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TABLE OF CONTENTS:

PAGE

Editorial H.K. Berger	1
News related to IWGO matters U. Kuhlmann	2
<i>Diabrotica virgifera virgifera</i> Le Conte found on the territory of Belarus Trepashko, L.I.; Golunov, I.A.; Nadtochaeva, S.V.	4
The 2010 <i>Diabrotica</i> situation in Brescia Province of Northwestern Italy Edwards C. R.; Agosti, M.; Michelon L.	5
First report of <i>Diabrotica virgifera virgifera</i>, LeConte in Albania Cota, E.	8
<i>Diabrotica</i> Distribution Map in Europe 2009 Edwards C. R.; Kiss, J., et al.	9
<i>Diabrotica</i> Abstracts I-XII 2009 <i>Compiled by Toepfer, S.</i>	10
<i>Diabrotica</i> Abstracts I-VI 2010 <i>Compiled by Toepfer, S.</i>	30

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IWGO – NEWSLETTER 30 / 1

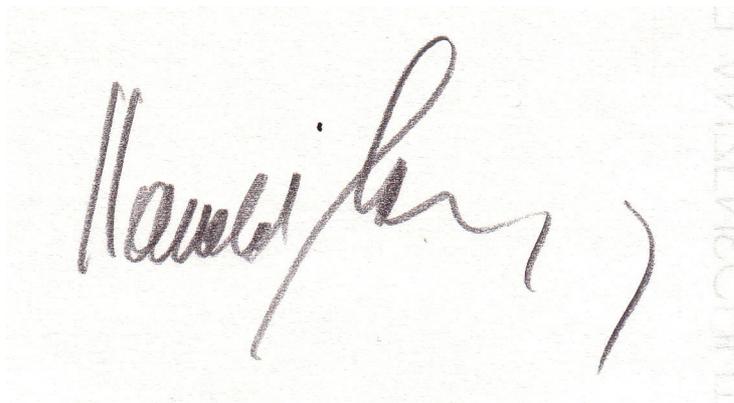
EDITORIAL

This issue of the **IWGO NEWSLETTER** appears later than planned but with excellent news that the special issue of IWGO has been published in the Journal of Applied Entomology in June 2010. This is the second time and we are very satisfied with the final outcome.

This time, the **IWGO NEWSLETTER** contains a large survey of the most recent publications on *Diabrotica* and other rootworms from 2009 and the first half year 2010. You will find articles about the first discoveries of *Diabrotica* in Belarus and Albania. Rich Edwards reports about the current situation of *Diabrotica* in the Brescia Province of northwestern Italy. In addition you will find the 2009 distribution map of *Diabrotica* for Europe.

The **IWGO NEWSLETTER** – which is planned as information for scientists working on different maize pests (and published since 1982) - provides the reader with papers which are sent to the editor (Berger) or to the convenor (Kuhlmann). We like to encourage you to use the opportunity. Also we welcome and are willing to publish personal information, such as retirement, change of job, change of address, etc. As we publish the NEWSLETTER in a digital format, photos can be easily added to your documents. So, please don't hesitate to send scientific publications, other information or personal notes any time to the editor or to the convenor.

Yours,

A handwritten signature in black ink, appearing to read 'Harald K. Berger', is written over a light-colored, textured background. The signature is fluid and cursive. To the right of the signature, there is a vertical stamp that reads 'I W G O NEWSLETTER'.

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IWGO – NEWSLETTER 30 / 1

News Related to IWGO Matters

- **Special Issue for IWGO in Journal of Applied Entomology published in June 2010**

In June 2010, IWGO has published the second time a special issue in the Journal of Applied Entomology (Vol 134 (5) 355-490). Twelve selected manuscripts presented during the Munich conference have been published. Included are two comprehensive perspective and review papers, the first perspective paper focuses on pests, pesticide use and alternative options in European maize production and its current status and future prospects (an outcome of the EU Project ENDURE), and the second review summarizes chemical control options for western corn rootworm (an outcome of the EU Project DIABR-ACT). The content of the special issue is as follows:

Perspective

Meissle, M; Mouron, P; Musa, T; Bigler, F; Pons, X; Vasileiadis, v p; Otta, S; Antivchi, D; Kiss, J; Palinkas, Z; Dorner, Z; van der Weide, R; Groten, J; Czembor, E; Adamczyk, J; Thibord, J-B; Melander, B; Cordsen Nielsen, G.; Paulsem, R T; Zimmermann, O; Verschwele, A; Oldenburg, E. Pests, pesticide use and alternative options in European maize production: current status and future prospects. Journal of Applied Entomology 134(5) 355-356.

Review Articles

van Rozen, K; Ester, A. Chemical control of Diabrotica virgifera virgifera LeConte. Journal of Applied Entomology 134(5) 376-384

Original Articles

Wessler, J; Fall, EH. Potential damage costs of Diabrotica virgifera virgifera infestation in Europe - the 'no control' scenario. Journal Of Applied Entomology 2010 134(5) 385-394

AU Dillen, K; Mitchell, PD; Tollens, E. On the competitiveness of Diabrotica virgifera virgifera damage abatement strategies in Hungary: a bio-economic approach. Journal of Applied Entomology 2010 134(5) 395-408

Dun, Z; Mitchell, PD; Agosti, M. Estimating Diabrotica virgifera virgifera damage functions with field trial data: applying an unbalanced nested error component model. Journal Of Applied Entomology 2010 134(5) 409-419

IWGO – NEWSLETTER 30 / 1

Miller, NJ; Richards, S; Sappington, TW. *The prospects for sequencing the western corn rootworm genome. Journal of Applied Entomology 2010 134(5) 420-428*

Xu, L; Wang, Z; Zhang, J; He, K; Ferry, N; Gatehouse, A M R. *Cross-resistance of Cry1Ab-selected Asian corn borer to other Cry toxins. Journal of Applied Entomology 2010 134(5) 429-438*

Lundgren, J G; Toepfer, S; Haye, T; Kuhlmann, U. *Haemolymph defence of an invasive herbivore: its breadth of effectiveness against predators. Journal of Applied Entomology 2010 134(5) 439-448*

Li, H; Toepfer, S; Kuhlmann, U *Flight and crawling activities of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae) in relation to morphometric traits. Journal of Applied Entomology 2010 134(5) 449-461*

Toth, M; Ujvary, I; Imrei, Z *8-Methyldecan-2-yl Acetate Inhibits Response to the Pheromone in the Western Corn Rootworm Diabrotica v. virgifera Journal of Applied Entomology 134(5) 462-466*

Toepfer, S; Burger, R; Ehlers, RU; Peters, A; Kuhlmann, U. *Controlling Western Corn Rootworm Larvae with Entomopathogenic Nematodes: Effect of Application Techniques on Plant-scale Efficacy. Journal of Applied Entomology 2010 134(5) 467-480*

Zhang, F; Babendreier, D; Wang, Z-Y; Kang, S-I; Zheng, L; Pyon, Y C; Bai, S X; Song, K; Ri, J O; Grossrieder M; Kuhlmann U. *Mass releases of Trichogramma ostrinae increase maize production in DPR Korea. Journal of Applied Entomology 2010 134(5) 481-490*

IWGO – NEWSLETTER 30 / 1

Article

***Diabrotica virgifera virgifera* Le Conte found on the territory of Belarus**

L.I Trepashko; I.A. Golunov & S.V. Nadochaeva

Institute of Plant Protection of Belarus

Western corn rootworm (WCR) was revealed for the first time on the territory of Belarus on August 20, 2009. It was discovered in the course of pheromone monitoring on 45 ha corn field in the direction of Brest-Tomashovka, located in 500 metres from the boundary crossing with Poland "Tomashovki". The identification of the caught individual was done by the Head of the Quarantine Expertise Department Mrs. Bychkovskaya who has proved that the caught individual is a quarantine object – Western Corn Rootworm *Diabrotica virgifera* Le Conte.

After the Western Corn Rootworm invasion into the neighboring states in the Ukraine and Poland, in 2006 at Entomology Lab of the Institute of Plant Protection of Belarus, researchers started on methodical supply and monitoring organization on the territory of the Republic for timely WCR invasions determination. The main method for this pest discovery is the use of the synthetic sex pheromones. The synthesis of WCR sex pheromones is accomplished by the workers of the Scientific-Research Elementary Synthesis Laboratory of the Belarussian State University. 680 samples of the synthetic sex pheromones have been produced, the attractiveness of which was evaluated in 2006-2009 in the focuses of mass pest development of the Trans Carpathian district of the Ukraine together with the scientific workers of the territorial quarantine centre. As a result of researches the most attractive synthetic pheromone entomological glue is revealed which is used with the PAL type traps for carrying out pheromone monitoring in places of the most likely quarantine pest invasion – in corn crops located in the boundary zone of Brest, Grodno, Gomel districts round the International airport "Minsk-2" and the airport "Gomel". 600 pheromone traps on the area of 364 ha were placed in 2007, in 2008 - 900 on 1052 ha, in 2009 - 1600 traps on the area of 2500 ha.

The favorable climatic conditions, a wide forage reserve and the natural enemies of this pest absence can facilitate its acclimatization. Good migratory activity and a high coefficient of the insect reproduction, a lot of transport highways – also the facts facilitating its spread in Belarus, a danger the pest brings to agriculture is extremely high. Corn in the Republic runs one of the first among the main agricultural crops in Belarus. It is grown on the area of more than 800 ths. ha. Special programs are developed directed to raising green mass and grain. To overcome the subsequent WCR entering on the territory of Belarus and based on the order of the European Commission 2003/766/EC "On immediate measures on prevention *Diabrotica virgifera* spread in the European Union, the additionally developed by the EPPCO regional standard PM 9/4(1) "*Diabrotica virgifera*: procedures of official control" and also the Position on the order of determination and designation of quarantine zone, imposition and removal of plant quarantine,

IWGO – NEWSLETTER 30 / 1

establishment and provision of the quarantine regime approved by the Council of Ministers of the Republic of Belarus decision, the scientific workers of the Institute of plant protection and the Chief State Inspection on Seed Production, Quarantine and Plant Protection prepare temporal recommendations on the western Corn rootworm spread restriction on the territory of Belarus.

Article

The 2010 *Diabrotica* Situation in Brescia Province of Northwestern Italy

C. Richard Edwards¹, Mauro Agosti² and Lorenza Michelon²

¹Department of Entomology, Purdue University, West Lafayette, Indiana, USA

² Consorzio di Difesa delle Colture Intensive della Provincia di Brescia, Brescia, Italy

Our work on *Diabrotica virgifera virgifera* LeConte continues in Brescia Province, Lombardy Region, northwestern Italy. Because of the presence of intensive livestock operations and the need for feed, maize represents the most important field crop in this area ($\approx 80\%$ of all arable land) and is grown mainly in monoculture. The western corn rootworm has been present in Brescia since 2002 moving across the Po Valley of the Lombardy Region from the 2000 initial infestation site near Milan Malpensa Airport (Boriani and Gervasini, 2000). It is likely, according to Miller et al. (2005), that this was a different introduction from North America from the original introduction discovered in the former Yugoslavia in 1992 near Belgrade (Baca, 1994) and later found in the Veneto Region, Northeastern Italy, in 1998 (Furlan et al., 1998).

During the past 5 years of our work, we have experienced the typical changes in population from low initial numbers to building and spreading of the population to yearly fluctuations in areas where established. The first economic activity was noted in the western area of the Province in 2006. Since its introduction, the western corn rootworm has spread across the Province and into neighboring regions. In 2009, more than in the previous years, a significant increase in the population was observed and numerous fields with economic root damage were noted. It was not uncommon last year to see root ratings of above 1, and in some fields greater than 2, using the 0-3 node-injury scale (Oleson et al., 2005).

Based on our research and observations, we believe that economic damage begins to occur at a root node-injury damage rating of around 0.75 in irrigated fields (under current cropping system dynamics and maize economics). Nevertheless, it is not uncommon to see extensive root regeneration in irrigated fields showing significant root damage. This regeneration greatly compensates for the damage caused by larval feeding, although it does not completely eliminate economic losses. However, for sure, it reduces those losses! As a comparison, in Indiana, USA, economic

IWGO – NEWSLETTER 30 / 1

damage normally begins at a root node-injury damage rating around 0.25. Indiana maize fields are mostly dryland, rain-fed fields and unless adequate rainfall occurs during and following the period of larval activity, plants have little opportunity to regenerate roots thus allowing for some compensation for larval damage. The fact that most fields in Brescia are regularly irrigated and receive water about every 7-10 days really helps in plant recovery and distinguishes these fields from most Indiana fields.

Field monitoring with Pherocon[®] AM (Trécé Inc., Adair, Oklahoma, USA) unbaited yellow sticky traps is one of the tools we use to predict the risk of economic damage occurring the following year in continuous maize fields and to help farmers to adopt the most appropriate management practices. Previously, according to Lombardy regional guidelines, it was recommended that 6-8 traps per field be used to monitor within-field populations. However, based on our and regional data, farmers should be able to use 3 traps per field, depending on field size, to obtain predictive-data. This will save a lot of time in trap monitoring, as well as reducing monitoring costs. It is more likely that we will be able to convince more farmers to do their own monitoring, if fewer traps are needed. It is obvious from the number of calls to the Consorzio di Difesa, that farmers are getting more interested in monitoring their fields.

There are two methods used to manage the rootworm in the Province. One is adult control when female beetles are in the early stages of egg laying. These controls are applied to reduce the number of eggs in fields to, hopefully, reduce the number of larvae in next year's maize. The other method is to use a soil insecticide at planting or ridging, depending on whether early or late maize plantings. Seed treatments were used prior to the 2009 growing season, but honey bee mortality issues from the dust from seed treatments resulted in their ban from use. Where possible, some farmers rotate out of maize into another crop to avoid the potential for larval problems the next year and to eliminate the added cost of control. As noted before, many of the farmers have livestock and may not be able to rotate all or some of their fields due to their need for maize.

A unique aspect of the farming system in the Brescia Province is that there are two, usually distinct, planting times for maize. About 70% of the maize is planted in late March through mid April. The time of this early planting and the time of rootworm larval hatch (early to mid May) poses some control issues for farmers. Soil insecticides at planting may not provide good control, if the time of application occurs 3-4 weeks or more before larval hatch. The characteristics of the soil insecticide and weather play a significant part in the length of insecticide activity. The issue with control in these early plantings can be negated some by the application of the soil insecticide at ridging. This occurs at about the V 6-7 plant growth stages. Ridging prepares most of the fields for surface irrigation, while some fields with overhead irrigation require no ridging. The remaining maize is planted after the harvest of rye. This planting occurs normally in early to mid May, depending on weather and rye harvest conditions. In regard to the presence of rootworm larvae, the early planted maize is usually well established before the rootworms begin to hatch in early May. For the late planted maize, plantings normally come at a time when larval activity is

IWGO – NEWSLETTER 30 / 1

just getting or well underway and damage can be significant. A soil insecticide applied at planting will normally work very well in late plantings, while an application at ridging would be too late to provide protection in most instances. In some very late plantings, like seen in 2010, the application of a soil insecticide was not needed.

It is too early (1 July as of this writing) to determine the outcome of rootworm activity in the Province for 2010, but overall it is certain that damage will be less than in 2009 and adult numbers will be generally lower. However, there is greater chance for economic losses in the eastern and southeastern part of the Province than noted for those areas in 2009. The rootworm-typical fluctuation in population linked to yearly differences in weather conditions, management practices, etc., is evident for this Italian Province, as is the case in other infested countries. Local differences do occur, even in bordering fields, and farmers must understand those differences to properly manage this insect. As *Diabrotica* researchers and managers know, the work goes on and new situations and surprises will surely follow.

