Successful application of entomopathogenic nematodes for the biological control of western corn rootworm larvae in Europe – a mini review

Erfolgreiche Applikation entomopathogener Nematoden zur biologischen Bekämpfung des Westlichen Maiswurzelbohrers in Europa – ein Mini-Review

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Summary

10 years of joint efforts in research and development have led to a nematode-based biological control solution for one of the most destructive maize pests, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Commercially mass-produced *Heterorhabditis* species of beneficial entomopathogenic nematodes are ready to use. They can be applied into the soil during sowing of maize for controlling the subsequently hatching larvae of *D. virgifera virgifera* thus preventing root feeding and damage to maize. Policy bodies, decision makers and farmers are advised to consider biological control as one of the alternatives to synthetic pesticides in maize production, and according to the EC Directive on the sustainable use of pesticides and implementation of integrated pest management.

Key words: *Diabrotica virgifera virgifera;* Chrysomelidae; insect parasitic nematodes; inundative biological control; *Zea mays*

Zusammenfassung

Nach 10 Jahren gemeinsamer Anstrengungen und Entwicklungen wurde eine biologische nematodenbasierte Möglichkeit zur Bekämpfung eines der gefährlichsten Maisschädlinge, des Westlichen Maiswurzelbohrers *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), gefunden. Nützliche entomopathogene Nematoden von *Heterorhabditis*-Arten wurden in Massenproduktion hergestellt und stehen zur Anwendung bereit. Sie können mit der Maisaussaat in den Boden eingebracht werden und dann die schlüpfenden Larven von *D. virgifera virgifera* bekämpfen und so Wurzelfraß und Maisschäden verhindern. Wir empfehlen politischen Gremien, Entscheidungsträgern und Landwirten die biologische Bekämpfung als eine Alternative zu synthetischen Pflanzenschutzmitteln im Maisanbau und entsprechend EU-Richtlinie über die nachhaltige Anwendung von Pflanzenschutzmitteln und die Umsetzung des integrierten Pflanzenschutzes zu prüfen.

Stichwörter: *Diabrotica virgifera virgifera*; Chrysomelidae; insektenparasitäre Nematoden; inundative biologische Schädlingsbekämpfung; *Zea mays*

1. Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most destructive pests of maize in North America (Levine and Oloumi-Sadeghi, 1991). It is a univoltine species with eggs that overwinter in the soil (Chiang, 1973). After maize has germinated, the eggs hatch, and its three larval life stages feed on maize roots, often causing plant lodging and yield losses. Adults can occasionally reduce yields through intensive silk feeding.

Over the last 25 years, *D. virgifera virgifera* has moved into Europe causing problems in maize (Ciosi *et al*., 2008; Ciosi *et al*., 2011; Szalai *et al*., 2011). In an effort to eradicate or contain the species, legislation has been put into place, which forces farmers to rotate their fields (thereby interrupting the life cycle of *D. virgifera virgifera*) and to apply granular soil insecticides or to use insecticide-coated maize seeds (to target the root feeding larvae) (Carrasco *et al*., 2010; DLZ, 2011). Additionally, broad-spectrum foliar insecticides are sprayed against the adults using high clearance spraying machinery (Rozen and Ester, 2010). However, the soil insecticides and seed coatings are potentially problematic due to non-target effects resulting in public concerns and in a partial ban on their use in maize (Gill *et al.,* 2012; Cressey, 2013). Also, the synthetic foliar insecticides are usually broad spectrum knock-down contact pesticides with considerable non-target effects. In an attempt to reduce insecticide usage against this invader, biological control options have been recommended for this pest (Babendreier *et al*., 2006; DLZ, 2011; Knuth *et a*l., 2011), and for European maize production in general (European Commission, 2009).

10 years of joint efforts in research and development for a nematode-based biological control solution are reviewed here and outcomes are presented.

