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## Editorial

Dear All

I am delighted to inform you that Tom Sappington, Wang Zhenying and I are making good progress in preparing the next 25<sup>th</sup> IWGO Conference, which will be held in Chicago, U.S.A., in the beginning of April 2014. Exact dates need to be confirmed but please reserve the following dates in your agenda for 2014: Sunday 6 to Wednesday 9 April plus Thursday 10 April 2014 or Sunday 13 April to Wednesday 16 April plus Thursday 17 April. Time period will be chosen according to hotel costs depending on dates.

The suggested schedule: Sunday evening through late Wednesday afternoon will be the IWGO Conference, with the usual reception on Sunday evening, and scientific programme Monday through Wednesday. We presume it could be the usual 12 sessions (with 2 or 3 sessions for the 4th International Conference on *Diabrotica* Genetics), and posters. Thursday would be a joint meeting of the North Central Corn Entomologist Technical Committees (NCCC46 and NC205) and the IWGO Conference, which anyone attending IWGO would be welcome to attend. The topics for discussion or presentation on Thursday may be more fluid, with decisions made at the committee meetings in late January 2014. This is an unique opportunity as it will combine for the first time into a single conference, U.S. corn expert entomologists with international scientists working on insect pests of maize of reciprocal concern to U.S. and Europe and potentially Asia. No doubt that the joint meeting facilitates opportunities fostering of new collaborative projects between scientists who have sometimes used different approaches to tackle similar problems.

If there is interest, we could arrange for tours of delegates before or after the IWGO meeting to visit laboratories of American colleagues within driving distance of Chicago, which probably could include visits to industry facilities as well (e.g. Pioneer in Iowa, Dow in Indianapolis, Monsanto in St. Louis; or outlying research stations like Syngenta in Iowa). These would involve driving, probably in one or two vans, and probably 2-4 overnight stays because of the distances involved, and depending on the itinerary. Wade French at Brookings, South Dakota, has indicated he would welcome visitors to the large rootworm rearing facility he manages there. However, please note that there are extra costs involved and that these activities are only associated to the IWGO Conference.

In addition we can report that Tom Sappington has submitted a Conference Grant proposal for submission to the USDA-AFRI (Agriculture and Food Research Initiative), Foundational Program to try and obtain funding to help underfunded colleagues with their travel to attend the IWGO meeting in Chicago. A travel grant will be also requested from IOBC-Global, however the grant is restricted to underfunded younger scientists (< 35 years old) holding an IOBC membership.

We will keep you updated on the process determining the date of the 25<sup>th</sup> IWGO Conference!

Ulli Kuhlmann  
IOBC-Global IWGO – Convenor

Tom Sappington  
IWGO Co-Convenor

Wang Zhenying  
IWGO Co-Convenor

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## News related to IWGO matters

- **International Conference on the German *Diabrotica* Research Programme, Berlin, 14<sup>th</sup>-16<sup>th</sup> November 2012**

An international conference was taking place in Berlin to highlight the research findings of two substantial research programmes, which were funded in cooperation with the German Ministry of Agriculture (led by the Julius Kühn Institute( JKI)) and the Free State of Bavaria (led by LfL). Objectives of the programmes aimed to obtain profound knowledge of the economic impact of *Diabrotica* establishment and quarantine measures, of sustainable chemical and non-chemical control of *Diabrotica* and to draft an expert report for quarantine and containment strategies for Germany. If you are interested in the oral and poster presentations, presentations are all available on the *Diabrotica*-Homepage:

<http://diabrotica.jki.bund.de/index.php?menuid=59&reporeid=129>

- **Current infestation of western corn rootworm in Germany**

Peter Baufeld, Julius Kühn-Institute, Institute for National and International Plant Health, Kleinmachnow, Germany, provided an update at the *Diabrotica* Conference about the current infestation/distribution of beetles in Germany. Peter Baufeldt reported that a total of 5,128 beetles were caught in 2012 (data based on catches until 03 September 2012). The highest number of beetles was detected in Baden-Württemberg in the containment zone with 4,807 beetles. This is a reduction of about one third compared to the previous year (2011: 6,119). In Bavaria the number of beetles caught were more or less stable with 293 in the containment zone compared to 2011 with 173 beetles. Furthermore, new outbreaks were reported from Baden-Württemberg in one location with 17 beetles, in Bavaria in two locations with one and four beetles, and in Rhineland-Palatinate in two locations with two and four beetles, respectively. Peter Baufeldt reported that eradication was successful in North Rhine-Westphalia in two locations, in Hessen in one location, in Rhineland-Palatinate in one location and in Baden-Württemberg in one location. After two years without any catches of beetles the eradication measures were finished in two locations in North Rhine-Westphalia.

- **GPS-assisted monitoring of *Diabrotica* & other pests using smartphones**

Manfred Rohrig, Information System for Integrated Plant Production (ISIP), Bad Kreuznach, Germany & Christian Kuhn, Central Institution for Decision Support Systems in Crop Protection (ZEPP), Bad Kreuznach, Germany reported about the development of a mobile assistant for the monitoring of the western corn rootworm using GPS capabilities of a modern smartphone. With this technology, the monitoring trap locations are determined and in the next step relocated again. The date and the number of trapped beetles can be entered directly into the device and transferred online to the server. For technological reasons a native Android application has been developed. The

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system has been tested experimentally in 2010 and was in practical use in 2011 in Rhineland-Palatinate and North Rhine-Westphalia. In 2012 the state of Saxony joined, contributing to the total number of 447 managed traps by September 2012. Due to the successful introduction of the system, further developments have two aims (1) to make the mobile application independent of the smartphone operating system of the smartphone and (2) to extend the range of monitored pests and diseases.

- **Population model for the western corn rootworm**

Tim Balschmitter, Julius Kühn-Institute, Institute for Strategies and Technology Assessment, Germany, reported about the development of a web-based simulation model of the western corn rootworm to plan the monitoring of the pest and to determine optimum treatment dates. A comprehensive survey of published literature was conducted and data analysed in order to identify and weight all relevant factors influencing the occurrence of the western corn rootworm. The relations between the influencing factors and processes in population dynamics important for the model (reproduction, mortality and ontogeny) were quantified and cross-linked to simulate population dynamics. Since spring 2011, the first version of the simulation model has been presented on the *Diabrotica* microsite of the Julius Kühn-Institute (<http://diabrotica.jki.bund.de> => Prognosemodell). The user gets predicted dates for a certain field through the use of simple GIS functions like map view, some navigation functions (zooming, moving) and site selection. Furthermore, the model offers a daily updated risk map of Germany. It is thus possible to estimate the development over the last seven days. The web-based model needs no installation, does not depend on any computer systems and may be used on mobile devices (like smartphones or a tablet PC).

- **dianem<sup>®</sup> – a product for the biological control of the western corn rootworm**

The product dianem<sup>®</sup>, based on the entomopathogenic nematode *Heterorhabditis bacteriophora*, significantly reduces root damage and adult emergence of *Diabrotica virgifera virgifera*. Field trials demonstrated that dianem<sup>®</sup> is equally effective or, with regard to adult mortality, even superior to neonicotinoide seed treatment or application of pyrethroide ganules. The nematode attacks only soil-dwelling insects, its use is environmentally friendly and exempted from regulation in most EU countries and the USA. Dianem<sup>®</sup> can be applied with conventional spraying technology. See the following website for more information: [www.e-nema.de](http://www.e-nema.de)

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## Article

### **Bioassay arenas for studying the biology and ecology of adult western corn rootworm**

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The western corn rootworm (*Diabrotica virgifera virgifera* LeConte; Coleoptera: Chrysomelidae) is one of the most destructive maize pests in the USA (Levine & Oloumi, 1991; Meinke et al., 2009). It has been in an invasive phase in Europe since the early 1980s (Guillemaud et al., 2010; Szalai et al., 2011), and is currently causing considerable economic problems in maize production of Central and South-eastern Europe, as well as northern Italy (Dillen et al., 2010; Wesseler et al., 2010).

*Diabrotica v. virgifera* also belongs to one of the best-studied insect pests (Levine & Chan 1990). Approximately 1440 grey and peer reviewed papers or conference proceedings have been published on this pest between 1910 and 2011 (CAB Abstracts 2012). There is considerable knowledge on the management of its larvae and adults (Levine & Oloumi, 1991; Vidal et al., 2005). This includes, for example, preventive control measures such as crop rotation or the choice of tolerant or transgenic maize hybrids (Ivezic et al., 2009), as well as direct control measures, such as biological control (Toepfer et al., 2009) or chemical control (Rozen et al., 2010). Also the pest's behaviour, ecology and population dynamics are well understood (Spencer et al., 2009; Meinke et al., 2009).

To support studies on this pest, several methods had been established for mass rearing *D. v. virgifera* adults, larvae and eggs (Branson et al., 1975; George & Ortman, 1965; Guss et al., 1976; Krysan & Miller, 1986). Consequently a number of different rearing cages are proposed, mainly based on different set-ups of gauze cages for adults and plastic trays with germinated maize only or with germinated maize in sterilised soil for the larvae.

