



# IWGO

International Working  
Group on *Ostrina* and  
other maize pests

## **22<sup>nd</sup> Conference** **Vienna, Austria**

**5-8 November 2006**



# ORGANIZING COMMITTEE

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## LOCAL ORGANIZATION

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# **SCIENTIFIC PROGRAMME**

# SCIENTIFIC PROGRAMME

## Sunday, 5 November 2006

16:00 *Welcome Coffee and Tea*

16:00 *Registration – Sissi Bar/ Empore*

- *Drop-off Point for Poster Presentations;*
- *Provisions for PowerPoint Oral Presentations on CD or USB Memory Stick*

19:00 *Welcome Reception and Buffet – Sissi Bar*

- *Welcome Address and Organizational Issues for the Meeting*  
*Ulli KUHLMANN, IWGO Convenor*

## Monday, 6 November 2006

07:30 *Late Registration – Foyer Elisabeth Salon*

08:00 *Welcome Address – Franz-Josef Saal*

- *C. Richard EDWARDS, IWGO Co-Convenor*

08:05 *Opening Address – Franz-Josef Saal*

- *Harald BERGER, Local Organizer*

08:15 *Scientific Session 1 – Franz-Josef Saal*

### **Session 1: Maize Insect Management with Genetically-Engineered Maize: Lessons Learned**

*Session Organizers: Rick HELLMICH, Iowa State University, Ames, Iowa, U.S.A and Blair D. SIEGFRIED, University of Nebraska, Lincoln, Nebraska, U.S.A.*

08:15	Rick HELLMICH & Blair SIEGFRIED	Introduction
08:20	Michael E. GRAY, Kevin L. STEFFEY, Ron ESTES & Jared B. SCHROEDER	Differential responses of nine transgenic (MON 863) maize hybrids to variant western corn rootworm larval injury
08:40	Christian H. KRUPKE & Larry W. BLEDSOE	Transgenic maize for rootworm control: Describing the relationships between refuge design and beetle movement
09:00	Thomas W. SAPPINGTON, Brendon J. REARDON & David L. DORHOUT	Factors affecting spatial distribution of European corn borer adults across the landscape
09:20	Douglas SUMERFORD, Blair SIEGFRIED, Richard HELLMICH & Leslie LEWIS	Understanding the inheritance of resistance to <i>Bt</i> proteins in the European corn borer: Genetic mapping and future directions
09:40	Zhenying WANG, Dongyan WANG, Kanglai HE, Shuxiong BAI & Bin CONG	Effects of transgenic <i>Bt</i> corn expressing Cry1Ab toxin on survival of the Asian corn borer (Lepidoptera: Crambidae)

**10:00 Coffee/Tea Break – Elisabeth Salon**

**10:30 Scientific Session 2 – Franz Josef Saal**

**Session 2: Compatibility of Insect-Resistant Transgenic Maize Plants with Biological Control**

*Session Organizers: Joerg ROMEIS, Agroscope Research Station ART, Zurich, Switzerland and Galen DIVELY, University of Maryland, College Park, Maryland, U.S.A.*

10:30	Joerg ROMEIS & Galen DIVELY	Introduction
10:35	Richard L. HELLMICH, Jörg ROMEIS, Jeff WOLT & Leslie C. LEWIS	Assessing non-target effects of <i>Bacillus thuringiensis</i> ( <i>Bt</i> ) maize: Lessons learned and new directions
10:55	Alan RAYBOULD, David STACEY, Demetra VLACHOS, Robert JOSEPH, Gerson GERSON & Mike MEAD-BRIGGS	Environmental risk assessment of maize expressing mCry3A for control of corn rootworm
11:15	Xavier PONS, B. LUMBIERRES, C. LÓPEZ, M. EIZAGUIRRE & R. ALBAJES	Indicator species for testing non-target effects of <i>Bt</i> maize on natural enemies: A proposal
11:35	Galen P. DIVELY & Robyn I. ROSE	Community-level effects of <i>Bt</i> sweet corn on non-target arthropods
11:55	Michael MEISSLE & Jörg ROMEIS	Hunting in <i>Bt</i> maize – business as usual for a web-building spider?

**12:15 Lunch Break – Wintergarten**

**13:30 Scientific Session 3 (Posters) - Kaisersalon**

**Session 3: State of the Art of All Things *Diabrotica*: Biology, Population Dynamics, Genetics, Monitoring, Thresholds, Refuge Management, and Current Control Practices**

*Session Organizers: C. Richard EDWARDS, Department of Entomology, Purdue University, W. Lafayette, Indiana, U.S.A. and Ulrich KUHLMANN, CABI Europe, Delémont, Switzerland*

- Display of Posters # 001 to # 028

**15:15 Coffee/Tea Break – Kaisersalon**

**15:45 Scientific Session 4 – Franz Josef Saal**

## 15:45 *Scientific Session 4 – Franz Josef Saal*

### Session 4: Classical Plant Breeding for Insect Resistance in Maize

*Session Organizers:* Bruce HIBBARD, USDA-ARS, Columbia, Missouri, U.S.A. and Marija IVEZIC, University of J.J. Strossmayer, Osijek, Croatia

15:45	Bruce HIBBARD & Marija IVEZIC	Introduction
15:50	Bruce HIBBARD	Breeding for resistance to corn rootworm larval feeding: History, techniques, and the Missouri USDA-ARS program
16:10	Marija IVEZIC, Emilija RASPUDIC, Mirjana BRMEZ & Ivana MAJIC	Results of 5-years investigations of corn hybrids tolerance to western corn rootworm larval feeding
16:30	Martin BOHN & Georgia DAVIS	Genomic evaluation of the defense response of maize against the western corn rootworm – How to use this information in breeding programs?
16:50	Kenton E. DASHIELL, B. Wade FRENCH, Mike M. ELLSBURY, D. A. PRISCHMANN & Bruce E. HIBBARD	Screening maize germplasm for resistance to western and northern corn rootworms at the North Central Agricultural Research Laboratory in Brookings, South Dakota, U.S.A.
17:10	Günter SEITZ	Developing hybrids with resistance to western corn rootworm. A seed company point of view

## 17:30 *End Scientific Sessions*

## 19:30 *Departure from Hotel and Transport to City Hall by Bus (15 min)*

## 20:00 *Reception at City Hall Vienna*

## Tuesday, 7 November 2006

## 08:00 *Daily Announcement – Franz Josef Saal*

## 08:15 *Scientific Session 5 – Franz Josef Saal*

### Session 5: Trophic and Ecological Interactions with Maize Insect Pests

*Session Organizers:* Stefan VIDAL, Department Crop Science, University of Goettingen, Germany and Ted TURLINGS, Institute of Zoology, University of Neuchatel, Switzerland

08:15	Stefan VIDAL & Ted TURLINGS	Introduction
08:20	Marlin RICE & David DORHOUT	Intraguild competition of Lepidopteran larvae in maize
08:40	Sylvie DERRIDJ & P. COUZI	Reduction of <i>Ostrinia nubilalis</i> Hbn. egg laying on maize by foliar application of soluble carbohydrates
09:00	Ivan HILTPOLD, Sergio RASMANN & Ted C.J. TURLINGS	Influence of insect-damaged roots on the host-finding behavior of the entomopathogenic nematode <i>Heterorhabditis megidis</i>
09:20	Kai GLOYNA & Thomas THIEME	Cereals and weeds as potential host plants for <i>Diabrotica v. virgifera</i>
09:40	Joachim MOESER, Bruce HIBBARD & Stefan VIDAL	Impact of plant colonizing soil fungi on the invasive root feeding herbivore <i>Diabrotica v. virgifera</i> mediated via the host plant

## 10:00 *Coffee/Tea Break – Elisabeth Salon*

## 10:30 Scientific Session 6 – Franz Josef Saal

### Session 6: New Biological Control Products to Control Maize Insect Pests: A Dream?

*Session Organizers:* Ralf-Udo EHLERS, Institute for Phytopathology, Christian-Albrechts-University, Kiel, Germany and ZHENG Li, Hengshui Tianyi Bio-Control Company, Ltd., Hengshui, Hebei, P.R. China

10:30	Ralf-Udo EHLERS & Li ZHENG	Introduction
10:35	Hans E. HUMMEL, J.T. SHAW & D.F. HEIN	MSD, a new bio-technical strategy for causing favorable population shifts of <i>Diabrotica v. virgifera</i> in maize
10:55	Miklós TÓTH, József VUTS, István SZARUKÁN, István JUHÁSZ & Ferenc MANAJLOVICS	Female-targeted semio-chemical baits for the western corn rootworm <i>Diabrotica v. virgifera</i> (Coleoptera: Chrysomelidae)
11:15	Christina PILZ, Rudolf WEGENSTEINER & Siegfried KELLER	Microbiological control of the western corn rootworm <i>Diabrotica v. virgifera</i> with entomopathogenic fungi
11:35	Stefan TOEPFER, Benedikt KURTZ, Ralf-Udo EHLERS & Ulrich KUHLMANN	Persistence of applied <i>Heterorhabditis bacteriophora</i> , <i>H. megidis</i> and <i>Steinernema feltiae</i> in maize fields and their effectiveness in controlling larvae of <i>Diabrotica v. virgifera</i>
11:55	Christos G. ATHANASSIOU & N. G. KAVALLIERATOS	Control of the larger grain borer, <i>Prostephanus truncatus</i> (Horn) (Coleoptera: Bostrychidae) in stored maize by using diatomaceous earth formulations

## 12:15 Lunch Break – Wintergarten

## 13:30 Scientific Session 7 (Posters) - Kaisersalon

### Session 7: State of the Art of All Things *Diabrotica* and Free Themes

*Session Organizers:* Stefan TOEPFER, CABI Europe c/o Plant Health Service, Hodmezovasarhely, Hungary and Iona GROZEA, Banat's University of Agricultural Sciences, Timisora, Romania

- Display of Posters # 029 to # 055

## 15:15 Coffee/Tea Break - Kaisersalon

## 15:45 Scientific Session 8 - Franz Josef Salon

## 15:45 Scientific Session 8 – Franz Josef Salon

### Session 8: Implementing Maize IPM using Agro-chemicals: New Products and Trends

*Session Organizers: Ibolya HATALANE ZSELLER, Plant Health Service, Hodmezovasarhely, Hungary and Mario BERTOSSA, Agroscope ACW, Contone, Switzerland*

15:45	Ibolya HATALANE ZSELLER & Mario BERTOSSA	Introduction
15:50	Renata BAZOK, Jasminka IGRC BARČIĆ, Tomislav KOS & Tanja GOTLIN ČULJAK	Optimal timing and efficacy of ecologically acceptable insecticides in the European corn borer ( <i>Ostrinia nubilalis</i> Hübn., Lepidoptera:Pyralidae) control
16:10	Gerald E. WILDE & Kraig L. ROOZEBOOM	The effect of seed treatments, Poncho® and Cruiser® on corn yield in the absence of insect pest populations
16:30	Wolfram ANDERSCH, Rolf Christian BECKER & Bernd SPRINGER	Poncho Pro – control of maize pests and its impact on the establishment of CRW populations
16:50	Géza VÖRÖS, Ibolya HATALA ZSELLÉR, Tamás HEGYI, Géza RIPKA, Béla TÓTH & László VASAS	Experiences of trials for control of adults of western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte) in Hungary
17:10	Bertalan TOTH, Tamas SELLEY & Domonkos LUKACS	New possibilities for the control of <i>Diabrotica v. virgifera</i> in Hungary

## 17:30 End Scientific Sessions

## 19:15 Footwalk from Hotel to Palmenhaus Schönbrunn (10 min)

## 19:30 Reception and Buffet by the Minister of Agriculture, Forestry, Environment and Water Management – Palmenhaus Schönbrunn

## Wednesday, 8 November 2006

## 08:00 Daily Announcement – Franz Josef Saal

## 08:15 Scientific Session 9 – Franz Josef Saal

### Session 9: The Role of Genetics in Fighting the Western Corn Rootworm

*Session Organizers: Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A. and Thomas GUILLEMAUD, Institut National de la Recherche Agronomique (INRA), Sophia Antipolis, France*

08:15	Tom SAPPINGTON & Thomas GUILLEMAUD	Introduction
08:20	Tom W. SAPPINGTON, Kyung Seok KIM & B. Wade FRENCH	Genetic diversity in wild populations and laboratory colonies of western corn rootworm in the US
08:40	Thomas GUILLEMAUD & Nick MILLER	Introduction routes of the western corn rootworm invading Europe
09:00	Blair D. SIEGFRIED & Analiza P. ALVES	Genomics of the western corn rootworm midgut: Identification and validation of potential target sites
09:20	Nick MILLER & Thomas GUILLEMAUD	Genome scanning to search for genetic variation associated with the circumvention of crop rotation in <i>Diabrotica v. virgifera</i>
09:40	Freydoun GARABAGI, Wade FRENCH, Art SCHAAFSMA & K. Peter PAULS	Exploring the role of a cGMP-dependent protein kinase homologous to the <i>Drosophila</i> foraging gene in rotation resistant western corn rootworm ( <i>Diabrotica v. virgifera</i> )

**10:00 Coffee/Tea Break – Elisabeth Salon**

**10:30 Scientific Session 10 – Franz Josef Saal**

**Session 10: *Trichogramma* Releases to Control *Ostrinia* World-wide: Old Fashion or Still Trendy?**

*Session Organizers:* WANG Zhen-Ying, Plant Protection Institute, Beijing, P.R. China and Dirk BABENDREIER, Agroscope Research Station ART, Zurich, Switzerland

10:30	WANG Zhen-Ying & Dirk BABENDREIER	Introduction
10:35	Dirk BABENDREIER & Franz BIGLER	Effects of <i>Trichogramma</i> on non-target species: Fact or fantasy?
10:55	Christine NORONHA & Kathryn DAU-SCHMIDT	Biological control of <i>Ostrinia nubilalis</i> in potatoes: Does plant architecture influence efficacy
11:15	Feng ZHANG, Ki Nam ZHANG, Zhen-Ying WANG, Yong Chol PYON, Li ZHENG, Won Ok KI, Kai SONG, Je Ok RI & Ulrich KUHLMANN	Case study: Augmentative release of <i>Trichogramma ostrinae</i> for control of Asian corn borer in DPR Korea
11:35	Michael P. HOFFMANN, Tze-Hei YONG, Jeffrey GARDNER, Thomas KUHAR & Mark WRIGHT	Biological control of <i>Ostrinia nubilalis</i> with <i>Trichogramma</i> in North America
11:55	Li ZHENG, Kai SONG, Shuhong ZHENG, Zhenying WANG & Jie SHI	A mass production system of <i>Trichogramma ostrinae</i> Pang et Chen (Hymenoptera: Trichogrammatidae) on <i>Sitotroga cerealella</i> (Olivier) eggs

**12:15 IWGO Business Meeting – Franz Josef Saal**

**12:30 Lunch Break – Wintergarten**

**13:30 End 22nd IWGO Conference and Departure**

# POSTER SESSION PROGRAMME

## Monday, 6 November 2006

### 13:30 Session 3 (Posters)

- **Display of Posters: Poster 001 to Poster 028**

Poster 001	<u>Mauro AGOSTI</u> , C. Richard EDWARDS & Silvia I. RONDON	Presence of gravid <i>Diabrotica v. virgifera</i> LeConte females in maize fields in northern Italy
Poster 002	<u>Franja BACA</u> , Snezana GOSIC-DONDO & M. Veskovic MAIZE	Effects of crop residues on plant lodging caused by larvae of <i>Diabrotica v. virgifera</i> LeConte and grain yield
Poster 003	<u>Franja BACA</u> , F. JOVANOVIC, Snezana GOSIC-DONDO & M. TOTH	Effects of different growing systems on attractiveness of maize crop to beetles of <i>Diabrotica v. virgifera</i> LeConte in Zemun Polje in 2003, 2004 and 2005
Poster 004	<u>Peter BAUFELD</u> ; Jozsef KISS; Jens-Georg UNGER; Peter HOFMANN; Gabor JAHN & IstvánTERPÓ	Attraction of light sources and kerosene to adults of western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte)
Poster 005	Sabine C. BECKER, <u>Lance J. MEINKE</u> & Erin E. BLANKENSHIP	Stage-specific development and mortality of western corn rootworm reared on transgenic event MON 863 vs. a non-transgenic isoline field corn hybrid
Poster 006	<u>Sven BREITENBACH</u> , Udo HEIMBACH, Karl-Fritz LAUER & Heinz-Willhelm DEHNE	Field tests on the host range of the larvae of the western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte, Coleoptera) in Europe
Poster 007	<u>Milena BREZIKOVA</u> & Jiri ZARUBA	Spread of western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte) in the Czech Republic
Poster 008	<u>Peter C. CATE</u> , Helmut KLAPAL & Otto WURM	Results of investigations on various aspects of monitoring of Western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte) (Coleoptera: Chrysomelidae) in Austria in the years 2004 and 2005
Poster 009	<u>Sharon CHEEK</u> , R.J.C. CANNON, R.H.A. BAKER, A. MACLEOD & D. EYRE	Assessing and managing risks posed by <i>Diabrotica v. virgifera</i> in the UK
Poster 010	Mike M. ELLSBURY, <u>B. Wade FRENCH</u> , Graham P. HEAD, Christopher W. NOBLE & Billy W. FULLER	Spatial considerations in a replicated plot study of ground beetles (Coleoptera: Carabidae) in rootworm-resistant maize
Poster 011	Silvia FERNANDEZ, Bruno TINLAND & Francesca TENCALLA	The corn rootworm protected maize expressing the <i>Bacillus thuringiensis</i> Cry3Bb1 protein: Summary of benefits
Poster 012	<u>Florian FISCHER</u> , Udo HEIMBACH & Stefan SCHRADER	Orientation of larvae of <i>Diabrotica v. virgifera</i> LeConte (Coleoptera) in different soil compositions and soil densities
Poster 013	<u>B. Wade FRENCH</u> & Laurence D. CHANDLER	Within and between field dispersal of <i>Diabrotica barberi</i> and <i>D. v. virgifera</i> in the South Dakota Areawide Management Site
Poster 014	<u>Lorenzo FURLAN</u> , S. CANZI, F. CHECCHETTO & I. DELILLO	The potential of the management of maize planting date in <i>Diabrotica v. virgifera</i> eradication-containment programmes
Poster 015	<u>Ioana GROZEA</u> & Laura UNIPAN	The potential impact of weather conditions on flight dynamics of <i>Diabrotica v. virgifera</i> adults
Poster 016	Hongmei LI, C. Richard EDWARDS & Dayong XUE	Predicting the possibility for establishment of the western corn rootworm, <i>Diabrotica v. virgifera</i> LeConte, in different world regions based on the CLIMEX model

Poster 017	<u>Hans E. HUMMEL</u> , Gregor UREK, S. MODIC, Mario BERTOSSA, D.F. HEIN & C. ULRICHS	<i>Diabrotica v. virgifera</i> en route towards Central Europe
Poster 018	<u>Jasminka IGRC BARČIĆ</u> , Renata BAŽOK, C. Richard EDWARDS & Tomislav KOS	Study of western corn rootworm adult movement and possible egg laying in fields bordering maize fields
Poster 019	Jozef KUBIK	Results of monitoring of western corn rootworm, <i>Diabrotica v. virgifera</i> , in Slovakia in 2005
Poster 020	Nora LEVAY, Mark SZALAI, Jozsef KISS, Peter BAUFELD & Stefan TOEPFER	Pilot studies to model population changes of western corn rootworm in dependence of regional share of continuous maize fields
Poster 021	<u>Lance J. MEINKE</u> , Xuguo ZHOU, Emily R. NEKL, Bruce E. HIBBARD, B. Wade FRENCH & Blair D. SIEGFRIED	Effects of egg hatch timing, root injury, and larval background infestation on western corn rootworm larval movement in field corn
Poster 022	<u>Špela MODIČ</u> , Matej KNAPIČ, Zoran ČERGAN & Gregor UREK	Spread and population dynamics of western corn rootworm, <i>Diabrotica v. virgifera</i> LeConte, in Slovenia
Poster 023	<u>David W. ONSTAD</u> & L. M. KNOLHOFF	Rotation-resistant rootworm: Monitoring, bioassays, and modeling
Poster 024	<u>Joachim MOESER</u> & Stefan VIDAL	Screening for German maize varieties resistant against <i>Diabrotica v. virgifera</i>
Poster 025	<u>Emilija RASPUDIĆ</u> , Marija IVEZIC, Mirjana BRMEZ & Ivana MAJIC	European corn borer attack intensity and damages caused by western corn rootworm through 5 years investigations
Poster 026	<u>Marlin RICE</u> , Carol PILCHER & Clinton PILCHER	Management of <i>Diabrotica</i> and <i>Ostrinia</i> in the United States corn belt: A farmer survey of cultural, chemical, and transgenic practices and perceptions
Poster 027	<u>Jon TOLLEFSON</u> & Patricia PRASIFKA	Failure of crop rotation to control <i>Diabrotica</i> in Iowa
Poster 028	<u>Lukas SCHAUB</u> & Mario BERTOSSA	PAL-trap attractivity

## Thursday, 7 November 2006

### 13:30 Session 7 (Posters)

- **Display of Posters: Poster 029 to Poster 055**

Poster 029	<u>Ivan SIVCEV</u> , S. STANKOVIC, M. ALLARA, M. FREDRIX, J. JIGGINS & T. CVRKOVIC	WCR management by crop rotation on risk assessed corn fields
Poster 030	<u>Franja BACA</u> , Jan TANCIK, Jan PLANCAK, L'udovit CAGÁN & Miklos TOTH	Attractiveness of sweet maize hybrids to western corn rootworm ( <i>Diabrotica v. virgifera</i> LeConte) beetles in the late sowing in 2005
Poster 031	<u>Stefan TOEPFFER</u> , Nora LEVAY & Jozsef KISS	What happens after an alien <i>Diabrotica v. virgifera</i> beetle is accidentally introduced?
Poster 032	<u>Mirjana BRMEZ</u> , Marija IVEZIC, Emilija RASPUDIC & Ivana MAJIC	Correlation between plant lodging caused by western corn rootworm and grain yield
Poster 033	<u>Ana BUTRÓN</u> , G. SANDOYA, R. SANTIAGO, B. ORDÁS & R.A. MALVAR	Recurrent selection for maize resistance to pink stem borer
Poster 034	Laura A. CAMPBELL & <u>Lance J. MEINKE</u>	Fitness of <i>Diabrotica barberi</i> Smith and Lawrence x <i>Diabrotica longicornis</i> (Say) hybrids (Coleoptera: Chrysomelidae)
Poster 035	Britta FUESER, Joachim MOESER, Anant PATEL, Heiner FLESSA & <u>Stefan VIDAL</u>	Potential for western corn rootworm larval orientation disruption using artificial carbon dioxide sources
Poster 036	<u>Ibolya HATALA ZSELLÉR</u> , Géza RIPKA & Géza VÖRÖS	Some economic aspects of western corn rootworm for the Hungarian maize production
Poster 037	<u>Yukio ISHIKAWA</u> , Yongping HUANG, Takuma TAKANASHI, Xiaoyan FU, Suguru OHNO, Jun TABATA, Mai FUKUZAWA, Sugihiko HOSHIZAKI & Sadahiro TATSUKI	Evolution of sex pheromone communication systems in the genus <i>Ostrinia</i>
Poster 038	<u>LI Dunsong</u> , HUANG Shaohua, ZHANG Baoxin, PAN Zhiping & FENG Li	The annual population dynamics of <i>Ostrinia furnacalis</i> and its dominant parasitoid in sweet corn field
Poster 039	<u>Yunhe LI</u> & Jörg ROMEIS	Assessing the impact of <i>Bt</i> maize pollen on adult green lacewings
Poster 040	<u>LU Xin</u> , LI Li-juan, ZHANG Guohong	Studies on low temperature storage for industrial production of <i>Trichogramma dendrolimi</i> Matsumura
Poster 041	<u>LU Xin</u> , TIAN Zhilai, ZHANG Guohong & LI Lijuan	Research on the flight capability and effective accumulated temperature of different voltine ecotype of Asian corn borer
Poster 042	Dirk BABENDREIER, Siegfried KELLER, Ulrich KUHLMANN, Jörg ROMEIS, Stefan TÖPFER, Ted TURLINGS & Franz BIGLER	Development of biological products for sustainable control of the western corn rootworm
Poster 043	<u>Samy Hussein MOHAMED</u> & Hussein Fahim OMAR	Use of some eco-biological aspects and statistical analyses in determining the life table parameters and the number of generations of the green bug, <i>Schizaphis graminum</i> (Rondani) (Homoptera: Aphididae) on maize
Poster 044	<u>Margarida M. PEREIRA</u> & A.M. MEXIA	Spider mites population changes in corn and the relationship to leaf damage
Poster 045	<u>Marco BORIANI</u> & Mauro AGOSTI	PHEROCON <sup>®</sup> CCB, a new trap for monitoring western corn rootworm, <i>Diabrotica v. virgifera</i> LeConte (Coleoptera: Chrysomelidae)

Poster 046	John T. MARGARITOPOULOS, Bill GOTSPOULOS, Zissis MAMURIS, Panagiotis J. SKOURAS, Kostas Ch. VOUDOURIS, Kostas D. ZARPAS, Argyro A. FANTINOU & John A. TSITSIPIS	Genetic variation among Mediterranean populations of <i>Sesamia nonagrioides</i> (Lefèbvre) (Lepidoptera: Noctuidae)
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# **ABSTRACTS**

Monday, 6 Nov 2006, Session 1, 08:20; GRAY et al.

## Differential responses of nine transgenic (MON 863) maize hybrids to variant western corn rootworm larval injury

Michael E. GRAY, Kevin L. STEFFEY, Ron ESTES & Jared B. SCHROEDER

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In 2005, with the cooperation of Monsanto Company, we evaluated the root protection offered by nine YieldGard Rootworm (MON 863) hybrids and a check (nontransgenic hybrid) that were all planted on 29 April (four-row plots, 15 meters in length, four replicates) into a plot area that had been planted to a trap crop (late-planted corn intercropped with pumpkins) in 2004. The experiment was established near Urbana, Illinois which is near the epicenter of the variant western corn rootworm, *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae). Since the mid-1990s, the variant western corn rootworm has circumvented the usefulness of crop rotation as a pest management tool by laying eggs in soybeans and other crops. The fidelity to maize as an ovipositional site has been relinquished by the variant western corn rootworm. Roots were dug and rated (ten roots for each replicate by treatment combination) for injury on 20 July and 10 August. All hybrids were selected by Monsanto Company, and we rated them without any knowledge of their genetic background. Root injury (0 to 3 scale, 1 = one node of roots destroyed, 2 = two nodes of roots destroyed, 3 = three nodes of roots destroyed) in the check treatment was significant with two nodes of roots destroyed (root rating = 2.09, 20 July). As of 20 July, the root protection afforded by hybrids A (0.47), B (0.39), E (0.16), F (0.19), H (0.2), and I (0.21) was very good to excellent. YieldGard Rootworm hybrids A, B, E, F, H, and I were commercialized in 2005. By 10 August, root ratings were generally greater in these hybrids; however, we suggest the increases were not of biological significance. Hybrids E and H were the same hybrids. This information was revealed to us by Monsanto Company after we had concluded our root evaluations. Root pruning in YieldGard Rootworm hybrids C (0.98, 20 July; 1.27, 10 August) and D (0.78, 20 July; 0.98, 10 August) was excessive with nearly one node of roots destroyed in each hybrid. Monsanto Company indicated that they were not surprised by these results because both of these transgenic hybrids had failed their in-house screens during 2004. Neither of these hybrids was moved into the commercialization phase. Of surprise to Monsanto Company was the performance of hybrid G, which was commercialized in 2005 and had considerable brace root pruning by 20 July (0.75,  $\frac{3}{4}$  node destroyed) and 10 August (0.93, approximately 1 node destroyed). Hybrid G had undergone an in-house screen. These results suggest that some variation in root protection exists among YieldGard Rootworm (MON 863) hybrids. We will continue this research with Monsanto Company during the 2006 growing season in several areas of Illinois with different levels of variant western corn rootworm infestations.

