

MINUTES – NCCC46 Annual Meeting, January 21, 2013: Development, Optimization, and Delivery of Management Strategies for Corn Rootworms and Other Below-ground Insect Pests of Maize.

Wyndham Riverfront Hotel, 701 Convention Center Blvd., New Orleans, LA

Attendees:

Apurba Barman (Purdue University)
Mark Boetel (North Dakota State University),
Larry Bledsoe (Purdue University),
Ed Bynum (Texas A&M University),
Eileen Cullen (University of Wisconsin),
Christina DiFonzo (Michigan State University),
Galen Dively (University of Maryland),
Billy Fuller (South Dakota State University),
Wade French (USDA-ARS, Brookings, SD),
Aaron Gassmann (Iowa State University),
Mike Gray (University of Illinois),
Ron Hammond (Ohio State University),
Rick Hellmich (USDA-ARS and ISU),
Bruce Hibbard (USDA-ARS, University of Missouri),
Andrea Hitchon (graduate student, University of Guelph),
Fangneng Huang (Louisiana State University),
Chuck Mason (University of Delaware),
Brad McManus (South Dakota State University),
Lance Meinke (University of Nebraska),
Ken Ostlie (University of Minnesota),
Adriano Pereira (University of Nebraska)
Pat Porter (Texas A&M University),
Steve Pueppke (NCCC46/NCC205 Administrative Advisor, Michigan State University),
Tom Sappington (USDA-ARS, Ames, IA),
Art Schaafsma (University of Guelph),
Blair Siegfried (University of Nebraska),
Joe Spencer (University of Illinois),
Jocelyn Smith (University of Guelph),
John Tooker (Pennsylvania State University),
Sarah Zukoff (Kansas State University).

MONDAY, JANUARY 21, 2013

8:00-12:00

Meeting called to order, 8:00am by Eileen Cullen, Chair

(Minutes recorded by Andrea Hitchon, graduate student, University of Guelph)

Welcome and review of agenda items

Local arrangement announcements - Fangneng Huang

A motion was made to approve minutes from 2012 annual meeting by Eileen Cullen. Seconded by Ron Hammond and Pat Porter. A vote was called and 2012 minutes were approved.

Additional items

- 1) Fangneng Huang and Jensen Young (LSU) invited participation at the **ESA South Eastern Branch meeting**, March 3-6 at Baton Rouge. There will be an optional Sunday visit to the New Orleans insectariums.
- 2) Tom Sappington discussed the timing for a joint meeting of NCCC46 and NC205 next year in Chicago with the International Working Group on *Ostrinia* and other Maize Pests (IWGO). There was consensus for early April 2014. Participants wishing to organize a session or who have ideas are invited to contact Tom Sappington by late Summer/early fall. Many interests are the same between the groups and new collaborative research could be carried out.
- 3) Pat Porter and Art Schaafsma volunteered to be the nominating committee and report nomination for secretary by the end of the day.
- 4) Entomological Society of America (ESA) Symposium 'Remember IPM? Risks and Benefits of Global Expansion of Transgenes and Insecticide Seed Treatments in Field Crops' was well attended and generated a lot of discussion. Peer-reviewed paper may be written. (Art Schaafsma).
- 5) Thank you and best wishes to Ron Hammond who is retiring before the 2014 meeting.

Report from Administrative Advisor (Steve Pueppke)

Key items:

A mid-term project review is approaching.

- Reports need to be submitted on time
- Impact statements are required (between now and end of year)
- Steve will submit recommendations/progress report to review committee by end of year
- Bob Nowierski (USDA) could not attend - no federal report

Aaron Gassmann presented progress related to WCR resistance monitoring sampling and bioassays (2012 beetle collections); discussion of coordinated multi-state bioassay effort.

- Assays were run from April - September of 2012
- Started to look at mCry3A resistance and its relationship with Cry3Bb1 resistance. - Results are consistent with suspected cross resistance between the two.
- Resistance ratio was used to make comparisons between assays (the resistance ratio is the corrected survival on Bt corn divided by the survival on non Bt corn, divided by average corrected survival for the susceptible populations. So, if the resistance ratio is 3, there is three times the survival of the population tested on Bt corn compared to susceptible populations).
- Survival of WCR on Bt corn was compared to survival in high dose cases (i.e., pink bollworm) to illustrate that for events which are not high dose, lower levels of resistance

can be very meaningful in the field. Identifying meaningful changes in pest susceptibility in the field is far more complicated for low dose products.