However, there are few publications that include bioassay arenas. For example, Knolhoff, (2006; 2009) used cylindrical bioassay arenas with different crop plants and an inverted funnel to force adult beetles to search for the food. This was, for studying the searching and feeding behaviour of rotation tolerant rootworm populations. For a comparable research question, O'Neal et al. (2004) used two-chamber emigration arenas to assess the propensity of adult populations to leave maize during or after

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anthesis. In another assay, they used a side-arm olfactometer to test whether the relative attraction of adult beetles to other crops such as soybean was influenced by maize phenology. Toepfer et al. (2005) used metal stands on an inverted plastic funnel, surrounded by a water filled petri dish, in a gauze cage to evaluate flight take-off responses of differently marked and unmarked *D. v. virgifera* adults. A similar device was used by Li et al. (2010) to study activity difference between *D. v. virgifera* populations. A vertical tube apparatus was used to investigate the crawling activity of *D. v. virgifera* adults in response to different diets (Mabry et al., 2004). Strnad and Dunn (1990) used square glass plates (930 cm<sup>2</sup>) with moist filter paper to record the searching behaviour of neonate *D. v. virgifera* larvae for maize roots and other potential host plants. Xie et al. (1992) modified this method by using sealed 90 mm plastic petridishes to test the behavioural responses of *D. v. virgifera* larvae to hydroxamic acids applied to maize roots on filter paper. However, Knutson et al. (1999) concluded from comparable behavioural trials, that such bioassays are little useful for differentiating hosts or orientation cues of *D. v. virgifera* larvae. Nowatzki et al. (2006, 2008) developed feeding-behaviour and lethal-time bioassays for adult beetles based on 90 mm plastic petridishes, filter paper, and toxin treated agars or cellulose membrane disks. These assays allowed assessing *D. virgifera* resistances to toxins.

Altogether, however, it appeared that there is no standardised bioassay arena available for *D. v. virgifera*. We therefore propose two bioassay arenas, one for studying single *D. v. virgifera* adults or pairs of *D. v. virgifera*, and one for studying a small standardised populations of up to 20 pairs. The first was used for studying phenotypic traits of individual beetles from different *D. v. virgifera* populations under standardised conditions (Li et al., 2009; 2010); but can be used for many other standardised studies on the biology, ecology and behaviour of single or paired *D. v. virgifera* adults. The second was used for standardised crossings and rearing of differentiated small populations of *D. v. virgifera* (Li et al., in prep.), but can be used for many other standardised studies on the biology and ecology of small populations of *D. v. virgifera* adults.

## **Small size bioassay arena**

The bioassay arena consists of two ca. 140 to 200 cm<sup>3</sup> transparent plastic cups (e.g. urinalysis cups of 48 mm diameter, 80 mm high, 145 cm<sup>3</sup>, Fig. 1), stacked one inside the other (herein referred to as the upper and lower cups). The upper cup has a lid with a mesh-covered hole of 10 to 15 mm diameter to provide adequate ventilation and to prevent the beetles from escaping the arena. The upper cup also has a 10 mm hole in the bottom to allow the female to gain access to a lower, soil-filled cup for egg-laying. The bottom of the upper cup and the lower 20 mm of the wall of the lower cup are painted black to provide a dark environment for oviposition (Sherwood et al., 1993).

For most research questions, it might be advisable to work with newly emerged male–female pairs. Emerged adults can be sexed according to antenna length (Kuhar & Youngman, 1995; Staetz et al., 1976) and tarsus characteristics (Hammack

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& French, 2007). Males that have emerged before females (see protandry in *Diabrotica* sp. in Branson, 1987) can be excluded from a study, when similar age of males and females is required in an experiment. Then, males and females emerging the same day can be transferred as single male–female pairs into the rearing arenas. Each individual adult can receive a unique identification number. Abundant food can be provided into each arena depending on the aim of the experiment. Li et al. (2009), for example, provided unlimited food, i.e. of two soft unripe kernels from organically produced sweet maize, one 13 x 13 x 13 mm cube of zucchini flesh, one 13 x 13 x 13 mm cube of pumpkin flesh, and 5 x 5 x 5 mm of artificial pollen diet (Branson & Jackson, 1988; Singh & Moore, 1999). A 10 x 5 x 5 mm cube of 15% aqueous agar served as a water source for the adults. In this case, food and agar are changed every 5–7 days. Whatever the aim of the experiments is, the pairs of *D. v. virgifera* in the bioassays can be provided with the same amount of food, as *D. v. virgifera* vigour and behaviour is known to be influenced by an individual's recent diet experience (Mabry & Spencer, 2003; Mabry et al., 2004). After 10 days of maturation (Branson & Johnson, 1973; Hill, 1975) two teaspoons, i.e. a 5 to 10 mm layer, of moist, sterile black and non-sticky field soil (sieved at 0.15 mm; 25–35 w% moisture) can be placed into the lower cup of the bioassay arena. *Diabrotica v. virgifera* prefers dark soil for oviposition, but sieved sand can be also used depending on the aim of the experiment. The lower cup of the bioassay arena (containing soil and eggs) can be regularly removed (e.g. every 7 or 14 days), and replaced with a new one to collect the soil for assessing adult egg laying. Care has to be taken that females are not removed through this process as they like to sit in the soil for oviposition. Collected soil can be sieved through a 0.2-mm sieve, and recovered eggs (they are of 0.4 to 0.6 mm size) can be counted. With this approach, the age-specific and cumulative fecundity and realized lifetime fecundity can be assessed for each individual female. Moreover, individual adult life span can be assessed by regularly checking the bioassay arena, i.e. the lower and upper cups for live and dead adults. The date of death can be recorded and dead adults removed.

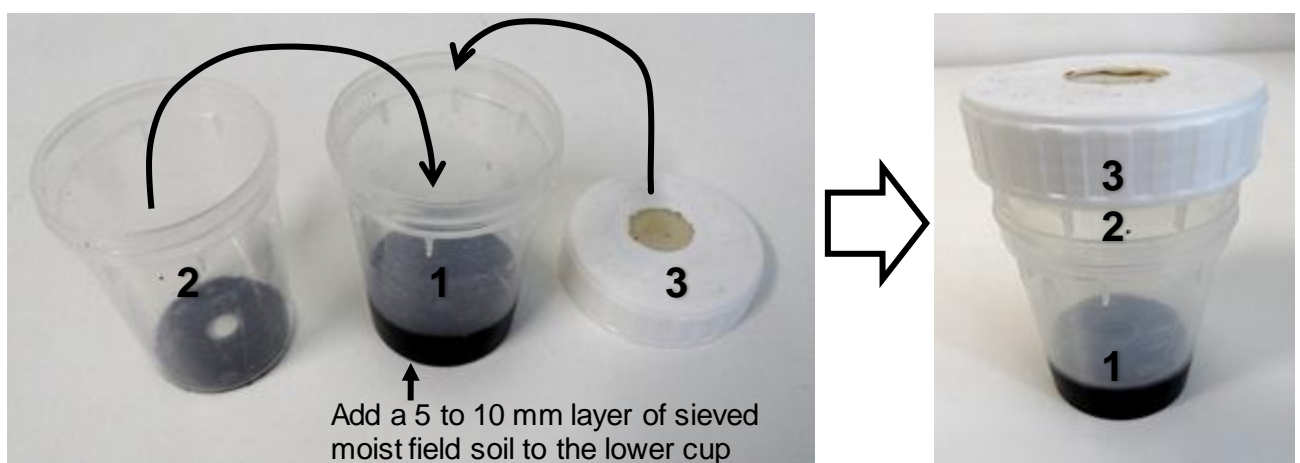


Fig. Small size bioassay arena for investigating and/or rearing single pairs of *Diabrotica v. virgifera* under standardised laboratory conditions.

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## Medium size bioassay arena

The bioassay arena consists of one ca. 1000 to 2000 cm<sup>3</sup> transparent plastic cylinder (e.g. a food cylinder of 110 mm diameter, 155 mm high, 1500 cm<sup>3</sup>, Fig. 2). The cylinder has a lid with a 60 to 80 mm mesh-covered hole to provide ventilation and to prevent the beetles from escaping the arena. A well-closing, soil-filled petridish is added into the bottom of the cylinder for egg laying (e.g. a 90 mm diameter petridish, 20 mm high, 160 cm<sup>3</sup>). The petridish has three 10 mm holes in the top to allow the females to gain access to the soil. The petridish contains four tablespoons i.e. a 10 mm layer, of moist, sterile, black and non-sticky field soil (sieved at 0.15 mm; 25–35 w% moisture). *Diabrotica v. virgifera* prefers dark soil for oviposition, but sieved sand can also be used depending on the aim of the experiment. Both, the bottom and the lower 25 mm of the cylinder, as well as the petridish lid are painted black to provide a dark environment for oviposition (Sherwood et al., 1993).

For most research questions, it might be advisable to work with newly emerged male–female pairs (see above). About 10 to 20 pairs of males and females, emerging the same day, can be transferred into the arenas. Food can be provided into each cylinder depending on the aim of the experiment. One could multiply the diet amounts proposed by Li et al. (2009) for single adult pairs (as above) with the number of pairs transferred into the arena. Food and agar should be changed every 5–7 days. All groups of *D. v. virgifera* pairs in the bioassay arenas can be provided with the same amount of food, and held under similar conditions. Every 7 or 14 days, the beetles are shortly removed with an aspirator device, and the petridish (containing soil and eggs) exchanged for a new one. Then beetles are returned to the arena. Soil can be sieved through a 0.2-mm sieve, and recovered eggs counted. With this approach, the age-specific and cumulative fecundity and realized lifetime fecundity can be assessed for a group of adults. Adult life span can be assessed by regularly checking the bioassay arenas for live and dead adults. Moreover, the transparency of the cylinder allows direct observations of adult behaviour. The medium size arena is also useful to conduct standardised crossings of males and females from different *D. virgifera* populations, and to collect their hybrid-offspring-eggs.