Monday, 6 Nov 2006, Session 1, 08:40; KRUPKE & BLEDSOE

## **Transgenic maize for rootworm control: describing the relationships between refuge design and beetle movement**

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Transgenic (*B.t.*) maize varieties are being rapidly adopted by producers throughout North America for protection against corn rootworms - *Diabrotica v. virgifera* LeConte and *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae). Grower compliance with the refuge requirements (i.e. 20% of acreage must be non-B.t. corn) associated with these technologies has been reported as high. However, there is a shortage of empirical data documenting the movement, mating and dispersal of adult beetles within these transgenic/refuge environments. These biological parameters have the potential to directly and dramatically impact the success of any insect resistance management (IRM) plan. We present experiments that quantify the movement of beetles within and between various refuge types (discrete block refuge vs. refuge strips planted throughout the field) to aid in determining the optimal configuration for a refuge. Using Pherocon AM traps placed at regular intervals throughout large (20 hectare) commercial fields we document movement of beetles within and between fields planted with various refuge configurations. This allows us to generate contour maps to visualize the changes in beetle density over space (field-scale) and time (growing season). We also describe differences in emergence times, sex ratios, and mean weights (Table 1) of beetles in transgenic vs. refuge environments. In light of these data, our aim is to quantify the "encounter rates" between transgenic and refuge beetles. Data comparing mating rates of beetles emerging from transgenic fields and those emerging from refuge fields are presented and the potential utility of these data for measuring effectiveness of various refuge configurations is discussed.

Monday, 6 Nov 2006, Session 1, 09:00; SAPPINGTON et al.

## **Factors affecting spatial distribution of European corn borer adults across the landscape**

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The European corn borer (ECB), *Ostrinia nubilalis* Hübner, is a major pest of maize across the US Corn Belt, and is a primary target of the Cry1Ab toxin in transgenic Bt-corn. There is concern that the ECB could evolve resistance to Bt-corn because of the high selection pressure the insect has been under with widespread adoption of the transgenic technology. The effectiveness of current insect resistance management strategies, as well as mitigation strategies to be implemented if resistance is detected, depends fundamentally on understanding adult dispersal, mating, and the relative timing of the two. However, much remains unknown about these behaviors. ECB adults are commonly found concentrated in grassy aggregation sites, where they rest during the daytime and mate at night. These sites are common in the rain-fed Midwestern US along roadsides outside of fields and in waterways within fields. We are conducting studies to elucidate factors that influence selection of sites for aggregation relative to landscape features. Sampling of roadside grass during the first flight in central Iowa suggests that some moths emerging from corn stubble may aggregate in adjacent grass, but that they redistribute themselves in the landscape within a short time. The presence or absence of adjacent corn was the overwhelming factor affecting spatial distribution of first-flight moths. Intensive mapping of first generation ECB larval damage in commercial cornfields suggests that spatial variation in plant height may be an important factor determining within-field spatial variation in oviposition site selection. Spatial distribution of moths in the grass surrounding a cornfield was not correlated with spatial distribution of first-generation larval damage, an index of oviposition sites. Intrinsic factors affecting behavior were tested by mark-release-capture experiments. Larvae reared on dye-impregnated artificial diet were allowed to pupate in corrugated cardboard rings, which were then placed in corn plots. Thus, marked adults emerged in large numbers from the corn plots, which were flanked by tiers of small-grain plots planted to serve as grassy aggregation sites. Wild moths were captured in the small-grain plots indicating that they were physically suitable for colonization, but very few of the newly-emerged marked moths were recovered in them. Given the size of our sampling arena, this indicates that most newly-emerged ECB moths disperse beyond at least 300 m before colonizing acceptable aggregation sites. How much further they disperse remains unknown. This unexpected finding suggests the possibility of an obligatory dispersal phase of ECB adults after eclosion from the pupa. We are testing this possibility using computer-interfaced flight mills, made possible by a new technique for tethering moths to the flight arm. Experiments are underway to examine differences in flight behavior between unmated males and females of 1, 3, and 5 days of age. Implications of our results for ECB resistance management will be discussed.

Monday, 6 Nov 2006, Session 1, 09:20; SUMERFORD et al.

## Understanding the inheritance of resistance to *Bt* proteins in the European corn borer: Genetic mapping and future directions

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Diagnostic-dose bioassays are used to phenotypically classify *Ostrinia nubilalis* individuals as resistant or susceptible. However, if the goal of resistance monitoring is to monitor changes in the frequencies of resistance alleles, a better genetic understanding of potential resistance traits is necessary. Unknown factors include how many genetic loci will impact resistance to Bt proteins in *O. nubilalis*, and do different populations have the potential to find different genetic solutions. We are using three laboratory-selected colonies of *O. nubilalis* as a starting point for a better understanding of the genetics of resistance to the Bt protein Cry1Ab. Previous research generated crosses of resistant and susceptible colonies of *O. nubilalis* to find doses of Cry1Ab that distinguished between resistant and susceptible phenotypes. The doses of Cry1Ab that best discriminated among the composite resistance phenotypes were incorporated into a genetic mapping project. The mapping of genetic factors will allow us to partition the composite resistance trait to regions of the *O. nubilalis* genome thus allowing us to determine if any of the phenotypic variance for Cry1Ab resistance is due to similar genomic regions among the different colonies. Genomic scans of *O. nubilalis*' linkage groups will initially be performed via linkage maps of AFLP markers. Future research would include isolating and cloning the actual gene(s) to determine the mechanism(s) of resistance. The ultimate goal will be efficient bioassays and genetic assays that will allow genotyping of individuals for loci with the greatest probability of influencing resistance evolution in wild populations of *O. nubilalis*. Such tools could be incorporated into F2 screens or other resistance monitoring approaches and could also be used in the assessment of fitness costs and the interactions of Bt resistance with ecological variables.

Monday, 6 Nov 2006, Session 1, 09:40; WANG et al.

## **Effects of transgenic *Bt* corn expressing Cry1Ab toxin on survival of the Asian corn borer (Lepidoptera: Crambidae)**

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The Asian corn borer, *Ostrinia furnacalis* Guenée, is the most destructive pest of corn in China. It causes 6 to 9 million tons yield loss in an ordinary year and even more in an outbreak year. Transgenic corn expressing Cry1Ab toxin from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner provides a new tool for managing the Asian corn borer. Effects of two Bt corn hybrids (event MON810 and Bt11) on controlling the Asian corn borer were evaluated in laboratory bioassay and in field conditions. The levels of the Cry1Ab toxin expressing in different tissues of two Bt corn hybrids were also carried out by using a quantification assay (ELISA) method. Results showed that the Asian corn borer neonates feeding on fresh whorl leaves, tassels, silks, husks, young kernels of two Bt corn hybrids died in laboratory bioassays. The amount of Cry1Ab protein expressed in whorl leaves, husks, ear tips and kernels of both Bt corn hybrids was highly correlated with the mortality of Asian corn borer neonates, except the tassels of MON 810 and tassels and silks of Bt11, which showed that the resistance of the Bt corn to the insect were also related to other factors besides the content of Bt toxin. None of surviving larvae is observed in two *Bt* corn plants when artificial infestation during the late whorl, tasseling and silking stage in the field, respectively. Significant differences are observed between two *Bt* corn hybrids and their non-Bt controls from the leaf-feeding scales, number of holes and the tunnel length per plant in tasseling stage, and the injured length per ear. The fourth-instar larvae feeding on Bt corn stalks were all died in laboratory tests. The corrected mortality was significantly higher on Bt corn plants than on non-Bt corns in 10 d. None of fourth-instar larvae pupates when feeding on Bt corn stalks, and the percentage of injured plants, the number of holes and the tunnel length per plant of two Bt corn hybrids were significantly lower than their controls under the field conditions. It was showed that the event MON810 and Bt11 had excellent resistance to the Asian corn borer during the growing season of corn.

Monday, 6 Nov 2006, Session 2, 10:35; HELLMICH et al.

## **Assessing non-target effects of *Bacillus thuringiensis* (Bt) maize: lessons learned and new directions**

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Ten years ago growers in the U.S. started to plant Cry1-expressing Bt maize, which has revolutionized control of European corn borer, *Ostrinia nubilalis* Hübner. Now Cry3-expressing Bt maize with resistance to corn rootworms, *Diabrotica* spp., is available and other types of biotech maize are on the horizon. Scrutiny of biotech maize, however, has been very high, particularly in Europe. Some scientists have questioned the ecological risk assessment process and others have questioned whether Bt maize negatively impacts biological control insects such as the common green lacewing, *Chrysoperla carnea* Stephens and butterflies such as the monarch butterfly, *Danaus plexippus* Linnaeus. Academic, government, regulatory and private-sector scientists have noted criticisms and are working to improve the science of risk assessment for all biotech crops with an emphasis on biotech maize. This talk will briefly summarize the science that has addressed Bt maize impact on green lacewings and monarch butterflies and highlight current activities by several scientists that should improve risk assessment of transgenic crops.

## **Environmental risk assessment of maize expressing mCry3A for control of corn rootworm**

Alan RAYBOULD<sup>1</sup>, David STACEY<sup>1</sup>, Demetra VLACHOS<sup>2</sup>, Robert JOSEPH<sup>2</sup>, Gerson GERSON<sup>2</sup> & Mike MEAD-BRIGGS<sup>3</sup>

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Syngenta has developed a variety of genetically modified maize, MIR604, that expresses a modified Cry3A protein (mCry3A) for control of corn rootworm. To assess the environmental safety of MIR604, we carried out laboratory studies of the toxicity of mCry3A to selected non-target organisms. This paper will report the design of the environmental safety testing strategy for MIR604, and the design and results of the laboratory toxicity studies. Finally, we will discuss the implications of the results, and whether laboratory studies are sufficient to demonstrate the environmental safety of MIR604. The implications of the results for genetically modified plants in general will also be discussed.

The paper will be in three parts:

1. **Problem formulation.** This part of the paper will discuss the design of the safety testing strategy, and in particular the reasons for basing the safety assessment of MIR604 on tier 1 methods: laboratory studies of the toxicity (hazard) of mCry3A to representative indicator species and estimates of worst-case environmental exposures to mCry3A. No higher tier studies (e.g. field studies) were planned unless risk was identified at tier 1. This section will also discuss the criteria for selection of species for laboratory testing; these included taxonomic relatedness to the target pest and likelihood of exposure in maize fields.
2. **Laboratory studies.** This section will discuss the design of the laboratory toxicity studies. The studies were designed to achieve long exposures of the most relevant test species to high concentrations of mCry3A. This required extensive method development of artificial diets and means to demonstrate exposure of the test species to mCry3A.
3. **Risk assessment.** This part of the paper will discuss the results of the laboratory studies and show how the data were combined with data on exposure to estimate the environmental risks of MIR604. We will discuss whether tier 1 laboratory studies are sufficient to demonstrate minimal risk of MIR604, or whether higher tier (field) studies are required. We will emphasize the relative power of tier 1 laboratory studies and field studies to test hypotheses that result from the problem formulation, and the importance of experimental design of tier 1 studies to achieve maximum power to extrapolate the results to species that have not been tested.

No adverse effects of mCry3A at concentrations greatly in excess of the expected environmental concentrations were detected in laboratory studies. It was concluded, therefore, that MIR604 poses minimal environmental risk.

Monday, 6 Nov 2006, Session 2, 11:15; PONS et al.

## **Indicator species for testing non-target effects of *Bt* maize on natural enemies: a proposal**

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Analysis of potential ecological impacts of GM maize on non-target organisms, particularly on pest natural enemies, is crucial to determine compatibility of insect-resistant transgenic maize with biological control. Most risk assessment approaches recommend a sequential process from laboratory tests in confined conditions to a more realistic environment in the field. Laboratory tests aim to screen a number of carefully selected organisms for their susceptibility to Bt toxins under worst-case conditions. Correct selection of indicator species for laboratory testing may save a lot of work in these preliminary phases of the risk assessment process. Criteria for selection of indicator species must include several aspects dealing with the significance and abundance of the species in the cropping system where GM crop has to be grown, species relevance for the ecological function concerned (biological control), intrinsic susceptibility of the species to the transgene product(s), likelihood of occurrence of the pathways through which the species may be exposed to the toxin in commercial and common growing conditions. After ten years of studying impact of insecticides and transgenic insect-resistant maize cultivars on predatory fauna in maize in Mediterranean conditions, we discuss suitability of the most abundant and frequently recorded species/groups of predators. It is concluded that *Orius* sp. (Heteroptera: Anthocoridae) is the most suitable predator for risk assessment in the laboratory. It is the most abundant group in maize in our conditions, it maintains on the crop for most of the season, it is exposed to the toxic through both direct (feeding on the plant and plant products) or indirect pathways (preying on a large spectrum of herbivores that potentially can acquire the toxic). Additionally, *Orius* sp. meets other practical requirements: it is relatively easy and cheap to rear and, in case of necessity, several species are commercially available. Advantages and disadvantages of *Orius* sp. in comparison with other potential indicator species of predators that are commonly found in maize in Mediterranean conditions are discussed.

## Community-level effects of *Bt* sweet corn on non-target arthropods

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A field study was conducted at two locations over two years to determine the effects of transgenic sweet corn containing a gene from the bacterium *Bacillus thuringiensis* (Bt) on the diversity and abundances of non-target arthropods. The hybrid 'Attribute GSS0966' (Syngenta Seeds, event BT11, Cry1Ab) and its non-transgenic isoline ('Prime Plus') were planted in plots consisting of 24 rows 30 m long. Plots were laid out in a 2 x 2 split plot design with four replicate blocks. The transgenic Bt hybrid was compared with transgenic and isogenic hybrids treated with lambda-cyhalothrin insecticide, and with an untreated isogenic hybrid as a control. Treated Bt plots received one insecticide application at 100% fresh silk, while the treated isogenic plots received five applications starting at early silk and repeated every three days. Direct plant inspections, yellow sticky cards, and pitfall traps were used to sample the foliage, aerial and surface-dwelling communities.

A total of 573,672 arthropods were enumerated, representing 128 taxonomic groups in 95 families and 18 orders. By functional group, 39, 32, 24 and 5% of the arthropod community was comprised of decomposers, predators, herbivores and parasitoids, respectively. Community-level responses in the Bt plots determined by principle response analysis were not significantly different from the responses in the undisturbed control community. All sampling methods showed no significant adverse effects from expression of the Cry1Ab protein on population densities of key non-target herbivores, decomposers, and natural enemies recorded at the family level. As expected, the insecticide lambda-cyhalothrin had broad negative impacts on many non-target arthropods. One insecticide application in the transgenic plots reduced communities of natural enemies by 21 to 48%. Five applications in the isogenic plots reduced natural enemy communities by 33 to 70%. All plant-dwelling predators, parasitoids, decomposers, and most herbivores were sensitive in varying degrees to the pyrethroid insecticide. The surface-dwelling arthropods were generally less affected due to the protective shelter afforded by the surface litter and lower amounts of insecticide residue that settled on the litter surface. Collembola, mites, and aphids tended to increase in numbers in the insecticide-treated plots. Non-target communities affected by Bt treated plots exhibited some recovery, but communities exposed to five applications showed no trends toward recovery during or after the crop cycle.

This study clearly showed that the non-target effects of *Bt* transgenic sweet corn on natural enemies and other arthropods were far less than the community-level disruptions of insecticide control, which have an accepted level of safety. The weight of evidence supports the general consensus that there are no unexpected ecological risks caused by transgenic lepidopteran-resistant corn on non-target organisms. The use of *Bt* sweet corn can result in significant reductions in conventional insecticides and less disruption to beneficial insects, which may lead to enhanced natural control of secondary pests.

## Hunting in *Bt* maize – business as usual for a web-building spider?

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Since its introduction in the late 1980s, the western corn rootworm (*Diabrotica v. virgifera* LeConte, Coleoptera: Chrysomelidae) is spreading rapidly over Europe. Especially maize growing regions not following crop rotation are vulnerable to this pest. Larvae feeding on maize roots ultimately cause plant lodging due to reduced nutrient and water supply.

One alternative to chemical pesticides is to grow transgenic maize, which expresses the coleopteran specific Cry3Bb1 toxin originating from *Bacillus thuringiensis* (*Bt*). The evaluation of potential environmental impacts on naturally occurring beneficial species like generalist predators is part of the risk assessment that precedes the commercialization of any transgenic crop. In our project, we assess the potential risk of Cry3Bb1 expressing maize for the spider *Theridion impressum* L. Koch (Araneae: Theridiidae). This species is common all over Europe and reproduces frequently in maize fields. By catching a broad range of insect pests in their space webs, the spider contributes to natural pest regulation.

As a first step for the risk evaluation, we estimate the potential exposure of the spiders to the Cry toxin in a *Bt* maize field. The toxin content of potential prey species, collected in experimental *Bt* maize plots in Germany, is determined using enzyme linked immunosorbant assays (ELISA). Using published data on the prey spectrum of *T. impressum* in European maize fields, a realistic worst case estimate of potential toxin exposure is calculated. To get a more realistic estimate of toxin uptake, toxin levels in field collected spiders are compared with the calculated values.

In a second step, we investigate the hazard of *T. impressum* being exposed to the toxin. Spiders are collected in the field and kept in the laboratory. Prey species that are known to contain large amounts of Cry toxin when feeding on *Bt* maize are offered to simulate a realistic worst case situation. Life table parameters (mortality, weight) of adult and juvenile spiders in the *Bt* treatment are compared with non-*Bt* controls.

Finally, potential exposure data are combined with results of the hazard experiments to estimate the overall risk that *Bt* maize poses for *T. impressum*. Although this risk evaluation procedure is conducted for one model species only, we expect that both the methodology and data can be used for further non-target risk assessments of transgenic plants.

## **Presence of gravid *Diabrotica v. virgifera* females in maize fields in Northern Italy**

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The continuous spreading of the western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae) in Northern Italy, is expected to reach economic populations in the near future. In some areas of the Padana Plane, damages could occur already in 2006. Maize is the most important agricultural crop in this region and is usually grown in monoculture. Crop rotation, a common WCR management tool used in many areas of Europe, is not common and is usually not well accepted by farmers because of the need for maize for livestock feed.

Among the different WCR management options available, adult control, which utilizes insecticide sprays to reduce egg laying to non-economic levels, could be one of the most interesting control possibilities. With this treatment, not only the WCR can be controlled, but also another major pest of maize, the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) is controlled at the same time.

The presence of WCR gravid females in a field is key to pinpointing the best time for insecticide application for control of egg laying.

In 2005 and 2006 in four maize fields in Northern Italy, WCR males and females were monitored weekly using different trap types from mid June to mid August. For each date, samples of captured females were dissected under a binocular microscope to evaluate ovarian development.

In 2005, the first gravid females were found at the end of June. By the second week of July, all females examined were gravid and a few presented full developed eggs. All captured females from mid July to the end of the sampling period were gravid and most with full developed eggs. The first females with spent ovaries appeared at the beginning of August. The number of females in the fields was quite low until July 10-12. From this date, however, the number increased rapidly. After the "blister" stage of maize development (2-3 weeks after full flowering), the number of females in the fields started to decline.

*In 2006, we intend to repeat the same fields observations. The results from 2006 will be combined with the 2005 data and will be reported at the IWGO meeting in Vienna, Austria.*

## **Effects of crop residues on plant lodging caused by larvae of *Diabrotica v. virgifera* and grain yield**

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Stover (CR) is mainly ploughed down in the maize production in Serbia, as animal keeping has been reduced, and feeding with stover is reduced to the minimum. Maize has been often grown in continuous cropping, or as today after the WCR appearance in repeated sowing. Stover ploughing down returns the organic matter of about 80 kg N/ha<sup>-1</sup>, and other nutrients for crops in succeeding years. As far as the soil is concerned, CR ploughing down increases its aeration and microbiological activity, while its structure is improved. Maize yield achieved in continuous cropping with the CR ploughing down is higher and more stable.

The aim of these studies was to check the effect of the stated changes made by harvest residues ploughing down on both, yields of maize grown in continuous cropping and WCR. The hypothesis was that: 1<sup>st</sup> the CR presence from the previous year, still in the stage of degradation during the succeeding spring, aggravates the optimal seedbed preparation and conditions for maize emergence are less favourable, 2<sup>nd</sup> during July and August when females lay eggs, depending on soil humidity, the CR presence can be an objective either difficulty or ease for egg laying, 3<sup>rd</sup> newly hatched larvae come across harvest residues and their search for food that has to be successful within a few hours after hatching is harder.

In order to check the stated assumptions, the analysis of nine-year studies on plant lodging, as a parameter of CR effects on WCR and yields, as a indicator of their effects on maize, was performed. Out of existing 54 combinations of mineral fertilisers (MF), manure (SM) and (CR), six variants of MF without application of SM were selected in combination with CR ploughing down and CR complete removal. The gained results are presented in Table 1.

**Table 1:** Grain yield and plant lodging between removal of CR and CR ploughing down over the combinations of MF rates in Zemun Polje 1997-2005.

Mineral fertilisers		Yield before WCR occurrence			Yield after WCR occurrence			Plant lodging in %		
Abr.	NPK kg/ha	CR0	CR2	CR2-CR0	CR0	CR2	CR2-CR0	CR0	CR2	CR2-CR0
MF1	0:0:0	6.172	6.675	0.503	4.162	4.732	0.570	4.66	10.63	5.97
MF2	135:132:74	9.239	9.820	0.581	6.627	7.465	0.838	12.71	13.28	0.57
MF3	270:246:148	9.130	9.709	0.579	6.694	7.356	0.662	14.77	14.07	-0.70
MF4	181:132:74	9.199	9.811	0.612	7.180	7.471	0.292	16.40	14.28	-2.12
MF5	316:246:148	9.239	9.743	0.504	6.418	7.423	1.005	22.46	13.32	-9.24
MF6	46:0:0	7.559	8.121	0.562	4.660	6.043	1.383	18.70	10.17	-8.53
<i>Mean</i>	<i>1 to 6</i>	<i>8.423</i>	<i>8.980</i>	<i>0.557</i>	<i>5.957</i>	<i>6.748</i>	<i>0.791</i>	<i>14.95</i>	<i>12.63</i>	<i>-2.32</i>

CR ploughing down prior to the WCR appearance resulted in the yield increase of 557 kg ha<sup>-1</sup>. After its appearance, during the succeeding nine years, a significant yield increase was registered. This increase amounted to 791 kg ha<sup>-1</sup>, with a positive difference of 234 kg ha<sup>-1</sup> or 42.0% in relation to the period prior to its appearance. At the same time, depending on the NPK combination, significant plant lodging decrease (up to 9.24%) was detected. The highest differences in obtained yields were detected in the combination with high rates of mineral fertilisers MF5 and the application of nothing else but nitrogen MF6.

According to everything stated, it can be concluded that maize continuous cropping on chernozem type of soil can be recommended only with CR ploughing down and the application of NPK fertilisers or merely nitrogens with application of insecticides for band treatment of soil.

## Effects of different growing systems on attractiveness of maize crop to beetles of *Diabrotica v. virgifera* in Zemun Polje in 2003, 2004 and 2005

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After appearance of western corn rootworm (WCR) in former Yugoslavia and Europe in 1992, an abrupt increase of the number of imagoes and territorial distribution were registered. These phenomena lasted till 2000 when abundance started to decrease. The following factors are considered the ones to cease decrease of abundance:

- unfavorable weather conditions with lack of rain during maize growing period (April - September), particularly in 2000.
  - abandoning maize in continuous cropping which is a principal generator of high abundance.
- Monitoring of imagoes in Serbia was initiated in 1996, systemic monitoring at Zemun Polje started in 1997. Results on monitoring of WCR beetles up to 2003 have been reported in national and international symposia.

Results on which this paper is based were obtained at Zemun Polje by monitoring of imagoes during the period July-September of 2003, 2004 and 2005 in the trial with the following combinations of maize cultivation:

- maize in continuous cropping, maize in two crop rotation 1<sup>st</sup> maize in the combination with wheat, 2<sup>nd</sup> maize in the combination with soybean and maize in three crop rotation in the combination with wheat and soybean.

Each of systems consisted of 16 maize rows of the total area of 448 m<sup>2</sup>, i.e. 224 m<sup>2</sup>/per a trap on the average. In each of systems, one pheromone (PhT) and one yellow stick trap (YsT) were placed with intermediate spacing maximally apart from the other. There were no imagoes in YsTs, while the results obtained in PhTs are presented in Tab. 1.

**Table 1:** The total number of WCR beetles registered in pheromone traps at Zemun Polje in the trial with maize growing systems in 2003, 2004 and 2005.

Years	Maize continuous cropping	Two crop rotation 1 M-W	Two crop rotation 2 M-S	Three crop rotation M-W-S	Total number	Rank
2003	188	223	205	199	815	1
2004	215	76	73	3	367	2
2005	115	34	20	14	183	3
Total	518	333	298	216	1365	
Index	100.0	64.3	57.5	41.7		

The migration of imagoes from maize in continuous cropping to maize in the variants with crop rotations varied over years. Hence in 2003, a greater number of imagoes were registered in the variants with crop rotations than in the variant with continuous cropping. It happened for the first time since the beginning of monitoring in this trial. A lower abundance of imagoes in variants with continuous cropping comparing to the variants with crop rotations was registered in 2004 and 2005.

Based on the presented results it can be concluded that the WCR abundance declined over years and that it was below the economic level in the variant with continuous cropping in 2005.

