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Review Article

Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe

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Abstract

The western corn rootworm, *Diabrotica virgifera virgifera*, was introduced to Europe from North America in the early 1990s. It spread rapidly from its site of introduction in Serbia and is now found over some 400,000 ha of southeastern Europe. The prospects for its classical biological control by introduction of natural enemies, including pathogens, nematodes and insects, from its area of origin are considered, based on a review of natural enemy–*Diabrotica* associations in the New World. The need for an integrated pest management (IPM) strategy is highlighted, and initial monitoring and management programmes in central Europe are described. Research looking at the possibility of using augmentation and conservation biological control techniques, and behavioural control measures based on attractants and pheromones being conducted at various centres in Europe are also described.

Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Col., Chrysomelidae) is the most important pest of maize in the USA and Canada causing yield losses and chemical control costs of up to one billion US dollars annually (Krysan & Miller, 1986; Chandler *et al.*, 1995). In the early 1990s it was accidentally introduced from the USA to southeastern Europe. The beetle was first observed in 1992 close to the Belgrade international airport, Serbia, occupying an area of only about 60 ha (Baca, 1994). By 1997 the pest had spread widely through Serbia, Bosnia-Herczegowina, Hungary, Romania and Croatia (also probably Bulgaria but this has not been confirmed), and occupies an area covering approximately 400,000 ha (H. K. Berger pers. comm., 1997). It is expected that in the future, the pest will colonize Slovenia, Bulgaria, Austria, Italy, Moldova, Ukraine and Slovakia, and eventually invade all maize producing countries in Eurasia.

About twenty thousand hectares of maize in Serbia were heavily infested and showed economic damage in 1997 (H. K. Berger pers. comm., 1997; W. A. C. M. van der Burgt unpublished data, 1997). In countries which already have the western corn rootworm, about 6.5 million hectares of maize are grown, while the total maize growing area in Europe is about 14 million hectares. Therefore the problem is only just beginning to manifest itself and requires timely interventions to minimize the impact of this invading pest.

It is assumed that *D. virgifera virgifera* and maize evolved together in the tropics or subtropics of Mesoamerica (Mexico/Central America) (Branson & Krysan, 1981). Important natural enemies may occur locally in areas of maize cultivation in southeastern Europe, particularly polyphagous predators, but specialized parasitoids, predators and pathogens may be lacking, because they have been left behind in the area of origin. In the absence of specialized natural enemies, *Diabrotica* populations in southeastern Europe are only limited by the availability of suitable food, the influence of abiotic factors, and the application of control treatments. Classical biological control has an important application in the management of invasive alien pest species in the agricultural crop environment, and allows the safe and selective control of pest species and thereby helps to protect local biodiversity. Biological control has the potential to reduce local and regional populations of western corn rootworm through the importation of specialized natural enemies from the region of origin of the pest.

In South and North America, corn rootworms are attacked by a range of pathogens, nematodes, predators and parasitoids, some of which appear to be rather specialized natural enemies of *D. virgifera virgifera* and related species in the soil. Recent reviews on the management strategies of *Diabrotica* species deal only briefly with natural control agents (Levine & Oloumi-Sadeghi, 1991; Baca *et al.*, 1995). This paper presents a detailed literature review on natural enemies–*Diabrotica* species associations in South and North America.

Natural Enemies of *Diabrotica* Species

Pathogens

Pathogens such as gregarine and microsporidian protozoa, fungi, bacteria and virus-like particles have been found in corn rootworms; however the role these biological control agents play in the field is mostly unknown (Levine & Oloumi-Sadeghi, 1991). Although the soil provides protection to biological control agents from desiccation and UV radiation, it also contains antagonistic microorganisms that may limit the effectiveness of these agents (Kinney *et al.*, 1989).

Protozoa

Brooks & Jackson (1990) reviewed studies on the eugregarines of *Diabrotica* species. Occasional sampling indicated that the incidence of gregarine infections in the field ranges from 5% to 100%. Gregarine infections have been shown to increase adult mortality and inhibit ovarian and fat body development in *D. virgifera virgifera*. It is concluded that eugregarines are debilitative and most virulent under suboptimal conditions or unnaturally high intensity levels of infection, but only a few species have potential as biological control agents (Brooks & Jackson, 1990).

