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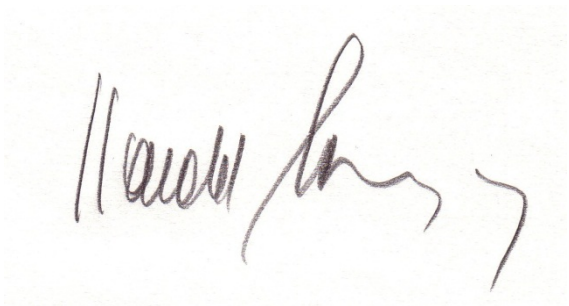
IWGO – NEWSLETTER 31 / 1

Editorial

This issue of the IWGO-NEWSLETTER contains the abstracts of the talks from the 24th IWGO meeting in Freiburg/Breisgau, Germany in October 2011. A second issue, will be published in the spring of 2012 and will contain the abstracts from the poster session in Freiburg and the list of participants. The meeting in Freiburg was attended by more than 115 participants from of 17 countries. Furthermore, this issue contains a portrait article of our newly appointed Co-Convenor Tom Sappington, an analysis of questionnaire regarding pheromone traps for the Z-pheromone strain of European corn borer prepared by Gabor Szöcs and Dirk Babendreier, and article about the development of genomics tools for the western corn rootworm – progress and promise compiled by Tom Sappington.

Unfortunately, the IWGO-NEWSLETTER could not be published earlier. The last issue was published in July 2010. There were no written contributions submitted to the editor or the IWGO convenor between July 2010 and now. We are able to publish any paper or "short information" piece dealing with pests and diseases in maize at almost any time so keep this in mind for future publication possibilities. Also, personal information, such as retirement, change of job or others is welcomed. So please don't hesitate to send any scientific paper, information or personal news to the editor at any time.

During the IWGO business meeting in Freiburg it was decided to hold the next (25th) IWGO conference in the USA (most likely in Chicago in April 2014). As IWGO is a global IOBC working group, it is definitely necessary to hold meetings globally and not just in Europe. We hope that the next meeting will also be attended by a large number of participants as in Freiburg.



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IWGO Proceedings in a special issue in the Journal of Applied Entomology

Ulli Kuhlmann

It was a great pleasure to meet you all in Freiburg, Germany, and I would like to thank you all, on behalf of the IWGO Convenors, for making the 24th IWGO Conference a great success through your participation.

Today I would like to follow up with regard to our discussions during the IWGO business meeting that the Editor-in-Chief of the Journal of Applied Entomology, Prof. Stefan Vidal, and the IWGO Convenors would like to encourage you to consider publishing your IWGO Conference paper in a special issue of the Journal of Applied Entomology.

In the past, IWGO published its own proceedings in which oral and poster presentations were included. However for the third time we will have the opportunity to publish selected manuscripts presented at the IWGO Conference in a special international journal issue. This journal publishes original articles on current research in all fields of entomology.

The language of the journal is English and all manuscripts will be evaluated in a peer-review process. This special issue will have up to about 14 to 20 papers that will be selected after the peer-review has been completed.

The journal offers an online submission system for authors and reviewers. http://www.wiley.com/bw/journal.asp?ref=0931-2048

Please submit your manuscript latest until Monday 30 January 2012. In addition, please ensure to note on the first page of the manuscript that this is a contribution from the IWGO Conference. The special issue of the IWGO Conference will be published at the end of 2012 if we all keep the deadline!

We would like for you to note that the submission of manuscripts, based on research results presented at the conference, to the Journal of Applied Entomology is optional and not obligatory however, this is an excellent way to get your up-to-date research published in a timely manner.

We would appreciate if you would use this opportunity to make the IWGO special issue of the Journal of Applied Entomology a success!

Please write me a short note if you would be willing to submit your paper as a manuscript (E-mail: u.kuhlmann@cabi.org).

Many thanks for consideration and contributions!

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Portrait of Dr. Thomas W. Sappington, the new IWGO Co-Convenor

I am a Research Entomologist in the Corn Insects and Crop Genetics Research Unit (CICGRU) of the USDA Agricultural Research Service, Ames, Iowa. My ongoing research projects encompass the ecology, behavior, management, and population genetics of European corn borer, *Ostrinia nubilalis*; western corn rootworm, *Diabrotica virgifera virgifera*; and western bean cutworm, *Striacosta albicosta*. Before transferring to Iowa in 2003, I served as Lead Scientist for four years in the Integrated Farming and Natural Resources Research Unit in Weslaco, Texas, where I conducted research on pests of cotton, especially boll weevil, *Anthonomus grandis*.

My formal training is as an insect ecologist, and my research interests are quite broad. For the most part, my current research is focused in the area of insect movement as it impinges on agriculture and in the context of insect resistance management. Dispersal of adult insects is inherently difficult to study, because it occurs over a wide range of spatial scales and emerges from a complex array of underlying behaviors that involve movement. Thus, no single technique can provide an adequate picture or explanation of a species' dispersal patterns. I routinely conduct mark-recapture, trapping, flight mill, and population genetics experiments, all techniques with strengths and weaknesses for elucidating patterns of movement at various spatial scales and in various ecological contexts.

In 2004, I co-organized, and continue to co-lead (with Thomas Guillemaud and Blair Siegfried) the international *Diabrotica* Genetics Consortium, a multidisciplinary group of 40+ scientists from 18 institutions in the U.S., Canada, France, Germany, Switzerland, Hungary, and Australia. Participants conduct research on any aspect of western, Mexican, or northern corn rootworm genetics or genomics, or on projects impacted by genetics. The goal of the Consortium is to accelerate progress in understanding this worsening pest complex through coordination of our genetics-related research activities and sharing of resources and information. I am currently involved in the international genome sequencing and transcriptome sequencing projects for the western corn rootworm. These are large multi-institution projects led and facilitated by a number of Consortium members. The last two International Conferences on *Diabrotica* Genetics have been integrated into the IWGO Conference, and I hope this synergistic relationship between our two groups will continue to flourish.

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Article

Analysis of questionnaire regarding pheromone traps for the Z-pheromone strain of European corn borer

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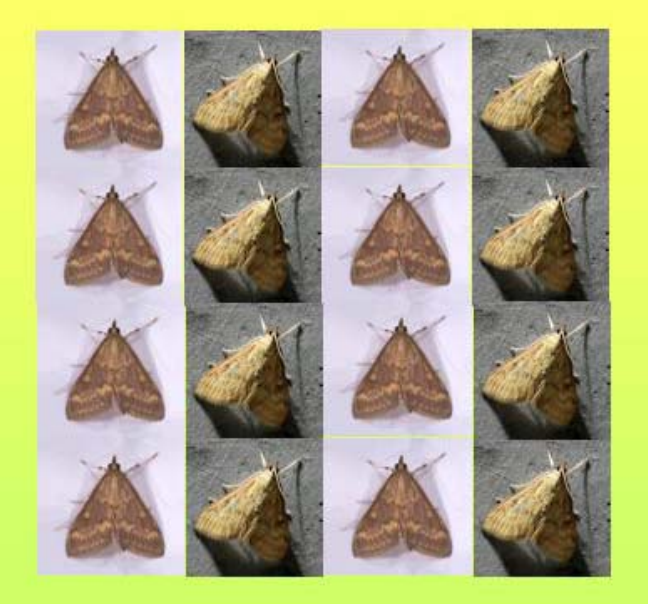
In the 24th IWGO Conference, a talk entitled „*The Ostrinia puzzle*“, given by Gábor Szőcs (PPI HAS, Budapest, Hungary) in Session 9 (Reproductive biology of maize pests and the implications on pest management) reported that pheromone traps for the Z-pheromone strain of the European corn borer (ECB), *Ostrinia nubilalis* (Lepidoptera: Pyraustidae), have long time been catching males only in insignificant numbers. Therefore they appear not to be reliable for monitoring population levels. In that talk numerous data, obtained in various regions of Hungary, were presented where pheromone trap captures (based on various experimental baits and traps prepared in his lab exclusively for the studies) were compared to those of light trap captures, and surveying adults either visually or by net-collecting. Literature references, published by others, were also cited reporting on similar cases obtained at field sites located in various countries in Central (temperate) Europe. The problem may differ sharply between regions as populations of ECB are known tovary with respect to pheromone strains, voltinism, and host plant preference. Given the importance of monitoring the seasonal flight pattern of ECB in regard of effectively timing control measures (e.g. releasing biological control agents such as egg-parasitoids), IWGO felt it is important to assess which geographical regions are affected by the above problem. Therefore, IWGO developed the following questionnaire to get a better understanding on where there is a problem in monitoring ECB with pheromones, and how severe it is for your research or the control of ECB in your region.



The *Ostrinia* puzzle: (photo: Gábor Szöcs)

From this questionnaire, we received 23 responses plus several more comments from IWGO members and the authors like to thank all participants once again. Responses were covering mostly central and Eastern or southeastern Europe (Germany: 5; Netherlands: 2; Austria: 2; Switzerland: 2; Croatia: 2; Slovenia: 1; Poland: 1; Romania: 1; Czech Repuplic: 1; Belarus: 1; Greece: 1; while one response also came from Spain and the UK respectively and two from the US. We also appreciated that apparently there was quite some ‚within country consultation' ongoing before submitting the responses.

1.) Is the European corn borer (ECB), *Ostrinia nubilalis,* an important pest in your region?

ECB apperently is a problem for most European contries except for the Netherlands and UK (where it was reported 2010 for the first time). In Germany, it can be shown that ECB is a major problem in southern parts while not in northern parts. ECB also is a problem in the NE of the United States.

2.) Do you regularly monitor ECB populations in your region?

Apparently, in most countries and regions from which responses were obtained, ECB is regularly monitored, except for Greece, Romania and the Czech Republic.

3.) If yes, by what kind of method(s) do you monitor ECB? Please, specify:

Often, more than one method is used at the same time for monitoring of ECB. In the responses to the questionnaire, light traps were mentioned 8 times, pheromone traps also 8 times, collection of egg masses 5 times while sweep-netting was mentioned once and also the use of *Heliothis* net traps was mentioned once (in the US).

4.) Do you know which ECB pheromone-strain(s) occurs in your region?

In most countries, either the Z-strain or both strains are occuring. Only in parts of Croatia, the E strain seems to be dominant and also the E/Z hybrid strain was observed. No information for this question was available from Czech Republic, Slovenia, Poland, Belarus as well as from those countries where ECB is not a problem (NL, UK).

5.) Do you know how many flights per season ECB has in your region?

Not astonishingly, more ECB generations can be observed in southern Europe compared to northern parts. For instance, 2 generations can regularly be observed in Romania, Croatia or Austria (east) and even three in Greece. However, it is apparent that a shift is occuring in certain areas in Southern Germany, Switzerland and in southern parts of Poland from only one to two generations.

6.) If you use ECB <u>pheromone</u> traps, what trap-body type do you use?

According to the responses, nobody is using sticky sheet type traps while other trap types are used in equal proportions (5 mentioned sticky delta traps, 5 mentioned funnel traps and 6 mentioned tent traps).

7.) If you use ECB <u>pheromone</u> traps, are you satisfied with the reliability of ECB pheromone traps for monitoring?

Most respondents indicated that they were not satisfied with pheromone trap catches. Actually, only three times the response was positive, i.e. one for Switzerland, one for northern Germany (the latter indicating that host density was anyway very low) and one from Spain. In contrast, 10 respondents mentioned that they were unsatisfied with trap catches from pheromone traps, including some respondents who indicated that they do not regularly conduct monitoring. This already points to the fact that pheromone trapping might have discontinued because of bad results.

8.) If you use ECB pheromone traps and are not satisfied with the reliability of ECB pheromone traps, then why are you not satisfied? Please, specify:

In all cases where respondents mentioned that they were not satisfied with pheromone trap catches, this occurred because of very low number caught. Often this coincided with high numbers found in light traps or a big number of egg masses directly collected from the plants. However, negative statements were not only referring to very low trap catches but also to low power for precise forecasting of ECB. This refers not only to the correct timing but also to wrong numbers that even might indicate the need for a treatment when in fact egg counts of ECB did not indicate such a need.

9.) If you use ECB pheromone traps and are not satisfied with the results, how big do you rate the problem for your research and the control of ECB in your region on a scale from 1 (minor problem) to 5 (severe problem)?

Although it might not be statistically sound, we were able to calculate a mean value of 3.2 from 9 respondends that provided a score. It appeared that biggest problems with poor trap catches of pheromone traps occur in Eastern Europe such as Belarus, Poland and Croatia.

10.) Comments / suggestions (detailed reasonings are highly appreciated)

From a practical point of view, the inefficiency of the pheromone traps does not cause huge problems since light trapping works well. However, light traps need electricity which may be difficult to manage in some regions and light traps raise environmental concerns as thousands of other insects are usually obtained in these traps, including red list species. Furthermore, Dichlorphos is necessary to run the light traps but is not easy to get anymore (besides being highly toxic) and finally there may be closely related pyralids in the light traps which are difficult to distinguish from ECB.

It was also mentioned that the source of the pheromone might be an important factor that should be followed up.

Preliminary Conclusions:

As preliminary conclusion, it is apparent that the problem of pheromone traps not being effective anymore is more pronounced in eastern parts of Europe as compared to western parts and the US. Altogether it seems that light traps – together with sampling of egg masses – is the standard procedure to monitor ECB and has replaced the pheromone traps, particularly in eastern European countries. Due to

above mentioned difficulties, however, more research and particularly coordinated research would be important to solve the *Ostrinia* puzzle and to make ECB monitoring and control easier and reliable based on effective pheromone traps. It is planned to follow up on this issue in subsequent IWGO newsletters.

