IWGO

International Working Group on Ostrinia and other maize pests

26th IWGO Conference

10 to 12 April 2017

National Agricultural Library
Chinese Academy of Agricultural Science

Beijing, P.R. China

The International Working Group on *Ostrinia* and other Maize Pests (IWGO)
is a Working Group of the International Organization of Biological Control – Global (IOBC - Global)

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SCIENTIFIC PROGRAMME

26th IWGO Conference

SCIENTIFIC PROGRAMME

Sunday, 9 April 2017

17:00 ***Registration, Friendship Hotel – Friendship Palace Building***

- *Provision of PowerPoint Oral Presentations on USB stick*

18:00 ***Registration & Welcome Drink, Friendship Hotel – Palace Building***

- *Welcome Address, Zhenying WANG, IWGO-IOBC-Global Co-Convenor*

19:00 ***Welcome Dinner - Friendship Hotel – Friendship Palace Building***

Monday, 10 April 2017

07:45 ***Registration & Drop-off Point Posters, CAAS National Agricultural Library***

08:30 ***Welcome Addresses - CAAS National Agricultural Library*** *(chaired by Z. WANG)*

- *Welcome Address, Deputy Director General, Dewen QIU, Institute of Plant Protection (IPP) of the Chinese Academy of Agricultural Sciences (CAAS)*
- *Welcome Address, Vice President, Kongming WU, Chinese Academy of Agricultural Sciences (CAAS)*
- *Welcome Address, Ulli KUHLMANN, IWGO-IOBC-Global Convenor*
- *Group Photo*

09:00 ***Scientific Session 1***

09:00 ***Scientific Session 1***

Session 1: Ecology of maize pests and implications for their sustainable management

Session Organizers: *Stefan TOEPFER, CABI c/o Plant Health Service, Hodmezovasarhely, Hungary & Ivan HILTPOLD, Department of Entomology and Wildlife Ecology, University of Delaware, Newark, U.S.A.*

09:00	Stefan TOEPFER & Ivan HILTPOLD	Introduction
09:05	Yu CHEN	Feeding can increase thermal adaptation of herbivorous insect: a case study with corn leaf aphid under acclimation temperature
09:25	Debora MONTEZANO	Immature stages of *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae): developmental parameters on Cry1F, Vip3A, and non-*Bt* maize
09:45	Julie A. PETERSON	Impact of perennial non-crop habitat at center-pivot irrigation corners on ecosystem services for adjacent maize fields
10:05	Ivan HILTPOLD	*Diabrotica virgifera virgifera* larval chemical ecology: scents in the darkness
10:25	Haicui XIE	Effects of elevated CO_2 and temperature on maize-*Tetranychus urticae* interactions

10:45 ***Coffee/Tea Break & Posters***

11:15 ***Scientific Session 2***

Session 2: Genomic and transcriptomic research applications in corn pest insects

Session Organizers: Nick MILLER, Department of Biology, Illinois Institute of Technology, Chicago, U.S.A. & Brad COATES, USDA-ARS, Corn Insects & Crop Genetics Research Unit, Iowa State University, Ames, U.S.A.

11:15	Nick MILLER & Brad COATES	Introduction
11:20	Bin YANG	Identification and targeted mutagenesis of odorant receptor genes in *Ostrinia furnacalis*
11:40	Xiangrui LI	microRNA expression profiling between Cry1Ab-resistant and susceptible European corn borer, *Ostrinia nubilalis* (Hübner)
12:00	Nick MILLER	RNA-Seq studies of corn rootworms
12:20	Brad COATES	The sequencing and assembly of the European corn borer genome
12:40	Marce LORENZEN	Use of genomic and transcriptomic resources for development of species-specific transgenes

13:00 ***Lunch Break***

14:00 ***Scientific Session 3***

Session 3: Environmental risk assessment of insect-resistant genetically modified maize

Session Organizers: Rick HELLMICH, USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, Iowa, U.S.A.& Zhenying WANG, Institute of Plant Protection, CAAS, Beijing, P.R. China

14:00	Rick HELLMICH & Zhenying WANG	Introduction
14:05	Jianwu WANG	The presence of Cry1Ab in *Bt* maize-aphid *Rhopalosiphum maidis* -ladybird *Propylea japonica* has no effects on insect life-history traits
14:25	Yinghua SHU	Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two *Bt* corn hybrids expressing Cry1Ab
14:45	Yunhe LI	Consumption of Cry1Ab/Cry2Aj-containing *Bt* maize pollen does not harm *Folsomia candida*
15:05	Rick HELLMICH	Environmental risk assessment of *Bt* maize in the U.S.: lessons learned
15:25	Huipeng PAN	Dietary risk assessment of v-ATPase A dsRNAs on ladybeetles

15:45 ***Coffee/Tea Break & Posters***

16:15 ***Scientific Session 4***

Session 4: Role of genotype by environment interaction in understanding innate resistance to maize pests

Session Organizers: Kanglai HE, Institute of Plant Protection, CAAS, Beijing, P.R. China & Xinzhi NI, USDA-ARS, Crop Genetics and Breading Research Unit, University of Georgia, Tifton, U.S.A.

16:15	Kanglai HE & Xinzhi NI	Introduction
16.20	Dan JEFFERS	Developing stress resilient maize for the southwestern China and as donor of special traits for temperate maize
16:40	Zhihong LANG	Transcriptome profiling analysis in maize responses to *Ostrinia furnacalis* and transcriptional regulation in indirect defense
17:00	Jingfei GUO	Exposure to herbivore-damaged maize leaves had an inhibitory impact on the fitness of the Asian corn borer *Ostrinia furnacalis* (Guenée)
17.20	Xinzhi NI	Influence of genotype by environment interaction on maize resistance to insect pests and aflatoxin accumulation

17:40 ***Invited Speaker: "Targeting cuticle re-modelling enzymes of Ostrinia furnacalis as a pest control strategy" by Qing YANG, Dalian University of Technology***

18:00 ***End of Day 1***

18:30 ***Social Event (to be announced)***

Tuesday, 11 April 2017

08:15 *Scientific Session 5a*

Session 5a: The evolution of resistance to insecticidal toxins by insect pests of maize

Session Organizers: Aaron GASSMANN, Department of Entomology, Iowa State University, Ames, U.S.A. & Brad COATES, USDA-ARS, Corn Insects & Crop Genetics Research Unit, Iowa State University, Ames, U.S.A.

08:15	Brad COATES & Aaron GASSMANN	Introduction
08:20	Aaron GASSMANN	Resistance to *Bt* maize by western corn rootworm
08:40	Tiantao ZHANG	The difference of *Bt* resistant relative genes in Asian corn borer
09:00	Michael CAPRIO	Remediation of resistance to crops expressing *Bt*-toxins
09:20	Marc NELSON	Cross resistance assessment of Cry1Ab and Cry1F in *Ostrinia furnacalis* (Guenée)

09:40 *Coffee/Tea Break & Posters*

10:15 *Scientific Session 5b*

Session 5b: The evolution of resistance to insecticidal toxins by insect pests of maize

Session Organizers: Aaron GASSMANN, Department of Entomology, Iowa State University, Ames, U.S.A. & Brad COATES, USDA-ARS, Corn Insects & Crop Genetics Research Unit, Iowa State University, Ames, U.S.A.

10:15	Aaron GASSMANN & Brad COATES	Introduction
10:20	Jianzhou (Joe) ZHAO	Laboratory-selected western corn rootworm colonies resistant to *Bt* and non-*Bt* transgenic corn plants
10:40	Yueqin WANG	Geographical and temporal variability in susceptibility to Cry1Ie toxin in China
11:00	Fernando ALVAREZ	Insect resistance monitoring of *Bt* maize in the EU: Results and challenges encountered after 18 years of cultivation
11:20	Mao CHEN	Control of *Ostrinia furnacalis* by *Bt* corn MON89034 and its IRM considerations in Asia

11:40 *Invited Speaker: "RNA-based biocontrol for use in pest management" by Geert PLAETINCK, Syngenta, Gent, Belgium*

12:00 *Lunch Break*

13:30 ***Scientific Session 6***

Session 6: Migratory pests of corn

Session Organizers: Xingfu JIANG, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, P.R. China & Tom SAPPINGTON, USDA-ARS, Corn Insects & Crop Genetics Research Unit Genetics Laboratory, ISU, Ames, U.S.A.

13:30	Xingfu JIANG & Tom SAPPINGTON	Introduction
13:35	Tom SAPPINGTON	Evidence for migratory behavior of western corn rootworm
13.55	Xingfu JIANG	Migratory immunity: density-dependent prophylaxis, immune response and host flight behavior manipulation in *Mythimna separate*
14:15	Baoping ZHAI	Migration patterns of armyworm moths in China
14:35	Miklós TÓTH	The *Ostrinia* trap which catches: comparing the performance of a new bisex corn borer lure with that of synthetic sex pheromone
14:55	Renata BAZOK	Changes in corn rootworm wing morphology related to development of pest resistance

15:15 ***Coffee/Tea Break***

15:45 ***End of Day 2 & Transfer to Hotel***

16:15 ***Social Event (to be announced)***

Wednesday, 12 April 2017

08:00 ***Invited Keynote Presentation: "Spread of fall armyworm in West Africa: need for an emergency response plan" by Victor CLOTTEY, CABI, Ghana***

09:00 ***Scientific Session 7***

Session 7: Regional suppression of lepidopteran pests by *Bt* corn

Session Organizer: Galen Dively, Department of Entomology, University of Maryland, College Park, U.S.A.

09:00	Galen DIVELY	Introduction
09:05	Kanglai HE	Efficacy and IRM studies of *Bt* corn events on the Asian corn borer in China
09:25	Galen DIVELY	Regional suppression of *Ostrinia nubilalis* due to *Bt* corn and its effects on other crops in the mid-Atlantic U.S.
09:45	Rebecca SCHMIDT-JEFFRIS	Effects of *Bt i*n the landscape on *Ostrinia nubilalis* in New York processing snap bean

10:05 ***Coffee/Tea Break & Posters***

10:35 ***Scientific Session 8***

Session 8: New research in rootworm basic biology and potential applications

Session Organizers: Bruce HIBBARD, USDA-ARS, Plant Genetics Research Unit, University of Missouri, Columbia, U.S.A. & Kent SHELBY, USDA-ARS, Biological Control of Insects Research Unit, Columbia, U.S.A.

10:35	Bruce HIBBARD & Kent SHELBY	Introduction
10:40	Yong YIN	Novel protein MOA for corn rootworm control
11:00	Darija LEMIC	The invasion of Europe by western corn rootworm results in changes to wing morphology
11:20	Fu-Chyun CHU	Emerging technologies for genetic manipulation of western corn rootworm
11:40	Bruce HIBBARD	Improved artificial diet for western corn rootworm assays
12:00	Kent SHELBY	Contributions of recovery and repair genes and microbiomes to *Diabrotica* ssp resistance management

12:20 ***Lunch Break & Posters***

13:30 ***Scientific Session 9***

Session 9: New generation traits for management of maize pests

Session Organizers: Joe ZHAO, Durability and Resistance Management, DuPont Pioneer R&D, Johnston, U.S.A. & Amit SETHI, Integrated Product Characterization & Development, DuPont Pioneer, Johnston, U.S.A.

13:30	Jianzhou (Joe) ZHAO & Amit SETHI	Introduction
13:35	Lu LIU	Novel insecticidal proteins from plants
13:55	Amit SETHI	New technologies for corn rootworm control
14:15	Kenneth NARVA	In-plant protection against *Helicoverpa armigera* by production of long hpRNA in chloroplasts
14:35	Yong YIN	Controlling corn rootworms: SmartStax PRO and beyond

14:55 ***Coffee/Tea Break & Posters***

15:30 ***Scientific Session 10***

Session 10: Biological control of maize pests

Session Organizers: Dirk BABENDREIER, CABI, Delémont, Switzerland & Li ZHENG, Plant Protection Institute, Shandong Academy of Agricultural Sciences, Jinan, P.R. China

15:30	Dirk BABENDREIER & Li ZHENG	Introduction
15:35	Westen R. ARCHIBALD	Survey of natural enemies for North American maize pest *Striacosta albicosta*
15:55	Zhenying WANG	Review of biological control for corn insect pests in China
16:15	Geoffrey JAFFUEL	The combined application of beneficial microorganisms to control the western corn rootworm
16:35	Julie A. PETERSON	Assessment of soil entomopathogens from maize fields of western Nebraska, USA: can they be useful for western corn rootworm control?
16:55	Lian-Sheng ZANG	Mass production of *Trichogramma* using eggs of *Antheraea pernyi* and field application in the suppression of corn borers in Jilin Province, China

17:15 ***IWGO Business Meeting***

18:00 ***End of Day 3***

18:30 ***Social Event (to be announced)***

Thursday, 13 April 2017

09:15 *Excursion - Visit to the Institute of Plant Protection (IPP) of the Chinese Academy of Agricultural Sciences (CAAS)*

09:15 Meeting in the lobby of the Friendship Hotel

09:30 Departure by bus and transfer to IPP-CAAS

10:00 Introduction to IPP-CASS by Director General, Xueping ZHOU

10:30 The Department of Entomology and its research priorities by Zhenying WANG

11:00 Introduction to the Biological Invasions national programme by Fang-Hao WAN

11:30 Guided tour through IPP-CAAS and its associated National Biosafety Research Centre

