



# IWGO

International Working  
Group on *Ostrinia* and  
other maize pests

**25<sup>th</sup> IWGO Conference &  
4<sup>th</sup> International Conference on  
*Diabrotica* Genetics &  
Open Meeting of  
NC205/NCCC46 Corn Insect  
Technical Committees**

**14 to 17 April 2014  
Chicago, U.S.A.**

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)



# **ORGANIZING COMMITTEE**

## **IWGO IOBC-GLOBAL CONVENORS**

Dr. Ulrich KUHLMANN (Convenor)  
CABI

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## **SCIENTIFIC SESSION ORGANIZERS**

Dr. William MOAR, Monsanto, St. Louis, Missouri, U.S.A.

Dr. Thomas GUILLEMAUD, INRA, Sophia Antipolis, France

Dr. Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A.

Prof. Blair SIEGFRIED, University of Nebraska, Lincoln, Nebraska, U.S.A.

Dr. Jon LUNDGREN, USDA-ARS, Brookings, South Dakota, U.S.A.

Dr. Stefan TOEPFER, CABI c/o Plant Health Service, Hodmezovasarhely, Hungary

Dr. Bruce HIBBARD, University of Missouri, Columbia, Missouri, U.S.A.

Prof. Stefan VIDAL, Georg-August-University, Göttingen, Germany

Dr. Mario SCHUMANN, Georg-August-University, Göttingen, Germany

Dr. Ivan HILTPOLD, University of Missouri, Columbia, Missouri, U.S.A.

Dr. Thomas E. HUNT, University of Nebraska, Concord, Nebraska, U.S.A.

Dr. Silvana PAULA-MORAES, EMBRAPA, Cerrados, Brazil

Dr. Zhenying WANG, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Dr. Feng ZHANG, CABI, Beijing, P.R. China

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Dr. Kanglai HE, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Dr. Kevin WANNER, Montana State University, Bozeman, U.S.A.

Prof. Mike GRAY, University of Illinois, Urbana, Illinois, U.S.A.

Dr. Ulrich KUHLMANN, CABI, Delémont, Switzerland

Dr. Michael ZELLNER, Bavarian State Research Centre for Agriculture, Freising, Germany

## **LOCAL ORGANIZATION**

Dr. Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A.

## **CONFERENCE ORGANIZER**

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# **SCIENTIFIC PROGRAMME**

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**4<sup>th</sup> International Conference on *Diabrotica*  
Genetics &**

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# SCIENTIFIC PROGRAMME

## Sunday, 13 April 2014

**17:00 Registration – 23<sup>rd</sup> Floor - Foyer, Allerton Hotel, Chicago, U.S.A.**

- Provision of PowerPoint Oral Presentations on CD or USB

**19:00 Welcome Reception & Snacks - 23<sup>rd</sup> Floor - TipTopTap Room**

- Welcome Address, Ulli KUHLMANN, IWGO-IOBC-Global Convenor

## Monday, 14 April 2014

**07:30 Registration & Drop-off Point Posters, 3<sup>rd</sup> Floor – Foyer of Buckingham Ballroom**

**08:00 Welcome Addresses – 3<sup>rd</sup> Floor - Buckingham Ballroom**

- Welcome Address and Organizational Issues for the IWGO Conference, Tom SAPPINGTON, IWGO-IOBC-Global Co-Convenor
- Welcome Address, Jonathan LUNDGREN, IOBC Nearctic Regional Section

**08:30 Scientific Session 1**

### Session 1: RNAi as a Pest Management Tool

*Session Organizer: William MOAR, Monsanto, St. Louis, Missouri, U.S.A.*

08:30	W. Moar	Introduction
08:35	B. Siegfried, A. Velez & X. (Joe) Zhou	RNA interference in insect pest management: assessing potential benefits and risks
08:55	E. Knorr, L. Bingsohn, M.R. Kanost & A. Vilcinskis	<i>Tribolium castaneum</i> as a model for high-throughput RNAi screening
09:15	H. Li, C. Khajuria, M. Rangasamy, B. Siegfried & K. Narva	Effectiveness of dsRNA versus siRNA in RNAi mediated knock-down in western corn rootworm ( <i>Diabrotica virgifera virgifera</i> )
09:35	W. Moar, G. Head, R. Bolognesi, J. Petrick & P. Bachman	Strategies to address corn rootworm control challenges
09:55	X. Zhou, H. Pan, X. Yang, A. Velez & B.D. Siegfried	Developing a framework for assessing of the <i>in planta</i> RNAi on non-target arthropods

**10:15 Coffee/Tea Break & Posters**

## 10:45 Scientific Session 2

### Session 2: Transcriptomics and Genomics of *Diabrotica*

*Session Organizers:* Thomas GUILLEMAUD, INRA, Sophia Antipolis, France & Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A.

10:45	T. Guillemaud & T. Sappington	Introduction
10:50	F. Legeai, A. Bretaudeau & <u>D. Tagu</u>	Bioinformatics Platform for Acroecosystems Arthropod (BIPAA) to assist agroecosystem insect genomics programs
11:10	H. Robertson	Sequencing the genome of <i>Diabrotica virgifera virgifera</i>
11:30	T. Guillemaud, E. Deleury, B. Coates, A. Gassman, W. French, N. Miller, B. Siegfried & T. Sappington	Western corn rootworm transcriptome project
11:50	L. Flage , R. Bansal, R. Kerstetter, M. Chen, M. Carroll, R. Flannagan, T. Clark, B. Goldman & A. Michel	Western corn rootworm transcriptome assembly and genomic analysis of population structure
12:10	N. Grubbs, Fu-Chyun Chu & <u>M. Lorenzen</u>	Use of genomic and transcriptomic data to facilitate germline transformation of the western corn rootworm

## 12:30 Lunch Break & Posters

## 14:00 Scientific Session 3

### Session 3: Western corn rootworm adaptation

*Session Organizer:* Blair SIEGFRIED, University of Nebraska, Lincoln, Nebraska, U.S.A. & Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A.

14:00	B. Siegfried & T. Sappington	Introduction
14:05	A. Eben	Diabroticina beetles: how can we explain their diversity?
14:25	D. Lemic, K. Mikac, H.A. Benitez & R. Bažok	Genotypic changes and wing shape variation associated with the invasion of southern Europe by western corn rootworm
14:45	<u>E. Lombaert</u> , M. Ciosi & T. Guillemaud	Colonization history of the western corn rootworm in North America inferred from microsatellite data
15:05	Chia-Ching Chu, J. Spencer, M. Curzi, J. Zavala & <u>M. Seufferheld</u>	Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm
15:25	S. Liu	Corn rootworm virus discovery from NGS and EST data

## 15:45 Coffee/Tea Break & Posters

## 16:15 Scientific Session 4

### Session 4: Ecologically based management of maize pests

*Session Organizers: Jon LUNDGREN, USDA-ARS, North Central Agricultural Research Laboratory, Brookings, South Dakota, U.S.A. & Stefan TOEPFER, CABI c/o Plant Health Service, Hodmezovasarhely, Hungary*

16:15	J. Lundgren & S. Toepfer	Introduction
16:20	<u>S. Koczor</u> , F. Szentkirályi & M. Tóth	Optimisation of a lacewing egg concentrator for common green lacewings ( <i>Chrysoperla carnea</i> species-complex; Neuroptera: Chrysopidae)
16:40	<u>X. Jiang</u> , L. Zhang, Y. Cheng & L. Luo	Ecologically based pest management of the oriental armyworm, <i>Mythimna separata</i> in China
17:00	<u>J.A. Peterson</u> , E.C. Burkness, J.D. Harwood & W.D. Hutchison	Predation of corn earworm <i>Helicoverpa zea</i> by the minute pirate bug <i>Orius insidiosus</i> in sweet corn
17:20	<u>K. Welch</u> & J. Lundgren	Predator behavioural responses to novel haemolymph defenses of western corn rootworm larvae
17:40	<u>S. Toepfer</u> , M. Glas, P. Knuth, M. Lichtenberg, J. Maier & K. Müller-Sämann	New application techniques for beneficial nematodes to better control rootworm larvae

## 18:00 End Scientific Sessions

**18:45 Departure from Allerton Hotel to group dinner by public transportation**

**19:30 Chicago-style stuffed pizza dinner at Giordano's Restaurant & Pizzeria in Greek Town, 815 W. Van Buren St., Chicago**

## **Tuesday, 15 April 2014**

### **08:00 Scientific Session 5**

#### **Session 5: Advances in and potential applications of rootworm/host interactions**

*Session Organizers: Bruce HIBBARD, University of Missouri, Plant Sciences, Columbia, Missouri, U.S.A. & Stefan VIDAL, Georg-August-University, Department of Crop Sciences, Agricultural Entomology, Göttingen, Germany*

08:00	B. Hibbard & S. Vidal	Introduction
08:05	<u>R. Ferrieri</u> , C. Robert, M. Erb & B. Hibbard	Understanding the physiological and metabolic basis for lateral root re-growth as a mechanism for crop tolerance to <i>Diabrotica virgifera</i>
08:25	<u>E. Bernklau</u> , B. Hibbard & L. Bjostad	Chemical ecology of host selection in <i>Diabrotica</i>
08:45	F. Dematheis, K. Smalla, B. Kurtz & <u>S. Vidal</u>	Interactions of western corn rootworm larvae with soil microorganisms
09:05	<u>L. Meihls</u> & B. Hibbard	Rootworm interactions with host plants, artificial diet, and <i>Bt</i>
09:25	M. Bohn	The genetic basis of host plant resistance in maize to the western corn rootworm

### **09:45 Coffee/Tea Break & Posters**

### **10:15 Scientific Session 6**

#### **Session 6: Innovative control strategies against maize soil pests**

*Session Organizers: Mario SCHUMANN, Georg-August-University, Department of Crop Sciences, Agricultural Entomology, Göttingen, Germany & Ivan HILTPOLD, University of Missouri, Plant Sciences, Columbia, Missouri, U.S.A.*

10:15	M. Schumann & I. Hiltbold	Introduction
10:20	<u>M.A. Brandl</u> , M. Schumann & S. Vidal	Modification of western corn rootworm larval behaviour with botanical based extracts
10:40	<u>M. Schumann</u> , B. Tappe & S. Vidal	Identification of resistance properties of conventional maize cultivars against western corn rootworm larvae in a soil - less bioassay
11:00	<u>G. Jaffuel</u> , I. Hiltbold & T.C.J Turlings	A root-produced quiescence factor may help to improve the shelf-life of entomopathogenic nematodes used in biological control
11:20	J. Maxwell, S.T. Jaronski, E.H. Clifton, M.W. Dunbar, M.A. Jackson & A.J. Gassmann	Interactions among <i>Bt</i> maize, entomopathogens and the western corn rootworm
11:40	L. Demarta, B.E. Hibbard & <u>I. Hiltbold</u>	The impact of the root architecture on foraging behavior biological control agents

### **12:00 Lunch Break & Posters**

## 13:30 Scientific Session 7

### Session 7: Invasive and emerging pests of maize

*Session Organizers:* Thomas E. HUNT, University of Nebraska, Concord, Nebraska, U.S.A. & Silvana PAULA-MORAES, EMBRAPA, Cerrados, Brazil

13:30	T.E. Hunt & S. Paula-Moraes	Introduction
13:35	<u>T. Guillemaud</u> , E. Lombaert & A. Estoup	Invasion biology: insights from evolutionary biology and population genetics
13:55	<u>S. Paula-Moraes</u> & A. Specht	<i>Helicoverpa armigera</i> in Brazil
14:15	<u>W. Hutchison</u> , D. Kriticos, M. Zalucki, D. Borchert & R. Venette	Risk and potential impact of <i>Helicoverpa armigera</i> in the U.S.A.
14:35	<u>J. Smith</u> , T. Baute & A. Schaafsma	The establishment and management of western bean cutworm <i>Striacosta albicosta</i> in Canada
14:55	<u>Z. Wang</u> , K. He, S. Bai & T. Zhang	Cropping system transformation makes <i>Athetis lepigone</i> to be a new insect pest of corn in China

## 15:15 Coffee/Tea Break & Posters

## 15:45 Scientific Session 8

### Session 8: Science-based knowledge transfer to improve maize production and enhance food security in developing countries

*Session Organizers:* Zhenying WANG, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China & Feng ZHANG, CABI, Beijing, P.R. China

15:45	Z. Wang & F. Zhang	Introduction
15:50	<u>D. Babendreier</u> , Z. Wang, Z. Li, F. Zhang, S.I. Kang & M. Grossrieder	Increased maize production in DPR Korea through <i>Trichogramma</i> mass production technology transfer from China
16:10	<u>F. Zhang</u> , Z.-Y. Wang, S.-X. Bai, H.-K. Tai, N.N. Htain, B. Soudmaly, L. Zheng, U. Wittenwiler, M. Grossrieder & U. Kuhlmann	Intra-regional transfer of biologically-based plant protection technology to improve livelihoods of smallholder maize farmers in the Greater Mekong Subregion
16:30	<u>B. Soudmaly</u> , Z.-Y. Wang, S.-X. Bai, F. Rodhe & M. Grossrieder	Maize production, pests status and potential solutions to strengthen productivity through IPM capacity building in Lao PDR
16:50	<u>Z.-Y. Wang</u> , F. Zhang, S.-X. Bai, H.-K. Tai, Z. Liu, L. Zheng, U. Wittenwiler, M. Grossrieder & U. Kuhlmann	Maize production, pests status and potential solutions through knowledge transfer of a <i>Trichogramma</i> based IPM approach: a case study from Yunnan Province of China
17:10	<u>U. Kuhlmann</u> & W. Jenner	Plantwise - a global alliance for plant health support: maize as an example

## 17:30 End of Sessions

## 18:00 Departure from hotel to baseball game by public transportation

## 19:10 Chicago White Sox vs. Boston Red Sox major league baseball game, U.S. Cellular Field, Chicago

## Wednesday, 16 April 2014

### 08:00 Scientific Session 9

#### Session 9: Behaviour and genetics of insect pests of maize

*Session Organizers:* Rick HELLMICH, USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, Iowa, U.S.A. & Kanglai HE, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

08:00	R. Hellmich & K. He	Introduction
08:05	J. Van den Berg, J. Marais & A. Erasmus	Possible explanations for resistance development of the African stem borer to <i>Bt</i> maize
08:25	A.M. Velez, A.P. Alves & B.D. Siegfried	Effect of Cry1F corn on the behavior of susceptible and resistant fall armyworm and European corn borer
08:45	H. Xie, K. He, Z. Wang & X. Lu	Effects of elevated atmospheric CO <sub>2</sub> on interactions between maize and the Asian corn borer
09:05	J. Spencer & S. Hughson	I fly from nearby: characteristics of airborne western corn rootworm beetles ascending from <i>Bt</i> cornfields
09:25	A. Gassmann, E. Clifton, M. Dunbar, A. Hoffmann, D. Ingber, S. Jakka, J. Petzold-Maxwell, M. Rudeen & R. Shrestha	<i>Bt</i> resistance in western corn rootworm: a model for understanding pest management with <i>Bt</i> crops that are not high dose

### 09:45 Coffee/Tea Break & Posters

### 10:15 Scientific Session 10

#### Session 10: Integrated approaches to control wireworms in maize & Free topics

*Session Organizer:* Kevin WANNER, Montana State University, Bozeman, Montana, U.S.A.

10:15	K. Wanner	Introduction
10:20	K. Wanner & A. Morales-Rodriguez	Wireworms, an increasing threat to small grain crops in the western United States
10:40	F. Mävers, M. Schumann, P. Humbert, M. Vemmer, W. Beitzen-Heineke, E. Hummel, J. Treutwein, H. Kleeberg, A. Patel & S. Vidal	First results of an <i>Attract-and-Kill</i> strategy to reduce wireworm infestation
11:00	J. Lundgren	Increasing predator diversity and the strength of trophic linkages to a target pest
11:20	C. Mogren & J. Lundgren	Evaluating the risks posed by RNAi crops to pollinators in an agricultural landscape
11:40	Y. Cardoza	Toxin expression in <i>Bt</i> transgenic corn roots, and western corn rootworm behavioral responses under two refuge mimicking scenarios

### 12:00 Lunch Break & Posters

### 13:30 Scientific Session 11

#### Session 11: New products/methods for IPM of *Ostrinia* and *Diabrotica* in modern maize production systems

*Session Organizers:* Mike GRAY, University of Illinois, Department of Crop Sciences, Urbana, Illinois, U.S.A. & Ulrich KUHLMANN, CABI, Delémont, Switzerland

13:30	M. Gray & U. Kuhlmann	Introduction
13:35	M. Rice	IPM tactics for Lepidoptera of maize in the southern United States
13:55	<u>E. Bynum</u> & P. Porter	Arthropod pest management within the complex IPM system of the Texas High Plains
14:15	<u>A. Aboul-Nasr Badawi</u> , H. Mesbah, N. El-Sayed & R. Mohamed	New approach of using endomycorrhizal fungi in the biological control of stem borer infestation in relation to the yield of corn plants
14:35	<u>V. Kaster</u> , M. Araba, D. Benson, T. Burd & I. Oyediran	Modern corn pest management: meeting the challenge with Syngenta solutions
14:55	M. Gray	Western corn rootworm resistance to <i>Bt</i> hybrids: management recommendations moving forward

### 15:15 Coffee/Tea Break & Posters

### 15:45 Scientific Session 12

#### Session 12: Free topics

*Session Organizer:* Michael ZELLNER, Bavarian State Research Centre for Agriculture, Freising, Germany

15:45	M. Zellner	Introduction
15:50	<u>S. Toepfer</u> , M. Zellner & U. Kuhlmann	Crop rotation remains the key to successful rootworm control in Europe
16:10	<u>C. Krupke</u> , E. Long, J. Holland & B. Eitzer	Recent research describing costs and benefits of neonicotinoid-treated seed: potential for non-target effects vs. effects upon pest damage and yield
16:30	D. Onstad	Economics of insect resistance management for western corn rootworm
16:50	<u>E. Schacht</u> , J. Barta & K. Ostlie	Performance of <i>Bt</i> -traits and insecticide at a <i>Bt</i> -resistant and a <i>Bt</i> -susceptible western corn rootworm ( <i>Diabrotica virgifera virgifera</i> ) site
17:10	<u>N. Miller</u> , J. Alouw & Z. Zhao	Transcriptional responses of western corn rootworms to benzoxazinoids

