Multi-Gene Insect Control Products & Refuge Issues - is more always better?

Illinois Corn Breeders School
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Outline

- Bt traits & Insect Resistance Management (IRM)
- Optimum® AcreMax™ 1 products – a case study
- IRM – evolving with the technology
IRM for Transgenic Insect Resistant Crops: A Brief History

- Regulated by U.S. EPA
- Framework developed more than a decade ago
- Initially harmonized across the industry
- Based on “High Dose – Refuge” assumptions
  - Cry1Ab and European corn borer
- Fifteen years later:
  - Much more data on many more pests
  - Many traits/pests may not fit “high dose” model
  - Initial “high dose” assumptions over-simplify adaptive responses in evolving populations (e.g. fitness costs & inbreeding depression, random mutations, etc.)
  - No longer harmonized across industry

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High Dose - Refuge Strategy

- High dose – kills >99%
- Low frequency of resistance genes
- Refuge produces susceptible insects to mate with rare resistant insects
- Essentially all SS and RS are killed
- Refuge placed to ensure random mating

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Optimum® AcreMax™ Insect Protection

Reduced & blended refuge
  – Optimum® AcreMax™ 1 – rootworm refuge
  – Optimum® AcreMax™ 2 – corn borer & rootworm refuge

Benefits:
  – Trait durability
  – Simplicity for growers
  – Agronomically acceptable to growers
  – Builds in refuge compliance
  – Reduced pesticide use on refuge acres

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Blended Refuge: Pros and Cons

Cons

- May be limited in size (%) due to unacceptable damage in high pest pressure regions
- May not be as durable as blocks/strips for single high dose toxins where older larvae may move readily between plants
- Requires more complex understanding of pest biology & field dose response

Pros

- Simple & efficient for growers
- Ensures refuge is planted
- Reduces insecticide use on refuge acres
- May be the most appropriate refuge for species with limited or no movement between adult emergence & mating
- Appropriate where high compliance rates are required to maintain durability

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Larvae are Exposed to Variable Concentrations

- Spatial variation occurs within 59122 roots
- Provides effective exposure
**Effect is More Chronic Than Acute**

Larvae Extracted After 17 Days of Exposure

*Initial feeding does not result in direct mortality*
Cry34/35Ab1 Makes 59122 a Less Acceptable Host

Positive Control (acceptable host)

Negative Control (no host)

Cry34/35Ab1 Treatment (unacceptable host)
Exposed Larvae Can Recover

- Neonate
- 2nd Instar
- 3rd Instar
- Recovery

<table>
<thead>
<tr>
<th>CRW Stage</th>
<th>Exposure Time</th>
<th>Adult Survival*</th>
<th>Average Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td></td>
<td>0.4%</td>
<td>-48%</td>
</tr>
<tr>
<td>2nd Instar</td>
<td></td>
<td>26%</td>
<td>-50%</td>
</tr>
<tr>
<td>3rd Instar</td>
<td></td>
<td>65%</td>
<td>-29%</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td>114%</td>
<td>+2%</td>
</tr>
</tbody>
</table>

*all values relative to the appropriate control

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Resistance to 59122 Does Not Appear to be Simply Inherited

Predicted Resistance Pattern

Observed Colony Response to Selection

Survival Rate

Selected Colony Generation

York-S
Rochelle-S

r = 0.0005
r = 0.001

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Life-History Traits of Beetles from Blends Similar to Refuge Beetles*

### Female WCR Egg Production

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eggs / Female</th>
<th>Eggs / Female / Day</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>59122_P250</td>
<td>114 a</td>
<td>3.5 a</td>
<td>30</td>
</tr>
<tr>
<td>Seed Blend</td>
<td>268 bc</td>
<td>7.8 b</td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>261 ab</td>
<td>7.7 b</td>
<td>29</td>
</tr>
<tr>
<td>Control_P250</td>
<td>356 c</td>
<td>9.8 b</td>
<td>30</td>
</tr>
</tbody>
</table>

Fishers’s Protected LSD Test, P<0.05

*Data generated by Meinke et al., University of Nebraska*
Simulation Models to Examine Trait Durability

