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Entomopathogens of three beetle species with agricultural importance (*Tanymecus dilaticollis*, *Oulema melanopus*, and *Diabrotica virgifera virgifera*): a review

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Abstract. Studying and applying biological control approaches using entomopathogens against important agricultural insect pests is a sustainable, environmentally friendly practice for controlling their high density in agricultural communities, especially in monocultures. In this review, three species of important beetles were considered – the grey maize weevil, *Tanymecus dilaticollis*, the cereal leaf beetle *Oulema melanopus* and the Western corn rootworm *Diabrotica virgifera virgifera*. They cause significant damage to crops in some regions of distribution. Characteristics related to pest infestations, plant damage, species compositions of entomopathogens, and bioassays with them against the targeted insect species were summarized. The presented list includes viral, bacterial, fungal, protozoan, and nematode species found and tested in these pests: seven in *T. dilaticollis*, twelve in *O. melanopus*, and thirty-two in *D. v. virgifera*.

Keywords: crop protection, grey maize weevil, cereal leaf beetle, Western corn rootworm, insect pests.

Introduction

Biological control of cultivated plants' insect pests to reduce pesticide dependence has been very important in the last few decades. Investigating the pathogenic spectrum of insect pests can help increase the use of biological agents such as microorganisms, fungi, and nematodes as a good alternative to chemical insecticides (Bamisile et al. 2021).

The bacterium *Bacillus thuringiensis* (Berliner, 1915), the fungi *Beauveria bassiana* (Balsamo) Vuillemin, 1912 and *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883 and some nematode species of genus *Steinernema* and *Heterorhabditis* are among the major pathogens that show promising efficacy against many insect pests (Lacey et al. 2015). They can be effective as biological control agents (Draganova et al. 2012, Lacey & Georgis 2012, Mazurkiewicz et al. 2019, 2021, Pilz et al. 2008, Toepfer & Kuhlmann 2004, Toepfer et al. 2005). This review presents data and characteristics related to the impact of the three important coleopteran pest species in agroecosystems and their entomopathogens, tested for susceptibility and efficacy in laboratory and field conditions.

Discussion

Tanymecus dilaticollis – infestation, damage, pathogens

The grey maize weevil, *Tanymecus dilaticollis* Gyllenhal, 1834 (Coleoptera: Curculionidae), is an economically important pest of maize in southeast Europe and southwest Asia (Georgescu 2023). This polyphagous species can cause serious damage to sunflower and sugar beet (Kirkov 1967, Popov 1969, Gerginov 1989, Sáringer & Takács 1994, Krasteva et al. 2006). In the spring, about 90% of adults appear on the soil surface three weeks after the average air temperatures reach 10 °C. Overwintering adults feed before reproduction and cause the most damage. At high densities, in the early stages of crop vegetation, they can even cause crop devastation. For example, each year in Romania, attacks were on approximately one million hectares cultivated with maize (Popov et al. 2007). Mating begins in early spring and lasts until the middle of June. Eggs develop in 10-12 days at a temperature of 20°C. Young adults hibernate in the soil (Paulian et al. 1969, Voinescu 1985, Barbulescu et al. 2001, Cristea et al. 2004, Popov et al. 2007).