### 17:30 IWGO Business Meeting

### 18:00 End of Sessions

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

## **Thursday, 17 April 2014**

### ***Joint Open Session, NC205/NCCC46 Corn Insect Technical Committees***

#### **08:15     *Welcome, background and introductions***

- Tom Hunt, University of Nebraska, U.S.A. (Chair NC205),
- Art Schaafsma, University of Guelph, Canada (Chair NCCC46),
- Steve Pueppke, Michigan State University, U.S.A. (Administrative Advisor, NC205/NCCC46) – *Merger of NC205 and NCCC46, and rewrite of NC205*
- Tom Sappington, USDA-ARS, Iowa, U.S.A. (Sec NC205, Past-chair NCCC46)

#### **09:00     *Genomics and genetics research on Ostrinia***

- *Brad Coates, USDA-ARS, organizer*
- Detection of *Ostrinia nubilalis* pheromone race, voltinism and resistance phenotypes using new genetic methods (Brad Coates, USDA-ARS, Ames, Iowa)
- Elucidating the molecular mechanisms of sex pheromone detection by moths using *Ostrinia* as a model (Kevin Wanner, Entomology, Montana State University)

#### **10:00     *Coffee/Tea Break***

#### **10:30     *Open discussion: moving forward with Diabrotica -omics research***

- *Tom Sappington (USDA-ARS), organizer*
- Bottlenecks, needs, ideas for progress on assembly
- Current and future relationships and roles among public-sector and private-sector scientists

#### **11:30     *Research updates on neonicotinoid insecticides***

- *Paul Mitchell (University of Wisconsin) & Terry Hurley (University of Minnesota), co-organizers*
- Market-Level Assessment of the Economic Benefits of Neonicotinoid Insecticides in the U.S. (Paul D. Mitchell, Agricultural and Applied Economics, University of Wisconsin)
- Assessing the Non-Pecuniary Benefits of Neonicotinoid Insecticides for U.S. Corn and Soybeans (Terry Hurley, Applied Economics, University of Minnesota)

#### **12:00     *Lunch***

#### **13:00     *Research updates on neonicotinoid insecticides (continued)***

- A meta-analysis approach to estimating the yield effects of neonicotinoid insecticides (Ken Frost, Plant Pathology, University of Wisconsin)
- Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem (Anders Huset, Entomology, Cornell University and Russell Groves, Entomology, University of Wisconsin)
- The hunt for real exposure at the North American hot-bed on the debate regarding neonic-use in field crops and their impact on pollinators - stakeholders, politics, economics, and science (Art Schaafsma, Tracey Baute\*, Victor Limay-Rios, Yingen Xue and Jocelyn Smith. University of Guelph, Ridgetown Campus, and \*Ontario Ministry of Agriculture and Food, Canada)

#### **15:00     *Final Comments & Adjourn***

# POSTER PRESENTATIONS

Poster 01	<u>A. Neupane</u> , S.-I. Eyun, H. Wang, B.D. Siegfried & E.N. Moriyama	Transcriptome analysis of western corn rootworm larvae and eggs
Poster 02	<u>T. Leaf</u> & K. Ostlie	Utility of five different sticky traps for use in risk assessment in <i>Bt</i> -susceptible and <i>Bt</i> resistant corn rootworm ( <i>Diabrotica</i> spp.) fields
Poster 03	D. Wangila & <u>L. Meinke</u>	Susceptibility of western corn rootworm populations collected in Nebraska to <i>Bt</i> corn events
Poster 04	<u>K. Ostlie</u> , E. Schacht, J. Barta & T. Leaf	Trait and insecticide efficacy against susceptible and resistant western corn rootworm populations: implications for resistance management
Poster 05	<u>M. Bertossa</u> , L. Colombi & H.E. Hummel	Cultural <i>Diabrotica</i> containment strategy in Switzerland an effective success by now
Poster 06	<u>M. Bredeson</u> & J. Lundgren	Corn insect communities of eastern South Dakota
Poster 07	<u>I. Hiltbold</u> & B.E. Hibbard	To resist or not to resist? An insect dilemma on <i>Bt</i> maize
Poster 08	<u>M. Schumann</u> , T. Schlecht, M. Brandl & S. Vidal	Spatial and temporal infection patterns of western corn rootworm larvae by two entomopathogenic nematode species
Poster 09	M. Zellner	Impact of tillage on the infestation with European corn borer
Poster 10	R. Bitzer, <u>R. Hellmich</u> , N. Schmidt & K. Carstens	Multivariate and ecological approach to assess non-target effects of <i>Bt</i> rootworm maize on ground beetles
Poster 11	<u>J.M. Beuzelin</u> , B.E. Wilson, M.T. VanWeelden & A.T. Showler	Mexican rice borer, <i>Eoreuma loftini</i> : a threat to corn in the southern United States?
Poster 12	T. Thieme, C. Buuk, K. Foltin & <u>M. Zellner</u>	Distributions of <i>Diabrotica virgifera virgifera</i> eggs in European corn fields
Poster 13	<u>X. Lu</u> , L. Chen, X. Chang, S. Zhoua, G. Zhang & Y. Ding	The effect of climate warming on the generation distribution of <i>Ostrinia furnacalis</i> in Jilin Province
Poster 14	<u>C. Mason</u> & M.A. Johnston	Optimum yield of female sex pheromone in <i>Ostrinia nubilalis</i> (European corn borer) during scotophase
Poster 15	<u>M.A. Brandl</u> , M. Schumann, M. Przyklenk, M. Vemmer, A. Patel & S. Vidal	An 'Attract & Kill'-approach against wireworms ( <i>Agriotes</i> spp.) in corn
Poster 16	<u>M. Tóth</u> , P.A. Viana, E. Vilela, M.J. Domingue, T.C. Baker & S. Koczor	KLP ('hat') trap with semiochemical lures suitable for trapping two <i>Diabrotica</i> spp. exotic for Europe
Poster 17	<u>M. Agosti</u> , L. Michelon & C.R. Edwards	<i>Diabrotica</i> management in Brescia province of northwestern Italy: the importance of developing a local program
Poster 18	<u>W. French</u> , L. Hammack & D. Tallamy	Mate choice and components of fitness in western corn rootworm in relation to Cry3Bb1 resistant and susceptible genotypes
Poster 19	<u>L. Rault</u> , N. Miller & B. Siegfried	Expression profile by Next-Generation Sequencing, of western corn rootworm ( <i>Diabrotica virgifera virgifera</i> ) neonates exposed to the <i>Bt</i> toxin Cry3Bb1
Poster 20	<u>K.S. Kim</u> , S.K. Choi, Y.-S. Lee, & T.W. Sappington	Population genetics studies of two major insect pests of corn in the U.S. Corn Belt
Poster 21	T.W. Sappington	Evidence from early accounts for reconstructing the mysterious origins of the bivoltine Z race of European corn borer in North America



**ABSTRACTS  
ORAL  
PRESENTATIONS**

## RNA interference in insect pest management: assessing potential benefits and risks

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Widely recognized as one of the premier functional genomics research tools, RNA interference (RNAi) has been used extensively in the post-genomics era to assign functions for genes annotated through small (expressed sequencing tags) or large (whole genome) scale sequencing efforts. Recently, the agricultural industry has recognized the potential to utilize RNAi as a mechanism to control the expression of target genes for pest control purposes resulting in a diversity of applications. Efficient delivery mechanisms, RNA stability, and RNA toxicity to the target organism remain as major technical challenges. However, a number of different approaches are being developed to overcome these challenges including transgenic crop plants that express RNAi traits (*in planta* RNAi). Although RNAi-based insect pest management technologies have yet to be commercialized, they are likely to become an important pest management tool that complements existing control practices including synthetic pesticides and *Bt* traits. This is especially important for target pest species, such as the western corn rootworm, where *Bt* traits are being challenged by resistance evolution. However, it is critical that the technology is used in a manner that is both sustainable and environmentally safe. The lack of a formalized/standardized ecological risk assessment (ERA) procedure remains as a major regulatory obstacle to integrate RNAi management approaches into sustainable pest management practices. An essential component of the ERA of RNAi plants involves *in vivo* RNAi toxicity testing under a defined worst-case scenario of exposure for both potential effects on non-target organisms and for resistance evolution. The studies described here are designed to answer questions directly pertaining to the risk of RNAi to non-target arthropods that are at greatest risk of exposure because of a shared environment and common molecular targets. In addition, studies to address the potential for resistance evolution are also described.

## ***Tribolium castaneum* as a model for high-throughput RNAi screening**

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Coleopteran insects are a highly diverse and successful order, and many beetle species are significant agricultural pests. New biorational strategies for managing populations of beetles and other insect species are needed as pests develop resistance to chemical insecticides and *Bt* toxins. There is now an opportunity to use genome sequence data to identify genes that are essential for insect growth, development, or survival as new targets for designing control technology. This goal requires a method for high-throughput in vivo screening of thousands of genes to identify candidate genes that, when their expression is disrupted, have a phenotype that may be useful in insect pest control. *Tribolium castaneum*, the red flour beetle, is a model organism that offers considerable advantages for such screening, including ease of rearing in large numbers, a sequenced genome, and a strong, systemic RNAi response for specific depletion of gene transcripts. The RNAi effect in *T. castaneum* can be elicited in any tissue and any stage by the injection of dsRNA into the hemocoel, and injection of dsRNA into adult females can even be used to identify phenotypes in offspring. A pilot RNAi screen (iBeetle) is underway. Several *T. castaneum* genes with promising RNAi phenotypes for further development as mechanisms for plant protection have been identified. Candidate genes identified in *T. castaneum* screens can then be tested in agricultural pest species (in which screening is not feasible), to evaluate their effectiveness for use in potential plant-based RNAi control strategies. Delivery of dsRNA expressed by genetically modified crops to the midgut of phytophagous insects is under investigation as a new tool for very specific protection of plants from insect pest species. The *T. castaneum* screening platform offers a system for discovery of candidate genes with high potential benefit.

## Effectiveness of dsRNA versus siRNA in RNAi mediated knock-down in western corn rootworm (*Diabrotica virgifera virgifera*)

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RNAi can be used for in-plant control of western corn rootworm (WCR, *Diabrotica virgifera virgifera*). Transcribed hairpin dsRNA in WCR resistant RNAi maize plants has been found to result in both long dsRNA and plant Dicer-processed siRNA populations. This raises the question whether the long dsRNA and/or the siRNA are active forms in the RNAi response. In this report, we studied the effectiveness of dsRNA versus siRNA targeted against the v-ATPase subunit C gene in both WCR larvae and adult beetles. In 9-day diet feeding assays, dsRNAs of at least 50 bp in length resulted in high levels of larval mortality. In contrast, 15-, 25-, or 27-bp dsRNAs and pooled 21-bp siRNAs did not cause mortality of WCR larvae. When WCR larvae were fed with the siRNAs, the v-ATPase C mRNA level did not change, whereas when fed with 174-bp dsRNA, the mRNA level was reduced by >20 fold in the treated WCR larvae relative to YFP dsRNA-fed negative control larvae. Similarly, 174-bp dsRNA caused 100% mortality of adult beetles while the mortality of beetles exposed to siRNA was similar to the negative control mortality. Further, when adult beetles were fed with siRNA, there was no effect on the level of v-ATPase C mRNA on day 5 after feeding, whereas WCR beetles fed with the 174-bp dsRNA showed ~35-fold reduction in v-ATPase C mRNA. Similar results were found with the 174-bp dsRNA/siRNA injections where we observed ~100-fold reduction in v-ATPase C mRNA level in the beetles injected with the 174-bp dsRNA and no change in the beetles injected with siRNAs. Our results suggest that only longer dsRNA or intact hairpin dsRNA in RNAi plants is effective in triggering knock down of v-ATPase C mRNA and causing WCR mortality. These results have implications for optimizing plant-delivered RNAi for rootworm control.

## Strategies to address corn rootworm control challenges

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The expression of dsRNA in crop plants, such as maize, to control damaging insect pests such as western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) represents a new class of insect control traits with a high degree of insect specificity. As with any new plant-expressed insect control trait, the potential for WCR to evolve resistance to dsRNA needs to be considered. This presentation will discuss information regarding pyramiding WCR-active dsRNA with *Bt* Cry proteins for increased efficacy and decreasing the potential for resistance development while maintaining a relative high degree of specificity in host spectrum.

## Developing a framework for assessing the risks of *in planta* RNAi on non-target arthropods

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Recently, ingestion of double stranded RNA (dsRNA) produced by transgenic corn plants has been demonstrated to be effective in triggering RNA interference (RNAi) in western corn rootworm (WCR, *Diabrotica virgifera virgifera*), providing potential novel opportunities for insect pest control. However, methods for environmental risk assessments of dsRNA against non-target arthropods have yet to be developed. Here we report preliminary assessments using two non-target coccinellid beetles, *Hippodamia convergens* and *Harmonia axyridis* and the pollinator, *Apis mellifera*. The insecticidal activity of a 400 bp dsRNA targeting the vATPase subunit A in WCR was designed based on the region of highest sequence similarity. Activity of both the WCR specific dsRNA (DVV-dsRNA) and the non-target species-specific dsRNAs were tested by oral ingestion at high concentrations. For *H. convergens* larvae, expression of vATPase A mRNA and protein was significantly reduced upon exposure to 4.0 µg/µl vATPase DVV-dsRNA and HC-dsRNA dsRNA in a droplet of sucrose solution. Importantly, the survival rate for both DVV and HC-dsRNA was significantly lower than control treatments. Similar trends were observed for *H. axyridis*, although differences in survival were not as pronounced. It is worth noting that the response of the two coccinellids was substantially different, suggesting differential susceptibility. In contrast to the results for lady beetles, *Apis mellifera* adults exposed to untreated diet, control dsRNA, and two doses of AM and DVV-dsRNA (1µg and 10µg) showed no significant effects on longevity or vATPase A expression at 48 hours after exposure. A slight reduction in adult emergence was observed when larvae were exposed to 10µg of AM-dsRNA, but no effects were detected when larvae were fed 1µg of AM-dsRNA. Differences in the response to dsRNA among the species tested indicate that sequence similarity/identify and taxonomic relatedness are informative in considering risk but not the only consideration. In addition, sublethal effects may be important in assessing risks, and assessments that provide a comprehensive view of various life-history traits may be important to future assessments.

## **Bioinformatics Platform for Agroecosystems Arthropod (BIPAA) to assist agroecosystem insect genomics programs**

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«Every genome deserves a home» as Dan Lawson (EBI) usually says. BIPAA provides an information system which is a home for arthropod genomes living in agroecosystems. BIPAA aims to assist genomics and post-genomics programs. It shares resources and standards critical to guarantee data integration and interoperability, and improves the coherence of the protocols set up by its various partners. Apart from giving access to the data, the main utility of BIPAA is to settle an environment allowing a larger community to elaborate complex genomics analyses, browsing, mixing or crossing heterogeneous data.

BIPAA uses open source software (such as GMOD tools) to browse genomes and display any genomics and post-genomics features computed by own workflows or provided by biologists such as transcripts, non-coding RNAs, proteins, polymorphisms, orthologs, transposons BIPAA provides also a gene curation tool (WebApollo) to enrich automatic annotation, and a synteny displaying tool (CMap). A Galaxy server has been implemented allowing users to analyse their data using various state-of-the-art tools and to extract knowledge. BIPAA is also able to work upstream of these services by assisting genome assembly, as well as automatic gene annotation.

BIPAA currently homes AphidBase (<http://www.aphidbase.org>) and LepidoDB (<http://www.inra.fr/lepidodb>) with the genomes of the pea aphid, the green peach aphid (under construction) and the fall army worm. Connection and interoperability is possible to other post-genomics information systems such as PhylomeDB (arthropod phylogenomics), CycADS (metabolic reconstruction) or GeneOntology.

BIPAA will be discussed as a possible home for the *Diabrotica* genome.

## Sequencing the genome of *Diabrotica virgifera virgifera*

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The genome of *Diabrotica virgifera* has been estimated at 2.6 Gbp. We have undertaken sequencing of this genome with USDA-NIFA support using ILLUMINA sequencing from multiple shotgun and mate pair libraries generated from an inbred strain maintained at the USDA facility in South Dakota. Preliminary assembly of the genome yielded 2 million scaffolds totalling 2.7 Gbp with a scaffold N50 of 53 kbp and longest scaffold at 1 Mbp. The high fragmentation of the assembly appears to result from the presence of numerous similar copies of long retrotransposons, both between and within genes. While this assembly is useful for some purposes such as studies of individual genes, we are exploring ways to improve this assembly to make it more useful to the community.

## Western corn rootworm transcriptome project

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Three years ago a large project to obtain genetic and genomic resources in western corn rootworm (WCR) started. The goal of this collaboration between American and European labs is to ultimately develop sustainable approaches to control the western corn rootworm populations. The goal of the project is three-fold: (i) to create a nearly complete inventory of the transcriptome of WCR by extensive sequencing of a pooled, normalized cDNA library representing various WCR life stages and tissues under normal and stressed developmental conditions; (ii) to compare specific gene expression profiles for tissues and physiological conditions related to behavior, larval metabolism of plant tissue, and adaptations to management practices; and (iii) to help assembling the WCR genome. During this presentation, we will present the results obtained so far, i.e. the quantitative and qualitative description of the reference transcriptome sequences and the de novo assemblies of the transcriptome. A short focus will be presented on the origin of the sequences (WCR, *Wolbachia*, parasites), the % of full length sequences, and the results of the automated annotation.

## Western corn rootworm transcriptome assembly and genomic analysis of population structure

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Western corn rootworm (WCR) is one of the most significant insect pests of maize in North America. WCR has dramatically increased its range in the last century, invading key maize production areas in the US and abroad. In addition, this species has a history of evolving traits that allow it to escape various control options. Improved genetic and genomic resources are crucial tools for understanding population history and the genetic basis of trait evolution. Here we produce and analyze a transcriptome assembly for WCR. We also perform whole genome population resequencing, and combine these resources to better understand the evolutionary history of WCR.