## **Attraction of light sources and kerosene to adults of western corn rootworm (*Diabrotica v. virgifera*)**

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The western corn rootworm has often been first recorded near airports. This indicates its introduction with airplanes from infested areas. It is not known in which way airports are attractive to the beetle and how it gets into a plane. For this reason, 2004 saw first experiments on the attractiveness of various types of lamps used at European airports. The experiments were carried out in South Hungary. The tests were continued in 2005 and extended to kerosene as a possible source of attraction. In 2004, the lamps had been mounted at a distance of 200 m to a heavily infested field, but did not show any attraction. For this reason the distance was shortened to 75 m in 2005. However, the lamps (mercury vapour lamp, natrium high pressure lamp) still did not attract beetles. During the entire season 16 beetles were trapped in 6 lamp traps (2.7 beetles/trap for both lamp types). Csalomon pheromone traps of the type PALs (3 pieces) caught 75 beetles (25.7 beetles/trap) at the same distance in the same period of time. The PALs traps captured 1,219.0 WCR adults/trap on the maize field during the same period. The mercury vapour lamps caught 11 beetles (3.7 beetles/trap) and thus had a higher trapping rate than natrium high pressure lamps with 5 beetles (1.7 beetle/trap).

Tests on kerosene (0 beetle/VARL trap) have shown that the fuel is not attractive and has rather a repellent effect.

The 2-year results lead to the conclusion that lamps used at European airports are not particularly attractive to the western corn rootworm. Aviation fuel kerosene is not attractive to this beetle, it has rather a repellent effect.

## **Stage-specific development and mortality of western corn rootworm reared on transgenic event MON 863 vs. a non-transgenic isoline field corn hybrid**

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A field experiment was conducted in 2004 and 2005 to determine if there are differences in stage specific larval development and mortality of western corn rootworm (*Diabrotica v. virgifera* LeConte, Coleoptera: Chrysomelidae) reared on transgenic event MON 863 vs. a non-transgenic isoline field corn hybrid (*Zea mays* L.). A randomized complete block design with 5 replications was used. Each block contained two treatments: transgenic (DKC 60-12, YieldGard Rootworm™: MON 863 + Poncho 250™ seed treatment) and non-transgenic isoline (DKC 60-15 + Poncho 250™ seed treatment) corn hybrids. Eggs were artificially infested at the V2-plant stage. Soil-plant sampling for larvae and standard sample processing techniques were annually performed twice a week over a two month period to recover western corn rootworm larvae. Beetle emergence was determined by monitoring single-plant emergence cages at 1 to 3 day intervals from the end of June to mid-September. A non-linear analysis approach was used to statistically analyze the cumulative number of larvae within each instar and adults retrieved over time in 2004 and 2005. In both years, a low level of rootworm injury occurred in each treatment; therefore, the experiments were conducted with only minimum potential for density-dependent factors to adversely affect development and mortality. Results indicate that the transgenic hybrid reduced total larval survival; most of the mortality appeared to occur early in the lifecycle. Nonlinear analyses suggest that larval developmental delays occurred in the transgenic plots. The adult emergence pattern was initially delayed in transgenic plots in relation to emergence patterns from isoline plots. The adult sex ratio was also more skewed toward females in the transgenic vs. isoline treatment. In the transgenic treatment, the level of larval developmental delay in larval stages does not explain the substantial initial delay in adult emergence unless the delays are compounded over each life-stage. The interaction of larval sex ratio and sex related developmental rates, plus, relative toxicity and exposure of each life-stage to Cry3Bb1 may have contributed to the observed patterns.

## Field tests on the host range of the larvae of the western corn rootworm (*Diabrotica v. virgifera*) in Europe

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Crop rotation is a commonly used and often cited method for eradication programs and integrated pest management of the western corn rootworm (WCR) *Diabrotica v. virgifera* LeConte in Europe. Research on alternative host plants except corn was mainly carried out in the United States. Until now, there are only laboratory data of possible alternative hosts plants for the larvae of the WCR, but no field data for the European region. Therefore a three-year field experiment was started in 2004 in Romania.

From 2004 to 2006 field trials were carried out in Lovrin, Romania, to test the host-suitability of different weeds and winter wheat for larvae of the WCR. The weeds tested in 2004 and 2005 were *Setaria glauca*, *S. viridis*, *S. verticillata*. Maize was used as positive control and *Sorghum halepense* (*S. halepense* is known to be toxic for larvae of WCR) as a negative control. In 2006 two more weed species and a variant with voluntary barely after winter barely and *S. glauca* as weed in winter barely were included.

In 2004 there were problems with *S. glauca* germinating in all tested variants, so that the results only indicated a possible suitability of *Setaria spp.* as host plants for the larvae of *Diabrotica*.

In 2005 for all three species of *Setaria spp.* development of larvae of the WCR was recorded. Compared to the maize control (set as 100%) there was 15% emergence of beetles in *S. viridis* and *S. verticillata* and 12% emergence in *S. glauca*. Furthermore the beetle emergence was delayed about two weeks compared to the maize control. Thus *Setaria spp.* seems to be suitable hosts for larvae of WCR in the field at least under Romanian conditions.

There was no emergence of WCR in winter wheat, but the plants were already dry and ripened when beetle emergence started. So there might have been no food resources for larvae of later larval stages, resulting in no emergence of adult beetles. In laboratory tests winter wheat and barley proved to be a suitable host, so that especially voluntary cereals should be analysed in further experiments.

The results show that crop rotation is a useful IPM and eradication strategy, but special care may need to be taken regarding weeds and voluntary grains in fields infested with eggs of WCR to prevent development of larvae.

## **Spread of western corn rootworm (*Diabrotica v. virgifera*) in the Czech Republic**

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Western corn rootworm was first observed in Serbia (Belgrade), Europe in 1992. It spread subsequently to other European countries: Hungary and Croatia (1995), Romania (1996), Bosnia and Herzegovina (1997), Bulgaria, Montenegro and Italy (1998), Slovakia and Switzerland (2000), Ukraine (2001), Czech Republic and Austria (2002), Slovenia, United Kingdom, Belgium and Netherlands (2003), Poland (2005).

In the Czech Republic, the first beetles were caught in July 2002. Nine males of *Diabrotica v. virgifera* were caught in maize fields at 6 localities in three districts (Hodonin, Breclav and Uherske Hradiste) in the south-east of the Czech Republic. The number of caught beetles has been increasing steadily, but beetles can't be considered as domesticated yet. Neither females nor juvenile stages (larvae or eggs) have been found so far. 510 males of *Diabrotica v. virgifera* were caught in 2005. 317 beetles were caught in three districts listed above. These districts have been the first source of the occurrence of *Diabrotica v. virgifera* in the Czech Republic. Western corn rootworm has expanded into the Czech territory to a distance about 80 km within 3 years.

The earliest occurrence of *Diabrotica v. virgifera* during the years 2002 – 2005 was recorded on 11<sup>th</sup> July 2003 and the latest capture on 1<sup>st</sup> October 2004.

The survey of occurrence *Diabrotica v. virgifera* has been carried out since 1999 using the standard PAL sex pheromone traps. In total, 204 sites were monitored in 2005. 133 survey sites were established in noninfested areas and 71 in infested areas. The average density of traps is one trap per 1424 hectares. The occurrence of western corn rootworm is being normally monitored from the beginning of July to the end of September. However, in some cases monitoring lasts longer because the end of monitoring depends on the term of harvest. Traps are being checked once a week.

The SPA diagnostic laboratory in Olomouc is in charge of diagnostics and also provides training and scientific advice. Adults are identified according to morphological characteristics mentioned in Diagnostic protocol for regulated pests PM 7/36 (EPPO Standards).

The SPA issued the Decision no 39/05/41 from 6<sup>th</sup> January 2005 regarding measure against spreading of *Diabrotica v. virgifera*. This document implements the provisions of Commission Decision 2003/766/EC in the Czech Republic.

## **Results of investigations on various aspects of monitoring of western corn rootworm (*Diabrotica v. virgifera*, Coleoptera: Chrysomelidae) in Austria in the years 2004 and 2005**

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The western corn rootworm (WCR) (*Diabrotica virgifera virgifera*) LeConte (Coleoptera: Chrysomelidae) was first recorded in Austria in 2002. In the course of monitoring the new pest, questions arose as to the efficacy of the methods used, in particular the recommended trap replacement schedule of 4 weeks.

### Trap replacement schedules.

Investigations were carried out in 2004 and 2005 on the effect of trap replacement schedules of 1, 2, 3 and 4 weeks on WCR beetle catches. Csalomon® PAL traps and pheromones of the Hungarian firm MTA Növényvédelmi Kutató-intézet were used in all studies. Investigations were carried out in fields of continuous corn around the village of Deutsch Jahrndorf in easternmost Austria. Four replicates were established in each corner of the 4-6 ha large rectangular fields. In each replicate the traps were set along the longer side of the field in the 10<sup>th</sup> row of corn and 25m apart, the first 25m into the field. The trap of each schedule was situated at a different position in each replicate. Beetles on the sticky traps were counted and removed weekly. Trap replacement followed the schedules of 1, 2, 3 and 4 weeks.

In both years the numbers of WCR beetles decreased sharply in the second, third and fourth week. Two-week old traps caught only 38% and three- and four-week old traps only 20% as many WCR beetles as one-week old traps. In all trap replacement schedules replaced traps caught equal numbers of beetles. Seasonal total numbers caught were also lower in traps replaced at longer intervals. In traps replaced every two, three and four weeks 62%, 37% and 36% resp. as many WCR beetles were caught as in traps replaced weekly.

### Sticky sheet replacement.

In 2005 the effect of replacing the sticky sheets at intervals of 2 and 3 weeks during a 4-week replacement schedule was also studied. In an identical experimental design the sticky sheets of 3 traps with a 4-week replacement schedule were replaced at 2-, 3- and 4-week intervals.

The results of this experiment were unexpected. After replacing the sticky sheets catch numbers increased sharply, irregardless of pheromone age on the trap. It seems that catch numbers are at least as well correlated to replacement of sticky sheets as to replacement of pheromones. Further experiments are necessary to validate these results and to separate effects of pheromone and sticky sheet replacement on WCR catch numbers.

### Pheromone age and efficacy.

In 2005 another experiment with 3 replicates comparing fresh pheromones with those 1 and 2 years old was carried out. No significant differences in catch numbers were seen between the pheromones of different age. "Old" pheromones can definitely be used for monitoring with the same efficacy as fresh ones, provided that they had been stored according to the recommendations of the production firm (temperature no higher than -5°C to -10°C).

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## **Assessing and managing risks posed by *Diabrotica v. virgifera* in the UK**

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*Diabrotica v. virgifera*, the western corn rootworm (WCR) has spread widely in central Europe since it was first found in (former) Yugoslavia in 1992. Further satellite outbreaks in Europe have strongly suggested a link with airports, and first reports of WCR in France near Paris airports in August 2002, raised further concerns in many northern European countries. New introductions of WCR were confirmed near airport locations in the Netherlands, Belgium and UK, for the first time in 2003. More extensive surveying in the UK in 2004 and 2005 has confirmed that the pest is confined to areas near to Heathrow and Gatwick airports.

Under current climatic conditions, WCR appears to be at the edge of its range in the UK. However, the production of maize in the UK has increased over the last decade to become an important crop within dairy and mixed farming systems. Predictions of the impact of the pest under UK climatic conditions are difficult, particularly for a pest where all immature life-stages occur in the soil, and may depend on the extent to which it can adapt to more northerly climates. In addition, future impacts need to consider climate change scenarios: data is presented which suggests that by 2050 a large area of the UK will be suitable for the establishment of this species. Assessing the pest potential of marginal pests such as WCR poses a particular challenge, as relatively small changes in either biotic and a-biotic conditions can change the outcome of the pest risk analysis, in terms of potential economic impact.

Pest management options aimed at eradication and containment of the pest are discussed, in the context of applying European Commission Decision (2003/766/EC). An economic analysis is also presented to illustrate the cost of measures in more marginal areas, such as the UK may exceed the economic damage caused by the pest.

## **Spatial considerations in a replicated plot study of ground beetles (Coleoptera: Carabidae) in rootworm-resistant maize**

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Ground beetles (Coleoptera: Carabidae) are of particular interest in agroecosystems because they are effective predators of soil and canopy-dwelling pests and also are considered positive indicators of sustainability.

There is concern that ground beetles may be adversely affected by plant-incorporated insecticides in Bt-maize for management of corn rootworms. In a series of field experiments, we tested hypotheses that ground beetles, as indicator species, are unaffected by the genetically-modified corn rootworm-resistant maize. Three experimental treatments, a resistant maize incorporating the Cry3Bb1 gene derived from *Bacillus thuringiensis* (Berliner) subsp. Kumamotoensis (Bt-based line), an insecticide-treated isoline, and an untreated maize isolines were included in a random complete block design with four replications. Each maize treatment plot was 2 ha and was bounded by soybean alleyways and borders of about 19 m. Crop-specific spatial distributions, influence of topography, and soil apparent electrical conductivity on beetle spatial distributions were apparent in georeferenced data taken from a grid array of pitfall traps (N=105) for ground beetles. There were no apparent effects of plant-incorporated insecticides (BT-maize) on ground beetle spatial distributions, thus further supporting the benign nature of these hybrids on non-target ground beetle communities.

## **The corn rootworm protected maize expressing the *Bacillus thuringiensis* Cry3Bb1 protein: summary of benefits**

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Corn rootworm is a major maize pest in North America with estimated 5.7 million hectares of maize in the US treated annually with organophosphate, carbamate, pyrethroid, and phenyl pyrazole insecticides to control this pest. Corn rootworm larvae damage maize by feeding on the roots which reduces the ability of the plant to absorb water and nutrients from soil and causes harvesting difficulties due to plant lodging.

In 1992, *Diabrotica v. virgifera* (WCR) was found for the first time in Serbia and is spreading rather rapidly in the region with additional foci found outside the region. Research indicates that WCR is likely to be established and persist wherever maize is grown in Europe.

*Bacillus thuringiensis* (*Bt*) is a naturally occurring bacterium that is found in soil worldwide that selectively kills insects. Different sub-species of *Bt* produce different proteins. The "Cry" proteins are a group of toxins produced in *Bt* which are classified based on their structure and which insects they control. Susceptible insects contain receptors in their midgut that bind to the specific Cry protein (e.g. Lepidoptera have receptors that bind to Cry 1 toxins, while Coleoptera have receptors that bind to Cry3 toxins). This leads to the creation of pores which interfere with ion transport systems across the midgut wall causing lysis of the midgut epithelium and, depending on the dose, subsequent paralysis of the gut or death of the insect.

Using the particle acceleration transformation method, Monsanto Company has transformed maize which is protected from damage caused by corn rootworm feeding. These plants contain a *Bt* gene coding for the Cry3Bb1 protein which is selective for coleopterans (*Chrysomelidae*) of the *Diabrotica* family, to which corn rootworms belong. Corn rootworm protected maize has been grown commercially in the US since 2003, with approximately 1.7 million Ha of the trait MON 863 (as a single trait or stacked with other traits) planted in 2005.

Corn rootworm protected maize offers many benefits to the grower and the environment. A few examples are:

- A new means to control corn rootworm, compatible with integrated pest management (IPM approaches);
- Increased economic benefits to farmers (\$231 million from yield gains and \$58 million in reduced insecticide use and time savings estimated for the US);
- Increased intangible benefits for farmers linked to the safety of not being exposed to insecticides, ease of use and handling, time and labor savings, as well as better pest control;
- A significant reduction in insecticide use (75.2% reduction in active ingredient use estimated for the US if transgenic rootworm maize were planted on 4 million hectares);
- Reduced impact on beneficial, non-target insects.

## **Orientation of larvae of *Diabrotica v. virgifera* in different soil compositions and soil densities**

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Finding the host is an important aspect of pest biology and therefore relevant as research task to find solutions for fighting the invasive maize pest *Diabrotica v. virgifera*. The eggs of the western corn rootworm (WCR) *Diabrotica v. virgifera* are usually not located close to the roots, so that hatching larvae first have to find the roots of their host. Freshly hatched larvae have a limited stock of energy, have to move in soil and to find the roots quite fast. So the question is how do larvae orientate to make host finding most effective. It is known, that carbon dioxide (CO<sub>2</sub>) shows an attractant effect on the larvae and this attractant effect was observed. At least 30 percent of the produced carbon dioxide in the soil results from root- and animal breathing. 70 percent is produced by microorganisms which exist in every soil. So a higher activity of microorganisms is often associated with larger amounts of organic matter and leads to an enhanced metabolism activity and thereby to a higher concentration of carbon dioxide in the soil. Therefore high concentrations of CO<sub>2</sub> may hinder larval orientation and host finding. To get more knowledge on the orientation of larvae different soil conditions were tested in laboratory experiments using two narrow glass-plates, which were kept on distance by a silicone string, fixed with large clips and filled with soil. Different soil compositions were established by varying the percentage of sand and of a gardening substrate with high content of organic matter. The soil density was varied by different compaction of the substrates in the test containers. By varying the relative density in the system also a change in the width of the pores was achieved. Plants were grown for a week in a certain part of the containers and then freshly hatched larvae were released into the system. By recovering the larvae at different locations between the glass-plates 24 hours after release, observation of the movement of larvae in the system in relation to the starting point and to the location of the roots was possible. The starting point, the direction and the final position of larvae in the system was recorded. Results are not finally analysed but indicate that more larvae reached the area of the roots in the test systems with higher content of sand.

**Within and between field dispersal of *Diabrotica barberi* and *D. v. virgifera* in the South Dakota area wide management site**

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Dispersal is a means by which organisms search for food, shelter, mates, oviposition sites, etc., and can ultimately result in gene flow among populations. We investigated the within and between field movement of *Diabrotica barberi* Smith and Lawrence and *D. v. virgifera* LeConte (Coleoptera: Chrysomelidae) in the 41.4 km<sup>2</sup> South Dakota Corn Rootworm Areawide Management site. These are two economically important pests of maize, *Zea mays* L., in the U.S. Corn Belt, and *D. v. virgifera* has become an invasive pest in many countries of Europe. We used emergence cages and Pherocon AM yellow sticky traps to capture these beetles within maize fields, and Pherocon AM yellow sticky traps to capture the beetles between fields of soybean (*Glycine max* L.), continuous maize, and first-year maize. *Diabrotica barberi* were captured in high numbers from continuous and first-year maize fields, whereas *D. v. virgifera* were captured in high numbers only from continuous maize fields. For both species, males began emerging in higher numbers than females early in the season, and then as the season progressed female emergence was greater than male emergence. This capture pattern also is reflected from the sticky traps placed within and between fields. Generally, more beetles of both species were captured between continuous and first-year maize fields than between maize and soybean fields. We discuss our results in relation to corn rootworm biology and areawide pest management.

## **The potential of the management of maize planting date in *Diabrotica v. virgifera* eradication-containment programmes**

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Maize rotations have provided good results in *Diabrotica v. virgifera* LeConte (WCR) eradication – containment programmes implemented in Italy. However, this measure, particularly when used in some areas, have a high social and economic impact and therefore farmers and/or nearby communities may not like it, especially if farm organization is based on the transformation of the maize plants into feed for livestock. Can the management of the maize planting date (appropriate delay) reproduce the effect of crop rotation reducing the negative impact of rotation on farmers? Can the available WCR development models give reliable information for management strategies, suitable for areas where WCR specimens detection is not feasible because of the very low population levels? Since for containment or eradication measures to be effective, these need to be based on control strategies providing >99% effectiveness, the effect of planting date management was evaluated assessing larval population levels and the number of beetles emerging from the fields.

### Materials and Methods:

Plot trials were carried out in Italy over a 3-year from 2003-2005 in Lombardy, Italy, on a monoculture maize field. Larval and beetle (also using emergence cages) densities along with root damage ratings were estimated at different localities. The experimental design was a randomized complete block with four replications of treatments (4 planting dates, from May to July).

*Larval sampling:* larval densities were determined in May – July digging up roots, including soil. Each root and soil sample was placed in a funnel, which included a vial filled with water.

*Adult sampling:* cages that covered the interrow width of each treatment were used to estimate absolute WCR adult emergence. Each cage enclosed three maize plants and was constructed of a wooden frame (100 X 50 cm) to which screening was stapled. A Pherocon AM trap was placed in the middle of the cage.

### Results:

Planting dates statistically impacted WCR population levels. It was possible to cultivate maize after maize producing a good amount of forage units avoiding any emergence of beetles from the fields.

Davis' development model precisely predicted the completion of WCR egg hatching and the time suitable for planting maize without any risk of WCR larval development. In each year planting maize after a DD accumulation of 320 to 350 (base 11-18 °C) avoid any beetle emergence and any root damage. Also lower DD accumulations (250-300) resulted in a significant reduction of beetle emergence which dramatically impacted WCR population dynamics. Generally in Italian conditions planting maize in late May was suitable for a good beetle containment while planting maize in the second part of June completely avoid beetle emergence (eradication).

## The potential impact of weather conditions on flight dynamics of *Diabrotica v. virgifera* adults

Ioana GROZEA & Laura UNIPAN

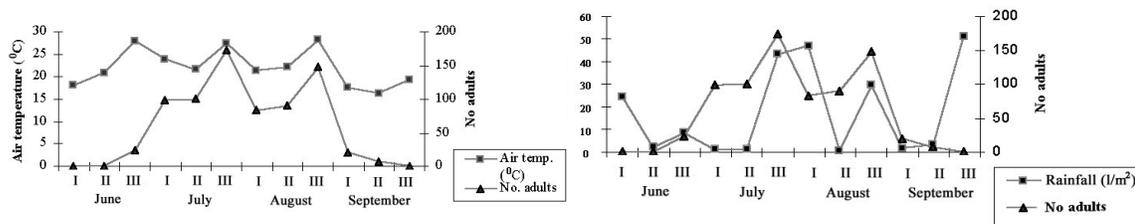
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The climate change and its influences on species behavior and distribution are becoming more and more apparent. In order to investigate the potential impact of weather conditions on pest populations a clear understanding of the nature and characterization of the pest is required. In general, most pest species are influenced by warm and humid conditions. The invasive *Diabrotica v. virgifera* Le Conte species has become a very important pest of maize growing areas from Europe.

Since western corn rootworm started to spread in the western part of Romania, many investigations were started to understand very well the ecobiology of the species in order to predict its evolution and its potential area of dispersion. Its habitat and survival strategies are strongly dependent on local weather patterns, having in mind results of the researches made in different monitored areas.

Based on our data from the years 2003, 2004 and 2005, we can emphasize a very serious influence of air temperature and rainfall on WCR flight dynamics in adults.

Dry and warm conditions promote growth of insects' number. Therefore, in 2003, July (III) the temperature raised to 26.5°C, led to increasing of adult's number (189). For 2004, the data shown a positive correlation between temperature and adults captured on pheromone traps (see figure). Similar aspects were recorded in 2005.



Excessive or insufficient rainfall is an important variable that affected adults' dynamics in maize fields, as displayed in figure (2004). The most observations, in all studied years, shown a decreasing number to rainfalls increasing.

**Predicting the possibility for establishment of the western corn rootworm, *Diabrotica v. virgifera*, in different world regions: based on the CLIMEX model**

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Western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), is known to occur and is spreading in parts of North America and Europe. This invasive species is a major threat to many of the corn producing areas of the world. Based on climatic conditions, areas potentially suitable for the establishment of the WCR were assessed using the CLIMEX model. Twenty-one temperature and moisture parameters were used to determine the response of the WCR in a global context. The simulated and verified results were highly consistent with the current distribution of WCR, and the data show that the pest might spread further in North America and Europe. The predicted results also showed that the potentially suitable areas for the WCR were from 55° N to 44° S, and covered the primary corn producing areas of the world. The values of the ecoclimatic index (EI) were divided into five levels to gauge the degree of suitability of a location for the establishment of the WCR. The more and highly suitable habitats represented the majority of potentially suitable areas according to the model. The predicted distribution represented different patterns in different continents based on temperature and moisture. Due to the interaction of temperature and moisture, potentially suitable areas for the WCR represent discontinuous or block-like distributions. The most important impact factors of the negative potential for WCR establishment in a given area was very low temperatures at the upper latitudes and very high temperatures and moisture levels in middle to low latitudes. In Europe, potentially suitable areas for WCR increase as latitudes decrease as long as temperatures and moisture levels are favorable to the WCR. Extremely dry conditions and very high temperatures are limiting factors for WCR in Africa. Low temperatures and dry climates are the limiting factors for its establishment in parts of Asia, North America and South America. For Australia, very high temperatures and very dry climates are important factors limiting WCR survival.

## ***Diabrotica v. virgifera* en route towards central Europe**

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Trapping experiments in 2004 and 2005 during the flight season of *Diabrotica v. virgifera* LeConte (WCR) (Coleoptera:Chrysomelidae) were undertaken in Slovenia and Switzerland. The monitoring was designed to document presence, to record abundance, increase, and spreading of WCR. Some emphasis was put on population movement in northerly direction towards Germany where WCR as of 2005 has not been recorded in spite of ongoing monitoring initiatives undertaken since 1999. The investigations focussed on terrestrial movement, without ignoring the possibility of new aerial introductions from the New World to Europe or simultaneous aerial crossmigration *within* Europe. The likelihood of new invasions assisted by mankind's traffic and trade activities has been experimentally proven by Miller et al. 2005 for at least 3 independent transatlantic immigration episodes.

### Materials and methods:

Monitoring traps were of the Metcalf sticky cup type (15 cents per piece) and were usually attached directly to the maize plants at ear (1 to 1.2 m) height. Readings were taken twice a week, with routine renewal of sex pheromone (8-methyl-decane-2-ol propanoate, 0.1 mg) and/or kairomone lures (4-methoxy-cinnamaldehyde, 10 mg), both dispensed on sturdy 5x5cm squares of chromatography paper.

### Results:

The Metcalf cup trap proved to be of highest sensitivity and, with sex pheromone bait, responded to the earliest beetles (usually males) and, with kairomone bait, also to the latest beetles, mostly females flying in September. Expectably, flight curves seen in Southern Switzerland and in Slovenia are quite similar in shape and in duration. A trend to further population increases is visible in both countries. However, there are also differences between Ticino and Slovenia. Crop rotation is mandatory and enforced in Ticino since 2003, resulting in a levelling off in WCR increases. In contrast, in the neighboring Swiss canton of Grisons, (up to 2005 without crop rotation) in the Misox valley steep increases were recorded in 2004 and 2005. In Slovenia, crop rotation does not fit well into farmers' growing practices and has not been in force. However, the population pressure in the eastern districts is still far below economic concerns, although significantly increasing during 2004 and 2005 in the border regions to Hungary and Croatia.

### Discussion and conclusions:

Both Southern Switzerland and Slovenia are countries with well developed traffic infrastructure and transalpine freeway connections to Northern Switzerland, Austria and Southern Bavaria, resp. Increasing WCR populations seen specifically along major traffic routes in the Ticino (Wudtke et al. 2005, Hummel et al. 2005) suggest to pay increasing attention to the advancing terrestrial expansion trend in the direction toward southern Germany and Austria.