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Furlan, L., M. Vettorazzo, A. Ortez, and C. Frausin. 1998. *Diabrotica virgifera virgifera* è già arrivata in Italia. *Informatore Fitopatologico* 12, 43-44.

Miller, N., A. Estoup, S. Toepfer, D. Bourguet, L. Lapchin, S. Derridj, K.S. Kim, P. Reynaud, L. Furlan and T. Guillemaud. 2005. Multiple transatlantic introductions of western corn rootworm. *Science* Vol. 310, 992.

Oleson, J.D., Y. Park, T.M. Nowatzki, and J.J. Tollefson. 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 98(1): 1-8.

IWGO – NEWSLETTER 30 / 1

Article

2010: *Diabrotica virgifera virgifera* LeConte found in Albania

Ejup Çota

Plant Protection Department; Faculty of Agriculture and Environment
Agricultural University of Tirana, Albania

The presence of *Diabrotica virgifera virgifera*, LeConte (Insecta: Coleoptera: Chrysomelidae: Galerucinae: Luperini: Diabroticina) has now been confirmed in Albania. Samples were collected from maize fields in July 8, 2010 in Gjegjan, Dedaj, Gojan, Dardhë, Iballë of Pukë District (County of Shkodër). On the basis of the morphological characteristics of the adults (male genitalia), pupae and larvae, and root damage, the pest was identified as *D. virgifera virgifera*, LeConte. This is the first report of *D. virgifera virgifera* in Albania.

The situation of *Diabrotica virgifera virgifera*, LeConte in Albania can be described as follows: Present, first recorded in 2010, found in Pukë District (County of Shkodër).

In addition, EPPO was informed about the presence of Diabrotica virgifera virgifera in Albania on July 8, 2010



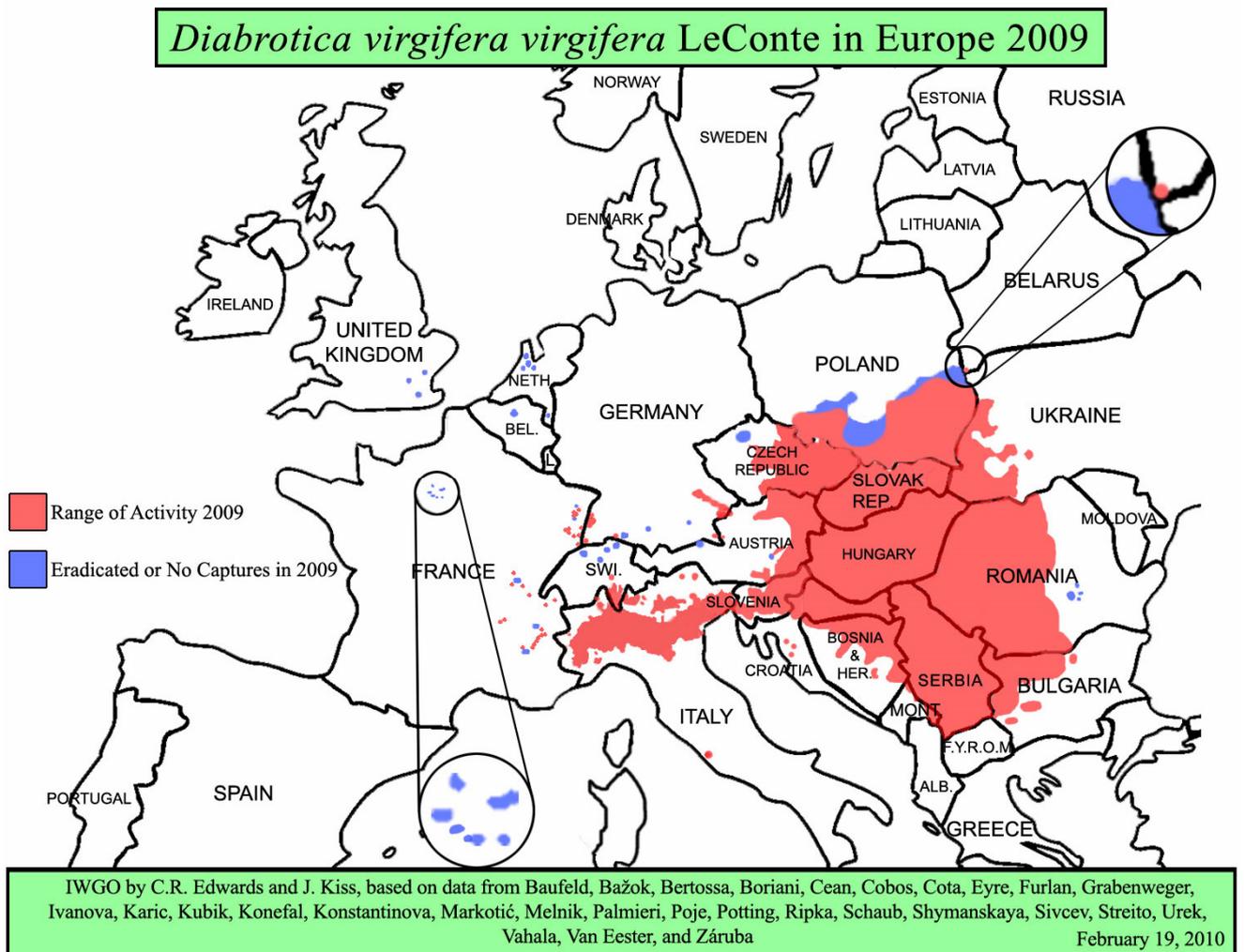
IWGO – NEWSLETTER 30 / 1

Article

DIABROTICA DISTRIBUTION MAP 2009

The following web site has the 2009 *Diabrotica* spread map for Europe (as seen below)

(<http://extension.entm.purdue.edu/wcr/>).



IWGO – NEWSLETTER 30 / 1

Diabrotica Abstracts 2009

Dispersal and Mating Behavior of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Bt Cornfields

AU Marquardt, PT; Krupke, CH

SO Environmental Entomology 2009 38(1) 176-182

AB Understanding of the mating and dispersal behavior of the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), is essential to predicting potential resistance to Bt corn technologies recently deployed to combat this pest. To quantify movement of male beetles, field studies were conducted during 2006 and 2007 in commercial Bt cornfields that included the Environmental Protection Agency-mandated 20% refuge acreage. Wing traps containing a single virgin female beetle were placed along transects throughout these fields. Male beetles were collected from the sticky bottoms of traps to compare the mean number of beetles captured at the different transect distances. Gut contents of each captured male were assayed with protein test strips to determine if the Bt protein (Cry3Bb1) was present. This provided an estimate of the distances traveled by males to reach virgin females. These data indicated that the mean number of males captured approximate to 200 m from refuge corn was not significantly different than the mean number of males captured close to the refuge (< 22 m). Complementary field observations were conducted to determine how far newly emerged female beetles moved before mating and the time of day mating occurs. Results indicated that females do not move far from the site of emergence before mating and do not mate within the first 4 h of adult life. The implications of these data for movement and mating patterns of adult rootworms in Bt/refuge environments are discussed.

Influence of Weight of Male and Female Western Corn Rootworm (Coleoptera: Chrysomelidae) on Mating Behaviors

AU Kang, J; Krupke, CH

SO Annals Of The Entomological Society Of America 2009 102(2), 326-332

AB We evaluated the effect of weight, collection site, and age upon mating behaviors of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) with multivariate analysis. Our laboratory study of *D. virgifera* revealed that female weight was positively associated with the likelihood of mating, although female weight was not associated with females' receptivity to aedeagus insertion. Furthermore, the relationship between male and female weight on mating was investigated at two field locations in Indiana, and evidence of nonrandom mating was found. The implications of these results for pest management are discussed, with particular focus upon using this information for the construction of robust viable resistance management systems in *Bacillus thuringiensis* corn, *Zea mays* L.

Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes

AU Kurtz, B, Hiltbold, I, Turlings, TCJ, Kuhlmann, U, Toepfer, S

SO Biocontrol 2009; 54(2), 255-262

AB As a first step towards the development of an ecologically rational control strategy against western corn rootworm (WCR; *Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) in Europe, we compared the susceptibility of the soil living larvae and pupae of this maize pest to infection by three entomopathogenic nematode (EPN) species. In laboratory assays using sand-filled trays, *Heterorhabditis bacteriophora* Poinar and *H. megidis* Poinar, Jackson & Klein (both Rhabditida: Heterorhabditidae) caused comparable mortality among all three larval instars and pupae of *D. v. virgifera*. In soil-filled trays, *H. bacteriophora* was slightly more effective against third larval instars and pupae, and *H. megidis* against third larval instars, compared to other developmental stages. In both sand and soil, *Steinernema feltiae* (Filipjev) (Rh.: Steinernematidae) was least effective against second instars. In conclusion, all larval instars of *D. v. virgifera* show susceptibility to infection by all

IWGO – NEWSLETTER 30 / 1

three nematodes tested. It is predicted that early application against young larval instars would be most effective at preventing root feeding damage by *D.v. virgifera*. Applications of nematodes just before or during the time period when third instars are predominant in the field are likely to increase control efficacy. According to our laboratory assays, *H. bacteriophora* and *H. megidis* appear to be the most promising candidates for testing in the field.

New polypeptide that enhances a *Bacillus thuringiensis* Cry protein, comprises a segment that binds the Cry protein; useful for inhibiting coleopteran, rootworm, boll weevil, carrot beetle, corn flea beetle, and Japanese beetle

AE Univ Georgia Res Found Inc

AU Abdullah M A F, Adang M J

PY 2009

AB Novelty A polypeptide that enhances a *Bacillus thuringiensis* Cry protein, comprising a segment that binds the Cry protein, where the segment comprises at least 85% sequence identity with a midgut cadherin ectodomain from a coleopteran insect, is new. **USE** - The polypeptide and methods are useful for inhibiting a coleopteran and a rootworm (all claimed). The polypeptide can also be used for inhibiting one or more coleopteran pests including boll weevil, carrot beetle, corn flea beetle, grape colaspis, northern masked chafer (white grub), southern masked chafer (white grub), northern corn rootworm, southern corn rootworm, western corn rootworm, wireworms, Mexican bean beetle, clover leaf weevil, rice water weevil, wireworms, cereal leaf beetle, *Phyllophaga crinita* (white grub), flea beetles including canola flea beetle, Japanese beetle, rice weevil, maize billbug, and sunflower beetle. **Advantage** - The invention provides a polypeptide that enhances a *Bacillus thuringiensis* Cry protein (claimed).

Detailed Description - Independent Claims are: (1) a method of inhibiting a coleopteran comprising providing the coleopteran with a *Bacillus thuringiensis* Cry protein for ingestion together with a polypeptide comprising a segment that binds the Cry protein, where the segment comprising at least 85% sequence identity with an insect midgut cadherin ectodomain, and where the coleopteran is other than a rootworm; and (2) a method of inhibiting a rootworm comprising providing the rootworm with a *Bacillus thuringiensis* Cry protein and a polypeptide for ingestion, where the polypeptide comprises a segment that binds the Cry protein, the segment comprising at least 85% sequence identity with an insect midgut cadherin ectodomain, and where Cry protein is selected from a Cry3 protein, a Cry34 protein together with a Cry35 protein, a Cry8 protein, and a Cry36 protein.

TF BIOTECHNOLOGY - Preferred Polypeptide: The polypeptide comprises at least 85% identity with any of SEQ ID NO. 2, 4, 7, and 10. Sequences not defined here may be found at <ftp://ftp.wipo.int/pub/publishedpctsequences/publication>. The coleopteran is a Colorado potato beetle, a grub or Japanese beetle. The Cry protein is selected from a Cry1Ba protein, a Cry11a protein, a Cry3 protein, a Cry 7 protein, a Cry8 protein, a Cry23 protein, and a Cry37 protein. Preferably, the coleopteran is of the genus *Diabrotica* or *Tenebrio*.

Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield

AU Ma, BL, Meloche, F, Wei, L

SO Field Crops Research 2009 111(3); 189-196

AB Corn rootworm (*Diabrotica* spp.) has become the most concern and widespread insect pest of corn (Zea mays L) production in North America. Two held experiments were conducted to assess the agronomic and yield performance of transgenic rootworm trait, *Bacillus thuringiensis* (Bt) Cry3Bb, seed-coating treatment, and a soil-applied insecticide under natural corn rootworm infestation. Experiment 1 compared a conventional corn hybrid with and without insecticide (Force 3G) with its near isoline Bt hybrid from 2003 to 2005, on a clay loam soil. Experiment 2 investigated the same treatments as in Experiment I plus an additional seed-coated Poncho treatment on a sandy loam in 2004 and 2005. Rootworm population before the crop anthesis, root node injury and root:shoot dry weight ratio during the early grain filling stage, and stalk lodging and grain yield were determined. Our data showed that rootworm population diminished over the 3 years owing to rootworm displacement and adverse weather conditions. At the clay loam site, both Force 3G and the Bt hybrid significantly

IWGO – NEWSLETTER 30 / 1

reduced the larval Populations, root injury and lodging score, and increased root:shoot ratio. Over the 3 years, grain yields of the Bt hybrid were 11-66% greater than the untreated non-Bt isolate hybrid; yield of the non-Bt hybrid treated with Force 3G was also significantly greater than the same untreated non-Bt hybrid in 2 of 3 years. Despite less root node injury in the first rows of non-Bt plants adjacent to the Bt plots was observed, yield benefit of this effect remained to be proven. On sandy loam soil, the larval population was very low and there were no differences in root node injury and plant lodging among all the four treatments in either 2004 or 2005. The yield of the Bt hybrid was 10% greater than its non-Bt isolate hybrid treated or not with an insecticide in 1 year. Our data showed that Bt rootworm seed technology was effective to control the rootworm larvae and protected grain yield under severe infestation. Furthermore, our data suggest that some of the gain in Bt hybrid yield may be attributed to the genetic transformation as observed in sandy loam soil experiment. In all cases, corn producers should be aware of the pest history, rootworm pressure in relation to economic threshold, soil type and the expected cost-to-benefit ratio before deciding to adopt any protective measures.

Possible applications of western corn rootworm's winterforecasting in precision agriculture

AU Tamas, Nemeth, Miklos, Nadasy, Jozsef Takacs

SO *Novenydelem* 2009 45(2) 69-72

AB Last years the Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte, has become one of the most significant pests in Hungary. Since its appearance in 1992 at Belgrad the insect has conquered almost the whole country and nowadays causes economical damage. The main point of sustainable development is the reduction of chemicals use in agriculture, which is possible with precision treatment. Environment friendly technologies would be supported forcefully with a sampling method and forecasting system, which can be used by precision agriculture.

Protease activities in the midgut of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

AU Kaiser-Alexnat, R

SO *Journal Of Invertebrate Pathology* 2009 100(3), 169-174

AB The Western corn rootworm is one of the most economically important pests in corn. One possibility for controlling this pest is the Cultivation of transgenic corn expressing *Bacillus thuringiensis* (Bt) toxins, such as Cry3A, Cry34Ab1/Cry35Ab1, and Cry3Bb1. However, widespread cultivation of the resulting Bt corn may result in the development of resistant pest Populations. The Bt toxins are processed by proteases in the midgut Of susceptible insects. Thus, protease activity studies were conducted using the midgut juice (pH 5.75) from third instars larvae of the susceptible Western Corn rootworm. As a result, the activities of the serine endopeptidases trypsin, chymotrypsin, elastase, cathepsin G, plasmin, and thrombin; the cysteine endopeptidases cathepsin L, papain, cathepsin B, and cathepsin H; the aspartic endopeptidase pepsin; the metallo endopeptidase saccharolysin; the exopeptidase aminopeptidase, and the omegapeptidase acylaminoacylpeptidase were detected. These results are of basic interest but also lead to reference systems for the identification of protease-mediated resistance mechanisms in potentially resistant individuals. (C) 2009 Elsevier Inc. All rights reserved.