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Article

Developing Genomics Tools for the Western Corn Rootworm – Progress and Promise

Thomas W. Sappington

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The *Diabrotica* Genetics Consortium was organized in 2003 as a way to enhance and encourage communication among scientists in North America and Europe conducting research on all aspects of rootworm genetics (Sappington et al. 2006). The initial impetus for organizing ourselves was the mutual discovery that five laboratories in the US and France were simultaneously engaged in, or about to engage in, development of microsatellite DNA markers for population genetics studies of the western corn rootworm, *Diabrotica virgifera virgifera*. Development of microsatellites is a costly and rather time-consuming endeavor. We agreed that we could all benefit from more efficient use of our resources by coordinating our efforts and freely sharing information and markers, thus reducing needless duplication of effort and unproductive competition. We further agreed to open membership in the Consortium to anyone working in the field of genetics of any *Diabrotica* species, as well as to anyone whose research was impacted by those fields. The response and interest were overwhelming. The Consortium has since grown to include more than 40 laboratories in seven countries in North America, Europe, and Australia. It also has expanded far beyond the relatively narrow field of population genetics.

Early on, the idea of eventually obtaining a genome sequencing project through leveraging the expertise and collective influence of the Consortium membership was discussed as something of a dream for the distant future. But with rapid advances in sequencing technology, a precipitous drop in the cost of sequencing, and concerted efforts within the Consortium to generate genomics tools and resources, the day of sequencing the genome is now upon us (Miller et al. 2010). In November 2008, members of the *Diabrotica* Genetics Consortium and other interested scientists convened a Western Corn Rootworm Genome Sequencing Workshop at the Annual Meeting of the Entomological Society of America in Reno, Nevada. The purpose of the Workshop was to review the genomic assets already available to the rootworm scientific community, identify which resources were still needed, and generate a plan to coordinate the development of the needed resources. The idea was to position ourselves as an international research community to submit one or more viable grant proposals within the following two years to fund a genome sequencing project for *D. v. virgifera*. Nick Miller (now an Assistant Professor in the Department of Entomology, University of Nebraska) summarized the workshop and strides forward in a talk at the Second International Conference on *Diabrotica* Genetics, held in conjunction with the 23rd IWGO Conference in Munich in April 2009, which was published in more detail

(Miller et al. 2010) as an article in the special IWGO issue of the *Journal of Applied Entomology* that came out in June 2010 (134:420-428). Considerable progress was made in the year after the Conference, and two large grant proposals were submitted in spring and summer 2010. One contained sequencing the genome as an objective (Hugh Robertson, PI), and the other involved sequencing the transcriptome (Thomas Guillemaud, PI). Both proposals were funded, and Nick Miller and Thomas Guillemaud presented goals and updates of the two projects at the Third International Conference of *Diabrotica* Genetics held in conjunction with the recent 24th IWGO Conference in Freiburg. In the remainder of this article, I will briefly summarize some of the background preparatory work that helped make these landmark projects possible from a technical standpoint, and where we are now.

Among the assets already available at the time of the 2008 workshop in Reno was the *Diabrotica* Genetics Consortium itself, which represents an organized international community of interested scientists. This is important not only for facilitating a coordinated effort to generate genomics tools and resources, but just as importantly for demonstrating a significant number of laboratories whose future research will utilize and benefit from the sequenced transcriptome and genome. We also have available a bacterial artificial chromosome (BAC) library, constructed by Blair Siegfried at the University of Nebraska, which consists of 110,592 clones containing long inserts of *D. v. virgifera* DNA. Two expressed sequence tag (EST) libraries have been constructed, one from the midgut (Siegfried et al. 2005) and one from the head (Knolhoff et al. 2010). ESTs are generated from genes transcribed during a particular stage, in a particular tissue, and under prevailing environmental conditions. We also had an estimate of *D. v. virgifera* genome size made by Spencer Johnston at Texas A&M University of 2.5 Gbp, but there was some uncertainty as to precision of the estimate. This was because no one expected the genome to be so large, and the estimate fell outside the largest size standard. Brad Coates (USDA-ARS, Ames, Iowa) has now confirmed the astonishingly large size of the *D. v. virgifera* genome as 2.58 Gbp (manuscript submitted).

Among the needed resources identified at the workshop was development of an inbred line of *D. v. virgifera*. Such an inbred strain is necessary to reduce the amount of variation in the genome due to alleles at a given locus. In model organisms like *Drosophila*, inbred lines are commonplace, but it was not an easy proposition to develop one in *D. v. virgifera* because of the difficulties inherent in rearing this insect. Wade French of USDA-ARS took on the challenge at the North Central Agricultural Research Laboratory rootworm rearing facility in Brookings, South Dakota. His team started several lines of *D. v. virgifera* from single-pair matings drawn from the partially inbred non-diapause colony, then nursed a number of families through seven generations of single-pair sibling matings. He now has an inbred line that is maintained by mass matings within the line. This is a major achievement that is critical to the success of sequencing and reassembling the genome, and these lines will be valuable for many other future applications as well. Both the transcriptome and genome sequencing projects are utilizing this inbred colony.

The BAC library is being used to provide information on the structure of the *D. v. virgifera* genome. Because of its large size, we anticipate the genome contains much repetitive DNA, which can complicate reassembly of the genome sequence. DNA preparations representing the entire library, organized into "pools" and "superpools," were screened for genes of interest by PCR. Each superpool contains DNA from 4,608 clones corresponding to twelve 384-well plates of library clones, giving a total of 24 superpools for the entire library. For each superpool, there is an associated pool plate containing 12 pools of DNA from each 384-well plate, 16 pools from each row of all the plates, 24 pools from each column of all the plates and 24 diagonal pools. This kind of arrangement allows efficient screening for particular genes of interest so that a BAC clone can be identified and sequenced in its entirety. Twenty-eight clones have been identified and selected by University of Nebraska and USDA-ARS (Iowa) scientists for sequencing because screening revealed they contain genes coding for important proteins such as receptors and enzymes that are often involved in insecticide resistance. DNA from these clones has been extracted, and is now being purified and prepared for complete sequencing. Seventy additional clones will be selected based on their relative position in the genome according to the recently completed linkage map.

A linkage map will be critical in assembling the genome sequence, as well as in identifying genes that are responsible for, or are associated with, phenotypic traits of interest. In a large ongoing project involving collaborators from University of Illinois, University of Nebraska, and USDA-ARS in Iowa, next-generation genomic sequencing technology (Illumina GoldenGate assay) was used to identify and verify large numbers of single nucleotide polymorphisms (SNPs) as molecular genetic markers from *D. v. virgifera*. Of 2,222 candidate SNPs that were determined to be potentially useful, 1,536 were chosen for further testing at the University of Illinois W. M. Keck Center for Comparative and Functional Genomics. Verified SNPs were integrated by Brad Coates (USDA-ARS, Iowa) into 10 consensus linkage groups of about 35 markers per group. The BAC library is being screened further with the validated SNPs, and 72 clones will be strategically selected for full sequencing in addition to the 28 already targeted as mentioned above. Many of the SNPs are already being used in population genetics studies as well.

A mechanism for sharing data through an integrated database of genetic maps, nucleic acid sequences, and related data will be a powerful catalyst to accelerate research progress on *D. v. virgifera*. The bioinformatics Co-PIs on the various grants are actively discussing the best way to integrate our efforts to make the oncoming flood of genomics data most useful to the research community. The genetic maps and associated molecular and other data generated by large-scale sequencing projects are generally similar in both content and scope to those in other genetic/genomic databases. The two leading possibilities being considered at this time as models for housing *D. v. virgifera* data are AphidBase, maintained by Denis Tagu and Fabrice Legai of INRA, Rennes, France, and SoyBase, the home of soybean genomics data developed and maintained by David Grant of USDA-ARS in Iowa.

One of our biggest needs has been to generate more EST sequence data, which are very valuable for assembling and annotating the genome sequence. The large international project being led by Thomas Guillemaud of INRA (Sophia-Antipolis, France) to generate a reference transcriptome of *D. v. virgifera* fulfills this need. The data generated will be based on 454 Titanium pyrosequencing of pooled libraries from whole bodies of all developmental stages, under both optimal rearing conditions and a variety of stressful environmental conditions (e.g., heat shock, sublethal doses of insecticide, entomopathogenic nematode challenge, etc.). The reference transcriptome will also be of great value in its own right as a resource for gene discovery, gene expression, and characterization of sequence variation.

All of these resources and the organized research Consortium make *D. v. virgifera* the most logical translational genomics model for species of underground insect pests of grasses proposed as biofuel crops, such as *Miscanthus* and switchgrass. Translational genomics is a field wherein genome sequence information in a model species is leveraged to solve practical problems in related, but genetically uncharacterized, species. Corn already serves as the translational genomics model of biofuel grasses. Protection of biofuel crops is a new priority of the USDA Agriculture and Food Research Initiative grant program. It is difficult to obtain funding solely to sequence an insect genome, but we were able to make the case in a multi-institution proposal led by Hugh Robertson at the University of Illinois that sequencing the *D. v. virgifera* genome will allow identification of genes involved in successful attack of roots by this species. With the decreasing cost of sequencing, we were able to embed an objective for genome sequencing within a larger project that will exploit that information to discover genes involved in colonization of roots by coleopteran larvae. The western corn rootworm will likely be a direct pest of *Miscanthus* (Spencer and Raghu 2009), but the role of *D. v. virgifera* as a translational genomics model for other insect pests will make the results of this study of even broader value.

These are exciting times for entomologists studying *D. v. virgifera* because of the amazing research tools rapidly becoming available. Whether involved directly with genetics research or not, all of us who study this pest and who seek ways to mitigate the damage and misery it causes will benefit from the new advances being made in unlocking the secrets of its genome. One of the most rewarding and exciting aspects of working on this insect is the extensive international cooperation that continues to make such rapid advances possible (Moeser and Guillemaud 2009).

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IWGO – NEWSLETTER 31 / 1

Abstracts of oral presentations
24th IWGO Conference
Freiburg / Breisgau, Germany
24 to 26 October 2011

Session 1: Does *Diabrotica virgifera virgifera* control pay? On the economic benefits and costs of regulations and control measures

Session Organizer: Justus WESSELER, Chair Agricultural and Food Economics, Center of Life and Food Sciences Weihenstephan, Technical University Munich, Freising, Germany

Costs and benefits of controlling pest *Diabrotica* in maize in the United States

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The *Diabrotica* (corn rootworm) complex of pest species (*D. virgifera virgifera*, *D. barberi*, *D. virgifera zeae*, *D. undecimpunctata howardi*) is the most damaging insect pest of maize in the U.S. All major maize producing areas in the U.S. commonly have economically damaging populations of one or more of these *Diabrotica* species. Only *Ostrinia nubilalis* (European corn borer) challenges *Diabrotica* as the insect pest of greatest economic significance for maize production, but populations of *O. nubilalis* have been regionally suppressed in many areas by the widespread planting of transgenic maize. *D. virgifera virgifera* recently became established in Europe, with populations now causing economic damage to maize in 15 European nations, further highlighting the growing economic importance of the *Diabrotica* pest complex.

Despite the economic importance of *Diabrotica*, few published regional or national scale assessments of yield losses and the costs and benefits of control measures exist. Furthermore, these older estimates of the costs and benefits of losses and control measures are seriously outdated, as maize prices and production have increased greatly in the U.S. and control has shifted to greater reliance on transgenic maize. The total value of maize produced in the U.S. increased from $18.5 billion in 2000 to $66.7 billion in 2010. Transgenic maize for control of *Diabrotica* was first commercially available in the U.S. in 2004. Though U.S. data on planted areas of the different transgenic hybrids are unavailable, 33% of the U.S. maize planted area contained transgenic genes for control of insect pests in 2004, which increased to 57% by 2011. Given these changes, this paper presents updated estimates of the costs and benefits of controlling pest *Diabrotica* in maize in the U.S. Estimates include the value of yield losses that occurred due to *Diabrotica* damage in 2010, as well as the cost of control measures and the value of yield losses prevented by these control measures.

Costs and benefits of plant health measures against *Diabrotica*: experiences and estimations for Germany

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Since the first detection of the Western Corn Rootworm in 2007 in Germany the official regulatory measures have caused high costs as well for the Regional Plant Protection Organisations (RPPO) of the Federal States and the maize growing farmers in the regions with *Diabrotica virgifera virgifera* infestation. While RPPOs can claim a part of their expenditures for eradication measures from the EU solidarity fonds economic impacts due to yield losses of maize growers are not compensated.

To evaluate the benefits of the phytosanitary measures on the spread of the beetle the effects of containment or, where still possible, eradication measures, on the dispersal of *Diabrotica* should be known on a spatially explicit basis. Distribution maps of the beetle for the different years of infestation show that the Western Corn Rootworm slowly spreads further to other regions. New introductions provide new starting points thus adding to the actual dispersal.

Based on the observed data costs and benefits of the application of plant health measures for various spread scenarios are estimated and conclusions from the experiences in Germany discussed. Previous modeling approaches for expected impacts and assumptions for spread rates are evaluated under consideration of the recent data.

The corn root worm in Bavaria – local relevance and the economic impact on single farms

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The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) was observed in Southern Bavaria for the first time in 2007. The aim of this project is to assess the possible regional significance of the pest and to determinate the economic impact of eradication and containment measures at farm level using a case study approach.

Farms in regions with a high portion of maize grown (>50%) and a strong area-related increase of maize production in recent years (2005 - 2009) have been selected. In addition to the portion of maize grown on the single farm and in the specific region also the farm types (dairy cattle, cashcrop production, bull fattening, pig production, etc.) and the willingness of the manager to participate in the survey were determining factors for the selection of case study farms.

For the evaluation of the economic implications a whole-farm simulation has been used to include indirect effects.