## **Study of WCR adult movement and possible egg laying in fields bordering maize fields**

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Western corn rootworm (WCR) larval damage in maize following soybean was observed in Croatia in 2003. Damage was recorded along the edges and within soybean fields which bordered continuous maize fields in 2002. The only explanation was that WCR adults moved from the continuous maize to the neighbouring soybean fields to lay eggs. Larval feeding damage in the first-year maize the following year was recorded up to a distance of 50 m into the fields. Farmers' fields in Croatia are commonly up to about 50 m wide and the above described situation could diminish crop rotation as a valuable tool for WCR management.

As a result of this situation, research was carried out to determine how far WCR adults will go into neighbouring fields to lay eggs. The WCR adult population was monitored in a continuous maize field in 2003 by using Pherocon<sup>®</sup> AM (PAM) traps in the middle and on the borders of the maize field and up to 50 m distance from the edge of the continuous maize field into neighbouring fields to the south, east and west. Larval presence and root damage ratings (ISU1-6 scale) were recorded at different locations within the field in 2004. The field was 120 m wide and was divided into 3 sections in 2005. On two sides, maize was planted and in the middle section soybean was planted. The WCR adult population was monitored using PAM traps in the middle and on the borders of the maize fields and each 5 m within the soybean field to a maximum of 40 m.

No significant differences in number of WCR adults on PAM traps located in the centre and on the edges of maize field were observed in 2003. The number of WCR adults on PAM traps located 50 m within neighbouring fields was significantly lower. Number of WCR larvae/plant was 3.5 in the centre and 2.25 and 2.75 on the west and east edges, respectively. The average root rating was 4.46 in the centre and 3.93 on the west edge. On the east edge of the field and at all observed distances eastward, lower root damage ratings were recorded (between 1.66 and 2.23). The average root damage ratings at 5, 10 and 15 m to the south were between 2.98 and 3.58. The average root damage rating to the south at 20 m was 2.4. At the distance of 50 m, no significant root damage was recorded in any direction. Maximum daily captures of WCR adults in 2005 in the centres of the maize fields and on the south edges of both maize fields did not significantly differ. At all other distances and on the north edge of the maize fields, maximum daily catches were significantly lower. Investigations will conclude after root ratings are taken in 2006. It appears that the so called "edge effect" could happen within distances of 15-20 m of maize field borders and that the direction of the prevailing winds can impact this movement.

## Results of monitoring of western corn rootworm, *Diabrotica v. virgifera* in Slovakia in 2005

Jozef KUBÍK

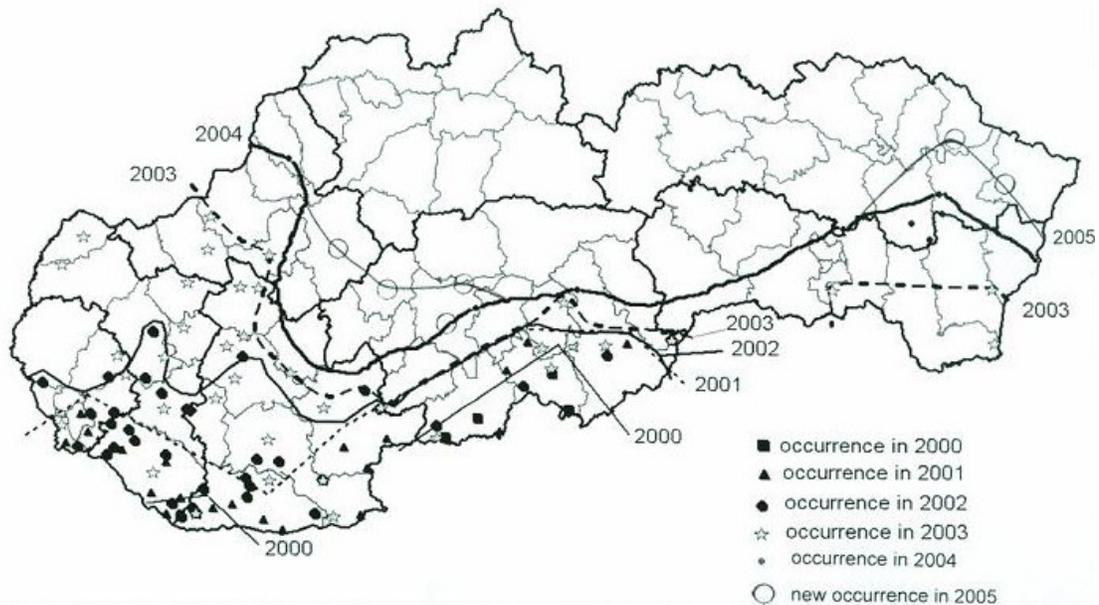
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The first occurrence of WCR in Slovakia was recorded in 2000. In 2005 the survey was focused mainly on the east part of Slovakia and its north border – border of WCR occurrence. The yellow traps for monitoring population density in infested areas were provided within FAO project. The survey was carried out by the phytosanitary inspectors of the Central Control and Testing Institute of Agriculture.

We used pheromone traps Csalomon and yellow traps Pherocon AM for survey. The monitoring points were set as in infested as in endangered corn belts. The pheromone and yellow traps were set together in infested areas. In endangered corn belts only the pheromone traps were set. The monitoring of WCR adults started in the second half of June.

In 2005 on the pheromone Csolomon PAL traps was caught 8782 pcs of WCR adults and on yellow Pherocone AM traps 2484 pcs. The highest number of adults captured within one week was on yellow trap Pherocone AM situated in Čiližská Radvaň. In areas with the highest population density of WCR were also reported losses. On the map you can see infested territory.

Spread of the Western Corn Rootworm - *Diabrotica virgifera virgifera* in the Slovak Republic in 2000 - 2005



## **Pilot studies to model population changes of western corn rootworm in dependence of regional share of continuous maize fields**

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The western corn rootworm (*Diabrotica v. virgifera* LeConte, WCR) has well established in several Central European countries. Thus, sustainable management options are needed for this pest. The main non-chemical control and management option is the rotation of maize to other crops. The rotation may be conducted each year or after two-three or more years upon estimating the adult WCR population in a maize field which is high enough for causing economic larval damage in subsequent year maize. However, in 2005, no economic damage occurred in Croatia and limited one in Serbia or Romania (Kiss et al. 2006). Thus, WCR populations are likely to be manageable with careful population estimation and relevant decision for rotations. WCR population levels in a maize field depend on population build-up over years and on emigration of adults (dilution effect) as well as on immigration of adults from surrounding "donor" fields. On one hand, the share of "donor" fields and their WCR population levels are key factors in influencing actual adult density on a maize field. On the other hand, tolerable share of continuous maize fields in a region prevents the development of rotation resistant strains (Kiss et al 2005).

This study aims to develop a model that helps to manage WCR populations on maize fields over regions and years under various farming conditions, i.e.: High percentage of continuous maize fields in a region; Lower percentage continuous maize fields in a region. Parameters such as beetle fecundity, generational mortality, rate of immigration into and emigration out of maize fields, economic and action threshold levels as an upper limit were incorporated into the model. Field experiments were conducted in summer 2006 in order to determine the level of migration of *Diabrotica v. virgifera* adults under various maize growing conditions, i.e. share of continuous maize fields.

## **Effects of egg hatch timing, root injury, and larval background infestation on western corn rootworm larval movement in field corn**

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A field study was conducted in 2003 and 2004 at the University of Nebraska Agricultural Research and Development Center, near Mead, NE, USA, to determine the effects of egg hatch timing, root injury, and larval background infestation on western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte, larval movement in field corn. This was accomplished by using an organophosphate resistance-associated esterase marker to track movement of organophosphate-resistant (OP-R) larvae under various background infestation levels of organophosphate-susceptible (OP-S) larvae. The study site was a silty clay loam soil under sprinkler irrigation. A randomized complete block design was used with 4 - 5 replications; main plot: sample date (4 sampling dates); subplot: factorial arrangement of 3 background levels of OP-S WCR eggs (0, 600, or 1800 viable western corn rootworm eggs per 30.5 row-cm [USDA-ARS Brookings, SD diapause colony] x 2 infestation dates of OP-R (NE R colony) WCR eggs. Each treatment combination subplot was 3 rows by 11 m with a blank row between each subplot. A single plant was infested in the center row of each subplot (600 viable OP-R eggs / plant). On each sampling date, 7 plants from each treatment combination were dug and live larvae were recovered from each plant (the plant infested with resistant eggs, four plants down the row, the closest plant across the row, and a plant across the row 1.4 m from the infested plant). This procedure was repeated in each subplot to record root damage rating for each plant.

Similar trends were observed in 2003 and 2004. A significantly larger number of OP-R larvae were recovered from the OP-R infested plant and immediately adjacent plants than plants farther away from OP-R infested plants. However, a small number of larvae were recovered up to 1.4 m from the OP-R infested plant; this occurred primarily in treatments with moderate to high larval background densities. Much of the larval movement away from the OP-R infested plant occurred during 2nd-3rd instar when significant root injury was recorded. This study increases our knowledge of WCR larval biology which will be useful as resistance management models are developed that address grass weed - transgenic plant interactions, or transgenic-isoline seed mix "refuge in the bag" concepts.

## **Spread and population dynamics of western corn rootworm, *Diabrotica v. virgifera* in Slovenia**

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*Diabrotica v. virgifera* LeConte (WCR) has occurred in Slovenia since 2003 in two areas as natural invasion from infested parts of neighbouring countries in the North-east (Austria, Croatia and Hungary) and West (Italy). Other as in some western EU countries, it did not enter the country via airports.

Surveys started in 1997 at 30 monitoring sites, each with one Csalomon® PAL pheromone trap and one yellow sticky trap. After the outbreaks of WCR in 2003, the number of sites was increased. In general, traps were monitored from the end of June until mid October (or maize harvest) by weekly inspections. In 2005, floral bait KLP traps, which attract mainly females, were also introduced.

The main criteria for selecting monitoring sites in non-infested areas were the density of maize fields and the presence of risk factors such as an airport, main roads from the North-east to the South-west and storehouses for corn. Earlier, monocultures of maize in non-infested areas were not targeted. In targeted locations about 30 % of maize was grown in monocultures. In 2006 the cropping history of monitored localities was analysed. About 30 % of fields, on which maize was grown in monocultures for at least 2 years, were included in the survey.

The WCR population increased from 2003 to 2005 in Eastern Slovenia dramatically. In 2003, 19 specimens were caught at 14 locations; in 2004, 386 specimens at 55 locations; and in 2005, 1349 specimens at 120 locations.

WCR spread in 2005 some 40 km westwards. This is the maximum predicted by a slow rate scenario of pest distribution (some 40 km/year). The pest apparently did not spread from the initially infested part near the Slovene-Italian border.

In 2005, WCR populations of adults emerged in the second decade of July and reached a peak in the second decade of August, one week earlier than in 2004.

Monday, 6 Nov 2006, Session 3, **POSTER 023**; ONSTAD & KNOLHOFF

## **Rotation-resistant rootworm: monitoring, bioassays, and modeling**

David W. ONSTAD & L. M. KNOLHOFF

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In the north central part of the United States, *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), circumvents crop rotation, an historically successful control measure, by ovipositing in soybean fields and in other crops in rotation with maize. Over the past several years, procedures for monitoring in soybean fields and computer modeling have helped us predict the geographic spread of the rotation-resistant phenotype. The modeling combines simulation of insect dispersal influenced by climate with geographic information systems (GIS) that represent the landscapes of the north central US. We have also developed a technique for distinguishing between rotation-resistant and wild-type behavioral phenotypes that may help determine the genetic basis of rotation resistance. The time between release into and departure from a bioassay arena was used as a measure of beetle activity to distinguish between behavioral phenotypes. Results from these assays indicate that *Diabrotica v. virgifera* females from regions where crop rotation is no longer effective are more active than females from regions where rotation remains effective. The geographic source of the beetle population was a main significant effect in trials done in both 2004 and 2005. Behavioral differences were more easily observed in a maize field rather than in the laboratory. Results were consistent with the hypothesis that a loss of fidelity to maize rather than any particular attractant is the cause of rotation resistance. Behavioral differences between populations of beetles in similar environments suggest that there is a genetic difference between rotation-resistant and wild-type *Diabrotica v. virgifera*, although no specific gene or genes have yet been identified. Monitoring, bioassays, and modeling can be used to better understand and manage this pest.

Monday, 6 Nov 2006, Session 3, **POSTER 024**;MOESER & VIDAL

## **Screening for German maize varieties resistant against *Diabrotica v. virgifera***

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Using bioassay and semi-field greenhouse experiments we were studying 30 German maize varieties from six breeders with regard to their potential resistance against *Diabrotica v. virgifera* LeConte (Coleoptera, Chrysomelidae). The selection of the varieties was based on finding hybrids which were morphologically as different as possible (ripening group, plant height, etc.), thus yielding a higher chance of encountering mechanisms of native resistance. As a second step three plant parameters are measured to explain differences in larval development and behaviour:

1. phytosterol content and composition,
2. fiber content of the roots,
3. content of hydroxamic acid.

The results from these analyses were correlated with the differences in feeding by the larvae on the distinct varieties. Additionally the larval performance and development are correlated with these plant parameters. The results are discussed with regard to possible resistance mechanisms.

## European corn borer attack intensity and damages caused by western corn rootworm through 5 years investigations

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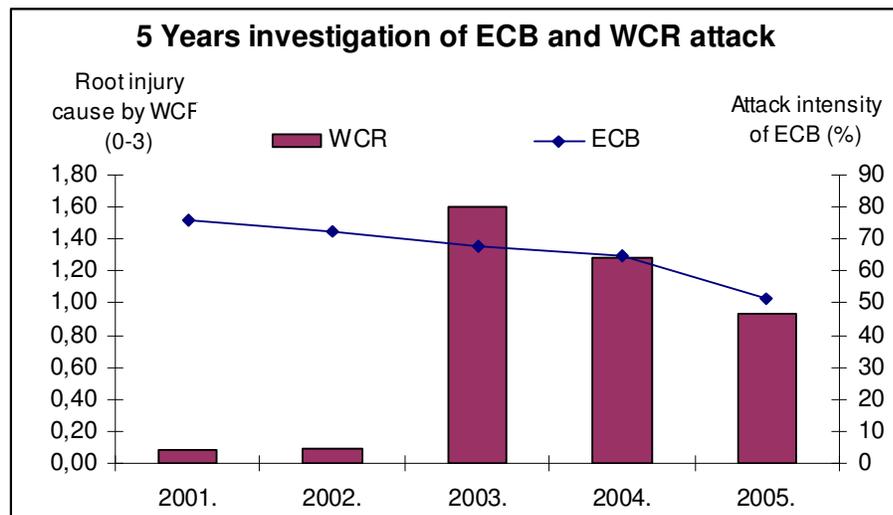
Corn is one of the major crop sown in Croatia. In the past, the main pest of corn in this area was European corn borer (ECB), but since 1995, western corn rootworm (WCR) occurred, and become new threaten in corn production.

The aim of this investigation was to determine attack intensity of European corn borer and damages on roots caused by Western Corn Rootworm.

Field trials were settled in Osijek area in 2001 – 2005. Seven commercial corn hybrids were evaluated in four replicates (25 plants in each replicates) in order to determine damages caused by both pests. Root damages to larval feeding of WCR were evaluated early in July according to Iowa Node Injury Scale (0-3). Intensity of attack (%) of ECB was evaluated in September, before harvest.

Results of Investigations for both pests are presented in figure 1.

**Figure 1:** European corn borer attack intensity and damages caused by western corn rootworm through 5 years investigations.



Statistical analyses showed significant differences between years of investigations, but not between hybrids, for both pests.

## Management of *Diabrotica* and *Ostrinia* in the United States Corn Belt: a farmer survey of cultural, chemical, and transgenic practices and perceptions

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Northern corn rootworms, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), western corn rootworms, (*Diabrotica v. virgifera* LeConte), and European corn borers, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) are annual pests of maize (=corn), *Zea mays* L. (Poales: Poaceae) in the central United States. *Diabrotica v. virgifera* larvae feed on maize roots, which causes plant lodging and yield losses. Adults feed on maize silks and may interfere with pollination. Cultural control (crop rotation) and chemical control (soil insecticides at planting) are common control methods for larvae. Transgenic maize that expresses the Cry3Bb protein for protection against *Diabrotica v. virgifera* larvae was first commercialized in 2003. Transgenic maize for controlling *Ostrinia* has been planted by farmers since 1996.

In 2006, we conducted a survey of farmers in the Corn Belt (five leading maize production states: Illinois, Indiana, Iowa, Minnesota, and Nebraska). Farmers in these states planted 18,858,000 ha of maize in 2005, or 57 percent of all U.S. hectares. The objective of the survey was to document farmer perceptions of these three pests and how this influenced their management decisions.

Farmers (n=1,250) were mailed a written survey. Farmers not responding were contacted by telephone. A total of 646 surveys (52%) were completed. Each state had a nearly equal response (range 120-141). Selected questions and responses are presented.

1. How many hectares of field corn did you plant in 2005? mean 244
2. How many hectares were continuous (mean 106) or rotated corn (mean 197).
3. In 2005, did you use a corn rootworm soil insecticide or seed treatment in a first-year cornfield?
  - Yes, 30.8% NCR, mean 165 ha. treated
  - Yes, 29.4% WCR, mean 182 ha. treated
4. In 2005, did you plant a corn rootworm transgenic corn hybrid in a first-year cornfield?
  - Yes, 13.5% NCR, mean 74 ha. planted
  - Yes, 11.1% WCR, mean 76 ha. planted
5. During the past five years, has corn rootworm damage in your maize fields increased, decreased, or stayed the same? (n=642)
  - Increased, 28.0%
  - Decreased, 11.5%
  - Stayed the same, 52.5%
  - Don't know, 7.9%
6. Estimate how many kilograms per hectare yield loss you experienced during the 2005 growing season from corn rootworms? Mean 879 kg/ha (n=220)
7. During 2005, what management practices did you use to prevent corn rootworm injury and how many hectares did you manage with each practice?

<b>Management Practice</b>	<b>Used Practice</b>	<b>Mean Hectares</b>
Crop rotation	76.8%	197 (n=446)
Soil-applied insecticide	43.7%	190 (n=257)
Seed-applied insecticide	26.2%	143 (n=151)
Transgenic rootworm corn	22.8%	76 (n=137)
Adult beetle spray (insecticide)	4.2%	100 (n=26)
Other (please specify)	1.1%	121 (n=5)

8. In 2006, will you increase, decrease, or maintain your current hectares planted to transgenic rootworm hybrids? (n=610)
  - Increase, 39.8%
  - Decrease, 4.3%
  - Stay same, 55.9%
9. In 2005, did you plant a transgenic corn hybrid for control of European corn borer? (n=642)
  - Yes, 65.7% If yes, how many hectares? 139 ha. (n=389)
  - No, 34.3%
10. In 2006, will you increase, decrease, or maintain your current hectares planted to transgenic European corn borer hybrids? (n=624)
  - Increase, 25.2%
  - Decrease, 6.1%
  - Stay same, 68.8%

## **Failure of crop rotation to control *Diabrotica* in Iowa**

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During the late 1980s, a variant of the northern corn rootworm, *Diabrotica barberii* (Smith and Lawrence) (Coleoptera: Chrysomelidae) that had a two-year lifecycle severely damaged northwest Iowa corn grown in annual rotation with soybeans. The distribution of the northern variant has gradually expanded throughout the state. During 1995 corn planted where soybeans had been grown the previous season in east central Illinois was seriously damaged by the western corn rootworm, *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae). The western corn rootworm variant defeated rotation by ovipositing in the crop planted the year before corn, usually soybeans. During the last decade, the distribution of the western rotation-resistant variant has expanded; finally reaching the Mississippi river on the eastern edge of Iowa. Also during the last decade the soybean aphid, *Aphis glycines* Matsamura (Homoptera: Aphididae), invaded Iowa and caused serious damage to soybeans. Sampling aphid populations in soybeans discovered numerous northern and western corn rootworm adults in soybeans throughout Iowa, raising the question "are both species laying eggs in soybeans?" The ultimate question is "are all 5,000,000 h of corn grown in Iowa, both rotated and corn grown in monoculture, susceptible to infestation by both species of corn rootworms. To answer this question, we conducted surveys throughout eastern Iowa during 2005 and 2006 to determine the extent and severity of corn rootworm infestations in rotated corn.

### Methods

Survival of northern and western corn rootworms in corn planted after soybeans was monitored by placing 10 emergence cages in rotated corn. The presence of adult rootworms in soybeans was monitored with 12 Pherocon™ AM yellow sticky traps per field. The cages and traps were in place from late July through August. To assess the severity of the problem, corn roots from rotated cornfields were dug and larval injury was rated using the Iowa State University 0-3 Node Injury scale.

### Results

The two-year, qualitative survey indicated that rootworm infestations in rotated corn continue to spread through out Iowa; both because of the increase in range of the extended-diapause northern corn rootworm and the presence of the rotation-resistant western corn rootworm in eastern Iowa. Injury caused by the rotation-resistant western corn rootworm is low at the present time. The rotation-resistant northern corn rootworm is a more serious threat to rotated corn than the western laying eggs in soybeans. It is recommended that yellow sticky traps be used to monitor the populations of corn rootworms in soybeans to decide if controls should be applied if corn will be planted the following season.

Monday, 6 Nov 2006, Session 3, **POSTER 028**; SCHAUB & BERTOSSA.

## **PAL-trap attractivity**

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PAL-trap attractivity was evaluated by releasing colour marked males at various distances of traps from within (5 – 50 m) and from outside (40 – 250 m) maize fields. Recapture rates of 2005 varied from 50% to 3%. Results of 2006 will be presented.

Monday, 6 Nov 2006, Session 4, 15:50; HIBBARD

## **Breeding for resistance to corn rootworm larval feeding: history, techniques, and the Missouri USDA-ARS program**

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There have been several serious research programs aimed at identifying and developing sources of maize with resistance to corn rootworm larval feeding. As far back as the 1930s and 1940s, researchers in Illinois were evaluating maize germplasm for reduced effects of rootworm larval feeding. Iowa State University has had a series of researchers working on corn rootworm resistance from the 1970s through today. The USDA-ARS laboratory in Brookings, South Dakota had an active program in the 1970s and 1980s and is becoming active again. In the early 1990s, a research group out of the University of Ottawa was active in pursuing native corn rootworm resistance. Programs actively pursuing native resistance to corn rootworm larval feeding today include those in Croatia, Serbia, and the USA. In the U.S., programs at the University of Illinois, Iowa State University, and the University of Missouri along with USDA-ARS programs in Missouri and South Dakota are currently active along with private interests. A number of techniques have been developed over the years and these will be discussed. The USDA-ARS program in Missouri includes screening new accessions from Central and South America, adapting selected lines to photoperiod, developing populations, population improvement via recurrent selection, and inbred line development - all under selection for reduced damage from corn rootworm larval feeding. Current goals include the mapping of resistance genes from CRW3, a population that has currently undergone six cycles of selection for reduced damage. Five superior plants were chosen from S3 progeny of CRW3(C3). Sub-populations were formed by crossing these S3 lines to LH51, a U.S. inbred, and deriving F2:3 families. Mapping requires accurate phenotypic data from a large population. In 2006, 230 entries from the CRW3 mapping population will be evaluated in Illinois, South Dakota, Iowa, and in two locations in Missouri. Since rootworm damage is inherently a variable trait, the more data, the better. Molecular marker data will be taken on the 230 entries in the fall of 2006 by simple sequence repeat markers and the composite families will be used to generate a map and conduct multiple interval marker analysis with QTL Cartographer. Sub-population size is large enough to estimate specific effects of allelic substitution for significant markers by best linear unbiased prediction (BLUP). The primary overall goal of this approach is to facilitate utilization of our strong source of native resistance by providing genetic information to enable transfer of corn rootworm resistance genes into elite germplasm with minimal introduction of unwanted genes.

*Monday, 6 Nov 2006, Session 4, 16:10; IVEZIC et al.*

## **Results of 5-years investigations of corn hybrids tolerance to western corn rootworm larval feeding**

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Investigations of corn tolerance to larval feeding were conducted in Croatia continuously through five years. A great number of Croatian commercial hybrids and Pioneer hybrids, as a standards for tolerance and susceptibility, were evaluated for corn tolerance by measuring root damage, root size and root regrowth.

Here are abstracted seven Croatian hybrids which were present throughout all five years at Osijek locality. Hybrids were in field trials in four replicates. Root injury was rated by using Iowa Node Injury Scale (0-3), and the hybrid tolerance to larval feeding was compared by evaluated root size and root regrowth. The Eiben 1-6 Scale, reversed, (Rogers 1975) was used to rate root size and regrowth. On the reversed scales a rating of 1 indicated a large root system or well developed secondary roots, and a rating of 6 indicated a small root system or poorly developed secondary roots.

Results showed significant increase of root damage in 2003 (1,67), 2004 (1,25) and 2005 (1,36) in compare to 2001 (0,07) and 2002 (0,10). Root size and root regrowth also differ significantly in years of investigations, while differences between hybrids occurred only in analyses of root regrowth. According to the root regrowth scale, hybrid OSSK 617 appeared to be the most tolerant through all years of investigation.

Monday, 6 Nov 2006, Session 4, 16:30; BOHN & DAVIS

## **Genomic evaluation of the defense response of maize against the western corn rootworm – how to use this information in breeding programs?**

Martin BOHN<sup>1</sup> & Georgia DAVIS<sup>2</sup>

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From the 1940s until today more than 12,000 maize accessions, comprising inbreds, synthetics, and open pollinated varieties, as well as maize relatives, like teosinte and *tripsacum*, have been screened for their level of resistance to western corn rootworm (*Diabrotica virgifera* LeConte, WCR) larvae feeding. After initial evaluations for a wide array of traits associated with different resistance mechanism active against WCR employing trap crop or artificial infestation techniques, 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 insect 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 of available methods comprising microarrays, protein and metabolite analysis. This integrative approach will guide us to genes involved in the wounding and defense responses of maize roots, the complex transcriptional changes caused by WCR feeding, the involved pathways and their crosstalk, as well as quantitative and qualitative changes in proteins and metabolites. How this knowledge impacts the design and efficiency of breeding programs to improve WCR resistance will be discussed. As a direct output of this project, molecular markers will be available to efficiently screen germplasm for novel defense response variants and to perform marker-assisted selection.

Monday, 6 Nov 2006, Session 4, 16:50; DASHIELL et al.