Fungi

Corn rootworms probably acquire fungal infections in the larval or pupal stage (Levine & Oloumi-Sadeghi, 1991). The fungus *Beauveria bassiana* (Bals.) Vuill. (Mitosporic fungi; formerly Deuteromycetes) can cause natural epizootics in corn rootworm populations (Maddox & Kinney, 1989). Heineck-Leonel & Salles (1997) carried out a survey of natural enemies of *Diabrotica speciosa* (Germar) in southern Brazil and found *B. bassiana* and *Metarhizium anisopliae* (Metsch.) Sorok (Mitosporic fungi). Tonet & Reis (1979) demonstrated the pathogenicity of *B. bassiana* to *D. speciosa* in the laboratory and factors influencing sporulation were investigated with a view to its use in biological control as biopesticides. Branson *et al.* (1975) mentioned the occurrence of *B. bassiana* in laboratory rearings of *D. virgifera virgifera*. This fungus occurs when the relative humidity is higher than 75% to 80% and the temperature varies between 24°C and 27°C. Soil moisture, soil type, soil fertility, fungal strain and presence of other microbes have been identified as factors greatly affecting the infectivity of *B. bassiana* in laboratory bioassays (Tonet & Reis, 1979; Kinney *et al.*, 1989). Kinney *et al.* (1989) studied *B. bassiana* for many years in the field, but its potential for reducing corn rootworm feeding damage has not been proven. Naranjo & Steinkraus (1988) collected cadavers of *Diabrotica barberi* Smith & Lawrence from the field in New York, USA, during 1985 to 1986 and found that *Diabrotica* beetles were infested with an unidentified entomophthoralean fungus (presumably *Tarichium* spp.; Zygomycetes).

Bacteria

Herrnstadt & Soares (1989) reported that spores and crystals of *Bacillus thuringiensis* (strain San Diego) had been used as an insecticide against insect pests of maize, and that pests including *Diabrotica virgifera* LeConte have been controlled with this agent. Other studies tested different strains of *B. thuringiensis*: five strains isolated from Sweden were toxic to *Diabrotica undecimpunctata* Mannerheim at high concentrations (Abdel-Hameed & Landen, 1994), *B. thuringiensis* EG4961 was toxic to larvae of *Diabrotica undecimpunctata howardi* Barber (Rupar *et al.*, 1991; Donovan *et al.*, 1992), and *B. thuringiensis* toxin CryIIIB2 exhibited activity against *D. undecimpunctata* (von Tersch *et al.*, 1994). Treatment of *D. undecimpunctata howardi* larvae and adults of *D. virgifera* and *Diabrotica longicornis* (Say) with *B. thuringiensis* and *Bacillus popilliae* showed that application of spores to the food substrate did not increase mortality of corn rootworms (Sutter, 1969). The bacterium *Pseudomonas aeruginosa*, isolated from laboratory cultures of *D. undecimpunctata* in South Dakota, was tested and found to have some value as a biological control agent against *D. undecimpunctata howardi* and *D. virgifera* (Hamilton, 1968). The bacteria associated with the intestine of *Diabrotica balteata* LeConte were surveyed in adults collected by Schalk *et al.* (1987); of the species identified *Proteus mirabilis* and *Pseudomonas aeruginosa* are insect pathogens. Applications of the ice-nucleating-active bacterium *Pseudomonas syringae* to *D. undecimpunctata* reduced its coldhardiness and Strong-Gunderson *et al.* (1992) proposed that ice-nucleating-active bacteria may be used as a biological insecticide for the control of insect pests in winter.

Viruses

Regarding viruses, in an ultrastructural study on the testes and spermatheca of adults of *D. virgifera virgifera*, large masses of 24- to 26-nm virus particles were found in the cytoplasm of spermatocyst cells (Degrugillier *et al.*, 1991). Degrugillier *et al.* (1991) showed that there was a high incidence of abnormal sperm but the virus did not appear to affect the chrysomelid adversely in terms of life span, motility and mating behaviour.

Nematodes

Tests to control *Diabrotica* spp. with nematodes have yielded diverging results. Laboratory tests have resulted in high mortality, but the results of field tests depend very much on environmental conditions (i.e. insufficient soil moisture is detrimental) and application techniques (Barbercheck, 1993; Jackson & Brooks, 1995).

Among the entomopathogenic nematodes, the Steinernematidae and Heterorhabditidae are the most studied species (Table 1). *Steinernema carpocapsae* (Weisser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982 was most effective against the second and third corn rootworm larval stages and in the pupal stage, where it was found to complete its life cycle (Jackson & Brooks, 1995). Larvae of *D. virgifera virgifera* were most susceptible to a Mexican strain of *S. carpocapsae* (Jackson & Brooks, 1989). In addition, *D. virgifera virgifera* was successfully parasitized by *S. carpocapsae*, showing a mortality rate of more than 90% under controlled conditions (Nickle *et al.*, 1994). In field and laboratory experiments *S. carpocapsae* caused 93.3% mortality of *D. balteata* and *D. undecimpunctata howardi* (Schalk *et al.*, 1993). Wright *et al.* (1993) confirmed that the same nematode species was successfully used in high concentrations against *D. virgifera virgifera* and *D. barberi*. Viable nematodes were found from seven until 28 days after the treatment in low concentrations (Wright *et al.*, 1993). In field experiments carried out by Jackson & Hesler (1996), adult emergence was reduced by 79% to 93% by adding 100,000 or 200,000 aqueous nematodes to a 15-cm diameter circle of soil at the base of a maize plant. These application rates held the root damage near the economic threshold (Jackson & Hesler, 1996), and it is suggested that *S. carpocapsae* should be applied when second and third instar corn rootworm larvae are predominant in the field (Jackson & Brooks, 1995).