2. Material and methods

Between 2004 and 2008, CABI, the University of Neuchâtel in Switzerland, the farmer association fenaco (UFA-Samen Beneficials) in Aesch in Switzerland, the Plant Protection Directorate in Hodmezovasarhely in Hungary, Agroscope Reckenholz-Tänikon in Switzerland, the University of Kiel in Germany, and the nematode producers e-nema at Schwentinenthal in Germany and Andermatt Biocontrol at Grossdietswil in Switzerland, laid the scientific base for nematode-based biological control products against *D. virgifera virgifera* (CABI, 2008). Between 2007 and 2008, a number of institutions reviewed biological control options against rootworms and proposed them to the European Commission (Diabr-Act, 2007; CABI, 2008). Between 2009 and 2012, the Landwirtschaftliches Technologiezentrum Augustenberg, Karlsruhe in Germany, Cult-tec Ltd. in Freiburg in Germany, the Austrian Agency for Health and Food Safety in Vienna in Austria, the Cereal Research Station in Szeged in Hungary, SAGEA Centro di Saggio S.r.l. in Italy, CABI, and others improved application technologies aiming for the farmer-friendliest and least-costly method (CABI, 2012).

3. Results and discussion

Review of biological control options against *D. virgifera virgifera*

Several natural enemy species or groups appeared promising candidates for control strategies with different ecological rationales. Research was proposed to pursue: (1) developing inundative biological control products, particularly mass-produced entomopathogenic nematodes and fungi, (2) understanding specific natural enemies of Diabroticina larvae throughout the Americas and of adults particularly in higher altitudes of Central America or northern South America including potential classical biological control agents against *D. virgifera virgifera*; (3) enhancing natural enemies through cultural practices, i.e. reduced tillage, reduced weed control, cover crops, diversified crop rotations or soil amendments. For complete lists of natural enemies of Diabroticina and further details refer to: Kuhlmann and Burgt, 1998; Diabr-Act, 2007; Toepfer *et al*., 2009; Pilz *et al.,* 2008).

Nematode screening in laboratory

Screenings experiments in Petri dishes on filter paper or in sand, as well as bioassays in containers with sand or soil and maize revealed that *Heterorhabditis bacteriophora, H. megidis, Steinernema feltiae, St. arenarium,* and *St. kraussei* are highly virulent against *D. virgifera virgifera* larvae. *St. abassi* was found intermediate. *St. carpocapsae* and *St. glaseri* appeared less virulent. For details refer to: Journey and Ostlie, 2000; Jackson, 1995; Toepfer *et al*., 2005, Kurtz *et al.,* 2009; Hiltpold *et al.,* 2009.

Nematode screening in the field

Plant scale field experiments with artificial *D. virgifera virgifera* infestation and into-soil applications of fluids of different nematode species during sowing in April or into and onto soil later in June, revealed that *H. bacteriophora* and *H. megidis* are highly effective against *D. virgifera virgifera* larvae (i.e. up to 81%), and in preventing damage to maize roots (i.e. up to 80%) (Fig. 1). *S. feltiae* appeared moderately effective. For comparisons with pesticides and further details refer to: Jackson, 1996; Jackson, 1997, Toepfer *et al.,* 2008; Pilz *et al*., 2009; Hitpold *et al*., 2009.