Effect of Wild Radish on Preimaginal Development of *Diabrotica balteata* and *Agrotis ipsilon*

AU McCutcheon GS, Simmons AM, Norsworthy, JK

SO *Journal of Sustainable Agriculture* 2009 33(2) 119-127

AB Aqueous extracts of wild radish (*Rhaphanus raphanistrum* L.) roots, stems, and leaves combined were examined for antibiotic effects on hatching and larval development of the banded cucumber beetle, (*Diabrotica balteata* Le Conte [Coleoptera: Chrysomelidae]) and black cutworm (*Agrotis ipsilon* [Hufnagel] [Lepidoptera: Noctuidae]). Hatching was delayed significantly in eggs of *D. balteata* exposed to the wild radish extract for 18 h. In some trials, the hatching percentage increased to levels similar to other treatments by the third and final day of hatching; in others, the percentage hatching remained significantly less among eggs exposed to wild radish extract for the duration of the study.

IWGO – NEWSLETTER 30 / 1

The mortality rate of *D. balteata* larvae was increased significantly by exposure to the wild radish extract. Hatching of *A. ipsilon* eggs was delayed significantly after an 18 h exposure to the aqueous wild radish extract 4 d after treatment in only one of the six trials. In that trial, all treatment results were similar by day 5. In other trials, hatching of *A. ipsilon* was decreased similarly in eggs exposed for 18 h in an aqueous wild radish treatment as well as the water control. Although this study shows an effect of aqueous wild radish extract on two insect species, supplemental studies which utilize methods that do not rely upon an aqueous solution may better define the insect suppression activity of wild radish.

Localized Search Cues in Corn Roots for Western Corn Rootworm (Coleoptera: Chrysomelidae) Larvae

AU Bernklau, EJ, Bjostad, LB, Meiiils, LN; Coudron, TA, Lim, E; Hibbard, BE

SO Journal of Economic Entomology 2009 102(2) 558-562

AB Cues that elicit a characteristic localized search behavior by neonate larvae of the western corn rootworm, *Diabrotica virgifera virgifera* Leconte (Coleoptera: Chrysomelidae), were extracted from living corn *Zea mays* L roots with acetone. Larvae were exposed to corn roots or to an acetone extract of corn roots and then transferred into a bioassay arena where their movements were tracked and recorded. After a 5-min exposure to live corn roots, larvae produced highly convoluted tracks that were indicative of a localized search behavior and these distinctive tracks were also produced by larvae exposed to an acetone extract of corn roots, larvae exposed to filter paper control moved in relatively straight paths that were indicative of ranging behavior. Larval tracks were recorded by means of a video camera and tracking software and four parameters of movement were used to quantify the tracks mean turn angle, mean meander total distance and maximum distance from origin. For every parameter measured, tracks resulting from exposure to the control were significantly different from tracks resulting from exposure to live corn roots and to all doses of the corn root extract. In a separate experiment, larvae exposed to corn root pieces or corn root juice exhibited the localized search behavior, but larvae exposed to oat root pieces and oat root juice (nonhost) exhibited ranging behavior.

European populations of *Diabrotica virgifera virgifera* are resistant to aldrin, but not to methyl-parathion

AU Ciosi, M; Toepfer, S; Li, H; Haye, T; Kuhlmann, U; Wang, H; Siegfried, B; Guillemaud, T

SO Journal Of Applied Entomology 2009 133(4) 307-314

AB The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest of cultivated corn in North America and has recently begun to invade Europe. In addition to crop rotation, chemical control is an important option for *D. v. virgifera* management. However, resistance to chemical insecticides has evolved repeatedly in the USA. In Europe, chemical control strategies have yet to be harmonized and no surveys of insecticide resistance have been carried out. We investigated the resistance to methyl-parathion and aldrin of samples from nine *D. v. virgifera* field populations originating from two European outbreaks thought to have originated from two independent introductions from North America. Diagnostic concentration bioassays revealed that all nine *D. v. virgifera* field populations were resistant to aldrin but susceptible to methyl-parathion. Aldrin resistance was probably introduced independently, at least twice, from North America into Europe, as there is no evident selection pressure to account for an increase of frequency of aldrin resistance in each of the invasive outbreaks in Europe. Our results suggest that organophosphates, such as methyl-parathion, may still provide effective control of both larval and adult *D. v. virgifera* in the European invasive outbreaks studied.

IWGO – NEWSLETTER 30 / 1

New isolated microsclerotia of entomopathogenic fungus comprising Metarhizium species, Beauveria species/Lecanicillium species, useful e.g. to control insects e.g. Reticulitermes, Diabrotica species and Tetanops myopaeformis

AE US Sec. of Agric. Jackson M A; Jaronski S T

AU Jackson M.A.; Jaronski S.T.

PY 2009

AB NOVELTY - Isolated microsclerotia (I) of an entomopathogenic fungus comprising Metarhizium species, Beauveria species or Lecanicillium species, is new. USE - (I) are useful for controlling insects including root weevils, soil grubs, rootworms, wireworms, fruit flies or root maggots, preferably subterranean termites (*Reticulitermes* and *Coptotermes* spp.), corn rootworms (*Diabrotica* spp.), black vine weevils (*Otiorhynchus sulcatus*), wireworms (larvae of family Elateridae), Citrus root weevils (*Diaprepes abbreviatus*), sugar beet root maggots (*Tetanops myopaeformis*), cabbage/turnip/onion/seed corn maggots (*Delia* spp.), carrot rust fly (*Psila rosae*), sweet potato weevils (*Cylas formicarius*), Japanese beetles (*Popillia japonica*) or European chafer (*Rhizotrogus majalis*). (I) are useful for applying to the bark and canopy of plants and trees to control plant foliage or tree bark inhabiting insects including emerald ash borer (*Agrilus planipennis*), gypsy moth (*Lymantria dispar*) or pecan weevil (*Curculio caryae*). (I) are useful, upon rehydration, germinate hyphally or sporogenically, to produce infective, aerial conidia (all claimed). The ability of (I) to kill insects was tested against *Tetanops myopaeformis*. The result showed that the microsclerotial granules exhibited a mortality value of 100% than the traditional conidia-covered corn grit granule in clay-loam and clay soils. **ADVANTAGE** - The entomopathogenic fungus is substantially pure. (I) are: desiccation tolerant and produced in high yield; storage stable; and survive at low-cost. (I) exhibit excellent shelf-life at room and refrigerated temperatures and can be processed to formulation sizes, which are compatible with conventional granular pesticide applicators. (I) are capable of germinating both sporogenically and vegetatively, and retain the insecticidal capabilities of their native or normal forms (i.e. hyphae, blastospores and/or conidia of the same entomopathogenic fungus).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a composition comprising (I), where (I), upon rehydration, germinate hyphally or sporogenically to produce infective, aerial conidia; (2) a method for insect control, comprising applying (I) to the locus of the insects; and (3) the preparation of (I).

BIOLOGY - Preparation (Claimed): Preparation of (I) comprises: inoculating a liquid culture medium comprising a carbon source (greater than 20 g/l) and a nitrogen source (8.1-50 g/l) with fungal propagules of the entomopathogenic fungus; incubating the propagules for a sufficient time to allow for the production of (I); and collecting (I). Preferred Components: The entomopathogenic fungus comprises a *Metarhizium* species, preferably *Metarhizium anisopliae*. Preferred Method: (I) are produced by liquid culture and are present at a concentration of at least 1×10^6 per gram of biomass in the recovered biomass. The applying step comprises applying (I) to soil or greenhouse soil less potting mix.

Initial Larval Feeding on an Alternate Host Enhances Western Corn Rootworm (Coleoptera: Chrysomelidae) Beetle Emergence on Cry3Bb1-Expressing Maize

AU Chege, PG; Clark, TL; Hibbard, BE

SO Journal of The Kansas Entomological Society 2009 82(1); 63-75

AB The potential for rootworm larvae to move between grassy weeds and transgenic maize may be important in resistance management of transgenic rootworm-resistant maize. An experiment was conducted under growth chamber and greenhouse conditions to determine the impact or initial feeding of rootworm larvae on alternate hosts, followed by switching host to transgenic maize, on the development of western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Two grassy weed species, rootworm-resistant Cry3Bb1 transgenic maize (MON863, YieldGard(R) Rootworm) and its isolate, were infested with 50 neonate larvae per container four weeks after planting. Large crabgrass (*Digitaria sanguinalis* (L.) Scop), and giant foxtail, *Setaria faberi* R. A. W. Herrm both in the Poaceae, were chosen because they were relatively good hosts in previous studies. On the 5(th), 10(th), and 15(th) d after infestation, the Tullgren funnel technique was used to facilitate larval movement (host-

IWGO – NEWSLETTER 30 / 1

switching) from the original host plants to the final hosts (MON863 or its isoline). Beetle emergence and fecundity were significantly impacted by the original host, the final host, and their interaction. The original and the final host plant also significantly impacted the sex ratio of the beetles. The host-switching date did not impact any of those three variables. Only the original host had a significant impact on egg viability. Significantly more reproductively fit beetles were produced from larvae that fed on an alternate host before moving to transgenic maize than from larvae fed exclusively on transgenic maize. Interactions with alternate hosts may influence transgenic maize resistance management models.

Agronomic assessment of Bt trait and seed or soil-applied insecticides on the control of corn rootworm and yield

AU Ma, B. L.: Meloche, F.; Wei, L.

SO Field Crops Research 2009 111(3) 189-196

AB Corn rootworm (*Diabrotica* spp.) has become the most concern and widespread insect pest of corn (*Zea mays* L) production in North America. Two held experiments were conducted to assess the agronomic and yield performance of transgenic rootworm trait, *Bacillus thuringiensis* (Bt) Cry3Bb, seed-coating treatment, and a soil-applied insecticide under natural corn rootworm infestation. Experiment 1 compared a conventional corn hybrid with and without insecticide (Force 3G) with its near isoline Bt hybrid from 2003 to 2005, on a clay loam soil. Experiment 2 investigated the same treatments as in Experiment 1 plus an additional seed-coated Poncho treatment on a sandy loam in 2004 and 2005. Rootworm population before the crop anthesis, root node injury and root:shoot dry weight ratio during the early grain filling stage, and stalk lodging and grain yield were determined. Our data showed that rootworm population diminished over the 3 years owing to rootworm displacement and adverse weather conditions. At the clay loam site, both Force 3G and the Bt hybrid significantly reduced the larval Populations, root injury and lodging score, and increased root:shoot ratio. Over the 3 years, grain yields of the Bt hybrid were 11-66% greater than the untreated non-Bt isoline hybrid; yield of the non-Bt hybrid treated with Force 3G was also significantly greater than the same untreated non-Bt hybrid in 2 of 3 years. Despite less root node injury in the first rows of non-Bt plants adjacent to the Bt plots was observed, yield benefit of this effect remained to be proven. On sandy loam soil, the larval population was very low and there were no differences in root node injury and plant lodging among all the four treatments in either 2004 or 2005. The yield of the Bt hybrid was up to 10% greater than its non-Bt isoline hybrid treated or not with an insecticide in 1 year. Our data showed that Bt rootworm seed technology was effective to control the rootworm larvae and protected grain yield under severe infestation. Furthermore, our data suggest that some of the gain in Bt hybrid yield may be attributed to the genetic transformation as observed in sandy loam soil experiment. In all cases, corn producers should be aware of the pest history, rootworm pressure in relation to economic threshold, soil type and the expected cost-to-benefit ratio before deciding to adopt any protective measures. (C) 2009 Elsevier B.V. All rights reserved.

Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae)

AU Lundgren, JG; Nichols, S; Prischmann, DA; Ellsbury, MM

SO Biocontrol Science and Technology 2009 19(3) 327-333

AB The diel and seasonal activity of epigeal predators associated with pre-imaginal *Diabrotica virgifera* was described. Due to its duration, the egg stage was exposed to more predators than the larval stage. Most predators were easily categorized into day- and night-active guilds. Seasonal and diel niche partitioning may contribute to the maintenance of this diverse and abundant predator community.

IWGO – NEWSLETTER 30 / 1

Minimizing structured rootworm refuge in maize plots comprises planting maize first variety in the plot, where the first variety of maize expresses corn rootworm-specific pesticidal proteins from *Bacillus thuringiensis*

AE Pioneer Hi-Bred Int Inc; Dupont De Nemours&Co E I

AU BINNING R; FLEXNER J L; LEFKO S; NOWATZKI T; ONSTAD D; THOMPSON S

PY 2009

AB NOVELTY - Minimizing structured rootworm refuge in a maize plot comprises planting a first variety of maize in a plot, where the first variety of maize incorporates event DAS-59122-7 (which is the expression of corn rootworm-specific pesticidal Cry34Ab1 and Cry35Ab1 proteins from *Bacillus thuringiensis*); and where the first variety of maize makes up at least 90% of the total maize planted in the plot.

USE - The methods are useful for minimizing structured rootworm refuges in maize plots and for minimizing structured pest refuges in crop plots. The methods are also used for evaluating the potential negative cross-resistance of compounds to resistant western corn rootworms (all claimed). The resistant western corn rootworm colonies are specifically used for developing negative cross-resistance strategies and improved resistance management strategies for crops.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method of minimizing structured pest refuge in a crop plot comprising: (a) planting a first crop variety in a plot, where the first crop variety comprises one or more plants that produces a first pesticidal agent; and (b) planting a second crop variety in the same plot, where the second crop variety comprises one or more plants that do not produce the first pesticidal agent; where the first pesticidal agent is active against a first pest; and where the first crop variety makes up at least 90% of the total crop planted in the plot; (2) a method of evaluating the potential negative cross-resistance of a compound to western corn rootworms resistant to event DAS-59122-7 comprising: (a) exposing a first group of one or more western corn rootworms to a compound, where the first group of western corn rootworms exhibits an increased tolerance to event DAS-59122-7; (b) evaluating the toxicity of the compound to the first group of one or more western corn rootworms; (c) exposing a second group of one or more western corn rootworms to a compound, where the second group of western corn rootworms comprises western corn rootworms that do not exhibit increased tolerance to event DAS-59122-7; and (d) comparing the toxicity of the compound to the first group of western corn rootworms with the toxicity of the compound to the second group of western corn rootworms to determine if the compound is more toxic to the first group of western corn rootworms than the second group of western corn rootworms; and (3) a compound selected according to the method of evaluating the potential negative cross-resistance of compounds to resistant western corn rootworms as described above.

BIOTECHNOLOGY - Preferred Method: In minimizing structured rootworm refuge in maize plots, the first variety of maize makes up at least 95-100% of the total maize planted in the plot. In minimizing structured pest refuge in crop plots, the first crop variety makes up at least 95-99% of the total maize planted in the plot. The source of the second crop variety is production error resulting in one or more packages of seed of the first crop variety to also comprise the second crop variety. In one case, the first and/or second crop varieties also produce a second pesticidal agent. In a second case, the first and/or second crop varieties are also exposed to a second pesticidal agent. The first pesticidal agent is derived from event DAS-59122-7. Specifically, the first pesticidal agent comprises Cry34 and Cry35 proteins. Evaluating the potential negative cross-resistance of compounds to resistant western corn rootworms further comprises selecting a compound for further development when the compound exhibits greater toxicity to the first group of western corn rootworms than the second group of western corn rootworms.