Steinernema feltiae (Filipjev 1934) Wouts, Mracek, Gerdin & Bedding, 1982 is mentioned as a potential biological control agent by Munson & Helms (1970), Gaugler (1981), Poinar *et al.* (1982), Steffey *et al.* (1987) and

Jackson & Brooks (1989). In other tests, infective stages of *S. feltiae* were applied together with liquid fertilizer at the time of planting of maize for the control of *Diabrotica* spp. (Poinar *et al.*, 1983). Significantly fewer beetles were found in the treated area compared with untreated and chlorpyrifos-treated areas. In addition, costs of the treatment with *Steinernema* were lower than the costs of the chlorpyrifos treatment (Poinar *et al.*, 1983). Thurston & Yule (1990) mentioned that they recorded a reduction in the number of first instar *D. barberi* larvae emerging following the application of *S. feltiae* at maize seeding time.

Successful tests with *Heterorhabditis bacteriophora* Poinar, 1976 were carried out by Jackson (1996). A *Heterorhabditis* species was discovered in larval cadavers of *D. balteata* in South Carolina, USA, and has promising potential as a biocontrol agent for this pest (Creighton & Fassuliotis, 1985).

A combination of *S. carpocapsae* and *H. bacteriophora* with a pesticidal soap (M-Pede) was effective against larvae of *D. undecimpunctata* in soil (Kaya *et al.*, 1995). Georgis *et al.* (1991) demonstrated that *S. carpocapsae* and *H. bacteriophora* significantly suppressed pest populations of *D. virgifera virgifera*, whilst not adversely affecting the numbers of non-target soil arthropods. In addition, Jackson (1996) evaluated *S. carpocapsae* (Mexican and All strains) and *H. bacteriophora* (Lewiston strain) and tebufos as control agents for *D. virgifera virgifera*. It was found that these treatments reduced root damage below the threshold for potential economic loss and adult emergence by 66% to 98%. Brust (1991a) used three cultural methods (tillage, weed control and soil irrigation) in an attempt to increase numbers of the endemic nematode *Heterorhabditis heliothidis* [= *H. bacteriophora*] to levels high enough to control artificial infestations of *D. undecimpunctata howardi*.

Mermithids of the genus *Hexamermis* were found in *Diabrotica speciosa vigens* Erichson from beans, maize and alfalfa in Peru (Nickle *et al.*, 1984) where infection rates from 5% to 90% were observed. Field and laboratory tests in the USA indicated a wide host range of *Hexamermis* sp., including *D. undecimpunctata howardi*, *Leptinotarsa decemlineata* (Say) (Col., Chrysomelidae), *Epilachna varivestis* Mulsant (Col., Coccinellidae) and other beneficial coccinellids (Nickle *et al.*, 1984).

Laboratory, greenhouse and field studies were carried out in South Carolina to evaluate the effectiveness of the mermithid nematode *Filipjevimermis leipsandra* Poinar & Welch in controlling larvae of *D. balteata* on maize. In cage tests in field microplots, an application of eggs of the nematode to the soil resulted in a parasitism rate of 50% to 100% (Creighton & Fassuliotis, 1983). A description of the biology, ecology and potential of *F. leipsandra*, together with information about rearing this nematode, is given by Creighton & Fassuliotis (1980, 1981, 1982) and Fassuliotis & Creighton (1982). Infection by *F. leipsandra* of *D. balteata* peaked at 30°C, and fell off sharply

Table 1. Entomophagous nematodes attacking *Diabrotica* spp. (Col., Chrysomelidae).