Various agrotechnical measures (crop rotation, tillage, sowing dates, favorable conditions for rapid seedling development, and plant density) and chemical treatment control are applied against the grey maize weevil (Kirkov 1967, Krasteva et al. 2006). The inclusion of suitable bioagents, in particular microbial insecticides, in integrated pest management can reduce the chemical treatment and the negatives associated with insecticide application. Theoretically, parasitoids, predators, and pathogens can reduce the population density of *T. dilaticollis* in infested regions, but data on their action and regulatory role are scarce. Entomopathogenic fungi are known as natural enemies of *T. dilaticollis* (Sáringer & Takács 1994, Čamprag & Sekulic 2002, Draganova et al. 2012, Takov et al. 2013, Fătu et al. 2019). According to Hajek & St. Leger (1994), entomopathogenic fungi are important for controlling beetle (Coleoptera) pests because viral and bacterial infections are rare among beetles. *B. bassiana* was isolated from adult individuals of *T. dilaticollis* in several localities in the Danubian Plain in Bulgaria (Draganova et al. 2012, Takov et al. 2013) and Romania (Fătu et al. 2019). *M. anisopliae* (isolate Ma (619) was reported by Draganova et al. (2012) from one locality in northern Bulgaria. In bioassays with three isolates of *B. bassiana* on adults of the grey maize weevil, high cumulative mortality of the test insects from the mycosis and lethality was reported shortly after treatment (Draganova et al. 2012). Additionally, during the study on entomopathogens of the grey maize weevil, Draganova et al. (2012) found the fungi *Fusarium* Link, *Aspergillus flavus* Link, and *A. ochraceus*

Wilhelm in smears, slide preparations, and cultures on SDAY.

Toshova et al. (2021) examined the effectiveness of two bioinsecticides, Naturalis® based on *B. bassiana* and the azadirachtin-based botanical product NeemAzal T/S® against *T. dilaticollis* adults under laboratory and field conditions. The results showed that under laboratory conditions, adults of the pests were more susceptible to B. bassiana than to azadirachtin. A significantly higher mean percent of pest mortality was registered in maize plots after two treatments with Naturalis® than in the control variants.

Takov et al. (2013) found an infection caused by a protozoan of the genus *Gregarina* (Apicomplexa: Sporozoa) in almost 30% of the studied *T. dilaticollis* individuals. Gregarines are parasites localized in the gut lumen of the host. They are less pathogenic but, through mechanical damage to the intestinal epithelium, favor the penetration of several toxins, metabolites, and microorganisms into the hemocoel of the affected insects (Lipa 1967). In addition, these protozoa favor susceptibility to other entomopathogens compared to uninfected individuals (Lopes & Alves 2005).

Toader et al. (2017) tested a bioinsecticide derived from the bacterium *Saccharopolyspora spinosa* Mertz and Yao, 1990, Laser 240-TS (spinosad 240 g/l active substance) and a commercial formulation of *B. thuringiensis* subsp. *kurstaki*, Bactospeine DF-TS, against *T. dilaticollis* and reported that the seed yield in plots with seeds treated with these products was high - 6676 kg/ha and 6447 kg/ha for plots with spinosad and *B. thuringiensis,* respectively. Treatments of maize plants during vegetation with 54% *B. thuringiensis* (Bactospeine DF, at a dose of 0.01%) showed 78% saved plants, while after vegetation treatment with spinosad (Laser 240 SC, at a dose of 250 mL/ha), the saved plants were 79% (Toader et al. 2020).

Recently Fătu et al. (2023) compared the effectiveness of native strains of *B. bassiana* and *B. pseudobassiana* Rehner et Humber with the bioinsecticide Naturalis® and the pure strain ATCC 74040 isolated from the commercial product against *T. dilaticollis* adults under laboratory conditions. They reported the lowest probability of survival and the highest virulence in the treatment with the commercial product Naturalis®. *B. bassiana* strain (BbTd1) and *B. pseudobassiana* strain (BbLy) were comparable in percentage of mycosis and virulence to the pure ATCC 74040 strain. In the field, the strains BbTd1 and BbLy applied as conidia multiplied on barley grains in the soil did not affect the *T. dilaticollis* density in maize crops.