The WCR transcriptome assembly presented here contains approximately 16,000 unigenes, many of which have high similarity to other insect species. Among these unigenes we found several gene families that have been implicated in insecticide resistance in other species. We also identified over 500,000 SNPs among 26 WCR populations. We used these SNPs to scan for outliers among the candidate genes, and to understand how population processes have shaped genetic variation in this species.

This study highlights the utility of transcriptomic and genomic resources as foundational tools for dealing with highly adaptive pest species. Using these tools we identified candidate gene families for insecticide resistance and reveal aspects of WCR population history in light of the species' recent range expansion.

## Use of genomic and transcriptomic data to facilitate germline transformation of the western corn rootworm

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Transformation-based applications such as transposon tagging, enhancer trapping and genome-wide mutagenesis have facilitated the genetic dissection of model species such as *Drosophila melanogaster*. Following this paradigm, we are developing a germline transformation system for the western corn rootworm, *Diabrotica virgifera virgifera*. We are currently using a *piggyBac*-based transformation system known to work in another coleopteran, the red flour beetle, *Tribolium castaneum*. However, unlike *Tribolium*, *D. v. virgifera* lacks eye-color mutants conventionally used to observe transgene expression. To overcome this obstacle we are using genome-editing tools (e.g. CRISPRs) to produce site-specific mutations (insertions/deletions) in a gene required for proper pigmentation of the eye. Moreover, in an effort to create a highly efficient transposon-based mutagenesis system we are attempting to introduce site-specific insertions of marked transposons. Our progress towards developing tools, techniques and protocols for *D. v. virgifera* germline transformation will be presented. Establishing transgenic technologies for this beetle is the first step towards bringing a wide range of transformation-based tools to bear on understanding *D. v. virgifera* biology, which can then be extended to other rootworm species.

## Diabroticina beetles: how can we explain their diversity?

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The subtribe Diabroticina encompasses almost 1,000 species mainly distributed in Mesoamerica and Brazil (Branson & Krysan 1981). All species are herbivorous but differ in diet breadth. The section Diabroticites and within this section the genus *Diabrotica* is the most diverse group. In order to understand which processes might have led to this diversity a phylogeny of a selected number of species was reconstructed (Eben & Espinosa de los Monteros 2004). Changes in host range were inferred from evolutionary scenarios based on the phylogenetic reconstruction. Polyphagous feeding and speciation co-occurred in the genus *Diabrotica*. The pattern of host use detected allowed us to hypothesize that host specialization is a transitory feature in the evolutionary history of this genus (Eben & Espinosa de los Monteros 2008). However, research on macroevolutionary patterns of diversity comparing species numbers within and between lineages needs to include a timeframe (Daza et al. 2010).

This study aimed to review possible processes that might have shaped the speciation patterns observed in three genera of Diabroticina. The sequences of five genome loci were compared for 44 species. A new phylogeny was reconstructed and times of potential isolation events were calculated based on coalescent analyses (Drummond & Rambaut 2007). With the results obtained we concluded that the onset of radiation in Diabroticina is not recent but probably occurred about 30 million years ago. Pharmacophagy, insect-host coevolution, competitive exclusion, geomorphological parameters, and agricultural practice are discussed as processes that could have influenced in the observed evolutionary-radiation patterns within the lineages studied (Eben & Espinosa de los Monteros 2013).

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## **Genotypic changes and wing shape variation associated with the invasion of southern Europe by western corn rootworm**

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Spatial and temporal genetic analyses of western corn rootworm (WCR) populations in southern Europe (Serbia, Croatia, Hungary and Italy) were conducted during the introduction and establishment/spread phases of WCRs invasion of this region. The results indicated that during the introduction phase genetic diversity and genetic structure were lower compared to the establishment/spread phase. Unusually high genetic differentiation was found between the Italy and southern European comparisons, including high differentiation between Italian populations separated by a short distance during the establishment/spread phase. Serbia was the geographic source of WCR to Croatia and Hungary in the introduction phase, while the U.S.A. was the source of WCR to Italy in 2001. Repeated introductions and admixture events in southern Europe have resulted in genetically diverse WCR populations that have attained 83% of all known alleles worldwide.

In addition to the genetic analyses conducted variation in hind wing shape was investigated using geometric morphometrics (GM). GM was used to examine the influence of soil type on WCR populations in Croatia that differed according to edaphic factors and climate and to investigate the potential presence of directional asymmetry on hind wings. Results indicated that WCR hind wing shape changed according to major soil type classifications in Croatia. The wing shape change found varied because of basal radial vein differences. These results indicate that WCR hind wing shape has the potential for use as a biomarker monitoring tool to differentiate populations. In an evolutionary context, the presence of directional asymmetry in WCR hind wings adds to the ever growing data available on the evolution of beetle wings.

Ongoing research by our group is focused on expanding our WCR wing shape database to include the majority of European populations and the inclusion of US source populations to confirm the utility of wing shape as a monitoring tool.

## Colonization history of the western corn rootworm in North America inferred from microsatellite data

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The western corn rootworm *Diabrotica virgifera virgifera* (WCR) was first described in 1867 in Fort Wallace, Kansas, close to the Colorado border, and was formerly named the Colorado corn rootworm. Since then, the species has been observed in a large part of North America, becoming a major pest of cultivated corn. Although the species is thought to be native from southern North America (Mexico or Guatemala), it was not described in this area before the 1950's. These few facts highlight a noticeable lack of knowledge about the precise origin and colonization history of the species in North America. Here, we used various population genetics methods, including Approximate Bayesian Computation, to retrace the colonization history of WCR in North America. This work is based on the analysis of the genetic variation measured at thirteen microsatellite loci of 917 individuals from 21 WCR populations. Our results confirmed that Mexico is probably the native area of the species, while the northern U.S.A. population corresponds to a single introduction followed by a large expansion. We also showed that the populations in southwestern U.S.A. (in Arizona, New Mexico and Texas States) were founded well before what was previously thought. Finally, the analysis of 62 individuals from two population samples of the Mexican Corn Rootworm, *Diabrotica virgifera zea*, suggested frequent gene flow between both subspecies. These results raise new questions about the evolutionary history of the *Diabrotica virgifera* species complex.

## **Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm**

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Insects are constantly adapting to human-driven landscape changes; however, the roles of their gut microbiota in these processes remain largely unknown. The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae) is a major corn pest that has been controlled via annual rotation between corn (*Zea mays*) and nonhost soybean (*Glycine max*) in the United States. This practice selected for a 'rotation-resistant' variant (RR-WCR) with reduced ovipositional fidelity to cornfields. When in soybean fields, RRWCRs also exhibit an elevated tolerance of antiherbivory defences (i.e., cysteine protease inhibitors) expressed in soybean foliage. Here we show that gut bacterial microbiota is an important factor facilitating this corn specialist's (WCR's) physiological adaptation to brief soybean herbivory. Comparisons of gut microbiota between RR- and wild-type WCR (WT-WCR) revealed concomitant shifts in bacterial community structure with host adaptation to soybean diets. Antibiotic suppression of gut bacteria significantly reduced RR-WCR tolerance of soybean herbivory to the level of WT-WCR, whereas WTWCR were unaffected. Our findings demonstrate that gut bacteria help to facilitate rapid adaptation of insects in managed ecosystems.

## Corn rootworm virus discovery from NGS and EST data

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Viruses are important insect pathogens and can be used as biological control agents for pest management. Viruses can also be used as tools for gene expression and toxin delivery. Nudivirus-like particles and piconavirus-like particles have been reported in *Diabrotica* (Kim & Kitajima, 1984, Degrugillier et al., 1991), but no viral sequences have been determined. To identify viruses from CRW, we analyzed genomic DNA sequence data for southern corn rootworm (SCR), northern corn rootworm (NCR) and western corn rootworm (WCR), and RNA-seq and EST data for WCR. A novel nudivirus (double-stranded DNA, dsDNA virus) was discovered from NCR genomic DNA sequence data. Nudivirus sequences were also found in SCR and WCR. In addition, sequences of a new ascovirus-like virus (dsDNA virus) were identified from SCR and NCR DNA sequence data. From the contigs assembled from WCR RNA-seq Illumina reads, we identified two novel positive sense, single-stranded RNA viruses. Additionally, on analysis of a WCR EST library, we identified Aphid lethal paralysis virus-like viral sequence. Our results indicate that CRW are infected by multiple DNA and RNA viruses, confirming earlier observation of viruses in *Diabrotica*. The newly discovered viruses may have potential for use in CRW management and for development of molecular tools for CRW research.

## Optimisation of a lacewing egg concentrator for common green lacewings (*Chrysoperla carnea* species-complex; Neuroptera: Chrysopidae)

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The common green lacewings (*Chrysoperla carnea* species-complex) are important predators of several pests, including aphids and different stages of other arthropods (e.g. moth eggs). This taxon is widely spread and comprises cryptic species the occurrence of which might vary with different regions. Some of these species are also used as biological control agents, and are commercially available. There are also efforts to maintain local populations (e.g. use of overwintering chambers for adult lacewings).

Adults of these lacewings feed on plant derived substances, and in accordance with their feeding habits several plant volatiles were suggested as attractant. Recently a ternary lure was developed which performed better in field experiments than previously known attractants.

The ternary lure attracted both males and females in field tests, furthermore, females were found to lay eggs in the vicinity of baits. Previous observations suggested that lacewings prefer to lay their eggs on the top of small protruding parts of plants, such as small spines, hairs. In the present study we aimed to test the effect of chemical and surface stimuli on oviposition site preference of green lacewings in field experiments.

The experiments were carried out in Hungary from 2008 to 2011. Artificial surfaces with different surface characteristics were compared for egg-laying preference of green lacewings.

The ternary bait showed a significant effect, lacewings laid significantly more eggs on surfaces where bait was also included. In the comparison of different surfaces spiny surface was preferred over smooth surface. This knowledge made possible to develop a lacewing egg concentrator in favor of enhancing concentrated egg-laying of common green lacewings on artificial surfaces together with the ternary bait.

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## **Ecologically based pest management of the oriental armyworm, *Mythimna separate*, in China**

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The oriental armyworm, *Mythimna separata* is one of the most important pests of agricultural crops such as rice, wheat and maize in China. Especially in recent years, with the increasing of the corn production area, it became the main pest of corn which caused severe yield production loss in China. This insect is very difficult to real time monitoring and accurately forecasting, resulting destroyed destruction of crops, because it undertakes a seasonal, long-distance, multigeneration roundtrip migration between southern and northern China. The regularities of migration, overwintering and occurrence of the species are well clarified by Chinese scientists. Based on these regularities, ecologically based monitoring, forecasting and management techniques were developed in China. Firstly, based on the insect pest long distance migration route across China, approximate 250 widely distributed monitoring station were built to collect the population dynamic information of the insect, and the emigrant and immigrant population dynamics forecasting model were developed based on the migration regularity; Secondly, because the oriental armyworm only overwintering in relative smaller place of South China, including the southern part of Guangdong, Fujian and Guangxi province, the production area of the wheat which is the main overwintering host of the species was significantly reduced, this ecological measure have changed the species overwintering habitat environment and decreased the total number of the oriental armyworm, therefore, have greatly reduced the population base of the overwintering generation, which determine the occurrence and damage level of the first generation of the species in central China. Thirdly, based on the species ecology and biology, some effective biological control measures including sex pheromone and light trap were also developed to control the species in China.

## **Predation of corn earworm *Helicoverpa zea* by the minute pirate bug *Orius insidiosus* in sweet corn**

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The corn earworm, *Helicoverpa zea*, is a polyphagous pest of many crops in North and South America. In the northern United States, moths that have migrated from overwintering sites in southern states will oviposit on sweet corn, where their larvae can cause significant economic damage to corn ears. However, biological control from a suite of natural enemies found in the upper Midwest can contribute to integrated management of this pest. The minute pirate bug, *Orius insidiosus*, a small hemipteran predator, has been shown to have an impact on pest populations in sweet corn (e.g., *Ostrinia nubilalis*) and other systems (e.g., soybean aphids in soybean). To determine the strength of this trophic interaction, *Orius* were collected from sweet corn fields in Minnesota before, during, and after the peak *H. zea* flight in 2011. The gut-contents of these predators were screened for the presence of *H. zea* DNA using species-specific PCR primers targeting the mitochondrial COI gene. Feeding trials determined that with this primer, the detection period of *H. zea* DNA in *O. insidiosus* was less than 8 h post-consumption of a single prey egg. Molecular gut-content analysis revealed that between 0% (on dates pre- and post-moth flight) and 32% (during peak moth flight in late August) of collected *Orius* were positive for *H. zea* DNA. Particularly given the short detection window of this molecular method, we believe these results indicate that *O. insidiosus* predation on *H. zea* is very strong during the period of peak moth flight, and could therefore contribute to controlling pest populations. The preservation or enhancement of *Orius insidiosus* and other generalist predator populations should therefore be considered in integrated pest management programs for sweet corn.

## **Predator behavioural responses to novel haemolymph defenses of western corn rootworm larvae**

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Many arthropod pests use defensive chemistry to discourage predators from attacking. This chemistry relies on the ability of predators to rapidly learn to recognize and avoid offensive stimuli. Western corn rootworm (WCR) employs multifaceted chemical defences in its haemolymph, which may contribute significantly to its success as a major economic pest. Here, we test the hypothesis that agrobiont predators can rapidly learn to recognize and avoid WCR larvae, and will thereby reduce their contribution to WCR suppression. In controlled feeding assays, the effectiveness of WCR haemolymph defences varied across three predator taxa (crickets, centipedes and ants). Centipedes were minimally affected by WCR defences, but crickets spent less time feeding on WCR relative to an undefended control prey, house fly maggots. However, we uncovered no evidence indicating that experienced crickets rapidly learn to avoid WCR larvae, indicating that haemolymph defences offer few, if any, survival benefits for WCR. Colonies of *Lasius* ants switched from solitary foraging tactics in initial attacks on WCR to group foraging tactics in subsequent attacks, indicating an attempt to overcome, rather than avoid, WCR haemolymph defences. These results suggest that a diverse community of natural enemies will show a diverse array of behavioural responses to toxic pest prey, and highlight the importance of behavioural diversity in driving the function of natural enemy communities.

## New application techniques for beneficial nematodes to better control rootworm larvae

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The use of soil insecticides and seed coating against the western corn rootworm, *Diabrotica virgifera virgifera*, is problematic due to non-target effects which resulted in a current ban from use in corn in most European countries. In an attempt to reduce insecticide use, a nematode-based biological control solution has been developed. Commercially mass-produced *Heterorhabditis* species of beneficial entomopathogenic nematodes are now available in several European countries. Recent studies aimed to find the optimal application technique for nematodes, i.e. being practical and effective at reasonable costs. Field studies revealed that nematodes can be applied into soil at sowing, this is, a few weeks before rootworm egg hatching; as well as into or onto soil along rows of young corn plants. Fluid and micro-granular applications as well as seed coating with nematodes achieve control of rootworm larvae as well as some root damage prevention. However, the easiest, and currently most promising technique is the fluid stream spray of a nematode-water suspension into soil at sowing with a low amount of water (200-400 l per hectare) through using sowing machines with applicators that apply nematodes behind the sowing or press wheel and prior the soil-closing wheels. Farmers may use their equipment for fluid soil insecticides, or may use the nematode-specific application tools such as LIQ-inject<sup>TM</sup> recently developed for the most common sowing machines. This allows to reduce the required nematode dose to  $2 \times 10^9$  per hectare, and thus the costs of this control technique. Nematode application together with fluid manure is also possible.

In conclusion, beneficial nematodes are ready to use against rootworm larvae in corn and are one of the first cases of a nematode-based biological control solution at field scale.

The studies were funded by the Ministry for Rural Areas and Consumer Protection of the State of Baden-Württemberg, Germany.

## Understanding the physiological and metabolic basis for lateral root re-growth as a mechanism for crop tolerance to *Diabrotica virgifera*

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Given the high resistance and adaptability of the western corn rootworm, *Diabrotica virgifera* to maize defense mechanisms, a promising strategy for sustainable pest management is the propagation of tolerant crop varieties that have the ability to maintain a high yield potential under herbivore pressure. Though research on crop resistance has been extensive extending back over decades, the underlying mechanisms for plant tolerance remain unclear even today. As a consequence, modern breeding programs cannot be leveraged in a rational way to generate new crop varieties with even greater tolerance traits. Here we explored the physiological and metabolic basis for ectopic branch root patterning as a tolerance response mechanism of maize plants to cope with root herbivore pressure using new investigational tools in plant science that include Positron Emission Tomography (PET Imaging) and radiometabolomic flux analysis that rely on the short-lived radioisotope <sup>11</sup>C (t<sub>1/2</sub> 20.4 min). Data will be presented that shows a strong temporal and spatial coordination between whole-plant carbon and nitrogen resource mobilization and allocation belowground with local root metabolism, and with pathway specific root auxin biosynthesis that tightly maps to lateral root growth. This research was supported by the Department of Energy, Office of Environmental and Biological Research.

## Chemical ecology of host selection in *Diabrotica*

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Attraction, host recognition and host acceptance are three chemically mediated behaviors involved in the process of host location by western corn rootworm larvae. After exposure to the roots of a host plant, larvae exhibit a unique tight-turning (localized search) behavior indicative of host recognition. Larvae exposed to roots of non-host plants do not exhibit this behavior.

We identified a blend of compounds from root extracts of *Zea mays* that elicits the tight-turning response by neonate larvae. The active blend contains monogalactosyldiacylglycerides (MGDG) and sugars (glucose, fructose, sucrose and myoinositol). The most abundant MGDG structures in the maize root extracts were determined, with LC-MS (positive ion scan), to be the 18:3-18:3, 18:3-18:2, 18:2-18:2 and the 18:2-16:0 species. In behavioral bioassays, activity of the isolated MGDG increased after subsequent exposures to groups of larvae, suggesting that the host recognition response is elicited by compounds that are produced after exposure of MGDG to larval salivary enzymes. In a follow up test, larvae exhibited the tight-turning behavior in response to synthetic blends of galactose, glycerol and free fatty acids, which are potential products of the enzymatic breakdown of MGDG. In the same experiment, larvae responded most strongly to blends containing only the 18:3 fatty acid.