Host	Nematode species/strain	Stage attacked	Reference
D. balteata LeConte	*Filipjevimermis leipsandra* Poinar & Welch, 1968	larva	Creighton & Fassuliotis 1980, 1981, 1982, 1983
		larva	Elsey 1989
	Heterorhabditis sp.	larva	Creighton & Fassuliotis 1985
	Steinernema carpocapsae (Weiser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982	larva	Schalk *et al.* 1993
D. barberi Smith and Lawrence	*Steinernema feltiae* (Filipjev, 1934) Wouts, Mracek, Gerdin & Bedding, 1982	larva	Thurston & Yule 1990
	Steinernema bibionis [= *S. feltiae*]	larva	Thurston & Yule 1990
D. speciosa (Germar)	*Hexamermis* sp.	larva	Nickle *et al.* 1984
	Micoletzkya vidalae Stock, 1993	larva	Stock 1993
D. undecimpunctata howardi Barber	*Heterorhabditis bacteriophora* Poinar, 1976	larva	Barbercheck 1993
	Heterorhabditis heliothidis [= *H. bacteriophora*]	larva	Brust 1991a
	Howardula sp.	larva	Elsey 1977
	Steinernema carpocapsae	larva	Barbercheck 1993
D. undecimpunctata Mannerheim	*Heterorhabditis bacteriophora*	larva	Kaya *et al.* 1995
	Steinernema carpocapsae	larva	Kaya *et al.* 1995
D. virgifera virgifera LeConte	*Heterorhabditis bacteriophora*	larva	Georgis *et al.* 1991
		larva	Jackson 1996
		larva, pupa	Jackson and Hesler 1996
		adult	van der Burgt *et al.* in press
	Heterorhabditis spp. HU-9	adult	van der Burgt *et al.* in press
	Heterorhabditis spp. Lu-II	adult	van der Burgt *et al.* in press
	Steinernema anomali [= *S. arenarium* (Artyukovsky, 1967) Wouts, Mracek, Gerdin & Bedding, 1982]	adult	van der Burgt *et al.* in press
	Steinernema carpocapsae	larva	Jackson & Brooks 1995
		larva	Nickle *et al.* 1994
		larva, pupa	Jackson & Hesler 1996
		larva	Wright *et al.* 1993
		larva	Schalk *et al.* 1993
		larva	Georgis *et al.* 1991
	Steinernema feltiae	larva	Gaugler 1981
		larva	Jackson & Brooks 1989
		larva	Steffey *et al.* 1987
		larva	Poinar *et al.* 1982
		larva	Poinar *et al.* 1983
		adult	van der Burgt *et al.* in press
	Steinernema spp. SZS.2	adult	van der Burgt *et al.* in press
Diabrotica sp.	*Howardula benigna* Cobb, 1921	larva	Fronk 1950

at warmer or cooler temperatures. No development, oviposition or infection occurred below a temperature of 20°C (Elsey, 1989).

In addition, a *Howardula* species (Allantonematidae) was found in parasitized adults of *D. undecimpunctata howardi* (Elsey, 1977), and Fronk (1950) mentioned that *Howardula benigna* Cobb, 1921 is sufficiently numerous to be important. The nematode *Micoletzkya* sp. is a facultative parasite of *D. speciosa* larvae in Argentina (Stock, 1993).

So far literature records have shown that it is mostly the larval stages of *Diabrotica* that are attacked by nematodes, with the single exception described in a paper by Elsey (1977). Recently a study was carried out to evaluate the potential of nematodes to control *Diabrotica* adults (van der Burgt *et al*., in press). Nematodes potentially attack beetles when they are emerging or crawling out of or into the soil to lay eggs (van der Burgt *et al*., in press). In laboratory tests, these authors found successful parasitism of *D. virgifera virgifera* beetles by *S. feltiae*, *Steinernema anomali* [= *Steinernema arenarium* (Artyukhovsky, 1967) Wouts, Mracek, Gerdin & Bedding, 1982], *H. bacteriophora* and different strains of *Steinernema* spp. and *Heterorhabditis* spp.

Predators

Predatory mites

Predatory mites such as erythraeids (Prostigmata) are abundant above ground in some agroecosystems (Welbourne, 1982; Santiago-Blay & Fain, 1994). Nevertheless, these mites are polyphagous and apparently feed on available prey items (Thompson & Simmonds, 1965). Manure management was followed by increased populations of mesostigmatids and presumably contributed to the observed larval population decline of two *Diabrotica* spp. in maize in Minnesota, USA (Chiang, 1970), but mites predatory on rootworms were not found under similar conditions in Canada (Dominique *et al*., 1984). In addition, Allee & Davis (1996) reported that numbers of mesostigmatid mites significantly increased in manured plots but no evidence of enhanced predation was reflected in larval counts. Brust & House (1988) found that the acarid mite *Tyrophagus putrescentiae* Schrank occurs in North Carolina, and it is an effective egg predator of the southern corn rootworm, *D. undecimpunctata howardi*, under no-tillage conditions. Based on unpublished observations by McCartney & Stinner (*in:* Stinner & House, 1990) microarthropod predation is a significant overwintering mortality factor for *Diabrotica* in the midwestern USA as well. In contrast, laboratory life table studies have shown that predatory laelapids have a very low intrinsic rate of growth, a low net reproduction rate, and are not specific to *Diabrotica* spp. or other leaf beetle eggs or larvae (Mihm & Chiang, 1976). Most of the evidence indicates that mites do not have a strong impact on leaf beetle pest populations (Risch, 1981).Their absence in many cases of sudden chrysomelid outbreaks suggests their minor role as natural control agents (Santiago-Blay & Fain, 1994).