12:30 Picnic lunch at IPP-CAAS

13:30 Departure to Friendship Hotel

14:00 Arrival at Friendship Hotel

14:00 End Excursion

POSTER PRESENTATIONS

Poster 01	Jianqing GUO	Effect of plant age and species on population growth of six *Rhopalosiphum maidis* (Fitch) lineages
Poster 02	Xing-Chuan JIANG	A potential application of an intercropping system for pest control: interference with Asian corn borer orientation and mating behaviour
Poster 03	Westen R. ARCHIBALD	Feeding damage from *Striacosta albicosta* at various infestation rates on *Bt* hybrids and implications for economic injury levels
Poster 04	Xing GE	Identification of putative olfactory related genes from yellow peach moth *Conogethes punctiferalis* (Guenée) antennae transcriptome
Poster 05	Debora MONTEZANO	Susceptibility of *Ostrinia nubilalis* (Hübner) and *Striacosta albicosta* (Smith) populations to pyrethroid in the United States
Poster 06	Stefan TOEPFER	Application techniques for beneficial nematodes against soil insect pests in maize
Poster 07	Pamela BRUNO	Survey of natural enemies of the western corn rootworm in Central Mexico
Poster 08	Hao CHEN	Release of *Trichogramma ostriniae* for biological control of *Ostrinia furnacalis* in summer corn region of China
Poster 09	Michael ZELLNER	Long-term effects of different insecticides on the wireworm population (*Agriotes* spp.) in the soil

ABSTRACTS
ORAL
PRESENTATIONS

Feeding can increase thermal adaptation of herbivorous insect: a case study with corn leaf aphid under acclimation temperature

Yu Chen[1,2], Yudong Quan[1], Kang-Lai He[1], Frederic Francis[2] & Zhenying Wang[1]

[1]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing, P.R. China, ychen_007@126.com
[2]Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, ychen_007@126.com

Climate change is predicted to increase the frequency and magnitude of extreme heat events, which poses great challenges for most ectotherms. The corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), is a worldwide agricultural pest throughout the temperate zone and therefore be most likely to experience extreme high temperatures. In this study, effects of feeding on thermal tolerance were examined with corn leaf aphid under acclimation temperature. The critical high temperatures were about 39.0°C for all instars nymphs and adults, except 3rd and 4th instars nymphs in feeding treatments, which were as high as to 39.5°C. The survival rates were decline as the temperature increased. The upper lethal temperatures (ULTs) of corn leaf aphid were significant different between feeding and no feeding treatments. The ULT_{50} values were from 39.51 to 40.56°C for the 1st to the 4th instar nymphs, apterous and alatae adults in no feeding treatments. In contrast, the ULT_{50} values were from 39.96 to 42.25°C for these insects in feeding treatments, which were significantly higher than that in no feeding treatments. In addition, the ULT_{50}s varied significantly among the insect development stages. The 4th instar nymphs had the highest value of ULT_{50}s, whereas the 1st instar nymphs had the lowest value of ULT_{50}s. These results illustrated that feeding can increase thermal adaptation of corn leaf aphid under acclimation temperature.

Immature stages of *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae): developmental parameters on Cry1F, Vip3A, and non-*Bt* maize

Debora Montezano[1], Thomas Hunt[1] & Julie Peterson[2]

[1]*Universty of Nebraska, Lincoln, Nebraska, U.S.A., deiagm@gmail.com*
[2]*University of Nebraska, North Platte, Nebraska, U.S.A.*

The western bean cutworm, *S. albicosta,* is a destructive insect pest that can cause severe yield loss in corn and dry beans (up to 40% and 10%, respectively). Current management practices in Nebraska rely heavily on planting transgenic *Bt* corn or treatment with conventional insecticides. The *Bt* proteins expressed in transgenic plants vary in their effectiveness against a suite of pests; Cry1F and Vip3A are the only proteins that target *S. albicosta*. However, the decrease in efficacy of certain transgenic hybrids has become a concern among researchers and growers in locations were this pest is a problem. This study was designed to detail the temporal and morphological parameters of the immature stages of *S. albicosta* feeding on Cry1F, Vip3A and non-*Bt* maize under controlled conditions (25 ± 1°C, 70 ± 10% RH and 14 hour photophase). For Vip3A, mortality was 100% after 48 hours. The viability of the larval, pupal and pre-pupal stages for Cry1F and non-*Bt* was 79 and 63%, respectively. The average duration of the larval and pre-pupal stages on Cry1F and non-*Bt* maize was 72 and 68 days, respectively. During the larval stage, 90 and 10% of larvae on Cry1F passed through the 6th and 7th instar, respectively, and 14, 84 and 2 % of larvae on non-*Bt* corn passed through the 5th, 6th and 7th instars, respectively. Mean larval growth rate for non-*Bt* was greater than on Cry1F. The non-*Bt* pupae were significantly larger and heavier than those on Cry1F. Differences in *S. albicosta* developmental parameters when fed on Vip3A and Cry1F compared to non-*Bt* indicated that Vip3A is extremely effective in controlling *S. albicosta* larvae. Non-*Bt* shows low mortality difference when compared to no Cry1F, however, it does show difference in development in immature stages of *S. albicosta.* Understanding pest biology and response to transgenes will aid in developing effective IPM and resistance management strategies for the target pests.

Session 1-T 3 - Ecology of maize pests and implications for their sustainable management

Impact of perennial non-crop habitat at center-pivot irrigation corners on ecosystem services for adjacent maize fields

Kayla A. Mollet[2], Débora G. Montezano[2] & Julie A. Peterson[1]

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[2]*University of Nebraska, Lincoln, Nebraska, U.S.A.*

Large-scale commercial agriculture can often lead to low diversity landscapes with few resources for wildlife and beneficial arthropods. However, enhancing plant diversity has been shown to increase populations of beneficial species, such as natural enemies, pollinators, and decomposers, as well as the ecosystem services that these communities provide. In the state of Nebraska (USA), a unique conservation program called Corners for Wildlife (CFW) allows farmers to receive cost-share assistance and rental income by planting a high diversity of trees, shrubs, and perennial plants on center-pivot irrigation corners. Our project seeks to understand whether pivot corners with higher plant diversity (those enrolled in CFW) compared to conventional lower-diversity corners (planted to maize or winter wheat) will provide greater ecosystems services of: 1) Biological control of pests in the adjacent maize fields; 2) Decomposition of dung from cattle grazing in the adjacent maize fields; and 3) Enhancement of recreational land value through support of game bird (pheasant and quail) populations. Results from field collections in pitfall traps indicate that activity-density of Carabidae (predatory ground beetles) and Scarabaeidae (dung beetles) is higher in high diversity perennial corners and adjacent irrigated maize. Frequency of pheasant and northern bobwhite quail calls was also higher in high diversity corners. Maize pest species (western bean cutworm *Striacosta albicosta* (Lepidoptera: Noctuidae) and western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)) were observed to utilize pollen and nectar resources from high diversity corners, but pest pressure was not elevated in adjacent maize fields. Sentinel egg cards were designed to mimic the egg masses of *S. albicosta*, a critical pest of maize, and deployed determine the strength of biological control pressure. These data indicate that during the period of early *S. albicosta* oviposition, predation pressure is highest in maize fields immediately adjacent to high diversity, perennial corners. Results indicate that this program has the potential to positively impact the abundance and diversity of beneficial organisms, leading to enhanced provisioning of ecosystem services.

Diabrotica virgifera virgifera larval chemical ecology: scents in the darkness

Ivan Hiltpold[1]

[1]*University of Delaware, Newark, Delaware, U.S.A., hiltpold@udel.edu*

In the darkness of the soil matrix, the larva of the western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte relies on chemical cues to locate and accept its plant host roots. For almost 40 years, entomologists and chemical ecologists have tackled the challenge of isolating and identifying active chemical compounds emitted by host plant roots (most of the research has been conducted on maize *Zea mays* L.) and used by the foraging insect pest larvae. Rewarding this research effort, a number molecules of interest have been documented. In the current arms race to manage WCR (and soil-dwelling pests), an in-depth understanding of the mechanisms underlying the insect foraging behavior is certainly critical to the development of highly effective but also sustainable pest control strategies. This contribution reviews the progress and identifies the gaps in our current knowledge on the chemical ecology of WCR larvae. It also proposes avenues (tested or theoretical) to eventually implement this knowledge in integrated pest management of this major maize pest, a critical approach as WCR has evolved resistance to several control strategies.

Effects of elevated CO2 and temperature on maize - *Tetranychus urticae* interactions

Haicui Xie[1]

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Climate modellers predict that the atmospheric CO_2 concentration will double and temperature will increase 1.8-4.0°C, globally by the end of this century, which will influence the growth of crop plants and herbivores. Effects of elevated CO_2 (eCO_2) and temperature (eT) on two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) feeding on maize, *Zea mays* L. (Gramineae), were examined in environmental chambers. eCO_2 (700-750 µl l^{-1}) significantly decreased N content, increased TNC content and TNC: N ratio in maize leaves, while elevated temperature (+3 °C) did not influence maize leaf chemistry. Compared with ambient CO_2 condition, the developmental time of deutonymph of *T. urticae* was extended under eCO_2.The developmental time of larvae, protonymph, deutonymph and immature stage were reduced at elevated temperature. Thus, eCO_2 mitigated the effects of eT on developmental time to some extent. The fecundity of *T. urticae* was declined under eCO_2 and eT. *T. urticae* had lower net reproductive rate (R_0) and shorter generation time (T) at eT, regardless of the atmospheric CO_2 levels. These results indicated that eCO_2-mediated decrease innutritional quality of maize leaves extended the development time of *T. urticae*. Taken together, with rising of CO_2 and temperature, the growth and development for *T. urticae* were accelerated feeding on maize, but its fecundity declined, which will decrease the damage of *T. urticae* on maize for single generation. However, presumably the number of generations will increase in high accumulative temperature regions, that may exacerbate their damage on maize.

Session 2-T 1 - Genomic and transcriptomic research applications in corn pest insects

Identification and targeted mutagenesis of odorant receptor genes in *Ostrinia furnacalis*

Bin Yang[1,2], Takeshi Fujii[2], Yukio Ishikawa[2] & Takashi Matsuo[2]

[1]*Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China, byang@ippcaas.cn*
[2]*The University of Tokyo, Tokyo, Japan, byang@ippcaas.cn*

Genome editing tools such as TALEN or CRISPR has been applied for various model organisms but not yet for agricultural pest insects. In this study, TALEN-mediated mutagenesis of the gene encoding odorant receptor co-receptor (Orco) of an important agricultural pest *Ostrinia furnacalis* (OfurORco) was carried out. Of the two pairs of TALEN constructs designed for OfurORco, one generated high somatic and germline mutation rates. Physiological and behavioral analyses using a gas chromatograph–electroantennographic detector system and a wind tunnel, respectively, revealed that antennal responses to sex pheromone components were decreased to trace levels, and behavioral responses were abolished in OfurOrco mutants. This study demonstrated that TALEN-mediated mutagenesis is applicable to pest insects, and these results will open the way for a better understanding of chemosensory systems in wild insects.

Session 2-T 2 - Genomic and transcriptomic research applications in corn pest insects

MicroRNA expression profiling between Cry1Ab-resistant and susceptible European corn borer, *Ostrinia nubilalis* (Hübner)

Tian Yu[2], Xiangrui Li[1], Brad S. Coates[3], Blair D. Siegfried[4] & Xuguo Zhou[2]

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Transgenic maize hybrids that express insecticidal *Bacillus thuringiensis* (*Bt*) crystalline (Cry) protein toxins effectively protect against the European corn borer, *Ostrinia nubilalis*. Field monitoring and laboratory selections have detected varying levels of *O. nubilalis* resistance to Cry1Ab toxin. MicroRNAs (miRNAs) are short non-coding RNAs that are involved in post-transcriptional gene regulation, but their potential roles in the evolution of *Bt* resistance remain largely unknown. Sequencing of small RNA libraries from the midgut of Cry1Ab susceptible and resistant *O. nubilalis* larvae resulted in the discovery of 277 miRNAs, 248 conserved and 29 novel. Comparison of miRNA expression levels between the two strains suggested that 26 and 9 transcripts were significantly up- and down-regulated in the Cry1Ab resistant strain, respectively. Computational predictions estimated 134,147 miRNA target sites within 3'-UTR regions of 9,059 different transcripts previously assembled within a reference *O. nubilalis* midgut transcriptome. Differentially-expressed miRNAs were predicted to affect transcripts involved in cell membrane components with functions in metabolism and binding, and putative *Bt* resistance genes aminopeptidase N and cadherin. These results lay the foundation for future investigation of the potential role of miRNAs in the evolution of *Bt* resistance.

RNA-Seq studies of corn rootworms

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"RNA-Seq" studies use high-throughput sequencing to deep-sequence the entire transcriptome of tissues or whole organisms under different conditions. RNA-Seq is a powerful tool for studying transcriptome-wide changes in gene expression, especially in non-model organisms for which genomics resources are scarce.

We have used RNA-Seq to investigate changes in gene expression in corn rootworm larvae to understand their responses to maize defensive secondary metabolites, resistance to *Bt*-maize and their response to different host and non-host plants. In all cases we have identified transcripts that are likely to be important to the fitness of the insect. Nevertheless, some significant challenges remain, including the lack of an annotated genome sequence and the problem of identifying the key genes of interest when hundreds or thousands of differentially-expressed transcripts are identified.