Larval response to the maize root extract is typically 80-95%, which is comparable to the response elicited by germinating maize roots (90-95%). Although the isolated active blend (MGDG plus sugars) elicits a response by at least 60% of larvae tested, the difference between the active blend and the root extract indicates that other active compounds are present in the extract. We have partially isolated other active components, and determined that they include compounds from three different chemical classes. The first class are polar compounds isolated in a water fraction from a reverse phase C-18 chromatographic column. The second class are less polar compounds isolated in a methanol fraction from reverse phase C-18. Compounds in the third class were isolated in a chloroform fraction from a Florisil column separation of a hexane partition of the original root extract. This fraction may contain neutral lipids, steroids, hydrocarbons and ester steroids.

## Interactions of western corn rootworm larvae with soil microorganisms

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Despite the importance of the rhizosphere microorganisms, little is known about the multitrophic interactions between plants, microbial communities in the rhizosphere and root feeding insects. The rhizosphere and endorhiza are dynamic environments in which plant, fungi, bacteria, viruses, nematodes and herbivore insects interact with each other, influencing the agro-ecosystem functionality, and thus the sustainability of crop production systems.

We used greenhouse experiments to investigate the effects of WCR larval root feeding on fungal and bacterial communities in the corn rhizosphere. The presentation will specifically address the following questions: (i) does WCR larval feeding, due to altered root exudations, result in shifts in the microbial communities of the corn rhizosphere; (ii) does soil type and cultivar effect the composition of microbial communities inhabiting the digestive tract of the WCR larvae; (iii) which are the most dominant gut-associated microorganisms in WCR larvae; (iv) are these microorganisms transmitted by transovarial process and do they originate from the rhizosphere; (v) which are the dominant microbial populations associated with WCR eggs; (vi) does *Glomus intraradices* mycorrhization affect the composition of microbial populations in the rhizosphere and endorhiza of corn; (vii) does *Gl* mycorrhization of corn roots affect WCR larval fitness; (viii) does the feeding of WCR larvae alter the microbial communities in the endorhiza and rhizosphere of mycorrhized and non-mycorrhized maize plants; (ix) does WCR larval feeding affect the incidence and severity of root colonization by the plant pathogen *Fusarium verticillioides* and does this in turn influence development?

We analyzed internal transcribed spacers (ITS) and 16S rRNA gene fragments, amplified from TC DNA extracted from samples of guts and eggs of WCR larvae, and the corn rhizosphere microorganisms were analyzed by denaturing gradient gel electrophoresis (DGGE). Dominant microorganisms from these environments were identified by cloning and sequencing of ITS and 16S rRNA gene fragments.

We show that the microbial environment of western corn rootworm larvae should be taken into account when trying to understand the exceptional high adaptability of this pest.

## Rootworm interactions with host plants, artificial diet, and *Bt*

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The recognition of western corn rootworm (WCR) field-evolved resistance to *Bt*-producing maize has led to an increase in the development of screening assays to evaluating potentially *Bt*-resistant WCR populations. Several methods for evaluating field populations for *Bt* resistance have been published, ranging from on-plant assays to artificial diet toxicity assays. Plant based assays differ greatly in the length of the assay, the amount of corn seed and WCR eggs required per experiment, and the ability of the assay to detect resistance in a WCR population. Diet toxicity assays differ with each *Bt* toxin, with each *Bt* having a unique artificial diet, egg treatment regimen, and toxin solution. The use of different screening assays makes direct comparisons between WCR populations impossible. Therefore, the development of a unified technique for evaluating WCR populations in both plant based assays and diet based assays is needed. Here we report progress towards evaluating available plant based and artificial diet based resistance monitoring assays for consistency and ability to detect resistant WCR populations.

## **The genetic basis of host plant resistance in maize to the western corn rootworm**

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During the last 60 years more than 12,000 maize accessions have been screened for their level of resistance to western corn rootworm (WCR) larvae feeding. Less than 1% of this germplasm was selected for initiating recurrent selection programs. In general, the selected genotypes were characterized by large root systems and superior secondary root development after root damage caused by WCR larvae. However, no non-transgenic maize cultivars with high level of resistance under moderate to high WCR pressure have yet been released. To overcome this problem, we are in the process of evaluating the defense response of maize to WCR feeding in a coherent framework, which includes gene expression and metabolite analyses. In addition, we investigate the genetic basis of WCR resistance in new maize materials with improved levels of resistance using linkage disequilibrium mapping approaches. We found genomic regions contributing to WCR resistance on all maize chromosomes, except for chromosomes 2 and 4. A model fitting all QTL simultaneously explained about 30% of the phenotypic variance for root damage scores in both mapping populations. This underlines the complex genetic structure of host plant resistance against WCR larvae feeding in maize. Interestingly, three of these QTL regions also carry genes involved in ascorbate biosynthesis, a key compound, we hypothesize, based on our metabolite studies, is involved in the expression of WCR resistance.

## Modification of western corn rootworm larval behaviour with botanical based extracts

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The western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a major pest in corn fields. Larval feeding may cause severe damage to roots; nutrient and water uptake is therefore disrupted, lodging may occur at high pest densities. Indigenous to Central America, WCR has been spreading in Europe since its invasion in the early seventies' of the last century. Different approaches to control this pest have focused on the larval stage to reduce WCR population density on one hand and provide direct crop protection on the other hand. In Europe the development of sustainable control strategies for an integrated pest management (IPM) approach is necessary because transgenic crops are not available for the farmers. Our studies aim at implementing IPM modules based on a modification of WCR larval behaviour. Semiochemicals of botanical origin may act as repellents and disturb larval host location. Consequently larvae are stressed and are more susceptible to infection by biological control agents such as entomopathogenic fungi (EPF).

Eleven botanical compounds were screened in laboratory bioassays with 2<sup>nd</sup> instar WCR larvae; four acted as potential repellents to WCR larvae. In further screenings the essential oil of turmeric (*Curcuma longa*) was evaluated as the most promising repellent. The application of turmeric in a rhizotron modified larval activity by an increased larval movement and decreased feeding activity during a 4 day period. Further bioassays with different application rates have shown that neonate WCR larvae were repelled by lower application rates of Turmeric than 2<sup>nd</sup> instar larvae, making it more suitable for a potential application in the field.

As a conclusion a modification of larval performance is possible with a botanical compound. Future experiments will look into the potential to combine botanical extracts with EPF to enhance EPF infection and subsequently help to establish effective IPM strategies.

This study is part of the EU Project InBioSoil (Innovative biological products for soil pest control; <http://inbiosoil.uni-goettingen.de/>)

## **Identification of resistance properties of conventional maize cultivars against western corn rootworm larvae in a soil - less bioassay**

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We tested a new bioassay to evaluate the performance of western corn rootworm (WCR) larvae on conventional maize cultivars. Surface sterilised maize seeds were placed on a glass bead layer which was previously coated with a transparent agar/nutrient mixture to enable root growth. This set up creates a transparent medium for a direct observation of WCR behaviour and an easy and quick recovery of the larvae from the maize roots. Neonate larvae were placed on roots of a 17 day old seedling and the larval fitness and root damage was evaluated after 11 days of larval feeding.

38 encoded cultivars were screened in a series of laboratory experiments. A number of tolerant cultivars were identified, mainly expressed through an increased root growth upon larval feeding, leading to lower root damage. These cultivars, however, favoured larval fitness and would therefore not be suitable for a reduction in larval density. Some cultivars, on the other hand, had a negative effect on larval fitness through an increased larval mortality or reduced larval development. In some cases, however, higher root damage was observed despite a reduced larval fitness. The larvae must have increased their feeding activity to compensate lower root quality.

The screening of these cultivars highlighted different resistance properties by conventional maize cultivars either through lower root damage or reduced larval fitness, but never both parameters combined. In the end resistance properties of conventional cultivars should aim to lower root damage and reduce larval fitness for an incorporation into IPM strategies. The rapid and efficient screening procedure of this bioassay could contribute to identify such properties.

## **A root-produced quiescence factor may help to improve the shelf-life of entomopathogenic nematodes used in biological control**

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Plant-nematode interactions are partially shaped by the exudation of secondary metabolites by roots. The root cap exudates of green pea (*Pisum sativum*) trigger a state of metabolic dormancy in various genera of nematodes. Originally shown to hinder plant parasitic nematodes, such exudates also induce quiescence in four species of beneficial entomopathogenic nematodes (EPN). We studied various aspects of the phenomenon in detail for the EPN *H. bacteriophora* in order to determine if it can be exploited in biological control. The induction strength of quiescence varied among the green pea cultivars that we tested, but it was evident that EPN infectiousness, mobility and lipid reserves were better maintained over time after they experienced quiescence during storage in root exudates. Interestingly, low concentrations of the exudate resulted in a significant increase in activity and infectiousness in EPN, but still induced quiescence in two plant-parasitic nematode species. One could speculate that these contrasting effects, under natural conditions, boost the effectiveness of EPN, but impair plant parasitic nematodes. To further explore the potential of exploiting the active compounds in the root exudates for pest control we developed a high-throughput extraction method for deep-frozen root tips from green pea and maize. We obtained highly concentrated extracts that reliably induced reversible quiescence in EPN, which remained infectious after recovery. Efforts to identify the quiescence inducing compounds are underway. We are confident that this research will eventually lead to new applications for the biological control of soil-dwelling root pests.

## Interactions among *Bt* maize, entomopathogens and the western corn rootworm

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How *Bt* crops interact with natural enemies, especially insect pathogens in below-ground systems, is not well understood, but provides a unique opportunity to study below-ground tritrophic interactions. We examined tritrophic interactions among *Bt* maize, the western corn rootworm (*Diabrotica virgifera virgifera*), and a blend of entomopathogens. In a greenhouse experiment, the effects of *Bt* maize producing Cry34/35Ab1 and entomopathogens on western corn rootworm mortality were tested in a fully crossed design (*Bt* maize and non-*Bt* maize crossed with presence and absence of a blend of entomopathogens). The community of entomopathogens significantly increased mortality of western corn rootworm, and *Bt* maize increased larval developmental time and mortality. Entomopathogens and *Bt* maize acted in an independent and additive manner, with both factors increasing the mortality of western corn rootworm. A follow-up two year field study was conducted to determine how a blend of entomopathogens interacted with *Bt* maize producing Cry34/35Ab1 to affect rootworm mortality, root injury to maize, and yield. *Bt* maize decreased root injury and survival of western corn rootworm and northern corn rootworm but did not affect yield. During year one of the study, when rootworm abundance in field soil was high, entomopathogens in combination with *Bt* maize led to a significant reduction in root injury. In year two of the study, when rootworm abundance was lower, entomopathogens significantly decreased injury to non-*Bt* maize roots, but had no effect on *Bt* maize roots. Yield was significantly increased by the addition of entomopathogens to the soil. However, entomopathogens did not decrease survival of corn rootworm species. The results of this study suggest that soil-borne entomopathogens can complement *Bt* maize by protecting roots from feeding injury from corn rootworm when pest abundance is high, and can decrease root injury to non-*Bt* maize when rootworm abundance is low.

## The impact of the root architecture on foraging behavior biological control agents

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As obligate parasites, entomopathogenic nematodes (EPN) rely on insects to complete their life cycle. EPN foraging behavior has been relatively well characterized, but little is known about the physical role of roots in this process. Therefore, the impact of root architecture on foraging *Heterorhabditis megidis* was assessed. Insect baits were attached to the bottom of metal-roots, with three degrees of architectural complexity, and placed in pots with moist sand. 36h after the EPN injection, the baits were recovered and stored for four days. The numbers of EPN-infected baits and the counts of mature nematodes inside cadavers were recorded. Root architecture impacted EPN foraging as more baits were infected in pots with metal-roots than in sand only. However, the number of EPN in baits significantly decreased with increasing root complexity, suggesting a negative effect of root architecture on host finding ability of *H. megidis*. Yet, the addition of (*E*)-BETA-caryophyllene, a root volatile attracting this EPN, resulted in a dramatic shift in the nematode response; more nematodes were found in baits when spiked with (*E*)-BETA-caryophyllene and this cue significantly counterbalanced the negative impact of root complexity on mature EPN counts. Natural root system architecture also impacted EPN behavior. The numbers of nematodes were significantly different at the bottom of two maize root systems with divergent architectures. These results underpin the importance of roots in EPN effectiveness. Root architecture is therefore not important for the plant only but also impacts higher trophic levels and potentially insect pest management.

## **Invasion biology: insights from evolutionary biology and population genetics**

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Insect invasion biology and agriculture are intimately related because many agricultural insect pests are recent invaders. In this presentation, we propose to summarize some of the main results that were recently obtained in ecology and evolutionary biology that are important to understand invasions. We also present population genetics studies on invasion routes whose conclusions lead to fundamental questions in ecology and in insect pest management. In particular, we focus on several aspects of the invasion process at the population level that might help understanding the success of invasion: multiple introductions, genetic bottlenecks, admixtures and the bridgehead scenario. To illustrate these points, we mainly focus on the western corn rootworm and the harlequin ladybeetle invasion history. The bridgehead invasion scenario, in which an invasive population is the source of other invasive populations, is evolutionarily and ecologically parsimonious, and may have played a crucial role in insect invasion biology. The question of the role of multiple introductions and admixture is discussed in an evolutionary context.

## ***Helicoverpa armigera* in Brazil**

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The Embrapa Cerrados's work with *Helicoverpa armigera* will be presented. In the crop season of 2012/2013 collections were done in the states of Bahia, Distrito Federal, Mato Grosso and Paraná, Brazil. Moths were obtained from larvae collected in cotton, corn, soybean crops and light traps. Identification was based on male genitalia and analysis of mitochondrial gene sequences of cytochrome B and the region *cox1-tRNALeu-cox2* from the same specimens. The male genitalia were compared with the morphological description in the literature, and the gene sequences with gene sequences deposited at GenBank. In both analyses the presence of *H. armigera* was confirmed. The detection of this species in Brazil was the first report on the American continents. Since then, several studies were initiated considering the economic damage observed in corn, soybean, cotton, tomato, and dry bean crops. The topics of research are related to taxonomy, ecology, behavior and development of the management tools for *H. armigera*. Special focus was directed on the characterization of the morphology of adults and immatures of *H. armigera* and *H. zea*; bionomics of *H. armigera*, including pupal diapause studies and host preference; landscape effects on *H. armigera* considering adult movement and the spatial and temporal effect of different host crops on its infestation; and interspecific competition and niche prevalence among newly introduced *H. armigera* and the key Brazilian polyphagous pests *H. zea* and *Spodoptera frugiperda*.

## Risk and potential impact of *Helicoverpa armigera* in the U.S.

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The Old World cotton bollworm, *Helicoverpa armigera*, is a highly polyphagous and damaging agricultural pest that has recently become established in Brazil. Historically, the pest has been limited in distribution to Old World countries, including China and India, some EU and African countries, and Australia. The pest feeds on over 180 plant hosts (> 45 families), including several major crops of global importance (corn, soybean, cotton, sorghum, tomato), and has a propensity for developing resistance to conventional insecticides and *Bt* crops. Given the availability of cotton, corn and soybean in Argentina, Brazil, Paraguay and Uruguay, further range expansion of the pest in South America appears likely. Also, given the mobility of the pest, the risk of subsequent establishment in Central and North America is a growing concern. In addition, via international trade, the U.S. has documented over 1023 detections of known *H. armigera* specimens at ports and borders since 1985 (avg. of ~36 interceptions/year). Given the long-term biosecurity risk of *H. armigera* to the Americas, we used CLIMEX, an ecological niche model, to assess the potential for *H. armigera* to establish and sustain population growth in the U.S. We used 30-year climate scenarios and population growth and stress parameters (e.g., heat stress, lower and upper temperature thresholds, moisture stress), to update a previously published model (Zalucki & Furlong 2005). The results suggest that based on the Ecoclimatic Index (EI) there is a high potential for establishment in the southeastern U.S. and western coastal states. These results are consistent with risk maps developed for *H. armigera* by USDA-APHIS in support of the Cooperative Agricultural Pest Survey Program. The annual Growth Index (GI<sub>A</sub>) also suggests, that assuming long-distance migration south-north (via low-level jet systems), that significant summer populations of bollworm could be active in the north central U.S. Corn Belt, as well as the northeastern U.S., reflecting production risks to corn, soybean, sweet corn, tomato and other vegetable crops. A preliminary economic impact on selected crops will be discussed.

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## **The establishment and management of western bean cutworm *Striacosta albicosta* in Canada**

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Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera, Noctuidae) is a pest of corn, *Zea mays* (L.) and beans, *Phaseolus* sp. (L.) historically known to inhabit the Great Plains region of the United States. The northeastern range expansion of *S. albicosta* into the Great Lakes region has been documented through a coordinated pheromone trapping network, field surveys, and overwintering studies. An overview of research on the biology and management strategies of this new corn pest in Canada will be presented.