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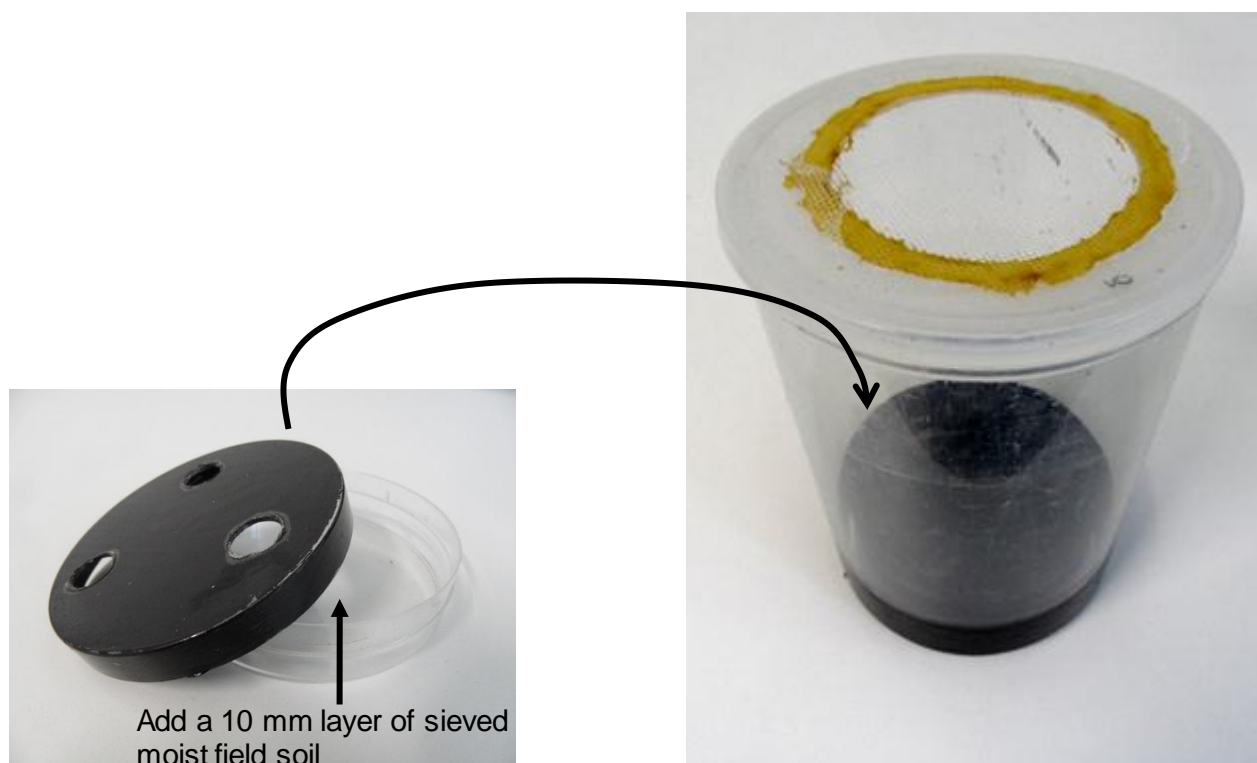


Fig. Medium size bioassay arena for investigating and/or rearing multiple pairs of *Diabrotica v. virgifera* under standardised laboratory conditions.

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## Article

### **European corn borer (*Ostrinia nubilalis* Hbn.) incidence in corn cultivated in different agroclimatic zones of Belarus**

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**Key words:** European corn borer, corn, agroclimatic zones, damage

#### **Abstract**

At present, the corn sowing areas which occupy up to 1 million hectares is expanding in Belarus. Climate warming is also promoting the European corn borer (*Ostrinia nubilalis* Hbn.) and, based on the results of research done in 2010 in the Gomel district, this species was found to affect 35 % (8601 hectares) of the surveyed area, with 0,03-1,0 caterpillars per one plant and 0,01 to 40 % of the plants colonized. In the Brest district, the European corn borer was found in 22 % of the inspected area, plant colonization by this pest from 1-10 %, with 0.01-0.1 caterpillars per plant. Corn stubble analysis carried out in 2011 in the centres of mass pest development (the southern regions of Belarus) revealed high levels of colonization by pest overwintered caterpillars (20 to 82.7 %). In the central areas this indicator did not exceed 3-5 %. During the vegetative period in the southern agro-climatic zone there was an increase of the European corn borer number and plant infestation level. Before harvest, the maximum plant affection reached 73-76 % and 6 to 34.3 % in the southern and central agroclimatic zones, respectively.

#### **Introduction**

Climate change is considered to be a threat to the ecological safety of Belarus. The mid-annual temperature for the last 120 years has increased by 1°C, resulting in a huge impact on agriculture, forestry and the water economy of the country. The rise in temperature has led to a 10-day increase of the vegetative period and a sum of temperatures of 200° C, which corresponds to for a shift of 150-200 km in latitude (to the north) of more southern environmental conditions. Such changes in the agro-ecological conditions influences the principle field crops located in the different soil-climatic zones. Now the corn sowing areas in Belarus have expanded to occupy up to 1 million hectares. The actual motivation for corn cultivation is for grain.

The European stem borer was registered for the first time in Gomel district in the Southern agroclimatic zone in 1962 as a hemp pest and did not cause any corn damage till now. However, with climate warming the situation has changed. Corn's overall prevalence in the structure of the sowing areas, its cultivation in monoculture, and the reduction of agro-technical measures has promoted pest accumulation in corn crops on the whole and for the European corn borer (*Ostrinia nubilalis* Hbn) in particular. European corn borers' wide prevalence, its rapid population growth under

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favorable agrometeorological conditions, and its high harmfulness merit its inclusion in lists of corn's noxious pests.

## Materials and methods

For objective corn agrocoenosis, the phytosanitary situation (i.e. the European corn borer incidence, number and harmfulness) was estimated using methods accepted in entomology. Corn organogenesis at different phenological phases and stages was studied at specific time intervals on the same experimental plots. Four distinct stages were defined for each phenological stage:

1. First phase if the fixed ontogenesis stage is revealed in no more than 5 % of plants;

2. The stage is beginning if it is marked in 20% of plants;

3. Mass stage is passing if it is marked in 50% of plants and more

4. The stage has ended if it is marked in 80% of plants and more.

Overwintering caterpillars and pupae were detected during autumn and spring by analyzing and dissecting stubble residues of 100 stalks selected at regular intervals diagonally across the field. At the 5-6 leaf stage, oviposition and caterpillar number were recorded by visually inspecting 10 samples each including 10 plants selected in the field diagonally. Vegetative samples were also collected and subsequently dissected.

## Results

Research on European stem borer biology has been determined that the low temperature threshold of its development is equal to 11°C, and the optimum value of the sum of active temperatures is 711°C. The rainfall necessary for pest development during summer months ranges from 200 to 300 mm. The analysis of environmental conditions of southern and central agroclimatic zones of Belarus shows that for summer months in both zones (Table 1) the amount of rain is enough. Concerning thermal resources, from the presented table it can be seen that in the central agroclimatic zone the sum of active temperatures often falls below the 100°C required for total completion of the European stem borer development, whereas, in the Southern agroclimatic zone this indicator exceeds the phytophage development threshold. Thus, it is possible to conclude that in the central agroclimatic zone the European corn borer can only develop in particularly warm years whereas in southern zone the phytophage forms a resistant incidence and harmfulness zone which is further demonstrated by our research.

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**Table 1.** Calculation of the effective temperatures sum necessary for European stem borer development in the southern and central agroclimatic zones of Belarus.

Agroclimatic zone point	Month	Average many years temperature, °C	Recorded effective temperatures sum, °C	Rainfall sum, mm
<b>Southern agroclimatic zone</b>				
Brest	May	14,0	93,0	55
	June	16,9	177,0	73,3
	July	18,2	223,2	85,3
	August	17,7	207,7	72,6
Total			<b>700,9</b>	<b>286,2</b>
Gomel	May	14,2	99,2	48
	June	17,5	195,0	84
	July	18,6	235,6	82
	August	17,6	204,6	59
Total			<b>734,4</b>	<b>273</b>
<b>Central agroclimatic zone</b>				
Minsk	May	13,2	71,3	65
	June	16,4	162,0	89
	July	18,5	232,5	89
	August	17,5	201,5	68
Total			<b>667,3</b>	<b>311</b>
Mogilev	May	12,9	58,9	53
	June	16,1	153,0	74
	July	18,1	220,0	81
	August	17,0	186,0	65
Total			<b>617,9</b>	<b>273</b>

In 2010 in the southern agroclimatic zone (Gomel area) the European corn borer was found on 35 % (8601 hectares) of surveyed areas. The pest numbered 0,03-1,0 caterpillars/plant, with 0,01 – 40 % of plants colonized. In other parts of the southern agroclimatic zone (Brest area) the European corn borer moth was found on 22 % (3221 hectares) of surveyed areas, and plant colonization by this pest was 1-10 %, with 0,01-0,1 caterpillars/plant.

The situation in corn agrocoenoses necessitated carrying out further European corn borer monitoring during the 2011 vegetative season. Analysis of corn plant residues in the southern areas of Belarus has revealed high levels of colonization (from 20 to 82.7%) by overwintered caterpillars. In the central areas, this parameter did not exceed 3-5 %. The observations carried out during the 2011 vegetative season have shown that at corn panicle formation, this phytophage numbered 0.16 caterpillars/plant with 17.5 % of the crop colonized. For this, a 14% plant infestation level was observed. At the corn milky-wax development stage (the second week of

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August) plants had an average of 1.1 caterpillars/plant, 63% of the plants were colonized, and 71% of the crops were infested. Before harvesting maximum plant infestation was 73-76%. Analysis of wintering stock in this zone indicates that 86 % vegetative residues were colonized by the European corn borer.