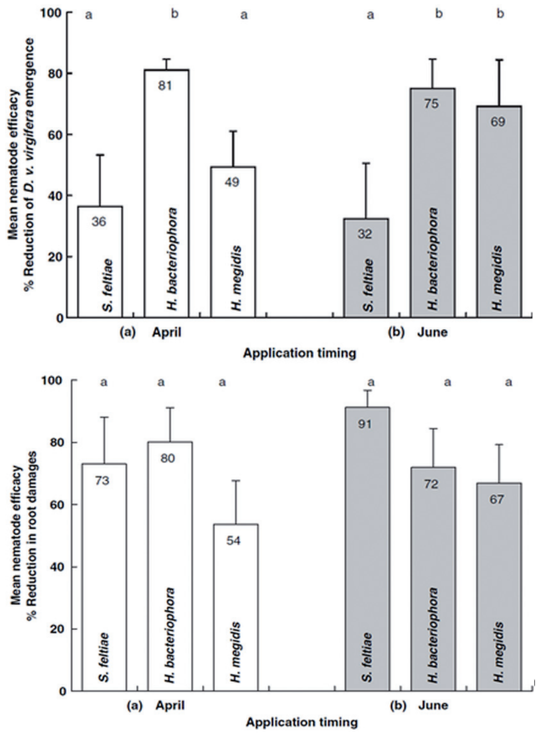

Fig. 1 Results extracted from Toepfer *et al*. (2008) on the efficacy of entomopathogenic nematodes in plant scale field trials in southern Hungary at reducing *D. virgifera virgifera* and at preventing root damage. Percent reduction of adult emergence and root damage (Node Injury scale-based) in comparison to the controls; application of 0.21 -0.26 Mill nematodes per row meter (ca. 2.8 – 3.5 x 10^9/ha) as a solid stream into the soil during sowing of maize (A) or onto the soil and along rows of young maize plants (B); Letters on *D. virgifera virgifera* bars indicate differences according to the LSD PostHoc test after ANOVA; letters on root damage bars indicate differences according to the M - Witney U test; Error bars=SEM).

Abb. 1 *Ergebnisse aus Töpfer et al. (2008) zur Wirksamkeit entomopathogener Nematoden in Feldversuchen in Südungarn zur Bekämpfung von* D. virgifera virgifera *und der Verhinderung von Wurzelschäden. Prozentuale Verringerung der Adulten und von Wurzelschäden (anhand der Knotenverletzung) im Vergleich zur Kontrolle; Anwendung von 0,21-0,26 Mio Nematoden je Reihenmeter (ca. 2,8 – 3,5 x 10^9/ha) als Flüssigkeit in den Boden während der Maisaussaat (A) oder auf den Boden entlang der Reihen junger Maispflanzen (B); die Buchstaben an den* D. virgifera virgifera*-Säulen zeigen Unterschiede gemäß LSD Post-Hoc-Test nach ANOVA; die Buchstaben an den Wurzelschädensäulen zeigen Unterschiede gemäß M – Witney U-Test; Standardfehler = SEM).*

3.1 Scientific pre-requisites

Instar susceptibility of target

Bioassays with different life stages of *D. virgifera virgifera* and different nematodes revealed that all larval instars and even pupae are effectively killed by *H. bacteriophora*, *H. megidis* and *St. feltiae*. Adults appeared less susceptible. For details refer to: Kurtz *et al*., 2009.

Orientation of nematodes to target

Nematodes were found to orient towards *D. virgifera virgifera* -damaged maize roots using the root-emitted organic volatile compound (E)-β-caryophyllene as an orientation cue to find *D. virgifera virgifera* larvae. Caryophyllene might be particularly important for *H. megidis* and less for *H. bacteriophora*. Other authors mention that caryophyllene is of little to no importance for nematodes. For details refer to: Rasmann *et al*., 2005; Hiltpold and Turlings, 2008; Kneight, 2010; Anbesse and Ehlers, 2013.

Maize hybrid importance

There are hardly any hints that the choice of maize hybrids is important for biological pest control with entomopathogenic nematodes. Some hybrids have lost the capability to emit the nematode-attracting (E)-β-caryophyllene; however, most European maize hybrids emit caryophyllene upon larval feeding. For details refer to: Rasmann *et al*., 2005; Hiltpold and Turlings, 2008; Hiltpold, 2010.

Establishment and persistence of nematodes

Field experiments revealed that applied nematodes establish at relatively low rates in the soil of maize fields; but, that they survive more than two months, which is long enough to effectively kill all three larval instars. Nematodes were found to propagate on *D. virgifera virgifera* larvae in the field, which is a big advantage over pesticides. For details refer to: Kurtz *et al*., 2007; Pilz *et al*., 2011a.