The sequencing and assembly of the European corn borer genome

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The European corn borer, *Ostrinia nubilalis*, is a major pest of maize in North America and Europe. A draft genome was assembled from an inbred line of the *O. nubilalis* biovoltine Z-pheromone strain using data from Illumina HiSeq libraries. The assembly consists of 455.7 Mbp across 8,876 scaffolds with a median size (N50) of 395 kbp, which represents ~99% of the ~458.7±3.2 Mbp flow cytometry estimated genome size. Preliminary annotation predicted 56,625 transcript isoforms that are encoded by 29,090 putative unique gene coding regions, which 72 odorant receptor genes. Annotated genes also include coding regions for aminopeptidase N, ATP binding cassette (ABC) transporter, alkaline phosphatase gene families that have been reported as conferring *Bacillus thuringiensis* (*Bt*) resistance in other species of Lepidoptera. The *O. nubilalis* genome will be a resource for future investigations into the biochemical basis of pheromone responses and resistance to *Bt* toxins.

Use of genomic and transcriptomic resources for development of species-specific transgenes

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Sophisticated manipulations of insect genomes using germline transformation, or similar mechanisms, frequently requires species-specific promoters to drive expression of transgenes. We are currently working to develop transgenic tools for the western corn rootworm (WCR), a major pest of maize. Here we report the use of Illumina's MiSeq platform to acquire transcriptomic data for WCR in order to build nearly full-length RNA-Seq contigs which are necessary for pin-pointing the location of useful promoters. Barcoded libraries were generated from WCR eggs, larvae, pupae, adult females, and adult males. Unlike our previous RNA-Seq methodologies, here we used paired-end sequencing of ~700-bp fragments on Illumina's MiSeq (2 x 300 bp). Contigs were assembled using DNASTAR's SeqMan NGen software and annotations generated using Blast2GO. Of the 23,296 contigs produced, 8,584 were over 2 kb, and most appear to represent full-length transcripts. Comparison of the WCR transcriptomic data to that from another coleopteran, the red flour beetle, *Tribolium castaneum*, also suggests these represent complete, or nearly complete transcripts. Full-length transcripts are critical because they define the precise location of a locus' transcription boundaries. The upstream boundary of each promoter of interest was identified by analyzing 0.5 to 1 kb of upstream genomic sequence using the Neural Network Promoter Prediction Program. Our progress towards the development and use of WCR-specific transgenes will be presented, along with some of the challenges we had to overcome along the way.

The presence of Cry1Ab in *Bt* maize-aphid *Rhopalosiphum maidis* - ladybird *Propylea japonica* has no effects on insect life-history traits

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Transgenic *Bacillus thuringiensis* (*Bt*) crops receive particular attention because they carry genes encoding insecticidal proteins that might negatively affect non-target arthropods. Here, laboratory experiments were conducted to evaluate the impact of Cry1Ab-expressing transgenic maize (5422Bt1 (Event Bt11) and 5422CBCL (MON810)) on life-history traits of non-target arthropod, aphid *Rhopalosiphum maidis* (Homoptera: Aphididae) and ladybird *Propylea japonica* (Coleoptera: Coccinellidae). We found no significant differences between *R. maidis* feeding on *Bt* maize and the near-isogenic line when offspring production, survivorship, longevity, intrinsic rates of natural increase (r_m), finite rates of increase and doubling times were compared. No significant differences were found between treatments for pre-reproductive and reproductive periods. No detrimental effects were detected on development (nymphs, adults, and progeny eggs), fecundity, longevity, and egg hatching of *P. japonica* on *Bt* maize aphids compared with non-*Bt* maize aphids.A tri-trophic assay revealed that there was a great decline in the detection of Cry1Ab toxin through the trophic chain (*Bt* maize leaves- aphid *R.maidis*- ladybird *P. japonica*) by immunological tests (ELISA). Along with the increasing generations, Cry1Ab protein concentrations in aphids significantly decreased, and Cry1Ab quantities in the 2nd and 3rd generation *P. japonica* preying on *Bt* maize exposed aphids were nil. Our results indicated that the non-target insect *R. maidis* and its predator *P. japonica* are exposed to Cry1Ab toxin from transgenic cry1Ab maize, but life-history traits of this predator was not affected by the toxin via fed-prey.

Responses of the cutworm *Spodoptera litura* (Lepidoptera: Noctuidae) to two *Bt* corn hybrids expressing Cry1Ab

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A major threat to the long-term application of *Bacillus thuringiensis*(*Bt*)corn is possible evolution of target pest resistance. Additionally, their efficiency for controlling some secondary lepidopteran pests has been questioned, raising concerns about potential outbreaks and their economic consequences. The common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), a serious crop pest with a strong migratory ability in the world. *S. litura* larvae are polyphagous with 389 host plants including >30 cultivated crops, such as cotton, corn, soybean, groundnut, vegetables, etc. To examine the responses of the secondary lepidopteran pest *Spodoptera litura* (Lepidoptera: Noctuidae) to two *Bacillus thuringiensis* (*Bt*) corn hybrids [5422Bt1 (Event Bt11), 5422CBCL (MON810)] expressing Cry1Ab, larval bioassays with Cry1Ab toxin, corn leaves or kernels and bagging on corn plants were conducted. The results showed that larvae displayed a similar performance when fed kernels rather than leaves of 5422Bt1, 5422CBCL and their near-isogenic non-*Bt* corn (5422). Significantly higher Cry1Ab amounts were detected in larvae fed leaves than kernels of both *Bt* hybrids, with different molecular weights of protein band in plants (72 and 90 kDa for 5422Bt1 and 5422CBCL, respectively), gut contents (65 kDa), feces (50 kDa), which indicated that larvae had lower ingestion, higher degradation and excretion of Cry1Ab when fed kernels not leaves of both *Bt* hybrids. Significantly higher levels of cadherin-like receptors and alkaline phosphatase transcripts were detected in larvae fed leaves than kernels of two *Bt* hybrids. Catalase, superoxide dismutase and glutathione-S-transferase activities in larvae fed 5422Bt1 leaves were significantly higher than that of 5422 treatments. Therefore, *S. litura* had low susceptibility to 5422Bt1 and 5422CBCLwhen larvae fed kernels not leaves of *Bt* corn. Additionally, *S. litura* presented a much stronger tolerance to 5422CBCL than 5422Bt1.

Consumption of Cry1Ab/Cry2Aj-containing *Bt* maize pollen does not harm *Folsomia candida*

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The common soil arthropod *Folsomia candida* can survive well when fed only maize pollen and thus may be exposed to insecticidal proteins by ingesting insect-resistant genetically engineered maize pollen containing *Bacillus thuringiensis* (*Bt*) proteins when being released into the soil. Laboratory experiments were conducted to assess the potential effects of Cry1Ab/Cry2Aj-producing transgenic *Bt* maize (Shuangkang 12-5)pollen on *F*. *candida* fitness. Survival, development, and the reproduction were not significantly reduced when *F. candida* fed on *Bt* maize pollen rather than on non-*Bt* maize pollen, but these parameters were significantly reduced when *F. candida* fed on non-*Bt* maize pollen containing the protease inhibitor E-64at 75μg/g pollen. The intrinsic rate of increase (r_m) was not significantly reduced when *F*. *candida* fed on *Bt* maize pollen but was significantly reduced when *F*. *candida* fed on non-*Bt* maize pollen containing E-64. The activities of antioxidant-related enzymes in *F. candida* were not significantly affected when *F. candida* fed on *Bt* maize pollen but were significantly increased when *F. candida* fed on non-*Bt* pollen containing E-64. The results demonstrate that consumption of *Bt* maize pollen containing Cry1Ab/Cry2Aj has no lethal or sublethal effects on *F. candida.*

Environmental risk assessment of *Bt* maize in the U.S.: lessons learned

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Growers from a generation ago would find many differences if they walked though fields with genetically-engineered (GE) crops. Now there are fewer weeds due to herbicide-tolerance traits and much less insect damage due to insect-resistance traits. On closer inspection they might even note, especially in maize, the quality of the grain has improved dramatically. These crops have led to reduced use of chemical insecticides and the use of more environmentally friendly herbicides. Biotechnology indeed has changed the shape of agriculture. Yet, despite the exciting potential of biotechnology, there are many challenges. Some scientists have questioned the ecological risk assessment process and others have questioned whether GE maize negatively impacts beneficial arthropods. Over the past two decades academic, government, regulatory and private-sector scientists in the U.S. have noted these criticisms and have worked to improve the science of environmental risk assessment for all biotech crops with an emphasis on biotech maize. This talk will provide an overview of lessons learned in the U.S. for assessing environmental risks of *Bt* maize on non-target arthropods.

Dietary risk assessment of v-ATPase A dsRNAs on ladybeetles

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RNA interference (RNAi)-based genetically modified (GM) plants targeting insects have been developed and offer a new approach for insect control. However, the lack of standardized environmental risk assessment (ERA) framework and a standardized ERA protocol limits the integration of RNAi technologies into sustainable pest management practices. The overall objective of this study is to assess the risks of RNAi-based GM crops on four nontarget ladybeetle species including *Hippodamia convergens*, *Harmonia axyridis*, *Coleomegilla maculata*, and *Coccinella septempunctata*, which could potentially be exposed to dsRNA-containing herbivores and pollen from GM crops. The primary risk hypothesis is insecticidal dsRNA targeting at the western corn rootworm, *Diabrotica virgifera virgifera,* has no adversely impacts on ladybeetles. Following a tiered approach, we tested this risk hypothesis using a well-designed dietary RNAi toxicity assay. To create the worst case scenario, full-length *v-ATPase A* cDNAs from four ladybeetle species were obtained and a 400 bp fragment with the highest sequence similarity was selected as the template to synthesize dsRNAs. For RNAi toxicity assay, newly hatch neonates were provisioned with *v-ATPase A* dsRNAs derived from *D. v. virgifera* and each of the four ladybeetles, respectively, a dsRNA generated from a plant control gene, and H_2O. In average, each neonate larva ingested approximately 16 µg of dsRNA during the assay, which is equivalent to an exposure of 1,600 times higher than the LC_{50} reported for *D. v. virgifera* larvae. The endpoint measurements included gene expression, and life history traits, such as survival rate, developmental time, and pupa and adult weight. Dietary RNAi had significant impacts on each of the four ladybeetles at both transcriptional and phenotypic levels, suggesting that ingested dsRNAs have significantly impacts on all of the four ladybeetles.

Session 4-T 1 - Role of genotype by environment interaction in understanding innate resistance to maize pests

Developing stress resilient maize for the southwestern China and as donor of special traits for temperate maize

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Subtropical and temperate maize production in China is principally under rain-fed conditions, and yield is impacted by many biotic and abiotic stresses, while much of the germplasm base available for breeders has limited genetic diversity to provide improved stress resilience. The Yunnan Academy of Agricultural Sciences-CIMMYT collaborative maize breeding program was established to broaden the germplasm base for improving yield and stress resilience for use in the subtropical region of southern China, and neighboring countries, and to provide source germplasm for China's temperate maize area. The introduced maize germplasm comes from international collaborative efforts in CIMMYT's breeding network, and includes biotic stress resistant inbreds developed under artificial inoculations and insect infestations for identifying disease and insect resistant sources, and abiotic stress screening to identify abiotic stress tolerant donors. The introduced lines are being evaluated for per se performance, and in hybrid combination, while special trait germplasm is being used in line recycling activities and for trait introgression into key public Chinese temperate inbreds.

Session 4-T 2 - Role of genotype by environment interaction in understanding innate resistance to maize pests

Transcriptome profiling analysis in maize responses to *Ostrinia furnacalis* and transcriptional regulation in indirect defense

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Pests cause severe yield losses in crop production worldwide. Crop plants counteract chewing insects by transcriptionally promoting a repertoire of defense gene products that are either toxic to, or attractive to the natural enemies of, pest insects. The genome-wide early responses in maize seedlings to Asian corn borer (ACB, *Ostrinia furnacalis*) and also to jasmonate (JA), the pivotal phytohormone controlling plant defense response against herbivory, were transcriptionally profiled. Clustering of differentially expressed genes (DEGs) along with functional enrichment analysis revealed important biological processes regulated in response to ACB infestation and jasmonate. Moreover, an AP2/ERF family transcription factor EREB58 was identified to promote the terpene synthases TPS10 gene expression by directly binding to the GCC-box within the region from -300 to -200 of the TPS10 promoter. Transgenic maize plants overexpressing EREB58 constitutively over-accumulate TPS10 transcript and also (E)-bfarnesene and (E)-a-bergamotene, two major sesquiterpenes produced by TPS10. The further works focus on the EREB58 function and regulation network in maize defense response.