Results show the economic impact of the containment measures seem to be small at farm level but with the exception of one region, the Rottal. The specific situation of the maize farmers in Rottal does result in substantial compliance costs. Nevertheless, most farmers assessed the adaptation measure 2/3 crop rotation to be of a minor problem.

Monitoring and current spread of *Diabrotica* in France: an increasing economic problem

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Corn root worm, *Diabrotica virgifera virgifera*, became a main maize pest in Europe. After its first settlement in intensive maize cropping around airports in Serbia and Italia, late outbreaks were observed in less intensive maize area close to airports in western part of Europe, mainly around Paris but extend to Ile de France area. It was quite easily eradicated in 2006. Spread in France is now under transport by road with settlement close to motorways and transit areas for trucks. Rhône-Alpes, Alsace and less Bourgogne area were the most concerned by catches in 2011. Few catches were detected in Aquitaine and alpine valley of PACA.

Pest management first done with insecticides on adults and localised rotation is now under extended rotation and use of insecticides on larva. Considering economic consequences and mycotoxins increase in wheat, for systematic rotation on a large area, complexity in the tools involved for pest management is the key for a sustainable control. If avoiding the settlement in France seems to be out of reach, it may be possible to greatly delay it. Catches in Alsace, seventeen time less than it was the case in the German Rhine side in 2011 show some possibilities to prevent spread with the strategy applied in France and have acceptable impact on the economy of the area.

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Session 2: Emerging concerns for western corn rootworm resistance development to *Bt* in the Corn Belt of the U.S.A

Session Organizers: Mike GRAY, Department of Crop Sciences, University of Illinois, Urbana, Illinois, U.S.A. & Joe SPENCER, Illinois Natural History Service, University of Illinois, Champaign, Illinois, U.S.A.

Response of western corn rootworm to planting of *Bt* maize in Iowa, USA

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Crops engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*) are planted on millions of hectares annually, reducing the use of conventional insecticides and suppressing pests. However, the evolution of resistance could cut short these benefits. A primary pest targeted by *Bt* maize in the United States is the western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). We report that fields identified by farmers as having severe rootworm feeding injury to *Bt* maize contained populations of western corn rootworm that displayed significantly higher survival on Cry3Bb1 maize in laboratory bioassays than did western corn rootworm from fields not associated with such feeding injury. In all cases, fields experiencing severe rootworm feeding contained Cry3Bb1 maize. Interviews with farmers indicated that Cry3Bb1 maize had been grown in those fields for at least three consecutive years. There was a significant positive correlation between the number of years Cry3Bb1 maize had been grown in a field and the survival of rootworm populations on Cry3Bb1 maize in bioassays. However, there was no significant correlation among populations for survival on Cry34/35Ab1 maize and Cry3Bb1 maize, suggesting a lack of cross resistance between these *Bt* toxins. This is the first report of field-evolved resistance to a *Bt* toxin by the western corn rootworm and by any species of Coleoptera. Insufficient planting of refuges and non-recessive inheritance of resistance may have contributed to resistance. These results suggest that improvements in resistance management and a more integrated approach to the use of *Bt* crops may be necessary.

Resistance of western corn rootworm from laboratory selection to multiple types of transgenic *Bt* corn

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Selection experiments with western corn rootworm on transgenic corn have been done with at least thirteen different populations of insects on four different proteins/products and in four different laboratories. Each of these selection

experiments have also had unselected colonies and some experiments include colonies with partial selection. In thirteen out of thirteen selected strains, resistance developed relatively quickly. Since the types of transgenic corn targeting rootworms are relatively low dose, high resistance ratios are not possible and have not been found. However, all colonies that have been evaluated in the field have been documented to have increased resistance not only in the lab, but also had field relevant resistance. Currently, western corn rootworm colonies with resistance to all currently registered and one unregistered transgenic event have been developed in our laboratory. My talk will include a quick summary of what is publicly known about all thirteen colonies along with a summary of recent data from resistant colonies in our laboratory.

Western corn rootworm behaviour and movement in refuge and transgenic corn

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Corn rootworm-protected *Bt* (*Bacillus thuringiensis*) transgenic corn hybrids have changed corn insect management in the USA Corn Belt. *Bt* hybrids provide efficacy equivalent to soil insecticides and simplify farming operations; they are also vulnerable to insect resistance. To slow the development of resistance to *Bt*, US growers of *Bt* crops must set aside a portion of each field as a non-*Bt* refuge. Refuges provide areas for western corn rootworm (*Diabrotica virgifera virgifera,* WCR) to develop without exposure to the *Bt* toxin expressed in the *Bt* crop, leaving them susceptible to this technology. It is expected that abundant *Bt*-susceptible male WCR beetles emerging from refuges will move into the *Bt*portion of fields and mate with potentially-resistant females. These pairings should dilute the potential for production of resistant offspring. In the Corn Belt, the EPA currently requires a 5% to 20% refuge within or adjacent to a rootworm-protected *Bt* cornfield.

Ironically, much of the rootworm biology on which we have based current refuge requirements originated with research conducted before the commercialization of any *Bt* crops. Our study updates information on the movement and mating behavior of WCR beetles within refuge and *Bt* corn deployed as blocks, strips and seed blends. WCR beetles do not always behave in ways that meet our long-held assumptions. WCR emergence and abundance varied with the configuration of refuge; WCR movement patterns in block refuges may not always provide adequate mixing between mate-seeking WCR from refuge and *Bt* corn. In contrast, blended refuges provided very uniform distributions of WCR adults and mating activity. Refuge configuration can dramatically influence WCR movement and mating in *Bt* cornfields. We expect that data on pest behavior, rates of compliance with refuge requirements, and the threat of pest resistance will continue to define grower options for deploying refuge corn well into the future.

Producer perspectives on *Bt* refuges: closer to greater compliance or resistance?

M. Gray
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The popularity of *Bt* usage by Illinois' producers was confirmed at the 2010 and 2011 Corn and Soybean Classics (University of Illinois Extension meetings). For the 2009 growing season, 96.7% (n = 530) of producers indicated they had planted a *Bt* hybrid. The following year (2010), a similar percentage of producers, 95.4% (n = 540), used a *Bt* hybrid. When producers were asked about their compliance with refuge requirements, 75.7% (n = 405) indicated they had established a 20% refuge in 2009 according to the suggested guidelines. Refuge compliance (2010 growing season) among those producers who attended the 2011 Classics was 78.6% (n = 444). In 2009, 73.3% (n = 376) of Illinois' producers who participated in the 2010 Classics indicated they chose to plant a *Bt* hybrid with knowledge that anticipated damage levels for European corn borers and corn rootworms were low. For the 2009 growing season, 39.8% (n = 210) of producers indicated they did not have access to elite non-*Bt* corn germplasm. Without the availability of this elite germplasm, even producers in southern Illinois, an area of the state not typically prone to economic infestations of western corn rootworms, were electing to use *Bt* hybrids. At the 2010 Classics, 80.4% (n = 533) of the producers indicated they would consider using a seed blend (*Bt* and non-*Bt*) as their refuge. Of those producers who expressed an interest in the seed mixture strategy, 90% (n = 503) indicated they would use a seed blend that contained non-*Bt* seed in the 2% to 5% range. In sharp contrast, the percentage of producers interested in a seed mixture approach to resistance management declined to 53.1% (n = 289) when the non-*Bt* seed increased to a 6% to 10% range. To date, the evolution of resistance within commercial fields by western corn rootworms to *Bt* maize has not been confirmed even though the use of *Bt* hybrids remains very high across the Corn Belt of the U.S.

Developing methods for RNA interference in western corn rootworms

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Corn rootworms (CRW), *Diabrotica* spp. are highly destructive pests of corn, and the western corn rootworm (WCR), *D. virgifera virgifera*, is arguably the most important rootworm pest throughout the U.S. Corn Belt. Perhaps the most significant recent development in the control of rootworms has been the introduction of transgenic corn hybrids expressing insecticidal proteins from *Bacillus thuringiensis* (*Bt*). This technology possesses many attributes that favor its adoption and that minimize the environmental consequences associated with traditional soil insecticides. Alternatives to *Bt* toxins for rootworm control are currently lacking and strategies involving a

different mode of action are critically needed to promote sustainability of a transgenic approach. One such alternative involves the use of RNA interference (RNAi). Recently, it has shown that ingestion of double-stranded (ds)RNAs supplied in an artificial diet triggers RNA interference (RNAi) in WCR larvae. In addition, transgenic corn plants engineered to express WCR dsRNAs show a significant reduction in larval feeding damage suggesting that the RNAi pathway can be exploited to control rootworms. Initial studies in our laboratory involved injection of dsRNA into rootworm larvae and showed silencing of genes involved with post-molt cuticular tanning, formation of peritrophic membrane, and cellulose digestion. In addition, we have been able to show that feeding dsRNA to rootworm adults silences the same gene previously shown to cause mortality in WCR larvae. This technique circumvents the inherent difficulties associated with exposing rootworm larvae to treated artificial diets. Using this approach, we have been able to knockdown the expression of a hydrolytic enzyme believed to be involved with organophosphate resistance and confirm that the enzyme is at least partially responsible for resistance. These experiments demonstrate that RNAi-mediated gene silencing is systemic in western corn rootworms and provides an important tool for discovery of novel target sites and potentially for direct pest management applications.

Session 3: Exploring gene flow in *Diabrotica* across space, time, and species

Session Organizer: Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A. & Thomas GUILLEMAUD, INRA, Sophia Antipolis, France & Blair SIEGFRIED, University of Nebraska, Lincoln, Nebraska, U.S.A.

Historical and contemporary genotypic changes associated with the invasion of Croatia by the WCR

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In 2008, genetic capabilities within the *D. v. virgifera* research group at the University of Zagreb were established and preliminary microsatellite genotyping at 6 loci for 6 WCR populations sampled across Croatia showed non-significant low levels of genetic differentiation indicative of high levels of individual movement with no barriers to dispersal at distances between 0.5-134km (unpublished data). This preliminary investigation has since expanded to include a temporal study of genetic structure over a 14 year period (historic versus contemporary genotypes, 1996-2009) from *D. v. virgifera* populations in Croatia and neighbouring countries (Serbia, Hungary, Italy). To understand how genetic structure has changed over time we have genotyped individuals from 43 populations from Croatia, Serbia, Hungary and Italy and the USA (suspected source population). Future work will focus on phenetic/metric variation

(geometric morphometrics), described by the geometry of wing vein intersections of individuals, to further estimate population structure and dispersal. During this talk we will discuss how the genetic variation found has enabled continued invasion success in southern Europe where *D. v. virgifera* was first detected and spread from into Europe.

Western corn rootworm dispersal in the Italian hybrid zone

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is one of the most destructive pests of corn and is invading Europe. The two main European invasive outbreaks of WCR are located in Northern Italy and Central Europe and they originated from independent introductions from North America. According to the WCR distribution maps a secondary contact probably occurred between these two expanding outbreaks in 2008. Here we used 13 microsatellite markers to conduct a population genetics study to demonstrate that this geographical contact resulted in a hybrid zone in North Italy in the Veneto area. Clinal variations of allelic frequencies along a West-East axis were found at most microsatellite loci (12 out of 13). By studying the shape of allelic frequency clines we obtained maximum likelihood estimates of dispersal parameters, including the mean square parent–offspring distance. The estimation results are presented, compared to other estimates of the WCR dispersion and are discussed in the context of the invasion of WCR.

Demography of the early phases of the invasion of central and south-eastern Europe and north-western Italy by the western corn rootworm

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The demographic characteristics of the early phases of a biological invasion are considered to have a major impact on the success of and invasion. The western corn rootworm (WCR) was independently introduced several times from Northern USA to Europe since the beginning of the 90's and is thus a model for which we have access to "spatial replicate" of introduction and invasion. The demographic events associated with the different phases of a biological invasion affect the genetic diversity of invasive populations and it is theoretically possible to infer demography from genetic data in return. The principal aim of this study was to infer, from genetic data, historical and demographic parameters of the two main WCR European invasive populations, i.e. in central and south-eastern (CSE) Europe and north-

western (NW) Italy. To do so, we have used genetic diversity data obtained at 13 microsatellites loci in both these European populations and in a Northern USA population. Based on these data, we have performed Approximate Bayesian Computation (ABC) analyses to i) quantitatively compare different demographic models of invasion, ii) to define the parameters that can be effectively and accurately estimated and iii) to estimate the demographic and historical parameters in WCR European populations. The use of temporal samples increased the power of ABC analyses to detect demographic expansion events and some parameters associated with the foundation of introduced populations could be accurately estimated. A population bottleneck was detected in both invasive WCR populations. Estimations of the bottleneck intensity (K) and the number of founder's individuals (Nf) were similar in both populations (K=0.32 & 0.40 ; Nf=44 & 28 for NW Italy and CSE Europe respectively). A demographic expansion was detected in CSE Europe only suggesting that demographic stochasticity is stronger in the NW Italian population.

Introgression between *Diabrotica barberi* Smith and Lawrence and *Diabrotica longicornis* (Say)

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[3]*Monsanto Company, Chesterfield, MO, U.S.A.;*
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Diabrotica barberi Smith and Lawrence and *D. longicornis* (Say) are considered to be sister species, and it has been proposed that the two species may hybridize under field conditions. The objective of this study was to examine genetic characters of *D. barberi* and *D. longicornis* for evidence of field introgression. Both species were collected from sympatric and allopatric areas. Amplified fragment length polymorphisms (AFLP) were used to examine variation within and among populations of *D. barberi* and *D. longicornis*. Relatively little of the overall genetic variation was explained by the putative species designation, and most of the genetic variation was found within populations. Additionally, genetic differences were not correlated with geographic location. Genetic data indicate that introgression occurs between the two species. *D. barberi* and *D. longicornis* demonstrate limited reproductive isolation and little genetic divergence, but may be isolated by mate recognition and habitat preferences. Further work on the ecology of both species and the genetic and morphological variation in *D. barberi* and *D. longicornis* should help to clarify their evolutionary relationship.