## **Screening maize germplasm for resistance to western and northern corn rootworms at the North Central Agricultural Research Laboratory in Brookings, South Dakota, USA**

Kenton E. DASHIELL<sup>1</sup>, B. Wade FRENCH<sup>1</sup>, Mike M. ELLSBURY<sup>1</sup>, D. A. PRISCHMANN<sup>1</sup> & Bruce E. HIBBARD<sup>2</sup>

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### Rootworm resistant varieties

In the hopes of lessening the current reliance on soil insecticides, researchers recently have been developing novel corn varieties that exhibit resistance and/or tolerance to corn rootworm larvae. However, before new corn lines can be integrated into rootworm control strategies, their susceptibility to feeding damage must be adequately evaluated. Here we report the results of a two-year ongoing research project assessing the resistance and tolerance of 14 maize genotypes to western corn rootworm larvae, *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae). Corn lines were planted in field plots previously managed under a four-year rotation of corn, soybeans, oats, and spring wheat to insure that experimental corn plots were not contaminated by surrounding rootworm populations. We used a randomized complete block design with 10 hand-planted seeds of each corn line per plot, replicated four times. Plots were mechanically infested with 1,000 *D. virgifera* eggs per 30 cm using Sutter and Branson's technique (1980). Rootworm eggs were suspended in an agar solution and were obtained from colonies maintained at the North Central Agricultural Research Laboratory in Brookings, SD. Resistance and tolerance to rootworms was evaluated using previously established methods, including: the Iowa 1-6 root damage rating scale, percent of plant lodging, adult rootworm emergence, and compensatory root growth ratings. In 2005, the genotype with the least damage had an average root damage rating of 2.10, while the genotype with the most damage had an average root damage rating of 3.95. We will also present data from 2006. Many historical evaluations of corn germplasm have focused solely on resistance to western corn rootworms, even though different rootworm species frequently co-exist. Therefore, maize genotypes also will be tested for resistance and tolerance to northern corn rootworm larvae, *Diabrotica barberi* Smith & Lawrence. While rearing methods for *D. virgifera* are well established, maintaining productive colonies of *D. barberi* has been challenging, and may limit the scope of our experiments.

### Improved rootworm egg infestation methods

We will also discuss modifications to Sutter and Branson's mechanical rootworm egg infester. The new apparatus is capable of delivering variable egg rates of multiple rootworm species. This will allow us to conduct more realistic experiments examining the susceptibility of corn lines to several rootworm species simultaneously, and to determine whether specific genotypes exhibit resistance and/or tolerance solely to *D. virgifera*, *D. barberi*, or to both. We will also explore interspecific competition between rootworm species, and the effects of initial infestation densities on this interaction in the field.

Monday, 6 Nov 2006, Session 4, 17:10; SEITZ

## **Developing hybrids with resistance to western corn rootworm. A seed company point of view**

Günter SEITZ

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Western corn rootworm (WCR, *Diabrotica v. virgifera* LeConte) has in recent decades developed into one of the most devastating pests in the central US Corn Belt causing significant economic damage due to reduced grain yields and increased root lodging.

Currently, the growth of genetically modified hybrids carrying the Cry3Bb gene from *Bacillus thuringiensis* (Bt) is considered the most reliable solution for the farmer to control damage. In 2004 the Monsanto MON863 gene was the first such transgenic resistance introduced in the market. In 2005 a severe drought in several WCR infested areas of the US Corn Belt showed that hybrids carrying the MON863 gene significantly outperformed either hybrids without the gene or protected by insecticides. As a result the use of transgenic hybrids to control WCR will increase significantly over the next several years. This development will be further accelerated by the availability of additional transgenic Bt based genes from Syngenta, Dupont and Dow. However, despite the increased availability of transgenic genes to control this pest, it is essential to further investigate alternative approaches since currently all transgenic events are based on the same mechanism of the Bt protein. Large scale growth of the Bt based genes will increase the probability that WCR will overcome the existing Bt genes.

Development of a sustainable multi-gene tolerance to WCR will help to increase the useful life span of the Bt genes, and potentially substitute for Bt when used in combination with seed treatments in areas with low infestation.

Past efforts to develop resistance to WCR with conventional breeding has had only limited success. The main reason being the lack of well known sources of resistance in adapted elite breeding germplasm. However, several recurrent selection programs utilizing a wide range of adapted and exotic germplasm have reported success improving tolerance to larvae feeding. The successful integration of this germplasm into commercial elite lines however is largely limited by difficulties in selecting for WCR resistance during the breeding process. High costs for evaluating large entry numbers combined with the low heritability for the trait are the major stumbling blocks for most applied breeding programs.

Molecular Markers combined with the Doubled Haploid technology provide important new tools to overcome these limitations. In 2005 AgReliant Genetics and the USDA-ARS initiated a collaboration to explore what level of native resistance to WCR could be achieved in Elite breeding populations by analyzing crosses between exotic and semi-exotic sources with increased tolerance to WCR and commercial elite inbred lines. The main goals of the study are to:

1. Analyze the available genetic variability for improving WCR resistance.
2. Identify major QTL's involved in WCR resistance.
3. Estimate quantitative genetic parameters that allow for the optimization of a Molecular Marker based selection program.

## Intraguild competition of lepidopteran larvae in maize

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Maize (=corn), *Zea mays* L. (Poales: Poaceae) in the central United States is annually attacked by several lepidoptera. Common species that feed on maize silks and kernels are the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), and western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae). Larvae of each species can cause economic yield losses but rarely does more than one species occur in a maize ear. The European corn borer and corn earworm occur throughout the Corn Belt while the western bean cutworm has historically been restricted to the western Corn Belt.

Transgenic maize for control of lepidopteran pests has been widely planted throughout the central United States, substantially reducing European corn borer populations and possibly the corn earworm (CEW). However, the western bean cutworm (WBC) is expanding its range. In 2000, large WBC populations (six per ear) were found for the first time in western Iowa, damaging up to 95 percent of maize ears. By 2005, the WBC had expanded its range 850 km eastward. A partial explanation for this species expansion maybe a change in intraguild competition between the CEW and WBC resulting from increased transgenic maize plantings.

The objective of our study was to measure the potential intraguild competition between CEW and WBC larvae. To evaluate intraguild competition, several mid-stage instars were used based on availability. Two larvae (one CEW and one WBC) were placed in 15 ml cups containing either a meridic or maize silk diet and allowed to feed for nine days. Silks from isogenic maize and transgenic maize (YieldGard) expressing the Cry1Ab protein were evaluated. In each experiment, two WBC (no CEW) in a cup were used as the control. Treatments were replicated 3-7 times.

Four different instar scenarios were tested on three diets. Percent survival of larvae is shown in Table 1. Competition between WBC and CEW in the meridic diet resulted in a mean survival of 10.7% for WBC and 96.4% for CEW, with the WBC control averaging 97.9%. On the isogenic maize silk, mean survival was 0% for WBC and 100% for CEW, with survival of WBC control at 91.7%. In contrast, larval survival on the transgenic maize silk was 100% for WBC and 0% for CEW, with 89.5% survival of WBC control. Based on these results, we believe that the CEW is more aggressive than the WBC and is likely to out-compete the WBC when both occur on isogenic maize. CEW is known to be cannibalistic and rarely does more than one larva occur in a maize ear. This aggressiveness also occurs toward the WBC as most larvae were killed and at least partially eaten by the CEW. In contrast, CEW mortality increased when feed transgenic maize silk while WBC was unaffected and survival was high. In increased planting of transgenic maize throughout the Corn Belt may be suppressing the CEW and reducing intraguild competition with the WBC, thereby allowing it to become more dominant and expand its range.

**Table 1:** Survival of western bean cutworm and corn earworm larvae during competition studies on meridic and maize silk diets.

Species (Instar)*	N**	Diet	WBC Survival	CEW Survival	WBC Control Survival
WBC(2)/CEW(3)	6	Meridic	16.7%	83.3%	100%
WBC(3)/CEW(4)	7	Meridic	0%	100%	100%
WBC(2)/CEW(4)	7	Meridic	0%	100%	100%
WBC(4)/CEW(3)	7	Meridic	28.6%	100%	92.7%
WBC(2)/CEW(3)	7	Isoline Silks	0%	100%	100%
WBC(3)/CEW(4)	7	Isoline Silks	0%	100%	83.3%
WBC(2)/CEW(3)	6	Transgenic Silks	100%	0%	100%
WBC(3)/CEW(4)	3	Transgenic Silks	100%	0%	75%

\* WBC=western bean cutworm, CEW=corn earworm

\*\*N for larvae in control is typically twice the number of larvae in the competitions

Tuesday, 7 Nov 2006, Session 5, 08:40; DERRIDJ & COUZI

## **Reduction of *Ostrinia nubilalis* Hbn. egg laying on maize by foliar application of soluble carbohydrates**

Sylvie DERRIDJ & P. COUZI

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Photosynthetic soluble carbohydrates coming from the apoplast, passing through the leaf cuticle are present on maize leaf surface in tiny quantities (ng per cm<sup>2</sup>) (Fiala et al 1990). *Ostrinia nubilalis* females after alighting on leaf surface, can detect them by gustatory sensillae localised on their legs and ovipositor, 10<sup>-3</sup> moles of fructose can be detected by one sensilla of the ovipositor (Derridj S. et al 1992). The composition (quantities and/or ratios) of the three sugars D-fructose, D-glucose and sucrose linked to the selective permeability of the cuticle (Stammitti L. et al 1995) and to the plant physiology are signals which stimulate *O. nubilalis* egg laying (Derridj S. et al 1989, 1996).

Modifications of these signals have been investigated to reduce *O. nubilalis* egg laying. Among them we have been trying the deposition of soluble carbohydrates on the leaf surface which after penetration into the plant may have multiple functions already known at the cellular level like regulation of gene expression of plant metabolism and in particular photosynthesis (Koch K.E. and Zeng Y. 2002). Thus by this way we could hope modifications of the leaf surface composition in soluble carbohydrates as signals and consequently of the insect behaviour.

On two maize hybrids LG 2447 and Anjou 258 grown in green houses, we sprayed soluble carbohydrate solutions of the three sugars (0.1, 1 and 10 ppm) on leaf 3 at the VT4 stage. On both hybrids 20 days after the treatments with fructose 0.1ppm and sucrose 10ppm when plants were at the VT 8-9 stage, *O. nubilalis* laid half less egg on treated plants vs. control in choice conditions. The distributions of eggs within the leaf positions on the whole plant showed also some modifications.

Foliar applications of soluble carbohydrates on one leaf (first end growing one) induced systemic changes in the plant growth and leaf surface composition in soluble carbohydrates less quantities of sucrose and modifications of the three sugar ratios.

Consequences on the free amino acid which are in relationship with *Diabrotica v. virgifera* Lecomte egg laying and which stimulate larval nutrition are analysed to define the opportunity of investigation of this treatment as a potential new management strategy to protect maize against the two pests.

Tuesday, 7 Nov 2006, Session 5, 09:00; HILTPOLD et al.

**Influence of insect-damaged roots on the host-finding  
behavior of the entomopathogenic nematode  
*Heterorhabditis megidis***

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Entomopathogenic nematodes are used in many integrated pest management and biological control programs. Infective cruiser juveniles have the capacity to actively search for their host in a complex below-ground system, using chemical and vibration signals. We have found that maize roots respond to feeding by *Diabrotica v. virgifera* LeConte (WCR) (Coleoptera: Chrysomelidae) larvae by releasing a chemical, (E)- $\alpha$ -caryophyllene. The emission of this sesquiterpene results in increased recruitment of the entomopathogenic nematode *Heterorhabditis megidis* Poinar, Jackson & Klein (Rhabditida: Heterorhabditidae). It seems that maize breeding has resulted in loss of the (E)- $\alpha$ -caryophyllene signal in some varieties, drastically reducing their attractiveness to *H. megidis*. Under field conditions, the infection rate of WCR by *H. megidis* were found to be fivefold higher on a maize variety producing the below-ground signal than on a variety that does not. Moreover, spiking the root system of a non-producing variety with synthetic (E)- $\alpha$ -caryophyllene decreased the emergence of WCR adults to less than half. The sesquiterpene diffuses into the soil through the air. Experiments in a semi-natural medium using a solid phase micro extraction (SPME) fiber have shown that (E)- $\alpha$ -caryophyllene diffuses faster and further at low moisture level. In addition, the amount of this chemical detectable 11 cm from the source decreases drastically with the soil humidity level; a fourfold greater amount is detected at a humidity rate of 1% than with 10% of water in the medium. However, it seems that a water layer is needed to avoid loss by vertical diffusion (evaporation); no (E)- $\alpha$ -caryophyllene is detectable when the medium is completely dry.

Tuesday, 7 Nov 2006, Session 5, 09:20; GLOYNA & THIEME

## **Cereals and weeds as potential host plants for *Diabrotica v. virgifera***

Kai GLOYNA & Thomas THIEME

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The preferred food source of the western corn rootworm (WCR), *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) is maize (*Zea mays* L.). But it is known that WCR is able to develop on other monocotyledonous plants too, e.g. weeds or cereals. These so called alternative host plants are of importance for eradication programs and our understanding of further spreading as well as for pest management. Especially crop rotation, as common IPM tool, and a possible development of resistance against transgenic Bt maize are related subjects of interest.

Therefore a number of common European weeds and cereal varieties were tested for their suitability as host plants for WCR larvae.

### Methods:

All trials were conducted in a greenhouse in a randomised block design at 25 °C, 60 % rh and 10:14 h L:D. Five replications per test plant were used. For infestation ten neonate larvae, not older than 12 hours were transferred into each pot using a fine artist brush. The final assessment was carried out 25 days after infestation. The soil was carefully sorted out by hand. Detected animals were staged, balanced and transferred into single glass tubes, filled with moistened soil and a seedling of the tested plant, to achieve a complete development if possible. For statistical analysis the number of recovered stages and the mean weight of recoveries are used.

### Results and discussion:

For six cereals (wheat, rye, barley, triticale, oat, and spelt) a total number of 18 varieties and 16 common European weed species were tested for their suitability as WCR host. All cereals tested allowed a complete development of WCR except of oat, where no development was detected. In some cases the food quality was comparable to that of the maize control. Differences between different cultivars of cereals became obvious.

In tests with wild grass species only quack grass, *Elymus repens* (L.) Gould; yellow bristle grass, *Setaria pumila* (POIR.) Roemer & J.A. Schultes; and bristly foxtail, *Setaria verticillata* (L.) BEAUV., were suitable for larval development of WCR.

The presented results are contradictory to the reported success of crop rotation in Hungary and first field studies. The different suitability of tested plants under field and greenhouse conditions could be due to the coincidence of WCR larvae and the developmental stage of host plants.

Further experiments are in preparation to elucidate possible implications for eradication programs or crop rotation as IPM tool to control WCR.

Tuesday, 7 Nov 2006, Session 5, 09:40; MOESER et al.

## **Impact of plant colonizing soil fungi on the invasive root feeding herbivore *Diabrotica v. virgifera* mediated via the host plant**

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Combining multitrophic interactions research with mechanisms that explain patterns in biological invasions we studied the impact of soil fungi on the invasive maize pest *Diabrotica v. virgifera* LeConte (WCR) (Coleoptera, Chrysomelidae). As modern agricultural practices reduce the chances of fungal colonization of maize by fungicide applications we put forward the hypothesis that WCR profits from the absence of systemically growing plant fungi in maize fields. This in turn may lead to a higher invasion speed and population build-up.

We conducted experiments to assess the impact of three different functional groups of fungi on the quality of WCR main host plant maize. We inoculated maize plants either with mycorrhiza (*Glomus intraradices*), an endophyte (*Acremonium strictum*) or a pathogen (*Fusarium graminearum*) in single, double and triple inoculations. We conducted bioassay experiments in the lab as well as field experiments under natural conditions in Missouri, USA in 2005. Results indicate a significant influence of the three different fungi depending on which fungus or which combination of fungi is present. Larval parameters like growth and development as well as plant parameters like damage and feeding were significantly altered by the inoculations. The corresponding plant growth stage from the field and lab trials yielded comparable results. The results from the field studies show a similar pattern with regard to root damage rating and adult emergence.

## **MSD, a new biotechnical strategy for causing favorable population shifts of *Diabrotica v. virgifera* in maize**

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*Diabrotica v. virgifera* LeConte (WCR) (Coleoptera: Chrysomelidae), an invasive rootworm pest of tremendous genetic versatility, is of concern both to the New and, recently, also to the Old World. To date, it successfully resists all attempts of eradication, in spite of numerous quite sophisticated approaches by entomologists, toxicologists, agronomists, and geneticists. WCR. is a powerful adversary against all human attempts to curb its spreading and to manage its impact on large scale maize production. Facing this situation, the search for new strategies in environmentally compatible and sustainable plant protection is mandatory. Here, we wish to propose a new biotechnical approach to population management which significantly reduces both WCR beetle populations in treated maize field sections and WCR eggs deposited in the soil beneath. The non-polluting strategy is called "MSD", short for a combination of *mass* trapping, *shielding* and *deflection*. It is characterized by establishing an invisible odor barrier which diminishes the flux of beetles across a high capacity trap line baited with WCR. kairomones, thus reducing both, population crossmigration, reproductive success, and/or oviposition within the shielded area. The new strategy is significantly different from the communication and mating disruption approach.

### Materials and methods:

*Zea mays* fields were located at Urbana (2003) and Champaign, Illinois, USA (2004). MSD barrier traps of the "Omni" or "Uni" or "Vario" funnel type were established at the field margins at distances of 10 to 20 m from each other and baited with 10 mg of 4-methoxycinnamaldehyde (MCA) kairomone as an attractant plus 100 mg of bitter cucurbitacin bait powder (as an arrestant, laced with a tiny trace of carbaryl insecticide for knockdown). Traps for analyzing the effects were established along the centerline of the field and consisted either of the above funnel traps or of "Metcalf type" sticky traps baited with 0.1 mg of WCR. sex pheromone. The soil washing procedure for counting WCR eggs was taken from Shaw et al. 1976.

### Results:

Significantly reduced daily beetle counts on maize plants within the shielded field sections were observed in comparison to unshielded control sections of equal size, both for the years of 2003 and 2004. Also, the numbers of WCR beetles attractable to kairomone or sex pheromone baited traps were reduced ( $p < 0.05$  to  $0.001$ ). Most striking was the reduction ( $p < 0.001$ ) of WCR. egg numbers encountered in soil after harvesting the maize in 2004. WCR. beetles actually mass trapped in the high capacity traps represented only a small fraction of the total estimated field population.

### Discussion and conclusions:

Unlike most other control strategies, MSD needs no "blanketing" of the treated area with an "area wide" carpet. Rather, it uses only a linear "curtain": A simple trap line impedes beetles from crossing over between field sections. On field sizes of 0.25 to 0.5 ha, the resulting population imbalance persists for the duration of the experiment. Thus, MSD works by redistributing beetles within the treated space and is reminiscent of Novak et al. 2001.

## Female-targeted semio-chemical baits for the western corn rootworm *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae)

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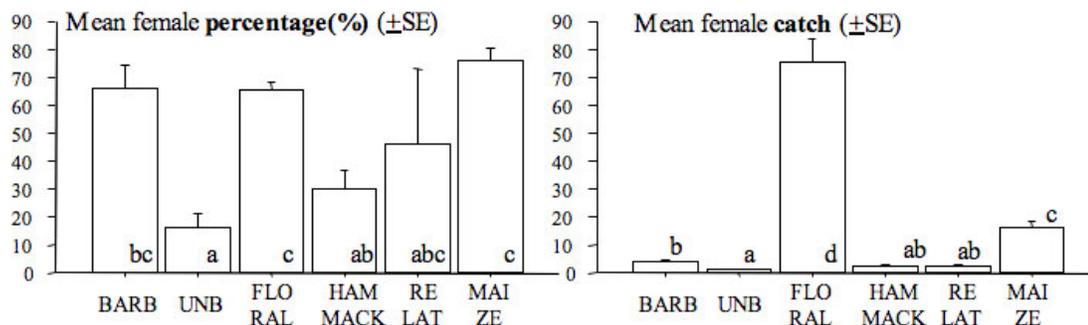
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In recent years emphasis of semiochemical research on the western corn rootworm (WCR) (*Diabrotica v. virgifera* LeConte, Coleoptera, Chrysomelidae) shifted towards the development of more female-specific lures. Through identifying volatile compounds from maize silk, Hammack et al (J. Chem. Ecol. 27:1373-1390, 2001) claimed that some combinations of their newly identified compounds were more powerful in attracting females than the conventional floral baits (based on 4-methoxy cinnamaldehyde).

Earlier we found that the newly developed "hat" trap design (CSALOMON® KLPflor+) when baited with the floral bait was especially suitable for the capture of female WCR. Encouraged by this we set out to compare efficiency of female-targeted *Diabrotica* baits. The following treatments were tested:

FLORAL: 4-methoxy cinnamaldehyde + indole (the conventional floral attractant for WCR; Metcalf et al., J. Chem. Ecol. 21, 1149–1162, 1995); HAMMACK:  $\beta$ -ionone + methyl salicylate +  $\beta$ -caryophyllene (attractant claimed to be more female-specific by Hammack et al, 2001); RELAT: 1-phenylethanol + 3-methyl eugenol + benzyl alcohol + benzyl acetate + geraniol (compounds known to be attractive to related beetles); MAIZE: phenylacetaldehyde + 2-phenylethanol + methyl anthranilate + eugenol + benzaldehyde (other compounds isolated from maize volatiles), BARB: 4-methoxyphenethyl alcohol + cinnamic alcohol + (*E*)-anethol + indol (the attractant described for *D. barberi*; Metcalf et al., 1995); and UNB: unbaited controls.

Female percentage in catches was equally high in treatments BARB, FLORAL, MAIZE and RELAT. It was surprising that HAMMACK (which had been described as a strong female attractant by US scientists) was not more attractive for females than unbaited controls. Among treatments more attractive for females, none produced significantly higher female percentages than FLORAL. However, FLORAL caught far more beetles than any of the other treatments. In conclusion, so far the most efficient female-targeted trap-bait combination for WCR is the conventional floral attractant applied in KLPflor+ traps.



**Figure 1:** Mean female percentages and catches of WCR in traps baited with different female-targeted bait combinations and in unbaited controls. Hajdúböszörmény, Hungary, JUL 5 – SEP 19, 2005. Means with same letter within a diagram not significantly different at P=5% by ANOVA, Games-Howell.

## **Microbiological control of the western corn rootworm *Diabrotica v. virgifera* with entomopathogenic fungi**

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The western corn rootworm *Diabrotica v. virgifera* is a serious corn pest recently introduced in Europe. It was first observed near the airport of Beograd in 1992. Subsequently, the insect spread quickly through central- and western Europe by human transport ways as well as by their flight capabilities. Attempts to eradicate this invasive pest are done by crop rotation, insecticide application and the use of pesticide coated seeds but could not hinder the spread and population increase.

In order to develop a sustainable control method based on indigenous natural enemies a survey for presence and importance of naturally occurring pathogens, mainly fungi and nematodes, was carried out in Hungary in 2005. Larvae and pupae were sampled in maize fields at 12 different locations and adults were collected at three locations in Hungary and at one location with four sampling plots in Austria adjacent to the Hungarian border. A total of 762 larvae and pupae were collected as well as about 37 200 adults. From the collections in Hungary five larvae, one pupa and three adult beetles were infected with *Metarhizium anisopliae* and one larva was infected with *Beauveria* sp. Additionally, soil samples from the same Hungarian maize fields were taken to determine presence and density of entomopathogenic fungi (EPF). EPF could be detected at all locations either by plating soil suspension on selective medium or by using *Galleria mellonella* and *Tenebrio molitor* as bait insects. Entomoparasitic nematodes (*Heterorhabditis* sp.) were found in nine larvae collected in Hungary. From 605 dissected beetles collected in pheromone traps in Austria three beetles, collected at a nematode treated plot, contained a few *Heterorhabditis* sp. No fungus-infected beetle was found.

Bioassays with the collected fungus isolates and with standard isolates were carried out to identify the most virulent ones. For this third instar *Diabrotica* larvae from a laboratory rearing were used. Infection rates of the individual isolates ranged from 0 to 47%. Isolates from *Diabrotica* were clearly more virulent than isolates from other hosts.

During the summer 2006 field trials will be done for the first time in Hungary. *M. anisopliae* will be applied at a soil depth of 8- 10 cm as fungal colonized barley kernels in small scale field experiments at two locations. Aims are to study the establishment and persistence of the fungus in the soil during summer and winter time in different soils and their impact on *Diabrotica*. Larvae will be collected from root systems and adult beetles will be caught in emergence cages in the different treatment regimes. Results of these field experiments will be discussed.

**Persistence of applied *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema feltiae* in maize fields and their effectiveness in controlling larvae of *Diabrotica v. virgifera***

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Larvae of the invasive alien *Diabrotica v. virgifera* (WCR) damage maize roots and are therefore a prime target for control actions. In order to avoid the use of soil insecticides or genetically modified *Bt*-maize, control using entomopathogenic nematodes (EPN) was tested under field conditions in Hungary during the last three years.

(1) Applications of *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema feltiae* on *D. v. virgifera* infested maize fields revealed that *H. bacteriophora* in particular can prevent 50 to 70% of economic root damage. (2) *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema feltiae* had short persistence periods in the soil of maize fields. However, an application persisted long enough for successful use during maize sowing periods in April (prior WCR larval hatch) or June (during 2<sup>nd</sup> instar larval stage).

In conclusion, the development of an EPN-based product against larvae of WCR appears to be possible. Such a product can be used in countries where (a) subsidies for maize production require IPM, good farming practices or organic farming, or (b) in the production of maize with higher economic value (e.g. polenta or seed maize production).

## **Control of the larger grain borer, *Prostephanus truncatus* Horn (Coleoptera: Bostrychidae) in stored maize by using diatomaceous earth formulations**

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The larger grain borer, *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) was accidentally introduced in Africa during the early 80's, where it became a serious pest of stored maize. As an internal feeder, this species is not very vulnerable to natural enemies, and it has developed a considerable level of resistance to traditional contact insecticides. Diatomaceous earths (DEs), the fossilized remains of diatoms (phytoplanktons), are very promising non-toxic alternatives to chemical insecticides in stored-product protection. In the present work, we evaluated several DEs for the control of *P. truncatus*. The DEs used were a) SilicoSec (a freshwater DE that contains >90 % SiO<sub>2</sub>), b) PyriSec (a DE that contains SilicoSec, natural pyrethrum and piperonyl butoxide) c) Insecto (a DE that contains approx. 90 % SiO<sub>2</sub> and 10 % food additives) and d) DEA-P (a mixture of DE with abamectin). In all cases, two doses were tested, 100 and 500 ppm. The tests were carried out at the laboratory at 30 °C and 70 % RH, while mortality was assessed after 7 and 14 d of exposure in the DE-treated maize. In all cases, after 14 d of exposure, mortality was <75 %, with the exception of DEA-P, where mortality was close to 100 % even after 7 d of exposure. Moreover, in maize treated with SilicoSec, PyriSec and Insecto, progeny production was not avoided. The results of the present study indicate that some DEs could be used with success against this serious maize pest.

## **Western corn rootworm (WCR) management by crop rotation on risk assessed corn fields**

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Crop rotation is the main WCR management option for the majority of farmers in Serbia. Several reasons are explaining that option but first of all is its efficacy and simplicity. However, WCR management by crop rotation is reducing corn acreage at national level.

Risk assessment method incorporated in agroecosystem analysis conducted in Farmers Field Schools appeared to offer solution to overcome such situation. The whole plant count technique and Pherocon AM sticky trap technique were applied by the trained farmers having a need for repeated corn sowing. This allows corn producers to decide to rotate corn with another crop only when there is a real risk of WCR damage.