## **Cropping system transformation makes *Athetis lepigone* to be a new insect pest of corn in China**

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*Athetis lepigone* (Möschler) (Lepidoptera: Noctuidae), is distributed across in Europe, as well as Japan and China. Reports about this insect are almost only the species records, even no host plants described before the larvae was damage on summer corn seedlings in Hebei of China in 2005. It has soon observed damage corn in Shandong in 2007. In 2011, an outbreak was reported in six provinces in summer corn region in China, covered an area about 2.2 million ha. The larvae are mainly as scavengers that feed on plant residues, such as fallen and decayed leaves. *Athetis lepigone* have four generations a year, but only the 2nd generation is the most seriously damage on summer corn seedlings in the non-tillage fields with thick stalks after wheat harvest. This generation larvae occur in the late June to early July. The 1st generation occurs mainly in winter wheat field. The larvae feed on the decayed or old wheat leaves covered the soil surface surrounding the wheat stalks. The peak time of 1st generation adult is just about the wheat harvest. As new cropping system transformation is adapted, e.g. summer corn directly sown with non-tillage after wheat harvested. The wheat straw and chaff covered the soil surface provide suitable habitat for *A. lepigone* as that the larval survival and adult reproduction prefer in a hidden and wet habitats. Large number of wheat grains remains in fields provides germinant wheat and volunteers as foods for *A. lepigone* larvae. When the larval density is high and the corn seedlings less than 3 leaves, the larvae make serious damage. Germinant wheat is the best food for *A. lepigone* development, survival and reproduction, and results in high population of 2nd generation. It could explain why only the 2nd generation larvae damage more seriously on summer corn seedlings. The 3rd and 4th generations occur in summer corn and other crop fields with less damage. The 4th generation larvae overwinter in various fields where high plant densities appear correlated with higher larval densities, especially soybean, cotton fields. Most of cotton fields do not be ploughed up in autumn in recent years, provide a suitable habitat for overwintering larvae. The higher survival of overwintering larvae provides a higher base for the 1st generation of *A. lepigone*. These cultural practices might favor *A. lepigone* to be a new pest of summer corn seedlings in China.

## Increased maize production in DPR Korea through *Trichogramma* mass production technology transfer from China

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Maize is the second most important crop in DPR Korea but yields are threatened by pest and disease attacks. The most serious insect pest is the Asian Corn Borer, causing yield losses typically in the range of 10-30%. Field experiments were conducted on three cooperative farms to evaluate the potential of *Trichogramma ostriniae* to successfully control this key pest in DPR Korean maize fields. In line with experiences in China and further countries, *T. ostriniae* releases reduced damage of maize plants and cobs caused by the Asian corn borer and increased grain maize production significantly. When released five times against the two corn borer generations (750,000 parasitoids/season), the increase in fresh yield was 28.2% for all farms and the two study years.

To increase maize yields on a larger scale, 24 facilities mass-producing *Trichogramma* were established in 21 counties of four different provinces in DPR Korea. Based on Chinese technology, a production design was developed, strongly reflecting local conditions such as limited supply of electricity. Supported by Chinese and international partners, the assembly of rearing equipment was done locally as much as possible. To improve the sustainability of operations, quality control procedures, owner agreements and a business plan were implemented and strong ownership of the County Farm Management Boards in DPR Korea ensured. Key to success was further the training of staff involved in rearing as well as farmers releasing the biological control agent. Towards the end of the three year's project, a total of 240 kg of *Trichogramma* were produced on a yearly basis, allowing mass releases in 13'440 ha of maize annually to keep the Asian corn borer under control. Based on this successful increase of yields without much additional input, the DPR Korean government decided to upscale this biological control based IPM approach even further to an area of 200,000 ha. With a potential yield increase of about 0.8 tons/ha, the *T. ostriniae* releases may contribute significantly to stabilize the country's maize production as well as support community efforts to avoid reversion into a food emergency situation.

**Session 8-T 2 - Science-based knowledge transfer to improve maize production and enhance food security in developing countries**

**Intra-regional transfer of biologically-based plant protection technology to improve livelihoods of smallholder maize farmers**

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Maize, along with rice and wheat, provides a third of the caloric intake for more than 4.5 billion people in 94 developing countries around the world. This includes 900 million poor consumers, 50-100 million poor farm families and a third of all malnourished children. As such, maize plays an important role in the livelihoods of low-income smallholder farmers in the Greater Mekong Subregion. Lepidopteran pests, such as the Asian corn borer (*Ostrinia furnacalis*) and the corn earworm (*Helicoverpa armigera*), are the most prevalent insect pests in maize cultivation in the tropics/subtropics. The rural smallholder farmers usually lack the resources and support to take appropriate plant protection measures. While the majority of smallholder maize farmers in Myanmar and southwestern China still use conventional pest control methods and rely heavily on broad-spectrum insecticides, farmers in Laos have virtually no access to any commercial plant protection products.

Funded by DG EuropeAid (DCI-ASIE/2011/261-127), a joint action involving agricultural research, development and extension institutions as well as a commercial biological control agent production company was initiated in the three target countries to strengthen intra-regional linkages and sharing of plant protection technology through south-south partnerships. Using an innovation systems approach, the action ensures that one of the weakest and often excluded groups, the smallholder maize farmers, will be brought into the mainstream of technology transfer. In addition, smallholder farmers will become key actors in establishing/strengthening effective grassroots organisations, which will be instrumental for providing biological control agent and other essential agricultural inputs and marketing services. Through the transfer of a biologically-based plant protection technology and the local production of *Trichogramma* wasps, the action will reduce dependency upon the use of chemicals and enhance the environmental sustainability of maize production. Furthermore, the development and promotion of a maize IPM Technical Guideline as a production standard will improve agricultural governance and support the large-scale implementation of sustainable agricultural practices in the region.

Session 8-T 3 - Science-based knowledge transfer to improve maize production and enhance food security in developing countries

## Maize production, pests status and potential solutions to strengthen productivity through IPM capacity building in Lao PDR

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Maize is currently produced on approximately 100 million hectares of land in 125 developing countries. Along with rice and wheat, it provides a third of the caloric intake for more than 4.5 billion people in 94 developing countries around the world. This includes 50-100 million poor farmers and as such, maize plays an important role in the livelihoods of millions of low-income smallholder farmers in Asia. In Lao PDR, maize is the most important crop after rice, being used as both animal feed and for human consumption. Annual total production for 2010 was calculated as 5,296,000 tons. It is estimated that over 90,000 farmers are involved in maize production on an area of 200,000 ha, with farm sizes ranging from 1-3 ha. Yields in the target countries are well below potential production levels and a realistic maize target in Lao PDR and Southeast Asia would be around 6 tons/ha.

Lepidopteran pests, such as the Asian corn borer (*Ostrinia furnacalis*) and the corn earworm (*Helicoverpa armigera*), are the most prevalent and destructive group of insect pests in maize cultivation in the tropics/subtropics. Within the scope of a DG DEVCO EuropeAid funded project supporting technology transfer through south-south partnerships, these major threats to maize production in the area have been addressed. The technology transfer has been accompanied by a strong capacity building component for the production of a bio-control agent, *Trichogramma* to be applied in an IPM approach. Knowledge transfer first focussed on issues like exposure and exchange in order to make communities and individuals aware of problems and possible solutions to the problem. During this project phase, an awareness creation tour was organised for participants of the local farming communities as well as for representatives of the policy level supposed to becoming involved in the activities. There were several steps of practical hands-on training in parallel to the establishment of a pilot rearing facility with a training room. This later on served for training of the local rearing staff, with the support of the Chinese specialist for *Trichogramma* production.

Besides the capacity building for *Trichogramma* production personnel, the project will as well focus on the training of farmers in the application of the bio-control agent.

However, due to the special situation in the Lao maize production within the project, and the fact that not all the communities are facing problems with ACB, the most important part of the training will focus on the transfer of skills and knowledge about maize IPM in general. Another important problem, the maize storage pest *Sitophilus zeamais*, the grain weevil can in this way be targeted too and important topics as store hygiene and handling of residues can be included in the training efforts. As described for the general IPM training, getting messages about maize storage into action will depend on very well comprehensible messages as well as practical hands-on activities in order to get improvements implemented at a large scale. As it is set up, with the support of the national extension system, maize IPM will reach numerous maize producing communities in Lao PDR and the application of maize IPM has a great potential of improving the situation of the maize producing smallholder farmers' families.

## Maize production, pests status and potential solutions to strengthen productivity through IPM capacity building in Yunnan province, China

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China is the second largest producer of maize in the world. Maize has become the first grain crop both in acreage planted and total yield in China since 2012. In 2013, 36.12 million hectares of maize were grown, producing a total yield of 218 MMT. Maize growers in China are farming relatively small plots. The total production area is shared among 100 million maize growers. The Asian corn borer (ACB), *Ostrinia furnacalis*, is the most serious insect pest of maize that occurs in most maize-growing areas in China. Annual loss due to this insect ranges from 6 to 9 MMT. The direct yield losses arise from ACB damage, whereby the large impact indirectly arises from larval feeding on silks and kernels that leads to ear rot, increasing mycotoxin contamination and reducing grain quality. Other lepidoperan pests of concern for maize in China include *Helicoverpa armigera* and *Mythimna separata*. *Trichogramma spp.* are widely used to control ACB in northeast China, and at a smaller scale against *H. armigera* in summer maize growing areas of North China.

Yunnan province, adjacent to Laos and Myanmar, belongs to Southwest Hilly Maize Region, the third most significant maize planting region in China. 1.46 million hectares of maize were planted with a total yield of 7 million tons in 2012, meanwhile, the total damaged area by maize insect pests was 767'000 hectares with yield losses of 250'438 tons in the same year. ACB and *H. armigera* are also the most important insect pests of maize in Yunnan. Yield losses due to ACB were 67'900 ton. Unlike maize production in other areas of China, where the maize is planted in flat land (e.g. North spring corn region and Huang-Huai-Hai Summer Corn Region), the maize in the Yunnan provinces mainly planted in hilly land. It is difficult for the smallholder maize farmers to control the maize insect pests by spraying pesticides. Some of the farmers do not take any control measures due to technical difficulties, and some of them use conventional pest control methods and rely heavily on broad-spectrum insecticides.

Funded by DG EuropeAid (DCI-ASIE/2011/261-127), a joint action involving agricultural research, development and extension institutions as well as a commercial biological control agent production company was initiated in the Greater Mekong Subregion, to strengthen intra-regional linkages and sharing of plant protection technology through south-south partnerships, including public/private partnerships. In Dehong District of Yunnan province, a pilot facility for *Trichogramma* wasps mass rearing adapted to the local condition was established in Mangshi. Another four facilities for *Trichogramma* wasps production are under construction in Yingjiang and Longchuan counties. At the same time, monitoring for the occurrence of the ACB in these areas are also carried out by using ACB sex pheromone traps in order to determine the time for *Trichogramma* release. When established, each facility will produce enough biological control agents to treat a total area of approximately 800-1200 ha in subsequent years. The facilities will be handed over to local grassroots organizations and operated by them. Through the transfer of a biologically-based plant protection technology and the local production of *Trichogramma* wasps, the smallholder maize farmers will get more benefits by reducing the yield loss caused by ACB and *H. armigera*. Furthermore, the biologically based maize IPM approach will be transferred in other regions of Yunnan and other provinces in the Southwest Hilly Maize Region of China. This is in compliance with the strategy of Green Control for Corn Borer in China recently promoted by the Ministry of Agriculture of China.

## **Plantwise - a global alliance for plant health support: maize as an example**

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Plantwise is a global programme, led by CABI, to increase food security and improve rural livelihoods by reducing crop losses. Working in close partnership with relevant actors, Plantwise strengthens national plant health systems from within, enabling countries to provide farmers with the knowledge they need to lose less and feed more. As Plantwise addresses all problems on all crops, it applies equally well to a specific crop like maize. This is achieved by establishing networks of plant clinics, based on a similar approach to human health clinics, where farmers can find advice to manage and prevent crop problems. Agricultural advisory staff trained as plant doctors learn methods to identify any problem on any crop brought to the clinics, and provide appropriate recommendations guided by national and international best practice standards. Plant clinics are reinforced by the Plantwise knowledge bank, a gateway to actionable plant health information with online and off-line resources for pest diagnostic and advisory services. Plant clinic records are collated and analysed to support the quality of advice given to farmers and inform decision-making. By putting knowledge into the hands of smallholder farmers, Plantwise is able not only to help them lose less and feed more, but also to gather data which can assist all stakeholders in the plant health system- from research, agro-input supply, extension and policy-making. Most importantly, Plantwise is a development programme which cooperates with a number of international and national organisations working to remove constraints to agricultural productivity. By enabling partners to embed and sustain networks of plant clinics to help farmers, national plant health systems are growing stronger and building functioning link between actors. Countries are now utilizing knowledge bank resources to improve national vigilance. As a result of the Plantwise programme, increased numbers of women and men working in agriculture will have access to appropriate, timely and locally-relevant knowledge on plant health management for generations to come.

## Possible explanations for resistance development of the African stem borer to *Bt* maize

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The African maize stem borer (*Busseola fusca*) developed resistance to *Bt* maize (Cry 1Ab) in South Africa, 7-8 years after release of *Bt* maize in the country. Several factors that may have contributed to resistance development have been identified. The insect resistance management (IRM) strategy recommended for stem borers is the high-dose/refuge strategy. Poor compliance to refuge requirements was blamed for resistance development after surveys showed that farmers only started to comply with requirements, 8 years after release of *Bt* maize. Non-compliance can however not be the only reason for resistance development. Resistance development highlighted huge gaps in knowledge of pest biology (for example migration patterns) and interactions in the wider agro-ecosystem (indigenous host plants) and resistance inheritance patterns. Seed mixture strategies that are being considered as IRM strategy have previously been considered not to be suitable for migrating pests. Results will be presented on migration patterns of *B. fusca* larvae inside different seed mixture treatments over time. Increased damage over time in seed mixtures is associated with migration of older larvae, indicating that the expressed dose does not kill larvae above a certain developmental stage. Migration of larger larvae late in the season may result in survival of individuals that developed on non-*Bt* plants and then migrated to *Bt*-plants, thereby contributing to survival of RS individuals and resistance development. IRM in Africa is faced by challenges unique to farming in subsistence and small scale systems where refuge requirements are unrealistic and difficult to manage and monitor. Furthermore, factors such as seed management practices, gene-flow to maize land races could be contributing factors to resistance development. Alternative IRM strategies such as seed mixtures may be more practical but have its own challenges and have not been proven successful yet.

## Effect of Cry1F corn on the behavior of susceptible and resistant fall armyworm and European corn borer

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Understanding the behavior of pests targeted with *Bacillus thuringiensis* (*Bt*) crops is important to define resistance management tactics. Particularly, the study of larval movement between plants is important to determine the feasibility of different refuge configurations. Studies suggest that exposure to *Bt* corn increases larval movement in several lepidopteran species. However, few studies have examined the potential for resistance to interact with behavioral responses to *Bt* toxins. Choice and no-choice experiments were conducted with *Spodoptera frugiperda* and *Ostrinia nubilalis* to determine if Cry1F resistance influences neonate movement. Leaf discs of Cry1F corn TC1507 and the corresponding isolate were used to characterize behavioral responses. In both experiments the location (on or off of plant tissues) and mortality of susceptible and Cry1F resistant neonates was recorded for five days. Analysis of larvae location was performed up to 7h after transfer to avoid mortality. Our results indicated that there is not a strong difference between resistant and susceptible phenotypes in *S. frugiperda* and *O. nubilalis*, although, a small percentage of susceptible neonates in both species abandoned corn tissue expressing Cry1F. However, significant behavioral differences were observed between species. *Ostrinia nubilalis* exhibited increased movement between leaf discs, while *S. frugiperda* selected plant tissue within the first 30 minutes and remained on the chosen plant regardless of the presence of Cry1F. *S. frugiperda* behavior observed in this experiment suggests that lack of larval movement may have implications to refuge configuration. This study represents the first step towards understanding the effects of Cry1F resistance on larval behavior. Information regarding behavioral differences between species could aid in developing better and more flexible resistance management strategies.

## Effects of elevated atmospheric CO<sub>2</sub> on interactions between maize and the Asian corn borer

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Due to the 'fertilization effect', elevated atmospheric CO<sub>2</sub> generally mediates dilution of folia nitrogen--a limiting nutrient for insect herbivores. In turn, herbivores may increase consumption rates to compensate. Understanding response of both plants and insects to elevated CO<sub>2</sub> atmosphere should help to elucidate the mechanistic bases of the effects of elevated CO<sub>2</sub> on interactions of insects with plants. The effects of elevated atmospheric CO<sub>2</sub> on growth development of Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), a key pest of maize in China, as well as its damage to maize plants were examined in the open-top chambers (OTC). Maize (XY335, DuPont Pioneer Hi-Bred) was planted in three levels of atmospheric CO<sub>2</sub> OTCs., i.e., ambient (~390 µL/L) and elevated CO<sub>2</sub> (550 and 750 µL/L). Plants were artificially infested with neonates of ACB either at V8 or silking stage. Plant growth and chemical compositions as well as damage and fitness of ACB larvae were estimated. Elevated CO<sub>2</sub> resulted in a negative effect on the N content, but a positive effect on the C content in maize plants. Compared to ambient CO<sub>2</sub>, the ratio of TNC: N was increased by 8.5-35.3% in two elevated CO<sub>2</sub>, the plant height and average kernels per ear increased 4.0-5.1% and 4.3- 4.5% in elevated (750 µL/L), respectively. The damage of 1<sup>st</sup>-generation ACB infestation significantly reduced plant height by 5.5-5.6% at ambient and 550 µL/L, but only 1.8% at 750 µL/L. Elevated CO<sub>2</sub> significantly reduced 2<sup>nd</sup>-generation larval survival by 16.5- 21.0%. The average weight gain per larva (2<sup>nd</sup>-generation, diapause) declined significantly 13.0-16.1% when larvae fed on plant grown at elevated CO<sub>2</sub> atmosphere. While the damage (number and length of tunnels) caused by ACB to plants did not been significantly influenced by elevated CO<sub>2</sub>. The results suggest that the nutritional quality of maize plants will decline while growing in the elevated atmospheric CO<sub>2</sub> environments; the development and survival of ACB larvae are adversely affected by CO<sub>2</sub>-mediated lower maize plant suitability; ACB larvae may increase food intake to compensate, however, this will be offset by both 'air-fertilizer' effect for the plant and lower fitness of the larvae on the plants.