- 3 is proposed as the threshold resistance ratio which confers resistance, and validates concerns about unexpected damage. An above threshold resistance ratio must be coupled with population density to determine in-field significance (further research ongoing).

Others' experience with the Gassmann assay:

- Most labs have been having success replicating and running the Gassmann assay (Meinke, Hibbard)
- Some concerns were raised about how different lab conditions (temperature, light availability, hybrid selection) and year-to-year variability affected assay results
 - o The importance of having a similar amount of root tissue was raised. More information (pictures/video) was requested about what the appropriate amount of root mass looks like (Meinke)
- Ken Ostlie found pupation was occurring after 17 days, and has shortened the length of his assays to 15 days to increase utility
- Do we want to use resistance ratio as indicator for resistance? If so, first we must characterize the appropriate baseline susceptibility (multiple reps and populations)
- In order to optimize survival a good genetic background hybrid pair must be chosen
- The assay has been most successful on lab-reared rootworms which have been on the seedling mat for the shortest amount of time. After a certain point of selection with seedling mat will lab reared populations become less suitable as controls for a whole plant assay? Wade French will try to send eggs from more recently collected diapausing colonies. Some labs may run experiments to look at differences in current lab colonies.
- What is the relative rate of gene expression in younger vs. older plant OR single plant vs. a seedling mat?
- Dose will dictate which assay is most appropriate (seedling may be more sensitive to 34/35 - may relate to mode of action of the protein, or affects on insect behavior – preference is not present for non-Bt over 3Bb1, but larvae avoid 34/35) (Blair Siegfried)
- Soil used in the assay should be standardized.

State reports

- Ken Ostlie (University of Minnesota)
- Pat Porter and Ed Bynum (Texas A&M)

1:30 – 5:00

State reports continue

- Mike Gray (Illinois)
 - Report is available online: ipm.illinois.edu/ontarget
 - Noted rootworm hatch was very early and truncated (by about 2 weeks)
- Eileen Cullen (Wisconsin)
 - MON863 (as a single trait) will be removed from DiFonzo and Cullen 'Handy Bt Trait Table' at the request of Monsanto seed company reps. (MON863 single trait is not sold much anymore, rather it is stacked or pyramided).

- Cullen and graduate student published a new Extension fact sheet on seedcorn maggot management in organic systems, focuses on corn planting time based on seed corn maggot degree days and spring cover crop incorporation date. On line temperature model now available for seedcorn maggot degree day accumulation in MN and WI, through UW Extension Ag Weather.

- Ron Hammond (Ohio)
 - Observed low rootworm numbers in the previous season
 - Noted a grub problem (Asiatic garden beetle) which has been occurring for the past 4-5 years in areas with sandy soils from Lake Erie towards lake Michigan. Insecticides are needed to address secondary pests.
- Billy Fuller and Brett (South Dakota)
 - Observed a shift from Northern → Western corn rootworms in some fields
- Blair Siegfried (Nebraska)
 - Discussed RNAi as a new technology which may replace some use of Bt traits. Current research is looking at mode of action and risk assessment for non-target organisms. In addition, work continues into examining expression differences within proteins, transcript profiling and cyclodienes.
 - A southern corn rootworm colony (non diapausing, used as proxy for westerns because they are easier to rear) has been established to study RNAi resistance through adult selection experiments.
- Aaron Gassmann (Iowa State)
 - Seen an increase in number and geographic scope of pest resistance to Cry3Bb1 and mCry3a. This resistance can develop in 3-6 generations.
 - A resistance ratio greater than three can result in meaningful field damage (10 fold increase in low-dose vs. 100 fold seen in high-dose events)
 - Some different IPM strategies/rotations were presented as possibilities
 - Ex. 1: soy, non-Bt corn
 - Ex. 2: soy, non-Bt corn, Bt-corn or non-Bt + soil insecticide
 - Ex. 3: soy, non-Bt corn, non-Bt corn + soil insecticide, pyramid, pyramid
 - Ex. 4: longer rotation involving two types of pyramids, Bt corn and soil insecticides
- John Tooker (Penn State)

John has been talking to a colleague at Penn State interested in studying the interactions between industry and individuals in this group and NC205. The question posed is: Would this group and 205 be willing to participate in a brief survey organized by Leland Glenna (<http://aese.psu.edu/directory/llg13>), a rural sociologist who studies the history of universities and their interactions with industry?