Oulema melanopus – infestation, damage, pathogens

The cereal leaf beetle *Oulema melanopus* (Linnaeus, 1758) (Coleoptera: Chrysomelidae) is a pest of various cereals, mainly attacking wheat, oats, and barley (Leibee & Horn 1979, Dosdall et al. 2011). Originally from Eurasia, *O. melanopus* is a common pest of cereals throughout Europe, especially in the Balkans (Kostov 2001). It was discovered as invasive species in North America in 1962 (Dysart et al. 1973) and has also been reported in North Africa, as well as in northern and western Asia, where it is classified as a major pest of various cereals, grains, and grasses (Bai et al. 2002, Hitchcox et al. 2000). In Europe, a higher abundance of *O. melanopus* has been observed since the 1980s (Kaniunczak 1988) as a result of intensified agriculture and the presence of large areas with monocultures. In addition, climate change (warmer winters and early spring) seems to favor the survival and development of beetles. In Europe, the economic level of damage is estimated at two to three larvae per shoot/stalk (Stilmant 1995). The beetle is reported to cause damage to cereals in Hungary, the countries of the former Yugoslavia, Romania, Bulgaria, and the southern regions of the former USSR (Hilterhaus 1965). The life cycle of *O. melanopus* is well known (Helgesen & Haynes 1972, Gage 1974, Casagrande et al. 1977). This species is univoltine. Adults hibernate in groups in sheltered places such as shelters in the field, in cracks in the bark of trees, or inside rolled-up leaves. They become active in the spring when the temperature reaches 10°C and feed initially on wild grasses. Egg-laying begins about 14 days after the appearance of adults. Over the next two months, each female can lay several hundred eggs. The larvae go through four instars, each lasting two to three days. Pupation occurs in the soil a few cm below the surface. Larvae and adults usually feed on the leaves of the host plants.

The economic impact of the cereal leaf beetle can be significant. Heyer (1977) estimated that one *O. melanopus* larva reduced yields by about 10%. The massive larvae attack reduces overall yields by up to 80% (Grala et al. 1991), causing losses of about one ton of grain per hectare. In a 2002 study, an attack of 13 to 126 (average 88) larvae per 100 stalks was reported (Hitchcox et al. 2002). These levels would lead to yield losses of about 2 to 4% of the crop. This raises the question of the status and economic impact of these species.

Pathogens of *O. melanopus* include fungi such as *Alternaria alternata* Keissler, *Isaria farinosa* (Holmsk.), and *Verticillium lecanii* (Zimmerman) Viegas (Machowicz-Stefaniak & Miczulski 1985). The entomopathogenic fungus *B. bassiana* is known to affect cereal leaf beetle populations (Paschke 1965). Adults treated with *B. bassiana* conidia showed a significant reduction in activity and feeding 48 hours after treatment at 26 °C (Paschke 1965). Studying the arthropod host cadavers in a research farm in central Denmark for the presence of entomopathogenic fungi, Meyling et al. (2011) recorded *Metarhizium flavoviride* Gams and Rozsypal from *O. melanopus*. However, few studies have been conducted to optimize the effectiveness of this pathogen, probably because the yield value is too low compared to the cost of these alternative biopesticides. The nematode species, *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Filipjev) (Steinernematidae) have also been reported as biocontrol agents for *O. melanopus* (Laznik et al. 2010a, 2010b, 2012). Laznik et al. (2010a) showed that *S. carpocapsae* strain C101 leads to greater than 80% mortality when overwintering adults of *the* cereal leaf beetle are treated with different doses of the nematode in laboratory conditions.

A microsporidian pathogen, *Nosema oulemae* Ditrich, Hostounsky, Lom, da Silva, Slemenda, and Pieniazek, 1995 has been found, and its SSU rRNA sequence was deposited in GenBank under accession U27359. Recently, the microsporidia *Vairimorpha hostounsky* sp. nov. was detected in the Blue shieldbug, *Zicrona caerulea* Linnaeus, 1758 (Hemiptera: Pentatomidae), showing 99.9% SSU rRNA sequence similarity to *N. oulemae* (Yıldırım 2021). Based on sequence similarity, the isolate from *Z. caerulea* was attributed to *O. melanopus* provisionally designated as *N. oulemae*.