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Session 4-T 3 - Role of genotype by environment interaction in understanding innate resistance to maize pests

Exposure to herbivore-damaged maize leaves had an inhibitory impact on the fitness of the Asian corn borer *Ostrinia furnacalis* (Guenée)

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Maize damaged by herbivorous insects is often induced defense responses that can negatively affect the subsequent insect fitness. Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), is a major devastating insect pest in maize, however, the impacts of ACB-induced maize resistance on ACB fitness are poorly understood. Here we chose maize inbred line Jingke968 to evaluate the impacts of ACB-induced maize resistance on ACB fitness. In the first part of this study, a 2-day feeding trial was conducted to assess the impacts of ACB-damaged maize resistance on the food intake and utilization of the 2nd instar ACB larvae. Results showed that efficiency of ingested food (ECI), and relative growth rate (RGR) were significantly lower on ACB-damaged maize leaves, but the relative consumption rate (RCR) and consumption index (CI) were significantly higher on ACB-damaged maize leaves. Approximate digestibility (AD) and efficiency of conversion of digested food (ECD) had no significant differences between ACB-damaged and control maize leaves. In the second part, we reared ACB with ACB-damaged maize leaves throughout larval development to investigate the impacts of ACB-damaged maize resistance on ACB demography using age-stage, two-sex life table. Significantly longer larval life-span, pupae and total pre-oviposition period (TPOP), as well as mean generation time (T) were observed in ACB fed on ACB-damaged maize leaves. The finite rate of increase (λ), intrinsic rate of increase (r) and net reproductive rate (R_0) were also negatively affected. Therefore, exposure to ACB-damaged maize leaves for ACB could have an inhibitory impact on their fitness.

Session 4-T 4 - Role of genotype by environment interaction in understanding innate resistance to maize pests

Influence of genotype by environment interaction on maize resistance to insect pests and aflatoxin accumulation

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Environmental conditions influence insect and disease resistance under the field conditions. When spatial and temporal patterns of aflatoxin contamination in relation to multiple insect damage was examined using a commercial corn hybrid in 2008 and 2009, edge effect was detected in aflatoxin contamination, maize weevil (*Sitophillus zeamais* Motschulsky) and stink bug [*Euschistus servus* (Say)] damage in the field, but not for corn earworm [*Helicoverpa zea* (Boddie)] damage. The two-year field data also varied. In addition, a set of 12 reciprocal experimental crosses was made using maize inbred lines with fall army resistance and high levels of kauralexins or zealexins to develop new maize germplasm lines with insect and disease resistance. These experimental crosses were evaluated under the field conditions in 2013 and 2014. In each year, the 12 breeding crosses and 4 commercial controls were evaluated for yield, insect and disease resistance, and aflatoxin contamination utilized the randomized complete block design. Fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] and corn earworm damage, and aflatoxin contamination, produced by *Aspergillus flavus* were screened with artificial inoculations. Natural occurrence of predator abundance and diversity in relation to fall armyworm damage at whorl stage and southern rust (*Puccinia polysora* Underwood) infection after flowering were also assessed. Significant difference was detected between the years, as well as among these crosses in yield, and fall armyworm, corn earworm, and southern rust resistance, as well as in the levels of aflatoxin contamination. Genotype by environment interaction is important spatially and temporarily, and it is critical to assess insect and disease resistance, natural enemy abundance, and aflatoxin contamination in multiple years and/or locations.

Invited Speaker

Targeting cuticle re-modelling enzymes of *Ostrinia furnacalis* as a pest control strategy

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Innovation of pesticide molecular targets is the key scientific issue for the development of novel pesticides. Chitin, a linear biopolymer composed of β-1,4-linked N-acetylglucosamine (GlcNAc), is an essential component in the skeleton of insects but is absent in human and mammals. Targeting chitin metabolism is therefore an eco-friendly strategy for pest control and management. Three family enzymes are closely related to chitin metabolism. They are chitin synthase for the polymerization of glucosamine (GlcNAc) and chitinolytic enzymes including chitinase (EC 3.2.1.14) and β-N-acetyl-D-hexosaminidase (EC 3.2.1.52) for the old chitin-component degradation. Chitinase first cut the chitin chain into shorter ones by an endo-acting manner, while β-N-acetyl-D-hexosaminidase acting as an exo-enzyme to release monosaccharide GlcNAcs from the ends of chitin chains.

Focusing on chitin degradation and synthesis, we have been working with the most destructive pest, *Ostrinia furnacalis*, for over ten years. The main achievements we have made with *Ostrinia furnacalis* include: 1) the discovery of a series of enzymes specially required for chitin degradation and synthesis; 2) the reveal of crystal structures of the key enzymes involved, namely the exo-acting chitinolytic enzyme OfHex1 and the endo-acting chitinolytic enzymes OfChtI, OfChtII and OfCht-H; and 3) the structure-guided synthesis and design of highly efficient and insect-specific inhibitors against these enzymes. These achievements provide promising targets for control *Ostrinia furnacalis*.

Resistance to *Bt* Maize by western corn rootworm

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The western corn rootworm, *Diabrotica virgifera virgifera,* is a serious pest of maize in the United States and is currently managed by planting transgenic maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*). Beginning in 2009, field populations of western corn rootworm were identified that had developed resistance to *Bt* maize producing Cry3Bb1. Subsequent bioassays and field studies found that resistance to Cry3Bb1 also conferred cross-resistance to transgenic corn that produced either mCry3A or eCry3.1Ab. Management of *Bt* resistance in western corn rootworm has focused on the refuge strategy in conjunction with pyramiding of multiple *Bt* toxins targeting rootworm. Delays in the evolution of resistance achieved by the refuge strategy are expected to be greater when resistance is inherited as a recessive trait and fitness costs are associated with resistance. Laboratory experiments with strains that contain field-derived resistance alleles indicate that resistance is not inherited in a recessive manner and that few fitness costs may be associated with resistance. It is possible that both of these factors may have contributed to the rapid development of *Bt* resistance by western corn rootworm. The results of this research are discussed in the context of applying insect resistance management to delay the evolution of resistance by agricultural pests to transgenic *Bt* crops.

IRM in *Bt* crops: the importance of pest migration behavior and utilization of wild host plants

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Resistance evolution threatens the sustainability of *Bt* crops. It is especially in Africa where insect resistance management (IRM) strategies are faced by unique challenges. The assumptions, on which current IRM strategies for stem borers are based, are not all valid for African maize stem borer species. The high dose/refuge strategy relies heavily on the presence of appropriate refuges (non-*Bt* plants) where pests are not under selection pressure and where sufficient numbers of *Bt*-susceptible individuals are produced to mate with possible survivors on the *Bt* crop. Desired characteristics of refuge plants are that they should be good pest hosts, implying that larval survival is high and that it produces sufficient numbers of high quality moths. Refuge plants should also have large cover abundance in areas where *Bt* maize is planted. While wild host plants may suffice in IRM strategies for polyphagous pests, this is not the case with stenophagous pests. Results of ecological studies and stem borer surveys conducted over the past decade shows that wild host plants are unsuitable for development and survival of sufficient numbers of stem borer individuals but that it may contribute to bollworm IRM in cotton. For stem borers, grasses rather act as dead-end-trap plants and do not comply with refuge requirements of producing 500 susceptible individuals for every one resistant individual that survives on *Bt* maize.

Session 5-T 3 - The evolution of resistance to insecticidal toxins by insect pests of maize

The difference of *Bt* resistant relative genes in Asian corn borer

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Cry1Ac and Cry1Ab are typical *Bt* toxins can effectively against Asian corn borer, *Ostrinia furnacalis*. In our lab, the ACB stains evolved more than 1000 times resistant to Cry1Ac or near 200 to Cry1Ab. To identify the resistant relative genes and evaluated the expression level between resistant and susceptible strains, quantitative comparisons were made among midgut expressed transcripts from *O. furnacalis* susceptible (ACB-BtS) and laboratory selected strains resistant to Cry1Ab (ACB-AbR) and Cry1Ac toxins (ACB-AcR). Transcriptome-wide expression estimated from RNA-seq read depths predicted significant down-regulation of transcripts for previously known *Bt* resistance genes, aminopeptidase N1 (apn1) and apn3, as well as a putative ATP binding cassette transporter group G (ABCG) gene in both ACB-AbR and -AcR. Compared with APN sequences of ACB-BtS, there were 9, 5, 10, and 12 amino acid mutations in the deduced protein sequences of Ofapn1, Ofapn2, Ofapn3, and Ofapn4 in ACB-AbR, respectively. In addition, at the non-translatable 3'-end region, a deletion of 11 nucleotides and an insert of 9 nucleotides occurred in the Ofapn2. Furthermore, we observed three mutant alleles in Ofcad gene in Cry1Ac resistant strains. This suggested the reduced susceptibility to Cry1Ab or Cry1Ac strains performance on the expression difference of resistance gene, and mutant as well.

Session 5-T 4 - The evolution of resistance to insecticidal toxins by insect pests of maize

Remediation of resistance to crops expressing *Bt*-toxins

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When transgenic crops are approved for release in the USA by the Environmental Protection Agency (US-EPA), the entities responsible for release must also present plans for the remediation of resistance. These plans must include options for identifying resistance and unexpected damage as well as options for remediation of resistance once it is identified. Unfortunately, the plans that have been proposed and submitted to the US-EPA have never been tested, even in simulation or modeling efforts. The goal of our research has been to test models of remediation and determine the conditions under which such remediation might succeed. It is clear that dispersal will be a key driver in any such model, and we paired research into dispersal of western corn rootworm (*Diabrotica virgifera virgifera*) with modeling and compared those results to models of an insect with higher dispersal rates (*Ostrinia nubilalis*). Direct field estimates of CRW dispersal were made (Joe Spencer) and compared to indirect estimates of migration via genetic differentiation (Tom Sappington) and combined into model parameters. Our modeling strategy simulated a core set of non-compliant fields (fields that did not implement resistance management) surrounded by fields compliant with IRM requirements. The expectation was that resistance would evolve first in the non-compliant fields, and one prominent remediation strategy would be to remove the relevant *Bt*-technologies from those fields and reduce the remaining resistant populations via sprays of conventional chemistry. This strategy could be successful if the resistance alleles were still concentrated in the non-compliant fields. The assumption is that a steep cline in resistance allele frequencies will exist between the non-compliant and compliant fields and treating the resistant allele enriched non-compliant fields will reduce the overall level of resistance. If, however, the cline is less steep, the resistance allele frequencies will be more uniform and there will little advantage to treating hot-spots of resistance. Thus, our goal, in its simplest terms, was to determine the slope of the cline in resistance allele frequencies extending out from the non-compliant core for both CRW and ECB. These results can also be used to estimate the area over which remediation efforts should occurs once a resistance hot-spot has been identified.

Cross resistance assessment of Cry1Ab and Cry1F in *Ostrinia furnacalis* (Guenée)

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Maize event TC1507 (Herculex 1, HX1®) expresses the Cry1F protein from *Bacillus thuringiensis* (*Bt*) for lepidopteran control. Since commercialization in 2003, HX1® has provided growers with an effective pest management tool against European corn borer (ECB), *Ostrinia nubilalis* (Hübner). However, the possibility of ECB adaptation to Cry1F exposure might compromise the utility of HX1® to growers in the future. Maintaining the durability of HX1® in North America initially relied on the deployment of non-traited structured refuge as a resistance management strategy. More recently, the refuge strategy was bolstered by the introduction of pyramiding lepidopteran-active traits providing more refuge options including reduced refuge size for slowing resistance development. The key aspect of the pyramid strategy that allows reduced refuge without compromising durability is that the pyramided traits act by distinct mechanisms or modes of action (MoAs) in killing targeted pest insects. For *Bt* toxins, this means that each active must rely on binding to distinct target sites or "receptors" in the insect gut to achieve toxicity. DuPont Pioneer is interested in promoting the long-term durability of HX1® against *Ostrinia furnacalis* (Guenée) (Asian corn borer; ACB) by pyramiding TC1507 (Cry1F) with MON810 (Cry1Ab). However, the relative MoAs of Cry1F and Cry1Ab have not been evaluated directly for this pest. Here we report data showing that Cry1Ab only partially shares binding sites with Cry1F in ACB midgut tissue which is very similar to the binding site relationship that has been reported for these toxins in ECB. These results support the strategy of prolonging HX1® durability against ACB by pyramiding with MON810.

Laboratory-selected western corn rootworm colonies resistant to *Bt* and non-*Bt* transgenic corn plants

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We used experimental or commercial maize events with high efficacy on western corn rootworm (WCRW) to develop laboratory-selected colonies highly resistant to transgenic corn traits expressing *Bt* (Cry34/35) or non-*Bt* (AfIP-1A/1B) proteins. Evident cross-resistance was detected for the laboratory-selected colonies to transgenic corn plants expressing Cry34/35 and AfIP-1A/1B, suggesting non-*Bt* insecticidal proteins can have same mode of action as *Bt* proteins. The resistant WCRW colonies provided valuable resources for research and development of novel traits without cross-resistance to the current products in commercial cultivation.

Geographical and temporal variability in susceptibility to Cry1Ie toxin in China

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The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), is one of the most important insect pests on maize in China. Transgenic *Bt* maize expressing Cry1Ie toxin from *Bacillus thuringiensis* has been developed in China, providing good levels of control against the primary lepidopteran pests as well as showing a great potential to deal with Cry1Ac resistance insects, such as cotton bollworm, *Helicoverpa armigera*, a major insect pest of cotton and maize. In order to establish the proactive insect resistance management (IRM) programs, it is essential to determine the baseline susceptibility of ACB to Cry1Ie toxin before the widespread implementation of Cry1Ie maize. The bioassays were performed by exposing neonates to the agar-free semi-artificial diet incorporated with Cry1Ie toxin. Thirty four populations of ACB sampled from 13 provinces through 2012 to 2016. The toxicity (LC_{50}) of Cry1Ie toxin ranged from 0.20 to 6.90 ug/g (Cry1Ie protein / diet). And the range of EC_{50} among the populations was 0.06 to 1.54 ug/g. All the tested larvae died at diagnostic concentration (299 ug/g). The results suggest that the field populations are still susceptible to Cry1Ie prior to the widespread deployment of *Bt* corn.