Gene flow among populations of western corn rootworm in its (almost) original home range in Colorado and Kansas

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Population genetic structuring is a function of gene flow between populations, and in principle both can be estimated using selectively neutral genetic markers. Understanding gene flow in the western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is of interest because it affects the rate of evolution and spread of adaptations such as insecticide and crop rotation resistance. However, both structuring and gene flow in WCR have proven difficult to measure in North America using microsatellite markers. This difficulty is probably a result of this species' recent range expansion from the Great Plains to the Atlantic Coast beginning about 60 years ago. In other words, there has not been enough time for genetic drift to lead to population differentiation. We are addressing this issue in two ways. First, in 2009 and 2011 we sampled WCR populations from Colorado and western Kansas, an area where the species has been resident since at least 1867 when it was first described. Although it is thought that this species ultimately originated in Meso-America and spread north with maize cultivation, it was probably resident on the Great Plains long before the 19th century. We reason that neutral genetic variation in populations from this area should not be affected by the range expansion. Second, we are genotyping individuals from these populations with large numbers of single nucleotide polymorphism (SNP) markers that we recently developed. Using several hundred markers should increase sensitivity enough to detect structuring if it exists. Preliminary genotyping of six populations of WCR collected in 2009 from Colorado, Kansas, and three states further east with over 800 SNPs indeed revealed differentiation among several of the populations. Simulation of subsamples of SNPs in assignment analyses indicates that a minimum of about 550 loci will be necessary to attain the same sensitivity. Temporal analyses of gene flow based on resampling of populations in 2011 is underway.

Session 4: Developing genetic and genomic resources for rootworms and their implications to sustainable pest management

Session Organizer: Thomas GUILLEMAUD, INRA, Sophia Antipolis, France; Blair SIEGFRIED, University of Nebraska, Lincoln, Nebraska, U.S.A. & Tom SAPPINGTON, USDA-ARS, Ames, Iowa, U.S.A.

Western corn rootworm transcriptome project

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera* is the most destructive pest of maize in the USA, and a new invasive pest in Europe. This project is part of a larger effort to substantially increase genetic and genomic resources to assist in the development of environmentally safe and sustainable approaches to managing the economic losses to maize production that result from western corn rootworm infestations. The goal of the proposed project is two-fold: (i) to create a nearly complete inventory of the transcriptome of WCR by extensive sequencing of a pooled, normalized cDNA library representing all WCR life stages and tissues under normal developmental conditions, and representing specific life stages exposed to environmental stresses that may elicit the expression of genes involved in adaptation; and (ii) to compare specific gene expression profiles for tissues and physiological conditions related to behavior, larval metabolism of plant tissue, and adaptations to management practices. We expect from this project to build resources for new gene discovery, for studies of gene expression and sequence variation, and for future WCR whole-genome assembly and annotation. These resources will open up many avenues of investigation in developing sustainable integrated pest management approaches, and in larger questions of ecology, evolutionary biology and genomics that would not otherwise be possible.

Characterisation and sequencing of the western corn rootworm genome

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The western corn rootworm, *Diabrotica virgifera virgifera* is a highly destructive pest of maize that is indigenous to North America and a recently introduced species in Europe. The economic impact, invasiveness and history of adaptation to control measures of the species make it an important target for both applied and fundamental research. The science of genomics offers unprecedented opportunities to accelerate and deepen our understanding of many aspects of an organism's biology. As a result of recent technological advances, genomic approaches are no longer confined to a few model species and the study of western corn rootworm genomics has become a reality. A project to provide a number of genomic resources for western corn rootworm is now nearing completion. This project identified and tested over 1500 Single Nucleotide Polymorphisms (SNPs) within coding genes and used them to construct a linkage map of the western corn rootworm genome. In the final phase of this project, several large segments of the corn rootworm genome are being sequenced and associated with the linkage map. A new project has just been started that will sequence the entire western corn rootworm genome and apply genomic techniques to understand the molecular basis of western corn rootworm larvae's ability to colonise their host plants.

RNAi as a tactic for rootworm management

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Currently, all commercialized transgenic approaches for managing western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, and northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, in the USA utilize a *Bt* as the insecticidal mode of action. While this approach has proven to be an excellent tactic for maize producers in the USA, additional modes of action will likely be required to ensure the long term success of transgenic approaches for rootworm management. We recently reported that ingestion of dsRNAs from selected target gene templates can induce larval stunting and mortality in artificial feeding assays. Furthermore, we demonstrated that maize plants expressing selected dsRNAs protect roots from rootworm feeding damage. Here, we demonstrate that *in-planta* dsRNAs alone and in combination with *Bt* significantly impacts multiple aspects of WCR life history while challenging these pests to a novel mode of action. Beyond root feeding protection, our results indicate that this approach may serve as an effective resistance

management option as we move forward with additional options for managing rootworms of maize.

Characterization of Cry34/35 resistance of a laboratory *Diabrotica virgifera virgifera* colony

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The aggressiveness of *Diabrotica virgifera virgifera* LeConte, western corn rootworm (WCR), as a pest of corn production in terms of yield losses, use of chemical insecticides, and history of adaptation to management tactics makes this species the most important pest of field corn throughout the U.S. Corn Belt. Transgenic corn expressing the Cry34/35Ab1 *Bacillus thuringiensis* insecticidal proteins (event DAS-59122-7) has gained widespread acceptance in the U.S. as a safe and effective control technology for use against *Diabrotica* spp., protecting corn roots and yield potential. However, there is a concern that the continuous expression of insecticidal proteins and the widespread use this technology will rapidly lead to development of resistance. One aspect critical to the success of resistance management strategies is understanding the inheritance of resistance. Field collected WCR populations were brought into the laboratory, crossed to a non-diapausing laboratory WCR colony, and the progeny selected for resistance to event 59122 expressing Cry34/35 insecticidal proteins. The objectives of this study were to characterize the inheritance of the resistance using an on-plant sub-lethal seedling assay and to determine the level of genetic variation between the field and laboratory colonies.
Traditionally, characterization of sex-linkage and dominance is conducted using dose-range bioassays in parental strains and F1 reciprocal crosses. However, because WCR is relatively insensitive to Cry34/35 in diet bioassays we attempted to use an on-plant seedling assay to estimate sex linkage and dominance of resistance after 22 generations of on-plant selection. In addition, genetic variation of the field and non-diapausing laboratory parental strains was compared with the various generations of the selected colony using microsatellite markers.

Inheritance of diapause in *Diabrotica barberi*

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Diabroticite corn rootworms are prominent pests of maize and have adapted to both cultural and chemical management methods. In response to a widely used corn-soybean crop rotation in the U.S. Corn Belt over several years, northern corn rootworm (NCR) populations adapted by increasing the proportion of eggs that diapause over two winters instead of one. The frequency of eggs diapausing for two years increased with time and prominence of the maize-soybean rotation in the landscape. We investigated the pattern of inheritance of egg diapause duration in relation to male and female parental phenotypes for diapause duration. We collected NCR as pupae from a maize field that had been in a maize-soybean crop rotation for several years. We sexed the pupae and maintained them individually. We also collected pupae from a NCR lab colony that had been selected for one year diapause for several generations. We established reciprocal F1 families from the extended diapause (ED) and one year diapause (D) lines. Eggs obtained from the females were provided two overwintering periods, one each for five months at 8º C. Eggs were allowed to hatch at 25º C for 45 days after each overwintering period. Eggs obtained from ED females had a significantly higher proportion of eggs with the ED trait compared to eggs obtained from D females ($F \geq 4.13$ $P \leq 0.015$; $h2 = 0.69$). With a strong female genetic influence on diapause duration in NCR, we can begin selecting for a non-diapausing line to facilitate research on this important pest.

Session 5: Genetically-engineered maize: current issues

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

An overview of current issues of GM maize in Spain

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GM maize with resistance to corn borers (*Bt* maize) has been cultivated in Spain since 1998. The surface devoted reached 76500 ha (23% of maize growing area) in 2010. During this period, several studies, funded by public organisms, have been developed to evaluate its environmental impact.

Monitoring of field resistance in target insect pests has been assessed and strategies for delaying resistance proposed. No consistent shifts in susceptibility for field populations of corn borer have been recorded.

A considerable effort has been made in assessing the impact of *Bt* maize on non-target arthropods, especially pests and natural enemies. We have dealt with subjects such as: 1) the identification of the most representative arthropod species in Spanish maize; 2) the assessment of the exposure of non-target species to *Bt*-toxin; 3) the assessment of changes in composition and abundance of non-target arthropods, and 4) the effects on insect performance. Results of effects of *Bt* maize on non-target lepidopterans are reported in another presentation of the conference. No economically population increases of other herbivores have been recorded and no detrimental effects of farm-scale *Bt* maize have been observed on natural enemies, suggesting that *Bt* maize is compatible with IPM strategies.

Genetically modified herbicide-tolerant (GMHT) varieties suppose the major surface of GM maize in the world. These varieties are not allowed for cultivation in Europe but they could be cultivated in the next future with a single trait or stacked with *Bt* traits. The GMHT cultivation may alter composition, abundance, and phenology of weed flora and consequently the components and relationships in the food web. We have assessed the potential impact of GMHT on herbivores and natural enemies and, therefore, on biological control functions. Arthropods responded to weed changes but in variable directions. No significant changes in the density of *Orius* sp., the most abundant predator in Spanish maize fields, may be expected for moderate alterations in weeds arising from the deployment of GMHT maize varieties.

In addition to generate data to contribute to environmental risk assessment and post-market environment monitoring in Europe, methods to standardise field trials may be derived from the studies conducted the last 13 years in Spain.

Arthropods in European maize fields: a database to support risk assessment

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Pre-commercial environmental risk assessment is part of the regulatory approval process for genetically modified (GM) plants. For insect resistant GM plants, such as *Bt* maize, potential adverse impacts on non-target arthropods are of major concern. For practical reasons it is not possible to test all arthropod species present in the crop. Therefore, appropriate species need to be selected as surrogates that represent taxonomic groups as well as the ecologically and economically important functions.

Currently, a Microsoft Access database on European arthropod species is being built within a project commissioned by the European Food Safety Authority (EFSA). For each species, information on taxonomy, ecological function, distribution, and abundance is compiled. Systematic literature searches are conducted to retrieve arthropod abundance records for maize, potato, oilseed rape, sugar/fodder beet, cotton, rice and soybean. Furthermore, detailed geographical information will allow comparisons of species composition in different geographic regions. For non-target risk assessment, this database can support and justify the selection of test species for laboratory studies, higher-tier studies, and case-specific monitoring.The current database is developed from a previous database that contains 968 plant- and ground dwelling arthropod species from 124 families and 16 orders in European maize crops (Knecht et al. 2010). Coleoptera, Araneae and Hemiptera are the orders with the highest numbesrs of species in the database. Carabidae, Staphylinidae, Cicadellidae and Linyphiidae are the families containing the highest numbers of species. Database records come from 109 references covering 18 European countries. Hungary and Germany reported the highest numbers of species.

Reference:
Knecht S *et al* (2010) A faunistic database as a tool for identification and selection of potential non-target arthropod species for regulatory risk assessment of GM maize. IOBC/WPRS Bulletin 52: 65-69.

Research on the molecular mechanism of cross-resistance in the Asian corn borer to four Cry toxins

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Genetically engineered maize that express cry proteins from *Bacillus thuringiensis* (*Bt*) can successfully control the Asian corn borer (ACB), *Ostrinia furnacalis*, which is the most sever pest of maize in China. Confined field trials showed that Cry1Ab-, Cry1A-, Cry1Ah-, Cry1Ac-, Cry1Ac-Cry1Ie-, and Cry1F-expressing maize have track records to significantly suppress ACB. However, concerns have been raised regarding the possibility of ACB evolving resistance to *Bt* maize, especially there were significant differences in susceptibility among the geographic distinct populations, which likely reflect natural Bt selection pressure. In the laboratory, ACB developed significant resistance to Cry1Ab and Cry1Ac toxins. In addition, susceptibility of Cry1Ab or Cry1Ac resistant strains to a number of other *Bt* toxins to which the selected strains had not previously been exposed, was dramatically varied. Understanding the molecular mechanism of cross-resistance in ACB is thus both of theoretical and practical interest for developing insect resistance management strategies. Heterologous competition binding assays were exploited to distinguish the binding sites of Cry1Ab, Cry1Ac, Cry1F, and Cry1Ie proteins on the brush border

membrane vesicles (BBMV) of ACB. Cross-binding between Cry1Ab and Cry1Ac was detected. Partial cross-bindings between Cry1Ab and Cry1Ah as well as Cry1F were notice. In contrast, no cross-binding were observed. This suggest that Cry1Ac and Cry1Ab share same binding site(s); besides Cry1Ah and Cry1F share a common binding site with Cry1Ab, there are also other binding sites of Cry1Ah and Cry1F; Cry1Ie does not share binding sites with Cry1Ab and Cry1Ac on the BBMV of ACB.