Diabrotica virgifera virgifera – infestation, damages, pathogens

In Europe, the species *Diabrotica virgifera virgifera* LeConte, 1868 was first discovered in 1992 near Belgrade airport in former Yugoslavia (Bača 1994) and became a very serious quarantine maize pest in Europe in the mid-1990s (Bažok et al. 2021). *D. v. virgifera* has one generation per year (Chiang 1973). Usually, overwintering eggs are concentrated up to 20 cm in the soil. The larvae hatch in May-June, depending on several environmental factors. Three larval instars feed on the roots of corn. As the larvae grow and their food needs increase, they burrow into the roots. Damage to larvae is usually most severe after the secondary root system is well developed and supporting roots are formed. They are often located in tunnels formed in the larger roots and occasionally in the plant's crown. The larvae are found close to the base of the plant, causing growth retardation or death. Soon after the plants have sprouted, root feeding begins. Early symptoms are expressed as drying or nutrient deficiency. The lodging of plants occurs later in plant development. The development of the larval stages depends on the temperature, which is optimal between 21 °C and 30 °C (Krysan 1986). In Central and Eastern Europe (e.g., Hungary and Serbia), the appearence of adults can begin in late June to early July, with peaks often occurring in July and even earlier in Croatia (Barčić et al. 2003) and later in Bulgaria – from the middle of July (Toshova et al. 2017). Adult beetles can be observed in the field for weeks. Females prefer to lay their eggs in moist soil. The pest can be spread actively and passively over long distances - for example, by the natural spread of flying beetles (carried by the wind) or possibly by supplies of corn. According to European studies, yield losses were assessed from 10% to 30% (Wesseler & Fall 2010).

Since its discovery, it has become the most important pest of this crop in several Central and Eastern European countries (Boriani et al. 2006) and may pose a potential threat to all EU maize production. Damage during feeding of *D. v. virgifera* larvae often causes economically significant losses in yield and quality of agricultural production. Area-wide studies showed that crop rotation is an effective strategy to interrupt the pest's life cycle and to keep *D. v. virgifera* populations below the damage threshold (Bažok et al. 2021, Furlan et al. 2022). The rationale is that egg-laying in most *D. v. virgifera* adult populations commonly occur in maize fields (Levine & Oloumi-Sadeghi 1991), and larvae develop mainly on maize roots (Moeser & Hibbard 2005). However, many farmers prefer not to alternate crops in their maize fields every year, as maize is one of Europe's most profitable field crops (Fall & Wesseler 2008), and it can be grown continuously for many years in the same field. Kuhlmann & van der Burgt (1998) reviewed the known associations of *D. v. virgifera* with natural pathogens and parasites, which include virus-like particles, bacteria, gregarines, fungi, and nematodes. Three RNA viruses, tentatively named *Diabrotica virgifera virgifera virus 1, Diabrotica virgifera virgifera virus 2,* and *Diabrotica virgifera virgifera virus* 3 (DvvV3) (genome sequences in GenBank under accession numbers KY070327, KY064174, and KY200663, respectively) were extracted and identified from the Western corn rootworm specimens collected from various locations in United States and Europe (Liu et al. 2017a, 2017b, 2017c). Recently, Liu et al. (2021) reported DNA sequences derived from a novel nudivirus (Nudiviridae) (DvvNV) embedded in adults of this pest collected from the United States.