IWGO – NEWSLETTER 30 / 1

Western corn rootworm, useful for genetic study, including e.g. designing new toxins or new versions of existing toxins, understanding the mechanism of resistance development, and a negative cross-resistance strategy

AE Pioneer Hi-Bred Int Inc

AU Binning R; Lefko S; Nowatzki T; Thompson S; Flexner J L; Onstad D

PY 2009

AB NOVELTY - Western corn rootworm exhibiting increased tolerance to event DAS-59122-7, is claimed. **USE** - The Western corn rootworm is useful: for genetic study, including designing new toxins or new versions of existing toxins, understanding the mechanism of resistance development, and assisting in determining how resistance may be delayed from genetic; in development of a negative cross-resistance strategy, resistance monitoring strategies, refuge deployment strategies, positive cross-resistance determinations, and determination of mechanisms of resistance; for determining how resistance may be delayed if there are multiple and independent traits in the same target pest that confer different levels of resistance. **ADVANTAGE** - The Western corn rootworm exhibits increased tolerance to event DAS-59122-7.

DETAILED DESCRIPTION - **INDEPENDENT CLAIMS** are included for: an egg, larva, pupa and beetle of the rootworm.

BIOLOGY - Preferred Components: The rootworm is from: the York selected colony; or the Rochelle selected colony.

New substituted pyrimidinyl aryl hydrazone compounds, useful e.g. to control insects e.g. Heliothis species, Helicoverpa species, Spodoptera species and Diabrotica species, and to protect plants against virus, fungus or bacterium diseases

AE Dow Agrosociences Llc

AU Brown A V; Guenthensperger K A; Hunter R; Johnson T C; Niyaz N M; Trullinger T K

PY 2009

AB NOVELTY - Substituted pyrimidinyl aryl hydrazone compounds (I) and their acid addition salts are new. **USE** - (I) are useful: in a composition for controlling insects (all claimed), preferably e.g. Lepidoptera (e.g. Heliothis spp., Helicoverpa spp. and Spodoptera spp.) and/or Coleoptera (e.g. Diabrotica spp., Leptinotarsa decemlineata and Oulema oryzae); to protect plants against virus, fungus or bacterium diseases; to protect textiles, paper, stored grain, seeds and other foodstuffs, houses and other buildings which may be occupied by humans and/or companion, farm, ranch, zoo, or other animals.

ADVANTAGE - (I) have broad spectrum activity against insects.

DETAILED DESCRIPTION - Substituted pyrimidinyl aryl hydrazone compounds of formula (I) and their acid addition salts are new. X1, Y1=H, halo, 1-6C haloalkyl, 1-6C haloalkoxy, 1-6C halothioalkyl or 1-6C haloalkyl (substituted with OH or 1-6C acyloxy); X2, Y2=H, halo, CN, 1-6C alkoxy, 1-6C haloalkyl, 1-6C haloalkoxy, 1-6C halothioalkyl, 1-pyrrolidinyl, 1-piperidinyl or 1-6C haloalkyl (substituted with OH or 1-6C acyloxy); Z=CH or N; L=H, halo or 1-3C (halo)alkyl; Q=H, halo, OR 3, 1-3C haloalkyl, SR3 or NR4R5; R1, R2=H or CH3; R3=1-4C alkyl (optionally substituted with one to the maximum number of Cl or F substituents); either R4=H or 1-4C alkyl; and R5=1-4C alkyl (optionally substituted with one to the maximum number of Cl or F substituents), or substituent comprising 1-4C alkoxy, 1-4C alkylamino or a pyridin-3-yl substituted in the 6-position of the pyridine ring with halo, 1-4C alkoxy or 1-4C haloalkyl; or NR4R5=substituted morpholin-4-ium moiety of formula (II); and R6, R7=H or CH3.

Provided that: at least one of X1 or Y1 is not H; and at least one of X2 or Y2 is not H. An

INDEPENDENT CLAIM is included for the preparation of (I).

ORGANIC CHEMISTRY - Preparation (Claimed): Preparation of (I) Comprises: contacting a 2,4,6-trichloropyrimidine compound of formula (A1) or 2,4,6-trifluoropyrimidine compound of formula (B1) with an aryl amine compound of formula (C1) in a polar aprotic solvent in the presence of a base to provide 2-arylamino-4,6-dichloropyrimidine compounds of formula (D1) and 4-arylamino-2,6-dichloropyrimidine compounds of formula (E1) or 2-arylamino-4,6-difluoropyrimidine compounds of formula (F1) and 4-arylamino-2,6-difluoropyrimidine compounds of formula (G1), respectively; contacting (D1) or (F1) with a nucleophile (Q) in a polar aprotic solvent in the presence of a base to

IWGO – NEWSLETTER 30 / 1

provide 2-arylamino-6-chloropyrimidine compounds of formula (H1) or 2-arylamino-6-fluoropyrimidine compounds of formula (J1), respectively, or contacting (E1) or (G1) with a nucleophile (Q) in a polar aprotic solvent in the presence of a base to provide 4-arylamino-6-halopyrimidine compounds of formula (K1) or 4-arylamino-2-halopyrimidine compounds of formula (L1), respectively; contacting (H1) or (J1) with a hydrazine compound of formula (RNHNH₂) (a) in a polar aprotic solvent to provide a 2-arylamino-6-hydrazine pyrimidine compound of formula (M1), or contacting (K1) with (a) in a polar aprotic solvent to provide a 4-arylamino-6-hydrazine pyrimidine compound of formula (N1), or contacting (L1) with (a) in a polar aprotic solvent to provide a 6-arylamino-2-hydrazine pyrimidine compound of formula (O1); and contacting (N1), (M1) and (O1), individually, with an aryl aldehyde compound of formula (VIII) in a polar solvent to provide (I).

A New Biotic Stress Factor in Maize Production: the Western Corn Rootworm (*Diabrotica v. virgifera* Le Conte)

AU Marton, CL; Nagy, E; Oross, D

CT 8th Alps-Adria Scientific Workshop; Apr 27-May 02, 2009; Neum, BOSNIA & HERCEGOWINA

SO Cereal Research Communications 2009; 37 189-192

AB The appearance of corn rootworm is one of the greatest plant protection challenges that European maize production has faced in its 500-year history. Since the first specimens were caught in 1992, the pest is to be found on all the major maize-producing areas. In several regions the damage it causes has reached the threshold of economic loss, making it the most problematic of all the biotic and abiotic stress factors faced by maize growers. The aim of the research was to determine the level of corn rootworm tolerance in various maize genotypes and to use selection methods to breed maize hybrids whose tolerance level provided satisfactory protection against this biotic stress factor. The present paper describes studies on the tolerance level of 43 Martonvasar hybrids at three locations in two years and that of the parental lines at two locations in one year. Significant differences were found in the tolerance levels of the hybrids, and a close positive correlation was revealed between root-pull resistance and yield, while there was a close negative correlation between root lodging and yield and between root-pull resistance and lodging. Root regeneration is also an important factor, good values of which were found for three hybrids. Inbred lines had smaller root diameter and lower root pull resistance than hybrids. The heterosis on root diameter was 140 %, while in case of root pull resistance the heterosis is 123 %.

Relationship between phenotypic traits and selected fitness components of *Diabrotica virgifera virgifera*

AU Li, HM; Toepfer, S; Kuhlmann, U

SO Entomologia Experimentalis Et Applicata 2009; 131(3) 254-263

AB *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is one of the most important and best-studied maize pests, yet little information is available regarding the basic relationships among its phenotypic traits, particularly those that reflect the overall fitness of this successful invader in Europe and North America. Such information is critical for studies on the invasiveness, behavioural ecology, and management of this pest. Phenotypic traits that change over the lifetime of the beetle were investigated (e.g., fresh body weight) as they can indicate changes in adult physiology or fitness of *D. v. virgifera*. Phenotypic traits that remain stable over the beetle's lifetime were also investigated (e.g., pronotum width, head capsule width, hind tibia length), as they most likely allow detection of genetic differences between populations. Furthermore, phenotypic traits were investigated that may best predict fecundity (e.g., fresh body weight, elytra width) and life span (e.g., elytra length), as these two factors influence the population growth of this highly invasive species. Finally, regression equations are provided for the age-specific oviposition and survival of the long-living *D. v. virgifera* adults, which may allow researchers to reduce the duration of their bioassays without losing information.

IWGO – NEWSLETTER 30 / 1

Technical Aspects of Machines Chosen for the Control of Western Corn Rootworm Adults

AU Poje, T; Godesa, T; Stajniko, D; Jejcic, V; Urek, G; Modic, S; Leskosek, G; Rak-Cizej, M; Sagadin, M; Lakota, M

ED Kosutic, S

CT 37th International Symposium of Agricultural Engineering; Feb.10-13, 2009: Opatija, CROATIA

SO Actual Tasks on Agricultural Engineering, Proceedings 2009 37 163-170

AB The appearance of western corn rootworm brings new challenges to machines used for pesticide spraying. The control of western corn rootworm is difficult due to the height of maize crop in July and August. The current paper presents the exploitation characteristics of mistblower with cannon and sprayer with high installed spraying boom and hose drops with distribution nozzles. The ventilator of mistblower is a big consumer of driving power (the first speed of multiplier is 31.4 kW; the second one is 54.9 kW). The sprayer presents a good distribution pattern, but it has a relatively small working width. Both applications provoke maize losses due to machine passage.

Analysis of the Spread and Population Density of *Diabrotica virgifera virgifera* LeConte and its Impact on Agricultural Practice in Slovenia during the Period from 2003 to 2007

AU Knapic, M; Urek, G; Modic, S

SO Cereal Research Communications 2009 37(2) 227-236

AB The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is one of the most important maize pests, and was introduced to Slovenia in 2003 by natural spread from both the East and West. In Slovenia, as in other European countries, different patterns of spread and population growth of the WCR have been observed in maize growing areas, depending on the density of maize fields in the area and the frequency of maize in the crop rotation. In this study, the spread, population growth and impact on agriculture practice of the WCR have been analysed using GIS tools. In the period from 2003 to 2007, the spread and population growth were observed to be different between western and eastern Slovenia due to differences in population pressure from neighbouring areas and the portion of maize in the area. In the West, the distribution and prevalence of WCR has remained approximately unchanged since the first year of introduction, while in the East, both spread and population growth have become more progressive. However, both parameters showed only moderate progress up to 2006, while in 2007, conditions became more favourable for WCR development and spread. Spread was influenced significantly, although there were only 45 captured imagos per positive PAL pheromone trap per year after 4 years of presence, on average. Moderate growth of the WCR population was a consequence of the implementation of area-wide containment measures in a delimited area, which resulted in a considerable decrease of mono-culture maize growth. This altered pattern of agricultural practice was confirmed with GIS analysis in areas that were demarcated in 2004 and 2005. In the maize growing area in the East, the portion of area under monocultural maize cultivation decreased from 28% to 5% in demarcated areas of WCR from 2004 and 2005, and decreased from 18% to 2% in 2007. In the area demarcated in 2004 in the West, the portion of monocultural maize is decreased in 2007 to only 3%, compared with 34% in 2003. Obviously Slovenian farmers have followed the legislation to a great extent and adopted crop rotation as the main WCR control measure. Implementation of area-wide containment measures from the first year of WCR infestation resulted in slower population growth and expansion.

Population density of *Diabrotica virgifera virgifera* LeConte beetles in Serbian first year and continuous maize fields

AU Sivcev, I; Stankovic, S; Kostic, M; Lakic, N; Popovic, Z

SO Journal of Applied Entomology 2009 133(6) 430-437

AB A 5-year field survey examined western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte) beetle density in Serbia from 2002 to 2006. First-, second-, third-, fourth- and fifth-year maize fields were sampled; they represented 64.61%, 21.66%, 9.45%, 3.53% and 0.75% of all sampled fields respectively. Results showed that the mean WCR beetle population density from 794 maize fields differed depending on cropping history. Minimum mean WCR/trap/day was 0.0 in the first-

IWGO – NEWSLETTER 30 / 1

year maize fields in 2002 and 2006. Maximum mean WCR/trap/day was registered in the fourth-year and the fifth-year maize fields (27.8 and 21.2 respectively). Mean population density of WCR adults increased with the number of years of continuous maize from 1.17, 4.61, 6.41, 10.30 up to 13.53 WCR/trap/day for first-fifth-year maize fields respectively. Mean WCR/trap/day \pm SE exceeded the economic population threshold of >6 WCR/trap/day in third-year continuous maize fields. Out of 794 maize fields, 697 (87.78%) registered a mean population density below the <6 beetles/trap/day threshold. In only 97 fields was WCR population density >6 beetles/trap/day, a finding that predicts a risk of economic damage to a subsequent maize planting. These data are representative of the Serbian situation from 2002 to 2006; they indicate that WCR are well dispersed across commercial maize fields in Serbia. These results provide new insight into the current low WCR population densities in maize fields managed by crop rotation, a finding that can help in creating long-term management strategy.

Conventional Screening Overlooks Resistance Sources: Rootworm Damage of Diverse Inbred Lines and Their B73 Hybrids Is Unrelated

AU Flint-Garcia, SA; Dashiell, KE; Prischmann, DA; Bohn, MO; Hibbard, BE

SO Journal of Economic Entomology 2009 102(3) 1317-1324

AB The western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), is a major pest of maize, *Zea mays* L., in the United States and Europe, and it is likely to increase in importance as a trend toward increased nonrotated maize favors larger rootworm populations. Options for rootworm management in nonrotated maize in Europe and in nontransgenic "refuge" areas in countries that permit transgenic maize are limited to insecticides. Development of additional options for growers would be helpful. Screening maize germplasm (e.g., landraces, populations, inbreds) for native resistance to western corn rootworm is labor-intensive and is usually conducted on unfinished germplasm and not on hybrid materials. However, we have recently observed that topcrossed (hybrid) materials tend to have reduced western corn rootworm damage. To formally test whether rootworm damage to inbreds and associated hybrids were correlated, we evaluated 25 diverse inbred lines and their B73 hybrids for western corn rootworm damage in seven environments. Overall, hybrids had significantly less damage than inbreds, but unfortunately, the correlation between inbreds and hybrids was not significant. These findings have important implications regarding screening germplasm for western corn rootworm resistance, namely, that inbred materials and perhaps populations should be topcrossed to form hybrid materials before screening for western corn rootworm damage to ensure that valuable sources of resistance to western corn rootworm are not missed during the screening process.

Susceptibility of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to the Entomopathogenic Fungus *Metarhizium anisopliae* when Feeding on *Bacillus thuringiensis* Cry3Bb1-Expressing Maize

AU Meissle, M; Pilz, C; Romeis, J

SO Applied and Environmental Microbiology 2009 75(12) 3937-3943

AB Genetically engineered maize producing the insecticidal protein Cry3Bb1 from *Bacillus thuringiensis* (Bt maize) is protected against corn rootworms (*Diabrotica* spp.), which are serious maize pests in North America and Europe. The aim of the present study was to investigate the interaction of Bt maize (event MON88017) and the entomopathogenic fungus *Metarhizium anisopliae* for controlling the western corn rootworm, *Diabrotica virgifera virgifera*. Exposure to Cry3Bb1 expressed in Bt maize seedlings resulted in decreased weight gain in *D. v. virgifera* larvae but did not influence susceptibility to *M. anisopliae*. Adult beetles were not affected by Cry3Bb1 in their food, but mortality when feeding on maize leaves was higher than when feeding on silk. Adults were more susceptible to the fungus than larvae. The results indicate that the effects of Bt maize and *M. anisopliae* on *D. v. virgifera* are additive and that Bt maize does not interfere with the biological control provided by entomopathogenic fungi.