Session 5-T 8 - The evolution of resistance to insecticidal toxins by insect pests of maize

Insect resistance monitoring of *Bt* maize in the EU: results and challenges encountered after 18 years of cultivation

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Genetically modified (GM) maize producing the insecticidal protein Cry1Ab, which confers resistance to the European corn borer (ECB) and the Mediterranean corn borer (MCB) has been cultivated in the European Union (EU) since 1998. In 2015, Spain leaded commercial production of maize MON810 with 107 749 ha.

The possible resistance evolution to Cry1Ab in insect target pests was identified by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) as a concern associated with the cultivation of maize MON810, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects. The EFSA GMO Panel therefore recommended that resistance and compliance monitoring is conducted routinely to allow the periodic evaluation of the adequacy and efficacy of insect resistance management (IRM) strategy, so that early warning signs indicating increases in tolerance in the field are detected; a timely detection of such signs enables actions to limit the spread of field-evolved resistance among populations.

The consent holder follows a two-pronged approach: (i) routine monitoring of ECB and MCB populations from the main Spanish geographical areas where maize MON810 is grown to assess changes in baseline susceptibility to Cry1Ab; and (ii) monitoring symptoms of unexpected field damage caused by ECB and/or MCB through a farmer alert system.

From 2005 onwards, the consent holder has submitted to the European Commission (EC) the results of resistance monitoring activities as part of the annual post-market environmental monitoring reports on the cultivation of maize MON810.

The results of the laboratory bioassays did not indicate a decrease in baseline susceptibility to Cry1Ab in the tested target pests from the field populations monitored in the different geographical areas. The EFSA GMO Panel has also provided several recommendations on the methodology followed for the monitoring activities, specifically, setting a maximum detection level of 3% of resistance allele frequency in ECB and MCB populations; focusing the sampling effort exclusively in those areas where field resistance is more likely to develop; and performing annual monitoring of ECB and MCB populations when adoption rate of maize MON810 is > 60%.

Control of *Ostrinia furnacalis* by *Bt* corn MON89034 and its IRM considerations in Asia

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Asian corn borer, *Ostrinia furnacalis*, is a primary corn insect pest in most of the Asian countries. *Ostrinia furnacalis* can cause significant feeding damage to corn plants at vegetative and productive stages. Small-holder farmers in most of the Asian countries suffered great economic impact because of *O. furnacalis* damage. *Bt* corn MON89034 expresses two *Bt* proteins: Cry1A.105 and Cry2Ab2. MON89034 baseline susceptibility and field efficacy data on *O. furnacalis* were collected in several Asian countries. The lab and field results indicate that MON89034 provides excellent control of *O. furnacalis*. Insect resistance management risks, such as efficacy level, larval movement, ovipositional behavior, of *O. furnacalis* to MON89034 were studied. Considerations and challenges for implementing effective IRM programs for *Bt* corn in small-holder farming systems are also discussed

Invited Speaker

RNA-based biocontrol for use in pest management

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RNA-based biocontrols provide a new mode of action based on the naturally-occuring process of RNA interference (RNAi) and can offer advantages in flexibility for pest control over traditional technologies. Because nucleotide sequence complementarity is a determining factor, RNA-based biocontrols can be designed to be selective for target pests whilst minimizing the potential for effects in non-target organisms. RNA-based biocontrols selective for Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*) have been tested over several seasons in field trials at multiple locations. The results demonstrate that the RNA-based biocontrol is as effective at protecting potato crops as a class-leading chemical pesticide. The potential for RNA-based biocontrol solutions to overcome resistance to conventional pesticides, provide low environmental impact and good crop tolerance, has also been evaluated. These results clearly validate RNA-based biocontrols ad effective solutions for crop protection. Corn rootworms (CRW) are devastating corn pests and are responsible for significant crop loss each year. Soil-applied RNA-based biocontrols are being developed for effective control of and the potential for high selectivity of CRW. Applying RNA-based biocontrol directly to soil leads to mortality of emerging Western CRW (*Diabrotica virgifera virgifera*) larvae and protects corn plantlets from root damage. This finding opens new possibilities to control CRW more broadly than current control methods. A particular challenge for the CRW project is to have sustained activity of the active RNAi ingredient (double stranded RNA) in the soil environment. Stink bug species are economically important pests across various crop systems and geographies. Applying sprayed, formulated RNA-based biocontrol protects 2 weeks old soybean plants against damage by Southern green stink bugs (*Nezara viridula*). Syngenta is developing these RNA-based biocontrols into commercially viable products, with new modes of action to support growers in their integrated pest management programs.

Session 6-T 1 - Migratory pests of corn

Evidence for migratory behavior of western corn rootworm

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is the most important pest of corn in North America and has invaded much of Europe. In the U.S., it is a major target of transgenic *Bt* and RNAi corn, but evolution of resistance to protective traits threatens product efficacy. Dispersal determines gene flow, and gene flow influences the rate of resistance evolution and the rate of its spread. There are multiple lines of evidence that long-distance flight by WCR occurs, especially of mated, gravid females, and is not rare. Evidence includes rate of range expansion and jumping ahead of the invasion front, high elevation flight, and tethered flight experiments. We are using genetic markers to estimate distances and frequency of long-distance dispersal by WCR. Preliminary results, along with older tethered flight data, suggest that most WCR adults reproduce very close to the natal site, within ca. 260 m. However, a small but significant portion, ca. 10%, disperse much longer distances, up to at least 275 km. Such a bimodal distribution of flight behavior would help explain many otherwise puzzling observations in the field.

Session 6-T 2 - Migratory pests of corn

Migratory immunity: density-dependent prophylaxis, immune response and host flight behavior manipulation in *Mythimna separata*

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The oriental armyworm, *Mythimna separata*, a long distance migratory insect pest undertakes a seasonal, gregarious and multigenerational roundtrip migration between southern and northern China annually. In the field the immigration population lay eggs in a short time and their gregarious offspring larvae shows varying degree of cuticle melanization, all the melanic larvae are not sensitive to the biological pesticide as well. Here, we identified whether density-dependent melanization and density-dependent prophylaxis existed in the species, how the host larvae defensed to the parasite and disease infection, and whether the host flight behavior changed as result to successful defense. Our results indicated that larval cuticle melanism of oplical density increased significantly as increasing of larval density, and the melanic larvae showed a stronger resistance to the challenge of a parasite, *Exorista civilis* and an entomopathogenic fungus *Metarrhizium anisopliae*. Increase of haemocyte number especially immunocyte and antibacterial activity in the haemolymph, combined with the increase of phenoloxidase levels in different organization were responsible for the enhancement of immune defense capacity in high larval density. Adults emerged from the larvae successfully defend to the infection of *M. anisopliae* showed significantly stronger flight capacity than those of no disease challenge.

Session 6-T 3 - Migratory pests of corn

Migration patterns of armyworm moths in China

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Insect migration process and patterns would change dramatically year by year, that has significant impact and ecological consequence on population processes. Case studies on migration process of armyworm moths based on the light-trap catches, field survey and numerical simulation were shown to reveal the influence of food resources allocation and meteorological backgrounds on the prominent annual fluctuation of the immigration events in China. Our results indicate that the extremely variable monsoon and the special synoptic weather systems and processes are the determinant shaping the moths' pathways and destination, by which, armyworm moths exhibit more complex seasonal migration patterns and intermittent population outbreaks distributed from western China to southeast Japan, and the obvious pied piper effect in north-eastern China. Conclusion: the migration patterns of armyworm moths will be governed by the synoptic weather on a continental scale.

Session 6-T 4 - Migratory pests of corn

The *Ostrinia* trap which catches: comparing the performance of a new bisex corn borer lure with that of synthetic sex pheromone

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The sex pheromone traps for the European corn borer *Ostrinia nubilalis* are notoriously problematic. In a European survey to assess the performance of traps baited with the synthetic pheromone, in 10 countries out of 13 the trap was reported to be not satisfactory (Szöcs and Babendreier, 2011). In has been known that phenylacetaldehyde also attracts both sexes of *O. nubilalis* (Maini and Burgio, 1990; Burgio and Maini, 1994). However, experience showed that the activity of this compound is also low and usually is not satisfactory for practical applications. Recently we discovered a synergist which, when added to phenylacetaldehyde, increased catches by 3-5-fold, and thus the development of a practically usable trap lure for *O. nubilalis* was possible (Tóth et al., 2016). The present research was undertaken to compare the performance of this new bisex *Ostrinia* lure with that of synthetic sex pheromone in 5 countries in Europe and the Middle East. In Hungary, Italy, Bulgaria and Turkey (sites where the *Z* pheromone strain predominantly occurs), the catches of the bisex lure highly exceeded those of the synthetic *Z* strain pheromone, which caught virtually nothing. A high percentage of moths caught by the bisex lure were females. In Slovenia, at sites where the *E* strain is present, there were also good catches in traps with the bisex lure, but these were not significantly higher than those in traps baited with the synthetic *E* strain pheromone. It is noteworthy that at one Slovenian site the experiment was conducted in a hop field (while in all other places tests were done in maize fields). In the future, a more detailed study on populations from Slovenia will be necessary. In conclusion, conventional funnel traps baited with the new bisex lure give the first practical opportunity to farmers to conduct successful detection and monitoring trapping of the European corn borer in Europe and neighbouring areas.

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Session 6-T 5 - Migratory pests of corn

Changes in corn rootworm wing morphology related to development of pest resistance

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The wing morphology of 358 western corn rootworm, *Diabrotica virgifera virgifera* LeConte, individuals from populations in Iowa (n=3 populations), Indiana (n=4) and Illinois (n=2), were investigated using standard geometric morphometric procedures based on the hind wing venation of 14 landmarks. The populations investigated comprised resistant (i.e. rotation and *Bt* maize strains) and non-resistant populations; data analysis was divided into two groups: 1) resistant versus non-resistant populations; and 2) rotation versus *Bt* maize strains. The results clearly demonstrated a morphological difference in resistant versus non-resistant populations and rotation versus *Bt* maize resistant strains of corn rootworm. This study further confirms the preliminary results of Mikac *et al*. (2013) who demonstrated evidence of wing shape and size differences in corn rootworm from rotated versus continuous maize in Nebraska, Missouri, Iowa and Illinois. The results of this study further show the utility of hind wing morphology as an inexpensive and accessible population biomarker for corn rootworm. With simple scientific equipment (camera mounted microscopes) and readily available free-software to capture and analyse land mark (shape and size) data, it is possible to cheaply monitor the pest resistance development and differences in rootworm populations.

Invited Keynote Presentation

Spread of fall armyworm in West Africa: need for an emergency response plan

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The *Spodoptera frugiperda* commonly known as the fall armyworm in the Americas has invaded Africa. There are two separate haplotypes currently known from samples taken from West Africa. The effect was greatly felt in 2016 although some have a strong conviction that it has been around since 2015 but was not properly diagnosed as others took it as a stem borer. The pest was almost simultaneous sighted across West Africa; from Nigeria in a western direction through the littoral countries and Burkina Faso from January – October 2016. The island of Sao Tome and Principe was also affected. Crops grown under irrigation during the dry season from November 2016 to March 2017 were also attacked showing that the pest, although listed as migratory, is exhibiting the tendency to stay in this belt for long. The pest also went through Central and East Africa in the southern direction towards the end of the year 2016 into early 2017. It has been found predominantly on maize and rice but also on cowpea and onions. There have been reports of it on some broad-leaf weeds in areas where cereals are not yet on the fields. Ghana has a little over 1.0 million hectares cultivated annually under maize since 2011. In the major season of 2016 alone, over 10000 ha were reported to be under attack constituting about 1% of the total crop area. This translates into about a minimum loss of 20000 MT in that season alone. In the northern part of the country and also in Burkina Faso where seed maize is grown under irrigation in the dry season, the attack observed pose a threat to the seed stock for the 2017 growing season. At this rate, we expect a more serious crisis at the end of 2017 if immediate short-term and long-term interventions to keep the pest population under a safe threshold are not put in place. When the pest was noticed, farmers used repeated chemical spraying to control it in the field but most could not bring it under control and abandoned their crops. The excessive use of chemicals is a recipe for the development of resistance by the pest and this must be curbed. A systematic introduction of biocontrol options in the integrated control strategies is desirable. Some trials have started, using *Bacillus thuringiensis* products on maize fields to see if that will be of help. However, to deploy existing and potential management options effectively, the following have to be in place: early detection techniques, identification of all other strains or species probably with different biological characteristics as farmers' observations suggest; knowledge on pathways of spread; complete catalogue of host plant range in this new habitat; and the in-country and sub-regional structures to deploy and monitor the management strategies.