Treatment of *D. v. virgifera* adults with *B. thuringiensis* showed that applying spores to the food substrate did not increase the mortality of corn rootworms (Sutter 1969). Later Herrnstadt & Soares (1989) reported that spores and crystals of *B. thuringiensis* (strain San Diego) had been used as an insecticide against insect pests of maize and that pests including *D. v. virgifera* have been controlled with this agent. Transgenetic maize hybrids, which produce insecticidal toxins of the bacterium *B. thuringiensis* (*Bt*), have been used to control *D. v. virgifera* since 2003. The first *Bt* hybrids selected to protect maize plants produced *Bt* toxic proteins such as Cry3Bb1, mCry3A, and Cry34/35Ab1 (Tabashnik & Gould 2012, Yin et al. 2020). The insecticidal proteins (Cry1, Cry2, Cry3) derived during the development of *Bt* have their essential role in protection against insects (Lepidoptera and Coleoptera). They form α-helical transmembrane pores on the insect gut epithelium, ultimately leading to pest mortality (Adang et al. 2014). They are classified as α-pore-forming proteins (α-PFPs) and β-pore-forming proteins (β-PFPs). As a final result, these proteins (Cry35Ab1, Cry34Ab1) cause toxicity to the western corn rootworm. As a fundamental approach to control *D. v. virgifera* in the USA, transgenic maize (expressing WCR-active proteins from *B. thuringiensis*) is cultivated (ISAAA 2017, Brookes & Barfoot 2015). Later, another *Bt* protein (Vpb4Da2) with a similar activity which was species-specific against WCR, was selected. Its effect is manifested in the fact that the grown transgenic maize has a better root defense against *D. v. virgifera* and a significant reduction in the population of the pest's beetles (≥ 97%) was also reported. All these data confirm that, under field conditions, transgenic maize provides the necessary protection (Yin et al. 2020).

Dematheis et al. (2012) presented a study providing new data about microbial communities associated with larval guts and eggs of *D. v. virgifera*. The bacterial compositions included species (*Wolbachia*, *Duganella* sp, *Herbaspirillum* sp., *Mortierella elongata*, *Pseudomonas* sp., *Azotobacter chroococcum*, *Lysobacter* sp, *Streptomyces* sp., *Rhodococcus* sp., *Tsukamurella* sp.) identified in the larval gut and/or in the eggs, but their biological role should be explored in the future investigations.

Pilz et al. (2008) studied pest populations from Austria, Italy, Romania, Serbia, and Hungary and, like Toepfer & Kuhlmann (2004), found a low rate of pathogenic infections in the studied individuals from all countries except Austria. Total infections with *M. anisopliae* and *Beauveria* spp. were found in 1.4% of larvae, 0.2% of pupae, and 0.05% of adult individuals collected from the field. At the same time, high levels of pathogen infection have been reported in maize fields in Hungary. *Beauveria brongniartii* (Sacc.) Petch and *M. anisopliae,* under laboratory conditions, reported that only one strain of *B. brongniartii* significantly influenced the mortality of the larvae after seven days. However, 21 days from the start of exposure of the larvae to spores, four strains of *B. bassiana*, two strains of B. *brongniartii,* and one strain of *M. anisopliae* significantly influenced mortality of the larvae with average mortality ranging from 63% to 87%. Jackson et al. (1986) and Brooks & Jackson (1990) reported eugregarines from this pest.

Pilz et al. (2008) also established entomopathogenic nematodes (EPN) of the genera *Heterorhabditis* and

Steinernema in the larvae of *D. v. virgifera* from Hungary. For biological control of this insect pest, in addition to the entomopathogenic fungi (*Beauveria* spp. and *Metarhizium* spp.), entomopathogenic nematodes (families Steinernematidae and Heterorhabditidae) are also used. Still, there are some critical aspects related to variable efficacy and high costs compared to insecticides and the impact on the local fauna (Ferracini et al. 2021). Results of laboratory screening tests with *Steinernema glaseri* (Steiner, 1929), *S. arenarium* (Artyukhovsky, 1967), *S. abbasi* Elawad, Ahmad & Reid, *S. bicornutum* Tallosi, Peters and Ehlers, *S. feltiae, S. kraussei* (Steiner, 1923), *S. carpocapsae* and *Heterorhabditis bacteriophora* Poinar against *D. v. virgifera* in small-volume arenas showed the highest potential in *H. bacteriophora*, followed by *S. arenarium* and *S. feltiae*, for further testing as candidate biological control agents (Toepfer et al. 2005). Toepfer et al. (2008) demonstrated that three entomopathogenic nematodes, *S. feltiae*, *H. bacteriophora*, and *H. megidis* Poinar, Jackson, and Klein significantly reduced *D. v. virgifera* independently, whether applied as a row spray with a solid stream into the soil at sowing or onto the soil along maize rows in June. *S. feltiae* appeared to be the least suitable EPN compared to *H. bacteriophora* and *H. megidis* (Toepfer et al. 2005, 2008). The choice of application technique in the field influenced the efficacy of the entomopathogenic nematode species *H. bacteriophora*. The highest density reduction (68%) of *D. v. virgifera* density was observed in the case of the application of nematodes to the soil together with maize sowing using a fluid-solid stream (Toepfer et al. 2010). Rainfall the day before applications and the type of soil also affect the mobility and effectiveness of the entomopathogenic nematodes (Toepfer et al. 2010)