IWGO – NEWSLETTER 30 / 1

Diabrotica virgifera Le Conte in Poland in 2005-2007 and Regulations in the Control of the Pest in 2008

AU Konefal, Tomasz; Beres, Pawel K.

SO Journal of Plant Protection Research 2009 49(1) 129-134

AB *Diabrotica virgifera* Le Conte has been present in Poland since 2005. The range of its distribution increased in 2006 and 2007. *D. virgifera* is known from areas of nine voivodeships in Southern and partially Central Poland. Current control strategy is based on containment measures to limit the spread of the pest from regions where it has occurred. This strategy includes the application of specific phytosanitary measures in defined parts of the country.

Damage Caused by Corn Rootworm (*Diabrotica v. virgifera* Le Conte) and the Dynamics of Seasonal Flight of the Beetles in Long-Term Fertilizer Application Trails

AU Tamas Arendas; Peter Bonis; Csaba Szoeki, Jozsef Vuts Miklos Toth

SO NoveNyvdelem 2009 45(6) 291-296

AB On an area infested with corn rootworm, investigations were made on the effect of forecrop, fertilisation and certain climatic factors on the presence of imagos and the damage suffered by maize. The population size was determined using CSALOMON (R) KLPfor+ traps from July to mid-October. The damage caused by larvae was quantified using root tests (force required to lift roots, root size and mass, Iowa scale). In maize sown after maize the swarming peak of the imagos was in late July or early August. In this treatment the total number of beetles trapped on fertilised plots was 60% greater than on unfertilised plots. When maize was sown after winter wheat the swarming peak was in mid-August, when the mean daily number of trapped imagos was 81.5 on unfertilised plots and 148.8 on fertilised plots. Within the population the sexual composition changed continuously over time, with almost 10% females in the first half of July and nearly 80% in the first third of September. The time shift in swarming peaks and the larger number of imagos in maize sown after wheat was clearly correlated with the fact that the roots of maize stands sown after maize were severely damaged by the larvae. These plants lodged intensely even before flowering and were less well developed. The imagos settled on better developed plants with less root damage, which thus had higher food value. In a year that was wetter than the mean over many years, imago feeding exhibited a close positive correlation with global irradiation, and a medium to close correlation with extreme daily temperature values. The activity of the beetles was influenced to a greater extent by weather factors during the final stages of swarming. Both the results of root analyses and the quantity of grain harvested proved the positive effect of crop rotation and fertilisation on areas infested with corn rootworm. The yield-increasing effect of fertilisation in maize sown after maize amounted to 86.2%, while after wheat this figure was 17.5%.

A review of Resistance Breeding Options Targeting Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

AU Ivezić, M; Raspudic, E; Brmez, M; Majic, I; Brkic, I; Tollefson, JJ; Bohn, M; Hibbard, BE; Simic, D

SO Agricultural and Forest Entomology 2009 11(3) 307-311

AB 1 This review presents the latest research regarding maize resistance breeding against western corn rootworm (WCR) in the U.S.A. and Europe. 2 Investigations in Europe on the development of maize cultivars possessing resistant mechanisms against WCR are just beginning. In 2003, the European Commission implemented measures aimed at slowing down the spread of the WCR in Europe. Nevertheless, this pest has already been found in 20 countries of the European region. To establish a sustainable production system, the evaluation of native (nontransgenic) resistance in maize cultivars is essential. 3 This review emphasizes the future challenges involved in the research of native resistance breeding in maize against the insect.

IWGO – NEWSLETTER 30 / 1

Impact of MON863 Transgenic Roots Is Equivalent on Western Corn Rootworm Larvae for a Wide Range of Maize Phenologies

AU Hibbard, BE, El Khishen, AA; Vaughn, TT

SO Journal Of Economic Entomology; 2009 102(4) 1607-1613

AB The effects of maize, *Zea mays* L., phenology on establishment, damage, and adult emergence of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, on MON863 transgenic maize expressing the Cry3Bb1 protein and its isolate was evaluated in field trials in 2002 and 2003. As expected, plant damage, western corn rootworm larval recovery, and adult emergence were significantly lower on MON863 than isolate maize. The average weight of larvae and adults recovered from MON863 and isolate maize was generally not significantly different. If western corn rootworm larvae were able to establish on transgenic rootworm-resistant plants, larval growth was relatively normal. Plant damage, the number of western corn rootworm larvae recovered, and adult emergence from MON863 did not significantly differ between egg hatch times from widely varying phenologies in either year of the study. Although the extractable level of Cry3Bb1 decreased significantly from vegetative (V)4 to V9 maize in previous studies, in the current study, the amount of Cry3Bb1 did not vary from V3 to R3 in a way that affected neonate survival by western corn rootworm larvae in the field.

Novel nucleic acid molecule isolated from *Bacillus thuringiensis*, useful for controlling or killing pest and protecting plant from pest including Coleopteran pest e.g. Western corn rootworm

AE Pioneer Hi-Bred Int Inc

AU ABAD A R; DONG H; LO S B; SHI X;

PY 2009

AB NOVELTY - An isolated nucleic acid molecule chosen from nucleic acid molecule comprising a defined sequence of 2190 nucleotides (SEQ ID No. 1), given in the specification or its full-length complement, nucleic acid molecule having at least 80% sequence identity to SEQ ID No. 1 or its complement, nucleic acid molecule which encodes a Cry toxin polypeptide having a defined sequence of 730 amino acids (SEQ ID No. 2), given in the specification, and nucleotide sequence that encodes polypeptide having sequence exhibiting at least 80% sequence identity to SEQ ID No. 2, is new. USE - New isolated nucleic acid molecule or pesticidal polypeptide encoded by the nucleic acid is useful for controlling or killing pest and protecting a plant from pest, preferably Coleopteran pest (both claimed) e.g. Western corn rootworm (*Diabrotica virgifera*). Can also be used for other insect pests chosen from e.g. pests of the order Diptera (e.g. corn blotch leafminer and midges), Hymenoptera (e.g. green leafhopper), Lepidoptera (e.g. armyworms, cutworms and loopers), Mallophaga, Homoptera and Hemiptera (e.g. planthoppers), Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera and Trichoptera, spiders and centipedes.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) DNA construct comprising the nucleic acid molecule; (2) host cell comprising the DNA construct; (3) transgenic plant comprising the host cell; (4) seed of transgenic plant comprising the DNA construct; (5) an isolated Cry toxin polypeptide having pesticidal activity, and chosen from polypeptide comprising amino acid sequence of SEQ ID No. 2, polypeptide comprising an amino acid sequence having at least 80% sequence identity to SEQ ID No. 2, polypeptide encoded by nucleotide sequence of SEQ ID No. 1, and polypeptide encoded by a nucleotide sequence that is at least 80% identical to the nucleotide sequence of SEQ ID No. 1; (6) composition comprising the polypeptide; (7) method for producing the polypeptide with pesticidal activity, involves culturing the host cell under conditions in which nucleic acid molecule encoding the polypeptide is expressed; (8) plant having stably incorporated into its genome a DNA construct comprising the nucleotide sequence mentioned above; and (9) method for protecting a plant from a pest, involves introducing into the plant or its cell at least one expression vector comprising the above mentioned nucleotide sequence that encodes the pesticidal polypeptide.

BIOTECHNOLOGY - Isolation: The nucleic acid molecule is isolated from strains of *Bacillus thuringiensis* that encodes polypeptide having pesticidal activity. Preferred Nucleic Acid: The

IWGO – NEWSLETTER 30 / 1

nucleotide sequence is optionally a synthetic sequence that has been designed for expression in a plant. Preferred DNA: The DNA construct further comprises a nucleic acid molecule encoding a heterologous polypeptide. Preferred Host Cell: The host cell is bacterial cell or plant cell. Preferred Plant: The plant is chosen from maize, sorghum, wheat, cabbage, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugar beet, sugarcane, tobacco, barley and oilseed rape. Preferred Polypeptide: The polypeptide further comprises heterologous amino acid sequences. Preferred Composition: The composition is chosen from powder, dust, pellet, granule, spray, emulsion, colloid and solution. The composition is prepared by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of *Bacillus thuringiensis* cells. The composition comprises 1-99 weight% of the pesticidal polypeptide.

New chimeric, insect-toxic crystal (Cry)34 protein, useful for controlling plant pests, preferably inhibiting rootworm

AE Dow Agrosciences Llc

AU SCHNEPF H E

PY 2009

AB NOVELTY - A chimeric, insect-toxic Cry34 protein comprising a front subdomain and a back subdomain, where the front subdomain comprises the front subdomain of a first Cry34 protein, and the back subdomain is a C-terminus of a second Cry34 protein, where the first Cry34 protein and the second Cry34 protein are different Cry34 proteins, is new.

USE -The chimeric protein is useful for controlling plant pests, preferably inhibiting rootworm (claimed). ADVANTAGE - The present invention provides modified, insecticidal Cry34 proteins with enhanced properties as compared to wild-type Cry34 proteins.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are: (1) a truncated Cry34 protein comprising residues 1-114 of a wild-type Cry34 protein and having at least one deletion after residue 114; (2) a polynucleotide that encoding the chimeric protein above; (3) a transgenic plant that produces the chimeric protein above; (4) a seed produced by the plant of (3); and (5) a method of inhibiting a rootworm by providing, for ingestion, to the rootworm the chimeric protein above.

BIOTECHNOLOGY - Preferred Chimeric Protein: The front subdomain is a Cry34B protein and the back subdomain is a Cry34A protein. The front subdomain is a Cry34A protein and the back subdomain is a Cry34B protein. The front subdomain comprises the first seven segments of the first Cry34 protein, where the front subdomain further comprises the 91-95 loop at residues 91-95 of the first Cry34 protein. The chimeric protein comprises residues 1-114 of the first Cry34 protein and the C terminus of the second Cry34 protein, where the chimeric protein comprises residues 1-118 of the first Cry34 protein. The position of the residues is shown in the specification. Preferred Truncated Protein: The truncated Cry34 protein comprises residues 1-118 of the wild-type Cry34 protein.

Enhancing capability of *Bacillus thuringiensis* insect-killing crystalline protein 3 (Cry3) for killing *Diabrotica virgifera virgifera* comprises modifying Cry3 by gene deletion and/or insertion

AE Univ Zhejiang

AU FANG J; SHEN Z

PY 2009

AB NOVELTY - Enhancing capability of *B. thuringiensis* insect-killing protein Cry3 for killing *D. virgifera virgifera* comprises deleting and/or inserting at least one polypeptide fragment, where length of the polypeptide fragment is 1-50 of amino acids, where the Cry3 is Cry3A or Cry3B. USE - The method is useful for enhancing capability of *B. thuringiensis* insect-killing protein Cry3 for killing *D. virgifera virgifera*. The plasmid is useful for preparing trans-gene pest-resistant corn (claimed).

ADVANTAGE - The present invention provides trans-gene pest-resistant corn having improved Cry3 protein, which has nice pesticide killing capability to the *D. virgifera virgifera*.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are: (1) an improved Cry3 obtained by the method above; and (2) a plasmid containing a nucleotide sequence for encoding the Cry3 protein.

BIOTECHNOLOGY - Preferred Method: The method for enhancing capability of *B. thuringiensis* insect-killing protein Cry3 comprises inserting at least one polypeptide fragment in 30th-33rd site of

IWGO – NEWSLETTER 30 / 1

amino acid sequences on N end of Cry3. The method comprises deleting the 1st-32nd site of amino acid polypeptide fragments on N end of Cry3. The method comprises replacing the 32nd-33rd site of amino acids into Gly-Pro-Gly-Lys. Preferred Cry3: The Cry3 is Cry3A, which contains the defined sequence of 565 or 599 amino acids (SEQ ID NO: 2 or 3), given in the specification. The Cry3 is Cry3B, which contains the defined sequence of 569 or 602 amino acids (SEQ ID NO: 4 or 5), given in the specification.

A multifaceted hemolymph defense against predation in *Diabrotica virgifera virgifera* larvae

AU Lundgren, JG; Haye, T; Toepfer, S; Kuhlmann, U

SO Biocontrol Science and Technology 2009 19(8) 871-880

AB The physical and chemical aspects of *Diabrotica virgifera virgifera* larval hemolymph were quantitatively assessed against two predatory beetle species in the laboratory. Adult *Poecilus cupreus* and *Harpalus pensylvanicus* (Coleoptera: Carabidae) were fed pupae, second or third instar *D. v. virgifera* or a palatable surrogate prey, i.e., *Calliphora vicina* or *Sarcophaga bullata* larvae (Diptera: Calliphoridae, Sarcophagidae, respectively) of equivalent size. The ethanol-soluble fraction of third instar *D. v. virgifera* hemolymph was extracted and suspended in a 0.24 M sucrose solution and offered to *H. pensylvanicus* (using a sucrose only control for comparison). The mean duration until first consumption was recorded for each predator, as was the amount of time spent eating, cleaning, resting, or walking for 2 min post-attack (or 5 min for the sugar assay). Maggots and *D. virgifera* larvae and pupae were attacked equally by both predators. But upon attack, *D. v. virgifera* larval hemolymph coagulated onto the mouthparts of the predators, which they began vigorously cleaning. Predators ate the sucrose solution for significantly longer than hemolymph + sucrose solution, indicating the presence of deterrent chemicals in the hemolymph. This research suggests that *D. v. virgifera* larvae are defended from predation by sticky and repellent hemolymph. We hypothesize that this defense partially explains the widespread success of *D. v. virgifera* as an invasive pest.

Comparative efficacy assessment of fungi, nematodes and insecticides to control western corn rootworm larvae in maize

AU Pilz, C; Keller, S; Kuhlmann, U; Toepfer, S

SO Biocontrol 2009 54(5) 671-684

AB All three larval instars of *Diabrotica virgifera virgifera* LeConte (western corn rootworm, Coleoptera: Chrysomelidae) feed on the roots of maize, *Zea mays* (L.). We assessed the efficacies of the following four agents in controlling these larvae: (1) the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae), (2) the nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Rhabditida), (3) a tefluthrin-based soil insecticide and (4) clothianidin-coated seeds. The agents were applied in field plot experiments in southern Hungary in 2006 and 2007. Efficacy was assessed by comparing the number of emerging *D. v. virgifera* adults and corresponding root damage among treatments and untreated controls. All agents significantly reduced *D. v. virgifera* numbers and root damage, but the relative success of each treatment was variable. On average across fields and years, the nematode and the two insecticides reduced *D. v. virgifera* by $65 \pm A 34\%$ SD, while the fungus reduced *D. v. virgifera* by $31 \pm A 7\%$. According to the node injury scale, the agents prevented 23-95% of potential root damage. Large-scale commercialisation of these biological agents could offer viable and practical control options against *D. v. virgifera*.