In the central agroclimatic zone the European corn borer incidence was not large. Analysis determined that 2-3% of the plant residues were colonized by overwintered caterpillars. During the vegetative season the European corn borer caterpillar numbers reached approximately 0,1 individuals/plant, with plant colonization up to 10%. Corn plant infestation ranged from 6 to 34.3%. Corn plant residues colonization by the European corn borer wintering caterpillars did not increase more than 1%

## Conclusions

Climate warming is promoting the European corn borer to expand to the north, which explains its colonization of corn areas increase in the central agroclimatic zone. However, in Belarus, the main area of the European corn borer incidence is located in the southern agroclimatic zone, where the centers with the highest pest number and corn plant infestation levels are found.

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## Article

### **Integrated system of corn protection against wireworms (*Elateridae*) in Belarus**

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**Keywords:** elater species, wireworms, click beetle, grain crops, corn, insecticide, pheromone trap.

## **Abstract**

In corn crops in Belarus three elater species *Agriotes sputator* L., *A. lineatus* L. and *A. obscurus* L. are widely spread. On non-rotated corn crops the aggregate population density can be 3-4 times higher than the threshold population density; and the number of affected plants can reach 33-57%. In order to improve the system of agricultural crop protection against elaters, the regulating role of agrotechnical methods was estimated. Long-term data was used as the basis for determining the assortment of effective seed dressings with insecticidal action. In order to forecast of elater numbers and their potential impact on field crops, a method for estimating wireworm population density was established using the number of pheromone-caught adults.

## **Introduction**

Wireworms (Coleoptera, Elateridae) are the primary group of soil-inhabiting pests causing considerable losses of agricultural crop yield in Belarus. In field crops in Belarus, there are 13 elater species: *Agriotes sputator* L., *A. lineatus* L., *A. obscurus* L., *Selatosomus aeneus* L., *S. latus* F., *Athous niger* L., *A. haemorrhoidalis* F., *Limonius aeruginosus* Ol., *Actenicerus (Corymbites) sjaelandicus* Muell., *Adrastus pallens* F., *Cardiophorus* sp., *Ampedus* sp., and *Hypnoidus (Cryptohypnus) sp.* (Purenok, 2005). Among them three species (*Agriotes sputator* L., *A. lineatus* L., and *A. obscurus* L.) are widespread. The number of insects varies with the proportion of crop fields to which crop rotation is applied. In non-rotated corn crops, the population density is 3-4 times high than the threshold one, and plant damage can reach 33-57%. Losses caused by elaters are expressed in crop thinning and damaged plants' depression. Losses directly depend on pest number. For the application of chemical measures against elaters to be highly effective and ecologically safe, the number of pests present must be taken into account. The labor-consuming method of soil excavation is traditionally applied to determine elater density. In other parts of the world, pheromone-based monitoring is used to forecast pest number, predict the potential harmfulness of pest populations and to plan economically proven protective measures (Parker & Howard, 2001; Milevoj et al., 2005; Vernon, 2004).

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## Material and Methods

The research was carried out on base farms of the Republic with demonstration plots of winter wheat, spring wheat, oats and corn cultivated following cultivations of other crops. Fields with unequal elater numbers were selected.

The number of soil insects was estimated by excavating at a depth of up to 30 cm using G.K. Pjatnitsky design handy drill with the working part diameter 11.3 cm (the area 0.01 m<sup>2</sup>). The invertebrates were extracted by hand layer by layer, putting soil aside using a palette-knife on an oilcloth. The collected insects were placed in test glasses in 4% concentration formalin solution (Pristavko, 1979).

On stationary grain crop fields soil samples were collected randomly from all areas of the field before sowing, before traps exposition, at the tillering stage, and after harvesting. In 10 places on each plot, dynamic records of plant damage were collected in parallel diagonally across the field on registration platforms 50×50 cm (0,25 m<sup>2</sup>) (Dolin, 1964). In isolated corn fields, the number of wireworms was recorded before sowing, before traps exposition, at the seedling stage, at the stem formation stage, and after harvesting. Before sowing and after harvesting, soil samples were selected diagonally in chessboard order. In plots of up to 100 ha, 16 soil samples were collected. In plots of more than 100 hectares, four additional soil samples were collected per every 100 ha.

Beginning with the corn seedlings stage, the number of elaters was recorded every one linear meter in 10 places. Simultaneously on these rows the dynamics of corn damage was determined where the total plants number was recorded, the number of dead and suppressed plants number was determined, and the percent of plants with damage was defined.

The average-weighted elater number taking into account the incidence was defined by formula:

$$Y = ((X*0) + (X_1*1)+(X_2*2)+ \dots + (X_n*n)) * 2 / N*n$$

where Y – average-weighted elater number per 1 linear meter;

X – number of samples without elaters;

X<sub>1</sub> – number of samples where elaters equal to 1;

X<sub>2</sub> – number of samples where elaters equal to 2;

X<sub>n</sub> – number of samples where elaters equal to n;

n – maximum number of elaters in a sample;

N – total samples number.

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Average-weighted elater colonization density before grain and other crops sowing considering the incidence was determined by formula:

$$Y = ((X*0) + (X_1*1)+(X_2*2)+ \dots + (X_n*n))*100/N*n$$

where Y – average-weighted elater number per 1 m<sup>2</sup>;

X – number of samples without elaters;

X<sub>1</sub> – number of samples where elaters equal to 1;

X<sub>2</sub> – number of samples where elaters equal to 2;

X<sub>n</sub> – number of samples where elaters equal to n;

n – maximum number of elaters in a sample;

N – total samples number.

Wireworm distribution by age category A.S.Kosmachevsky's, S.Rubelja's and V.G.Dolina's scales were used (Trepashko, 1997). Wireworms definition by species was done by B.M. Mamayev's determinant (Mamaev, 1972).

Elater impact was evaluated by applying a chemical control method to model plots with different degrees plant damage as per the E. Judenko method (Zubkov, 1983). In each crop, stationary platforms with different degrees of plant damage were selected: in grain crops – the area 0.25 m<sup>2</sup> (50x50 cm), in corn – on 1 row linear meter. In the same fields, soil was excavated near the stationary platforms in order to establish the number of elaters present. Each plot was replicated eight times. The crop from each platform was harvested separately and recalculated in cwt/ha. Wireworm adults were captured using "Estron"-type traps. The trap is made of polystyrene, in the form of a hollow cone closed from below by a cover. In the top part of a trap there is 2,5 cm<sup>3</sup> chamber where the pheromone source is located. The chamber has cracks for synthetic attractant evaporation. Assembled trap height is 7 cm, the diameter of the base is 16 cm, and it weighs 140 g (Kolesova, 1987). The Organic Chemistry Chair of Belarus State University supplied the pheromones. For monitoring a sexual pheromone of dominant elater species was synthesized AGVABATE (25VO) which has shown to be highly attractive activity to sowing (*A. sputator* L.), striped (*A. lineatus* L.) and dark click beetles (*A. obscurus* L.). An optimum pheromone concentration (10 mg/dispenser) and the carrier (an orange sponge) were used.

Traps were located at a soil surface level, 30-50 m. from a headland. In grain crops, the traps were located randomly. In corn the traps were place along the rows with 100 m distance between traps. The collected biological material was exposed to faunal processing. Definition of elater specific structure of click beetles was done by V.G. Dolina's (Trepashko, 1997), E.L. Gurevoj's (Gurieva, 1979), D. Tarnawski's (Tarnawski, 2000), and Z. Tóth's determinants.

## Results and Discussion

To improve the agricultural crop protection system against elaters, the regulating role of agrotechnical techniques was evaluated. The most effective methods were crop rotation, soil tillage (in autumn after grain crops and perennial grasses harvesting, fall

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ploughing), and double cultivation or disking before sowing in spring, nutrient balance, the observance of optimal times of sowing and seed planting depth. These methods reduced elater number by 40 to 75%, especially for the 1st and 2nd years of larval life.

In fields with a high density of elater populations it was necessary to apply insecticides. It was found that the most ecologically friendly and economically effective insecticidal action preparations were seed treatments.

Based on long-term data, the assortment of seed dressings with effective insecticidal action were identified. In corn, 12 formulations with different active ingredients are registered: imidacloprid (Gaucho, SC, Agrovital, SC, Commander WSC, Nuprid 600, SC, Pikus, SC, Taboo, WSC, Aulsal, SC, Coyote, SC), thiametoxam (Cruiser, SC), bifethrin ( Semaphore, FP), cepermethrin (Signal, 30% ES), and clotianidin (Poncho, SC). In grain crops 9 preparations are registered, with imidacloprid active ingredient (Gaucho, SC, Agrovital, SC, Commander WSC, Nuprid 600, SC, Aulsal, SC, Coyote, SC, Pikus, SC), thiametoxam (Cruiser, SC) and thiametoxam + fludioxanyl + difenoconazole active ingredient (Tselest Top, SC). The biological effectiveness of the preparations corresponded with a 45-60% reduction of elater number and a 78-95.6% reduction in damage per plant.

Insecticidal seed dressings against elaters are applied taking into account the economic thresholds of harmfulness calculated for concrete crop and their biological efficiency. The economic thresholds for spring and winter grain crops of different economic appointment and for corn for grain and green mass were calculated (Table 1).

**Table 1.** Economic thresholds of harmfulness (ETH) for elaters in corn and grain crops.

Crop	Economic thresholds of harmfulness, individuals/m <sup>2</sup>
<b>Corn</b>	
For grain	20-25
For green mass	25-30
<b>Spring grain crops</b>	
Barley for grain and forage	30-35
Brewing barley	20-25
Wheat for food purposes	20-25
Oats	25-30
<b>Winter grain crops</b>	
Wheat for food purposes	25-30
Triticale	30-35
Rye	45-50

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With the application of insecticidal seed dressings and taking into account elater ETH, it is possible to get higher economic return by using plant protection. For working out the forecast of elater number and harmfulness in field crops, the methods of formed larvae populations density definition is prepared by elater beetle quantity caught by pheromone traps. It was found that the quantity of beetles caught by pheromone traps depends on elater population density before sowing and by the number of four year old wireworm larvae. The higher number of larvae of a given age, the higher the trap catch. Analysis of elater age population structure shows that of the proportion of younger larvae (1 and 2 years of life) depends on soil type, the crop currently cultivated and the previous crop in the rotation (from 7.7 to 42.2% , the 3rd and 4th years of life – from 57.8 to 92.3% from total elater number). The collected biological material was statistically analysed. A close correlation between elater number in the soil and beetles caught by pheromone traps was found, and the conversion factors for estimating elater number were determined. The density of elater formed populations is defined by multiplication of caught beetles per one trap by corresponding conversion factor (Table 2).