## **'I fly from nearby': characteristics of airborne western corn rootworm beetles ascending from *Bt* cornfields**

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A portion of the western corn rootworm (WCR) beetle population that emerges in a cornfield will eventually disperse from their natal field. Scaffolding towers (7 m tall) were used to collect Champaign Co., Illinois WCR as they ascended from *Bt* cornfields with 5% block or seed blend refuges. Flying insects, along with insects collected in the cornfield canopy below collection towers, were collected and preserved for measurements and analyses. We found that the high-flying WCR population is dominated by recently-mated females. Analyses of Cry proteins in gut contents revealed that 43% of flying WCR originated from the field beneath the collection tower; a similar proportion originated from other nearby fields. Many characteristics of flying WCR differ from WCR collected in the canopy. Combined with in-field measurements, studying WCR that ascend from cornfields may improve our understanding of the movement and long distance dispersal potential of all WCR populations—including those with rotation resistance and resistance to *Bt* corn hybrids.

## ***Bt* resistance in western corn rootworm: a model for understanding pest management with *Bt* crops that are not high dose**

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The western corn rootworm, *Diabrotica virgifera virgifera*, is a major pest of maize in the United States and is currently managed with maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*). Recent cases of field-evolved resistance to *Bt* maize by this pest offer insights into the challenges that can arise when a *Bt* crop does not produce a high dose of toxin. Additionally, interactions between *Bt* maize and western corn rootworm provide a model for considering how pests might be managed with *Bt* crops that are less than high dose. In this talk, I will address ecological and evolutionary factors affecting interactions between western corn rootworm and *Bt* maize, and approaches for managing this pest. Central themes will include the evolution of *Bt* resistance in the field by western corn rootworm; tritrophic interactions among *Bt* maize, larval western corn rootworm and soil entomopathogens; fitness costs of *Bt* resistance; and the application of *Bt* maize as part of an integrated pest management strategy for western corn rootworm.

## **Wireworms, an increasing threat to small grain crops in the western United States**

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Prior to the “insecticide era” beginning in the 1950s, wireworms were important historical pests. After the 1950s their populations in the soil were suppressed by the use of conventional organophosphate and organochlorine insecticides that created a low pest status, resulting in little integrated pest management (IPM) research for nearly 40 years. Since the removal of lindane as a seed treatment in 2007, small grain producers in Washington, Idaho and Montana have reported increasing levels of crop damage by wireworms. Current challenges to their management will be reviewed, including the lack of basic pest biological and ecological knowledge. Field trial results conducted in Montana during the last three years will be summarized, including a statewide survey of the species causing damage.

## First results of an 'Attract-and-Kill strategy' to reduce wireworm infestation

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Wireworms are the larvae of click beetles and an important group of soil dwelling pests. They attack the subterranean parts of a wide range of crop plants and have recently become a serious problem in many cultures, mainly due to the lack of proper control options for this soil-borne pest. ATTRACT is a project that focusses on the development of an innovative technology, taking advantage of the fact that wireworms orientate towards their host plants via detecting root CO<sub>2</sub>-exudates. Providing an alternative CO<sub>2</sub>-source ('attract'-component) combined with a biocide such as Neem or Quassia as 'kill'-component may be the key to efficient wireworm pest control, especially since insecticides in susceptible crops like potatoes and maize are no longer available. First experiments performed on a laboratory scale have shown that 'Attract-and-Kill'-formulations affect wireworm vitality and have the potential to kill them. These preliminary results have been confirmed in field experiments conducted at three sites in Lower Saxony. The damage level of wireworm infestation in organically managed potato fields can be reduced by the application of 'Attract-and-Kill'-formulations. Our current aim is to improve present formulations and enhance the attractiveness of the capsules by adding phagostimulants, this leading the wireworm towards the capsule and elicit a bite reflex.

The development of an 'Attract-and-Kill'-strategy to control wireworm infestation will contribute to reduced pesticide inputs in sustainable agricultural-horticultural systems and offer potential savings for growers.

## **Increasing predator diversity and the strength of trophic linkages to a target pest**

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There remains some debate over whether increasing predator diversity promotes or detracts from biological control of a focal pest within agroecosystems. Practices that conserve generalist predators within agroecosystems often inherently increase the diversity of predators within a habitat. Although increased predator abundance and diversity likely inflicts greater mortality on a focal pest, this also likely increases intraguild interactions that could weaken potentially important trophic linkages within the pest-based food webs. The corn rootworm, *Diabrotica virgifera virgifera*, has recently been targeted in a conservation biological control program that involves implementing winter cover crops to aggregate predators within the corn agroecosystem (relative to leaving the soil bare during the fallow period). Predator abundance and diversity increased substantially in the cover-cropped system. We examined how predator diversity and conservation efforts affected the strength of trophic interactions between the target pest and its natural enemies using qPCR-based gut analysis of the predator community. There remains some debate over whether increasing predator diversity promotes or detracts from biological control of a focal pest within agroecosystems. Practices that conserve generalist predators within agroecosystems often inherently increase the diversity of predators within a habitat. Although increased predator abundance and diversity likely inflicts greater mortality on a focal pest, this also likely increases intraguild interactions that could weaken potentially important trophic linkages within the pest-based food webs. The corn rootworm, *Diabrotica virgifera virgifera*, has recently been targeted in a conservation biological control program that involves implementing winter cover crops to aggregate predators within the corn agroecosystem (relative to leaving the soil bare during the fallow period). Predator abundance and diversity increased substantially in the cover-cropped system. We examined how predator diversity and conservation efforts affected the strength of trophic interactions between the target pest and its natural enemies using qPCR-based gut analysis of the predator community.

## Evaluating the risks posed by RNAi crops to pollinators in an agricultural landscape

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RNA interference (RNAi) is the process by which small RNA molecules destroy a specific mRNA in order to inhibit expression of a particular gene. Currently, this process is being incorporated into corn so that plants will be able to produce insecticidal RNAs targeting the western corn rootworm (*Diabrotica virgifera virgifera*). While the technology promises to be highly specific towards the target pest, there are several hazards associated with RNAi-based GM crops to beneficial insects that have yet to be assessed, including non-target binding, off-target binding, saturation of a non-target organisms' RNAi machinery, and elicitation of immune responses in non-target organisms. Off-target effects arguably pose the greatest risk to pollinators even with the development of highly specific RNAi-based crops. Susceptibility may be further affected by the presence of other stressors in the environment, such as other plant incorporated pesticides that pollinators encounter while foraging. Studies evaluating the importance of habitat diversity and dietary breadth in pollinator health and fitness, as well as the potential transfer of RNAi within the landscape, will be crucial as RNAi crops near commercialization. *In silico* identification of potential off-target sites within the genomes of exposed pollinators will also assist with identifying at-risk species. This will allow for proactive steps to be taken to mitigate non-target effects in corn and soy monocultures, as occur in the Midwestern United States.

## **Toxin expression in *Bt* transgenic corn roots, and western corn rootworm behavioral responses under two refuge mimicking scenarios**

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Corn rootworms are one of the most serious corn pests in the Americas and Europe. Transgenic corn expressing *Bacillus thuringiensis* (*Bt*) toxin targeting rootworms has only been available since 2003, but resistance development by *Diabrotica virgifera virgifera*, the Western corn rootworm (WCR), has already been reported. The factors or mechanisms involved in WCR resistance to *Bt* corn are unknown, but resistance is likely a combination of insect and plant modulated factors. We investigated WCR larval responses to non-*Bt* and *Bt* corn roots under scenarios mimicking a block/strip and a seed mix refuges. We also investigated expression of *Bt* toxin in corn roots grown under both scenarios to determine if larval responses could be explained by differences in toxin expression along the root. Results from this study show that rootworm behavior can indeed be modulated in response to corn line and planting scenario. Moreover, WCR larvae show distinct settling preferences for different root regions, which are different for non-*Bt* and *Bt* corn, but cannot be explained by toxin expression levels. Rootworm feeding had significant effects on toxin expression, which were dependent on planting scenario, an aspect that may have applied implications for *Bt* resistance development in these insects. Importantly, our findings shed light on a novel aspect of refuge scenario affecting *Bt* toxin expression in genetically-modified corn roots: plant-plant interactions.

Session 11-T 1 - New products/methods for IPM of *Ostrinia* and *Diabrotica* in modern maize production systems

## IPM tactics for Lepidoptera of maize in the southern United States

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Maize in the southern United States is annually attacked by a complex of leaf-, stalk-, and ear-feeding Lepidoptera, including southwestern corn borer (*Diatraea grandiosella*), fall armyworm (*Spodoptera frugiperda*), and corn earworm (*Helicoverpa zea*). Integrated pest management necessitates the use of multiple tactics, such as planting date, insecticides, and host plant resistance to effectively keep injury below economically damaging levels. Data will be presented that addresses management of southern maize lepidopteran larvae.

## Arthropod pest management within the complex IPM system of the Texas High Plains

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The Texas High Plains has a unique and complex IPM system for corn compared to other regions within the United States. The Texas High Plains is a semi-arid region with a historical annual rainfall of 45.7 to 61 cm. Therefore, for corn to be grown in this environment, fields are watered with center pivot irrigation systems from the 'Ogalla' underground water aquifer. The heavy use of water from the aquifer for the past 60 years has caused significant decline in water levels by as much as 150 feet (92 meters) in some locations. This decline of water availability and the semi-arid growing conditions of high evapo-transpiration water losses and persistent drought conditions puts a strain on the system to be able to economically produce corn. These irrigation costs and other production expenses impact the decisions farmers must make for planting and for arthropod management. They have to account for their irrigation pumping capacity in order to meet the water demands for corn and the costs for *Bt* technology corn seed for above ground lepidopteran pests, the western corn rootworm and protection of the non-*Bt* refuge. The refuge requirements in the Texas High Plains is divided into a North Zone which follows the corn belt refuge requirements and a South Zone which uses refuge requirements for corn grown in the cotton belt. Other complexities to the IPM system are that different lepidopteran pests profiles and corn rootworm pressures exist in the North and South zones. Another complication to the system is that spider mites pose a constant threat to corn in both production zones. Rapid population increases occur during the grain developing growth stages and during moisture stress conditions that are common under this semi-arid region. And, the use of pyrethroid insecticides to control lepidopteran pests are almost guaranteed to cause mite infestation outbreaks. Practices that would conserve water or better manage one pest can adversely affect the status of another pest.

## **New approach of using endomycorrhizal fungi in the biological control of stem borer infestation in relation to the yield of corn plants**

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Three field experiments were carried out at the experimental farm of the Faculty of Agriculture (Saba-Basha), Alexandria University, at Abees region, Alexandria, Egypt, during two subsequent seasons of 2009 / 2010 and 2010 / 2011 to evaluate the treatment of arbuscular mycorrhizal fungus, AMF (*Glomus intraradices*) and rhizosphere bacterium (*Bacillus mycooides*) under two current types of organic and mineral fertilizers on the lignin content in stems of treated corn plants (var. Th.w. 310) and the incidence of corn stem borers infestation.

The obtained results, showed lower mean values of estimated levels of infested corn plants by stem borers, accompanied by higher value of lignin content in the stems of inoculated corn plants with AMF plus *B. mycooides*, under both adopted above mentioned fertilization types, compared to the un-inoculated control. NPK uptake in inoculated corn plants increased and the level of stem borers infestation decreased. The treatment of *G. intraradices* + *B. mycooides* was the most profitable treatment as it gave also the highest means of all examined yield parameters such as ear length (cm), weight of ear (g) and weight of grains (g).

Moreover, inoculation of corn plants with mycorrhizal fungus and *B. mycooides* elucidated the negative strong relationship between the estimated parameters of yield components and the measured levels of stem borers infestation.

## Modern corn pest management: meeting the challenge with Syngenta solutions

Von Kaster<sup>1</sup>, Miloud Araba<sup>2</sup>, Dirk Benson<sup>2</sup>, Tony Burd<sup>3</sup> & Isaac Oyediran<sup>2</sup>

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Syngenta is a leading global developer of agrochemicals and seeds that provide a broad range of solutions for management of major insect pests. Corn growers have many options for control of above and below ground insects, but they increasingly rely on insect-protected hybrids as a foundation for their pest management programs.

Syngenta's Agrisure Duracade™ trait is a proprietary technology that features a novel mode of action against corn rootworm and is distinct from its previously released Agrisure® RW trait. Agrisure Duracade™ will only be available stacked with a second corn rootworm trait, and offered as a 5 percent integrated, single-bag refuge product.

As part of a robust trait package that controls corn rootworm and preserves technology durability, the Agrisure Duracade™ trait will serve as the cornerstone of a more effective corn rootworm management system that includes traits, best-in-class seed care and industry-leading crop protection products.

Long-term CRW management will require corn growers to adopt a multi-year, whole-farm approach, while striking an important balance between CRW control, yield protection and resistance management. Effective CRW management will require the integration of multiple control measures, not a singular technology or approach.

## Western corn rootworm resistance to *Bt* hybrids: management recommendations moving forward

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The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) migrated from western Corn Belt states into Illinois in 1964. The primary management practice in non-rotated maize was to apply a planting-time soil insecticide. Initially, rotating soybeans with maize was highly effective in preventing economic losses caused by larvae feeding on roots. In the mid-1980s, root injury in rotated maize was reported in east central Illinois. In 1995, widespread damage in rotated maize occurred across east central Illinois and portions of Indiana due to significant root pruning and lodging. Western corn rootworms had adapted to crop rotation with a segment of the population capable of laying eggs in the soil of soybean fields. The use of soil insecticides increased significantly and now included rotated and non-rotated maize. In 2003, corn rootworm *Bt* hybrids expressing the Cry3Bb1 protein were commercialized. Subsequently, other *Bt* proteins (Cry34/35Ab1, mCry3A, eCry3.1Ab) also targeted at the western corn rootworm were brought into the market place. These new events were considered low to moderate dose. In spite of this, the refuge requirements (North Central Region U.S.A.) initially were the same as that of the high dose lepidopteran maize *Bt* events - a 20% structured refuge. In 2011, the evolution of field resistance (lowa) by western corn rootworms to the Cry3Bb1 protein was confirmed. Shortly thereafter, resistance to this protein also was confirmed in some Illinois field populations. In 2013, producers in northeastern Illinois reported severe root injury in rotated maize fields that had been planted to *Bt* hybrids expressing the Cry3Bb1 protein. Prior to these observations, *Bt* failures within Illinois had occurred exclusively in non-rotated maize. Because of escalating concerns of *Bt* resistance, producers are increasingly returning to the use of planting-time soil insecticides in conjunction with *Bt* rootworm hybrids. Integrated pest management practices (IPM) have been largely abandoned in favor of insurance approaches. Moving forward - new integrated management strategies will be required to prolong the usefulness of currently registered *Bt* proteins for the western corn rootworm.

## Crop rotation remains the key to successful rootworm control in Europe

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As the larvae of the univoltine western corn rootworm, *Diabrotica virgifera virgifera*, are largely restricted to corn, crop rotation has long been a powerful management tool for this major corn pest. In some regions of the U.S.A., western corn rootworm however started to adapt to the crop rotation system by more frequently laying eggs into non-host crops, such as soybean, where corn is grown the following year which then allows larval development. Such scenarios have so far not been reported from Europe. Multiple-choice field cage experiments were used to clarify to what extent rootworm adults use non-corn crop habitats as alternative food sources or oviposition sites in situations of non-corn/corn rotations typical for Europe. Results indicate that the polyphagous nature of rootworm adults is, under field situations, not as important as often stated. The generational, i.e. annual growth rate of populations, an indicator of crop habitat quality for food and oviposition, appeared highest when the entire multiple-choice cage had been planted with corn (populations doubled). When Sudan grass and Sorghum millet were combined with corn, a slight population growth was still possible. When corn was combined with any other habitat type, populations decreased from year to year, suggesting that non-corn crop habitats are suboptimal, and their role as alternative food sources under field conditions might be overestimated. As for oviposition, also corn was found to be the most attractive. Of medium proportional attractiveness were Sorghum millet, Sudan grass, and ploughed bare soil. Harvested and grubbed winter rape with some regrowth, harvested and grubbed or not grubbed winterwheat with regrowth, potatoes and soybean were comparatively less attractive for oviposition.

In conclusion, crop rotation, regardless of the chosen crop combination, remains an effective control measure for rootworm populations in Europe. It is hypothesised that an adaptation of rootworms in Europe remains unlikely because of a) the diversity of crop production systems in terms of crops, different rotation schemes (time), and field sizes (space), and b) the frequent changes in agricultural legislation and consequently agronomic practices.

This study was funded by the Bavarian State Ministry of Food, Agriculture and Forestry, Germany.

## **Recent research describing costs and benefits of neonicotinoid-treated seed: potential for non-target effects vs. effects upon pest damage and yield**

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In recent years, a great deal of attention has focused on the decline of pollinators and the potential factors contributing to their loss. Among other factors, synthetic pesticides have been highlighted as key risk factors for the health of bees and other non-target insects. Maize is planted over more acres globally than any other crop, and in North America virtually all maize seed is treated with neonicotinoid insecticides, which are highly toxic to honey bees. It has been established that honey bees can be exposed to neonicotinoid insecticides used as seed treatments via contact with contaminated planter exhaust dust that arises during planting of neonicotinoid treated seeds. However, the spatial patterns and concentration levels of neonicotinoids surrounding fields have remained largely undescribed. We present work aimed at quantifying the levels of neonicotinoid-contaminated dust that occur in the environment surrounding commercial maize fields during planting. We also describe our research documenting the potential benefits (i.e. pest management efficacy and yield) of seed treatments across multiple years and locations within Indiana.

## **Economics of insect resistance management for western corn rootworm**

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We used a published biological model and published economic algorithm to evaluate western corn rootworm (*Diabrotica virgifera virgifera*) IPM for growers over a 15 year period beginning after significant adoption of insecticidal corn (*Zea mays*). The primary focus of our analysis was the economic evaluation of transgenic insecticidal corn expressing Cry34/35Ab1 as event 59122 and its refuge planted continuously year after year (continuous corn). We chose the reference scenario for economic comparison to be the use of soil insecticides on continuous, conventional corn. The model simulated the evolution of rootworm resistance to transgenic insecticidal (*Bt*) corn; but did not simulate resistance to soil insecticides. We evaluated refuge sizes of 5-50% for single-trait *Bt* corn and 5-20% for pyramided *Bt* corn with two traits targeting western corn rootworm. We considered the role of block and blended (seed mixture) refuges for insect resistance management (IRM). Results demonstrated that, for pyramided *Bt* corn, block refuges planted in the same location within a field year after year gave the greatest overall economic returns. If growers relocated their block refuge annually (which is the most common practice), then a 5% blended refuge gave the greatest return. For single-trait *Bt* corn, 10-20% blended refuges gave greater economic return compared to block refuges ranging from 5% to 50%. Single-trait *Bt* corn with 5-20% block refuge (with no insecticide) was superior to soil insecticide use alone in all cornfields.