Session 7-T 1 - Regional suppression of lepidopteran pests by *Bt* corn

Efficacy and IRM studies of *Bt* corn events on the Asian corn borer in China

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Lepidopteran insects, such as the Asian corn borer (ACB), *Ostrinia furnacalis*, the oriental armyworm (OAW), *Mythimna separata*, and cotton bollworm (CBW), *Helicoverpa armigera*, are important pests of maize in China. *Bt* maize targeting these lepidopteran insects has been studied and developed in a couple of universities, academic institutions, as well as private sectors. *Bt* hybrids expressing Cry1Ab/2Aj (Shuangkang12-5), Cry1Ab (C0030.3.5), and Cry1Ah (GH5112E-117C) toxins, respectively, and their non-*Bt* isohybrids were evaluated for control these corn borers. The maize hybrids were planted in late spring and earlier summer represented spring-maize and summer-maize, in Beijing. Plants were infested artificially with 1st instar larvae of OAB at V6-V8 stage, CBW at silk stage, and ACB at both V6-V8 and silk stages. Meanwhile, fresh whorl leaves and silks were sampled in the same plots, which were used to do tissue bioassay with neonates of these corn borers. In addition, a line producing Cry1Ac (BT38) and a line producing Cry1Ie (IE034), as well as their non-*Bt* isolines were used to producing a hybrid with pyramided two genes and non-*Bt* isohybrid, which were evaluated with tissue bioassays using susceptible and resistant ACB strains for potential of insect resistance management for ACB. Hybrids of events Shuangkang12-5 and C0030.3.5 provided good control of ACB, OAW, and CBW. Event GH5112E-117C hybrid provided good control of ACB and CEB, but did not provided good control of OAW. Both molecular stack (Shuangkang12-5) and breading stack hybrid expressing Cry1Ac and Cry1Ie toxins showed a higher efficacy against resistant ACB larvae than hybrid only expressing either Cry1Ac or Cry1Ie toxin, which would have increased potential for managing or delaying the evolution of ACB resistance to *Bt* maize plants.

Regional suppression of *Ostrinia nubilalis* due to *Bt* corn and its effects on other crops in the mid-Atlantic U.S.

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There is strong evidence of major declines in European corn borer (ECB) populations with the increase in *Bt* corn adoption in the corn belt. One study published in Science by Hutchison et al (2010) was the first to report an economic analysis of this reduction, showing benefits to farmers planting non-*Bt* refuge corn. Other studies from cotton growing areas have reported declines in pink bollworm, cotton bollworm and cotton budworm populations where *Bt* cotton adoption is high. Corn is the primary reproductive host plant of ECB in the mid-Atlantic U.S. and the major source of moths colonizing several vegetable crops, causing yield losses and contamination problems. *Bt* corn hybrids were commercially introduced in 1997 and adoption of the technology by 2013 ranged from 57% to 99% of the corn acreage across the mid-Atlantic region. Since these hybrids provide 100% control of ECB, population recruitment has been significantly reduced.

Results reported here show significant declines in ECB infestations on green bean, peppers, and sweet corn crops and major changes in insecticide control recommendations, using databases of moth activity from light trap networks and naturally occurring field infestation levels during pre- and post-periods of *Bt* corn adoption. Moth activity in light traps significantly dropped 72% in Maryland, 77% in Delaware, and 56% in New Jersey, since the introduction of the *Bt* corn technology. For pepper, the percentage of damaged fruit averaged 35.4 % and 21.8 % during the pre- and post-periods, respectively, which amounted to a 38.5 % reduction in field populations. For sweet corn, the percentage of damaged ears averaged 59.2% and 23.0% during the pre- and post-periods, respectively, which amounted to a 61.1% reduction in field populations. Using existing treatment thresholds based on moth activity data, the probability of recommended control actions declined by 87% for green beans, from an average of 2.6 applications per acre during the pre-*Bt* period compared to 1.7 applications per acre during 1997-2006 and 0.3 applications per acre during 2007-2014. Similarly, recommended control actions on pepper averaged 4.5 applications per acre during the pre-*Bt* period, compared to 2.4 applications during 1997-2006 and 0.1 applications during 2007-2014. This represents a significant 98% reduction in recommended control actions as a result of the regional suppression of ECB by *Bt* corn. These results provide the first evidence that the *Bt* corn technology has led to changes in European corn borer control practices in other crops, resulting in potential economic and environmental benefits.

Effects of *Bt* in the landscape on *Ostrinia nubilalis* in New York processing snap bean

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Ostrinia nubilalis is a major pest of snap bean in the Great Lakes region of the United States. Because larvae are considered contaminants in pods, the tolerance for this pest is very low. Therefore, even though incidence of *O. nubilalis* contaminated snap beans in New York has become nearly zero, growers continue to apply insecticides proactively for control. The number of pesticide applications made per season is partially determined by *O. nubilalis* pheromone trap catch in nearby sweet corn fields, reported by the New York State Integrated Pest Management Program. One potential cause of decreased *O. nubilalis* contamination is the widespread adoption of *Bt* field corn, which can suppress *O. nubilalis* populations. Snap bean fields located where *Bt* field corn has been intensively grown in time and space may have reduced risk for *O. nubilalis* infestation, which if identified, could allow growers to reduce insecticide applications. Our goal was to determine if corn planting intensity or *O. nubilalis* trap catch in sweet corn could be used to predict *O. nubilalis* populations in snap bean in New York (2014-2015). Numbers of *O. nubilalis* adults captured in snap bean fields in “high” and “low” corn intensity areas were not different; this suggests that *O. nubilalis* does not respond to local levels of *Bt* corn in the landscape. The widespread reduction in *O. nubilalis* (likely caused by *Bt* adoption) may mask local suppression effects. There was also no relationship between *O. nubilalis* trap catch in snap bean and nearby sweet corn traps. The sweet corn traps caught 8× (2014) and 26× (2015) more moths than snap bean traps. Therefore, snap bean growers should not use sweet corn trap catch as an indicator of risk to snap bean as this method will cause overestimation of risk. Overall, the study indicates that risk of *O. nubilalis* infestation in snap bean is very low, as ~80% of traps caught zero moths. Growers can reduce or eliminate pesticide applications that target this pest.

Session 8-T 1 - New research in rootworm basic biology and potential applications

Novel protein MOA for CRW control

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One of the most devastating pests of corn in the United States is the corn rootworm. Rootworm larvae feeding upon the roots inhibit the corn plant's ability to take up water and nutrients, decrease its ability to develop and remain upright, and ultimately cause yield loss, depending on the amount of damage inflicted on the roots by the feeding pests and the growing conditions. Since 2003, transgenic corn plants expressing different *Bt* proteins toxic to corn rootworm have become the preferred agronomic approach for corn rootworm control in the US. This presentation will introduce a novel insecticidal protein toxic to corn rootworm having a unique mode of action (MOA) compared to toxin proteins currently in use in transgenic plants for next generation below-ground trait.

Session 8-T 2 - New research in rootworm basic biology and potential applications

The invasion of Europe by western corn rootworm results in changes to wing morphology

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An analysis of the wing morphology (size and shape) within and among western corn rootworm, *Diabrotica virgifera virgifera* LeConte, populations over a large geographic scale in Europe was conducted. For this study 500 *D. v. virgifera* were collected over an area of 160, 000 km^2 of agricultural corn production and analysed using standard geometric morphometric procedures based on the hind wing venation of 14 landmarks. The data presented here represents a major morphological investigation of one invasive species, during the introductory phase of their invasion of Europe. The results showed phenotypic changes across a major geographic corridor of invasion by this pest into corn growing areas in Europe. Both principal components analysis and the two distance measures (Procrustes Distances and Mahalanobis values that represent multivariate shape differences) used to analyse the data showed that hind wing shape and size could be reliably used, as there were discernible patterns in the data related to the invasion process of *D. v. virgifera*. Also, these morphological patterns were found to correspond to the findings from previously conducted population genetic studies of the same populations. Findings overall showed that population differences found using microsatellite markers could also be detected at the same geographic locations using morphometric techniques. The results of this study have important implications for invasive species monitoring and management, particularly where genetic capabilities and monetary investment in such techniques maybe not be feasible. This study presents an affordable and accessible genetics-alternative technique that reliably demonstrates that hind-wing shape and size differences can be used to reveal populations differences and thus can be used as a population biomarker when investigating biological invasions.

Session 8-T 3 - New research in rootworm basic biology and potential applications

Emerging technologies for genetic manipulation of western corn rootworm

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The western corn rootworm (WCR) is a major pest of maize and is notorious for rapidly adapting biochemically, behaviorally, and developmentally to a variety of control methods. Since transformation-based applications, such as transposon tagging, enhancer trapping, and genome-wide mutagenesis, have facilitated the genetic dissection of model species like *Drosophila melanogaster*, we have followed this paradigm and developed a germline transformation system for WCR. We have demonstrated that both *piggyBac*- and *Minos*-based transformation systems work well in WCR. However, unlike *Drosophila*, WCR lacks eye-color mutants conventionally used to observe transgene expression. To overcome this obstacle we used the CRISPR/Cas9 system to create a white-eyed mutant strain that greatly enhances the ability to screen for eye-specific expression of fluorescent markers. We are also in the process of creating a system that would enable genome-wide mutagenesis of the WCR genome by simply crossing two strains. We have already produced the first half of the system, the “helper” strain. These beetles express *piggyBac transposase*, so are expected to remobilize marked *piggyBac* elements within “donor” strains. Our progress towards developing tools, techniques and protocols for WCR functional genomics will be presented, along with some of the challenges we had to overcome along the way. Taken together, this combination of transposon- and CRISPR-based technologies is expected to bring a wide-range of transformation-based tools to bear on understanding WCR biology.

Session 8-T 4 - New research in rootworm basic biology and potential applications

Improved artificial diet for western corn rootworm assays

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Advances in management of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, are hindered by the lack of an artificial diet for larval rearing. In addition, seed companies use differing proprietary diets, so direct comparison of diet assay data evaluating commercial toxins originally from *Bacillus thuriengensis* (*Bt*) cannot be made. Our group has developed an improved diet for western corn rootworm larvae and have been able to test toxicity of all four current *Bt* toxins which have been transformed into maize and are commercially available for western corn rootworm management. Diet contamination has been an ongoing problem with western corn rootworm larval artificial diets, but we have also nearly eliminated diet contamination for these 10 day assays. Finally, we have been able to rear the western corn rootworm all the way to beetle emergence, but our diet is still vastly inferior to maize roots. We will continue to refine the diet. We have a long-term goal of being equal to corn roots in terms of developmental time and reproductive success. If we reach this goal, we believe a greater understanding of western corn rootworm nutrition will lead to additional management options and to better methods for evaluating toxins. Research progress toward this long-term goal will be discussed.

Contributions of recovery and repair genes and microbiomes to *Diabrotica* ssp resistance management

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Bt toxin binding to the midgut epithelial cells breaches that vital barrier, resulting in fatal septicemia. Avoiding or mitigating these acute effects of *Bt* intoxication may require a defense-in-depth, including host microbiomes, tissue repairs, and destruction of invading microbes. To identify genes involved in this process we used RNA-Seq to analyze gene expression during acute exposure and recovery from *Bt*-intoxication. Resistant and susceptible western corn rootworm (WCR) neonates were allowed to feed on *Bt* and isoline maize seedlings for 12 and 24 hours. Whole and midgut de novo transcriptomes were assembled using Trinity, and differential gene expression was examined using EdgeR. Results from the assemblies and annotation indicate that WCR neonates from the resistant colony express a small, specific suite of up and downregulated genes following *Bt* intoxication. By contrast susceptible WCR neonates express a very wide range of transcripts in response to intoxication. We here report our findings on the transcripts most closely associated with early, acute responses in resistant larvae.

Session 9-T 1 - New generation traits for management of maize pests

Novel insecticidal proteins from plants

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Many lepidopteran insect species are economically important pests of crops worldwide. The loss can be in billions of US dollars annually due to reduced crop productivity and increased pest management costs. Transgenic crops developed with *Bacillus thuringiensis* (*Bt*) insecticidal protein encoding genes have been widely adopted and they offer benefits to both farmers and the environment by increasing productivity and reducing reliance on conventional pesticides. However, rapid and wide spread adoption of these transgenic crops has increased the risk of evolution of pest resistance to *Bt* proteins and in certain instances it has resulted in reduced effectiveness. We report here the discovery of a new family of potent insecticidal proteins from plant species. These proteins show activity against lepidopteran insect pests including corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), soybean looper (*Chrysodeixis includens*) and velvetbean caterpillar (*Anticarsia gemmatalis*). Transgenic soybean (*Glycine max* L. Merill) and corn (*Zea mays* L.) plants expressing those proteins show protection from feeding damage by these insect pests. This family of novel proteins is useful for developing new transgenic crops for lepidopteran pest control.

Session 9-T 2 - New generation traits for management of maize pests

New technologies for corn rootworm control

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Western corn rootworm (WCR), one of the most devastating corn insect pests in the United States, has the potential to cost farmers as much as $2 billion yield loss annually if not controlled. Transgenic traits based on proteins from *Bt* (*Bacillus thuringiensis*) have been a key tool for controlling this pest over the last decade and efforts to prolong their durability have included refuge deployment, pyramiding of traits, and emphasis on integrated pest management. Nevertheless, reports of WCR resistance to *Bt* corn have heightened the urgency for developing new traits that will sustain the technology. Thus, DuPont Pioneer has worked to identify new sources of corn rootworm actives including screening non-*Bt* microbes for proteins, and RNA interference (RNAi) based WCR gene targets that can be developed into effective traits. These efforts have identified a protein from *Pseudomonas chloraphis* that is highly efficient in preventing root damage caused by WCR when expressed in transgenic plants. This protein represents a novel mode of action against WCR compared to current commercial traits and highlights the potential of non-*Bt* sources for future insect control traits. Based on RNAi technology, transgenic plants expressing DvSSJ1 dsRNA provided significant plant protection against WCR, by suppressing production of smooth septate junction (SSJ) membrane protein located within WCR intestinal lining, leading to growth inhibition and mortality.