Using genomics to addressing *Ostrinia* population dynamics and control in maize

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The European corn borer, *Ostrinia nubilalis*, is destructive to corn plants in North America and Europe that is a pest targeted by maize hybrids that express transgenic crystalline (Cry) toxins derived from the gram positive soil bacterium *Bacillus thuringiensis* (*Bt*). Genomic tools comprised of a whole genome sequence (WGS), a reference transcriptome, and molecular genetic markers are being developed and applied. Specifically, preliminary data from single nucleotide polymorphism (SNP) markers has successfully estimated the degree of genetic structuring and gene flow among *O*. *nubilalis* populations. Additionally, a SNP marker-based genetic linkage map has been constructed, and applied for the isolation of quantitative trait loci (QTL) that are linked to Cry1Ab and Cry1F toxin resistance traits of *O*. *nubilalis* larvae. The QTL for Cry1Ab and Cry1F toxin resistance are not linked in the *O. nubilalis* genome, and are likely determined by independent genetic mechanisms. RNA-seq data was used to uncover differentially-expressed genes within the midgut of *O*. *nubilalis* that co-segregating with *Bt* resistance traits, and indicate that mutations that are linked to resistance can involve gene regulatory pathways. The future completion of a WGS for *O*. *nubilalis* will assist in the identification of genes within the *O*. *nubilalis* QTL we have identified and may allow the rapid dissection of complex genetic traits. The application of genomic technologies hold the promise to elucidate similarly control issues that are associated with corn rootworm, *Diabrotica virgifera virgifera*, feeding damage in genetically-engineered maize.

Opportunities and challenges with seed blends for managing insect resistance to *Bt* maize

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Bacillus thuringiensis (Bt) maize has been commercially available to growers in the U.S.A for fifteen years and fortunately the primary lepidopteran pest, European corn

borer, *Ostrinia nubilalis*, has not evolved resistance. The high-dose/refuge strategy is used to manage insect resistance. The assumptions of this strategy are plants express a high dose of toxin, genes that confer resistance are rare, and there are many susceptible insects to mate with resistant insects. These assumptions are met with many lepidopteran pests of maize, but certainly not all; and the assumptions may not be met with the coleopteran pest, western corn rootworm, *Diabrotica virgifera virgifera*. With this strategy susceptible insects are produced through the use of structured or unstructured refuges. Until recently U.S.A. growers were mandated to grow at least 20% non-*Bt* maize as refuge when plants expressed a single *Bt* toxin. Now a new generation of transgenic maize that combines two or more toxins with different modes of action (pyramids) is in production. Generally these plants have a wider spectrum of insect control and because of the "redundant killing" properties growers are allowed to plant reduced amounts of structured refuge and in some cases blends of *Bt* and non-*Bt* seed. Previously seed mixtures were not recommended because interplant movement of larvae could violate high-dose assumptions. This paper will provide an overview of the use of maize seed blends in the U.S.A. and an update of studies with European corn borer to determine behavioral and genetic factors that influence larval movement among *Bt* and non-*Bt* maize plants.

Session 6: Wireworm biology and control strategies in maize

Session Organizer: Udo HEIMBACH, Julius Kühn Institute (JKI) - Institute for Plant Protection, Braunschweig, Germany

Effect of insecticides on four different wireworm species

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Wireworms of *Agriotes lineatus, A. obscurus, A. sputator* and *A. sordidus* were exposed to insecticide treated soil applied using two different control methods. One method comprised the application of insecticides diluted in water at doses of 50, 100, 200 and 300 g a.i. per ha. The other method consisted of a bait treatment at doses of 0.01, 0.1, 1 and 10 g a.i. per ha. At both methods the insecticides were homogeneous divided throughout the soil. Four insecticides were tested; fipronil efficacy was compared to the neonicotinoïds thiamethoxam, imidacloprid and clothianodin. In the soil treatment trial, chlorpyrifos at one dose was added as a standard insecticide treatment. Each trial contained an untreated control to check viability of the wireworms. The tests between methods and the various species were conducted at different dates, but at equally controlled conditions. Mortality was

observed after one and two (bait treatment) or three (soil treatment) weeks of exposure. Fipronil was highly lethal to each of the wireworm species tested, regardless of the method used. In general, mortality was higher compared to the neonicotinoïds tested, the latter showing low or no mortality at the given dose and exposure variants. Applying fipronil in a bait formulation may decrease the amount of active ingredient per ha considerably, therefore baits may have interesting environmental benefits.

Studies on the influence of soil moisture on the vertical distribution of wireworms

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The larvae of the click beetles, called wireworms, are developing in soil over several years. The polyphagous larvae need live vegetable tissues. As a result of increasing cultivation of corn and potatoes they become a huge problem in Germany's agriculture.

Wireworms do have several damaging phases caused by intense feeding. The damaging phases are depending on seasons and in relation to this on soil temperature and soil moisture. If one of both is not comfortable for larvae, they have the tendency to migrate into soil levels with suitable conditions. In consequence of a drying out soil, they move in deeper levels and in relation to increasing soil moisture they move back in upper soil levels (FURLAN 1998). Larvae only cause damages, when they stay in upper soil levels what means next to roots or crop of field culture.

The migration of wireworms in different soil conditions were tested in experiments. In field experiments analyses on the occurrence of wireworms in relation to soil moisture and soil temperature were done over a period of 2 years. Therefore traps were buried in soil and the appearance of larvae was analysed. In laboratory the migration caused be drying out soil was tested with 4 soil types and 6 replications with 20 wireworms each. Correlations between the percentage of occurred wireworms and soil moisture were analysed.

The results build the basis for a prediction model, which appraises the risk of damages on field culture caused by wireworms in relation to soil moisture and soil temperature. With logistic and non-linear regressions a first approach of a prediction model could be developed. One output binary displays the risk for damages. With a correct prediction in over 80 per cent of the cases the binary category was predicted correct. The second output displays the percentage of occurred wireworms in a special soil type in relation to soil moisture. With a R^2 from 0.81 till 0.88 the percentage of occurred wireworms could be predicted well.

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First approaches to forecast first appearance and flight-activity of selected *Agriotes*-species

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The number of wireworms and click beetles on agricultural crop land has been rising since a couple of years. Wireworms are the larvae of the click beetles, which spend three till five years in the soil until they evolve to beetles. The adults feed on pollen and are accordingly less damaging compared to their larvae, which causes in part large damages on economic plants. Measures to combat the wireworm are limited at present. An option to reduce the wireworm population in soil is to control the click beetle. A monitoring to determine the chronological sequence of the first appearance and flight-activity of this beetle was realized.

In 2008, 2009, 2010 and 2011 there were 228 pheromone traps placed on 40 fields to catch the most important species *Agriotes obscurus, A. sordidus, A. sputator, A. lineatus and A. sordidus.* The exposition of the traps was done from the beginning of March to the middle of July. A total of 59.273 beetles were trapped. The collected beetles were identified and the species separately evaluated.
The determined database was randomly split. One half was used for modelling, the second half of data was used for an impartial validation. With binary logistic regressions a first approach of a prediction model could be developed. Based on cumulated precipitation and cumulated temperatures (starting from 1. February) the model is able to forecast the first appearance and the flight-activity of the most important *Agriotes*-species in agriculture. If the model calculates an occurrence probability lower than 0.5, the phase is defined as first appearance. Is the probability of occurrence greater than 0.5, more than 50 % of the male population showed flight-activity. With more than 73 %, the majority of cases were correctly predicted by the model. Statistical differences between the species could not be found.

A two year click beetle monitoring with pheromone traps in Germany: species distribution, trap specificity and activity pattern

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Due to an increasing number of reports on damage caused by wireworms in German potatoes and corn fields a survey on click beetle species distribution and occurrence was initiated in 2009. Using pheromone traps for *A. lineatus*, *A. obscurus*, *A, sputator*, *A. ustulatus* and *A. sordidus* adults were collected at 40 different sites in

2009 and more than 70 sites in 2010, located in most parts of Germany. Abundance consistently varied between regions, with *A. lineatus* and *A. obscurus* being more abundant in northern parts, whereas *A. ustulatus* and *A. sputator* are more confined to warmer regions. The Mediterranean species A. sordidus is abundant in the Rhine valley, did already colonise some region further to the north. The specificity of the pheromone traps was sufficiently high for *A. ustulatus, A. lineatus* and *A. sputator*, but was very low for traps provided with the *A. obscurus* pheromone. Flight activities were unimodal for most species, but bimodal for *A. sputator*. The occurrence and abundance of the species is discussed with regard to climatic, landscape pattern, and crop rotation pattern at the sites the traps were located.

Three years of wireworms monitoring with pheromone traps in France

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Since a long time damages caused by wireworms are known by researchers, advisers growers and even gardeners. They were firstly described by Linnea during the second half of XVIII century.
During 50 years wireworms damages were limited due to use of agrochemical and mainly lindane. Since 1998 this active substance is not allowed anymore and populations of wireworms increased a lot. Lot of crops are now damaged by these pests. In France in 2005 and 2006, Arvalis-institut du végétal estimated losses on maize respectively at 500 000 and 700 000 tons because of soil pests amongst which wireworms play a key role. On top of yield losses wireworms are also damaging the quality of harvest on crops such as potatoes or melons. Lot of observations also shows that there is a change in the ratio of main species since last century because of agricultural practices, rotations and may be climatic change. In these conditions it is necessary to better monitor these pests in order to better understand the risk and to better manage populations. For these reasons Syngenta implemented during 3 years a large scale monitoring using the pheromone traps developed by Dr Furlan.

This paper is presenting the main results of this monitoring and the selectivity of the traps used. The repartition of the four main species in France is presented. The Yartlor F trap is a very interesting tool to monitor populations but the specificity of each pheromone has to be considered Up to now there is no relation between number of adults trapped and crop damages but if collection of data continues it will be possible to link plots with risk and level of adults collected in the traps. Mapping of species shows that *Agriotes sordidus* (Illiger, 1807) is now widespread. Now the question raised is why this specie is now dominant although there is 50 years rarely found it in Northern part of France.

Wireworm and click beetle species distribution in field crops in Lower Saxony

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Herbivorous wireworms are problematic pests in several crops, but especially the situation in maize is difficult in Germany, due to the loss of neonicotinoid seed treatments. In general *Agriotes* species are named as the most damaging group of wireworms, but also species from other genera may damage. Often the wireworm species accounting for damage are not recorded at all. Therefore many questions concerning wireworm biology are still open. For example it is not clear if there are differences in individual species preference for certain crops and in their potential for economic damage.

The adults of 5 *Agriotes* species of economic importance have been monitored (*A. lineatus, A. obscurus, A. sputator, A. sordidus, A. ustulatus*) with pheromone traps at several sites in Lower Saxony and the species composition has been determined. Additionally wireworms have been caught with bait traps and by manual collection at these sites.

For the understanding of the efficacy of pheromone traps more knowledge on the radius of pheromone trap catches is needed. Therefore click beetles were released in the field and trapped at different distances.

Session 7: New products/methods for IPM of *Ostrinia* and *Diabrotica* in modern maize production systems

Session Organizer: C. Richard EDWARDS, Purdue University, Lafayette, U.S.A.

Risk of dust drift during sowing of pesticide treated seeds

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Many seeds are treated with insecticides to protect seedlings against pests. In 2008 during sowing of maize in Germany bee poisoning in about 12000 hives was observed. Insecticides abraded in dust from coated maize seeds drifted into neighboring flowering crops. Different maize seed bag batches were sieved, up to about 25g of fine dust <0.5mm per 100000 seed (for about 1ha) were measured, about 25% of a.s. in the dust varying between <10 and >50% were detected with higher seed treatment rates resulting in higher percentage. Dust amounts sieved

from bags of other crops were small for sugar beet (about 0.1g/100000 seed), up to 4.8g dust /700000 seed for oil seed rape coated in 2007 and for cereals higher than for maize. As standardised abrasion method the Heubach Dustmeter was used. Max. Heubach values were set to minimise dust emission, e.g. for methiocarb treated maize in Germany max. 0.75g dust /100000 seed is fixed. For oil seed rape treatment facilities in Germany agreed voluntary on 0.5g/700000 and for sugar beet on 0.25g/100000 seed. The content of a.s. in dust needs to be taken into account if seed treatment methods change. Future work should aim to reduce dust abrasion as well as content of a.s. Field experiments using sowing technology with 90% drift reducing deflectors in maize and seed batches of cereals, oil seed rape and maize resulted in detectable residues between less than 0.1 up to 0.4% of the applied field rate in petridishes placed off crop in 1 m distance. Residues in an area directly neighbouring to sowing of maize were 2.5–5 times higher in oil seed rape plants than in petridishes exposed on bare soil. More than 1g a.s./ha analysed in directly neighboring flowering rape explains increased mortality of honey bees exposed in semifield and field during the experiments. The emission of a.s. during sowing depends on dust abrasion intensity, % of a.s. in dust, sowing technology, soil condition (e.g. wet soil reduces drift), wind direction and speed.

Maximal weekly vs. average daily capture on PhAM as a tool for WCR risk assessment

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Risk assessment (RA) for western corn rootworm (WCR) was done in the USA by various authors but till now few focused on European and a Croatian condition. Small scale (0.5-5ha) and high level of repeated maize require a better approach in providing RA for WCR in Croatia.

The aim of the investigation was to develop a model for RA in continuous maize for WCR. For the study, the relationship between WCR adult population density on Pherocon AM (PhAM) traps in the field and the average number of larvae (ANL) per plant, root damage ratings (RR) and plant lodging the following year were investigated. Adult population densities were determined over a 74-day period (24th to 35th week of the year) each year consecutively from 2006 to 2009 in 30 corn fields per year. Trap inspections were conducted weekly. Soil and root sampling was carried out to determine the ANL per plant, as well as larval root damage ratings according to the Iowa State University scale of 1-6. The percentage of partially and fully lodged plants was visually determined.

Significant interactions for maximal weekly (MWC) and average daily (ADC) capture of adults on PhAM and ANL and RR were determined. Climatic conditions (CC) were found to have an impact on these interactions. MWC of 66 and ADC of 2.32 predict an average 0.5 larvae per plant. MWC of 78 and ADC of 4.55 predict a common RR of 3.5 (ISU 1-6). Significant interactions for MWC and ADC of adults on PhAM and partially or fully lodged plants were not determined. CC had no impact on these relationships. MWC of 62 and ADC of 4.01 both predict a 10% chance of partially lodged plants in continuous maize fields. Fully lodged plants could not be predicted by MWC or ADC. Regression coefficients for the relationship of adult MWC and ADC and ANL per plant, RR and plant lodging were also calculated, compared and discussed. Results from this investigation are beneficial as management guideline of integrated maize production in Croatia.