**Table 2.** Conversion factors for determining elater populations density.

Crop	Conversion factors	
	at beetle peak flight	for the whole season
Spring grain crops	0.17	0.13
Winter grain crops	0.45	0.38
Corn	0.08	0.06

If it is established that the number of elaters caught by the pheromone traps is lower than the number corresponding the economic threshold of harmfulness in agricultural crops which will be cultivated the next year, planning of protective measures is inexpedient. When the number of elater caught in the pheromone traps is above the threshold value, it is predicted that elater populations density will be high enough for of the application of chemical controls to be expedient. Plant damage was estimated using regression models based on the number of elaters found. Later on, based on the percent of damaged plants, field crop yield losses were estimated using elater harmfulness parameters.

The proposed technique allows the number of elaters to be estimated in order to predict beforehand their harmfulness in field crops, and thereby to guide the rational use of chemical plant protection measures to lower the pest density to economically acceptable levels. Validation of the proposed technique under production conditions has shown high reliability, resulting in yield increases in winter grain crops up to 83.7%, spring grain crops – up to 78.0%, corn – up to 77.1%.

## Conclusions

For agricultural crop protection system improvement against elaters, the regulating role of agrotechnical methods which can decrease the pest number for 50-60% were evaluated. The assortment of effective insecticidal action seed dressings includes 13

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preparations in corn and 9 formulations in grain crops. On the average, application of insecticides reduced elater numbers by 45-60 %, and plant damage was reduced by 78-95 %. Economic thresholds of elater harmfulness in corn cultivated for green mass and grain, spring and winter grain crops were calculated. The methods of forecasting wireworm larvae is defined by the number of elater beetles caught by pheromone traps which enables forecasting of their harmfulness to field crops and in turn supports the rational use of chemical pesticides.

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## Abstracts of poster presentations 24<sup>th</sup> IWGO Conference Freiburg / Breisgau, Germany 24 to 26 October 2011

### Poster 01

#### Distribution of genes and repetitive elements in *Diabrotica virgifera virgifera*: prelude to assembling a large, repetitive genome

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The corn rootworm, *Diabrotica virgifera virgifera*, is destructive to corn plants in North America and Europe where control remains challenging due to evolution of resistance traits that allow survival when exposed to chemical and transgenic toxins. Genome sequencing of an inbred non-diapausing strain will soon be underway with the intent of devising novel tactics for control of this insect. A BAC library, DwBAC1, containing 109,486 clones with 104.4±34.5 kb inserts was created, which has an ~4.56X genome coverage based upon a 2.56 Gb (2.80 pg) flow cytometry-estimated haploid genome size. Paired end sequencing of 1037 BAC inserts produced 1.17 Mb of data (~ 0.05X coverage) and indicated ~ 9.4 and 16.0% of reads respectively encode endogenous and transposable element (TE)-derived protein coding genes. Sequencing of BAC full inserts and complete genes demonstrated that TE densities are high within intergenic and intron regions, and contribute to the increased gene size. Comparison of homologous genome regions indicated that TE movement causes haplotype variation within the inbred strain. Our data indicates that the *D. v. virgifera* genome is large and highly repetitive. Gene coding regions can be assembled from single-end 454-pyrosequencing read data, but repetitive DNA regions can cause gaps within contig assemblies. The BAC library, DwBAC1, and end sequence data presented here will be valuable resources for whole genome reassembly and future *D. v. virgifera* genomic investigations.



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## Poster 02

### Optimization of tools to monitor the development of Cry34/35 corn rootworm resistance

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The *Diabrotica virgifera virgifera* LeConte, western corn rootworm (WCR), is one of the most important pests of field maize throughout the U.S. Corn Belt both in terms of crop losses and synthetic insecticide use, and is now a major invasive pest in Europe. Crop rotation and chemical insecticides applied in sprays, in furrow or via seed treatments are some of the control techniques currently used in WCR management. Transgenic maize event 59122 expressing the Cry34/35Ab1 *Bacillus thuringiensis* insecticidal protein has gained widespread acceptance in the U.S. as a safe and effective control technology for use against *Diabrotica* spp., protecting maize roots and yield potential. Cultivation of this maize in Europe would provide growers with an alternative to managing this pest. An important component of stewardship of transgenic maize is the ability to detect changes in pest susceptibility. The purpose of this study was to establish the baseline susceptibility of WCR populations from Europe to 59122 maize using a plant-based assay. The sub-lethal seedling assay (SSA) was used to assess within and among population variation in susceptibility of neonate WCR to 59122 maize from six populations across Europe over two years. The SSA consists of exposing rootworm populations to either 59122 or near isoline (non-*Bt*) maize seedlings under controlled environmental conditions to maximize the potential for sub-lethal effects on the larval stage. The response of the WCR populations on the isoline root mats was used to account for possible differences in development across populations assayed at different times due to environmental effects. The results of the study and the applicability of the SSA method for monitoring resistance to 59122 in WCR are discussed.

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## Poster 03

### **Fecundity and longevity in female northern corn rootworm relative to body size and copulation frequency**

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A single mating usually provides female insects with enough sperm to fertilize a lifetime of eggs; however, many females mate repeatedly during their lifetime. It is believed that female northern corn rootworm (NCR) mate only once throughout their life. To investigate, we paired combinations of large, small, and average sized male and female NCR in Petri dishes. Copulating females were caged individually with plenty of food, water, and an oviposition dish and placed in growth chambers. Each week until death, females were provided with oviposition dishes and the opportunity to mate with an average sized male. Of 201 copulating females, 63 mated twice and five mated three times, but neither the proportion of females that copulated more than once nor their age at first copulation varied with cross type. Females tended to re-mate at a younger age when originally mated to small males. We found no differences in longevity or in total and viable egg numbers with cross type. Females mating more than once lived longer than singly mated females. These females also laid more eggs and viable eggs. Should greater longevity of multiply mated females and thus their greater fecundity occur under field conditions, then reproductive success of these females should exceed that of singly mated females. Female corn rootworms resistant to *Bt* corn could enhance offspring survival by maximizing fertilization of their eggs by *Bt* resistant males. More information on the reproductive biology of corn rootworms is needed to fully understand the evolution of *Bt* resistance.

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## Poster 04

### Diapause dynamics, cold hardiness, and seasonal phenology of the Asian corn borer

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Besides the necessary of a high population density of Asian corn borer (ACB) *Ostrinia furnacalis*, the most destructive insect on maize in China, and being coincided with favorable environmental conditions, higher proportion of good quality larvae within population plays an important role in promoting an outbreak. One of the criteria for good quality larvae is the ability of larval cold tolerance, especially diapause larvae. ACB larvae were collected just before or after corn harvest from 10 geographic locations throughout the country. In addition, Larvae were collected monthly from Guangzhou for two year. Their supercooling points were tested. Larvae after supercooling were reared at 26°C and 70% RH until pupae /die. Development and fecundity of uni- & bivoltine ecotypes from Jilin were compared. Average supercooling points varied among populations (diapause larvae) from 10 geographic locations, with a significant decrease from -26.0°C of Guangzhou in the south, where performing multivoltine, to -27.8°C of Jilin in the northeast, univoltine. It is about 1°C lower of uni- than bivoltine from Jilin. The supercooling points of population from Guangzhou fluctuated during the year, with an increase from -24.0°C in Dec to -10.2°C in May. However, the proportion of larvae with the supercooling point below -19.0°C, to be considered as diapauses larvae, were 40.5, 83.3, 86.5, 30.3, and <14.8% in Nov, Dec, Jan, Feb, and other months. This indicated that some of larvae were diapause during the winter even though it could develop continuously during the year. Diapause larvae from Jilin were able survive freezing and developed to pupae indicated that cold adaptation of ACB was freeze tolerance rather than freeze avoidance.

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## Poster 05

### Two-year field study demonstrates no negative impact of GM maize expressing Cry3Bb1 toxin on non-target arthropods

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Potential adverse impact of genetically modified (GM) plants on non-target arthropods is one of the concerns addressed in a pre-commercial environmental risk assessment of them. GM maize MON 88017 expressing the Cry3Bb1 is known as resistant to the target pest - Western corn rootworm (WCR, *Diabrotica virgifera virgifera*), quarantine pest rapidly spreading in Europe. It also contains CP4 EPSPS synthase gene which enables to plant tolerance to herbicides based on glyphosate. To verify environmental suitability of GM maize, it was planted along with a non-GM isogenic cultivar in two treatments, one with the insecticide Dursban 10G with chlorpyrifos as an active substance and the other without it, and with two reference cultivars KIPOUS and PR38N86. Each treatment was planted on five 0.5 ha plots (25 plots in total) on 14 ha field trial. The content of Cry3Bb1 in MON 88017 quantified by ELISA test depended on the maize tissue and varied during growing season. The occurrence of WCR was monitored with 10 pheromone traps Csalomon PAL which were placed on plants at the margins of the experimental area. The region infested with WCR is still growing and in 2010 WCR was found only 90 km far from the field trial. According to our monitoring WCR has not yet dispersed in the field trial region. The diversity and the abundance of flying predators and parasitoids (mainly hoverflies, lady birds, ichneumons, braconids) plant dwelling (mainly thrips, aphids, pirate bugs) and epigeic arthropods (spiders, ground and rove beetles) were monitored twice, four times, and six times per season, respectively. Multivariate analysis and analysis of variance did reveal any differences in neither species composition nor abundance of the non-target arthropods during the two years of study. This study was supported by the National Agency for Agriculture Research (grant QH91093) and was facilitated by research infrastructure built in the 7FP project MOBITAG (REGPOT-2008-1, GA 229518).