Predatory insects

Most reports on insect predator–*Diabrotica* spp. associations are limited to only mentioning the relationship between pest and predator. These data recorded in the literature are summarized in Table 2. Stoewen & Ellis (1991) conducted studies to determine predators feeding on eggs of *D. virgifera virgifera*. They found a large number of egg predators, i.e. ground beetles, hister beetles, rove beetles, dermestids, centipedes, and spiders (see Table 2). It was concluded that egg predation is not a significant factor worth monitoring for pest management purposes (Stoeven & Ellis, 1991). The importance of ground beetles as predators of *Diabrotica* spp. in maize has been documented (Tyler & Ellis, 1979; Brust *et al*., 1986) but other workers found no indication that ground beetles are effective predators (Best & Beegle, 1977; Kirk, 1982). In contrast, an ant, *Lasius neoniger* Emery (Hym., Formicidae) (Ballard & Mayo, 1979; Kirk, 1981), significantly reduced *D. virgifera virgifera* larval populations, presumably by feeding directly on larvae (Kirk, 1981), but did not reduce egg numbers (Ballard & Mayo, 1979). Branson *et al*. (1982) concluded that predators seem to play a greater role in the population dynamics of corn rootworms in Mexico than these biological control agents play in the population dynamics of the western corn rootworm in the cornbelt of the USA. Nonetheless, polyphagous predators are not considered for introduction as biological control agents because it is likely that native predators which prey on the western corn rootworm already exist in southeastern Europe. A comparison of mortality factors caused by generalist predators in North America and Central Europe should be carried out.

Parasitoids

Tachinids

Three tachinid (Dipt.) parasitoids in the genus *Celatoria* are known from literature records to parasitize adults of single or related genera within the Galerucinae or Alticinae (Cox, 1994). Regarding *Diabrotica* species, *Celatoria bosqi* Blanchard parasitizes *D. speciosa* in Argentina, Uruguay and southern Brazil (Herting, 1973; Guimaraes, 1977; Heineck-Leonel & Salles, 1997, W. Cabrera Walsh pers. comm., 1998). The maximum proportion of parasitism found by Cabrera Walsh (pers. comm., 1998) has been 18.2%. *Celatoria diabroticae* (Shimer) attacks *D. undecimpunctata* in Oregon, USA (Herting, 1973), *D. undecimpunctata howardi* in Virginia, South Carolina and Mississippi, USA (Herting, 1973; Arnauld, 1978), and *D. balteata* in South Carolina, USA (Elsey, 1988). Summers & Stafford (1953) reported that almost 35% of adult *D. undecimpunctata howard*i were found to be parasitized by *C. diabroticae* in southern Mississippi in 1951 and 19% parasitism was determined in 1952. It was concluded that this tachinid species may give important control of *D. undecimpunctata howardi* in this area. The third tachinid, *Celatoria setosa* (Coquillett), parasitizes *D. undecimpunctata* and *D. undecimpunctata howardi* in Indiana, USA (Arnauld, 1978). Bussart (1937) reported that 5.4% adults of *D. undecimpunctata howardi* were parasitized by *C. setosa* during summer 1933. In general, it seems that *Celatoria* species are more host specific than many other tachinids. It would seem that this is due to the elaborately modified piercing ovipositor of the females (J. O'Hara pers. comm., 1997). According to Bussart (1937), who studied the biology of *Celatoria setosa*, it is a primary solitary ovilarviparous endoparasitoid which lays its eggs under the elytra of adult *Diabrotica*. The first instar larva hatches immediately after oviposition and penetrates through the intersegment skin into the beetle. A female has a potential fecundity of approximately 100 eggs and presumably deposits a single egg during each oviposition. Larval development lasts 18 to 27 days. The mature larva leaves the beetle and forms its puparium on the plant close to the dead beetle or on the soil surface. Adult emergence from the puparia occurs after five to seven days during summer but occurs for up to 20 days in October. The larvae of *C. setosa* hibernate in the host tissue of the *Diabrotica* beetles. According to Bussart (1937) and Gould (1944), *C. setosa* can have four or five generations a year.

Table 2. Predators of *Diabrotica* spp. (Col., Chrysomelidae).