 - Discussion raised concerns that outcomes may be harmful to improving relationships, but there are potential benefits that this research involving a rural sociologist may have.

Vote: Should we involve the rural sociologist? Postponed. John and Steve will talk to Dr. Glenna; he will prepare a statement to clarify the goals and design of the research to submit to the Committee so everyone can make a more informed decision. The issue will be reconsidered at the March meeting.

Nominating committee (Pat Porter and Art Schaafsma) nominated John Tooker for secretary. A vote was called and the Committee was in favour. John Tooker accepted the nomination and will be the next secretary.

State reports continued

- Bruce Hibbard (University of Missouri)
 - Cross resistance studies (mCry3A, Cry3Bb1)
 - Behavioral assay (watch larvae under microscope for 30 mins and quantify behavior)
 - Bag experiments (no soil, paper growth medium), working on hydroponic methods

Progress update regarding the western rootworm genome. (Tom Sappington, ARS) The genome has been sequenced but assembly has been challenging due to large introns, lots of transposable elements, and lots of repetitive sequence. Hugh Robertson (Univ. of Illinois) is leading this project with involvement of several on this Committee. The transcriptome is being sequenced in France, with assembly of the reference genome underway. The next part of the project is to use the reference transcriptome in RNA-seq experiments to determine which genes are differentially expressed or regulated during different stages or under different stresses. The project is led by Thomas Guillemaud of INRA in France, with several Committee members as CoPIs and collaborators.

Discussion: Which is a good germplasm to use for standard test for rootworm resistance? B73MO17 was suggested because it is maintained publicly by many, has moderate resistance to rootworm, and has been around for awhile (and hopefully this means it will be around for a while longer). Discussion to be continued.

- Larry Bledsoe (Purdue)
 - Research projects and areas include:
 - The use and potential benefits of neonicotinoids as seed treatments
 - The likelihood of random WCR mating
 - Volunteer corn and its possible role in rootworm resistance
- Chris DiFonzo (Michigan State University)
 - Collected one rootworm population from a field with greater than expected damage. Described problematic interactions with local seed rep regarding that field.
- Jocelyn Smith and Art Schaafsma (University of Guelph)
 - Collected three field populations from fields with greater than expected damage
 - Workshop on resistance management will be held with the Canadian Food Inspection Agency on Feb. 14th in Ottawa
- Mark Boetel (North Dakota)

- A few additional cases of unexpected rootworm damage have been observed
- New student will be working on a species composition study/determining resistance

Discussion: Why do we need a lab bioassay? What are the problems with defining resistance?

- Low dose products make it more difficult to differentiate between susceptible and resistant populations.
- This difference requires a paradigm shift in how we think about resistance to low-dose products when compared to our previous high-dose mentality.

Discussion: Impact statements are needed for mid-project review.

Impact statements should focus on:

- Science communication/information on rootworm resistance
- Bioassay progress
- Accessibility of the group to EPA/farmers/public/industry
- Genome work
- Letter submitted to EPA on Cry3Bb1 resistance and its impact on terminology and protocols currently used by the EPA

State reports were circulated to all participants.

5:00 NCCC46 MEETING ADJOURNED

MINUTES – NCCC46/NC205 Joint Meeting, January 22-23, 2013
Wyndham Riverfront Hotel, 701 Convention Center Blvd., New Orleans, LA

TUESDAY, JANUARY 22, 2013

Purpose: Inform stakeholders of activities and outcomes of October 23-24, 2012 meeting in Minneapolis, MN and planned activities; discuss resistance monitoring sampling and assay methods with registrants, present updates on NCCC46 sampling and assay efforts, and develop conclusions related to developing a common standard for sampling and assays.

--- OPEN SESSION 8:00 to 11:00 am ---

Meeting called to order 8:00am (Eileen Cullen and Tom Hunt, Co-Chairs)

(Minutes recorded by Andrea Hitchon, graduate student, University of Guelph)

Recap of NCCC46/NC205 October 23-24, 2012 Interim Meeting (Eileen Cullen and Tom Hunt)

Jeannette Martinez presented EPA perspectives on resistance management. Her talk was a recap of an October 2012 BPPD talk to the joint Committees in Minneapolis and one at the IPM symposium at the ESA meeting in November. Stakeholder's motivations were highlighted, as well as everyone's common interest in delaying resistance.