Farmers Field School methodology was used for season long training of participating farmers. Over a 4 year period trained farmers checked 719 fields in order to estimate the potential risk of repeated corn sowing. In the majority of first year corn fields chosen by farmers risk was assessed to be absent. Out of 462 checked first year corn fields only 3 were at risk (< 1%) since the WCR adult population was high. In case of 157 checked 2nd year corn fields the situation is significantly changing but the training of the farmers and their subsequent work in the field was worthwhile since the majority of corn fields were not at risk allowing the farmers to repeat corn sowing for the third year. Farmers determined that only 25 second year corn fields (16%) were under the risk of subsequent damages from WCR larvae. In the majority of the assessed corn fields farmers found a clear situation allowing them to make safe and confident decisions.

Concerning WCR adult density in 3rd year corn fields participating farmers determined that 32 out of 73 checked corn fields (44%) is under the risk. In 27 checked 4th year corn fields 22 fields (82%) were under the risk. Our results showed that crop rotation is efficient when applied in WCR management and does not necessarily mean reduction in corn acreage and production. Trained farmers participating Farmers Field Schools were able to make their own decision based on action threshold and to manage WCR by crop rotation only when it was necessary. In order to share these information's within community participating farmers created village maps showing distribution of corn fields with and without risk. Such village maps are a visual tool to present, share and discuss data collected in fields around the village to learn from field experience and make final decisions and planning for following years. These maps represent a powerful tool for collaboration at community level that can also be used for purposes not limited to WCR management.

## **Attractiveness of sweet maize hybrids to western corn rootworm (*Diabrotica v. virgifera*) beetles in the late sowing in 2005**

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The sweet maize production is performed with a successive sowing from the beginning of April until the end of May-the beginning of June and as stubble crop sowing. Sweet maize is usually harvested prior to mass migration of WCR imagoes in search for food. Crops of later sowing dates or stubble crops are very attractive to migrating imagoes because they are rich in pollen and fresh silk. Considering that this production is performed under regular irrigation, besides a greater plant attractiveness, soil is softer and more suitable for egg laying.

The aim of this study was to confirm effects of a hybrid and a sowing date on crop attractiveness to WCR imagoes and to estimate efficiency of two types of traps in monitoring of the population and risk levels for the repeating maize sowing in the following year.

The plant test material consisted of two commercial sweet maize hybrids that were sown on four dates. The following hybrids were sown as follows: Royalty (May 24, and July 2 and 17), Victoria (May 30). Each hybrid was sown on the area of 20 ha. The trial was carried out under irrigation conditions on the plot of over 100 ha in NW Vojvodina, the location of Ruski Krstur. The following traps were used to monitor the WCR beetles and check attractiveness of the stated hybrids and their combinations with sowing dates: yellow sticky traps Pherocon AM (YsT) and cylindrical traps with cucurbitacine as an attractant (CuT). The traps were set on maize plants on the 1.5-m stakes on August 11, 15 and 17. Checking of traps started on August 15, each subsequent every second day and finished on August 22.

A total of 384 WCR beetles were trapped during eleven days of monitoring. Out of this number, 250 WCR beetles were trapped on yellow Pherocon AM traps (YsT) in the following ratio: hybrid Royalty sown in May 24: 5, in July 2: 95 and in July 17: 84 WCR beetles. A total of 134 WCR beetles were trapped on cucurbitacine traps (CuT) in the ratio: hybrid Victoria sown in May 30: 58 and hybrid Royalty sown in July 2: 29, in July 17: 42 WCR beetles. The greatest number of beetles on YsT, i.e. CuT was registered in the last five days of monitoring: in August 19 on YsT: 86, on CuT: 26 or 14,3 and 3,25 imago/trap/day. On August 22 on CuT 78 were registered on YsT: 57, or 6,3 and 6,5 imagoes/trap/day, respectively.

## **What happens after an alien *Diabrotica virgifera virgifera* beetle is accidentally introduced?**

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Mark - release - recapture experiments were undertaken in order to investigate the movement of adult *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) from accidental introduction points, such as airports, towards suitable habitats, such as maize fields. In Hungary in 2003 and 2004, nine mark - release - recapture experiments were carried out in a grass steppe area and lucerne field, in which two small maize fields (10 x 10 m) were established 300 m distant from the central release point. After each release of 5500 to 6000 marked *D. v. virgifera*, beetle recaptures were recorded three times using non - baited yellow sticky traps (Pherocon AM) placed on 30, 105, 205 and 305 m radii around the release point.

In seven out of 15 recapture periods (46%), beetle populations performed non-directional movements; and their movements towards any particular habitat cannot be predicted. During five recapture periods (33%), beetle populations showed a uni-directional movement, and in three cases (20%) a bi-directional movement was observed. In 10 out of 15 recapture periods (67%), the released populations moved in a direction that was comparable with the mean wind direction during these periods; thus, the beetle movements were slightly correlated with the wind directions. On average over sites and years, beetles were not preferentially moving towards the two small maize fields (located 300 m from the release point) compared to other directions. However, beetles moved significantly more frequently in the direction of naturally occurring maize fields within a radius of 1500 m than towards other habitats. Beetles stayed more frequently within flowering lucerne fields out to a radius of 300 and 600 m than in non - flowering lucerne or other habitats. On average, 2.8 % of all recaptured beetles arrived in one of the two small maize fields located 300 m from their release point indicating that there is a high risk of a founder population establishing. Habitat management, like prohibiting maize fields, cannot be suggested as a the only mean to prevent the beetle's initial dispersal because its movement was usually non-directional, and alternative food plants were used prior to reaching maize.

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Tuesday, 7 Nov 2006, Session 7, **POSTER 032**; BRMEZ et al.

## **Correlation between plant lodging caused by western corn rootworm and grain yield**

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Western corn rootworm (WCR) (*Diabrotica v. vigifera* LeConte) occurred in Croatia in 1995, and since then progressively increased in population. Many cornfields had great percentages of lodged plants which caused economic losses. The aim of this investigation was to determine correlation between plant lodging caused by WCR and grain yield. Field trials were settled in Osijek area in 2005. Investigation was done on twelve Croatian commercial hybrids, in four replicates. Twenty five plants in each replicates were ratified in five categories of lodging according to lodging angle: (1 - lodging angle 0°; 2 – lodging angle 1-15°; 3 – lodging angle 16-30°; 4 - lodging angle 31-45°; 5 – lodging angle >45°). Grain yield was estimate by weighting of corn cobs for each plant separately. Correlation between grain yield and plant lodging was done as well as correlation of lodged plants and ratings of root damage according to Iowa node injury scale (0-3).

Results showed that there were no statistically significant differences between correlation of grain yield and plant lodging although plants with no lodging had the greatest values of grain yield. Nevertheless, linear trend decreased in line with larger lodging angle. The results also showed strong negative correlation ( $r = -0,480^{**}$ ) between plants lodged over 45 degree angle and root damage ratings. That indicates how root damage ratings done earlier in the vegetation period (in the beginning of July) could serve as reliable parameter for determination of plant lodging.

## **Recurrent selection for maize resistance to pink stem borer**

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The pink stem borer, *Sesamia nonagrioides* Lefèvbre (Noctuidae) is the most important pest of maize (*Zea mays* L.) in the Mediterranean area. The maize composite EPS12, has been chosen as the base population for a breeding program for resistance to pink stem borer; the main selection criterion was resistance to stem tunneling. Yield was taken as a secondary selection criterion to avoid unwanted negative correlated response on this character. The aims of the present study were (1) to determine direct and indirect responses of the maize population to selection, (2) to study the effect of selection on larvae growth and mortality, (3) to study variance changes across selection, (4) to monitor the impact of selection for resistance to pink stem borer on the genetic structure of EPS12, and (5) to identify loci at which allelic frequencies changed significantly due to directional selection. For achieving the objective 1, cycles of selection per se, selfcrossed, and crossed to certain inbred lines were evaluated for agronomical and damage related traits under infestation with *S. nonagrioides* and *Ostrinia nubilalis* Hübner (Crambidae) eggs at two locations in two years. The three cycles of recurrent selection slightly reduced damage by *S. nonagrioides* and *O. nubilalis*, and unfavorable changes were not observed on agronomical traits, such as yield, although inbreeding showed a tendency to increase. The small response of EPS12 to selection could be consequence of the positive correlation between both traits of selection, stem tunneling and yield. Several bioassays at environmentally controlled conditions were planned to evaluate changes on maize antibiosis against pink stem borer larvae across selection. Although variability for antibiosis was present, leaf sheath, and pith stem antibiosis against pink stem borer larvae has not been significantly improved over the selection process for less stalk tunneling. Two designs were used to study the variance changes, one was based on the field evaluation of S<sub>1</sub> families from the original and the last cycles of selection and the other on a North Carolina design I involving the same cycles of selection, and both showed that there is enough variability to continue the selection scheme. Finally, the effects of selection for resistance to pink stem borer on allele frequency at 70 simple sequence repeat (SSR) markers were monitored to study the impact of selection for resistance to pink stem borer on the genetic structure of EPS12, and to identify loci at which allelic frequencies changed significantly due to directional selection. Genetic diversity was reduced over the selection process, but not significantly so. Although the loss of genetic variation was generally consistent with that expected in a model in which random genetic drift acts alone on neutral alleles, directional selection was acting on loci umc1329 and phi076, suggesting the presence of QTLs for tunnel length under artificial infestation with *S. nonagrioides* to lie on the long arm of chromosome 4. As conclusion, a slight response to selection for resistance to *S. nonagrioides* has been detected and understanding on the mechanisms and genetics involved has been gained.

Tuesday, 7 Nov 2006, Session 7, **POSTER 034**; CAMPBELL & MEINKE

## **Fitness of *Diabrotica barberi* Smith and Lawrence x *Diabrotica longicornis* Say hybrids (Coleoptera: Chrysomelidae)**

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*Diabrotica barberi* (Smith & Lawrence) and *Diabrotica longicornis* (Say) (Coleoptera: Chrysomelidae) are sister species that feed on roots of grasses. *D. barberi*, the northern corn rootworm, is an important pest of corn in the United States Corn Belt; its range includes the entire US Corn Belt and extends north and east into the northeastern US and Canada. *D. longicornis* is a nonpest species that occurs in grassland habitats in the southwestern U.S. The species' ranges overlap in eastern and central Nebraska and Kansas, and observational data suggest that some habitat overlap occurs as well. *D. barberi* and *D. longicornis* are not separated temporally, but do respond to different stereoisomers of the same pheromone. Hybrids have been created under lab conditions in previous studies, and hybridization between the two species may occur under field conditions, but the viability and behavior of hybrids is unknown. Lab-reared hybrids, behavioral analyses, and molecular techniques are currently being used to examine the role of hybridization in this system. This poster will focus on the fitness of *D. barberi* x *D. longicornis* hybrids. Adults of both species were collected from allopatric areas in 2004; virgin males and females were crossed to form hybrids and also to maintain parental lines in 2005. Eggs of hybrid and parental lines will hatch in 2006, and various life history traits will be measured that directly or indirectly may contribute to overall fitness. Percentage hatch for each cross, total development time (neonate larva to adult emergence), and larval survival will be recorded. Adult sex ratio, longevity, and fecundity will also be documented. Hybrid adults will be mated in 2006 to other hybrid individuals or backcrossed to individuals of the parental species to evaluate mating success and the fitness of offspring. Results will be discussed within the context of 1) the current knowledge of *D. barberi* and *D. longicornis*, and 2) the potential for hybridization to affect the evolution of these two species.

Tuesday, 7 Nov 2006, Session 7, **POSTER 035**; FUESER et al.

## **Potential for WCR larval orientation disruption using artificial carbon dioxide sources**

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Larvae of the western corn rootworm orientate towards the roots of their host plants by using the carbon dioxide emitted by growing roots (Bernklau et al. 2004). We tested whether artificial CO<sub>2</sub> – sources could be used to disrupt orientation of the larvae within the soil. In behavioural bioassays larvae were offered either maize plants or CO<sub>2</sub>-releasing capsules in boxes containing standardized soil. Maize plants released relatively steady amounts of CO<sub>2</sub> over time, whereas the potential of the CO<sub>2</sub>-releasing capsules exceeded the CO<sub>2</sub> release of the maize roots but decreased rapidly over time. In all no-choice-treatments, about 35 % of the larvae stayed in the segment where they had been released, 30 % were found near the artificial CO<sub>2</sub> source and 35 % near the maize roots, respectively. On the other hand, when given a choice 20 % of the larvae were recovered in the segment where the maize plant grew as compared to about 15 % in the opposite direction with the capsules. However, more than 50 % did not move at all, indicating that both CO<sub>2</sub>-sources might have counteracted their attractive potential when offered alone. Potential improvements in this method for stronger attraction of the WCR larvae towards artificial CO<sub>2</sub>-sources will be discussed.

## **Some economic aspects of western corn rootworm for the Hungarian maize production**

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In Hungary the most profitable arable crop is the maize. It covers about 1-1.3 million hectares yearly. After some years new maize pest, western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae) was found. The input of maize production increased with costs of control of WCR as well.

However plant protection specialists directed farmers' attention to danger of WCR the farmers have not taken into consideration this warning for some years. WCR problem was concealed for 4-5 years till economic population developed in an area and under extremely hot summer conditions larvae of cotton bollworm (*Helicoverpa armigera* Hübner, Lepidoptera: Noctuidae) caused serious direct damage and loss in maize.

First signs of the drastic damages caused by the larvae of WCR appeared in 2000 in the South-Eastern regions and later on the expansion was observed in other regions to. Nowadays farmers consider WCR as an important pest and they try to keep it under economic threshold. As part of the WCR management system they avoid the long monoculture and use different measures applying pesticides to prevent serious damages.

Estimation of the profitability of maize production many aspects have to be taken into account.

Crop rotation reduces the income of the farmers – there is no compensation.

Maize production even in case of first year crop became very risky without control of WCR larvae or adults or both in the areas with established population.

Control of larvae by insecticide application (seed dressing or soil application) is carried out at the time of planting without extra cost of application. Pesticide cost is about 50-75 Euros/ha.

Costs of the adult control consist of the price of insecticide and the application. The prices of the most frequent used insecticides fluctuate 8-20 Euros/ha. Aerial application costs about 20-25 Euros/ha. Cost of foliar application by high clearance tractor takes approximately 12 Euros/ha.

Entirety of damage and/or costs of control of WCR can cause equal about 1-1,5 t/ha loss according to our practical experience.

## Evolution of sex pheromone communication systems in the genus *Ostrinia*

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The genus *Ostrinia* (Lepidoptera; Crambidae) contains 21 closely related species with diverse host plant ranges, providing an excellent system for studies in evolutionary biology. Among all *Ostrinia*, eight species, i.e., *O. furnacalis* (Asian corn borer), *O. orientalis*, *O. scapulalis*, *O. zealis*, *O. zaguliaevi*, *O. palustralis*, *O. latipennis* and *O. ovalipennis*, are found in Japan. Analyses of the sex pheromones of all these species, conducted by our group, revealed substantial diversification among species (Table 1). The evolution of the sex pheromone communication systems in *Ostrinia*, however, is not resolved at present, since our effort to clarify the phylogenetic relationships among *Ostrinia* species based on mitochondrial DNA sequences has encountered difficulty due to their unusual closeness. Fortunately, experimental cross hybridization between *Ostrinia* species is possible in several combinations, and offspring are viable. Analyses of the sex pheromones of F1, F2 and backcross progeny provide useful information on the genetic basis for the divergence of the sex pheromones. In this presentation, we summarize the present knowledge on the sex pheromone communication systems in *Ostrinia*, and discuss the evolution of the systems.

**Table 1:** Female sex pheromone components and their ratios in *Ostrinia* species.

Species	Sex pheromone component					
	Z9-14:OAc	E11-14:OAc	Z11-14:OAc	E12-14:OAc	Z12-14:OAc	E11-14:OH
<u>Simple or bifid uncus species group</u>						
<i>latipennis</i>	-	-	-	-	-	100
<i>palustralis</i>	-	99	1	-	-	-
<u>Trilobed uncus species group</u>						
<i>furnacalis</i> (Matsudo)*	-	-	-	38	62	-
<i>furnacalis</i> (Nishigoshi)**	-	-	-	46	54	-
<i>orientalis</i>	-	2	98	-	-	-
<i>scapulalis</i> (Z type)	-	3	97	-	-	-
<i>scapulalis</i> (E type)	-	99	1	-	-	-
<i>scapulalis</i> (I type)	-	63	37	-	-	-
<i>nubilalis</i> (Z type)	-	3	97	-	-	-
<i>nubilalis</i> (E type)	-	99	1	-	-	-
<i>nubilalis</i> (hybrid)	-	63	37	-	-	-
<i>zealis</i>	70	22	8	-	-	-
<i>zaguliaevi</i>	45	5	50	-	-	-

\* Matsudo (35.8°N, 139.9°E). A similar component ratio was reported for the population from South Korea (Boo and Park, 1998).

\*\* Nishigoshi (32.9°N, 130.7°E). A similar component ratio was reported for the populations from the Philippines (Klun et al., 1980), from the People's Republic of China (Cheng et al., 1981) and from Taiwan (Kou et al., 1992).

Tuesday, 7 Nov 2006, Session 7, **POSTER 038**; LI et al.

## **The annual population dynamics of *Ostrinia furnacalis* and its dominant parasitoid in sweet corn field**

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The Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée) is the most destructive insect pest on sweet corn in south China. Egg parasitoid plays an important role in controlling ACB damage. Knowledge of ACB and its dominant parasitoid population dynamics is essential for developing sustainable protection strategies. The annual population dynamics of ACB and its dominant parasitoid in sweet corn field were investigated through systematic survey. Over two years, ACB egg masses could be found in sweet corn through March to December. Annual-average number of egg masses per hundred plants profile demonstrated a peak in June with serious infestation from May to August. The spatial-dynamics showed that the female moths prefer to lay eggs on plants at tasseling stage. It was found that ACB egg parasitoid species included *Trichogramma ostrinia* Pang et Chen and *T. chiloni*. *T. ostrinia* was the dominant species in this region, its parasitism was up to 80%. The annual-parasitism dynamics of *T. ostrinia* demonstrated the similar profile as ACB with a peak of abundance from June to October. It was accorded with the phenomenon that the enemy followed the pest. The investigation showed that *T. ostrinia* was a key factor affecting occurrence of *O. furnacalis* and had a good prospect for controlling the ACB.

## **Assessing the impact of *Bt* maize pollen on adult green lacewings**

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The planting of transgenic crops in the world has increased dramatically since the commercialization of *Bacillus thuringiensis* (Bt) maize in the mid-1990s. An advantage of Bt plants is that the reduced need for conventional insecticides, conferring benefits for the environment and human health. This notwithstanding, some environmental concerns about potential effects of Bt crops on nontarget organisms have still been raised during recent years due to the high and consistent expression of Bt toxin in most plant parts throughout the whole plant growth.

The common green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), has extensively been studied in respect to potential Bt maize effects. However, previous studies have focused on the predatory larvae stage of *C. carnea* and not on the adults that mainly feed on pollen and honeydew. Pollen from certain Bt maize events such as event176 and Mon88017 are known to contain measurable amount of toxin, Cry1Ab and Cry3Bb1, respectively. Maize pollen is abundant during anthesis and readily consumed by adult *C. carnea*. Therefore, adult lacewings are exposed to the Bt toxin containing in the pollen grains and it is therefore necessary to assess the impact of Bt maize pollen on adult *C. carnea*.

In this study, we have investigated the utilization of maize pollen by adult *C. carnea* and established an effective laboratory bioassay to investigate the potential dietary effects of transgenic pollen. In a subsequent study, adult lacewings were fed pollen from transgenic maize (expressing Cry1Ab or Cry3Bb1) or from corresponding non-transgenic maize varieties together with a 1 M sucrose solution. Over a period of 4 weeks, different life-table parameters (fecundity, fertility, survival) were recorded.

The research will allow to assess whether the deployment of Bt maize will pose a risk on adult *C. carnea*.

Tuesday, 7 Nov 2006, Session 7, **POSTER 040**; LU et al.

## **Studies on low temperature storage for industrial produce of *Trichogramma dendrolimi* Matsumura**

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Under constant temperature 3°C a long-term storage test was performed to undiapause *Trichogramma dendrolimi* of six stages of growth including young larva, middle larva, old larva, prepupa, initial pupa, middle pupa. The results indicate that the storage effect was influenced by different stage of growth. The middle larva is the best stage and young larva is better stage for the storage. The storage time of young larva and middle larva may not exceed sixty days, the other may not exceed fifteen days. With the storage time extending, the number of *Trichogramma dendrolimi* in the single host egg was reduced within the twenty days. The storage time was lengthened after the twenty days, the number of *Trichogramma dendrolimi* in the single host egg was no longer change. Storing young larva resulted in the number of *Trichogramma dendrolimi* in the single host egg being reduced greatly. With the storage time become longer and shorter, change of the number of the single female adult oviposition was not marked. But initial pupa and middle pupa were stored, the number of the single female adult oviposition were reduced greatly.

Tuesday, 7 Nov 2006, Session 7, **POSTER 041**; LU et al.

## **Research on the flight capability and effective accumulated temperature of different voltine ecotype of Asian corn borer**

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Research was conducted to evaluate the flight capability and effective accumulated temperature of univoltine and bivoltine ecotypes of Asian corn borer distribution in the east, west and middle area of Jilin province. The results are as follows: the moth of univoltine ecotype has more flight capability than the bivoltine ecotype; the flight of female moth has more capable than the male moth in the same voltinism; the threshold of development temperature of univoltine larve is higher than of the bivoltine larve; the effective accumulated temperature of the univoltine ecotype is higher than of the bivoltine ecotype.

## **Development of biological products for sustainable control of the Western Corn Rootworm**

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To prevent major economic losses due to the Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera*, ecologically sound and economically competitive control strategies are urgently needed. In this project, we aim to develop biological control products consisting of entomopathogenic nematodes and fungi and to integrate biological methods into sustainable control strategies of WCR. Critical factors that are being investigated for several nematode species include establishment potential and virulence as well as application techniques. In order to optimize efficacy of nematodes, we are investigating and make use of the attraction of nematodes by different maize varieties. A variety of fungal species and strains including those isolated from WCR were tested in order to select the strain with highest virulence and probability of controlling the soil dwelling larvae of WCR. As another management option, transgenic Bt-maize will be evaluated with special emphasis on non-target effects. Since the overall aim is to develop biological products, the compatibility of Bt-maize with above mentioned biological control agents will be studied. A comparative risk assessment, which also includes pesticides, will be performed in order to select the most environmentally friendly pest control system. For more details on this project, see presentations in sessions 2, 5 and 6 of this meeting.

**Use of some eco-biological aspects and statistical analyses in determining the life table parameters and the numbers of generations of the green bug, *Schizaphis Graminum* (Rondani) (Homoptera: Aphididae) on maize**

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The life table parameters temperature threshold, thermal units, reproduction rates, generation time, population doubling time, intrinsic rate of increase, finite rate of increase and number of generations of the green bug, *Schizaphis graminum* (Rondani) which reared on maize leaves were determined by using the statistical analyses and some eco-biological aspects.

Data revealed that the duration period of the nymphal stage was 11.52, 8.13, 6.22 and 4.31 days and the pre-viviparity period were 2.01, 0.74, 0.45 and 0.41 days at temperatures of 15, 20, 25 and 29 °C, respectively. Temperature threshold for the development of the nymphal stage was 8.82 °C and the thermal units necessary for the development was 90.05 day-degrees (DD).

Life table parameters were as following: The reproduction rates (R<sub>0</sub>) were 11.53, 30.81, 45.25 and 40.66; mean generation time (GT) was 23.67, 21.35, 13.28 and 12.67 days ; population doubling time (DT) of the greenbug was 6.91, 4.23, 2.44 and 2.38 ; intrinsic rate of increase (r<sub>m</sub>) was 0.104, 0.157, 0.284 and 0.289 and finite rate of increase (λ) was 1.105, 1.171, 1.331 and 1.335 at 15, 20, 25 and 29°C, respectively.

Number of generations of the green bug which could develop in one season on maize plants under Assiut conditions was about 14 generations.

## **Spider mites population changes in corn and the relationship to leaf damage**

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Fields studies were conducted during three growing seasons in Odemira (Portugal) in order to evaluate the abundance of spider mites populations (*Tetranychus* spp.) and its evolution in maize fields, and, at the same time, to determine the best relationship between mite population (female abundance, number of females per leaf) and plant infestation (percentage and number of leaves infested, percentage of the leaf area damaged). Data were measured from tasseling to grain-filling growth stages (Gavadour Cargill scale), considering five stratification levels: all plant (L1), ear leaf (L2), lower, middle and upper third (L3, L4 and L5). Several variables were compared between levels and growth stages. The seasonal female abundance was similar in levels 1 and 4, but the higher populations were recorded at level 4, so mite population evaluation could be restricted to counting the number of females on the middle one-third of maize plant. Populations always decline in early September. Regression analysis were used to compare plant infestation variables with the mite population variables, for each stratification level. Data were transformed and the results of this regression analysis demonstrated a good relationship between the female abundance and the percentage of leaf damage recorded either the same or the previous growth stages, for levels 2 and 4. Combining independent variables did not increase  $R^2$  values. The best models were obtained with a breaking point and some of the  $R^2$  values obtained were higher than 0.80 (for example: the regression between the number of females at level 4 and the percentage of the leaf area damaged at the same level as a  $R^2 = 0.83$ ). These results indicate that studies quantifying population dynamics could be restricted to the middle one-third of the plant and its possible to estimate the number of females using the percentage of leaf area damaged, for all stratification leaves considered, although with different levels of explanation.

## **PHEROCON<sup>®</sup> CCB, a new trap for monitoring western corn rootworm, *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae)**

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An improved design of the Pherocon<sup>®</sup> CRW trap (Trécé, Inc., Adair, Oklahoma, USA), with a yellow bottom and a more tapered top section, was effective in capturing western corn rootworm (WCR) beetles in Italy. This trap, referred to as the Pherocon<sup>®</sup> CCB, was previously tested only in the southeastern United States to evaluate kairomone formulations for their effectiveness as attractants for luring three species of cucumber beetles in cucurbit and sweet potato fields. In this USA study, the new trap was also compared to the clear plastic Pherocon<sup>®</sup> CRW trap.

The Pherocon<sup>®</sup> CCB trap was first evaluated on WCR beetles looking at trap color, with and without lures. In one study, the unbaited Pherocon<sup>®</sup> CCB trap was compared to the Pherocon<sup>®</sup> AM trap. In another study, the unbaited Pherocon<sup>®</sup> CCB trap with yellow bottom and with a clear plastic bottom were compared to evaluate the possible role of color and also the “stun pill” that is used to attract and kill beetles that enter the trap. In this study, clear plastic baited Pherocon<sup>®</sup> CCB traps were also utilized.