VOTiVO (*Bacillus firmus* I-1582) – a new biological product for suppression of nematode damages and plant growth enhancement in corn

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*Bacillus firmus*I-1582 is a natural occurring, non-transgenic spore-forming bacterium developed by BayerCropScience for suppression of nematode damages after seed-treatment (VOTiVO®) or for soil-application (Flocter®, Nortica®). The bacteria are cultivated in large-scale fermenter under controlled conditions. The dormant spores of the bacteria are harvested and used for formulation. VOTiVO® formulations as fluid suspension have demonstrated to have a shelf-life of more than 2 years. VOTiVO® can be applied to seeds like conventional insecticide/fungicide seed treatments.

Seed treatment of maize with VOTiVO® led to a colonization of the root-surfaces immediately after seed germination. The bacteria obviously grow and multiply by using the organic compounds, excreted by the plants. Root exudates are considered to be involved in the orientation of various nematode species towards their host plants. Spores of *Bacillus firmus* I-1582 are also able to colonize on nematode eggs and perforate the egg-shell, ceasing the development of the juveniles. No direct activity has been observed on J2-larvae.

Seed treatment with VOTiVO® has also demonstrated enhanced vigor and growth of seedlings, resulting in increased shoot-masses in comparison to the untreated plants. *Bacillus firmus* is known for its ability to form the phytohormone indol-acetic acid (auxins) or to solubilize phosphorous from soil constituents leading to a more vigorous seedling growth (Datta, et.al, 1982). In corn, the performance of VOTiVO® has been evaluated as a single product as well as in combination with the insecticidal seed treatment Poncho. Field trials demonstrated that Poncho/VOTiVO is capable of

reducing plant damage originating from a wide range of nematodes. In the majority of field trials, Poncho/VOTiVO® treated seeds produced higher yields.

*Bacillus firmus*I-1582 is already registered in USA and has been launched as Poncho®/ VOTiVO® for seed treatment on maize in 2011. The development for further crops and geographies is ongoing.

Evaluation of an "attract & kill" strategy for western corn rootworm larvae

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Western Corn Rootworm larvae (WCR) use CO_2 to locate maize roots over longer distances. This behaviour can be used to lure the larvae to an insecticide with artificial CO_2 emitting capsules → Attract & Kill (A&K). We evaluated the A&K approach with a combination of the capsules and the soil insecticide Force 1.5G in thin soil layer arenas (50 x 30 cm; soil layer: 6mm), where movement and behaviour of the larvae could be observed. The A&K approach was tested at maize growth stages BBCH 13-14 and BBCH 17-18. We applied the A&K components (capsules + Force 1.5G) 30 cm and 50 2nd instar larvae 15 cm from the plant base. The number of larvae with knock down symptoms (writhing, curling) were recorded 4 hours and then every 24 hours past inoculation.

At both growth stages larvae with knock down symptoms could be observed directly at the capsules. With the equivalent of a normal field application rate of Force 1.5G, up to 50 % of the inserted larvae were targeted with A&K. In the future, field application of different capsule systems and their potential to reduce insecticides in WCR management need to be tested.

Insecticides and cucurbitacins: compatibility of Invite EC with insecticides with different modes of action.

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is one of the most serious insect pests of continuous maize cultivation in the USA and parts of Europe.

Management strategies focus either on the control of the root feeding larvae, i.e. by crop rotation, seed treatments, soil insecticides or genetically modified maize hybrids or on the control of adult beetles by spraying foliar insecticides. Environmental

concern over the use of soil insecticides and seed treatments, the occurrence of insecticide resistance and the development of a behavioural adaptation to crop rotation in Illinois and Indiana since the 1990s renewed the interest in the effectiveness of combinations of foliar insecticides and cucurbitacin feeding stimulants (i.e. Invite EC^{TM}).

Cucurbitacins are extremely bitter secondary plant compounds specific to the Cucurbitaceae. These substances cause compulsive feeding and arrestant responses of Diabroticite beetles. When added to foliar insecticide sprays it allows a remarkable reduction of the application rates to about 10 % usually recommended. In laboratory experiments we evaluated the compatibility of five common insecticides (Avaunt, Biscaya, Karate-Zeon, Reldan22, and SpinTor), each with a different mode of action, with Invite EC^{TM}. The objective was to determine which insecticide is the most suitable for mixing with cucurbitacins.

Honorary Lecture: Behaviour and ecology of belowground herbivores on maize

From a blank canvas a picture emerges of WCR in Brescia Province, NW Italy

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It is unusual for an artist to start what he/she hopes will become a masterpiece on something other than a blank piece of canvas. However, because of circumstances often beyond their control, entomologists must often respond after the fact and “that” canvas may not be so blank. Occasionally, however, a new situation may present itself and one may be in the right place at the right time to begin from the beginning. Much was the case for us in regard to the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, in Brescia Province (BP), Lombardy Region, NW Italy. WCR was discovered in the Po Valley of the Lombardy Region near Malpensa Airport in 2000 (Boriani & Gervasini, 2000). According to Miller et al. (2005), this was a separate introduction of WCR into Europe from the one that was first recorded by Baca (1994) near Belgrade. Because of the importance of maize and potential impact of WCR on maize production in BP, a team was assembled to plan and carry out research studies; apply, test and improve the monitoring program designed by the Regional Government; develop management strategies; and establish a technical service to advise and assist farmers, if initial studies showed a real threat to maize production in the province. Because of the importance of livestock and diary production, and now also biogas in the province, over 80% is continuous maize and about 70% of all maize is irrigated. About 70% of the maize is planted early (March/April) and the remainder is planted in May after rye grass harvest. As a result

of this unique cropping system and the environment it operates within, it was hypothesized that differences would likely be noted in the biological development and impact of the pest on maize in BP when compared to WCR literature from other regions of Europe and North America. This, in fact, is exactly what was observed. The presentation will discuss the important findings and how this information is being used to manage this pest in BP.

Session 9: Reproductive biology of maize pests and the implications on pest management

Session Organizers: B. Wade FRENCH, USDA-ARS, NCARL, Brookings, South Dakota, U.S.A. & Panagiotis MILONAS, Benaki Phytopathological Institute, Kifissia, Greece.

Oviposition of *Diabrotica* in non-maize crops and implications on crop rotation

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Adults of the maize pest *Diabrotica virgifera virgifera* (western corn rootworm, Coleoptera: Chrysomelidae) are known to perform inter-field movements to crops other than maize, mostly to access protein-rich food sources. Crop rotation experiments aimed to investigate to what extent dispersing adults may also lay eggs into fields of 10 different crop habitats, and consequently reduce the efficacy of rotation as a control measure when maize is planted in the following year. Release and recapture in crop rotation experiments at two study sites in southern Hungary revealed that a proportion of the entire population in the infested maize fields disperses towards uninfested crop habitats. Maize was the most attractive crop for the dispersing *D. v. virgifera* among the 10 crop habitats investigated. A proportion of the population also emerged when maize was planted the following year, which is a result of oviposition of the dispersing adults into uninfested crop habitats. To date, however, no conclusion can be made as to whether the proportion of dispersing and ovipositing *D. v. virgifera* outside their natal maize fields may endanger the efficacy of crop rotation. All experiments are ongoing and repeated at two sites over four years.

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The *Ostrinia*-puzzle

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It is more than 40 years ago that the main pheromone component of the European corn borer (ECB), *Ostrinia nubilalis* (Lep.,Pyraustidae) has been identified, followed by revealing the pheromone strains. Shortly after that, the distributions of strains were clarified in North-America and in Europe. ECB has become a popular target species of ecological, physiological and genetic studies, and pheromone traps have been developed and used World-wide.

In contrast to this, there is a growing number of alerts, typically from Central-Europe, that the pheromone traps for the Z-strain catch males only in insignificant numbers, and therefore they are not reliable for monitoring. Studies aimed to reveal the problems pointed out the need for chemically pure, optimal lure composition and favorable trap design, but did not result in a practical solution.
In order to study why Z-ECB traps do not catch in Hungary, we conducted the following studies in the past fifteen years: 1.) blend ratio; 2.) blend dose; 3.) batches of synthetics; 4.) possible synergists; 5.) dispenser type; 6.) design of trap; 7.) placing traps at various height of the canopy; 8.) layout of traps inside or around the corn field; 9.) ultrasound communication; 10.) molecular markers.

No break-through was achieved in1-8., however, we showed in both fight tunnel and semi-field trapping tests that an aliquot of a pooled ovipositor extract of calling Z-females, in which the main pheromone component was quantified, attracted significantly more males than the optimal synthetic mixture at the same dose level. Moreover, we revealed a special ultrasound emission of courting males (same pattern in both strains), and described a pheromone-strain specific molecular marker expressing in larvae. The *Ostrinia*-puzzle has not been solved yet, albeit we have now more tools to track morphologically identical populations of the species, called as *Ostrinia nubilalis*.

Diabrotica barberi Smith and Lawrence diapause: potential impacts on management in Nebraska, USA

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The chrysomelid beetle *Diabrotica barberi* Smith and Lawrence (common name: northern corn rootworm) is an important insect pest of maize in the western Corn Belt of the United States. Some *D. barberi* populations exhibit extended diapause where eggs remain in diapause for two or more years in the soil. This allows eggs to circumvent annual crop rotation (i.e., from a *D. barberi* larval host to non-host crop) as a management practice which may lead to larval injury in first-year maize fields.

As part of a larger effort to more clearly understand the biology and pest potential of *D. barberi* in Nebraska, several studies were conducted to confirm that the extended diapause trait is present in Nebraska and to measure the frequency of extended diapause in populations of *D. barberi* from eastern Nebraska. In an initial study, *D. barberi* eggs were obtained from adults that emerged from first-year corn in Saunders County, NE and held on moist soil under appropriate temperature profiles to facilitate optimal egg survival, diapause development, and diapause termination. Some eggs hatched in all egg cohorts each year for four simulated summer-winter temperature cycles, documenting that extended diapause is present at the Saunders Co. location. A second study was conducted to measure the frequency of *D. barberi* extended diapause from various sites across eastern NE. The extended diapause trait was found to occur in all populations included in the study. However, significant variation in the frequency of extended diapause was observed among sites. Geographically the highest incidence of extended diapause was found in a north-south transect in the eastern-most counties of Nebraska. Results will be discussed within the context of *D. barberi* management and resistance management.

Mating success in European corn borer and female remating behaviour

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Direct and indirect fitness benefits (good genes and Fisherian selection) have a central role in mate selection theory. Although recent reviews suggest that on average direct effects appear to be greater than indirect effects, the empirical literature in the Lepidoptera suggests that the situation is complex. Mating success of male European corn borer *O. nubilalis*, in relation to their mating history and age, and consequent fitness parameters for their female mates, were investigated. Male age had no significant influence on mating success for *O. nubilalis* males: 0-day-old virgin males were as likely to mate as 3-, 6- and 9-day-old males. Lifetime fecundity was highest for females that mated with 3-day-old virgin males, which was related to greater female longevity. Mating experience significantly increased mating success for *O. nubilalis* males. Mating with experienced males significantly reduced lifetime fecundity and longevity of females. These results suggest that direct benefits from males are insufficient to account for the observed female mating preferences for different-aged males. Male persistence, female remating, or some other compensatory mechanism may account for female acceptance of experienced males. Females may accept these lower quality males to ensure they have obtained at least one mate, and/or to avoid the costs of resisting these males. In addition, if females can mate with another male, they may be able to compensate for mating with a low quality male. We tested this hypothesis by measuring mating propensity for females that mated with a low quality (3-day-old experienced) male versus a high quality (3-day-old virgin) male. Female propensity to mate was related to the quality

of the first mate. Females were more likely to remate when they mated with low quality males than when they mated with high quality males. A second mating with a young virgin male increased female longevity when their first mate was a low-quality experienced male.

Areawide suppression of European corn borer via *Bt* maize: impact of low densities on sex ratio, mating frequency and the pathogen, *Nosema pyrausta*

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Historically, the European corn borer (ECB), *Ostrinia nubilalis,* has been the most widespread insect pest in the U.S.; damage and control costs were typically estimated at more than $1billion per year. Due to the difficulty in monitoring for the pest, or timing insecticide applications, most growers acquiesced and accepted annual yield losses. Beginning in 1996, transgenic maize, engineered to express insecticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt*) became one of the most widely adopted technologies in U.S. agriculture. In 2010, *Bt* maize was planted on more than 22.2 million ha, constituting 63% of the U.S. crop. Using statistical analysis of per capita growth rate estimates (for 3 states; 1996-2009), we found that areawide suppression of ECB was significantly associated with *Bt* maize use.

Cumulative net revenues over 14 years, for 5 major maize producing states (Illinois, Iowa, Nebraska, Minnesota and Wisconsin) were conservatively estimated at $6.9 billion (U.S.). Moreover, ~$4.3 billion, or ca. 62% of the total benefit accumulated on the *non-Bt* maize hectares. Non-*Bt* maize growers benefit from pest suppression via increased yields and reduced input costs, by not having to pay a *Bt* technology fee for seed (savings of ~$10-15/ha). Of equal importance, the non-*Bt* maize serves as the key refuge element of the Insect Resistance Management (IRM) plan in the U.S. With high efficacy (high-dose) *Bt* maize, in tandem with non-*Bt* refuge plantings, there is no evidence of field-evolved resistance by ECB to *Bt* maize. These results affirm theoretical predictions of pest population suppression and highlight economic incentives for growers to maintain non-*Bt* maize refugia. Given the origins of ECB, and damage potential, these results also suggest substantial economic benefits for European maize growers as well.