Session 9-T 3 - New generation traits for management of maize pests

In-plant protection against *Helicoverpa armigera* by production of long hpRNA in chloroplasts

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Helicoverpa armigera is a major insect pest for a wide range of agricultural crops including cotton, soybean, and maize. *H. armigera* larvae cause economic loss due to feeding damage, especially to the reproductive parts of host plants. In addition to chemical insecticides, transgenic crops such as *Bt* cotton are highly effective in controlling *H. armigera*. However, field derived Cry1Ac, Cry2Ab, and Vip3A resistance alleles signal a threat to *Bt* crop technology for *H. armigera* and other important crop pests. Novel technologies to control *H. armigera*, as well as other lepidopteran insect pests, are needed to counter the threat of selection for resistant insect populations in the field. Transgenic RNAi, where double stranded RNA is expressed to silence essential genes in insect pests, is an emerging technology for crop protection, with a mode of action that is not cross resistant to *Bt* crops. However, despite the successful development of RNAi for Coleopteran pests such as corn rootworm, development of transgenic RNAi for Lepidoptera has yet to achieve commercial levels of insect control. We hypothesized that the dicing of dsRNA by plant RNAi RISC complexes might be one reason for the low-level pesticidal efficacy of RNAi in Lepidoptera. In this study, we expressed dsRNA specific for *H. armigera* acetylcholinesterase (ACE) in the chloroplast genome of *Nicotiana benthamiana.* Because chloroplasts lack RNAi machinery, the chloroplast-expressed dsRNA were not processed into siRNA in transplastomic events, resulting in protection against *H. armigera* feeding damage. This correlated with larval growth inhibition and knock down of ACE activity. These findings suggest that chloroplast-expressed dsRNA can provide protection against feeding damage by lepidopteran insect pests.

Controlling CRW: SmartStax PRO and beyond

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Corn rootworm is one of the most devastating maize pests in North America, as below-ground damages caused by larvae feeding on the roots can lead to significant yield loss. Since 2003, transgenic corn plants expressing different *Bt* proteins toxic to the insect pest have become the preferred agronomic approach to protect against corn rootworm in the US. This presentation will discuss SmartStax PRO and future generation traits providing new modes of action for corn rootworm control.

Session 10-T 1 - Biological control of maize pests

Survey of natural enemies for North American maize pest *Striacosta albicosta*

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Western bean cutworm (WBC) *Striacosta albicosta* (Lepidoptera: Noctuidae) is a major pest of maize (*Zea mays*) and dry beans (*Phaseolus vulgaris*) in the United States. Between 1999 and 2010 western bean cutworm range expanded into parts of southern Canada and the eastern United States. Current management strategies for *S. albicosta* focus on insecticides and *Bt* maize hybrids with Cry1F and/or VIP3A proteins. There is decreased efficacy for some WBC management tactics; for example, populations have shown a 5.2-fold decrease in susceptibility to Cry1F proteins. Some counties in Nebraska have reported of decreased efficacy of pyrethroid (i.e. bifenthrin) insecticides. The decreased efficacy of some management tactics has created a need for new tactics. Biological control may be important for the management of *S. albicosta*. To identify natural enemies of WBC, predator surveys were conducted in maize fields in western Nebraska during the 2015 and 2016 field season. The predators were keyed to family or species and their DNA was extracted. A species-specific PCR primer was developed for molecular gut content analysis to determine the frequency of predation on *S. albicosta*. The most abundant predators in the fields were Coccinellidae species *Hippodamia convergens* and *Coleomegilla maculata*, as well as *Orius insidiosus* (Hemiptera: Anthocoridae) and Chrysopidae (Neuroptera) larvae. A feeding comparison of *H. convergens* and *C. maculata* showed a statistically significant difference (P-value <.001) for both the frequency of predation and the average number of prey eaten. For *H. convergens*, 45% of specimens fed on eggs, 18% fed on larvae, and the average egg consumption was 12 in 2 hours. For *C. maculata*, 82% fed on eggs, 87% fed on larvae and the average egg consumption was 17 in 2 hours. The literature shows that maize pollen consumption may decrease egg consumption. An alternate food source study tested how *C. maculata* responds to WBC eggs with maize pollen as an alternate food source. We found that maize pollen did not statistically decrease the number of eggs eaten (P-value= .188) and that time exposed to eggs increases the number of eggs consumed. It is possible that *C. maculata* is a viable candidate for the biological control of *S. albicosta* eggs and that biological control can be an important part of *S. albicosta* management.

Session 10-T 2 - Biological control of maize pests

Review of biological control for corn insect pests in China

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Corn (*Zea mays* L., Poaceae) is ranked first as food crop in planting area and in total yield production in China. Total plant acreage of corn was 37.94 million hectares with total of 226 million of yield in 2015. Insect pests are one of the key limiting factors in the corn production, especially, the Asian corn borer, *Ostrinia furnacalis* (Guenée), the most serious insect pest of corn, which occurs in most corn-growing areas in China, with annual loss due to this insect ranges from 6 to 9 million tons. Other major lepidopteran pests of concern for corn in China include *Helicoverpa armigera* (Hübner), *Mythimna separate* (Walker), *Conogethes punctiferalis* (Guenée). The direct yield losses arise from these insect pest damage, whereby the largest impact indirectly arise from larval feeding on silks and kernels that leads to ear rot, increasing mycotoxin contamination and reducing grain quality. *Trichogramma* wasps have been released for control of Asian corn borer in China since 1970's. Microbial pesticides, such as *Beauveria bassiana* and *Bacillus thuringiensis* (*Bt*) have been also used for control these lepidopteran insect pests of corn in China. The release of *Trichogramma* and application of *B. bassiana* and *Bt* for controlling Asian corn borer and other lepidopteran pests became one of the key measures in corn IPM in China with total of 14.17, 3.58 and 3.77 million ha, respectively, between the years of 2011 and 2015 in China. It would be a new opportunity for biological control of corn insect pests as the Action for decrease of chemical fertilizer and chemical pesticide in China.

Session 10-T 3 - Biological control of maize pests

The combined application of beneficial microorganisms to control the western corn rootworm

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Maize plants (*Zea mays* L.) suffer from root herbivory by larvae of the Western corn rootworm (WCR), *Diabrotica virgifera virgifera*, which is the most important pest of maize in the North America and in several countries of Central and Eastern Europe. Larvae are the most damaging stage of this pest. They feed on root hairs and cortical tissue and tunnel inside roots, leading to pruning and destruction of root sections of the plant. As part of a research consortium that explores how soil health can be improved by applying ecological and rational approaches (NRP68: Biology), we study how different beneficial organisms (entomopathogenic nematodes -EPNs-, *Pseudomonas* bacteria and arbuscular mycorrhizal fungi –AMF-) can be better exploited to control WCR. We hypothesized that the application of beneficial soil organism will contribute to improve maize growth and limit the damage caused by WCR larvae. We also expected that the combined application (nematodes, bacteria and fungi) will have a stronger positive effect than their individual applications. In a field trial, we recovered three-fold fewer WCR larvae from plots inoculated with *Pseudomonas* bacteria than from control plots. Moreover, the recovered larvae were considerably smaller. On the other hand, root damage was lowest in the presence of EPNs. Overall, *Pseudomona* bacteria had a strong negative impact on WCR larvae, whereas EPNs had a positive impact on root health. But, we did not find any clear synergistic effect of the combination of the different microorganisms. Nonetheless, as the different microorganisms tested in this study had distinctly different positive effects, the results indicate that applying combinations of beneficial microorganisms can enhance crop protection.

Session 10-T 4 - Biological control of maize pests

Assessment of soil entomopathogens from maize fields of western Nebraska, USA: can they be useful for western corn rootworm control?

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In west central Nebraska, USA, continuous maize is a common agronomic practice and for that reason, the western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is the state's biggest soil pest. Resistance issues to transgenics and insecticides are present in areas of the state and other areas of the Corn Belt, limiting the control options for this pest. Biological control is an under-explored option for the management of WCR in the United States. Our goal is to identify key entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) from irrigated commercial maize fields that can be incorporated as biological control agents as a part of integrated pest management programs. We surveyed five maize fields in the growing seasons of 2014 and 2015 to document the complex of EPF and EPN species. In the laboratory, we isolated EPF and EPN species from soil samples using a baiting technique with *Galleria mellonella* and *Tenebrio molitor*. EPF were detected in every field site, with the most prevalent genus being *Metarhizium*.In the laboratory, pathogenicity assays are being conducted to determine if the isolated strains of *Metarhizium*, *Beauvaria* and other entomogenous fungiare efficacious against WCR larvae. Describing the natural enemy community from WCR-infested fields is the first step for the implementation of a biological control program. EPF and EPN community results will be discussed in the context of their potential efficacy as biological control agents for the management of this devastating pest.

Mass production of *Trichogramma* using eggs of *Antheraea pernyi* and field application in the suppression of corn borers in Jilin Province, China

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The Asian corn borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Pyralidae), is the most important agricultural insect pest in corn crops in north-eastern China. Without any control measures, this pest can cause 10% grain damage in this region. *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae), one of the dominant native egg parasitoids in the locality, exhibits good biocontrol potential against the corn borers. Since the 1980s, *T. dendrolimi* has been mass-produced using the large eggs of the Chinese oak silkworm, *Antheraea pernyi* Guérin-Méneville (Lepidoptera: Saturniidae) as a factitious host and released on a large scale in Jilin Province, China. In the last decade, the production process of *Trichogramma* using the large silkworm eggs has been greatly improved in terms of mechanization. Several devices have been developed for harvesting host eggs from female abdomens, drying the host eggs, separating parasitized and unparasitized eggs, and packaging parasitized eggs. Also, a diapause technique has been developed to rationalize the mass production of *T. dendrolimi*. It allows to extend storage time to 90 days from the original 30 days, while safeguarding the quality of the stored *Trichogramma* with an over 95% of diapause rate, diapause termination rate and emergence rate of parasitized eggs. The cumulative area covered by releases of such diapause *T. dendrolimi* asps from 2011 to 2016 was approximately 1,100,000 hectares. Since 2004, the total release area with *Trichogramma* for the control of the corn borer has increased up to 20 million hectares. The total financial contribution towards this program so far (including 2014) is about US$ 95million, which is almost entirely supported by the government. Since 2012, the release area with *Trichogramma* reared on *A. pernyi* eggs comprised 2.3 million hectares annually, covering approximately 60% of the cultivation area of corn in Jilin Province. The total number of parasitoids generally released per hectare is approximately 450,000, split into two batches of 225,000 wasps. The approximate cost per hectare is US$ 5.8. With the inundative release of *T. dendrolimi*, the percentage parasitism of *O. furnacalis* egg masses in the field exceeded 70% for each year of 2004-2016. Overall, it can be concluded that good success has been achieved in the biological control of corn borers in China using *T. dendrolimi,* based on what is probably one of the largest mass production and release programs worldwide.

ABSTRACTS
POSTER
PRESENTATIONS

Session 1-P 1 - Ecology of maize pests and implications for their sustainable management

Effect of plant age and species on population growth of six *Rhopalosiphum maidis* (Fitch) lineages

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Corn leaf aphid *Rhopalosiphum maidis* (Fitch) can feed on various cereal crops and transmit viruses that may cause serious economic losses. To test the impact of host plant species and age on *R. maidis*, we identified the survival and reproduction speed of six *R. maidis* populations (MS, GZ, DY, LF, BJ, HF) on 10 cm maize seedling, 50 cm maize seedling and 10 cm barley seedling via direct observation method for seven days in laboratory. Results showed that all the populations performed the best on barley seedling, but all of them could not survive on 10 cm maize seedling. All six populations can survive on 50 cm maize seedlings, however, the population growth speed was significantly slower than on barley seedlings especially from 4th day to 7th day. BJ population had the best performance both on 50 cm maize and barley seedling and the mean aphid numbers reached up to 57 and 134 on 7th day respectively, while the mean number were 18 and 78 of MS population. Our study revealed that 10 cm maize seedling is fatal to corn leaf aphid which may due to secondary metabolite synthesized by maize seedlings.

Session 1-P 2 - Ecology of maize pests and implications for their sustainable management

A potential application of an intercropping system for pest control: interference with Asian corn borer orientation and mating behavior

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The Asian corn borer (ACB, *Ostrinia furnacalis*) is one of the most serious insect pests of maize (*Zea mays*). Intercropping maize with various other crops is a practical adaptation to control pests. Sugarcane (*Saccharum officinarum*) is one of the successful partner plants used for intercropping with maize in Southwest of China. However, this maize and sugarcane intercropping system cannot spread to other temperate zones, because sugarcane cannot grow well except in tropical or sub-tropical areas. A better understanding of the principles of selecting proper partner plants in an intercropping system may of most importance, Thus, the present study examines the electrophysiological responses of both male and female Asian corn borers to different concentrations of extracts from sugarcane, maize or intercropping system of sugarcane and maize, and how these partner extracts interfere with the orientation and mating behavior of this pest. The results showed that the ACB moth could detect the difference between sugarcane and maize volatiles via physiological and behavioral results. Interestingly, the intercropping of sugarcane and maize still easily disrupts the orientation of both male and female ACB moths. Our results also indicated that the ACB moths do not specifically choose a special host plant field as mating area. These results indicate the similarity of volatiles between partner plants and host plants may interfere with insect pest behavior (such as orientation, mating, oviposition) to increase the yield of crops. More intercropping systems between crop-crop combination, or crop-weed combination, and different intercropping scales need to be further tested in future.