Insect i.e. corn rootworm beetle, capturing apparatus for monitoring insect load, has frame comprising top panel, and flexible sealing structure creating seal along portion of plant at plant slot on top panel to prevent egress of insect

AE Syngenta Participations Ag

AU CASSELMAN D; MEGHJI M; WHITE J;

PY 2009

AB NOVELTY - The apparatus (10) has a frame (12) comprising an open bottom and a removable top panel (30), where the top panel includes a plant slot to receive a portion of a plant (PS) and a capture

IWGO – NEWSLETTER 30 / 1

chamber opening to provide passage into a capture chamber (40). A flexible sealing structure (26) seals the plant slot on the panel. A securing mechanism (28) secures the structure to the top panel such that opening of the structure is aligned with the plant slot of the panel, where the structure creates a seal along the portion of the plant at the slot on the top panel to prevent egress of an insect (AI). USE - Apparatus for capturing insects i.e. corn rootworm beetle, around a plant i.e. transgenic corn plant, for monitoring insect load from soil.

ADVANTAGE The apparatus accurately measures and monitors the insect load in the field around the plants. The damage to the apparatus caused by wind and storm is minimized. The apparatus allows efficient retrieval of trapped insects by utilizing an easily removable and replaceable capture chamber, and efficiently creates the seal along the portion of the plant at the plant slot on the top panel to prevent egress of insects by the flexible sealing structure.

DETAILED DESCRIPTION – INDEPENDENT CLAIMS are also included for the following: (1) a method for assembling an apparatus for capturing insects around a plant (2) a method for capturing, monitoring and/or studying plant insects.

Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol

AU Toepfer, S; Zhang, F; Kuhlmann, U

SO Biological Control 2009 51(1) 26-33

AB *Celatoria compressa* (Wulp) (Diptera: Tachinidae), a parasitoid of adult chrysomelid beetles in the subtribe Diabroticina in North America, has been selected as a candidate for classical biological control of the alien invader, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae: Galerucinae), into Europe. We conducted host specificity testing to evaluate the fundamental host range of *C. compressa* and potential risks to native European coleopteran species. Nine potential non-target beetles were tested for host selection with *D. v. virgifera* in no-choice tests, sequential no-choice tests, choice tests and sequential choice tests in small experimental arenas in a quarantine laboratory. The nine representative non-target species were selected for experimentation based on (1) ecological host range information of *C. compressa*, (2) ecological similarities to *D. v. virgifera*, (3) close phylogenetic/taxonomic relationships, (4) safeguard considerations, (5) morphological similarities, geographical distributions, overlap of temporal occurrences with *D. v. virgifera* and *C. compressa*, and (6) accessibility and availability. Of the potential nine non-target hosts tested, gravid *C. compressa* only parasitized a few red pumpkin beetles, *Aulacophora foveicollis* (Chrysomelidae: Galerucinae), regardless of the presence or absence of *D. v. virgifera*. However, *C. compressa* significantly preferred *D. v. virgifera* (44.6% parasitized) over *A. foveicollis* (2.7%) in choice tests. Of the 1110 *A. foveicollis* tested among all experiment types, only 23 were parasitized and only one *C. compressa* successfully developed from the parasitism, demonstrating that *A. foveicollis* is a poor host. In conclusion, *C. compressa* has a fundamental host range restricted to the subtribes Diabroticina and Aulacophorina, and would therefore be unlikely to have a direct impact on indigenous species in Europe.

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DNA Isolation from *Diabrotica virgifera zea* Krysan and Smith and *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) by a CTAB Simplified Procedure

AU Barragan-Valencia, G; Almaraz-Abarca, N; Alvarez-Zagoya, R; Delgado-Alvarado, AE; Perez-Dominguez, JF

SO Southwestern Entomologist 2009 34(3) 289-294

AB Microsatellite markers are used in genotypic studies of *Diabrotica* spp. Usually commercial kits are used for DNA extraction from preserved tissues. A simplified procedure, based on the CTAB, was used to obtain DNA from tissues preserved in 75% ethanol and from fresh samples of *Diabrotica* spp. from Durango and Jalisco, Mexico. Significantly more DNA was obtained from fresh than preserved tissues (926 and 827 ng/ μ L, respectively). However, the quality of DNA was similar ($A(260\text{nm})/A(280\text{nm})$ mean value = 1.93) between the two kinds of tissue. The CTAB procedure was used to isolate similar quality and quantity of DNA as obtained by using commercial kits but was less

IWGO – NEWSLETTER 30 / 1

time consuming and expensive. The DVV-D2 loci, a characteristic microsatellite of *Diabrotica* genus, was amplified.

Research Regarding *Diabrotica virgifera virgifera* Le Conte (the Western Root Worm) Control in Sustainable Agriculture

AU Ciobanu, C; Sandor, M; Ciobanu, G; Domuta, C; Samuel, AD; Vuscan, A; Chereji, I

SO Romanian Agricultural Research 2009 26 79-84

AB *Diabrotica virgifera virgifera* Le Conte has been present in Romania for over 100 years (1996) and has become a very dangerous pest for corn. Its spreading on a large area was favored by the ecological conditions and by the considerable share of corn in the crop structure, especially by corn monoculture. Taking into consideration that almost all the developing stages of the pest, except the adults, live in soil, limitation of area extension and of produced damage can only be achieved by crop management measures together with chemical methods. The research performed during the period 2005 - 2007 in long term field experiments (since 1990) in various crop rotation types, in which corn is present in monoculture, in two and three years rotation (under irrigation and without irrigation) and in six years crop rotation (non-irrigated), has shown that monoculture is the most important factor responsible for the pest multiplication. In corn monoculture the larvae number on the roots ranged between 4.91 and 8.23 and root attack degree in IOWA scale (with marks from W to P in which maximum attack is 6), had values between 3.84 and 5.62. The frequency of attacked plants with the symptom "goose neck" ranged between 16.4 and 31.2%, larval aggressiveness being higher under favorable soil moisture in irrigated conditions. The rotation with other crops interrupts the biologic cycle of the pest. Planting corn later and utilizing lower plant density contributed to prevention of pest multiplication, while earlier sowing at high density favored the larvae developing. The results obtained in this field experiment showed that chemical control of larvae was necessary at moderate to high infestation pressure best control being obtained by utilizing granulated insecticides for soil at sowing (Force 1.5 G) or seed treatment (ST 280, Cruiser 350, Poncho 510). For chemical control of adults the best efficacy was obtained utilizing the insecticides : Actara 25 WG, Karate 5 EC, Calypso 480 SC and Talstar 10 EC.

Stereoselective synthesis of (R)-10-methyltridecan-2-one, the sex pheromone of the southern corn rootworm, using (4S)-benzylthiazolidinethione as a chiral auxiliary

AU Lu, CF; Zhang, SB; Li, Y; Yang, GC; Chen, ZX

SO Tetrahedron-Asymmetry 2009 20(19) 2267-2269

AB The stereoselective synthesis of (R)-10-methyltridecan-2-one, the sex pheromone of the Southern corn rootworm, was carried out in 20.7% overall yield based on (4S)-benzylthiazolidinethione (five steps). In the crucial step, the stereogenic center was generated by an asymmetric Michael addition using enantiomerically pure (4S)-benzylthiazolidinethione as a chiral auxiliary.

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Likelihood of Multiple Mating in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)

AU Kang, J; Krupke, CH

SO Journal Of Economic Entomology 2009 102(6) 2096-2100

AB We evaluated the mating ability of male western corn rootworms, *Diabrotica virgifera virgifera* Leconte (Coleoptera: Chrysomelidae), for 20 d after initial mating, using a series of laboratory experiments. Males mated an average of 2.24 times within 10 d after their first mating and averaged 0.15 matings; between days 11 and 20 after their first mating. Because estimating the mating frequency in Bt/refuge cornfields is critical to developing robust and reliable models predicting Bt resistance development in this pest, we discuss how these laboratory findings may influence development and evaluation of current and future insect resistance management plans.

IWGO – NEWSLETTER 30 / 1

Native Resistance to Western Corn Rootworm (Coleoptera: Chrysomelidae) Larval Feeding: Characterization and Mechanisms

AU El Khishen, AA; Bohn, MO; Prischmann-Voldseth, DA; Dashiell, KE; French, BW; Hibbard, BE
SO Journal Of Economic Entomology 2009 102(6) 2350-2359

AB Seven maize, *Zea mays* L., genotypes selected for native resistance to western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), larval feeding damage (SUM2068, SUM2162, CRW3(S1)C6, NSS1XCRW3(S1)C6, PI583927, CRW2(C5), and AR17056-16) were evaluated along with three control maize genotypes in the field for plant damage, larval recovery, adult emergence, root size, and root regrowth. Larvae recovered were further analyzed for head capsule width and dry weight and adults for dry weight. All factors evaluated with the exception of adult dry weight varied significantly among maize genotypes. Control genotypes included a highly susceptible hybrid, B37XH84, a transgenic rootworm-resistant hybrid expressing the modified Cry3A protein (MIR604), and the untransformed modern hybrid with the same genetic background as the MER604 we used (isoline) as a second susceptible control. In general, the genotypes previously selected for resistance to western corn rootworm larval feeding had less damage, fewer larvae recovered, smaller larvae recovered, and fewer adults recovered than the susceptible controls. SUM2162 was significantly less damaged than all other native sources of resistance. Western corn rootworm larvae recovered from SUM2162 and SUM2068 were significantly smaller in terms of head capsule width and average weight than larvae recovered from all other maize genotypes, indicating that antibiosis is a mechanism of resistance for these two hybrids.

***Dolatia coriaria* (Kraatz) (Coleoptera: Staphylinidae) as a Pest of Laboratory and Greenhouse Colonies of the Western Corn Rootworm (Coleoptera: Chrysomelidae)**

AU Meihls, LN; Hibbard, BE

SO Journal of the Kansas Entomological Society 2009 82(4) 311-315

Response of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) to 1,4-Dimethoxybenzene and Analogs in Common Bean Crop

AU Marques, FD; Wendler, EP; Macedo, A; Wosch, CL; Maia, BHS; Mikami, AY; Arruda-Gatti, IC; Pissinati, A; Mingotte, FLC; Alves, A; Ventura, MU

SO Brazilian Archives of Biology and Technology 2009 52(6) 1333-1340

AB Several synthetic and commercial analogs of 1,4-dimethoxybenzene, a kairomone of *Diabrotica speciosa*, along with other compounds already shown to be attractive to other species of *Diabrotica*, were tested as attractant to *D. speciosa*. Yellow cup traps were lured with the compounds and installed in a common bean field. Assessments were conducted 24 h later. 1,4-dimethoxybenzene lured traps caught significantly more beetles than the control traps. Captures of traps lured with 1,4-dimethoxybenzene analogs did not differ from the control traps. Results showed that position and nature of the substituents on the aromatic ring played a crucial role in the activity of the natural compound. The aromatic ring was also very important to the activity of the kairomone.

Studies on the Tolerance of Maize Hybrids to Western Corn Rootworm (*Diabrotica virgifera virgifera* Le Conte)

AU Marton, LC; Szoke, C; Pinter, J; Bodnar, E

CT 21st Conference on Maize and Sorghum Breeding in the Genomics Era; Jun 21-24, 2009 Bergamo, ITALY

SO Maydica; 2009 54(2-3) 217-220

AB The corn rootworm, which has been introduced from North America, is becoming an increasingly serious problem for maize producers in Hungary. In several regions the damage it causes has reached the threshold of economic loss, making it the most problematic of all the biotic and abiotic stress factors faced by maize growers. The aim of the research was to determine the level of corn rootworm tolerance in various maize genotypes and to use selection methods to breed maize hybrids whose tolerance level provided satisfactory protection against this biotic stress factor. The present paper describes Studies on the tolerance level of 41 Martonvasar hybrids at three locations in two years and

IWGO – NEWSLETTER 30 / 1

that of the parental lines at two locations in one year. Significant differences were found in the tolerance levels of the hybrids, and a close positive correlation was revealed in 2007 between root-pull resistance and yield. Root regeneration is also an important factor, good values of which were found for three hybrids. Inbred lines had smaller root diameter and lower root pull resistance than hybrids. The heterosis on root diameter was 140%, while in case of root pull resistance the heterosis is 123%.

Nucleic acid sequences from *Diabrotica virgifera virgifera* LeConte and uses thereof

AE Monsanto Technology LLC

AU Andersen, Scott E.; Anonymous; Hicks, Glenn R.; Huesing, Joseph E.; Romano, Charles P.; Vetsch, Clayton S.

SO Official Gazette of the United States Patent and Trademark Office Patents

PY 2009

AB Expressed Sequence Tags (ESTs) isolated from the Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte, are disclosed. The invention encompasses nucleic acid molecules that encode *D. v. virgifera* protein homologs and fragments thereof. In addition, antibodies capable of binding the proteins are encompassed by the present invention. The disclosed ESTs have particular utility in isolating genes and promoters, identifying and mapping the genes involved in developmental and metabolic pathways, and determining gene function. The ESTs provide a unique molecular tool for the targeting and isolation of novel genes for plant protection and improvement. The invention also relates to methods of using the disclosed nucleic acid molecules, proteins, fragments of proteins, and antibodies, for example, for gene identification and analysis, and preparation of constructs.

***Diabrotica virgifera virgifera* Le Conte Larval Size may be influenced by Environmental Conditions in Irrigated Maize Fields in Northwestern Italy**

AU Agosti, Mauro; Michelon, Lorenza; Edwards, Richard

SO Entomologia Croatica 2009 13(2) 61- 68

AB Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae were collected in different irrigated maize fields in Brescia Province in Northwestern Italy in 2006, 2007 and 2008. Head capsule widths, considered a valid indicator of larval instar stage, were measured for 2063 specimens and data for the three years were combined and grouped in increments of 20 μm width sizes. Frequency of larvae within each grouping was graphed as a histogram and three distinct peaks, interpreted to represent the peak frequency of the head capsule widths of the three larval instars, were observed. Assuming a normal distribution of head capsule widths, a multiple nonlinear Gaussian Curve regression was applied to the frequency histogram. The peaks of the fitted normal curves were $227 \pm 3 \mu\text{m}$, $353 \pm 6 \mu\text{m}$, and $519 \pm 3 \mu\text{m}$ for the first, second and third instars, respectively. These values are higher than those reported by other authors in similar studies. The possible impact of irrigation and other environmental factors is discussed.

Extraction methods and concentrations for the insecticidal effect of *Ruta graveolens* L., *Artemisia verlotorum* Lamotte, and *Petiveria alliacea* L. against *Diabrotica speciosa* Germar.

AU Barbosa, F. S., Leite, G. L. D.; Martins, E. R.; Guanabens, R. E. M.; Silva, F. W. S.

SO Revista Brasileira de Plantas Mediciniais 2009 11(3) 221-229

AB Extraction methods and concentrations for the insecticidal effect of *Ruta graveolens* L., *Artemisia verlotorum* Lamotte, and *Petiveria alliacea* L. against *Diabrotica speciosa* Germar. The aim of this work was to determine the best extraction method and concentration for *Ruta graveolens* L. (Rutaceae), *Artemisia verlotorum* Lamotte (Asteraceae) and *Petiveria alliacea* L. (Phytolaccaceae) concerning their insecticidal effect against *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae). Extracts using commercial soybean oil as at least one of the solvents resulted in higher *D. speciosa* mortality in controls (solvent = oil) of the three studied plants, relative to their treatment concentrations. *R. graveolens* extract in water, at 5% concentration, showed higher *D. speciosa* corrected mortality (32.5%) than *A. verlotorum* extracts in water (10% concentration) (20.3%) and *P. alliacea* in ethanol (2% concentration) (12.5%). The extraction method with water is simple and can be used by small

IWGO – NEWSLETTER 30 / 1

farmers. *R. graveolens* is an easily cultivated plant and constitutes, therefore, a good alternative to control this plague.