Host	**Predator**	**Family**	**Prey stage**	**Reference**
D. balteata LeConte	*Labidura riparia* Pallas	Labiduridae	adult	Waddill 1978
D. longicornis (Say)	*Carabus nemoralis* O.F. Müller	Carabidae	egg	Tyler & Ellis 1979
	Androlaelaps sp.	Laelapidae	egg, larva	Mihm & Chiang 1976
	Clivina fossor L.	Carabidae	egg, larva	Tyler & Ellis 1979
	Pterostichus melanarius (Illiger)	Carabidae	egg, larva	Tyler & Ellis 1979
	Stratiolaelaps sp.	Laelapidae	egg, larva	Mihm & Chiang 1976
	Bembidion quadrimaculatum oppositum Say	Carabidae	larva	Tyler & Ellis 1979
	Harpalus pennsylvanicus (De Geer)	Carabidae	larva	Tyler & Ellis 1979; Kirk 1973
	Tachys sp.	Carabidae	larva	Tyler & Ellis 1979
	Trechus apicalis Motschulsky	Carabidae	larva	Tyler & Ellis 1979
	Phymata erosa (L.)	Phymatidae	adult	Dominique & Yule 1984
D. speciosa (Germar)	*Chrysopa* sp.	Chrysopidae	adult	Bercellini & Malacalza 1994
	Eriopis sp.	Coccinellidae	adult	Bercellini & Malacalza 1994
	Lebia concinna Brullé	Carabidae	adult	Milanez 1984; Bercellini & Malacalza 1994; Hohmann 1989
	Nabis sp.	Nabidae	adult	Milanez 1984; Bercellini & Malacalza 1994
	Orius sp.	Anthocoridae	adult	Bercellini & Malacalza 1994; Hohmann 1989
	–	Class: Arachnida	adult	Bercellini & Malacalza 1994
	Cycloneda sanguinea (L.)	Coccinellidae	–	Hohmann 1989
	Doru lineare (Eschscholtz)	Forficulidae	–	Milanez 1984
	Scymnus spp.	Coccinellidae	–	Hohmann 1989
D. undecimpunctata howardi Barber	*Lasius* spp.	Formicidae	egg, larva	Brust 1991c
	–	Formicidae	egg, larva	Brust 1991b; Brust 1991c; Brust & House 1990
	Tyrophagus putrescentiae Schrank	Acaridae	larva	Brust & House 1990
	–	Cantharidae	larva	Brust 1991c; Brust & House 1990
	–	Order: Chilopoda	larva	Brust 1991c; Brust & House 1990
	Apiomerus crassipes (F.)	Reduviidae	adult	Morrill 1975
	Misumena vatia (Clerck)	Thomisidae	adult	Lockley *et al.* 1989
	Phidippus audax (Hentz)	Salticidae	adult	Young 1989
D. undecimpunctata Mannerheim	*Phidippus audax* (Hentz)	Salticidae	adult	Johnson 1996
D. virgifera virgifera LeConte	*Hypoaspis aculeifer* Canestrini	Laelapidae	egg	Stoewen & Ellis 1991
	Lasius neoniger Emery	Formicidae	egg	Ballard & Mayo 1979; Kirk 1981
	–	Dermestidae	egg	Stoewen & Ellis 1991
	–	Histeridae	egg	Stoewen & Ellis 1991
	–	Order: Chilopoda	egg	Stoewen & Ellis 1991
	–	Order: Araneae	egg	Stoewen & Ellis 1991
	Androlaelaps sp.	Laelapidae	egg, larva	Mihm & Chiang 1976
	–	Carabidae	egg, larva	Stoewen & Ellis 1991; Brust 1991c; Brust & House 1990; Brust *et al.* 1986; Best & Beegle 1977; Kirk 1982
	Stratiolaelaps sp.	Laelapidae	egg, larva	Mihm & Chiang 1976
	–	Staphylinidae	egg, larva	Stoewen & Ellis 1991; Brust 1991c; Brust & House, 1990
Diabrotica spp.	*Chauliognathus marginatus* (F.)	Cantharidae	–	Branson *et al.* 1982
	Harpalus erraticus Say	Carabidae	–	Kirk 1974
	Pheidole bicarinata longula Emery	Formicidae	–	Ballard & Mayo 1979
	–	Ameroseiidae	–	Chiang 1970; Mihm & Chiang 1976; Stoewen & Ellis 1991
	–	Rhodacaridae	–	Chiang 1970; Mihm & Chiang 1976; Stoewen & Ellis 1991