David Andow discussed IRM definitional issues. Two main questions were raised:

- 1) Can definitions be adopted so that field resistance can be determined more quickly and reliably?
- 2) In the case of confirmed resistance, are there intermediate remedial steps that could be taken before that of product withdrawal?

A short paper is in progress addressing these issues from the viewpoint of corn entomologists, and a draft will be ready for circulation before the March 2013 joint Committee meeting in Minneapolis.

Agricultural Biotechnology Stewardship and Technical Committee (ABSTC) (Elizabeth Owens)

- Annual Bt corn reporting updates*: Grower IRM compliance (Compliance Assurance Program) and Lepidoptera monitoring to Cry1 proteins (ECB, SWCB, CEW) was reviewed.
- Activities related to Bt crops and environmental safety (non-target organisms, risk assessments)
- Comments on ABSTC CRW population collections on behalf of member companies. Nine populations are collected and distributed to member companies. There is a move

to begin using sentinel fields to improve the collection efficacy. Some concern exists about integrated refuge making collection more challenging. In addition, interest exists in evaluating and selecting a universal diet for assays.

* These reports are available in EPA IRM docket.

Discussion: CRW Resistance Monitoring Bioassay (All)

Why don't we establish Gassmann assay as standard? (Rick Hellmich)

In response to discussion Aaron Gassmann presented a review of his methodology and results obtained to date. It was suggested that experiments which mix resistant and susceptible populations be completed in an attempt to calibrate the assay. Blair Siegfried asked whether any method will be sufficient for monitoring small changes in resistance? We need to have a clear purpose in mind to choose the best assay (routine monitoring or responding to problem fields, a 'yes/no' or a more sensitive result?)

Clint Pilcher (DuPont Pioneer) reviewed the methodology behind the sub-lethal seedling bioassay (SSA).

- Pioneer plans to continue using the SSA (but would consider using another assay in addition)
- Assay requires third party validation
- The need for increased transparency among industry groups was identified.

What are the objectives of the bioassay?

Industry: a tool to determine shifts in resistance which can trigger insect management response, and thus lead to increased durability of the technology

A comparison between the Gassmann and SSA shows a difference between the sample sizes tested. What role does the sample size play in determining the assay's ability to measure resistance?

- How do individual traits impact different populations? Is death always the measure? Can we compare across traits?
- Question was raised regarding where this year's assays should be focused? (diet, sub-lethal, whole plant) Eggs are very limited and this large number of assays makes it impossible to do everything well.

--- CLOSED SESSIONS 11:00 am to 5:00 pm ---

Monsanto (Graham Head, Matt Carroll, Luke Samuel, Tom Clark, Ron Flannagan)

Pioneer (Ana Alves, Clinton Pilcher, Marlin Rice, Susan Moser)

Syngenta/Pioneer (Tony Burd, Isaac Oyediran, Clinton Pilcher, Marlin Rice, Susan Moser)

Syngenta (Isaac Oyediran, Tony Burd)

Dow AgroSciences (Nick Storer)

5:00 ADJOURNED

WEDNESDAY, JANUARY 23, 2013 – MORNING

--- OPEN SESSION 8:00 to 9:30am ---

Purpose: Follow-up discussion as a group of previous day's topics and emerging issues, follow-up as a group. Wrap up discussion of CRW resistance monitoring sampling and assays, resistance definitional issues, and recommendations to preserve trait technology.

There is thought that the Gassmann assay may be more effective at measuring resistance to single gene events while the SSA may be more effective when it comes to polygenic events. ABSTC is working to develop a standardized bioassay across industry. There is concern that required toxins might not be available to perform the diet assay, and that the results are very dependent on egg handling and egg quality (Hibbard).

Discussion: Is it worthwhile to test 'random' rootworm populations [current EPA requirement] or should efforts be targeted to high risk areas? What is the best allocation of resources?