TRE9950 (500 mg of indole and 750 mg of 4-methoxycinnamaldehyde) was more effective lure than the original TRE 8275 (1500 mg of 4-methoxycinnamaldehyde) commercial formulation. Traps baited with kairomone formulations were more effective in capturing WCR, but the number of beetles captured in the unbaited Pherocon<sup>®</sup> CCB and Pherocon<sup>®</sup> AM traps were comparable. WCR beetle captures were also observed in the unbaited Pherocon<sup>®</sup> CCB traps made of clear plastic. This means that beetles outside the trap enter the clear plastic unbaited trap and walk up also attracted the stun pill inside the trap.

Growers and technicians are averse to using sticky traps, primarily because of the issue of handling the sticky material. Thus, the unbaited Pherocon<sup>®</sup> CCB trap can be effective and useful in monitoring programs for WCR. This study suggests that there is no need to use a bait as a lure when the yellow bottom trap is used. So the user needs only to check and empty the trap contents weekly to monitor WCR population levels. This trap can also be used effectively as a baited or unbaited trap. The relationship of beetle numbers captured to subsequent larval damage caused by WCR will have to be investigated. Regardless, enough information exists to support use of the unbaited Pherocon<sup>®</sup> CCB trap for monitoring WCR beetles as part of an areawide pest management program in Italy.

## **Genetic variation among Mediterranean populations of *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae)**

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Restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA segments of mitochondrial DNA (COI and) was used to examine genetic variation in *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) populations from the Mediterranean basin. Four populations were collected from central and southern Greece, and five from northern latitudes from Greece, Italy, France, and Spain. Lower level of intra-population polymorphism was found in the northern populations than in southern ones. The UPGMA phenogram based on Nei's raw number of nucleotide differences separated the populations in two major groups, i.e. one with the northern (40.6° N - 43.4° N) and the other with the southern populations (37.3° N - 39.2° N). All pair wise comparisons between northern and southern populations resulted in high and significant  $F_{ST}$  values (overall  $F_{ST} = 0.604$ ). The populations do not seem to have experienced a strong historical bottleneck. Long distance migration of *S. nonagrioides* seems to be a rare event. The occurrence of a few widespread haplotypes and the genetic similarity of the northern populations could be attributed to a historical expansion of certain haplotypes, tolerant to severe winters, from the south towards the northern borders of the species distribution area.

## **Impact of maize sowing date on the level of ECB (*Ostrinia nubilalis* Hbn.) infestation, rate of plant damage and grain yield**

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Field studies were conducted at Zemun Polje in the period 1995-2005 to determine the effects of six maize hybrids, of FAO 300 to 700 maturity groups with different dynamics of phenologies and six sowing dates on plant attractiveness for female of European corn borer (ECB), *Ostrinia nubilalis* (Hbn.), belonging to the first and second generations and a subsequent degree of infestation and larval injury. Results of the same study performed at the period 1986 – 1994, were published (Baca et al 1995) females to egg laying by female.

Each of the six hybrids were sown on six different dates; April 15 and 25, May 5 and 25th and June 5 to assess the use of early- and late- sown maize as means to attract female of both generations for egg laying. Percentage of attacked plants was recorded twice each year; first by the end of July beginning of August and secondly at the end of September beginning of October. General damage rating (1 – 10 scale, where 1 = resistant, 10 = susceptible) was recorded at the second evaluation. Yield data were usually collected in October. Data on weather conditions were registered at the Meteorological station located close to the experimental fields.

Average results of eight and/or nine year evaluation of the total attack and the attack structure per generations, as well as 10 year yield data are presented in table 1.

Table 1: *Average result on ECB Ostrinia nubilalis Hbn. plant attack and grain yield over the sowing dates in Zemun Polje 1995 – 2005.*

Criteria	Sowing date						Average
	April 15	April 25	May 5	May 15	May 25	June 5	
Total attack	56.32	54.76	51.72	55.32	54.81	46.71	52.64
1st Gen. attack	42.15	36.43	28.17	17.46	11.98	8.36	23.97
2nd Gen. attack	14.17	18.33	23.55	37.86	42.83	38.34	28.55
Damage Rating	2.53	2.63	2.57	2.85	2.83	2.72	2.69
Grain yield	11.139	11.285	11.042	9.983	9.424	8.350	10.204
Yield Index	98.7	100.0	97.8	88.5	83.5	74.0	

Significant differences in the levels of ECB infestation, rate of damaged plants and grain yield occurred among sowing dates, hybrids and the years. The total attack by both ECB generations ranged between 46.71 and 56.75% of attacked plants for sixth and first sowing date, respectively. The first generation attack ranged from 8.36 to 42.15%. Females of the first generation laid much more eggs in the early sowing, while the second generation females laid more eggs on the plants in the late sowing. In average, the total attack was 52.64%, attack of the first and the second generation was 23.97% and 28.55%, respectively. Plant damage was on the average 2.69 and slightly differed over the sowing dates. A significant difference of total plant damage by ECB larvae was recorded over the years.

Yield results indicate that the most suitable sowing period for maize in the Vojvodina province is between April 15 and May 5, but the most suitable date is the beginning of the third decade of April, when the soil temperature and the humidity are optimal.

Tuesday, 7 Nov 2006, Session 7, **POSTER 048**; STEINER & GARCIA-ALONSO .

## **Agrisure™ RW: Syngenta's solution to the corn rootworm pest**

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Syngenta has produced a genetically modified corn, Agrisure™ RW (MIR604), which expresses a protein that controls corn rootworms (CRW). This protein, a modified Cry3A (mCry3A), is produced from the mcry3A gene, a synthetic gene related to a cry gene from the naturally occurring soil bacterium, *Bacillus thuringiensis* (Bt). The modified Cry3A protein has activity against corn rootworms whereas the native cry3A protein does not. The modification does not enhance activity of the protein against non-target organisms like ladybird beetles and lacewings. The selectable marker in Agrisure™ RW is the phosphomannose isomerase (PMI) protein, a novel, safe and environmentally friendly selectable marker. Syngenta has conducted numerous studies to demonstrate that Agrisure™ RW corn is not materially different in composition, safety, or any relevant parameter from corn now grown, marketed and consumed. Thorough studies have been undertaken to demonstrate that the inserted transgene is expressed as a single copy, the expression levels and CRW activity are stable across generations, no mammalian toxicity was observed even at very high protein doses and that the allergenicity potential of mCry3A has been assessed and the data indicate that the mCry3A protein is labile to heat and will be readily digested as conventional dietary protein. Agrisure™ RW significantly reduces corn root damage compared to non-transgenic controls and provides equivalent or better control when compared to commonly used chemical control actives. Syngenta's Agrisure™ RW maize's built-in protection against corn rootworms will provide safe and effective control of the corn rootworm pest that is now rapidly invading Europe.

## **Experiences of several years' of control of western corn rootworm larvae in Hungary**

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Invasive corn pest, western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae) was detected first time in Hungary in 1995. Development of economic population needed about 4-5 years depend on ecological conditions in the maize growing areas after the first occurrence of WCR.

However, all insecticide treatments offer a less effective alternative to crop rotation but may provide useful components to some pest management programs and could be used in areas where the crop rotation is uneconomic.

Investigations were performed for registration insecticides for larvae control as well.

Experiments started in 1996 in Serbia and later on they were carried out in the South part of Hungary. At the beginning the effect of insecticides for seed treatments (imidacloprid, bifenthrin) and soil insecticides (carbofuran, tefluthrin, terbufos, chlorpyrifos) were tested against the WCR larvae at those times registered in maize crop for control soil-born pests.

Efficacy of measures altered against the WCR larvae depending on the weather of the given vegetation period, the level of the infestation, sowing time, soil conditions and hybrids.

Our investigations resulted official registrations of new active ingredients for seed dressing for control of WCR larvae (clothianidin, thiametoxam) in maize. Seed dressing is suggested in locations with a moderate WCR population and first year maize in regions with economic population of the pest. One can mentioned among the advantages of seed treatment beside the effect on rootworm larvae control of soil-born and early season pests, no extra costs for application, less use of pesticide less burden of the environment.

Insecticide soil treatments were effective against WCR larvae in areas with economic population applying in furrow in planting time. Granule formulations (tefluthrin, terbufos) provided better results than liquid ones (carbofuran, carbosulfan, chlorpyrifos). Incorporation of insecticides at emergence of larvae does not intensify the effect but carries extra costs for the application. Soil treatments can prevent the economic damage, slow down the development of economic population and means a relatively low pesticide use.

We intend to provide data on results of several years' of investigations and demonstrate the effects of different factors influencing on efficacy against WCR larvae.

## Control of western corn rootworm larvae in maize using seed treatments (three years of Pioneer project)

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In 2003, 2004 and 2005 efficacy trials on large plots were carried out with seed treatment products in counties Baranya, Bács-Kiskun, Békés and Tolna (Hungary) at five locations per year. In 2003, 2004 and 2005 the treatments were Cruiser 350 FS (thiametoxam) 1.25 mg a.i./kernel, Gaucho 600 FS (imidacloprid) 1.35 mg a.i./kernel, and Poncho 600 FS (clothianidin) 1.25 mg a.i./kernel (hybrid PR 36R 10); Cruiser 350 FS 2.85 µl/kernel + Force 20 CS (tefluthrin) 2.0 µl/kernel; Gaucho 600 FS 1.35 mg a.i./kernel, and Poncho 600 FS 1.25 mg a.i./kernel (hybrid PR 36R 10); and Cruiser 350 FS 1.0 mg a.i./kernel + Force 20 CS 0.4 mg a.i./kernel and Poncho 600 FS 1.25 mg a.i./kernel (hybrid PR 36R 10), respectively. The effect of insecticides were evaluated for several parameters, e.g. plant counting, root damage (on 10 x 2 plants per plot according to Hills and Peters (1-6) scale (year 2003) and Oleson and Tollefson (0-3) scale (years 2004, 2005), plant lodging, stem breaking, crop weight, phytotoxicity, germination.

### Results and conclusions

Seed dressing products can reduce the root damage in some degree, but in case of severe infestation (e.g. according to Iowa scale root damage on untreated plot > 5, or according to the 0-3 scale root damage on untreated plot > 1.5) it is probably not enough to prevent the plant lodging and yield loss. In 2003 and 2004 the effects of the three seed treatments were very similar 3.04, 3.0, 3.05; and 0.88; 0.91; 1.08, respectively. The yield was considerably higher on the treated plots, especially in the very dry year 2003.

**Table 1:** Root damage rating in 2003-2005 (average of 5 trials per year).

<b>2003</b>	Untreated	Gaucho 600 FS	Poncho 600 FS	Cruiser 350 FS
Average (Hills and Peters scale)	4.51	3.04	3.00	3.05
<b>2004</b>	Untreated	Gaucho 600 FS	Poncho 600 FS	Cruiser 350 FS+Force 20 CS
Average (Oleson and Tollefson scale)	1.69	0.91	1.08	0.88
<b>2005</b>	Untreated	-	Poncho 600 FS	Cruiser 350 FS+Force 20 CS
Average (Oleson and Tollefson scale)	1.14	-	0.34	0.49

**Table 2:** Crop volume in 2003-2005 (kg/ha yield) (average of 5 trials per year).

<b>2003</b>	Untreated	Gaucho 600 FS	Poncho 600 FS	Cruiser 350 FS
Average	3507.8	4931.6	4797.6	5255.0
<b>2004</b>	Untreated	Gaucho 600 FS	Poncho 600 FS	Cruiser 350 FS+Force 20 CS
Average	7109	8046	7722	7884
<b>2005</b>	Untreated	-	Poncho 600 FS	Cruiser 350 FS+Force 20 CS
Average	9551.3	-	10552.3	10475.7

## **Seed dressing against the larvae of western corn rootworm, *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae)**

Béla TÓTH<sup>1</sup> & Ibolya HATALA ZSELLÉR<sup>2</sup>

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The possibility of application into the practice of insecticide seed treatments had been investigated by the plant protection network since the 1950ies without significant results. The background of the failure was composed of the biological inefficiency, phytotoxicity, untimely degradation, persistency, and destructive effect on the environment.

Application of synthetic pyrethroids as zoocide seed dressing (1985) and the appearance of active ingredients of neonicotinoids (1992) started a new chapter in control of soil-born and early season pests. Two products of the mentioned pesticide classes FORCE 5, 10 CS (tefluthrin) and GAUCHO 350 FS (imidacloprid) were tested as seed treatments in Yugoslavia luck of suitable field for tests in Hungary.

Since 2000 examinations have been started with the different dosages of the two mentioned ingredients and CRUISER 350 FS (thiametoxam), MOSPILAN 70 WP (acetamiprid), PONCHO FS 600 (clothianidin), SEMAFOR 20 ST (bifenthrin) were also tested under domestic conditions.

The obtained results showed excellent efficiency of tested products against soil-born and early season pests but they were not enough to press down the damage caused by WCR larvae under economic threshold (<3 of Iowa scale value). Effect of seed dressing treatment decreased the root damage with 1-1,5 of Iowa scale value which is slight in case of a high damage level (>4.5 of Iowa scale value) one can expect plant lodging and yield loss (15-30%).

Seed treatments can be important in maize growing (monoculture) areas where the density of the pest was in low level and the adult population settled below the risky stage in the previous year, adult control took place in the field, the stubble of the preceding crop weedy (egg-laying: next year plant lodging sporadically) and in second year maize crops.

Results of numerous trials demonstrated the increase of efficacy against the WCR larvae (decrease the root injury with 2.5 of Iowa scale value) by the combination of the synthetic pyrethroid and neonicotinoid ingredients. Application as seed dressing of FORCE 20 CS + CRUISER 350 FS showed good efficiency. The results were near equal to the effect of granules applied into furrow. Costs of the insecticide combination used as seed dressing is about 60 Euros/ha it can be less than the soil treatments, it is a simple and convenient solution for farmers, and favorable from aspect of human and environment.

## **Seed dressing against the larvae of western corn rootworm, *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae)**

Veronika TÓTH<sup>1</sup>, Béla TÓTH<sup>2</sup> & Ibolya HATALA ZSELLÉR<sup>3</sup>

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## **The nutritive value of senescing maize and *Setaria* roots for western corn rootworm development.**

Kurt J. OLMER<sup>1</sup> & Bruce E. HIBBARD<sup>2</sup>

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It has been previously demonstrated that combinations of grassy weeds (*Setaria* and *Digitaria* species) with transgenic corn produce more western corn rootworm adults when the weeds are sprayed with herbicide 5 day after larval hatch than either the transgenic corn alone or the weeds alone. However, it is unknown how long dying root tissue supports corn rootworm development. The purpose of the current study was gain insight as when larvae may be “forced” to abandon senescing tissues. In addition to a *Setaria* species, maize roots were killed by either chopping the plant below the growing point or with herbicide to simulate adult emergence studies and volunteer corn respectively. Since the overall goal was to determine when dying maize and foxtail plants are no longer nutritious, initial weight of the larvae were recorded and weight gain on each treatment recorded for both neonate insects and second instar western corn rootworm larvae after 5, 10, or 15 days of feeding. The treatments for maize were: 1) living plant control infested with 30 neonate or, 2) 10 2<sup>nd</sup> instar larvae, 3) maize infested the day the plants are cut infested with 30 neonates or, 4) 10 2<sup>nd</sup> instar larvae, 5) maize that is cut 5 days before infestation and infested with 30 neonates or, 6) 10 2<sup>nd</sup> instar larvae, 7) maize that is cut 10 days before infestation and infested with 30 neonates or, 8) 10 2<sup>nd</sup> instar larvae, 9) maize planted five days early and cut five days before infestation with 30 neonate larvae or, 10) 10 2<sup>nd</sup> instar larvae, 11) maize planted 10 days early and cut ten days before infestation with 30 neonate larvae or, 12) 10 2<sup>nd</sup> instar larvae. The treatments for foxtail were the same as that used for maize except foxtail were sprayed with glyphosate to kill the plants on the day of infestation and only 8 2<sup>nd</sup> instar were used. The foxtail trial also had a living maize control for both neonates and 2<sup>nd</sup> instar larvae (treatments 13 & 14). Finally, the maize experiment was repeated, but sprayed with glyphosate to kill the plants rather than cutting below the growing point. Each of the three experiments were repeated a minimum of twice with five replications each set up in a randomized block design. In addition to larval recovery there was an adult emergence pot for each treatment and replication. Plants used for larval recovery were planted in 3.8-liter clay pots containing 2:1 (vol: vol) mixture of autoclaved soil/peat-based growing medium (15 pots per treatment/trial). Plants used for adult emergence were planted in larger (15 L) plastic pots using the same soil/peat-based growing medium (5 pots per treatment/trial). The number of larvae and weight gain and head capsule width gain were analyzed for each treatment along with adult emergence. Early results indicate that adults were recovered from maize infested with 2<sup>nd</sup> instars on all treatments, but only from the living *Setaria*.

## **Seed dressings as a useful IPM-strategy against the larvae of the western corn rootworm (*Diabrotica v. virgifera*)**

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Seed dressing is a common technique to prevent larval feeding damage to the roots of maize by the western corn rootworm (WCR), widely used in the United States and Europe. Compared to soil granules, seed dressings exposes mainly the roots of plant to insecticides and will therefore affect non-target soil organism less than any broadcast or in furrow treatments. Furthermore the amount of active substances is reduced compared to soil granules.

In 2005 and 2006 field experiments were carried out in the region of Lombardy, North Italy, where already a moderate to high infestation of WCR is present and damage to corn was reported for some years. Emergence cages were dug into the soil near to the stem-base of the maize plants in unrotated maize fields and the emerging beetles were recorded over an emergence period from the beginning of July to the end August. In 2005 we tested two concentrations of Clothianidin (0.5mg and 1.25mg a.i./kernel), a common seed dressing used against larval damage of WCR. An untreated control was also tested.

In 2006 we extended the trial with the insecticidal granule Aztec (Tebupirimphos (Phostebupirim) & Cyfluthrin, 0,576g/m<sup>2</sup>, Bayer CropScience), a widely used soil granule used for pest control against the WCR in the United States.

In 2005 a low to moderate infestation of WCR occurred in the field tested. Both products were able to reduce the emergence of adult beetles by nearly 50%, but there were no significant differences between the two rates used. Furthermore almost no goose-necking plants were observed in the treated variants compared to about 5 % in the control, which indicates less root damage and a bigger and healthier root system in the treated variants. Regarding a low to moderate infestation with WCR seed dressing seems to be a suitable method within an IPM-strategy for controlling and reducing populations of WCR. For fields with high infestation of WCR the results of such experiments may differ. Plants with seed treatment may be well protected from larval damage first and the root system will develop better than in control plots. But on the other side plants with better root system may allow more larvae to finish their development at later stages, during which the active substance degraded and the concentration is reduced. In such situations higher rates also might show better efficacy in reducing the number of emerging WCR.

## **EU-Project DIABR-ACT: Harmonising strategies to control *Diabrotica v. virgifera***

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In recent years the Western Corn Rootworm (WCR - *Diabrotica virgifera virgifera*) invaded central Europe more rapidly than expected in the past. Its rapid spread together with the establishment of continuous populations will seriously impact the European high intensity maize production and will result in problems throughout Europe. Several research activities in the EU member states either aimed at avoiding an introduction and spread in non-infested areas or finding integrated strategies for reducing WCR populations below threshold levels in already infested areas. However, there is an urgent need for harmonising and concentrating these activities both on a scientific and administrative level to establish a community-scale research and action plan. Thus the main goal of DIABR-ACT is to establish a harmonised and sustainable control strategy for continuously established and discontinuously emerging WCR populations. On the same time DIABR-ACT aims at minimizing the impact of these measures on biodiversity and environment, to meet the European Union politics. Control strategies to be established should be adapted to the situation of each country involved and should take into account the situation of the farmers and the economic chains build upon the maize crop (i.e. biological and integrated control, plant resistance traits, the adaptation of biotechnological approaches, cultural techniques, respectively). DIABR-ACT will specifically evaluate short and long term costs/benefits of containment and eradication strategies at the micro or macroeconomic level.

The outputs of DIABR-ACT will benefit the European Union by the following main results:

- a coordinated European Research Plan: identifying the priority research areas and minimising parallel research
- a coordinated European Action Plan: harmonising and improving control and prevention build up of high Western Corn Rootworm populations in Europe;
- the project will promote increased awareness and understanding of the problems caused by this pest species encountered by plants breeders, farmers, and plant protection services.

Tuesday, 7 Nov 2006, Session 8, 15:50; BAZOK et al.

## **Optimal timing and efficacy of ecologically acceptable insecticides in the European corn borer (*Ostrinia nubilalis*, Lepidoptera: Pyralidae) control**

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*Ostrinia nubilalis* Hübn., European corn borer (ECB) is one of the most important pest in Croatia. In spite of its importance, chemical control is often carried out only in maize for seed production or in sweet corn. When pesticides are applied, the following problems could appear, how to detect optimal application time; how to choose the most acceptable and most effective insecticide and how to apply it. The use of pheromone traps is the most common and most practical method for establishing proper application time. There are 2 strains of ECB pheromone traps, E and Z with different attractiveness on various ECB populations. Ecologically acceptable insecticides suitable for ECB control (especially in sweet corn production) belong to the three main groups: microbiological insecticides (*Bacillus thuringiensis* var. *kurstaki*- B.t.k.), insect growth regulators (IGR) and naturalytes (spinosad).

In order to establish the most attractive pheromone product and optimal time for applying insecticides and to establish the efficacy of different insecticides against ECB, a three year investigation was carried out in period 2002-2004 in corn fields in central part of Croatia. Three types of pheromones produced by «Isagro» E, Z and E/Z were set up in the field at the beginning of the moth eclosion. The insecticides were sprayed 14-20 days after the maximal daily catch in 2002 and 2003. In 2004, three different trials were set up, one trial with one early treatment, one with one late treatment and one with 2 treatments. In the trials B.t.k., IGR, spinosad and classical chemical insecticides were applied. The percent of infested plants was established by visual method before and after the spraying. Ten days after the insecticides application 10-20 plants per plot were dissected and the % of infested plants, number of tunnels per plant and number of larvae in the sample was established. Intensity of attack was calculated. Based on the collected data efficacy was calculated either by using Henderson- Tilton or Abbot formula.

The significant difference in the number of moths on different types of pheromone trap was recorded in 2002 and 2003 while in 2004 the total number of caught moths was too low for accurate conclusions. Pheromone trap, type E was the most attractive in two years of investigation. Efficacy of insecticides depended on the proper application timing. Very high efficacy (between 80 and 96%) was obtained with spinosad applied in the doses of 0.2 and 0.3 l/ha. Spinosad is very suitable for IPM programs, especially for sweet corn production because of very short safe period. Efficacy between 70 and 80% was reached with application of B.t.k. in the doses of 0.75 and 1.0 kg/ha. Efficacy of classical chemical insecticides depended on the year and insecticide. OP insecticides and pyrethroids gave moderate to good results and imidacloprid didn't reach significant efficacy. IGR insecticides resulted with moderate efficacy what implicates the need for earlier application timing. The results shows that one treatment on proper (earlier) time resulted with the same efficacy as two treatments. One treatment conducted too late resulted in very poor efficacy.

## The effect of seed treatments, Poncho® and Cruiser® on corn yield in the absence of insect pest populations

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In 2004, field plots were established in first year corn at 19 locations across Kansas to evaluate the effect of seed treatments on corn yield in the absence of insect pest attack. Treatments were Poncho® 250 at 0.25 mg/seed, Cruiser® at 0.25 mg/seed and an untreated check. Plots were two rows wide 20 ft in length with four replications in randomized complete block design. Results are presented in Table 1. There were no significant differences in yield at most locations. The average yield for each treatment was Poncho, 185 bu./acre, Cruiser 185.7 bu./acre, and untreated 188.9 bu./acre.

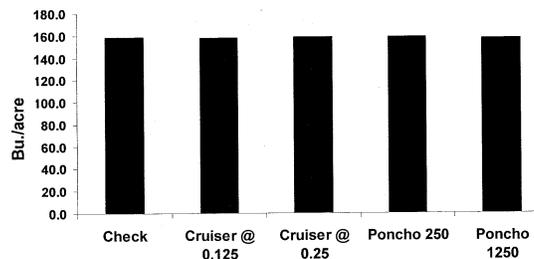
In 2005, field plots were established in first year corn at nine locations across Kansas to further evaluate the effect of seed treatments on corn yield. Plots were two rows wide and 20 ft in length replicated eight times. Yield from plots treated with two rates of Poncho® (0.25 and 1.25 mg/seed) and Cruiser® (0.125 and 0.25 mg/seed) on two hybrids (Pioneer 33R78 and DKC 6-15) were compared to the untreated check of each hybrid. Since there was no interaction between treatments and hybrids, data were pooled and are presented in Fig. 1. There was little and no significant difference in yield between treated and untreated corn when averaged across the nine locations and no significant difference within locations.

Our tests in two years suggest that seed treatments do not result in a yield increase or prevent yield loss in first year corn not affected by noticeable insect attack. These tests will be repeated in 2006 to better ascertain the effect of seed treatments on corn performance.

**Table 1:** Yield of two corn hybrids seed treatment study at 19 locations, Kansas. 2004.

	Check	Poncho	Cruiser	CY	LSD	Avg
Clay Center(I)	207.4	198.2	185.0	8.6	19.5	211.2
Severance(D)	221.8	202.8	213.8	5.8	18.5	228.5
Hays(D)	84.8	102.3	78.6	12.2	18.8	91.7
Ottawa(D)	168.2	177.6	167.5	8.6	19.3	160.9
Tribune(D)	155.7	161.9	162.1	7.7	17.3	159.8
Imman(I)	157.7	141.7	176.5	9.2	30.2	234.1
Manhattan(D)	241.8	234.0	244.7	5.6	18.8	242.5
Hutch(I)	188.3	171.4	173.0	5.8	14.8	181.9
Centralia(D)	197.7	202.7	204.3	5.7	16.0	203.0
Manhattan(D)	241.5	224.9	231.0	4.8	18.4	237.2
Hutch(I)	113.6	115.0	122.1	4.1	7.3	126.5
Belleville(D)	249.8	245.4	217.3	5.3	17.7	240.8
Scandia(I)	215.9	214.0	224.3	7.0	20.1	205.8
Topeka (D)	246.0	235.1	221.0	7.8	26.0	239.0
Topeka (I)	38.9	46.2	45.9	16.1	14.7	64.0
Colby (D)	271.2	240.2	257.1	5.8	21.8	271.6
Colby (I)	168.0	163.0	163.0	3.5	8.4	164.0
Hesston(D)	200.5	197.5	197.8	5.1	15.8	221.7
St. John(I)	243.3	243.2	243.3	7.2	25.2	249.0
Garden City(I)	188.9	185.0	185.7	7.0	18.3	196.5

I = Irrigated  
D = Dry land



**Figure 1:** Yield of two corn hybrids seed treatment study averaged across nine locations, Kansas. 2005.

Tuesday, 7 Nov 2006, Session 8, 16:30; ANDERSCH et al.