As of 2011, ECB populations continue to remain very low, with 1stand 2ndgeneration moth flights peaking at <40 moths/night (light traps) at most central U.S. Corn Belt locations (Midwest region). We were therefore curious about the viability of ECB populations at low densities. Via collaborators in five Midwest states, we collected moths during 2008-2009 to measure sex ratio, mating frequency (based on the number of spermatophores/female) and the incidence of a pathogen, *Nosema pyrausta*, a microsporidian that historically, is known to have a significant impact on

ECB populations. Results to date indicate, that despite low densities, ECB females show a high degree of mating frequency, generally >85%; this frequency is similar or higher than mating estimates recorded prior to ECB suppression. Interestingly, however, most of the locations indicated a shift toward a female-biased sex ratio (mean proportion; 0.56F; 18 locations), ranging from 0.53-0.74 female in 2010, compared to an assumed 50:50 ratio. With fewer male moths available for mating, females apparently remain efficient in attracting males. The role of *Nosema* as a biological control agent in the ECB system may be critical to maintaining sustainable ECB management that does not rely solely on *Bt* maize. For 2009-2010, we found a relatively high frequency of *Nosema* infected female moths (mean: 0.38; range: 0.06-0.87). In addition there was a weak but positive relationship between increasing *Nosema* infection with increasing frequency of female biased sex ratios. The high level of *Nosema* infection was somewhat surprising given the low overall ECB population densities, as larval-larval (horizontal) *Nosema* transmission is likely to have been quite low, particularly during the past 8 years when larval infestations have been historically low (<10 larvae/100 plants) during the annual autumn surveys. Additional analyses are underway to assess the impact of current mortality factors on ECB population dynamics.

Session 10: Population dynamics of maize pests and implications for pest management

Session Organizers: Michael ZELLNER, Bavarian State Research Centre of Agriculture, Freising, Germany and Stefan TOEPFER, CABI c/o Plant Health Station, Hodmezovasarhely, Hungary

The influence of flooding on the mortality of larvae of *Diabrotica virgifera virgifera* under Bavarian conditions

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In Bavaria there are areas which are regularly flooded by rivers. In these specific areas intensive maize growing has tradition. Out of 35,000 ha of maize in monoculture in Bavaria about 20,000 ha (57%) are located in high water endangered areas (Zellner, personally communication 2008). That makes sense. The maize has high tolerance against flooding contrary to other crops like cereals. In these flooding areas there is a higher level of fungal infestation (e.g. *Fusarium* of cereals) on others than maize crops. Up to now no information was available on the influence of flooding on the mortality of larvae of the Western corn rootworm under Bavarian conditions. It was supposed that a longer period of flooding could have an impact on the mortality of the larvae. This impact is supposed to be stronger on the first larval stage than on the second or third larval stage. Therefore the time of the year and the duration of flooding could influence the result.

Flooding of artificially infested maize plants with 600 eggs per plant were studied in climate chambers under quarantine conditions. The flooding data of June high water from the typical year 2009 in South Bavaria were used for the trial. Under these conditions flooding for 24 hours and for 96 hours with a water temperature of 13 °C in mid of June was simulated. At that time the larval stages two and three are present which mostly can be found in the roots. The first year results from 2010 presented no significance in the number of hatched beetles. The first year results were a surprise and had not been expected. The time of flooding and the water temperature could have strong influence on the result. This influence could be stronger on the first larval stage than on the second or third larval stage. Furthermore the larval stages two and three are mainly in the roots where they are tunneling within root. It is assumed that the tunnels might have stored air which helps larvae to survive.

The repetition of the trial but with a reduction of the egg number of 200 eggs per plant is still running in 2011 and will be prepared in 2012 as well. Also the results of the second year will be presented.

On the host-plant specificity and the impact of insecticides and tillage on *Diabrotica* populations

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All trials presented have been carried out in fields around Timişoara, Romania, where *Diabrotica virgifera virgifera LeConte* (Col.: Chrysomelidae) is present in significant population densities. Three years studies in cages covering 1 m² ground surface, showed no hatching of *D. virgifera* larvae in winter wheat and spring barley. Corn seed treatment with clothianidin and the application of insecticidal-granules containing tefluthrin or thiacloprid reduced the number of adult beetles compared to the untreated control plot. Results out of two years trials indicated an influence of different cultivation methods (plough, grubber, disc harrow) in autumn and spring on the population density of *D. virgifera*. The results have to be verified in the next trial season.

Simulating maize rotation strategies to develop IPM for *Diabrotica v. virgifera* in Europe

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A discrete spatiotemporal simulation model was developed to investigate the effect of maize rotation strategies against the maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. The modelled agricultural landscape was simplified into a latticewhere fields became cells defined as continuous maize, first year maize or non-maize crop. The yearly update of cells according to the rotation strategy was applied for ten consecutive years, and was determined by the proportion of maize in the modelled agricultural area, the proportion of first year maize among all maize fields (% of rotation), and the presence/absence of policy restrictions to grow maize for not more than 3, 4 or 5 consecutive years. In these lattices of cells *D. v. virgifera* adults only emerged in continuous maize fields and some dispersed among neighbouring maize fields for oviposition. In the subsequent year, i.e. after the update, a part of the population was eliminated due to rotation, and a generational growth rate was applied where maize was grown again. The model output was the proportion of maize fields reaching densities above a defined economic threshold level. Sensitivity analysis was conducted to identify key input factors among the 20 input factors of the model.

Most influential input factors appeared to be the % of rotation (a factor of rotation strategy) and the generational growth rate of *D. v. virgifera*. Astonishingly, the proportion of maize was not a key input factor in case that at least 20% and up to 60% of the fields were maize. Simulation results indicated a general pattern of model output having a low flat stage after a turning point. When at least 80% rotation was applied, the proportion of maize fields with population densities above the threshold level was low, i.e. less than ~5%, among all maize fields even at different levels of other input factors. The presence of policy restrictions could shift this turning point towards an even lower % of rotation.

Modelling of population dynamics of *Diabrotica virgifera virgifera* – examination of various containment measures

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The population development of *Diabrotica virgifera virgifera* adults was calculated using a model which compares various control measures. These measures were examined for their efficiency as containment measures. The calculation is based on a

median oviposition of 486 eggs per female and a median mortality from egg to adult of 97%. This results in an annual growth rate of 7.505 without control measures. When a control measure is taken the abundance of the population reduces according to its efficacy. The calculation covers a period of nine years.

The model calculation shows that a proportion of at least 75% maize in crop rotation resulted in a rapid increase of the population within several years. While the control of the Western corn rootworm larvae could not avoid population growth, the control of the adult beetles showed to do so. However, the efficiency of this control measure is heavily dependent on weather and environmental conditions and therefore not suitable for containment. The calculation for a proportion of 66% maize in crop rotation shows a slow increase of the Western corn rootworm abundance which could be compensated in several years when weather conditions are unfavorable. Calculations for a combination of 66% maize in crop rotation with chemical control measures in the first of year maize cultivation resulted as well in a decline of the population. Furthermore, proportions of 50% and 33% maize led to a decrease of the population also without chemical control measures.

The calculation recommends the following measures for containment:

- 66% maize with chemical control measures at least in the first year of maize,
- a proportion of maize not exceeding 50%,
- a proportion of 66% maize when the population development was controlled by continuous monitoring, and additional measures were done in case of increasing densities.

Could *Bt* maize deployment change the relative abundance of the Lepidoptera pests of maize in Spain?

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Maize crop in Spain is attacked by several Lepidoptera larvae which relative abundance might be affected by *Bt* maize deployment. *Bt* maize based on the event MON810 is highly efficient against the maize borers, *Sesamia nonagrioides* and *Ostrinia nubilalis*, but less efficient against the ear worm *Helicoverpa armigera* and the leaf-eating *Mythimna unipucta*, and not efficient at all against the cutworm *Agrotis sp*. Moreover, previous studies of our group demonstrated antagonistic effects of some of the pheromone components of several of the above mentioned species for the male pheromone perception of the others. This interference in pheromone perception may lead to changes in maize Lepidoptera community depending on the occurrence of host plants other than maize and some biological features as overwintering site and number of generations per year. Strong suppression of maize borers by *Bt* varieties may cause that the other Lepidoptera take benefit and increase

their densities. The present work analyses the potential outcome of these complex interactions in terms of the relative abundance of the 4 Lepidoptera species.

Session 11: Corn borer control and *Trichogramma*: new research and implementation insights

Session Organizers: Dirk BABENDREIER, CABI, Delémont, Switzerland & Bernd WÜHRER, AMW Nützlinge GmbH, Pfungstadt, Germany

Quality of mass reared *Trichogramma* to control the European corn borer - from laboratory to field

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The successful control of pest insects with beneficials is highly depending on the quality of the released, generally mass reared beneficials. Methods were developed to select the best species/strains of *Trichogramma*, parasitoids of the European Corn Borer (*Ostrinia nubilalis*). They include host preference, searching ability, tolerance to climatic conditions, life span and egg laying capacity. Producers are hardly working on keeping the positive attributes in mass production: rearing/passages on eggs of the target pest, treatment with extreme temperature and humidity, recollection after field releases, etc. But does all these investigations guarantee efficacy in the field? Many additional factors, like transport, storage, dates of releasing are interfering.

About 30 years ago, a network of producers, distributers, users and plant protection services was established to exchange knowledge and experience in the use of *Trichogramma*. Extensive research was investigated to simplify the test methods to control the quality of mass reared *Trichogramma*. Meanwhile approximately 20.000ha of corn are treated with *Trichogramma brassicae* in Germany – more than 4 Billion of individuals were released, but only a few ones can be checked …

Much more research is needed, especially in regions, where *Ostrinia* is newly established. Although we are talking about an “old”, well-known pest insect and an established method to control it, governmental support for further investigations is essential!

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Implementation of integrated management of Asian corn borer in DPRK and farmer participatory training for widespread dissemination

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Maize is a major cereal crop in DPR Korea (DPRK) with Asian corn borer, currently the most important pest causing an average yield loss of around 10-30%. Field studies were conducted between 2005 to 2009 at four Cooperative-Farms (10ha field plots in each farm) to evaluate the use of an integrated pest management package (IPM) to control ACB. Key components of the IPM package included hand removal of root stalks to kill overwintering larvae, light traps to capture adults in spring, releases of the egg parasitoid *Trichogramma ostriniae* and spraying of *Bacillus thuringiensis (Bt).* In each experimental field plot, after harvesting, maize straw was chopped and stored for animal feed and the root stalks were collected and burnt to kill larvae overwintering there. At the end of May when ACB adults appear, 2-3 light traps were set per hectare to trap the adults. Augmentative releases of *T. ostriniae* began when 1 or 2 egg masses of ACB were found per 100 plants. For the 1st generation of ACB, 3 releases (100,000, 120,000 and 80,000 wasps) were made at 4-6 day intervals and 2 releases (100,000 wasps each time) for 2nd generation of ACB. In addition, *Bt* was applied in the experimental plots. 1-2g of Bt granules mixed with 70kg of sawdust and placed in the medulla of the plant 8-10 days before tasseling to control 1st generation larvae and placed on the ear silk to control 2nd generation larvae. Chemical control plots were also installed, 0.3kg of deltamethrin mixed with 10kg of soil (2.5 % deltamethrin) was placed in all maize plants in 1ha 8-10days before tasseling to control 1st generation larvae and put on the ear silk to control 2nd generation larvae. Efficacy was evaluated through the parasitism of ACB egg masses, number of larval tunnels on the stem and yield increase in treated and untreated field plots. There were significant differences between IPM and chemical control plots. Parasitism rates on egg masses in IPM plots were 68.6% for 1st generation, 92.3% for 2nd generation while in chemical control plots 26.3% and 48.3% respectively. Yield increase was 21.2% in IPM plots and 6.3% in chemical plots. Therefore, it is concluded that IPM, incorporating the augmentive release of *Trichogramma*, is the most promising management tool for DPRK maize production. To ensure effective implementation of the technology in the field subsequent training focused on training farmers, with a farmer's manual on maize IPM, in Korean, produced to support this activity. Farmer participatory training was carried out and trained farmers. This should support the adoption and sustainable implementation of the ICM package for maize in DPRK.

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Establishment of mass production for *Trichogramma*: rehabilitation of Asian corn borer biological control programme in DPR Korea

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Maize (*Zea mays* L.) is a major cereal crop in DPR Korea (DPRK). A major constraint to increased production is the impact of the Asian Corn Borer (ACB), *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae). Infestation rates of ACB in DPRK can reach 100% and can typically result in yield losses of between 10 - 30%, though extremely high damage of up to 80% may occur. Previous attempts to control the ACB using *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae), the dominant natural parasitoid of the ACB in DPRK, had been initiated in the mid-1980s but had collapsed by 1995. In 2004, with funding from the Swiss Agency for Development and Cooperation (SDC), CABI Europe – Switzerland (CABI E-CH) and the Plant Protection Institute, Academy of Agricultural Sciences, Pyongyang (AAS-PPI) initiated a project to re-establish an effective maize IPM project based on inundative release of *T. ostriniae* for Asian corn borer control in DPRK. Here we will present an overview of the rehabilitation process from the initial efficacy testing of *Trichogramma* in the field, through to the introduction of Chinese technology and its adaptation to local conditions within DPRK, through to the re-establishment of *Trichogramma* rearing facilities at County level with sustainable mass production and high quality standards. In addition, the role of capacity building at all levels will be illustrated. Initial focus was on the training of *Trichogramma* rearing personnel, with a manual being produced in Korean. Finally, the extent of implementation and scale-up to date, following further financial support from SDC and EuropeAid will be presented and the current impact on maize production highlighted. It is concluded that the re-establishment of biological control of ACB with *Trichogramma* has been successful and has contributed significantly to enhanced production and food security in DPRK.