Session 1-P 3 - Ecology of maize pests and implications for their sustainable management

Feeding damage from *Striacosta albicosta* at various infestation rates on *Bt* hybrids and implications for economic injury levels

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Western bean cutworm (WBC) *Striacosta albicosta* is a North American lepidopteran pest of maize (*Zea mays*). Some maize fields with western bean cutworm infestations have reported up to a 40% yield loss. Late instar larvae damage the ear by consuming aborted and harvestable kernels on the ear tip or by burrowing into the side of the ear to feed on harvestable kernels. Current IPM strategies for WBC rely on insecticides and Bt maize with Cry1F and Vip3A traits. Economic injury level (EIL) and economic thresholds (ET) were developed for non-*Bt* maize in 2013 in Nebraska for western bean cutworm infestations; the recommended ET for non-*Bt* maize is 5-8% infestation. However, it is important that EILs are developed using *Bt* maize because of its common usage in nearly all maize growing regions and the incomplete control observed for Cry1F proteins. In western Nebraska, USA, 56 plots were planted with 4 types of maize that provide varying levels of control: non-*Bt*, which provides no control; Cry1A.105+ Cry2Ab2, with unlikely WBC control; Cry1F, provides moderate WBC control; and Vip3A, provides near 95% control. For 25 plants in the center of each plot, natural infestations were adjusted by removing egg masses or augmenting infestation by restricting live moths on the plants to achieve infestation levels of 0, 8, 16 or 32%. Larval survival rate was determined at 20 days after infestation. At crop maturity, 10 ears were harvested from each plot and assessed for damage (cm^2/ear). Of the 560 ears collected, 13.7% had WBC feeding damage to harvestable kernels. Seed type (P-value< .0001) and infestation rate (P-value= .0287) had a significant effect on the WBC feeding damage in each plot. However, the interaction between both factors was not significant. The mean damage to harvestable kernels was significantly higher in non-*Bt* maize at 16 and 32% than any of the *Bt* maize hybrids. Maize hybrids with Cry1A.105+ Cry2Ab2 had significantly higher mean feeding damage at 16% infestation rate than the other *Bt* maize hybrids. There was not a significant difference between the mean feeding damage on harvestable kernels for Cry1F and Vip3A. This data will be used to develop economic injury levels for *Bt* maize. Accurate EILs will allow growers to use insecticides more judiciously, and thus save money, conserve natural enemies and reduce environmental damage.

Session 2-P 4 - Genomic and transcriptomic research applications in corn pest insects

Identification of putative olfactory related genes from yellow peach moth *Conogethes punctiferalis* (Guenée) antennae transcriptome

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The yellow peach moth, *Conogethes punctiferalis* (Guenée), is an extremely important polyphagous insect throughout tropical and eastern Asia and Australia. Since the early 1990s, *C. punctiferalis* has become the main insect pests of corn in some areas, even made more serious damage than the major corn pests of *Ostrinia furnacalis* (Guenée) in China. Management of the pest by insecticides is not only undesirable, but also ineffective and expensive. The olfactory systems play important role in insect survival, which is the primary sensory perception modality to detect odor molecules in the environment. Our study aims to identify olfactory related genes for potential applications in behavioral responses of *C. punctiferalis*. By transcriptomic analysis of male and female antennae, 118 putative olfactory genes including 20 odorant binding proteins, 13 chemosensory proteins, 62 odorant receptors, 11 ionotropic receptors, 10 gustatory receptors and 2 sensory neuron membrane proteins were identified. Expression levels of 50 odorant receptor genes were further evaluated by quantitative real-time PCR in antennae. All the ORs tested were detected in antennae and some of which were associated with sex-biased expression. This result provides a comprehensive resource of the foundation in semiochemicals driven behaviors at molecular level in *C. punctiferalis*.

Session 5-P 5 - The evolution of resistance to insecticidal toxins by insect pests of maize

Susceptibility of *Ostrinia nubilalis* (Hübner) and *Striacosta albicosta* (Smith) populations to pyrethroid in the United States

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The European corn borer, *Ostrinia nubilalis*, and the western bean cutworm, *Striacosta albicosta*, are key pests of maize in the United States. *Ostrinia nubilalis* is an important pest in the global production of maize and *S. albicosta* is important primarily in the Corn Belt. Management practices rely on transgenic maize and applying chemical control. In response to reports of reduced efficacy of pyrethroids in areas of Nebraska and Kansas, and problem popcorn fields in north central Nebraska, the present research is designed to establish baseline susceptibility to the commonly used pyrethroid bifenthrin. To access *O. nubilalis* susceptibility, a laboratory population was used to compare with field populations collected in 2016. Due to the absence of a *S.albicosta* laboratory population, only resistance ratios between problem and non-problem fields were compared with populations collected in 2016. Concentration-response bioassays with neonate were performed to estimate the susceptibility of populations collected from different locations in Nebraska. Differences based on confidence intervals overlap were recorded in *O. nubilalis* between field populations. The highest LC50s for *O. nubilalis* were observed in populations from Fillmore County, NE, Saunders County, NE and Antelope County, NE (81.9, 63.0, and 57.8 ng/cm^2, respectively), with lowest LC50s for populations from Faribault and Lancaster counties (6.64 and 13.3 ng/cm^2 respectively). For *S. albicosta*, no significant differences between problem and non-problem fields were observed. The highest LC50s for *S. albicosta* were observed for populations from Lincoln County and Hall County, NE (76.56 and 62.85 ng/cm^2, respectively), with lowest LC50s observed from Scotts Bluff County, NE (50.10 ng/cm^2). This study suggests that there might be a difference in susceptibility to bifenthrin in *O. nubilalis* for field populations of Nebraska. More studies are necessary to determine if these differences are due to a change in susceptibility generated by multiple years of exposure to pyrethroid insecticides. Due to the absence of baseline data for *S. albicosta*, conclusions for this species are limited. However, no difference between problem and non-problem fields might indicate that the highly mobile behavior of *S. albicosta* might play a role in resistance evolution.

Application techniques for beneficial nematodes against soil insect pests in maize

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In an attempt to replace insecticides against soil insect pests, biological control solutions have been developed. For example, commercially mass-produced *Steinernematid* and *Heterorhabditis* species of beneficial entomopathogenic nematodes are available in most world regions. However, their use on a larger scale such as in field crops like maize is still limited. Recent studies aimed at developing optimal application techniques for nematodes in field crops, i.e. being practical and effective at such a scale at reasonable costs. Field studies revealed that nematodes can be applied into the soil at sowing as well as into the soil along rows of young plants depending on the crop. As for the sowing period, fluid and micro-granular applications as well as seed coating with nematodes are, in principle, possible. However, the easiest and currently most promising technique is the fluid stream spray of a nematode-water suspension into soil at the moment of sowing or during mechanical weed control. This method requires a relatively low amount of water (200-400 l per hectare) compared with onto-soil applications. In maize for example, sowing machines are used that have simple fluid applicators that spray nematodes behind the sowing or press wheel into the furrow prior the soil-closing wheels. Farmers may adapt their equipment for fluid soil insecticides, or may use the nematode-specific application tools recently developed for common sowing machines. This allows reducing the required nematode dose to between 2 and 3 x 10^{9} nematodes per hectare, and thus the costs of this control technique. Dose - efficacy response curves are currently developed as well as nematode-pest density responses. In conclusion, beneficial nematodes are ready to be used for the biological control of soil insect pests in field crops.

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Survey of natural enemies of the western corn rootworm in Central Mexico

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The western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a root-herbivore that specializes on maize (*Zea mays* L.). It is native to North America where it causes tremendous crop losses yearly, but also in parts of Europe where was recently introduced. Biological control strategies have been unsuccessful so far and there is an urgent need for more effective biocontrol agents. We screened for natural enemies of the root herbivore in Central Mexico, including parasitic flies, wasps and entomopathogenic nematodes (EPN). We examined adult beetles of the subtribe Diabroticina for the occurrence of the parasitoids *Celatoria compressa* Wulp (Diptera: Tachinidae) and *Centistes (Syrrhizus) diabroticae* Gahan (Hymenoptera: Braconidae), and we tested the virulence of the field-collected EPN *Heterorhabditis bacteriophora* against WCR larvae. This is a first step to obtain information and material for the development of an effective biocontrol agent against WCR.

Release of *Trichogramma ostriniae* for biological control of *Ostrinia furnacalis* in summer corn region of China

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Trichogramma ostriniae is the predominant parasitoid of attacking eggs of Asian corn borer, *Ostrinia furnacalis* in China. However, the application of this parasitoid is practically rarely reported because its production efficiency is comparatively low, by rearing with eggs of either *Corcyra cephalonica* or *Sitotroga cerealella*. Benefit by the equipment renewal, the production of *T. ostriniae* using *S. cerealella* has been improved greatly in Institute of Plant Protection, Shandong Academy of Agricultural Sciences, such as the process of host moths gathering, host eggs gathering and cleaning, and production packaging.

From 2015 to 2016, corn fields applied with *Trichogramma* for control of *O. furnacalis* were over 5,000 hectares in Shandong and Henan Province. Our survey results showed that egg parasitism rates in fields with *T. ostriniae* application (85.85%-97.43%) were higher than fields with pesticides application (15.67%-31.25%) significantly. The damage rates of corn plants in fields releasing *T. ostriniae* (5.68%-7.35%) were significantly lower than that using pesticides (10.90%-16.72%). We can conclude from our results that releasing *T. ostriniae* can achieve a better effect than pesticides application for the control *O. furnacalis.*

The success achieved by releasing *T. ostriniae* for biological control *O. furnacalis* can be summed up in 5 aspects. 1) Good choice of *Trichogramma* species. *T. ostriniae* is the predominant *Trichogramma* species for control *O. furnacalis* in Huang-Huai-Hai summer corn region. 2) Local *T. ostriniae* are used. We collected local *T. ostriniae* individuals outdoors as the parents for mass production. 3) High quality of products. Good environment control system, rejuvenation technique, efficient decives and protective package system, make a high emergence rate (over 95%) of *T. ostriniae* in field. 4) Good application strategy. Inoculative releases strategy was used. We released *T. ostriniae* twice, at the egg period of second and third generation of *O. furnacalis*, which can provide sufficient number of *T. ostriniae* in field in August, when *O. furnacalis* can cause serve damage. 5) Good application time and sufficient parasitoids. We released *T. ostriniae* at the early egg period of *O. furnacalis* and 150,000 individuals per hectare each time.

Long-term effects of different insecticides on the wireworm population (*Agriotes* spp.) in the soil

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Wireworms, larvae of click beetles (Coleoptera: Elateridae) belong to the most dangerous pests in corn. Yield and quality loss can also be monitored with other cultures. The most common click beetles within the genus *Agriotes* in Germany are *Agriotes lineatus, A. obscurus, A. sputator, A. sordidus* and *A. usulatus*. The click beetles' life cycle, from egg, to the different larval stages to the adult beetle, takes between two to five years depending on the *Agriotes* spp. Due to the larval state that takes several years and the hidden lifestyle in the soil, the click beetle is hard to control.

The insecticide active ingredients (ai) “Fipronil” (trade name: Goldor Bait), “Clothianidin” (Santana) a combination of “Thiamethoxam” and “Tefluthrin” (Force Zea) and “Thiacloprid” (Sonido) were tested in field studies on their reducing effects on wireworm damage on corn plants. Goldor Bait (5 kg/ha with 5 gai/kg Fipronil) and Santana (7 kg/ha with 0,7% Clothianidin) were applied as a granulate in the seed row. Force Zea (Thiamethoxam with 27.5 gai/ha + Tefluthrin with 11 gai/ha) and Sonido (Thiacloprid with 100 gai/ha) were used for seed treatment. To check whether the insecticides have reduced the wireworm population in the soil, corn was planted on the same plots the following year as well, but without a new insecticide treatment. For evaluation of the field trial plant damage at a height of 1.5m was monitored in every trial year.

During the year of insecticide treatment the plant loss was the lowest with Goldor Bait (40%) followed by Force Zeta (55 %), Santana (60%) and Sonido (70%). Within the untreated control 90% of the plants were destroyed. This shows that none of the approved insecticides is able to prevent harvest losses if there is a high wireworm population. The year following the treatment also showed that Goldor Bait achieved the best results with a plant loss of 24%. Plants treated with the other insecticides, belonging to the group of neonicotinoids (Santana, Force Zea, Sonido) suffered losses of around 70%. The untreated control only showed a loss of 50%.This leads to the conclusion that only Goldor Bait can achieve a reduction of wireworm populations in the soil. All neonicotinoid based insecticides only showed a repellent effect of about two month, but there was no significant killing of wireworms. Potato cultivation has already brought similar experiences. A seed tuber treatment with e.g. Imidacloprid another neonicotinoid based insecticide did not lead to a decreasing degree of wireworm damage in the daughter tubers.

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