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## Poster 06

### **IDIAM –PROJECT- Analysis of genetic variability and identification of genes involved in rootworm damage tolerance in maize\***

C. Lanzaova, N. Berardo, P. Valoti, H. Hartings, A. Torri, M. Motto & C. Balconi

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Western corn rootworm (WCR) (*Diabrotica virgifera virgifera*), is a devastating maize pest in North America and recently in Europe. The major damage to maize plants is caused by larval feeding on roots; the adult stage can cause silk clipping with low fertility of the ear and reduced production. Plants resistant to insects lead to a reduction in production losses, a decrease of the costs of insecticide treatments and improved food safety for animal feed and human. The most important methods to control this pest are crop rotation, insecticide application, use of resistant maize varieties by classical plant breeding, or use of transgenic approaches. Maize expressing *Bacillus thuringiensis* (*Bt*) toxins or the Caryophyllene synthase gene, responsible for (E)-  $\beta$ -Caryophyllene production in maize, were used as protection from pests. The identification of genes and molecules underlying the defensive plant response against the corn rootworm is of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae. The main topics of our research involve: i) analysis of genetic variability and identification of hybrids with reduced-radical damage ii) identification of genes underlying the plant response to damage by corn rootworm iii) validation of candidate genes and polymorphisms mapping. In our laboratory, experiments to set up an artificial WCR eggs inoculation method on B73x Mo17 hybrid are addressed to obtain root samples for differential gene expression analysis in comparison with controls. In addition, the artificial inoculation method could be useful for a preliminary analysis of genetic variability for rootworm tolerance in maize.

\* Research developed within the project: "IDIAM- Strategies to reduce the spread and damage by Western Corn Rootworm (WCR *Diabrotica virgifera virgifera*) in maize Italian crop", funded by the Italian Ministry of Agricultural Food and Forestry Policies".

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## Poster 07

### Integrated system of corn protection against elaters in Belarus

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In Belarus in corn crops three elaters species *Agriotes sputator* L., *A. lineatus* L. *A. obscurus* L are widely spread. The insects number is essentially differs in the share of rotation fields of cultivated crops. In separate corn crops the density of formed populations 3-4 times increases the threshold one, plant damage can reach 33-57%. Losses caused by elaters are expressed in thinning of plants, inhibition and death of damaged plants and directly depend on the pest number.

The agrotechnical techniques role is evaluated which can decrease the pest number from 40 to 75%, in particular the 1-st and 2-nd year of life larvae. It is necessary to apply insecticides in the fields with high formed elaters population density. It is determined that the most ecological friendly and economically effective is the pre-planting seed dressing by the insecticide-action preparations.

Based on perennial data, an assortment of the effective insecticide action seed dressers is formed which includes 13 preparations in corn, 9 – in grain crops. The insecticides application decreased the elaters number, on the average, for 45-60%, the plant damage for 78-95%.The economic thresholds of the expediency of insecticides application against elaters in corn cultivated for green mass and grain, spring and winter grain crops are calculated.

For the development of elaters number and harmfulness forecast in field crops the methods of determination the formed larvae populations density is prepared by the number of caught click beetles by pheromone traps. The objective evaluation of elaters number allows to forecast beforehand their harmfulness in field crops, calculate the rational rate of preparations application, differentially apply chemical plant protection products. The monitoring is accomplished using the synthesized sex pheromone which has shown high comparable attractiveness activity in relation to dominant click beetles species.

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## Poster 08

### Efficiency of pheromone traps for monitoring *Diabrotica*

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Mark-release-recapture trials with male western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) beetles were conducted to better understand capture data of pheromone traps produced in Europe during monitoring programmes. Median recapture rate in maize fields in Hungary, Italy and Switzerland was 10%. Two types of sex pheromone traps (sticky sheet and non-sticky container traps), placed inside or outside maize fields, showed no differences in efficiency. Logit analyses of recapture data in maize showed that at distances of less than 1 m, fewer than 20% of beetles ended up in the traps. A beetle in a 1 ha maize field would have about a 5% chance of being caught in a trap placed in the centre of the field.

## Poster 09

### Evaluation of western corn rootworm larval performance on conventional maize cultivars in a soil - less bioassay

M. Schumann, K. Reibe & S. Vidal

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We tested a new bioassay to evaluate the performance of western corn rootworm (WCR) larvae on conventional maize cultivars. In ordinary glass tubes surface sterilised maize seeds were placed on a glass bead layer (3 – 4 cm). The glass beads were previously coated with a transparent agar/nutrient mixture (Phytigel™) to enable root growth. This set up creates a transparent medium for a direct observation of WCR behaviour and an easy and quick recovery of the larvae from the maize roots. Three neonate larvae were placed on roots of a 17 day old seedling and larval development and fitness parameters (fresh and dry weight) were measured on day 3, 7 and 11 after insertion. At these time intervals larval performance could be determined at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> larval instar stage, respectively. 78% (~2.3 larvae/sample) of the larvae could be recovered from the medium for analysis. Differences in larval weight and development between the cultivars were detected at every instar stage. Overall we could group the suitability of the tested cultivars according to larval performance.

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## Poster 10

### **A new method for efficacy testing of control measures against adult *Diabrotica* in maize**

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Efficacy of control measures against pest insects is often assessed with relative sampling methods, like the employment of traps over a given time. However, these methods do not show the direct effect of the control measure to be tested, e.g. the mortality caused by the insecticide. In fact, they show the abundance of alive insects, which is influenced by the tested product together with many other factors, including mobility of the test species. This may be a challenge with highly mobile study subjects, like the western corn rootworm, *Diabrotica virgifera virgifera* (WCR).

In the presented poster, a field method to determine the mortality of adult WCR after treatment of maize fields with an insecticide is described. Fields with continuous corn and heavy WCR infestation were treated with a neonicotinoid insecticide and compared to untreated control fields. Efficacy of the treatment was assessed with yellow sticky traps and with a newly developed method for mortality assessment. The latter consisted of cotton panels mounted between the stems of four corn plants of two neighbouring rows, in order to collect dead beetles dropping from the plants beyond the covered area.

After insecticide application, the number of dead beetles collected with these panels in the treated plots was significantly higher than those in the control plots only 1 day and 3 days after application. However, no differences were found 7, 14 and 21 days after application. At the same time and in the same fields, the number of beetles caught with yellow sticky traps dropped significantly after insecticide application. Differences between treated plots and control plots were significant 1, 3 and also 7 days after treatment. Results of yellow sticky traps therefore suggested longer activity of the insecticide than proved by the mortality assessment with panels. Direct mortality assessment methods may therefore be important tools for the validation of control measures in field tests.



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## Poster 11

### Preliminary results on the western corn rootworm management in northwestern Italy

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most important pest species of maize in several countries of central and eastern Europe, and first reported in Italy in 1998.

The project “IDIAM- Strategies to reduce the spread and damage by Western Corn Rootworm (WCR *Diabrotica virgifera virgifera*) in maize Italian crop”, funded by the Italian Ministry of Agricultural Food and Forestry Policies, was set up with the aim of evaluating the effectiveness of different control strategies in NW Italy under natural infestation.

Field experiments were performed in the two-year period 2010-2011 in Lombardy and Piedmont regions, using the commercial maize hybrids Pioneer PR32G44 and P1758 in 2010 and 2011, respectively. The experimental design was a randomized complete block with four replications of each treatment. Different planting times, granular/liquid soil insecticides (Teflutrin/Clorpirifos) applied at planting and at earthing up, and insecticide-coated seeds (Clothianidin) were compared to untreated plants.

Surveys were made in order to obtain data about the larval/pupa infestation, the root damage (using the Node Injury Scale), the production of new roots and the grain yield. Monitoring of adults was made using yellow sticky traps (Pherocon® AM/NB trap), replaced weekly from the first adults' appearance for 6 consecutive weeks. Preliminary results and the further progression of the work are described and discussed.

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## Poster 12

### The *Diabrotica* infestation episode of 2007 at the eastern shore of Lake Constance, Germany

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In its quest for conquering major parts of Southern, Eastern and Central Europe, *Diabrotica v. virgifera* (Col: Chrysomelidae) (WCR) attempted to settle in Southern Germany which until 2007 had no reported occurrence of WCR. Suddenly, during the summer of 2007, however, not only Bavaria and the upper Rhine Valley, but also the area around the eastern shore of Lake Constance near Salem reported WCR in some of the pheromone monitoring traps.

While the monitoring of the State of Baden-Württemberg was still ongoing, additional Metcalf traps were established in the vicinity of Salem, Rickenbach, Frickenhausen and Friedrichshafen. These traps yielded five more WCR adults during August of 2009 which were not picked up in the regular monitoring traps. We reported and added these counts to the official tabulation of WCR monitoring within the official state monitoring program.

Fortuitously, the newly detected infestation of 2007 was recognized in time for initiating an emergency spraying program with Biscaya R. Its success was evident from the fact that in the following summer 2008 no more beetles were detected in the immediate vicinity of Lake Constance while the spreading of WCR both in the Danube and in the upper Rhine Valley continues.