Soil importance

Field trials showed that *H. bacteriophora* can effectively kill *D. virgifera virgifera* larvae in a wide range of soils in maize fields. As *D. virgifera virgifera* larvae are usually most damaging in dense soils, control efficacies of nematodes were also often found higher in dense soils than in light, e.g. sandy, soils. For details refer to: Grabenweger *et al.,* 2010; Toepfer *et al*., 2010d; Pilz *et al.,* 2011a.

Non-target effects

Entomopathogenic nematodes are restricted to arthropods, thus there is no danger to plants or humans. Nematodes are known to be only slightly host specific on certain insect groups. However, field experiments revealed only minor effects of treatments on non-target populations, suggested to be a result of the generally poor arthropod diversity in soils of intensive field crops such as maize, as well as of the application of nematodes into relatively narrow soil areas and close to the target. For details refer to: Georgis *et al.,* 1991; Gaugler, 2002; Babendreier *et al*., 2014.

3.2 Application of nematodes

Where

Nematodes were successfully applied through fluid solid stream sprays, microgranules or seed coating into soil at sowing, or through fluid solid stream sprays or granules into soil next to young maize plants, or through fluid narrow flat sprays applied with lots of water over rows of small plants. For details and more options refer to: Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c; Knuth *et al*., 2011; Hiltpold *et al*., 2012.

When

Nematodes were successfully applied into soil at sowing (Mid April to early May in Central Europe), this is, a few weeks before *D. virgifera virgifera* eggs hatch; as well as into or onto soil along rows of young maize plants (mid to late May in Central Europe). Field applications against adults, i.e. in July or August, have never been attempted. For details refer to: Toepfer *et al.,* 2010c.

Formulation

Nematodes can be applied against *D. virgifera virgifera* larvae preferably just diluted in water. Microgranules, seed coatings, capsules and other options may need further research. For details refer to: Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c; Knuth *et al*., 2011; Hiltpold *et al*., 2012.

Need of water

Field experiments revealed that the need of water during application is variable and depends on the soil type, wheather conditions, and application techniques. Currently a minimum of 200 to 400 litres water/ha are advised for fluid stream sprays of nematodes into soils, and a minimum of 800 to 1000 litres for narrow stream sprays onto the soil or plants. Details through SAGEA Centro di Saggio S.r.l. (2010, pers. comm.) or in Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c.

3.3 Farmer friendly application techniques

Fluid and micro-granular applications as well as seed coating with nematodes appeared technically possible with the available farmer machineries; and all can achieve control of *D. virgifera virgifera* larvae as well as some root damage prevention. Currently most promising and most used is the fluid stream spray application into soil at sowing, using sowing machines with applicators that apply nematodes behind the sowing wheel and prior the soil-closing wheels (Fig. 2). For details refer to: Toepfer *et al.,* 2010c, SAGEA Centro di Saggio S.r.l, 2010, pers. comm.; Cult-tec 2012; Knuth *et al.,* 2011.

Fig. 2 Currently most promising and most used application technique for entomopathogenic nematodes against *D. virgifera virgifera* larvae: fluid stream spray application into the soil at sowing using sowing machines with applicators that apply nematodes behind the sowing wheel and prior the soil-closing wheels. Examples from left to right: LIQ-Inject M1 (Cult-tec company, Germany) on a Monosem NG Plus sowing machine (USA); Self-made application tube for fluids on Pneumasem sowing machine of Nodet Gugis(France); John Deer with fluid applicator on White sowing machine (USA).