Seasonal Flight of Western Corn Rootworm (*Diabrotica virgifera virgifera* Le Conte) in a Dry Year in Martonvasar

AU Arendas, T.; Bonis, P.; Szoke, C.;

SO *Novenyvdelem* 2009 45(12) 645-650

AB The occurrence and effect of western corn rootworm on maize grown in a monoculture in a long-term fertilisation experiment was examined in 2009, when the dry weather retarded plant development. Changes in the number of imagoes on plots with no, non-intensive or intensive fertiliser application were estimated using KLPflor traps containing floral baits and KLPfero traps containing pheromone baits. The nature of the swarming patterns recorded with the different types of traps was similar, but differences of an order of magnitude were observed in the intensity, in favour of pheromone traps. The first swarming peak was noted on July 3(rd) or 6(th), depending on the type of trap, and the second on July 17(th). Due to the unfavourable weather, the maize plants started withering earlier than usual, so the populations were less stable than in favourable years. Correlation analysis revealed that the catches recorded in pheromone traps were more closely related to meteorological factors. In dry years, the weather was thus found to have a greater influence on the sexual activity of the males than on the feeding patterns of the imagoes. Unlike the situation in wet years, the latter could not be reliably predicted from changes in the weather under dry conditions.

Studies on the Tolerance of Maize Hybrids to Western Corn Rootworm (*Diabrotica virgifera virgifera* Le Conte)

AU Marton, Cs. L.; Szoke, Cs.; Racz, F.; Pinter, J.

SO *Novenyvdelem* 2009 45(12) 698-702

AB The corn rootworm, which has been introduced from North America, is becoming an increasingly serious problem for maize producers in Hungary. In several regions the damage it causes has reached the threshold of economic loss, making it the most problematic of all the biotic and abiotic stress factors faced by maize growers. The aim of the research was to determine the level of corn rootworm tolerance in various maize genotypes and to use selection methods to breed maize hybrids whose tolerance level provided satisfactory protection against this biotic stress factor. The present paper describes studies on the tolerance level of 41 Martonvasar hybrids at three locations in two years (2007-2008) and that of the parental lines at two locations in one year. Significant differences were found in the tolerance levels of the hybrids, and a close positive correlation was revealed in 2007 between root-pull resistance and yield. Root regeneration is also an important factor, good values of which were found for three hybrids. Inbred lines had smaller root diameter and lower root pull resistance than hybrids. The heterosis on root diameter was 140%, while in case of root pull resistance the heterosis is 123%.

Maize Tolerance to Western Corn Rootworm Larval Feeding: Screening through Five Years of Investigation

AU Ivezic, Marija; Raspudic, Emilija; Brmez, Mirjana; Majic, Ivana; Dzoic, Drazen; Brkic, Andrija

SO *Agriculturae Conspectus Scientificus* 2009 74(4) ISSN 1331-7768(print) 1331-7776(electronic)

AB Research on maize tolerance to rootworm larval feeding was conducted in Osijek, Croatia in period from 2001 to 2005. Seven Croatian commercial maize hybrids were evaluated by measuring traits associated with resistance: root injury, root size and root regrowth. Hybrids were grown in monoculture in field trials in four replicates. Root injury was rated according to Iowa Node Injury Scale (0-3), and the Eiben 1-6 Scale, reversed, was used for root size and regrowth assessment. No significant differences were observed for root injury between the hybrids. However, injury was significantly different across the years and more damaged roots were observed in 2003 (1.606), 2004 (1.281) and 2005 (0.940) compared to 2001 (0.081) and 2002 (0.099). Root size and root regrowth differed significantly across the years of investigation, while differences between the hybrids occurred only for root regrowth. Hybrids OsSK 617, OsSK 602 and OsSK 596 appeared to be the most tolerant through all years of investigation measured by root regrowth.

IWGO – NEWSLETTER 30 / 1

Diabrotica Abstracts 2010

(January – June)

Dispersal kernels of the invasive alien western corn rootworm and the effectiveness of buffer zones in eradication programmes in Europe

AU Carrasco, LR, Harwood, TD; Toepfer, S; MacLeod, A; Levay, N; Kiss, J; Baker, RHA; Mumford, JD; Knight, JD

SO Annals of Applied Biology 2010 156(1) 63-77

AB Europe is attempting to contain or, in some regions, to eradicate the invading and maize destroying western corn rootworm (WCR). Eradication and containment measures include crop rotation and insecticide treatments within different types of buffer zones surrounding new introduction points. However, quantitative estimates of the relationship between the probability of adult dispersal and distance from an introduction point have not been used to determine the width of buffer zones. We address this by fitting dispersal models of the negative exponential and negative power law families in logarithmic and non-logarithmic form to recapture data from nine mark-release-recapture experiments of marked WCR adults from habitats as typically found in the vicinity of airports in southern Hungary in 2003 and 2004. After each release of 4000-6300 marked WCR, recaptures were recorded three times using non-baited yellow sticky traps at 30-305 m from the release point and sex pheromone-baited transparent sticky traps placed at 500-3500 m. Both the negative exponential and negative power law models in non-log form presented the best overall fit to the numbers of recaptured adults (1% recapture rate). The negative exponential model in log form presented the best fit to the data in the tail. The models suggested that half of the dispersing WCR adults travelling along a given bearing will have travelled between 117 and 425 m and 1% of the adults between 775 and 8250 m after 1 day. An individual-based model of dispersal and mortality over a generation of WCR adults indicated that 9.7-45.3% of the adults would escape a focus zone (where maize is only grown once in 3 consecutive years) of 1 km radius and 0.6-21% a safety zone (where maize is only grown once in 2 consecutive years) of 5 km radius and consequently current European Commission (EC) measures are inadequate for the eradication of WCR in Europe. Although buffer zones large enough to allow eradication would be economically unpalatable, an increase of the minimum width of the focus zone from 1 to 5 km and the safety zone from 5 to 50 km would improve the management of local dispersal.

Microarray Analysis Yields Candidate Markers for Rotation Resistance in the Western Corn Rootworm Beetle, *Diabrotica virgifera virgifera*

AU Knolhoff, LM; Walden, KKO; Ratcliffe, ST; Onstad, DW; Robertson, HM

SO Evolutionary Applications 2010 3(1) 17-27

AB As pest species may evolve resistance to chemical controls, they may also evolve resistance to cultural control methods. Yearly rotation of corn (*Zea mays*) with another crop interrupts the life cycle of the western corn rootworm beetle (*Diabrotica virgifera virgifera*, Coleoptera: Chrysomelidae), but behavioral resistance to crop rotation is now a major problem in the Midwest of the USA. Resistant adult females exhibit reduced fidelity to corn as a host and lay their eggs in the soil of both corn and soybean (*Glycine max*) fields. Behavioral assays suggest that the adaptation is related to increased locomotor activity, but finding molecular markers has been difficult. We used microarray analysis to search for gene expression differences between resistant and wild-type beetles. Candidates validated with real-time polymerase chain reaction exhibit predicted patterns from the microarray in independent samples across time and space. Many genes more highly expressed in the rotation-resistant females have no matches to known proteins, and most genes that were more lowly expressed are involved in antimicrobial defense.

IWGO – NEWSLETTER 30 / 1

Harmonic Radar: Assessing the Impact of Tag Weight on Walking Activity of Colorado Potato Beetle, Plum Curculio, and Western Corn Rootworm

AU Boiteau, G; Vincent, C; Meloche, F; Leskey, TC

SO Journal of Economic Entomology 2010 103(1) 63-69

AB The impact of electronic dipole tags on the walking activity of three insects was determined using video tracking software. Results varied within and between the three species studied. The heaviest tag mounted on the pronotum of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), reduced its mean horizontal speed slightly but significantly. The mean horizontal speed of plum curculio, *Conotrachelus nenuphar* (Herbst), was significantly reduced by a light tag but not by heavier tags. The mean horizontal speed of the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), increased slightly when a tag was attached. A tag mounted to the dorsum of the Colorado potato beetle and the plum curculio slightly changed their climbing speed. Changes in the walking speed were variable but remained well within the range of values recorded in the literature. The impact of the additional tag weight was within the range of constraints to movement (e.g., slopes, barriers, etc.) normally experienced by insects in their environment. The results confirm that tags presently available will have minimal impact on the walking behavior of Colorado potato beetle, provide an estimate of the optimal tag weight for the plum curculio and show that a lighter tag and a better attachment method are required for the western corn rootworm. Because of the ability of insects to adapt to a wide range of landscapes and to their own body weight changes, the additional tag weight had a limited impact. Compared with the value of the ecological information gathered using this technology.

Density-Dependent and Density-Independent Mortality of the Western Corn Rootworm: Impact on Dose Calculations of Rootworm-Resistant Bt Corn

AU Hibbard, BE; Meihls, LN; Ellersieck, MR; Onstad, DW

SO Journal Of Economic Entomology 2010 103(1) 77-84

AB The percentage of viable eggs of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, which survived to the adult stage was evaluated for the effect of egg density in 2005 and 2007 in central Missouri. In 2005, each plot was 2.44 by 3.05 m and contained 64 maize (corn), *Zea mays* L., plants. In 2007, plots were 3.05 by 3.05 m and again contained 64 corn plants. Seven egg densities (2,400, 1,200, 600, 300, 100, 50, and 25 viable eggs per 30.5 cm) were evaluated with four to six replications in each year in a completely randomized design. In 2007 only, all additional row was infested near each plot to evaluate plant damage. In both years, there was no correlation of infestation level and percentage of emergence between infestation levels of 25-600 viable eggs per 30.5 cm, indicating that density-dependent mortality did not occur at these egg densities. In 2005, 8.04% of the viable eggs established on a corn plant and produced an adult at these lower infestation rates. In 2007, this value was 2.9%. Regardless of egg density, approximately 92-97% failed to establish and produce adults (density-independent mortality). In 2005 and in the combined analysis, as viable egg densities increased from 600 to 2400 per 30.5 cm there was a significant decrease in percentage of emergence. In a broken line analysis of the 2005 data, the point where density-dependent mortality began in the combined analysis was 851 eggs per 30.5 cm with a 95% confidence interval from 678 to 1,024. That year density-dependent mortality was important at high infestations and killed 54.4% of those larvae that successfully established on a plant at the highest egg density. However, little or no density-dependent mortality occurred at infestation levels <850 viable eggs per 30.5 cm in either year of the study. Combining data from both years with all previously published data in a broken line analysis indicated that density-dependent mortality began at approximately 800 viable eggs per 30.5 cm. These data are discussed in terms of dose calculations for products targeting the western corn rootworm.

Evaluating Western Corn Rootworm (Coleoptera: Chrysomelidae) Emergence and Root Damage in a Seed Mix Refuge

AU Murphy, AF; Ginzal, MD; Krupke, CH

SO Journal Of Economic Entomology 2010 103(1) 147-157

AB Resistance management is essential for maintaining the efficacy and long-term durability of transgenic corn engineered to control western corn rootworm (*Diabrotica virgifera virgifera* Le Conte).

IWGO – NEWSLETTER 30 / 1

Theoretically, a refuge can be provided by growing susceptible (refuge) plants in either a separate section of the field adjacent to resistant (transgenic) plants, or as a seed mixture. We examined the effects of varying the structure of a 10 and 20% refuge between currently approved structured refuges (block or strip plantings), as well as deploying the refuge within a seed mix, on adult emergence timing and magnitude, root damage and yield. Our 2-yr field study used naturally occurring western corn rootworm populations and included seven treatments: 10 and 20% block refuge, 10 and 20% strip refuge, 10 and 20% seed mix refuge, and 100% refuge. Beetles emerging from refuge corn emerged more synchronously with those emerging from transgenic (*Bacillus thuringiensis* [Berliner] Bt-RW) corn in seed mix refuges when compared with block refuges. The proportion of beetles emerging from refuge plants was significantly greater in a block and strip refuge structure than in a seed mix refuge. More beetles emerged from Bt-RW corn plants when they were grown as part of a seed mix. We discuss the potential benefits and drawbacks of a seed mix refuge structure in light of these findings.

New Syntheses of 1,7-Dimethylnonyl Propanoate, the Western Corn Rootworm Pheromone, in Four Different Ways via Cross Metathesis, Alkylation and Coupling Reactions

AU Mori, K

SO *Bioscience Biotechnology and Biochemistry* 2010 74(3) 595-600

AB A mixture of the four stereoisomers of 1,7-dimethylnonyl propanoate, the female sex pheromone of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), was synthesized in four different ways by employing one of the following four reactions as the key step: (i) cross metathesis using the Grubbs I catalyst, (ii) cross metathesis using the Grubbs II catalyst, (iii) alkylation of an alkynide anion, and (iv) Grignard coupling in the presence of dilithium tetrachlorocuprate. Although the cross metathesis approaches enabled two short syntheses (4 or 6 steps) of the pheromone to be achieved, the cheapest and most efficient synthesis was possible via Grignard coupling to give the desired pheromone in a 40% overall yield based on 2-methyl-1-butanol (8 steps).

Isolation of transcripts from *Diabrotica virgifera virgifera* LeConte responsive to the *Bacillus thuringiensis* toxin Cry3Bb1

AU Sayed, A; Wiechman, B; Struewing, I; Smith, M; French, W; Nielsen, C; Bagley, M

SO *Insect Molecular Biology* 2010 19(3) 381-389

AB Crystal (Cry) proteins derived from *Bacillus thuringiensis* (Bt) have been widely used as a method of insect pest management for several decades. In recent years, a transgenic corn expressing the Cry3Bb1 toxin has been successfully used for protection against corn rootworm larvae (genus *Diabrotica*). The biological action of the Bt toxin in corn rootworms has not yet been clearly defined. Because development of resistance to Bt by corn rootworms will have huge economic and ecological costs, insight into larval response to Bt toxin is highly desirable. We identified 19 unique transcripts that are differentially expressed in *D. virgifera virgifera* larvae reared on corn transgenic for Cry3Bb1. Putative identities of these genes were consistent with impacts on metabolism and development. Analysis of highly modulated transcripts resulted in the characterization of genes coding for a member of a cysteine-rich secretory protein family and a glutamine-rich membrane protein. A third gene that was isolated encodes a nondescript 132 amino acid protein while a fourth highly modulated transcript could not be further characterized. Expression patterns of these four genes were strikingly different between susceptible and resistant western corn rootworm populations. These genes may provide useful targets for monitoring of Bt exposure patterns and resistance development in pest and non-target insect populations.

IWGO – NEWSLETTER 30 / 1

How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the western corn rootworm?

AU Hiltbold, I; Toepfer, S; Kuhlmann, U; Turlings, TCJ;

CT Symposium on Chemical Communication Within, Among and Around Plants; Nov. 07-08, 2008

CL Genval, BELGIUM

SO Chemoecology 2010 20(2) 155-162

AB Because the ferocious maize pest *Diabrotica virgifera virgifera* LeConte can adapt to all currently used control strategies, focus has turned to the development of novel, more sustainable control methods, such as biological control using entomopathogenic nematodes (EPN). A good understanding of the biology and behaviour of these potential control agents is essential for their successful deployment. Root systems of many maize varieties emit (E)-beta-caryophyllene (E beta C) in response to feeding by larvae of the beetle *D. v. virgifera*. This sesquiterpene has been shown to attract certain species of EPN, thereby enhancing their control potential. In this study, we tested the effect of this root-produced volatile on the field efficacy of the three EPN *Heterorhabditis bacteriophora*, *Heterorhabditis megidis* and *Steinernema feltiae* against *D. v. virgifera* larvae in southern Hungary. By comparing beetle emergence and root damage for two maize varieties, one that emits E beta C and one that does not, it was found that root protection by *H. megidis* and *S. feltiae* was higher on the emitting variety, but this was not the case for *H. bacteriophora*. Overall, all three nematode species showed good control potential. We conclude that, if properly applied and in combination with the right maize variety, the release of these nematodes can be as effective as other control methods.

Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe - the 'no control' scenario

AU Wessler, J; Fall, EH

SO Journal Of Applied Entomology 2010 134(5) 385-394

AB The Western Corn Rootworm (WCR or Dvv., *Diabrotica virgifera virgifera* Le Conte) was first detected in Europe in the early nineties in Serbia. Since then the beetle has spread to more than 15 European countries. We assess the potential damage costs of the invasive species *Diabrotica virgifera virgifera* LeConte (Dvv.) in Europe under a 'no control' scenario. While previous studies considered benefits and costs at country level, this study explicitly investigates the external benefits of control in one country for other countries. The assessment considers the spatial and temporal aspects of invasion considering a number of scenarios developed together with experts. The results indicate enormous economic benefits can be gained by controlling further spread of Dvv. The economic benefits of control range between 143 million Euro in the best case and 1739 million Euro in the worst case scenario. The most likely scenario results in average annual economic benefits of 472 million Euro. Even in countries that do not face high damage costs control can be justified as this will reduce the speed of spread of the WCR and generate a positive externality for other regions with higher damage costs.

On the competitiveness of *Diabrotica virgifera virgifera* damage abatement strategies in Hungary: a bio-economic approach

AU Dillen, K; Mitchell, PD; Tollens, E;

SO Journal of Applied Entomology 2010 134(5) 395-408

AB *Diabrotica virgifera virgifera* Le Conte or western corn rootworm (WCR) is a major pest of cultivated maize, *Zea mays* L., introduced into Europe in 1992. Since then, the beetle spread through Central Europe, leading to a continuous pest population in 11 European countries. This article presents an economic assessment of different damage abatement strategies against this invasive species in the Hungarian maize sector. A bio-economic model, using Monte Carlo sampling, estimates the potential damage from WCR under no control and the value created by Bt maize, seed treatment, soil insecticides and cultural control. At the same time, potential market shares for the different damage abatement options under perfect information are deducted. The potential damages under a no control scenario are estimated at euro176/ha for grain maize farmers on average, which points out the need for well-designed damage abatement strategies. For land-constrained farmers cultural

IWGO – NEWSLETTER 30 / 1

control is a valuable damage abatement strategy, being the optimal choice in 69% of the cases. In monoculture Bt maize is the best option as it creates the highest value in 78% of the cases. However, as Bt maize active against WCR is not deregulated in the European Union, soil insecticides in 54% of the cases and seed treatment in 46% of the cases are the rational choices. As the value created by Bt maize is positive, not deregulating Bt maize in Hungary leads to benefits foregone ranging from euro16/ha for land-constrained grain farmers to euro49/ha in the case of silage maize under monoculture. Finally, the results of the sensitivity analysis can be used to develop a multi-criteria tool to aid farmers in applying the appropriate damage abatement strategy. This could decrease the dependency of farmers on scouting techniques and economic thresholds of WCR presence.

Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model

AU Dun, Z; Mitchell, PD; Agosti, M

SO Journal Of Applied Entomology 2010 134(5) 409-419

AB We apply the double-nested unbalanced panel data model developed by Antweiler [J. Econometrics 101 (2001) 295] to estimate a damage function for western corn rootworm (*Diabrotica virgifera virgifera*) using commonly available field data. These data are from experiments collecting maize yields and measures of maize root injury due to rootworm larval feeding for different treatments, with multiple replicates at many locations over several years, which creates nested panel data. The nested panel becomes unbalanced when the number of replicates, locations or years of data differs during the course of the study. We use Antweiler's (2001) method to estimate damage functions with data from four irrigated locations in northern Italy from 2006 to 2008 and from four dryland locations in the state of Illinois from 2005 to 2007 to predict the expected percentage of yield lost based on the observed node injury scale of Oleson et al. [J. Econ. Entomol. 98 (2005) 1]. Estimated coefficients imply that a one unit difference in the node injury scale is on average associated with a 17.9% yield loss for the Illinois locations and a 2.9% loss for the locations in Italy. We attribute the lower yield loss for the Italian locations to the use of irrigation. Estimated variance components were relatively large, indicating the tremendous variability in losses observed for plots with similar node injury scale differences. Given the large variation in observed yield losses and the large estimated random effects, the damage function is more appropriate for larger-scale, longer-term estimates of yield loss from the western corn rootworm rather than for field-scale estimates. These estimates are the first damage function estimates for the node injury scale in maize and should be considered preliminary, an initial baseline for comparison and refinement as additional data are collected and analysed in the US and Europe.

The prospects for sequencing the western corn rootworm genome

AU Miller, NJ; Richards, S; Sappington, TW

SO Journal of Applied Entomology 2010 134(5) 420-428

AB Historically, obtaining the complete sequence of eukaryotic genomes has been an expensive and complex task. For this reason, efforts to sequence insect genomes have largely been confined to model organisms, species that are important to human health and representative species from a few insect orders. This situation is set to change as a number of 'next generation' sequencing technologies are making large-scale DNA sequencing both affordable and accessible. Sequencing the genome of the western corn rootworm, *Diabrotica virgifera virgifera*, is likely to become a realistic proposition within the next 2 years. In the meantime, there is an active community of *Diabrotica* geneticists and biologists who are working to assemble the resources that will be needed for a genome sequencing project. A western corn rootworm genome sequence will be an invaluable resource that will facilitate research into the genetics, evolution and ecology of a major pest of maize agriculture in North America and Europe.

IWGO – NEWSLETTER 30 / 1

Flight and crawling activities of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in relation to morphometric traits

AU Li, H; Toepfer, S; Kuhlmann, U

SO Journal of Applied Entomology 2010 134(5) 449-461

AB Morphometric traits and body weight are often used to study changes in fitness. For the invasive alien maize pest, *Diabrotica virgifera virgifera* LeConte, little information is available regarding the possible relationship between morphometric traits and adult activity, which reflects the ability to disperse and invade. Flight and crawling activities of *D. v. virgifera* adults were investigated in relation to six different morphometric traits as well as body weight, sex and age. This laboratory study revealed that flight activity of *D. v. virgifera* differed between sexes and changed with age. Young adults of both sexes flew more frequently and took off faster than mature adults. Males flew more frequently and took off faster than females, regardless of age-class. No such differences were found for crawling frequency, but young males crawled faster than young females. Further analysis revealed that fresh body weight and morphometric traits of young adults were better predictors of flight and crawling activity than the same measurements made on mature adults. Particularly pronotum and elytra measurements on young adults are recommended for bioassay studies on activity parameters of *D. v. virgifera*.

8-Methyldecan-2-yl Acetate Inhibits Response to the Pheromone in the Western Corn Rootworm *Diabrotica v. virgifera*

AU Toth, M; Ujvary, I; Imrei, Z

SO Journal of Applied Entomology 134(5) 462-466

AB Compounds that are structurally closely related to the western corn rootworm (WCR) (*Diabrotica v. virgifera*, Coleoptera: Chrysomelidae) pheromone were prepared and screened for biological activity in the field, presented alone or in combination with the pheromone 8-methyldecan-2-yl propanoate. None of the synthetic compounds showed attraction when presented alone. However, when presented in combination with the pheromone, catches in traps containing 8-methyldecan-2-yl acetate as a second component were dramatically reduced, suggesting strong inhibitory activity for this compound. The addition of the inhibitory acetate to the known floral WCR lure (4-methoxycinnamaldehyde plus indole) did not influence male (or female) catches suggesting that the inhibitor interferes in the perception process of the pheromone and not by exerting repellency per se. To our knowledge, this is the first report on an inhibitor of response to pheromone in WCR. 8-Methyldecan-2-yl acetate has previously been described as a sex attractant of *Diabrotica cristata*, so its inhibitory activity towards males of WCR may reflect a role in maintaining reproductive isolation between the two taxa.

Controlling Western Corn Rootworm Larvae with Entomopathogenic Nematodes: Effect of Application Techniques on Plant-scale Efficacy

AU Toepfer, S; Burger, R; Ehlers, RU; Peters, A; Kuhlmann, U

SO Journal of Applied Entomology 2010 134(5) 467-480

AB The three larval instars of western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) feed on the roots of maize, *Zea mays* (L.). The effects of six application techniques on the plant-scale efficacy of the entomopathogenic nematode species, *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), in controlling *D. v. virgifera* populations were assessed in seven field plot experiments in southern Hungary between 2004 and 2007. Approximately 230 000 nematodes were applied per row metre using four different stream spray techniques; or, alternatively 400 000 nematodes per square metre using two different flat spray techniques. Nematode efficacy was assessed by comparing the number of emerging adult *D. v. virgifera*, and root damage between treatments and untreated controls. All tested nematode application techniques reduced *D. v. virgifera* density by at least 50% (on average across fields and years). The highest reduction in *D. v. virgifera* density was 68% and occurred when nematodes were applied into the soil together with maize sowing using a fluid solid stream. Rainfall, the day before application likely increased the control efficacy of *H. bacteriophora*. Using the 0.00-3.00 node injury damage rating scale, we estimated that potential root damage was prevented by 25-79% when *H. bacteriophora* was applied. Although, *H. bacteriophora* can effectively be applied with all of the techniques tested, for optimum performance

IWGO – NEWSLETTER 30 / 1

and minimum costs, it is suggested that the nematodes be applied as follows: (i) as a stream requiring 8-10 times less volume of water than flat sprays, or as a granule requiring no water, and (ii) into the soil when sowing maize, requiring less water than soil surface sprays and avoiding the destruction of nematodes by UV radiation and additional machinery use.

Chemical control of *Diabrotica virgifera virgifera* LeConte

AU van Rozen, K; Ester, A

SO Journal of Applied Entomology 134(5) 376-384

one of the most important pest species of maize in several countries of Central and Eastern Europe. This pest insect has invaded from the USA and is mainly controlled by insecticides in the EU. In the USA reliance on insecticides seems to decrease due to transgenic maize production. The EU project Diabr-Act aims at establishing a harmonized and sustainable control strategy for western corn rootworm populations in Europe. In this respect, current chemical control strategies used in the USA and EU are described. Seed, soil and foliar treatments as well as transgenically protected maize are discussed. Specific attention is given to insecticide resistance. Seed treatment has clear environmental benefits, but factors influencing the efficacy should be declared. Soil and foliar treatments have perspectives, though new technologies limiting environmental hazards should be considered. Transgenic maize may have serious perspectives.

New polynucleotide comprising specific nucleotide sequence encoding protein having caryophyllene synthase activity, useful for establishing or enhancing resistance against herbivore in plants e.g. resistance against rootworm in maize plant

AE Max Planck Ges Foerderung Wissenschaften; Univ Neuchatel

AU Crocoll C; Degenhardt J; Gershenzon J; Hiltold I; Koellner T; Turlings T

PY 2010

AB NOVELTY - A polynucleotide (P1) comprising a specific nucleotide sequence encoding a protein having caryophyllene synthase activity, is new. USE - For establishing or enhancing resistance against herbivore in plants; and for growing the plant. The herbivore is a root-damaging insect, and the plants are maize plants and the root-damaging insect is the corn rootworm (claimed). ADVANTAGE - The polynucleotide provides conferred or increased resistance against herbivore to a plant.

DETAILED DESCRIPTION - A polynucleotide (P1) selected from (a) polynucleotides comprising a nucleotide sequence encoding a polypeptide having amino acid sequence of SEQ ID No. 2 (SEQ ID No.: not defined) or SEQ ID No. 4 (SEQ ID No.: not defined) as given in the specification; (b) polynucleotides comprising the nucleotide sequence of SEQ ID No. 1 containing 1665 base pairs or SEQ ID No. 3 containing 1655 base pairs, as given in the specification; (c) polynucleotides comprising a nucleotide sequence encoding a fragment of the polypeptide encoded by a polynucleotide of (a) or (b), where the nucleotide sequence encodes a protein having caryophyllene synthase activity; (d) polynucleotides comprising a nucleotide sequence the complementary strand of which hybridizes to the polynucleotide of any one of (a) to (c), where the nucleotide sequence encodes a protein having caryophyllene synthase activity; (e) polynucleotides encoding a polypeptide having an amino acid sequence which is at least 50% identical to the amino acid sequence of a polypeptide encoded by a polynucleotide of any one of (a) to (c) and having caryophyllene synthase activity; and (f) polynucleotides comprising a nucleotide sequence that deviates from the nucleotide sequence defined in (d) or (e) by the degeneracy of the genetic code, is new.

INDEPENDENT CLAIMS are included for the following: (1) new polynucleotide (P2) having a length of at least 15 nucleotides which specifically hybridizes with a polynucleotide (P1) or with their complementary strand; (2) a recombinant nucleic acid molecule (M1) comprising the polynucleotide (P1) or (P2) and expression control sequences operably linked to the polynucleotide; (3) a vector (V1) comprising the polynucleotide (P1) or (P2) or the recombinant nucleic acid molecule (M1); (4) a host cell (C1) which is genetically engineered with the polynucleotide (P1) or (P2), the recombinant nucleic acid molecule (M1) or the vector (V1); (5) preparation of the polypeptide (P1) or (P2); (6) a binding molecule specifically recognizing the polypeptide (P1) or (P2); (7) a transgenic plant cell (C2) which is genetically engineered with the polynucleotide (P1) or (P2), the recombinant nucleic acid molecule

IWGO – NEWSLETTER 30 / 1

(M1) or the vector (V1); (8) a transgenic plant (T1) or plant tissue comprising the plant cells (C2); (9) propagation material (M2) or harvestable parts of the transgenic plant (T1) comprising the plant cells (C2); (10) conferring resistance or increased resistance against a herbivore to a plant involving the step of providing a transgenic plant in which polypeptide (P1) or (P2) is expressed; and (11) use of the transgenic plant (T1) or the propagation material (M2) together with entomopathogenic nematodes for growing the plant.

AGRICULTURE - Preferred Nematodes: The entomopathogenic nematode is *Heterorhabditis megidis*.

BIOTECHNOLOGY - Preparation (Claimed): Preparation of the polypeptide (P1) or (P2) involves cultivating the host cell (C1) under conditions allowing for the expression of the polypeptide, and isolating the polypeptide from the cells and/or the culture medium. Preferred Components: The expression control sequences comprise a root-specific promoter.

How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the western corn rootworm?

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AB Because the ferocious maize pest *Diabrotica virgifera virgifera* LeConte can adapt to all currently used control strategies, focus has turned to the development of novel, more sustainable control methods, such as biological control using entomopathogenic nematodes (EPN). A good understanding of the biology and behaviour of these potential control agents is essential for their successful deployment. Root systems of many maize varieties emit (E)-beta-caryophyllene (E beta C) in response to feeding by larvae of the beetle *D. v. virgifera*. This sesquiterpene has been shown to attract certain species of EPN, thereby enhancing their control potential. In this study, we tested the effect of this root-produced volatile on the field efficacy of the three EPN *Heterorhabditis bacteriophora*, *Heterorhabditis megidis* and *Steinernema feltiae* against *D. v. virgifera* larvae in southern Hungary. By comparing beetle emergence and root damage for two maize varieties, one that emits E beta C and one that does not, it was found that root protection by *H. megidis* and *S. feltiae* was higher on the emitting variety, but this was not the case for *H. bacteriophora*. Overall, all three nematode species showed good control potential. We conclude that, if properly applied and in combination with the right maize variety, the release of these nematodes can be as effective as other control methods.