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## Poster 13

### European corn borer incidence (*Ostrinia nubilalis* Hbn.) in corn cultivated in different agroclimatic zones of Belarus

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The corn sowing areas in Belarus are nearly 1 mln. ha. Corn is mainly cultivated for grain in the Southern and Central regions of the country the agroclimatic conditions of which are favorable for a noxious corn pest development – European corn borer (*Ostrinia nubilalis* Hbn.).

On the territory of the Republic the European corn borer was registered in 1962 in Gomel district as hemp pest. Rise in temperature facilitated the European corn borer population number increase and based on the results of researches carried out in 2010 in Gomel district this species was discovered on 35% (8601 ha) inspected area at 0.03-1.0 caterpillar per plant and plant colonization 0.01 up to 40%. In Brest district the European corn borer was discovered on 22% (3221 ha) inspected area, plant colonization by pest has made 1-10%, at 0.01-0.1 caterpillar per plant number.

In the focuses of mass development (Brest and Gomel districts) stubble colonization by hibernated European corn borer caterpillars was high – from 20 to 82.7%. High air humidity facilitated the rapid pupation completion (in the course of 20 days), moderate warm weather in June-July in spite of lack of precipitation promoted mass caterpillars hatching. As a result of it, at corn panicle stage – start of anthesis the crops damage more than 46% was marked.

In the Central agroclimatic zone in 2010 the European corn borer was met only on 5% of the inspected area, the colonization has made 1-5% of plants at 0.01-0.02 caterpillars per plant number. In 2011 the European corn borer was discovered on 12 % of the inspected area at corn plants colonization 1-2%.

So, the main area of the European corn borer incidence is located in the Southern agroclimatic zone of Belarus. Corn crops colonization increase in the Central agroclimatic zone testifies to deepening the European corn borer area to the North, what makes the necessity of monitoring organization, development of a measures system on the pest number and incidence restriction on the territory of Belarus.

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## Poster 14

### **Western corn rootworm: experiments on the melioration of the monitoring at low population densities**

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To evaluate the existing containment measures, a control of monitoring is essential. Few experiences are available for monitoring at low population densities as currently observed in Germany. This project focuses on different aspects like types of traps used so far, other types of traps as well as the position of the traps in the field. Another part of the project investigates the efficiency of pheromones stored over a longer period.

Therefore large-scale field trials in Bavaria and Upper Austria were designed in 2009, 2010 and 2011:

- Experiment: Comparison of different types of traps (pheromone traps, traps with a biological agent, sticky traps, insecticidal traps, yellow sticky traps, different combinations)
- Experiment: Investigations of the position of traps in the maize field (1., 7., 14. row of the border of the maize field)
- Experiment: Investigation of the efficiency of pheromones stored during winter period

Significant differences in the catchability of different types of traps were identified.

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## Poster 15

### Survival analysis of adult *Diabrotica v. virgifera*

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In order to develop models on the population dynamics of the maize pest *Diabrotica virgifera virgifera* (western corn rootworm, Coleoptera: Chrysomelidae) survival rates of its eggs, three larval instars, pupae and adults need to be known. In contrast to the already studied and reported survival rates for immature stages, the survival of *D. v. virgifera* adults had not been entirely clarified under field conditions. Particularly the understanding of the likely main period for oviposition of *D. v. virgifera* populations in the field (after a pre-oviposition period) would help to understand the timing for interventions by direct control measures against the adults. Therefore, the survival of *D. v. virgifera* adults was studied in two field sites between 2009 and 2011. Between 8 and 22 large gauze cages (ca. 4 x 2 x 2 m) were placed into each of the two study fields, and 50 newly emerged female and 50 male adults released in each cage (usually in mid-July) of each year. Survival was recorded weekly until no beetles were found any more, i.e. usually in September. Survival analysis is currently developed and discussed for their use in understanding and modelling population dynamics of *D. v. virgifera*.

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

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## Poster 16

### Current monitoring, distribution and strategies for eradication and containment of *Diabrotica virgifera virgifera* LeConte in Baden-Württemberg 2011

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At the end of 2010 in Baden-Württemberg the western corn rootworm, *Diabrotica virgifera virgifera* LeConte was present in several districts along the Rhine. From the southern border to Switzerland up to the Ortenaukreis. In total 308 beetles were caught in 65 pheromone traps (PAL) from July to September. In this area eradication and containment zones in the districts were enforced as follows: Lörrach eradication and observance, Ortenaukreis and Emmendingen containment zone, Breisgau-Hochschwarzwald plus City of Freiburg merged with the existing containment zone from neighbouring districts Ortenaukreis and Emmendingen. *D. virgifera virgifera* LeConte appears mainly in the western parts of these districts in the Rhine plains where arable crop production takes place. A minor number of beetles were found in the eastern parts where grassland is dominating the agriculturally cultivated hills of the Black Forrest. On 19 July 2011 a small number of beetles were found in PAL traps that were positioned in the district of Rastatt close to the borders of the containment zone of the Ortenaukreis. These beetles are part of the natural expansion. Therefore the existing containment zone will be expanded to encompass the district of Rastatt. Already in July 2011 the high number of more than 1000 beetles was caught. This supports the modelled high population increase in fields where maize follows maize. In the containment zone the cultivation of maize is allowed twice in any period of three consecutive years (2:1 crop rotation). 2011 will be the last year for the containment zone in Konstanz if no beetle is found during the course of this year. The catches in year 2011 will have to tell if the expected growth rate of 7.505 per generation will be reached, how effective the containment strategy 2011 will be, whether the containment zone of Konstanz can be abrogated or regions hitherto free of the western corn rootworm will be infested.

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## Poster 17

### Root damage caused by WCR larval feeding used as a predictor of maize plant lodging and yield loss

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It is proposed that western corn rootworm (WCR) larval damage can be an indicator of plant lodging and yield loss in Croatia. The aim of the investigation was (1) to find out if WCR larval damage correlates with plant lodging, (2) to determine the regression curve between WCR larval damage and percent plant lodging and (3) to determine if the root damage rating can predict plant lodging and yield loss. Ten maize fields were selected for this study in 2010 in the region of northwest Croatia. In each field, four areas containing 100 plants were inspected. Plants were grouped in one of the following three categories: up straight plants (USP), partially lodged plants (PLP) - plants lodged up to 45° when compared to USP and fully lodged plants (FLP) - plants lodged between 45-90° when compared to USP. For each category, roots were rated according to the Oleson et al. (2005) 0-3 Node-Injury Scale. Based on the average root rating (RR) of each plant category and on the ratio of each plant category for each field, an average RR was calculated. In each of three selected fields from each grouping, four samples containing ten plants were harvested. The average yield was determined for each category and based on the ratio of each category in a particular field the average yield was calculated. The yield loss was calculated by comparing obtained yields with the yields of USP plants in each of the selected fields. The average RR of USP was 0.42 and did not significantly differ from those of PLP (0.88), while the average RR of FLP (1.78) was significantly higher. The correlation between plant category and RR can be described as strong ( $r=0.7413$ ,  $P=0.0001$ ). We found differences between fields in the percentage of FLP, as well as in average RR. A significant ( $p=0.0001$ ), strong correlation ( $r=0.8938$ ) between the average RR and percentage of FLP was determined. The regression curve was linear and the regression coefficient was 37.45. The yield loss in the field where 65.5% of the plants were lodged ranged from 0 to 57.16%.

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### Development of a phenology model of *Ostrinia* in Switzerland

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A phenological model of the 1st-generation European corn borer, *Ostrinia nubilalis* is being developed. The model includes a time-distributed delay and is based on observations and literature data of developmental duration of juvenile stages depending on temperature. After calibrating temperature, the model provides a good simulation of observations at one location in Switzerland over 30 years. The model will be compared to other observations not used for calibration and necessary adjustments to the model will be made.

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### A decade of forced cohabitation with the invasive pest species *Diabrotica v. virgifera* in South Switzerland

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



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increase was quite different from exponential growth during the trial period. Nevertheless, root damage and goose neck symptoms were observed in three years in the monoculture field.

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

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### **Influence of primary tillage on population size of *Ostrinia nubilalis***

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The European corn borer (*Ostrinia nubilalis*) is the most important pest in maize and causes enormous problems to more and more farmers in Germany. In case of a massive infestation, substantial losses in yield and quality do appear. The most promising precaution is the deep ploughing of the maize stubbles and the maize straw.

To test the influence of ploughing on the corn borer mortality, a field experiment with emergence cages was conducted, where the ploughing-down of maize straw was simulated. The experiment was set up at two different dates, as well as with two different treatments; one with straw cover on the surface and one without.

A high number of *Ostrinia* larvae can leave the ploughed-down maize straw and colonise the straw which covers the soil. The main period for the colonisation is in autumn, but it can drag on until pupation. A clean ploughing-down of the maize straw is the most effective prevention; no other measures are necessary. The time of primary soil tillage doesn't seem to have an influence on the mortality rate. Dry and warm autumn weather conditions enhance the corn borer population.

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### **Does root feeding of WCR larvae influence the rhizosphere microbial communities?**

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This study aimed at investigating the effect of root feeding of Western Corn Rootworm (WCR) larvae on the microbial communities inhabiting the maize rhizosphere. Greenhouse experiments under quarantine conditions were performed with four maize cultivars grown in three different soil types, in presence and in absence of WCR larvae. 16S rRNA gene and ITS fragments PCR amplified from the total rhizosphere community DNA were analyzed by denaturing gradient gel electrophoresis (DGGE) in order to investigate changes in the bacterial and fungal community structure, respectively.

16S- and ITS-DGGE analysis showed that WCR larval feeding affects, in a soil type and cultivar dependent manner, the fungal and bacterial populations inhabiting the maize rhizosphere. Bacterial communities DGGE patterns showed, upon WCR larval attack, an increased abundance of a specific bacterial population in the rhizosphere of most of the cultivars in all soil types. The consequences of such shifts on the rhizosphere microbes induced by WCR larval feeding remain to be explored.