Abb. 2 *Derzeit vielversprechende und meist genutzte Applikationstechnik für entomopathogene Nematoden gegen Larven von* D. virgifera virgifera*: Flüssigstrahlapplikation in den Boden bei der Aussaat mit Hilfe einer Sämaschine mit einer Vorrichtung zur Applikation von Nematoden hinter dem Särad und vor dem Bodenschließrad. Beispiele von links nach rechts: LIQ-Inject M1 (Cult-tec company, Deutschland) an einer Sämaschine Monosem NG Plus (USA); selbstgebautes Applikationsrohr für Flüssigkeiten an der Sämaschine Pneumasem von Nodet Gugis (Frankreich); John Deer mit Flüssigapplikator an einer White-Sämaschine (USA).*

3.4 Field scale efficacy and dosage-efficacy response

Field scale trials using farmer machinery revealed that *H. bacteriophora* is able to reach control efficacies of *D. virgifera virgifera* larvae to the same extent and at similar variability as soil insecticides and insecticide seed coatings. On multiple year, site and machinery average, control efficacies are approximately between 30 and 80%. Nematodes can also prevent root damage, on levels close to soil insecticides and insecticide seed coatings. A dose-efficacy response curve is not finally established, but preliminary results suggest that the optimal dose of nematodes might be somewhat between 2 and 3 billion per hectare maize field depending on local conditions. For details refer to: Pilz *et al*., 2009; Toepfer *et al*., 2010b; Pilz *et al*., 2011b.

3.5 Products

Heterorhabditis bacteriophora- and *H. megidis*-based products are available from several biocontrol companies, and can be applied, without restrictions, in countries where entomopathogenic nematodes do not need registrations and where the products consider species that are native, e.g. currently in Germany. One of the products (Dianem ™) is, for example, also registered in Austria.

3.6 Legislation

With the banning of several insecticides for seed coatings due to their bee toxicity, and with recent discussions on a number of soil pesticides in maize, farmers need alternative products. Moreover, the European Directive on sustainable use of pesticides requests from EU member countries to prefer alternative pest control options. Entomopathogenic nematodes are exceptionally safe biocontrol agents; thus they are exempted from registration in many European countries; in some they need registration. For details refer to: Ehlers, 2003; European Commission, 2008, 2009; Delos *et al*., 2011; Gill *et al*., 2012; Cressey, 2013.

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References

Anbesse, S. and R. U. Ehlers, 2013: *Heterorhabditis* sp. not attracted to synthetic (E)-β-caryophyllene, a volatile emitted by roots upon feeding by corn rootworm. J. Appl. Entom., **137,** 88-96.

Babendreier, D., M. Baroni, P. Jeanneret, Ch. Pilz, and S. Toepfer, 2014: Non-target effects of insecticides, entomopathogenic fungi and nematodes when applied against western corn rootworm larvae into the soil of maize fields. Bicontrol (in. prep.)

Babendreier, D., S. Keller, J. Romeis, , F. Bigler, U. Kuhlmann, S. Toepfer, T. Turlings, and L. Schaub, 2006: New approaches for controlling the Western Corn Rootworm. Agrarforschung, **13**, 80 - 83. (in German)

CABI 2008: Biological control of western corn rootworm, *Diabrotica virgifera virgifera*. http://www.cabi.org/default.aspx?site=170&page=1017&pid=19982

CABI 2008: Harmonizing the strategies for fighting the maize pest *Diabrotica*. http://www.cabi.org/default.aspx?site=170&page=1017&pid=19984

CABI 2012: Developing a biopesticide for *Diabrotica* control in Hungary, Austria and Germany. http://www.cabi.org/default.aspx?site=170&page=1017&pid=19986

Carrasco, L. R., T. D. Harwood, S. Toepfer, A. MacLeod, N. Levay, J. Kiss, R. H. A. Baker, J. D. Mumford, and J. D. Knight, 2010: Dispersal kernels of the invasive alien western corn rootworm and the effectiveness of buffer zones in eradication programmes in Europe. Annals Appl. Biol., **156,** 63-77.

Chiang, H. C. 1973: Bionomics of the northern and western corn rootworms. Annual Rev. Entom., **18,** 47-72.

Ciosi, M., N. J. Miller, K. S. Kim, R. Giordano, A. Estoup, and T. Guillemaud, 2008: Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Mol. Ecol., **17,** 3614-3627.

Ciosi, M., N. J. Miller, S. Toepfer, A. Estoup, and T. Guillemaud, 2011: Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, *Diabrotica virgifera virgifera.* Evolut. Appl., **4,** 54-70.