Braconids

A new braconid (Hym.) species, *Centistes gasseni* Shaw, has recently been discovered in southern Brazil and obtained mainly from *D. speciosa* (Shaw, 1995; Heineck-Leonel & Salles, 1997), but reared rarely from *Diabrotica limitata* (Stahlberg) and *Acalymma bivittula* (Kirsch) (Col., Chrysomelidae) (W. Cabrera Walsh pers. comm., 1998). The percentage parasitism for this braconid in the Brazilian collections ranged from 3.3% to 22.3% (W. Cabrera Walsh pers. comm., 1998). It is currently being studied in the USA as a potential biological control agent for the *Diabrotica* pest-complex (Shaw, 1995; W. Cabrera Walsh pers. comm., 1998). As far as is known, all *Centistes* species are solitary koinobiont endoparasitoids of adult beetles, particularly in the families Curculionidae, Chrysomelidae, Coccinellidae, Anthicidae and Carabidae (Loan, 1972; Shaw, 1985). Only one other species, *Centistes diabroticae* (Gahan), is recorded as parasitizing adult stages in the subtribe Diabroticina (Gahan, 1922). It attacks the striped cucumber beetle, *Acalymma vittatum* (F.). Some *Centistes* have been reported to leap onto the host elytra (Gahan, 1922) or grasp the host beetle with their legs during oviposition (Loan, 1964, 1972). The dense layer of mesosternal setae in *C. gasseni* may be an adaption to similar host-mounting behaviour, but so far its biology has not yet been studied (Shaw, 1995). Gahan (1922) studied the biology of *C. diabroticae* and stated that it deposits eggs into the thorax of adult beetles, apparently through one of the sutures near the base of the elytra. The parasitoid larva feeds internally. The mature parasitoid larvae emerges from the host and pupates just below the soil surface in a closely woven silken cocoon. Pupation lasts about ten days (Gahan, 1922).

Conclusions

Biological techniques are useful elements of a strategic approach to the control of the western corn rootworm in southeastern Europe, leading to an integrated pest management (IPM) strategy which is likely to incorporate the use of pest monitoring systems, the release of biological control agents, cultural techniques to enhance the conservation of natural control, crop rotations and orientation disruption of *Diabrotica* adults.

Recently, a permanent monitoring network and a trapping for containment and control programme on *D. virgifera virgifera* became operational in Hungary, Croatia, Romania and Bosnia-Herzegovina (Edwards *et al.*, 1998a). In the containment and control programme, Hungarian pheromone and MultigardTM yellow sticky traps are used to capture both males and females (males are attracted to the pheromone, while both females and males are attracted to the MultigardTM traps). The purpose of this trapping activity is to determine the feasibility of trapping as many western corn rootworm beetles as possible (Edwards *et al.*, 1998a). It is hoped that this trapping method will slow down or possibly stop the build-up of smaller isolated *D. virgifera virgifera* populations. The other major activity is the area-wide management to eliminate western corn rootworm beetle populations or greatly limit the area of infestation to a more manageable unit by using Slam®. The product Slam® uses the 'attract and kill' concept whereby cucurbitacin (from the buffalo gourd root, *Cucurbita* spp.) acts as a feeding attractant/arrestant and is mixed with carbaryl as a toxicant (Edwards *et al.*, 1998a). Slam® applied by air within the known range of the infestation and some distance beyond as a buffer is being tested in Hungary (Petro *et al.*, 1998). There is an urgent need to look for alternatives to chemical control of *D. virgifera virgifera* since the main component of Slam® is carbaryl, a highly toxic carbamate chemical, the use of which is banned in several European countries (H. K. Berger pers. comm., 1997) where *D. virgifera virgifera* is expected to become established within a few years (Baufeld & Enzian, 1997). In addition, aerial applications of this commercial bait will not be permitted in several European countries (H. K. Berger pers. comm., 1997).

The need to develop an IPM strategy for western corn rootworm in Central Europe is obvious. A variety of control techniques should be considered which maximizes the use of biological and cultural control interventions and minimizes the widespread application of toxic baits. Successful biological control results from the suppression of pests to a level at which they no longer cause economic damage to a crop through an enhancement of the action of natural enemies (Greathead, 1986). In general there are several different methods that can be applied for the biological control of western corn rootworm in southeastern Europe: (1) the importation and establishment of exotic natural enemies from the region of origin of the pest that has invaded from another geographic region, to provide long-term reduction in pest damage on a regional scale, (2) the periodic release of artificially-reared natural enemies to augment populations for immediate reduction of pests on a localized scale, and (3) the conservation of natural enemies through changes in management practices to sustain a reduction in pest damage on a localized scale. There are opportunities for each of these approaches in western corn rootworm management in southeastern Europe.

As the western corn rootworm is not indigenous to the region, biological control has the potential to reduce regional populations of *D. virgifera virgifera* through the importation of specialized natural enemies from Mesoamerica, the region of origin of this pest. In this area, the western corn rootworm is attacked by a complex of natural enemies, some of which appear to be well adapted to attack corn rootworms and related species in the soil. Such natural enemies should be studied in greater detail with a view to selecting candidate biological control agents for introduction into Europe. It would seem that these native natural enemies do not regulate populations of the beetle effectively in the USA, but well selected species may have a significant effect upon the abundance of *D. virgifera virgifera* in southeastern Europe, i.e. such species could become more effective after their establishment. The fact that well selected natural enemies can be important mortality factors is well documented in reports on successful biological control programmes (Waage & Hassell, 1982). Parasitoids like the braconid *Centistes gasseni* and tachinid species in the genus *Celatoria* as well as fungal pathogens like *Beauveria* spp. should be considered for introduction with the aim of reducing western corn rootworm population densities in southeastern Europe. With respect to the safety of biological control, the 'Code of Conduct' for the release of biological control agents should be followed (FAO, 1997), which recommends that governments should evaluate information on each candidate biological control agent including: (1) accurate identification of the biological control agent, (2) a summary of all available information on its origin, distribution, biology, natural enemies and impact in its area of origin, and (3) an analysis of the host range expansion of the biological control agent and any potential hazards posed to non-target hosts.