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### Western corn rootworm development under constant and varying temperature regimes

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Prediction of Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) hatch and occurrence in the field depends on models using experimentally determined degree days (°D). For constant temperature regimes this sum is a reliable parameter to predict the hatch and development of WCR larvae. We evaluated in our experiment the effects of varying day-night temperature regimes compared to adequate constant temperature regimes in climate cabinets. The experiment resulted in significant differences of in the amount of the required temperature sums for hatching and larval development, indicating that varying day-night temperatures regimes need to be taken for calculating models on WCR development and optimal timing of control strategies. The effect of temperature depending plant growth, interacting with WCR larval development, will be discussed.

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### Observations of alternative host plants in fields in Italy

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*Diabrotica virgifera virgifera* LeConte is considered to be specialized on maize (*Zea mays* L.), but various research with artificial infestation has shown that larvae of the beetle can survive on some plants other than corn and, in some cases, adults emerge. In order to better understand the role of some alternative hosts to support the life cycle of the western corn rootworm in the field, some preliminary data were acquired. In Lombardy (Italy), where the pest is present since 2000, three fields with a high infestation were sown with possible host species. In 2010 two fields were sown with 16 species and in 2011 one field was sown with 11 species. The host plants were both cereals and forage species, chosen among species never tested before or species that showed contradictory results in previous tests. Surveys for the presence of larvae and pupae in the soil and in the roots were made each week within the preimmaginal period. Width of the head capsule was measured to ascertain the instar of the larvae. In order to observe the adults' presence, emergence traps were placed in the fields and checked weekly. In both years immature stages were found on the same 3 species, while in the second years *Diabrotica* was found also on 4 other species. No adults emerged from the alternative

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hosts. This preliminary information suggests that larval development can occur on other gramineous species also in the field, and these species have to be taken into account within management programs.

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### **Harnessing the pre-existing training infrastructure within DPRK to support the knowledge transfer of Asian corn borer IPM technology**

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Maize, *Zea mays* (L.) is a major crop in DPR Korea (DPRK) which plays an essential role in ensuring food security. A major constraint to production is the Asian Corn Borer (ACB: *Ostrinia furnacalis*). Yield losses due to ACB can be significant, with infestation rates reaching 100% in many instances. Conventional control options are unavailable and/or ineffective, and thus integrated pest management (IPM) has been developed as an alternative strategy, within a project funded by the Swiss Agency for Development and Cooperation (SDC). To support the implementation and adoption of the IPM approach in maize production throughout DPRK, rapid and efficient knowledge transfer is essential. Based on their extensive, pre-existing training channels, the agricultural universities of DPRK were selected as main partner for full implementation of this knowledge transfer.

Training was mainly conducted by the Pyongyang Agricultural University, Kim Il Sung University, Pyongyang (PAU) supported by CABI Europe-Switzerland (CABI E-CH), between 2008 to 2011, focusing on knowledge transfer to professors/lecturers, students and farm engineers. Training followed a logical progression. Initially, 5 professors/lecturers of the Department of Plant Protection, PAU (PAU –DPP) were trained as master trainers. These master trainers subsequently trained 15 professors/lecturers of the departments of plant protection from 5 provincial level agricultural universities and 1 national Further Education Centre. These agricultural universities have trained approximately 1000 students in ACB IPM in 2010 and will continue to do so in subsequent years

Use of pre-existing university training channels enabled a rapid knowledge transfer across a broad spectrum of stakeholders within the educational and applied farming communities. This rapid transfer of knowledge to the field, the future farm engineers and trainers/educators at all key educational stages should support the future implementation of IPM within the maize production system of DPRK. The associated development of educational material and training packages linked to maize IPM will be presented. In addition, new training packages, through the county level agricultural colleges are planned with the aim of improving training and knowledge transfer at the farm level.

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### Tritrophic interactions among maize, western corn rootworm, and soil entomopathogens

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Agricultural systems often provide a model for testing ecological hypotheses, while ecological theory can enable more effective pest management in agricultural systems. One of the best examples of this is the interaction between host-plant resistance and natural enemies. With the advent of crops that are genetically modified to produce insecticidal toxins from the bacterium *Bacillus thuringiensis* (*Bt*), a new form of host-plant resistance has been introduced to agro-ecosystems. How *Bt* crops interact with natural enemies, especially insect pathogens in below-ground systems, is not well understood, but provides a unique opportunity to study below-ground tritrophic interactions. We report the results of laboratory and greenhouse studies that test interactions among a community of entomopathogens, maize engineered with event DAS-59122-7 that expresses the insecticidal *Bt* protein Cry34/35Ab1, and larval western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), an obligate root feeder and a serious pest of maize. Laboratory preference test revealed that western corn rootworm larvae prefer to feed on non-*Bt* maize, while greenhouse experiments found that larval development is delayed on Cry34/35Ab1 maize. In two separate experiments, one in a greenhouse and one in a growth chamber, we tested effects of tritrophic interactions with a fully crossed design consisting of two maize treatments (Cry34/35Ab1 maize and non-*Bt* maize) and two entomopathogen treatments (present or absent). The entomopathogen community included both entomopathogenic nematodes and entomopathogenic fungi. The community of entomopathogens significantly increased mortality of western corn rootworm, and *Bt* maize increased larval developmental time and mortality. Entomopathogens and *Bt* maize acted in an independent and complimentary manner to reduce survival of western corn rootworm. Results from this study suggest that entomopathogens may complement host-plant resistance factors to reduce herbivore survival in some below-ground tritrophic interactions.

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### ***Sorghum, Miscanthus* & Co: energy crops as potential host plants of western corn rootworm larvae**

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One of the most important crops grown for the production of biomass is maize. To reduce negative effects of large scale and continuous cultivation of maize a number of alternative plant species have been suggested. Although there exists a considerable number of studies evaluating economic, environmental and social aspects of their cultivation, direct or indirect effects on agricultural pests are rarely investigated. Especially whether these plants are suitable host plants for the larvae of the western corn rootworm (WCR) is largely unknown.

Here we present results of a series of greenhouse experiments that evaluated the ability of WCR larvae to develop on plant species proposed as alternatives to maize in order to avoid large scale, year after year maize monocultures and the build up of high rootworm densities.

The development of WCR-larvae was monitored on 49 plant species/varieties, including 18 *Sorghum* species/varieties, 16 field grasses (including a number of *Lolium* and *Festuca* varieties), 6 switch grass varieties, *Miscanthus xgiganteus* and 5 other *Miscanthus* species/varieties and 3 broadleaf species. To assess the host quality of these plants the number of surviving larvae, larval head capsule widths and dry weights were recorded. A susceptible maize variety was used as control.

Some of the field and switch grasses were suitable host plants for WCR larvae, but the number of larvae that survived and their dry weights were significantly less than that recorded when fed on maize plants. The roots of most of the 18 *Sorghum* species or varieties tested were unsuitable for larval development. The number of larvae that could be recovered, their weight and head capsule widths when tested on *Miscanthus xgiganteus* were the same to those recorded for larvae reared on the maize control. Most of the other *Miscanthus* species/varieties examined were less suitable for WCR larvae. In accordance with previous studies no larvae developed on the three broad leaf species.

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### **Entomopathogenic nematodes for the biological control of soil-borne maize pests in DPR Korea: development of a locally adapted mass production system**

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Soil-borne insect pests are a major constraint to agricultural production in DPR Korea, particularly in maize. As most of these pests severely damage the early growth stages, particularly the seedlings of maize, they can significantly impact on the final yield. Controlling soil-borne pests is a considerable challenge due to their concealment below-ground, which makes detection difficult. Since DRPK generally lacks technologies to control such pests, an effective and locally feasible control strategy is urgently required. Therefore, this four year project aims to enhance the control of key soil pests using entomopathogenic nematodes that are mass-produced locally through self-reliant and sustainable procedures. Here we present the development of the locally adapted mass production system as it progresses from the initial steps of understanding the dynamics of the key pest species in maize and other crops within DPRK, and the identification of indigenous entomopathogenic nematodes through in-country surveys. In parallel, the development of experimental production facilities and the development of a locally adapted system is currently being pursued. The role of capacity building in-country and abroad is also emphasized. The future development of the locally adapted mass production system and its projected impact on crop production is highlighted in view of improved food security in DPRK.

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### Species composition of main natural enemies of maize pests in DPR Korea

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Maize is the second most important cereal crop and covers approximately 50% of cultivated area in DPR Korea. Asian corn borer (*Ostrinia furnacalis*), Oriental armyworm (*Mythimna separata*), Black cutworm (*Agrotis ypsilon*), Scarab beetles (*Holotrichia diomphalia*, *Maladera orientalis*) and corn aphid (*Rhopalosiphum maidis*) are the main pests of maize. It is very important to control these pests to obtain high yield.

To evaluate the importance and species composition of natural enemies of above mentioned pests, extensive surveys were conducted in DPR Korean maize fields. Natural enemy insects identified were from 8 Orders, 26 Families, 105 Genus and 162 Species. Of these, 56 species (34.6%) are parasitoids and 106 species (65.4%) are predators. Natural enemies found are targeting eggs (19 species, 11.7%), larvae (114 species, 70.4%), pupae (19 species, 11.7%) and adult stages (10 species, 6.2%). 224 species, including these 162 species of main insect natural enemies of maize pests were covered in the book “Colored book of natural enemy insects of agricultural crops” (first edition), published in DPRK. “Identification program of natural enemy insects” to enable identification of these insects was developed.

Further extensive surveys of natural enemies and research work on their biological and ecological characteristics is planned to enhance the role of natural enemies in integrated pest management in DPR Korea, particularly in the fields of conservation biology, mass rearing technology and application technique.



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