## **Poncho Pro – control of maize pests and its impact on the establishment of corn rootworms populations**

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Corn rootworms (*Diabrotica spp*) are the most serious pest in maize in the USA. During the last decades corn rootworms control was dominated by crop rotation of maize followed by soybeans and/or the application of conventional soil-applied insecticides. However, the positive effects of crop rotation with soybeans have become of limited value since the western corn rootworm (*D. virgifera virgifera*) has started laying eggs into soybean fields ('the variant') whilst the Northern corn rootworm (*D. barberi*) has adapted to the widespread corn-soybean rotation by an extended diapause.

Two new technologies for control of corn rootworms were introduced in the USA during the last two years. Beside the Bt-transgenic maize varieties, seed treatment of maize with an appropriate, 'state of the art' insecticide is becoming the alternative technology to substitute the 'old fashioned' soil insecticides - a measure safer for the user, environmental friendly, highly economic and extremely convenient for the farmer.

Clothianidin (Poncho Pro) is a new insecticide out of the chemical class of the neonicotinoids, which was successfully introduced as a seed treatment into the North American and some European markets since 2003 for control of corn rootworms and a wide range of major early season pests. The spectrum of Poncho Pro seed treatment includes wireworms, cutworms, *Tanymecus spp.*, seed corn maggots, *Oscinella frit* as well as various sucking pests like aphids and leafhoppers after systemic uptake through the roots.

Poncho Pro has performed very consistent against corn rootworms under various soil and weather conditions at moderate infestation levels. Due to its adequate physical-chemical characteristics clothianidin is desorbed easily from the seed after sowing and starts to form a halo around the growing rooting zone of the maize plants. Model-like experiments with radio-labeled C14-clothianidin demonstrated that all parts of the root system and even the tips of the youngest roots far distant from the kernels contained an amount of active ingredient sufficient to effect the *Diabrotica* larvae.

Poncho Pro reduces the potential corn rootworms root damage below the economical threshold. Additionally, clothianidin even at low dosages causes stress to corn rootworms resulting in an inhibition of the development and, consequently, remaining smaller in their size. Furthermore, uptake of sub-lethal dosages of clothianidin led to an anti-feeding behavior to corn rootworms forcing the larvae to search for an alternative food source. Our investigations have also demonstrated that the seed treatment of maize with clothianidin has drastically reduced the number of adults emerged from their larval stages. Taking this into consideration seed treatment with Poncho Pro should also be regarded as a useful integrated tool in European efforts to reduce the spread of corn rootworms across Europe and to limit its economical impact in the future.

## Experiences of trials for control of adults of western corn rootworm (*Diabrotica v. virgifera*) in Hungary

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The western corn rootworm appeared in 1995 in Hungary and became a dangerous pest in the corn belt of the south region (Counties Csongrád, Baranya, Békés, Tolna, Bács) within a few years. No registered insecticides were available against this new pest. It was evident claim to carry out official insecticide trials for registration pesticides against WCR adults.

Biological efficacy of all products was evaluated by use uniform methodology: counting adults on plants, using pheromone (CSALOMON) and yellow sticky (PHEROCON AM) traps, as well. Efficiency was calculated by the Henderson-Tilton formula.

At the beginning (2000-2001) mainly the pesticides registered against the European corn borer (active ingredients of methyl parathion, quinalphos, malathion, dichlorvos, chlorpyrifos and endosulfan) were investigated by aerial application and they provided very good efficacy and persistence (7-14 days) against the adults of WCR. Disadvantage of these pesticides was their increased danger on bees, beneficials and environment.

Since 2002 the developments have been aimed at introduction of ingredients with environment and bee save nature. The new ingredients (acetamiprid, thiacloprid, bensultap) proved excellent efficiency with long-term effect on invasive beetles in the flowering maize. At the same time a new technology shown up cucurbitacin + chlorpyrifos. This combination allowed with reduced insecticide ratio a very effective adult control.

A number of pyrethroids got registration after trials (lambda-cyhalothrin, beta-cypermethrin, esfenvalerate, alphamethrin, zeta-cypermethrin) but these products have disadvantages: short term effect and toxicity on beneficials.

From 2004 high-clearance tractors are also used for foliar applications to optimize the adult control of WCR. Promising results were obtained by using products contained active ingredients of spinosad, gamma-cyhalothrin, zeta-cypermethrin, deltamethrin, and thiacloprid.

## New possibilities for the control of *Diabrotica v. virgifera* in Hungary

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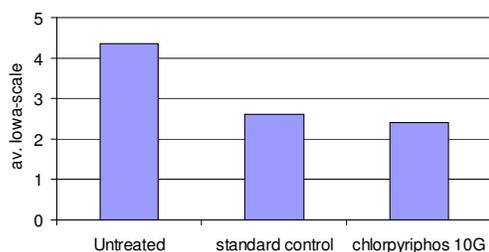
Corn is one of the biggest and most profitable crop in Hungary. Western corn rootworm (*Diabrotica v. virgifera*, Coleoptera: Chrysomelidae) became one of the most important pests in this crop after more than a decades of appearance.

Although in some of the corn-growing counties crop rotation is yet common, the results of the surveys carried out by the authorities show that the population of the adults was the highest in last year within a 9-year period.

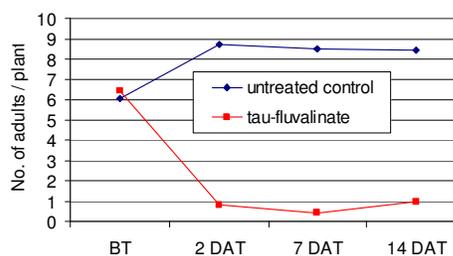
Taking these facts into consideration it is evident that we need better efficacy in controlling this pest. One of the possible solutions is if we attack the pest's life cycle at both sites: using soil treatments against larvae and field treatments against the adults.

Trials were conducted using granules of microencapsulated chlorpyrifos as soil treatment in furrow at sowing. The root damage was examined using the lowa-scale before the flowering of corn. According to the results, this treatment can effectively decrease the root damage by 55% comparing to the untreated control (Fig.1.).

The trials against the adults were carried out with tau-fluvalinate. Using the benefits of these ai. treatments could have been performed during the flowering period of corn. The spraying were done by self-propelled sprayer and the number of WCR adults per plant were counted before treatment as well as on the 2<sup>th</sup>-, 7<sup>th</sup>- and 14<sup>th</sup> day after treatment. The result shows that tau-fluvalinate had good effect on decreasing the number of the adults on the second day and kept this efficacy for the following two weeks.



**Figure 1:** Effect of chlorpyrifos granules on the damage of WCR larvae.



**Figure 2:** Effect of tau-fluvalinate on WCR adults.

## Genetic diversity in wild populations and laboratory colonies of western corn rootworm in the US.

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The western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte, (Coleoptera: Chrysomelidae) is a major insect pest of maize in North America causing over \$1-billion in losses each year in the US, and is spreading in Europe. Populations of this insect in the US have developed resistance to crop rotation and to many insecticides, and it is feared they may become resistant to transgenic ("Bt") corn as well. Designing effective strategies to prevent development of resistance to Bt corn requires an understanding of this insect's population genetic characteristics and the rate of gene flow between populations. Seven polymorphic microsatellite DNA markers were surveyed to characterize the amount and patterns of genetic variation in WCR populations across ten widely separated populations in nine U.S. states, from northwestern Texas to New York. All populations showed high levels of genetic diversity, with mean allelic diversity ranging from 7.3–8.6, and mean expected heterozygosity ranging from 0.600–0.670. Although there was sufficient variation in these markers to conduct population studies, WCR populations exhibited little genetic differentiation as a whole across the geographic range sampled, with a global  $F_{ST}$  of only 0.006. Pairwise  $F_{ST}$  estimates also revealed little genetic differentiation among populations. Most pairwise  $F_{ST}$  values were nonsignificant, except for those estimated between the Texas population and all others. There was a positive correlation between genetic distance and geographic distance as a whole, but no significant correlation for populations from Kansas to the East Coast. There was no evidence for a genetic bottleneck in any WCR population sampled. Phylogenetic and principal component analyses support the picture of high genetic similarity over much of the U.S. Although high migration rates could produce the same pattern and cannot be ruled out, it seems more likely that the WCR populations sampled have not had time to drift apart genetically since this insect began expanding its range eastward across the U.S. from the Great Plains about 50 years ago. Nevertheless, given the high genetic diversity among microsatellite loci, it should be possible to obtain good estimates of gene flow anywhere in the U.S. by examining temporal changes in allele frequencies or by employing spatial analyses on a finer geographic scale. The USDA-ARS maintains a number of laboratory colonies of WCR at the Northern Grain Insects Research Laboratory in Brookings, South Dakota. The main diapausing colony has been continuously reared since 1986 without introduction of wild genetic material. The non-diapausing colony has been continuously reared without outcrossing to wild individuals since the early 1980s after selection for the nondiapausing phenotype in 1976. Both colonies are used extensively by entomologists for field and laboratory studies. Assessment of genetic variation in this and other colonies maintained at Brookings using microsatellite markers indicates that observed heterozygosity and alleles per locus are similar to wild populations, although there has been a small loss of variability in the nondiapause line. Thus, researchers can expect a similar response to selection in these lines as would occur in natural populations.

## **Introduction routes of the western corn rootworm invading Europe**

Thomas GUILLEMAUD & Nick MILLER

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The western corn rootworm (*Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae)) is a pernicious pest of maize in North America where it causes huge economic losses. It was first detected in Europe in 1992 near Belgrade airport and has since spread through much of Central and south-eastern Europe. Several outbreaks of the beetle were subsequently detected in Western Europe, in locations disconnected from this primary outbreak (Italy, Switzerland, France, Belgium, the UK, the Netherlands). Until recently, it has been generally assumed that the Western European outbreaks were founded by insects from Central Europe. The genetic study of outbreaks of the western corn rootworm detected in Europe during the last 10 years shows this assumption to be false. In 2005, we analysed the genetic diversity of insect populations collected from five secondary European outbreaks (the two Paris outbreaks detected in 2002 and 2004, the outbreak from eastern France detected in 2003, the outbreak detected in 2000 in north-western Italy, and that from north-eastern Italy, detected in 2003). We then compared these genetic characteristics to those of beetles trapped in Central Europe and North America. We showed that among the five secondary outbreaks analysed, the only one to originate from Central Europe was the north-eastern Italian outbreak. The eastern France outbreak originated from the 2002 outbreak detected near Paris. Unexpectedly, the outbreaks detected near Paris in 2002 and in north-western Italy in 2000 were caused by separate introductions from North America. Overall, among the six European outbreaks that were analysed, at least three resulted from separate introductions from North America.

In 2006, we obtained samples from additional European outbreaks. We analysed the genetic variability at 8 microsatellite loci of samples from the UK, France, Italy and Switzerland. These analyses are ongoing and the expected results will provide a more complete view of the European invasion.

Wednesday, 8 Nov 2006, Session 9, 09:00; SIEGFRIED & ALVES

## **Genomics of the western corn rootworm midgut: identification and validation of potential target sites**

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The western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), is arguably the single most important pest of field corn in terms of crop losses and the use of synthetic insecticides. Managing corn rootworm populations to minimize risk of economic loss is becoming increasingly difficult given the insect's apparently unlimited capacity to evolve resistance and because regulatory actions are restricting or phasing out the use of certain broad-spectrum insecticides. WCR has recently become the target for insect resistant transgenic crops that express toxins from *Bacillus thuringiensis*. This technology has switched the focus for identifying insecticide targets from the insect nervous system to the midgut. Novel control methods that exploit the insect midgut as a target site have been widely recognized for their potential to suppress insect pest populations. We believe that the rootworm midgut offers unlimited opportunities for development of rootworm specific target sites that can be exploited in a manner, which confers selectivity and environmental safety.

We have recently described an Expressed Sequence Tag (EST) strategy for the identification of candidate targets in the WCR midgut and the relationship of these ESTs to known sequences from insects and other animals as well as their potential biological and molecular functions. A collection of 691 unique sequences from the western corn rootworm midgut was identified, 27% of which predict proteins with no matches in current databases. Of the remaining sequences, most predict proteins with either catalytic (62%) or binding (19%) functions, as expected for proteins expressed in the insect midgut. The utility of this approach is illustrated by the isolation of the first coleopteran cadherin gene which encodes a putative Bt receptor and also a description of the diversity of digestive enzymes expressed in the rootworm midgut which have previously been identified as potential insecticidal targets or resistance mechanisms.

In order to validate putative target sites from the rootworm midgut, we are currently developing RNA interference (RNAi) assays to determine the effect of silencing these putative target genes. While phenotypic consequences from RNAi might not precisely mimic insecticidal effects on a given target, the severity of defect by RNAi is a quickly obtainable indicator of target utility. The gene chosen for preliminary tests is the laccase 2A gene from *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) which encodes a phenol oxidase involved with tanning of the larval integument and which produces visible mutants in *T. castaneum* larvae subjected to RNAi. Injection of dsRNA specific for WCR laccase 2A into the body cavity of 2<sup>nd</sup> instars resulted in visible and persistent suppression of cuticular tanning of both the head capsule and terminal abdominal segment. This research will provide the basis for conducting large-scale identification of genes related to pathways important to midgut function and that represent potential insecticide target sites.

Wednesday, 8 Nov 2006, Session 9, 09:20; MILLER & GUILLEMAUD

**Genome scanning to search for genetic variation associated  
with the circumvention of crop rotation  
in *Diabrotica v. virgifera***

Nick MILLER & Thomas GUILLEMAUD

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Crop rotation is an important tool for controlling the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) both in the United States and Europe. It exploits the univoltine WCR life-cycle and the fidelity of females to maize as an oviposition site. First-year maize is protected from damage caused by WCR larvae because the crop present in the same field the year before was not an attractive oviposition site.

Unfortunately, crop rotation has become ineffective in a growing region of the United States Corn Belt, centred on east-central Illinois since the mid-1980s. This failure appears to be due to selection for a loss of or reduction in ovipositional fidelity to maize. Consequently eggs are often laid in rotated crops and the resulting larvae can hatch into maize fields where they can feed and cause damage. This variant behaviour is presently not well understood at the level of individuals. To date, no phenotypic or genetic markers have been identified that permit individual WCR to be identified as "variant" or "wild-type".

We present here preliminary results of a genome scanning study aimed at detecting genetic variation linked to the rotation-circumventing phenomenon. We collected three replicate population samples of variant WCR from first year maize (following soybeans) in Illinois and three wild-type samples from continuous maize in Iowa. Approximately 50 pupae were collected for each sample. Collecting pupae ensured that individuals had not dispersed since the egg stage and thus their field of origin was known. The Amplified Fragment Length Polymorphism (AFLP) technique was used to genotype the sampled individuals for a large number of polymorphic genetic markers. Although the majority of the AFLP markers were expected to be selectively neutral, one or more could be fortuitously linked to genes involved in the variant phenotype. We therefore sought to identify markers exhibiting abnormally high levels of differentiation when comparisons were made between variant and wild-type samples.

Wednesday, 8 Nov 2006, Session 9, 09:40; GARABAGI et al.

**Exploring the role of a cGMP-Dependent protein kinase homologous to the *Drosophila* foraging gene in rotation resistant western corn rootworm (*Diabrotica v. virgifera*)**

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Altered egg-laying behavior by variants of western corn rootworm (WCR) into non-cornfields has allowed these insects to circumvent crop rotation. No morphological or genetic characteristics have been defined to differentiate between the normal and variant biotypes. The inability to easily identify the variant WCR makes monitoring the incidence and spread of the variant form difficult and complicates pest-control strategies. Cyclic GMP-dependent protein kinases (PKG) have been implicated in the regulation of behaviors in the vertebrates, insects, and nematodes, including foraging behavior in *Drosophila* and food search behavior in honeybees. We have cloned a cDNA homolog of the *Drosophila* foraging gene from WCR (namely Dvfor1). The deduced DvFOR1 protein is approximately 70% similar to FOR proteins in *Drosophila*, silkworm and honeybee. It contains a coiled coil region of amino acid residues and two tandem cyclic nucleotide-binding domains at the N-terminus of the protein, a serine/threonine kinase catalytic domain in the C-terminus, and a serine/threonine kinase catalytic domain extension. All of these motifs are characteristically found in PKG proteins. Real-time PCR assays of foraging transcript levels in heads of normal and rotation adapted females of WCR obtained from lab-reared insect colonies indicated that the variants had higher levels (25%) of gene expression than normals. The magnitude of this increase is similar to that observed in *Drosophila* rover phenotypes compared to sitter phenotypes. However, *Diabrotica* contains at least two different foraging gene transcripts, which complicates establishing a direct link between the level of gene expression and insect behavior.

## Effects of *Trichogramma* on non-target species: fact or fantasy?

Dirk BABENDREIER & Franz BIGLER

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Egg parasitoids of the genus *Trichogramma* have been demonstrating their potential to control stem borers in maize for more than 30 years. For instance, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) is being released annually for control of the European corn borer in European countries with still increasing numbers especially in France. With increasing concerns for non-target effects of biological control agents in general, these releases have been questioned for their potential to cause detrimental effects on populations of non-target species such as butterflies. It is argued that generally polyphagous biological control agents are the most hazardous ones and most trichogrammatids in fact have a broad host range.

In this presentation, I will summarize data generated in a project to assess the risks of *T. brassicae* releases retrospectively. The first step in a risk assessment for exotic species used in inundative biological control is to study overwintering abilities and we found that *T. brassicae* has the potential to establish in Central Europe. Dispersal from the target habitat and into sensitive non-target habitat may also be important and we showed that wasps moved only several m per day and survived for about 2 days under warm field conditions. We furthermore evaluated to what degree non-target lepidopteran species are parasitized. High parasitism rates of *T. brassicae* on eggs of many lepidopteran species (including endangered species) were observed under laboratory conditions while low parasitism rates were found in experiments carried out under semi-field or field conditions. This observation was partly explained by a low searching efficiency of *T. brassicae* in several non-target habitats. Especially in meadows egg parasitism decreased by more than an order of magnitude as compared to maize, the target habitat.

In order to investigate potential factors underlying the low searching efficiency in non-target habitats, the behaviour of individual *T. brassicae* females was observed on common meadow plants in a third step. Significant differences in mean walking speed, turning angles and number of wasps leaving the plant were found between maize and four meadow plants. In addition, other factors such as the structural complexity of the plant and the whole habitat may also play a role in determining searching efficiency of these small parasitoid wasps. Altogether, we conclude that due to low dispersal rates and low parasitism rates outside the maize field, mass released *Trichogramma* have a very limited potential to cause non-target effects and thus constitute an environmental benign pest control strategy.

Wednesday, 8 Nov 2006, Session 10, 10:55; NORONHA & DAU-SCHMIDT

## **Biological control of *Ostrinia nubilalis* in potatoes: does plant architecture influence efficacy**

Christine NORONHA & Kathryn DAU-SCHMIDT

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The European corn borer *Ostrinia nubilalis* is now an established pest in potatoes in Atlantic Canada. Since the 1980's, damage to potato stalks by this pest has been steadily increasing and spreading on Prince Edward Island. In 2004, the majority of the potato fields on the island were infested, with levels ranging from 10-100 percent. Although insecticides are effective and are the main method used to control this pest, precise timing of application is essential. Inclement weather can often delay the application of an insecticide which could result in crop damage and maybe yield loss.

We studied the efficacy of a commercially available species *Trichogramma brassicae* to reduce the damage to potatoes by *O. nubilalis*. *T. brassicae*, has been recorded as a biocontrol agent of *O. nubilalis*. In field trials *T. brassicae* adults were released into potato fields during peak oviposition by *Ostrinia*. Results indicate that *T. brassicae* was efficient in reducing the number of larvae per stem and number of holes per stem when compared to the control. However, in a field cage trial with sentinel eggs, we found that *T. brassicae* was unable to find the majority of the egg masses on the plant. Does the potato plant structure influence searching ability of *T. brassicae* to find *O. nubilalis* eggs?

*Trichogramma* wasps have been used effectively in reducing *O. nubilalis* damage in corn. However, the architecture of a corn plant is much simpler than that of a potato plant. In addition to being more complex architecturally, the potato plant also possesses varying degrees of pubescence on the stems and leaves where *Ostrinia* females lay their eggs. Several studies have shown that plant structure influences the host finding capacity in parasitoids, and host finding by *Trichogramma* parasitoids has been found to decrease with increasing complexity of the plant. Studies are ongoing to determine the influence of the potato plant structure and pubescence on the host finding capability of this parasitoid, results will be discussed.

## Case study: augmentative release of *Trichogramma ostrinae* for control of Asian corn borer in DPR Korea

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Maize, *Zea mays* (L.) is a major cereal crop in the DPR Korea. Yield losses are serious due to the damage of Asian corn borer, *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae), as infestation rates can reach up to 100%. Field studies were conducted in 2005 and 2006 at four Cooperative-Farms (at each farm 3 or four 0.25 ha release field plots) to evaluate the potential use of augmentative releases of *Trichogramma* wasps for Asian corn borer management. At each Co-Farm, three releases of *Trichogramma ostrinae* Pang et Chen (Hymenoptera: Trichogrammatidae) were conducted at a 4-day interval during the first generation of Asian corn borer, and two releases during the second generation. Based on laboratory and field *Trichogramma* quality control data, the targeted field release rates were on average 150,000 *T. ostrinae* wasps per hectare for each release, and 37 to 42 egg-cards were placed in each field plot. Efficacy of *T. ostrinae* releases was evaluated through the number of Asian corn borer egg masses laid in field plots, egg parasitism by *T. ostrinae* released, number of host larvae, larval tunnel lengths, proportion of damaged ears and yield between release and non-release field plots. No significant difference was found in the number of egg masses laid by Asian corn borer during the first and second generations between release and non-release plots. Egg parasitism by *T. ostrinae* released during the first generation were significantly higher in release plots compared to non-release plots, and up to 90% parasitism was determined in a release field plot. However, no significant difference of egg parasitism during the second generation was found between release and non-release plots, which is probably due to the restricted number of replicates, heavy rain during the releases and catch-up of natural parasitism. In comparison to non-release field plots, the number of Asian corn borer larvae in release plots was significantly reduced reaching an average of 63.6% in the first and 64.4% in the second generation. In addition, larval tunnel lengths and ear damage rate decreased on an average of 66.2% and 53.5% in the first and second generation, respectively. Maize yield was significantly increased by 22.9% (fresh yield) and 30.9% (dry yield) in *T. ostrinae* release plots compared to non-release plots. Accordingly maize dry yield could be potentially increased by a mean of 1986 Kg per hectare if biological control would be applied. Therefore, it is concluded that *T. ostrinae* augmentation is a promising management tool in DPR Korean maize production, which will increase maize production and thereby enhance sustainable agriculture and food security in DPR Korea.

Wednesday, 8 Nov 2006, Session 10, 11:35; HOFFMANN et al.

## Biological control of *Ostrinia nubilalis* with *Trichogramma* in North America

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In North America, the use of genetically engineered (Bt) varieties is relatively common for management of *Ostrinia nubilalis* in field (grain) corn, whereas in sweet corn for human consumption, insecticides are still widely used. Because of regulatory changes affecting pesticide use and because of an expanding organic market, non-pesticide options for control of *O. nubilalis* are sought. Despite the apparent need, the use of *Trichogramma* for biological control of *O. nubilalis* is very limited. Research over the past decade has shown that one species, *Trichogramma ostrinae*, is an effective biological control agent of *O. nubilalis*, in corn and other crops. In sweet corn, early season inoculative releases of 75,000 *T. ostrinae*/ha generally result in up to 75 percent parasitism of egg masses and 50 percent reduction in damage. It also shows considerable promise in sweet peppers and potatoes. *T. ostrinae* is effective because it disperses rapidly and persists through the season even if fields are treated with insecticides. In addition, *T. ostrinae* successfully parasitize eggs of *O. nubilalis* throughout most of their embryonic development, parasitize almost all eggs in an egg mass, are easily reared and do not lose fitness when reared on factitious hosts. Taken together, these and other factors help explain the success of *T. ostrinae*. Concerns related to the release of a generalist biological control agent are sometimes raised but *T. ostrinae*'s potential for non-target effects appear small, despite a broad physiological host range. Permanent establishment of *T. ostrinae* also appears to be constrained by a lack of suitable overwintering hosts, particularly in northern climates. Despite the potential for *T. ostrinae* as a biological control agent it is not yet produced commercially due in part to a lack of an adequate market. More marketing, demonstrations of efficacy against additional pests and economic assessments are needed to assure commercial availability of *T. ostrinae* or other potentially effective species of *Trichogramma*.

## **A mass production system of *Trichogramma ostrinae* Pang et Chen (Hymenoptera: Trichogrammatidae) on *Sitotroga cerealella* (Olivier) eggs**

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The Asian corn borer, *Ostrinia furnacalis*, is one of the most important insect pests in China. *Trichogramma ostrinae* Pang et Chen is the dominant natural parasitoids on the Asian corn borer in China. In order to controlling the 2<sup>nd</sup> and 3<sup>rd</sup> generation of the Asian corn borer which seriously damages the summer corn in Hebei province, wasps of *T. ostrinae* were mass produced. A mass production system was developed and established in Hengshui, Hebei. *Sitotroga cerealella* (Olivier) eggs were used as host. An efficient *Sitotroga* production line was developed, and mass-production of *Sitotroga* eggs was realized. An effective *T. ostrinae* annual rearing system was designed and put into practice. For preventing the degeneration of *Trichogramma* due to continuously developing inside the same host eggs, the rearing system were developed by utilizing the diapause behavior of *T. ostrinae*. In this system, natural *T. ostrinae* was collected annually from corn fields in September as original stock wasps. They were reared on *Sitotroga* eggs for 3-4 generations at 25 ± 0.5, 75 ± 5% RH in incubator before diapause introduction. In mid-November, those stock wasps were introduced into diapause and were stored at 2-5. After 2-5 months storage, they were transferred into the incubator at 25 ± 0.5, 14L:10D for terminating diapause before enlarging the stock population. For stock rearing in spring and summer time, the parasitization of *Trichogramma* wasps on *Sitotroga* eggs was at 25 ± 0.5, 75 ± 5% RH in the incubator and the development of *Trichogramma* was in fluctuated temperature in thermometer screen outdoor. For mass production, *Trichogramma* were mass reared in the rearing room at 25-27, 75 ± 5% RH. *T. ostrinae* were developed on *Sitotroga* eggs for less than 12 generations before released. Approximately 200 million wasps were produced by using this system. Field releases were conducted during the oviposition period of the 2<sup>nd</sup> and 3<sup>rd</sup> generation Asian corn borer. Efficacy of *T. ostrinae* releases was evaluated. Asian corn borer larvae, larva tunnels and damaged ears were assessed in wasp release and non-release fields separately. Results showed that the number of larva, larva tunnel and damaged ear was reduced by 78.3%, 82.5% and 94.54% respectively.



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