Cressey, D., 2013: Reports spark row over bee-bothering insecticides free. Nature News, **494**.

Cult-tec, 2012: Nematec for nematode application into the soil. http://www.cult-tec.de/nematec-projekt.html

Delos, M., L. Folcher, N. Eychenne, A. Weissenberger, and C. R. Roger, 2011: Integrated pest management relation with the sustainable pesticide use Directive 2009/128/EC - interest and limits. 740-751.

Diabr-Act, 2007: Survey on units interested in biological control of western corn rootworm in Europe http://diabract.vitamib.com/documents

Diabr-Act, 2007: Report on the integration among biological control techniques against western corn rootworm and other pests in maize. http://diabract.vitamib.com/documents

Diabr-Act, 2007: Literature and project review to adapt biopesticides for control of western corn rootworm in Europe http://diabract.vitamib.com/documents

Diabr-Act, 2007: Farmer training manual on enhancing natural enemies for sustainable maize management. http://diabract.vitamib.com/documents

Diabr-Act, 2007: Final report on biological control options of western corn rootworm in Europe. http://diabract.vitamib.com/documents

DLZ, 2011: Aus fuer Mais nach Mais. DLZ agrar magazin, **3,** 20-25. (in German)

Ehlers R.U., 2003: Entomopathogenic nematodes in the European biocontrol market. Commun. Agric. Appl. Biol Sci., **68**, 3-16.

e-nema, 2012: Biologische Bekämpfung des Maiswurzelbohrers mit dianem®. http://www.e-nema.de/maiswurzelbohrer.php (in German).

European Commission, 2008: Comission Decision concerning the non-inclusion of certain active substances in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances. December 2008. Official J. European Union, **L 333/11,** 4 pp.

European Commission, 2009: Sustainable Use Directive (Directive 2009/128/EC). Official J. European Union, **Directive 2009/128/EC,** 4 pp.

Gaugler, R., 2002: *Entomopathogenic Nematology*. CABI, Wallingford, UK.

Georgis, R., H. K .Kaya, and R. Gaugler, 1991: Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environ. Entom., **20,** 815-822.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine, 2012: Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature, **491,** 105-108.

Grabenweger, G., C. Pilz, and U. Heimbach, 2010: Influence of soil texture and density on the rate of survival of larvae of of the western corn rootworm (*Diabrotica virgifera virgifera*). eds, pp. 188. Julius Kühn Institut, Bundesforschungsinstitut für Kulturpflanzen, Quedlinburg.

Hiltpold, I., B. E. Hibbard, B. W. French, and T. C. J. Turlings, 2012: Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. Plant and Soil., **358,** 11-25.

Hiltpold, I., S.Toepfer, U. Kulhmann, and T. Turlings, 2009: How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the western corn rootworm? Chemoecology, **20,** 155-162.

Hiltpold, I. and T. C. J. Turlings, 2008: Belowground chemical signaling in maize: when simplicity rhymes with efficiency. J. Chem. Ecol., **34,** 628-635.

Jackson, J. J., 1995: Pathogenicity of entomopathogenic nematodes for third instars of the western corn rootworm. J. Nemat., **27,** 504.

Jackson, J. J., 1996: Field performance of entomopathogenic nematodes for suppression of western corn rootworm (Coleoptera: Chrysomelidae). J. of Economic Entom., **89,** 366-372.

Jackson, J. J., 1997: Field efficacy and ecology of three entomopathogenic nematodes with the western corn rootworm. J. Nemat., **29,** 586.

Journey, A. M. and K. R. Ostlie, 2000: Biological control of the western corn rootworm (Coleoptera:Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae.* Environmenal Entom., **29,** 822 - 831.

Knight, K., 2010: Nematodes vanquish billion dollar pest. Science daily, **6**.

