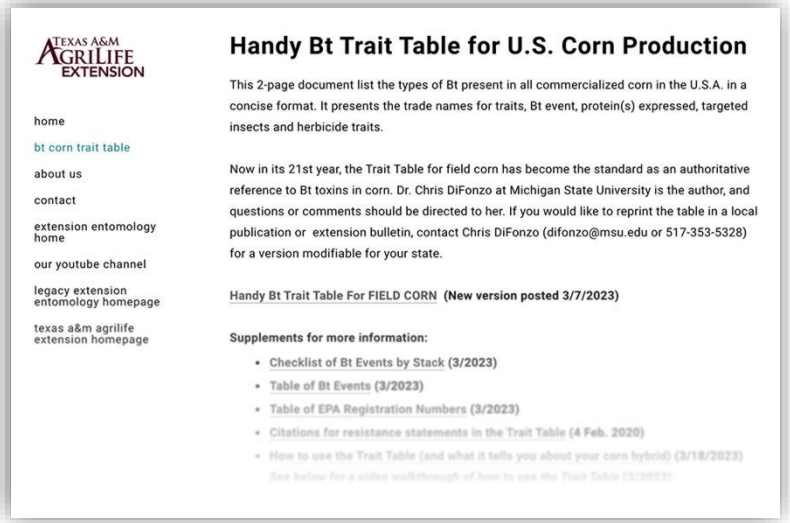
Production information

go.Illinois.edu/cropcentral



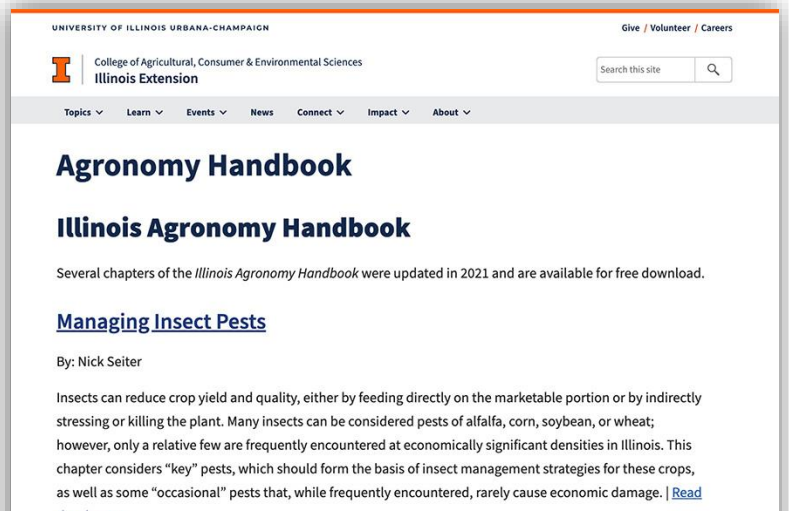
Handy Bt trait table

www.texasinsects.org/bt-corn-trait-table.html



Illinois Agronomy Handbook

go.Illinois.edu/AgronomyHandbook



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