- Tool for comparing IRM strategies
- Helps identify most important parameters
- Part of the ‘weight of evidence’ regulators use in assessing risk

“…a good RM strategy should maintain population susceptibility to a transgenic insecticidal crop for longer than 10 years.” (ILSI, 1999)
Adult Corn Rootworm Movement

Beetle “RR”

20% Refuge Field

80% Bt Field

Beetle “SS”

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Block Refuge Creates a Gradient for Male Dispersal - 20% Block (Red=Females Blue=Males)
Block Refuge Creates a Gradient for Male Dispersal- 5% Block (Red=Females Blue=Males)
Parameters for Beetle Movement

WCR mark-recapture study

- Quantify how far WCR beetles move within the cornfield
- Determine where mating occurs relative to their emergence site
- Larvae marked through feeding on rubidium-treated corn plants
- Beetles captured in traps at fixed distances from the field center
Modeling Approach: 59122 & Integrated Refuge

- Spatially explicit, landscape model
  - Includes beetle dispersal, probability of mating between refuge males and Bt females, and oviposition.
  - Supports up to a 2,500 hectare landscape (1 ha cells)
- Worst Case Assumptions:
  - simple recessive di-allelic resistance (not what we found in our selected colony)
  - continuous corn cropping system
  - 90% of females oviposit in their natal field

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Modeling Durability

Start

Parameter initiation
- General parameters
- Break field into cells
- Year 0 adult information

Initialize egg density

Start year

Start simulation

Next year

Yes

Year ≤ simulation length?

No

End

Larval sub model

- Overwinter survival of eggs
- Early larval movement survival
- Late larval toxin survival
- Density-dependent survival
  - Assume all surviving larvae complete pupa stage

Adult sub model

- Emerging of adults
- Dispersal of male adults
- Mating
- Mated female dispersal and ovipositing
- Egg density update
### Sensitivity of Refuge Size with a Single Di-Allelic Recessive Locus

#### Model Response to Refuge Size and Configuration

<table>
<thead>
<tr>
<th>Refuge Proportion</th>
<th>Relative Rate of Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.0</td>
</tr>
<tr>
<td>0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>0.1</td>
<td>0.46</td>
</tr>
<tr>
<td>0.20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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## Predicted Durability of Different Refuge Options

<table>
<thead>
<tr>
<th>Refuge Deployment Strategy</th>
<th>Worst Case</th>
<th>Benchmark Parameters</th>
<th>Best Case</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Resistance Allele Frequency = 0.01 Dominance Value = 0.1 surv-ss = 0.025 ovip-ss = 0.32 ovip-rr = 1</td>
<td>Initial Resistance Allele Frequency = 0.001 Dominance Value = 0.05 surv-ss = 0.0125 ovip-ss = 0.32 ovip-rr = 1</td>
<td>Initial Resistance Allele Frequency = 0.0005 Dominance value = 0.025, surv-ss = 0.0125 ovip-ss = 0.32 ovip-rr = 0.32</td>
</tr>
<tr>
<td>No refuge</td>
<td>3 years</td>
<td>5 years</td>
<td>9 years</td>
</tr>
<tr>
<td>20% block with variable locations and 70% compliance rate</td>
<td>4 years</td>
<td>8 years</td>
<td>13 years</td>
</tr>
<tr>
<td>5% blend</td>
<td>5 years</td>
<td>10 years</td>
<td>&gt;20 years</td>
</tr>
</tbody>
</table>

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Case Study Summary

- 59122 is not high dose against CRW
- Characteristics of 59122 are compatible with integrated refuge
- Realistic data on CRW beetle movement have been incorporated into the model
- New modeling shows that a 5% blend is more durable than a 20% block
- OAM1 builds in grower compliance with CRW refuge requirements
Conclusions

- Insect control technologies are diversifying
- “one size fits all” IRM not appropriate
- Each trait x target needs individual consideration
- Growers need choices

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“…the latest knowledge is usually the best. Moreover, knowledge grows or dies. It cannot live in cold storage. It is perishable and must be constantly renewed.”

~ Henry A. Wallace, co-founder of Pioneer Hi-Bred
Former U.S. Secretary of Agriculture & Commerce
Former Vice President of the United States