Investigations on the mass rearing and field relese of *Bracon brevicornis*, an ectoparasitoid attacking larvae of *Ostrinia nubilalis*

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In Germany the European Corn Borer (ECB) is controlled by releases of the egg parasitoid *Trichogramma brassicae* since 30 years. Since the ECB shows a second generation in some german regions and it has a tendency to a longer activity period it seems to be necessary to optimize the biological control strategy.

There is a wide range of natural enemies which could support *Trichogramma* in controlling the Corn Borer. We have selected the larval parasitoid *Bracon brevicornis* as the most promising candidate. It has regularly been found on maize fields in Germany. But the natural abundance and the level of parasitized ECB larva is quite variable. In 2003 maximum natural parasitization levels of 58 %and 89% have been reported from Saxonia / Germany. Laboratory results indicate that *B. brevicornis* has a high longevity and a preference for the ECB. As it is an ectoparasitoid and paralyses the host larvae it leads to an immediate feeding stop of the pest larva and the parasitoids offspring can multiply in the field.

Initial field trials have shown that the combination of *Trichogramma* and *B. breviconis* reduced the damage level. Though in the mass rearing after more than three years inbreeding effects and the mating strategy (single locus sex determination mechanism, slCSD) have negative effects on life table data, sex ratio and efficacy in the field. The mass rearing requires field collected individuals and a specially adapted rearing method to maintain the quality of this larval parasitoid.

Session 12: Biological control of *Diabrotica* using entomopathogenic nematodes: current progress and remaining challenges

Session Organizers: Giselher GRABENWEGER, Institute for Plant Health, Austrian Agency for Health and Food Safety, Vienna, Austria & Peter KNUTH, LTZ Augustenberg, Stuttgart, Germany

Efficacy of nematodes in biologically controlling *Diabrotica* larvae

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The use of soil insecticides and seed coating against the western corn rootworm, *Diabrotica virgifera virgifera,* is problematic due to non-target effects which resulted in a current ban from use in maize in several European countries. In an attempt to reduce insecticide use against this invader, several projects aimed between 2004 and 2011 to develop a nematode-based biological control product against the larvae of *D. v. virgifera* for Europe. Laboratory bioassays revealed that several nematode species and strains were highly virulent against *D. v. virgifera* larvae. Three nematode species tested in field experiments in Hungary also significantly reduced the number of *D. v. virgifera* and its damage. In particular, the nematode species *Heterorhabditis bacteriophora* showed a promising effect in reducing damage on

maize roots, regardless of whether applied as granules, seed coating, or fluid stream into the soil at sowing (23 field scale efficacy trials between 2004 and 2011). An increase in nematode application rate and/or an increased rainfall during the period of applications increased the efficacy of *H. bacteriophora*. The nematode survived longer than two months in field soils which is long enough to control *D. v. virgifera* larvae. Moreover, laboratory screening revealed that nematodes were able to effectively kill all three larval instars and the pupae of *D. v. virgifera*. By 2011, a nematode product based on *H. bacteriophora* was made commercially available in Germany.

The studies were funded by CTI Innovation Promotion Agency of the Federal Office for Professional Education and Technology in Bern, Switzerland (CTI P-No. 7485.1 LSPP-LS) and Landi REBA in Basel, Switzerland; and are currently funded by the Land Baden Württemberg via the LTZ Stuttgart and the Ministry of Agriculture of Germany via the JKI Braunschweig.

Persistence of entomoparasitic nematodes in maize fields with and without corn rootworm infestation

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Entomoparasitic nematodes (EPNs) are important biological control agents of arthropod pests. Several species of *Steinernema* and *Heterorhabditis* infect the larvae of the Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera*. Timing and method of the application are important factors influencing the field efficacy of EPNs against rootworm larvae. The application of EPNs is most practical when carried out during sowing of maize. This implies a considerable time lag between application and emergence of host larvae from overwintering eggs. First instar WCR larvae hatch several weeks after sowing and EPN application. Later instars, which are the most suitable for EPN propagation, occur even later. The ability of EPNs to persist in soil until suitable host stages occur is of crucial importance for control efficacy.

The persistence of a promising EPN species for corn rootworm control, *H. bacteriophora*, in maize fields during the period from corn rootworm egg hatching and larval development until adult emergence was tested. Nematodes were applied during sowing mid April in fields with continuous corn, naturally infested with WCR eggs in epidemic densities, and fields with 1st year corn, virtually free of natural WCR infestation. Additionally, small plots within the latter were infested artificially with a defined number of laboratory-reared WCR eggs after EPN application. Soil samples of these 3 variants (naturally infested, non-infested and artificially infested plots) were

taken at regular intervals within a 4 months period. They were examined for the presence of active *H. bacteriophora* by adding live *Tenebrio* larvae as bait hosts.

Results show that *H. bacteriophora* persisted in the soil of maize fields during the entire season, even when applied during sowing. Percentage of soil samples with nematode-infected *Tenebrio* larvae decreases with time, but remains on a level of up to 40% even 3 months after application. Influence of the presence of the target host and the soil type on EPN persistence ratios is discussed.

Technical and economic aspects of the use of EPN against *Diabrotica virgifera virgifera*

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The entomopathogenic nematode *Heterorhabditis bacteriophora* has been tested successfully against larvae of the Corn Rootworm (*Diabrotica virgifera virgifera*) for the last 5 years in Hungary, Austria and Italy. When applied at a dose of 1 x 10^9 nematodes per ha the results have been comparable to those obtained with chemical seed dressing with Neonicotinoids or application of granular insecticides containing the pyrethroide Tefluthrin. At higher dose of 2 x 10^9 the results were more stable at control between 70 and 90%. Although the differences are remote, in comparison to chemical insecticides the nematodes usually provided higher reduction of adults whereas less root damage was recorded for chemical insecticides. Possible reasons for this effect are discussed. The effect of nematodes is equally high whether applied during sawing of the maize or at occurrence of the larvae approximately 6 weeks later. Different application techniques have been tried and the problems with seed dressing and granular application will be discussed. Liquid applications into the drill with 200-400 litre water have provided optimal conditions for nematode establishment and persistence until the occurrence of the larvae. With the implementation of the new EU regulation 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and replacing Directive 91/414/EEC since 14 June 2011 the legal conditions favour biological control measures. Article 55 explicitly implies the promotion of the use of non-chemical and natural alternatives. Directive 2009/128/EC aims to achieve the sustainable use of pesticides Article 14 lines out that the Member States shall take all necessary measures to promote low pesticide-input pest management, giving wherever possible priority to non-chemical methods, so that professional users of pesticides switch to practices and products with the lowest risk to human health and the environment. Biological control industry is preparing to supply the markets with the necessary amounts of the entomopathogenic nematode *Heterorhabditis bacteriophora*. In 2010 the first products based on this nematode was introduced.

Different schemes for the practical application of entomopathogenic nematodes for controlling larvae of the western corn rootworm

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In 2009 the Department of Rural Areas and Consumer Protection of the state of Baden-Württemberg, has launched a project focusing on the use of the entomopathogenic nematode *Heterorhabditis bacteriophora* for the biocontrol of WCR-larvae. The aim was to develop techniques for a feasible application of the nematodes (liquid, in granular form or as nematode seed coating). 1. The liquid application, directly given into the open furrow during maize drilling, requires a water tank with agitator and an injection share. The share should insure the direct insertion of the nematode suspension before closing the seed furrow. For the application experiments in 2011 a special injection share was developed to fit to the Monosem-seed drilling machine. In all tested cases the nematode suspension could be injected into the furrow without any difficulty. 2. The application of the granule formulation along with the maize sowing was feasible using farmers own Microsem devices. However, the poor flowability of the granulates in the Microsem devices was found to cause an uneven distribution of the nematodes in the treated soil. 3. Seed Coating with nematodes was tested in 2011 for the first time. This scheme is likely to offer a new technique for nematode application against WCR-larvae. The approach is based on moistening the maize seeds and mixing them with the nematodes immediately before maize seeding starts. In conclusion, the liquid application scheme using a special injection share provides a reliable method for the successful biological control of the WCR-larvae with entomopathogenic nematodes. The project is carried out in close cooperation with the Austrian Plant Protection Service (ARGE) and CABI Europe-Switzerland in Hungary. Colleagues from these countries report on their own results on this topic.

Capsules containing EPN as a Trojan horse approach to control western corn rootworm larvae

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The western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte is a severe maize pest both in Europe and in the USA. Various strategies have been developed to control its larvae, the stage that causes root damage. These strategies include chemical pesticides, transgenic plants that produce a bacteria-derived toxin, or cultural approaches. WCR is able to evolve resistance to each of these approaches. During the last decade, efforts have been made to evaluating entomopathogenic nematodes as biological control agent. These insect-killing micro-organisms show high potential in controlling WCR larvae. The nematodes are actively recruited by

WCR-damaged maize roots, which emit a chemical alarm-signal into the soil. Using the right variety of maize and an adequate species of nematode may lead to an efficient control of the pest, comparable to chemical control. Unfortunately, application of nematodes in the field remains costly and difficult to handle by farmers. In order to solve these drawbacks, we are developing nematode-containing capsules that may easily be applied in the field. By incorporating feeding stimulants and attractants into the shell of the capsules we achieved that the larvae are as attracted by the capsule as they are by maize roots. When in contact with the right shell coating, the larvae bit small but significant amounts of the outer shell. First laboratory results are promising but the approach still needs to be fully tested in the field.

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The 24th IWGO Meeting in Freiburg/Breisgau, Germany 24 – 26 October 2011

Harald Berger

The meeting was perfectly organized by the IWGO Convenor Ulli Kuhlmann and by *KCS* Convention Service, together with the local organizers Hansjörg Imgraben, Regierungspräsidium Freiburg and Reinhard Albert LTZ Augustenberg, Stuttgart. The meeting took place in Freiburg/Breisgau, Germany and the scientific sessions were perfectly hosted in the "Schwarzwaldsaal" of the Regierungspräsidium Freiburg. Many thanks again to our local organizers!

"Historisches Kaufhaus"

The meeting was opened by the Vice-President of the Regierungspräsidium Freiburg Klemens Ficht and the IWGO Co-Convenor C. Richard Edwards.

At the end of the first day of scientific sessions (see abstracts), the group was invited by the Baden-Württemberg Ministry for Rural Area and Consumer Protection to the "Historisches Kaufhaus" (a historic shopping mall) in the center of the city of Freiburg. Mr. Thomas Berrer from the Ministry opened the "session" by cordially welcoming the IWGO participants. Dr. Kassemeyer from the State Institute for Viticulture and Enology presented different wines from the state wine institute for tasting.

Wine tasting in the „Historisches Kaufhaus"

Welcome reception with Wang Bing, He Kanglai, Wang Zhenying, Kang Song Il and Feng Xiaoping (from left to right)

C. Richard Edwards with the Croatian delegation: Tomislav Kos, Darija Lemić and Renata Bažok (from left to right)

During the second day of the scientific sessions, C. Richard Edwards presented an honorary lecture. As he is retiring as IWGO Co-Convenor, Ulli Kuhlmann presented him with a certificate as a token of thanks for 16 years of dedicated service to IWGO. C. Richard Edwards attended his first IWGO meeting in Graz, Austria, where the appearance of *Diabrotica virgifera virgifera* in Yugoslavia (now Serbia) was discussed for the first time. About 20 participants attended the Graz meeting. Over the next 10 years, IWGO meetings concerning *Diabrotica* were attended by more than 100 colleagues in several European locations and C. Richard Edwards served a Convenor of this group.

C. Richard Edwards presenting the Honorary Lecture

IWGO – Convenor Ulli Kuhlmann (right) handed an honorary gift over to C. Richard Edwards

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Tom Sappington inviting IWGO to Chicago

A delegation of scientists from DPR Korea presented two talks and two posters during the IWGO Conference (from left to right): Yu Kwang Song (CABI Project Liaison Officer), Pyon Yong Chol (Researcher, AAS-PPI), Kim Kwang Guk (Lecturer, Department of Plant Protection, PAU) and An Song Su (Researcher, AAS-PPI)

During the IWGO-Business Meeting, it was decided to hold the next meeting, which will be the 25th (our jubilee!!), in the USA: Dr. Tom Sappington, who was elected as the new IWGO Co-Convenor invited the group to Chicago for 2014 (most likely in April).

IWGO Convenors (from left to right): Ulli Kuhlmann, C. Richard Edwards and his successor Tom Sappington and Wang Zhenying

Group photo of young scientists who received funding from IOBC Global to facilitate the participation in the IWGO Conference (from left to right): Hanna Pranko (Priluki, Minsk Region), Mario Schumann (Göttingen), Ivan Hiltpold (Neuchâtel), Anne Wilstermann (Göttingen), Tomislav Kos (Zagreb), Darija Lemić (Zagreb) and Márk Szalai (Gödöllö)

During the business meeting, several books on subjects of interest to maize scientists were given to the winners of the best poster contest. There was a tie in the voting and the winners were Alois Egartner, Stefan Toepfer, Renata Bažok, Melanie Acker and Oxana Habustová. All received books.

The winners of the poster prices (from left to right): Alois Egartner (Vienna, here represented by his colleague Giselher Grabenweger) and Stefan Toepfer (Delémont) share the first price, Renata Bažok (Zagreb) and Melanie Acker (Freising) won the second price and Oxana Habustová (České Budějovice) won the incentive price for voting

A buffet dinner at the “Greifeneck Schlössle” in downtown Freiburg closed the day’s activities.

On the last day of the meeting, a public transport system strike in Freiburg made the transfer to the meeting place a little bit complicated. But, our excellent organizers provided the group with a bus transfer from the hotels to the meeting place and back.

The meeting ended on October 26 with a farewell reception.

See you in Chicago in 2014!