Knuth, P., Pilz, C. and G.,Grabenweger, 2011: Wuermer gegen Bohrer. DLZ agrar magazin, **44-47,** 20-25. (in German)

Kuhlmann, U. and W. A. C. M.,Burgt, 1998: Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. Biocontrol News Inform., **19,** 59 - 68.

Kurtz, B., I.,Hiltpold, T. C. J.,Turlings, U. Kuhlmann, and S. Toepfer, 2009: Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes. BioControl, **54,** 255-262.

Kurtz, B., S. Toepfer, R. U. Ehlers, and U. Kuhlmann, 2007: Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. J. Appl. Entom., **131,** 420-425.

Levine, E. and H. Oloumi-Sadeghi, 1991: Management of diabroticite rootworms in corn. Annual Rev. Entom., **36,** 229-255.

Pilz, C., J. Enkerli, R. Wegensteiner, and S. Keller, 2011a: Establishment and persistence of the entomopathogenic fungus *Metarhizium anisopliae* in maize fields. J. Appl. Entom., **135,** 393-403.

Pilz, C., U. Heimbach, and G. Grabenweger, 2011b: Entomoparasitic nematodes for control of the Western corn rootworm, *Diabrotica virgifera virgifera*. IOBC WPRS Bulletin, **66,** 275-279.

Pilz, C., S. Keller, and U. Kulhmann, 2009: Comparative efficacy assessment of fungi, nematodes and insecticides to control western corn rootworm larvae in maize. BioControl, **54,** 671-684.

Pilz, C., R. Wegensteiner, and S. Keller, 2008: Natural occurrence of insect pathogenic fungi and insect parasitic nematodes in *Diabrotica virgifera virgifera* populations. BioControl, **53,** 353-359.

Rasmann, S., T. G. Kollner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, , J. Gershenzon, and T. C. J. Turlings, 2005: Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, **434,** 732-737.

Rozen, K. v. and A. Ester, 2010: Chemical control of *Diabrotica virgifera virgifera* LeConte. J. Appl. Entom., **134,** 376-384.

Szalai, M., J. P. Komáromi, R. Bažok, J. I. Barčic´, J. Kiss, and S. Toepfer, 2011: Generational growth rate estimates of *Diabrotica virgifera virgifera* populations (Coleoptera: Chrysomelidae). J. Pest Sc., **84,** 133-142.

Toepfer, S., R. Burger, R. U. Ehlers, A. Peters, and U. Kuhlmann, 2010a: Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. J. Appl. Entom., **134,** 467-480.

Toepfer, S., C. Gueldenzoph, R. U. Ehlers, and U. Kuhlmann, 2005: Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. Bull. Entom. Res., **95,** 473-482.

Toepfer, S., I. Hatala-Zseller, R. U. Ehlers, A. Peters, and U. Kuhlmann, 2010b: The effect of application techniques on field-scale efficacy: can the use of entomopathogenic nematodes reduce damage by western corn rootworm larvae? Agric. Forest Entom., **12,** 389-402.

Toepfer, S., T. Haye, M. Erlandson, M. Goettel, J. G. Lundgren, R. G. Kleespies, D. C. Weber, G. C. Walsh, A. Peters, R. U. Ehlers, , H. Strasser, D. Moore, S. Keller, S. Vidal, and U. Kuhlmann, 2009: A review of the natural enemies of beetles in the subtribe Diabroticina (Coleoptera: Chrysomelidae): implications for sustainable pest management. Biocontrol Sc. Techn., **19,** 1-65.

Toepfer, S., P. Knuth, A. Peters, and R. Burger, 2010c: Insect pathogenic nematodes against Western corn rootworm. Mais, **37,** 68-70. (in German)

Toepfer, S., B. Kurtz, and U. Kuhlmann, 2010d: Influence of soil on the efficacy of entomopathogenic nematodes in reducing *Diabrotica virgifera virgifera* in maize. J. Pest Sc., **83,** 257-264.

Toepfer, S., A. Peters, , R. U. Ehlers, and U. Kuhlmann, 2008: Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. J. Appl. Entom., **132,** 337-348.