## **Performance of *Bt*-traits and insecticide at a *Bt*-resistant and a *Bt*-susceptible western corn rootworm (*Diabrotica virgifera virgifera*) site**

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As concern grows about trait resistance in corn rootworm, a better understanding of the responses of *Bt* traited hybrids to resistant populations must be gained. A direct comparison between a susceptible and a resistant population provides a unique opportunity to gain new insight on how resistance affects a variety of variables. This study compared two sites with different susceptibility to corn rootworm active *Bt* traits; Hills, MN (resistant) and Rosemount, MN (susceptible). Resistance to Cry3Bb1, Cry34/35 and Cry3A in the Hills population was confirmed through artificial diet bioassays. Eighteen treatments were represented; six pure-stand hybrids and three trait-refuge blends with or without an insecticide overlay (tefluthrin). All *Bt* traits commercially available at the time were represented; Cry3Bb1, Cry34/35, mCry3a, and a pyramid of Cry3Bb1 and Cry34/35. Randomized complete block studies were planted at two on-farm sites, each with a history of continuous corn and a corn rootworm population dominated by western corn rootworm (*Diabrotica virgifera virgifera*). Performance of traits and insecticide overlay were evaluated through adult emergence, lodging, root injury and yield. Overall yield, root injury and adult beetle emergence were significantly higher at the Hills site, supporting bioassay results indicating resistance. Results of this study, particularly adult emergence data provide insights that will allow a more detailed investigation of how resistance could impact management practices and resistance management.

## **Transcriptional responses of western corn rootworms to benzoxazinoids**

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Benzoxazinoids, especially DIMBOA are secondary metabolites found in young corn plants that have known insecticidal properties. Because western corn rootworms are specialist corn herbivores, it is likely that they possess mechanisms to detoxify benzoxazinoids. To identify transcripts with a potential role in benzoxazinoid detoxification, neonate western corn rootworm larvae were infested on mutant corn seedlings that were deficient in benzoxazinoid synthesis and closely-related wild-type seedlings. Messenger RNA from whole larvae was used in an RNA-Seq experiment to identify transcripts that were differentially expressed between larvae feeding on wild type and mutant corn. A small number of differentially expressed transcripts were identified including a putative cytochrome P450 monooxygenase and a putative cathepsin protease.



**ABSTRACTS  
POSTER  
PRESENTATIONS**

## Transcriptome analysis of western corn rootworm larvae and eggs

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The western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is one of the most devastating pests of corn causing nearly a billion dollars of financial loss both in terms of yield loss and treatment costs. Although Coleoptera is the most diverse order of insects comprising more than 400,000 species, only a few coleopteran genomes and transcriptomes (e.g., from *Tribolium castaneum* and *Dendroctonus ponderosae*) have been published to date. The genome size of haploid *D. v. virgifera* is estimated to be ~2.58 GB, one of the largest among beetle species. Its complete genome sequence is currently in the draft stage. In this study, in order to identify the gene sets expressed in their larval stages (when the most damage to corn is caused) and to contribute to improving the genome assembly, we have sequenced and assembled transcriptomes from egg, neonate, and third-instar larval stages of *D. v. virgifera* using next-generation technologies. In total ~700 gigabases were sequenced. *De novo* transcriptome assembly was performed using four different short read assemblers for individual and pooled sets of reads. Hybrid assembly using both Illumina and 454 reads was also performed. After examining the assembly quality based on contig length and annotation effectiveness with similarity search, we chose the Trinity assembly from the pooled dataset including 163,871 contigs (the average length: 914 bp) as the most inclusive. We identified and annotated genes encoding chemoreceptors, gamma-aminobutyric acid (GABA) type A receptor, and glycoside hydrolase families. Compared to the sequences found in the draft genome, we observed variations in sequences as well as in the number of introns. We also examined conservation of gene structures in chemoreceptors from closely related insect lineages. Our transcriptome sequences can contribute toward improved quality of the *D. v. virgifera* genome assembly and annotations.

## Utility of five different sticky traps for use in risk assessment in *Bt*-susceptible and *Bt* resistant corn rootworm (*Diabrotica* spp.) fields

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Control of corn rootworms (*Diabrotica* spp.) has been a focal point of growers for decades due to the injury the larval stage inflicts on corn (*Zea mays* L.) roots. While many control tactics have been developed, these tactics are often overcome by corn rootworm's high degree of adaptability. Corn hybrids producing an insecticidal toxin from *Bacillus thuringiensis* Berliner (*Bt*) are the most recent control tactic overcome by corn rootworms. Scouting is a useful tool for monitoring the success or possible failure of *Bt* hybrids and other corn rootworm control tactics. Sticky traps and whole plant counts have been used in the past to assess risk and adjust management tactics, but criteria for management decisions have not been updated in more than 20 years. This study explored the utility of five different yellow, non-baited, sticky traps for effectively monitoring corn rootworm populations. Sticky traps were placed in 12 locations at three different sites. These sites differed in their apparent rootworm susceptibility to corn rootworm-active *Bt* toxin and the hybrid used (Site 1: Refuge hybrid planted in *Bt* susceptible population, Site 2: Single-trait hybrid with insecticide overlay planted in suspected *Bt* resistant population, Site 3: Pyramided hybrid planted in suspected *Bt* resistant population). Sticky traps differed significantly in their ability to capture corn rootworms with the commonly used unbaited Phercon AM sticky trap capturing less beetles than other traps at all three sites. These data comprise the initial step for making recommendations to crops consultants and growers for use in risk assessment and management decisions.

## Susceptibility of western corn rootworm populations collected in Nebraska to *Bt* corn events

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To formally characterize the susceptibility of Nebraska western corn rootworm (*Diabrotica virgifera virgifera*, LeConte) (WCR) populations to rootworm-active *Bt* corn events, a study was conducted to evaluate the susceptibility of WCR field populations from Cry3Bb1 history fields to each commercial rootworm-active protein (i.e., Cry3Bb1, mCry3A, Cry34/35Ab1) and appropriate non-*Bt* isolines. In 2012, nine WCR field populations were collected of which six were from fields with a Cry3Bb1 history and had unexpected root injury. Six WCR colonies that had never been exposed to Cry toxins and three field populations from sites not experiencing unexpected injury to rootworm *Bt* hybrids were used as controls. In 2013, single-plant assays were performed in the laboratory by infesting each potted plant with 12 F1 neonates at V5 growth stage and then recovering larvae after 17 days. For each population, 12 replicates of each *Bt* protein and associated non-*Bt* isolate were included in a randomized complete block design. Larval survivorship was significantly affected by a significant interaction between field type and hybrid for populations reared on Cry3Bb1 and mCry3A but not Cry34/35Ab1. Mean survivorship of larvae from problem Cry3Bb1 history fields when reared on Cry3Bb1 or mCry3A was not significantly different than mean survivorship on respective isolines without Bt. This pattern was not evident when larvae were reared on Cry34/35Ab1 and associated non-*Bt* isolate. Our results suggest that WCR resistance to Cry3Bb1 has evolved in response to selection pressure and that a possible cross-resistance relationship exists between Cry3Bb1 and mCry3A. Results also suggest that WCR Cry3Bb1 resistance has evolved independently in multiple areas of Nebraska.

Session 3-P 4 - Western corn rootworm adaptation

**Trait and insecticide efficacy against susceptible and resistant western corn rootworm populations: implications for resistance management**

Ken Ostlie<sup>1</sup>, Elizabeth Schacht<sup>1</sup>, Jeremy Barta<sup>1</sup> & Trisha Leaf<sup>1</sup>

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Development of resistance by western corn rootworm to Cry proteins expressed in *Bt* maize has left growers scrambling to adapt. While several management options exist, their utility from plant protection, population and resistance management perspectives is unclear. On-farm research exploring the utility of management options against susceptible and resistant populations was conducted from 2010-2013 across southern Minnesota. These studies examined trait performance with and without a soil insecticide overlay in a variety of on-farm situations. This poster focuses on the population management dimension of these management studies and explores its implications for resistance management, mitigation and remediation.

## Cultural *Diabrotica* containment strategy in Switzerland an effective success by now

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After multiple introductions by airplanes and establishing itself firmly with considerable growth rates in several countries of Europe, a small population of western corn rootworm (WCR), *Diabrotica v. virgifera* Le Conte (Coleoptera: Chrysomelidae), has been detected during summer of 2000 in the Canton of Ticino, the southernmost part of Switzerland, adjacent to the Italian border. In response, Swiss and Cantonal authorities decided to contain or even eradicate the pest solely by the application of a mandatory crop rotation strategy. During the twelve years of severe and controlled prohibition of more than one year corn cropping, and, despite annual northbound migrations from the Lombardy region of Northern Italy, never any damage was observed. To validate the rotation strategy, an eight year comparison between a continuous corn field vs. four rotated corn fields in the surroundings has been carried out. Results showed a clear mitigating effect of the rotation treatment of up to a factor of 8:1. The populations in crop rotated fields are believed to have migrated from near Italian foci, beetle appearance occurred regularly after a 10 to 15 day delay compared to the population in the monoculture field. The impact of mortality factors affected population growth even under monoculture conditions, so population increase was quite different from exponential growth during the trial period. Nevertheless, root damage and goose neck symptoms were observed in three years in the monoculture field.

Crop rotation is confirmed to be an effective, economic and ecological method to contain WCR. The strategy permits to contain effectively WCR populations in the southern part of the Alps and at the same time prevents establishment of WCR populations in northern Switzerland, where occasionally few beetles are caught, mainly along transportation routes.

## Corn insect communities of Eastern South Dakota

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In 2013 corn was planted to over 95 million acres of farmland in the US, and represents the most commonly grown crop in the US. Corn also dominates the farming landscape in the state of South Dakota (more than 6 million acres). In the present study, 53 farms in eastern South Dakota were surveyed in the years 2010 and 2011. At each of the study sites, 50 corn plants were destructively harvested during anthesis. All arthropods found on corn plants were identified and counted. The data was analyzed to create a clear picture of the fauna found within corn fields, including distributions and infestation severities of key corn pests. The data collected from this study will guide scientists in recommending management strategies to farmers, and also direct which corn insect species should be focused on in future research projects.

## To resist or not to resist? An insect dilemma on *Bt* maize

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Resistance management has become a more pivotal component of long-term insect pest control. The commercialization of genetically-engineered plants producing *Bt*-toxins resulted in high selective pressure on many insect pests. Products targeting the western corn rootworm (WCR) *Diabrotica virgifera virgifera* produce levels *Bt*-toxins which cannot be considered high dose. *Bt*-resistant WCR have evolved in the field, in part, due to the lack of a high dose. As *Bt*-maize plants attacked by resistant WCR suffer more damage, it is likely that inducible plant defenses are elicited to a greater extent in these roots, resulting in the emission of volatiles that recruit entomopathogenic nematodes. These nematodes quickly kill the insect pest and effectively reduce root damage when used in combination with appropriate maize cultivars. In the laboratory, *Bt*-roots of particular cultivars induced with *Bt*-resistant WCR larvae recruited entomopathogenic nematodes. In the field, significantly less damage by *Bt*-resistant WCR was measured on those plants than on *Bt*-cultivars emitting an inaccurate blend or inappropriate doses of volatiles. This induced fitness cost to *Bt*-resistance offers additional and sustainable approaches to resistance management plans. These results underline the importance of understanding root-mediated interactions and their potential in the development of sustainable agroecosystems.

## Spatial and temporal infection patterns of western corn rootworm larvae by two entomopathogenic nematode species

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The use of entomopathogenic nematodes (EPN) has been evaluated as a promising approach for the management of western corn rootworm (WCR) larvae in recent years. Due to the below ground feeding of WCR larvae the understanding of EPN infection on WCR larvae is lacking but could help to improve the efficacy of this management strategy. This study therefore assessed the temporal and spatial infection pattern of *Steinernema feltiae* (*Sf*) and *Heterorhabditis bacteriophora* (*Hb*) in a non-destructive observation device which enables to directly observe EPN infection in WCR larvae.

The infection rate of WCR larvae was significantly higher with *Hb* than *Sf*. Furthermore, the time of first infection was observed 48 hours earlier with *Hb* than *Sf*. The highest rate of *Hb* and *Sf* infection was observed 7 and 10 days respectively after the EPN were released into the observation device. The majority of *Sf* infected WCR larvae were found in one location in the observation device up to 5 cm around the inoculation point of *Sf*. The *Hb* infected WCR larvae were found in multiple locations across the whole observation device up to 25 cm from the point of *Hb* release. These spatial infection patterns reflect the different foraging strategies of these EPN species. *Hb* has been categorised as a “cruiser” and actively searches for its host in the soil. *Sf* on the other hand exhibits a “sit and wait” foraging strategies where the EPN waits for a host to pass by and does not actively move in the soil. The observation device used in this study is therefore suitable to directly observe EPN infection in WCR larvae and quantify temporal and spatial infection patterns. *Hb* caused higher infection rates in WCR larvae than *Sf* mainly due to its cruiser activity which underlines the potential of *Hb* as a promising biological control agent for WCR larvae.

## Impact of tillage on the infestation with European corn borer

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The European corn borer (*Ostrinia nubilalis* Hbn.) is one of the most important pests in maize. The larvae of the European corn borer overwinter in crop residues of maize plants. To pupate the larvae need maize stubbles remaining on the soil surface. Yield losses occur mostly on very light and shallow or very heavy soils where ploughing is not possible. For economic and soil protection reasons more and more farmers want to turn to ploughless tillage even outside of these areas.

In five years of field experiments it was analysed how working maize straw into the soil at different depths results in reducing corn borer moths. Maize stubbles containing corn borer larvae (100 per test plot) were buried 25 cm deep into the ground (common depth of ploughing). Above hatching cages were set up to assess the number of moths developing. In the control plots not infested maize stubbles on the surface were added to check whether and to what extent larvae get to the soil surface and pupate. Furthermore it was assessed when larvae leave maize stems buried in the soil and move to maize stubbles on the soil surface. For that purpose, from December to May, stubbles lying on the surface were collected once a month, present larvae were counted and not infested straw was replaced. In order to find out if the point in time has an influence on the control effect.

If maize stubbles were chopped and then worked into the soil by a plough after harvesting 0 to 15% of the larvae developed into moths and were found in the cages. Remaining larvae either died in the soil or developed moths could not reach the soil surface. If however, additional maize stubbles lay on the soil surface 19 to 89% of the larvae developed into corn borer moths. This proves that the corn borer is dependent on intact maize stem and stubble residues on the soil surface to pupate and to provide the best chance of survival for the eclosing moth. With regard to effect of control measures it is essential to work maize straw and stubbles into the soil or shred or crush stubbles and stems thoroughly so that pupation is no longer possible at the soil surface.

With the temperature above long term average from October to April the number of moths increased in both variants, with and without stubbles on the soil surface. In contrast, with temperatures below long term average a maximum of only 1% of larvae developed to moths in the variant without stubbles. In the variant with stubbles it were 40%.

Regarding the number of moths the date of ploughing immediately after harvest or four weeks later was not relevant.

## Multivariate and ecological approach to assess non-target effects of *Bt* rootworm maize on ground beetles

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We conducted a non-target field study that was both a practical assessment of the potential treatment effects of rootworm *Bt* maize and an ecological investigation of how several environmental variables may affect carabid beetle assemblages. We then examined how the environmental variables influenced the treatment effects.

There were three main purposes of the study:

1. Assess the potential effects of rootworm *Bt* maize on carabid beetle assemblages throughout the growing season.
2. Compare carabid beetle assemblages between rootworm *Bt* maize and the positive control insecticide treatments.
3. Compare any treatment effects with those of ecological and environmental factors such as beetle phenology, elevation, and prey abundance.

Carabid beetles and other arthropods were sampled with pitfall traps in 60×60-foot plots. The experimental treatments included Herculex® RW rootworm-resistant *Bt* maize (event DAS-59122-7, Cry34/35Ab1) and its corresponding non-Bt isolate. Within both of these *Bt* treatments were three insecticide subtreatments (all applied at labeled rates): check, seed treatment (clothianidin, Poncho® 1250), and granular soil insecticide treatment (tefluthrin, Force® 3G) to serve as a positive control and to assess the relative strength of the effects of *Bt* and insecticide. Data were analyzed with canonical correspondence analysis. Overall, insecticide effects on arthropods were significantly greater than *Bt* maize; and no significant biological effects were observed between *Bt* maize and isolate controls. Our most substantial finding was that insecticide treatment effects were small compared with ecological and environmental factors such as beetle phenology, elevation and prey availability. Considering such environmental factors may help account for the natural variation that makes running ecological field studies so challenging.

## Mexican rice borer, *Eoreuma loftini*: a threat to corn in the southern United States?

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The Mexican rice borer, *Eoreuma loftini* (Dyar), is an insect indigenous to Mexico that has become an invasive pest of grass crops in the Gulf Coast region of Texas. After expanding its range through the Texas rice production area, *E. loftini* was first detected in Louisiana in 2008 and in Florida in 2012. Because *E. loftini* has become a primary corn pest in Mexico and has the ability to survive sub-freezing temperatures, corn in the southern United States may soon be impacted as the pest continues spreading. Thus, assessing pest status and developing management plans for *E. loftini* is now critical.

A field study was conducted in Louisiana in 2013 to assess *E. loftini* pest severity under natural infestations. Five transgenic *Bt* corn hybrids and three associated non-*Bt* near isolines were evaluated. The *Bt* hybrids expressed Cry1Ab (*Bt*11 event), Cry1F (TC1507 event), Cry1Ab and Cry1F (MON 810, TC1507 events), Cry1A.105 and Cry2Ab2 (MON 89034 event), and Cry1Ab and Vip3A (*Bt*11, MIR162 events). When plants attained the R1 and late R5-R6 stages, stalks were collected and examined for *E. loftini* injury, larvae, and pupae.