- Running assays is expensive and time consuming.
- We should look where selection is applied and use a colony as a control/standard baseline instead of collecting a susceptible which may or may not have had a shift
- If the resistance is based primarily in one field testing other fields is less critical
- Resistance alleles might not be rare
- Movement into fields is suggested when resistance occurs sooner than a few years of corn on corn (the spread is still playing a role). The history of spreading (after introduction, variant spreading) should be considered. Rotated fields can be infested in one year (evidence of movement).
- Potentially after establishment spread becomes more important
- John Thompson has seen 200 miles movement vs. typically 50-70miles [this makes spread an unlikely explanation]
- Females disperse more at higher densities (since resistant fields often have higher numbers there is likely greater dispersal)
- Look at OP resistance (still around, low fitness costs)
- **How do these considerations impact the necessary monitoring?**

We might need to think of two ways/protocols to respond.

1. Responding to problem fields
2. Routine monitoring for shifts in populations

Discussion: Do we need an 'insect reserve' for study? (Chuck Mason)

Planting a public hybrid without Bt traits might be more indicative of year to year shifts. This might also help with pheromone research and provide additional information for models. ABSTC is planting non-Bt fields across the Corn Belt (like sentinel plots for honey bees) which might be able to serve some of this purpose. Trap crops of longer maturity hybrids or non-Bt corn will be most successful in regions with an aggregation of continuous corn.

Discussion: Do we have a bioassay to detect low levels of resistance?

We do not really have an assay which can reliably test less than 5 fold changes in susceptibility. A molecular diagnostic is needed but due to slow development of this tool will probably not be available until we are using a new technology (Siegfried). There are also concerns about the appropriateness of the various assays for testing northern corn rootworms – plant based might be preferable due to poor performance on diet based assays.

Data gaps:

- Cause and information on northern corn rootworm declines, how to overcome severe technical limitations collecting colonies for monitoring
- Diet for northern and western rootworms
- Standard protocol for responding to problem fields
- Standard protocol for collecting and rearing rootworms

Discussion: What is the current status of seed blends in the Southern US?

- Risk assessment didn't consider earworm (how differential expression may create more favourable conditions for the development of resistance)
- Terms have since been added to the terms and conditions – more discussion will follow at branch meeting
- Concerns exist over how much industry will adopt this technology

Bt soy is being considered for the Southern US by Pioneer. There is debate over whether it would be a single or dual mode of action product. If this happens it is likely other companies will follow suit.

EPA is behind on releasing info from western corn rootworm industry reports.

There is thought the recommendation for fringe areas should be different than main areas (they need to be rotated and not to triple stack) (Chris DiFonzo). Point sources should be targeted before spread occurs.

- Is it too late when evidence of a problem has already been seen? (it has had enough time to spread into neighboring fields)
- How to catch and deal with early is difficult. What is the containment value? How big a radius needs to be wiped?
- To prevent spread from a field in a 'fringe' region, field personnel must be highly trained, better than they are now.
- People are scared to talk about the situation / it should be treated as a significant issue (Art Schaafsma)

Data gaps

- After rotation to a non host crop in a field with high resistance, how soon will resistance redevelop? This will depend on surrounding areas and the density.
- What are the potential consequences where rotation resistance and Bt resistance overlap?

Discussion: Should we recommend adulticides in fields with resistant beetles?

- Reviewing area wide eradication efforts in Europe may help address this question

- Suitable products with sufficient residual are not really on the market
- If recommended, proper timing must be emphasized so that females are killed
- There would need to be a large increase in consultant and grower education if adulticides are recommended more frequently.

--- CLOSED SESSION 9:30am – 12:00am ---

EPA (Alan Reynolds)

Final Progress Summary

- 1) Bioassays – progress is slow but occurring, it will be best to get industry to buy into a standardized assay
- 2) Position paper – in progress
 - a. Discussed need for the paper to have a clear objective
 - b. When does IPM apply? Does it stop applying once unexpected damage occurs and there is a new goal to minimize gene flow (eradicate) or does this still blend with economic concerns?
 - c. How do other pests (e.g., bollworm) fit into the framework laid out?
- 3) Focus groups – progress is slow but a leader has been selected and things should get underway
- 4) Plan for March – discussion points: Monsanto research grants announced in February, research updates from academics to industry, mitigation effects of management, 2013 grower survey results

12:00 – NCCC46/NC205 Joint Meeting ADJOURNED

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