In addition to the regional management of western corn rootworm through the importation of specialized parasitoids and pathogens from the area of origin, localized management using natural enemies can be achieved through natural enemy augmentation. This is particularly important where regional control proves to be inadequate. On-going work at the Department of Entomology, University of Wageningen, the Netherlands, and at the Biocontrol Laboratory, Hódmezövásárhely, Hungary, is dealing with the use of parasitic nematodes for the control of *Diabrotica*. In addition, the use of fungal pathogens of the beetle as biopesticides (i.e. *Beauveria* and *Metarhizium* spp.) incorporated into the soil deserves study as a potential alternative to agrochemicals. This technique has been used successfully to control soil-dwelling beetle pests with similar ecologies (chafer grubs) in other parts of Europe (M. G. Hill pers. comm., 1997). The potential of augmentative biological control has been demonstrated in maize for the corn borer, *Ostrinia nubilalis* Hübner (Lep., Pyralidae), in Europe (Wajnberg & Hassan, 1994), but the main constraints to their more widespread adoption are the costs and quality of the natural control agents. Unlike many of the broad-spectrum insecticides, biopesticides based on natural enemies are characterized by their short duration of activity in the field which necessitates both more accurate timing and more effective coverage by applications. A number of natural enemies, including parasitoids, nematodes and microbial products, can be mass-produced and a number are commercially available. Concerns about reliance on insecticides in European pest management are likely to enhance interest in the use of augmentative biological control in the not too distant future. There is considerable potential for the application of such techniques, but additional experimental research is required to develop the more effective means to augment the use of natural enemies in pest control.

The conservation of native biological control agents in maize is a particularly important and valuable aspect of biological control. Such natural mortality factors of the western corn rootworm need to be studied within its area of introduction in southeastern Europe. A first study to assess the predators and parasitoids acting as mortality factors of *D. virgifera virgifera* has been started in Croatia (M. Maceljski pers. comm., 1997). Once these are known it may be possible to use cultural practices to enhance their effectiveness as mortality factors. The importance of ground beetles as predators of *Diabrotica* spp. in maize has been disputed in the USA, but carabid beetles are considered as beneficial insects in European agriculture. More extensive agricultural production systems with an adequate number of natural ecological compensation areas are required to enhance the effectiveness of such predators (Luka, 1996).

Levine & Oloumi-Sadeghi (1991) reported that the practice of growing maize in rotation with small grain, hay, clover or alfalfa provided excellent control of western corn rootworm in the USA because *D. virgifera virgifera* eggs are laid almost exclusively in cornfields, and larvae must feed on maize roots the following season for complete development. Currently the production of continuous maize remains a popular option in many parts of southeastern Europe owing to the fact that maize is grown mostly on small farms. There have been some instances in the cornbelt of the USA where western corn rootworm has been a problem in maize following soyabeans (Edwards *et al*., 1998b). A behavioural change in some western corn rootworm populations is threatening the benefits of crop rotation, which has been one of the great success stories for controlling *D. virgifera virgifera* in the USA (Edwards *et al.* 1998b). Nonetheless, there is still an opportunity to practise crop rotation in southeastern Europe because soyabeans are mostly not yet grown within the area so far colonized by *D. virgifera virgifera.*

Recently, a large number of volatile compounds that are highly attractive to western corn rootworm adults have been identified; many have been found in the ancestral host plants of the Diabroticites – the Cucurbitaceae (Levine & Oloumi-Sadeghi, 1991). These plant kairomone mimics, first described in detail by Metcalf & Metcalf (1992), offer a promising alternative solution to controlling adults of *Diabrotica* by a behavioural method. Western corn rootworms are strongly attracted to sticky traps in maize fields permeated with the kairomone mimic 4-methoxycinnamaldehyde, a powerful attractant for both sexes and superior to the natural, female-secreted sex pheromone 8-methyl-10-decanol propoanoate which affects only males (Hummel *et al*., 1998). Methoxycinnamaldehyde is easy to synthesize and less expensive than the female sex pheromone. Hummel *et al.* (1998) reported that there is still a need to optimize this method by using higher application rates, longer lasting and better formulations, and by increasing the treated areas in order to suppress immigrating populations originating from outside the fields. This method offers an alternative behavioural control approach in contrast to conventional toxicants used against *D. virgifera virgifera,* and it is compatible with the use of biological control agents in an IPM approach for *D. virgifera virgifera* in Europe.

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