Pheromone trapping revealed that *E. loftini* moths were active throughout the duration of the experiment. However, *E. loftini*-bored stalks were not observed for plants at the R1 stage, and only one *E. loftini* neonate was observed in the leaf sheath of a plant expressing Cry1F. Near the end of the growing season, non-*Bt* hybrids at the late R5-R6 stage exhibited a greater proportion of *E. loftini*-bored stalks than *Bt* hybrids. Non-*Bt* hybrids were in general more infested than the *Bt* hybrids. Larvae or pupae were recovered from the three non-*Bt* hybrids but also from the two hybrids expressing Cry1A.105 and Cry2Ab2, and Cry1Ab and Cry1F. Stalks of the hybrid expressing Cry1Ab and Vip3A were free of *E. loftini* injury and infestation.

This study confirms that corn is susceptible to the invasive *E. loftini*. Thus, the pest represents a threat to corn grown in the southern United States and may require management. Results suggest that commercially available transgenic corn expressing *Bt* proteins targeting lepidopteran pests is effective in reducing *E. loftini* injury and infestations.

## Distributions of *Diabrotica virgifera virgifera* eggs in European corn fields

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To forecast damage caused by *Diabrotica virgifera virgifera* Le Conte and take appropriate control measures the egg laying behaviour of this species, including where they lay, number and dispersion of their eggs, are of interest. Most data available on this topic was collected in the U.S.A. where the conditions important for agriculture, e.g. climate and type of soil, differ from those in Europe. Due to these differences the damage threshold for Europe was defined by combining data on egg laying behaviour with that on damage to maize plants.

In spring 2013 soil samples were collected from seven locations in Austria with a permanent maize crop infested with *Diabrotica*. The samples were collected from ten plots, each 10\*10 m, of which five plots were at the edges and five in the middle of fields. Eggs of *Diabrotica* were washed out of the samples using an egg washing machine similar to the one used in the U.S.A. The eggs recovered in this way were counted and used to predict the expected number of larvae. In August the roots of maize plants in the plots sampled in spring were assessed for damage caused by *Diabrotica* larvae and goose neck symptoms were assessed in September.

The results indicate that female *Diabrotica* did not prefer to lay eggs in any particular part of a field. It was not possible to determine the exact damage threshold from trial data but only confirm that a threshold of one larva per plant is valid for maize grown under dry conditions. Even three larvae per plant don't seem to have an adverse effect on maize if there is enough water. To confirm and refine the results further studies are needed.

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## **The effect of climate warming on the generation distribution of *Ostrinia furnacalis* in Jilin Province**

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Through the system investigation and data analysis of adults oviposition period, larval development progress, and pupation rate of different regions of *Ostrinia furnacalis*, we clarified pupation and the adults oviposition beginning period, full incidence period, last period in 20 countries or cities which were in the east, middle or west ecological area of Jilin Province. The result showed that the generation number of *O. furnacalis* in Jilin Province has changed due to climate warming, east ecological area near middle area has changed from one generation to one and a half generations, while east mountainous area was still one generation; the middle ecological area has changed from one and a half generations to two generations or two and a half generations; west ecological close to south area has change from two generations to two and a half generations, while west ecological close to north area was still two generations.

## **Optimum yield of female sex pheromone in *Ostrinia nubilalis* (European corn borer) during scotophase**

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Females of *Ostrinia nubilalis*, European corn borer, (ECB) produce sex pheromones during scotophase to attract males. ECB has two pheromone strains, E and Z, in the U.S., where Z is the most prominent. These strains differ by the dominant proportions of E and Z 11-tetradecenyl acetate isomers that are produced. This study was conducted to determine which two hour interval during scotophase yields the most pheromone quantity for the Z-strain. Our data indicate that the greatest quantity is obtained when pheromone glands are excised early in the 7<sup>th</sup> hour of scotophase.

## An 'Attract & Kill'-approach against wireworms (*Agriotes* spp.) in corn

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Wireworms, larvae of clickbeetles (Coleoptera: Elateridae) are polyphagous worldwide pests. Their prolonged and cryptic lifecycle requires comprehensive strategies to target this pest. The implementation of an integrated pest management (IPM) strategy will become mandatory in the future because of limited chemical control options and increasing damage. In this regard, biological control agents gained more interest in recent years. *Metarhizium brunneum*, a well-studied entomopathogenic fungus (EPF), could become a major player to control wireworms.

We set up a field trial in corn aimed at reduction wireworm densities by implementing an "Attract & Kill"-approach. Two agents were applied in the field; yeast (*Saccharomyces cerevisiae*), acting as the "Attract"-component by generating CO<sub>2</sub> and *M. brunneum* (strain ART2825) acting as the "Kill"-component. Both components were applied as formulated products to enhance stability and viability in the field. Plant germination rate was measured up to four weeks after sowing. Furthermore soil samples were taken once a month in July, August, September and October to identify wireworm densities per plant. No corn damage was observed, despite the fact that wireworm densities were above the economic threshold (> 2 wireworms/m<sup>2</sup>). Wireworm densities were reduced within the "Attract & Kill"plots, but no significant differences were measured. Future field studies will be set up this year to further evaluate the "Attract & Kill" approach with *M. brunneum* as a potential IPM option for wireworm control in corn.

This study is part of the EU Project InBioSoil (Innovative biological products for soil pest control; <http://inbiosoil.uni-goettingen.de/>)

## **KLP ('hat') trap with semiochemical lures suitable for trapping two *Diabrotica* spp. exotic for Europe**

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The KLP (“hat”) trap baited with pheromone or floral lures is a highly efficient non-sticky trap for the western corn rootworm *Diabrotica v. virgifera*. We tested this trap design whether it is suitable for the related *D. speciosa* and *D. barberi*, baited with their respective lures. Both species are exotic to Europe: the former inhabits South America and the latter occurs in some parts of North America.

In screening tests performed in Brazil several synthetic floral compounds and their combinations were found to be attractive to *D. speciosa*, however, best effect was recorded by the known 1,4-dimethoxybenzene. When the most active compounds in the preliminary tests, 2-phenylethanol, methyl anthranilate, eugenol or benzaldehyde were added to 1,4-dimethoxybenzene, no synergistic effect was observed. When 1,4-dimethoxybenzene was formulated in three types of polyethylene (PE) dispensers, PE bag dispensers were superior to two types of PE vial dispensers and caught hundreds of *D. speciosa* in KLP traps. Unbaited traps caught negligible amounts of beetles.

As an interesting side capture, KLP traps with 1,4-dimethoxybenzene caught large numbers of the fly *Euxesta exoleta*, which is known as a maize pest. To our knowledge this is the first report of an attractant for this pest.

As for *D. barberi*, the pheromone and a potent floral lure are already known. In our tests with KLP traps we found that both pheromonal and floral lures can be applied in the same trap. Thus both male and female numbers captured are maximized.

In conclusion, for first detection programs in Europe the application of KLP traps baited with 1,4-dimethoxybenzene in PE bag dispensers could be recommended for use in programs for *D. speciosa*, and KLP traps with dual (pheromonal and floral) lures for *D. barberi*. In the case of *D. barberi* one should note that the lures also show some attraction for *D. v. virgifera*, and ratio of *D. barberi* vs. *D. v. virgifera* in the catch will be predominantly determined by the relative population densities at the given site.

## ***Diabrotica* management in Brescia province of northwestern Italy: the importance of developing a local program**

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When the western corn rootworm (WCR) was first detected in Brescia province in 2002, no management programs were available for Northern Italy and only eradication programs and initial field observations were being carried out in other areas within the region. In Brescia province, corn is the most important field crop and it is primarily grown in monoculture (corn on corn). Because the area was declared infested in the same year and no eradication programs were applied, farmers needed useful and practical information to correctly manage this invasive pest.

Initially, we monitored the WCR population to gain an understanding of adult and larval dynamics within and among the fields in Brescia province, where corn is mostly irrigated and root regeneration can be an important factor in the reduction of the economic impact of root-feeding organisms.

Several trap designs and field schemes were studied to obtain reliable indications of adult populations in fields and to develop local thresholds to guide in choosing and applying available management strategies.

Many field studies were carried out to determine and better understand the best practices to use to reduce the impact of larval feeding where corn rotation was not feasible or economic to be applied. Studies were conducted on the field dynamics of adults and females, in particular, so as to develop strategies to control egg laying and thus reduce the number of larvae the following year.

All studies and observations were designed and carried out in such a way to develop sustainable and successful management strategies for the control of *Diabrotica* within the province and to help farmers preserve the value of their corn crop.

Several differences, compared to what has been reported in the literature, were found when the biology and economic impact of WCR were studied under local corn growing conditions. These findings reinforce the importance of developing local strategies and programs for optimum management of this pest.

## **Mate choice and components of fitness in western corn rootworm in relation to Cry3Bb1 resistant and susceptible genotypes**

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The success of transgenic (*Bt*) maize to control corn rootworms is dependent on high-dosage, a refuge of non-*Bt* maize, and random mating between resistant and susceptible beetles. Cry3Bb1 resistant populations of western corn rootworm (WCR) have easily been selected for in the lab and currently are being observed in maize fields throughout the U.S. Corn Belt, perhaps related to lack of fitness costs due to lower dosage than expected and/or non-random mating between resistant and susceptible beetles. Non-random mating occurs through male-male competition and/or female choice. To ascertain whether female WCR prefer to mate with males of similar genotype, we paired combinations of laboratory selected Cry3Bb1 resistant and susceptible males and females. Neither the proportions of pairs that copulated successfully (42% overall) nor courtship durations varied significantly with genotypes; however, copulation duration was shortest for resistant males crossed with susceptible females. Although the genotype of the mating partner had no apparent effect on female longevity, fecundity, or viable egg production, body weight was highly correlated among successfully copulating pairs but not among non-copulating pairs. Therefore, with the newly marketed, high-dose pyramid *Bt* maize available for corn rootworm control or with future high-dose events, the survival of corn rootworms on transgenic crops may correlate with smaller body size, which could promote positive assortative mating among resistant beetles, enhance the spread of resistance, and decrease the durability of the transgenic maize. Ultimately, this new essential knowledge of WCR biology and behavior will lead to better corn rootworm resistance management plans.

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**Expression profile by next-generation sequencing, of western corn rootworm (*Diabrotica virgifera virgifera*) neonates exposed to the *Bt* toxin Cry3Bb1**

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is a major pest of corn in the United States. This is due, in part, to its ability to adapt to control measures including synthetic insecticides, crop rotation and most recently, transgenic *Bt* corn. Resistance to corn expressing the *Bt* toxin Cry3Bb1 has been documented in several US states during the last few years. We are investigating the molecular basis of this resistance, using next generation sequencing to compare the transcriptomes of Cry3Bb1-resistant and -susceptible WCR larvae as they colonize Cry3Bb1 corn or its near isoline.

## Population genetics studies of two major insect pests of corn in the U.S. Corn Belt

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In a series of previous studies, we developed neutral molecular genetic markers for two major insect pests of corn, Western corn rootworm (WCR, *Diabrotica virgifera virgifera*) and European corn borer (ECB, *Ostrinia nubilalis*), and applied them to gain insight into patterns of gene flow and genetic differentiation among populations of these species across the U.S. Corn Belt. Examination of population genetic structuring of WCR and ECB using microsatellite markers revealed very low and non-significant  $F_{ST}$  values across locations from New York to Kansas for WCR and from Illinois to Nebraska for ECB, indicating almost no differentiation over these large geographic regions. Consequently, a significant relationship between genetic distance and geographic distance, i.e. a pattern of isolation by distance (IBD), was not detected in either pest across large segments of the Corn Belt. In the case of WCR, lack of differentiation is probably because migration-drift equilibrium has not yet had time to develop since its eastward range expansion from the Great Plains that began in the mid 20th-century. The ECB also expanded its range across the Corn Belt beginning in the early 20th century, spreading westward from the East coast to the base of the Rocky Mountains. However, results of temporal population genetic analyses suggest that the initial geographic scale of sampling along transects was too small relative to gene flow over even the greatest distances tested (720 km). Furthermore, when geographic samples of ECB were extended from New York to Colorado, we could detect a significant IBD for ECB, enabling us to calculate Wright's genetic neighborhood area for this insect. The radius of this area is an estimate of typical per-generation net dispersal distance.

We have initiated a study to estimate the genetic neighborhood area for the WCR, and eventually to evaluate the normal spatial scale of individual reproductive dispersal from the natal field. Our strategy is to sample in the native range of this insect in eastern Colorado and western Kansas where migration-drift equilibrium should be less disturbed than in areas colonized during its range expansion. Knowing the radius of the neighborhood area of WCR within its larger continuous distribution will be valuable in parameterizing future models designed to predict rate of resistance development to transgenic corn and the rate of spread after it develops.

## **Evidence from early accounts for reconstructing the mysterious origins of the bivoltine Z race of European corn borer in North America**

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The European corn borer (ECB), *Ostrinia nubilalis*, is an invasive lepidopteran (Crambidae), introduced to the northeastern U.S. and southeastern Canada from Europe in the early 20th century. After its introduction, it eventually spread westward across the Corn Belt, reaching the foot of the Rocky Mountains by the late 1970s. However, the full story is far more complicated and far more interesting. The complications relate to a number of sympatric ECB races that are partially reproductively isolated. The Z-pheromone race is widespread east of the Rockies, while the E-pheromone race is restricted to eastern North America, with only low proportions of E moths as far west as Ohio. Complexed with pheromone race are partially isolated voltinism races. Diapause induction is facultative for the bivoltine (=multivoltine) (B) race, and obligate for the univoltine (U) race. The B race is now widespread in North America except perhaps in the northernmost part of the ECB range. The U race is present in the northern tier of states and Canada, often in sympatry with the B race. Combinations result in three races: BZ, UZ, and BE (there is no UE race). Evidence from the early ECB literature on voltinism, host use, and range expansion suggests that the BZ race of ECB, currently the most widespread and destructive race in North America, was introduced independently from Europe sometime in the mid to late 1930s. This was long after the original introductions of the UZ (~1918) and presumed BE (~1910) races and their subsequent slow geographic spread. Infestations by more-recently introduced BZ race ECB anywhere in New England where the BE race was established could easily have gone unnoticed. If the BZ race was more invasive than the BE and UZ races, a late introduction would explain the sudden appearance of bivoltine ECB populations in formerly univoltine areas, and the sudden rapid westward expansion of ECB so surprising to entomologists at the time.

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## Successful IWGO meeting in Chicago

Following a long standing tradition, 104 scientists from 15 countries met in Chicago, U.S.A. to attend the 25<sup>th</sup> Conference of the International Working Group of *Ostrinia* and other maize pests (IWGO) from Sunday 13 to Thursday 17 April 2014. IWGO is a global working group of the International Organisation of Biological Control (IOBC). Participation was observed from North America, Europe, Asia, South America and Africa but with higher than usual attendance of scientists from the United States. For the first time, Dr Ulrich Kuhlmann, Convenor of IWGO, could welcome participants from Argentina, Brazil, South Africa, South Korea and Laos, clearly demonstrating the still high and even growing global interest in this IWGO Conference and the scientific programme. The 25<sup>th</sup> IWGO Conference was linked again with the 4<sup>th</sup> International Conference on *Diabrotica* Genetics, and as pointed out by Dr Tom Sappington, Co-Convenor of IWGO and local organiser, was notably unique in being jointly held for the first time together with the North Central Corn Entomologist Technical Committees (NCCC46 and NC205). This allowed United States maize experts to exchange information with international scientists working on insect pests of maize of reciprocal concern to the U.S.A., Europe and elsewhere. An impressive number of 60 professional oral presentations were given during the 12 scientific sessions, resulting in a packed but exciting programme. Remarkably, even the very late talks stimulated substantial and lively discussions, indicating not only the high quality of the talks but also that the conference spans an interesting, not too wide, scientific area of common concern. Furthermore, 21 posters presented additional new information on the conference subjects. These posters were visited and discussed extensively, facilitated by their close proximity to the well-equipped coffee break area. Last but not least, the final day of the conference was used to exchange information in a more flexible way, focusing on ideas for future directions and coordination of rapidly expanding ‘-omics’ research on *Ostrinia* and *Diabrotica*, as well as the latest information regarding the biological and economic risks and benefits of neonicotinoid seed treatments used in maize.

Even after long days, conference participants activated energy reserves to explore a bit of downtown Chicago and particularly to join the IWGO “Take Me Out to the Ballgame” night, a major league baseball match between the Chicago White Sox and the Boston Red Sox! For many of the Non-Americans this was the first baseball match seen live and was highly appreciated, at least when ignoring the freezing temperatures. An IWGO entomologist even caught a home run ball while enjoying a beverage behind the right-field fence!

The well-organized conference was clearly successful in exchanging the newest information on innovative management of maize pests, both above and below ground; of particular interest was to see progress in science and developing products against the Western Corn Rootworm where resistance against *Bt*-traits is of increasing concern. There is no doubt the joint meeting facilitated opportunities to foster new collaborative projects between scientists who are using a variety of different approaches to tackle similar problems.



Conference hall in the Allerton Hotel, Chicago (photo: Tom Sappington, USDA-ARS)



Kelton Welch, Geoffrey Jaffuel, Mario Schumann, Christina Mogren and Mike Bredson (from left to right) received the IOBC Global Travel Fund. Not pictured: Darija Lemic and Jennifer Maxwell who also benefited from the IOBC Global Travel Fund (photo: KCS, Delémont)

## CONFERENCE ANNOUNCEMENT

The next meeting will be organized by Dr Wang Zhenying, Co-Convenor of IWGO, in Beijing, China.



# IWGO

International Working  
Group on *Ostrinia* and  
other maize pests

## 26<sup>th</sup> IWGO Conference & 5<sup>th</sup> International Conference on *Diabrotica* Genetics

10 to 12 April 2017  
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)