A biological control product was developed based on the entomopathogenic nematode *H. bacteriophora*. It is available in several European countries, such as Germany, Italy, Austria, and Hungary (Toth et al. 2020). Investigation of the efficacy of the following four bioinsecticide products on the larval stages of the pest in field trials in Hungary was conducted: the entomopathogenic fungus *M. anisopliae* strain BIPESCO 5 (also known as F52) (Novozymes Biologicals BioAg Group, France), the entomopathogenic nematode species – *H. bacteriophora* (product of e-nema, Germany), the soil insecticide (tefluthrin) and clothianidin – an insecticide which is used for treating maize seeds (Pilz et al. 2009). As a result of this study, the authors believe that the effectiveness of the nematode product is comparable to that of chemical insecticides (Pilz et al. 2009). It achieved a 60% reduction in the adult population density, just like the results reported in the United States (Georgis et al. 1991). Records about bacterial, protozoan, and viral species were summarized by Toepfer et al. (2009). Combinations of entomopathogens offer new perspectives for biological control of the Western corn rootworm (Jaffuel et al. 2019). Table 1 summarizes the list of natural entomopathogens and tested bioproducts containing entomopathogens against the grey maize weevil, the cereal leaf beetle, and the Western corn rootworm.

Table 1. List of entomopathogens (detected and tested) against *T. dilaticollis, O. melanopus,* and *D. v. virgifera*, and the cited studies

Pathogens	Cited studies
Tanymecus dilaticollis	
Bacillus thuringiensis	Toader et al. 2017; 2020
Aspergillus flavus	Draganova et al. 2012
Aspergillus ochraceus	Draganova et al. 2012
Beauveria bassiana	Draganova et al. 2012; Takov et al. 2013; Fătu et al., 2019; Toshova et al. 2021; Georgescu et al. 2022; Fătu et al. 2023
Beauveria pseudobassiana	Georgescu et al. 2022; Fătu et al. 2023
Metarhizium anisopliae	Draganova et al. 2012; Georgescu et al. 2022
Fusarium sp.	Draganova et al. 2012
Gregarina sp.	Takov et al. 2013
Oulema melanopus	
Bacillus thuringiensis	Meissle et al. 2012
Nosema oulemae	Yıldırım 2021
Alternaria alternata	Raizada 1976; Machowicz-Stefaniak & Miczulski 1985
Beauveria bassiana	Paschke 1965; Kher et al. 2012
Fusarium sp.	Gallo 2007
Metarhizium flavoviride	Meyling et al. 2011
Verticillium lecanii	Kher 2014
Heterorhabditis bacteriophora	Laznik et al. 2010a; 2010b; Jaffuel et al. 2019; Mazurkiewicz et al. 2021
Heterorhabditis megidis	Kreft et al. 2013; Mazurkiewicz et al. 2019
Steinernema arenarium	Kreft et al. 2013
Steinernema carpocapsae	Laznik et al. 2010a; 2010b, 2012; Kreft et al. 2013
Steinernema feltiae	Laznik et al. 2010a; 2010b; Kreft et al. 2013; Mazurkiewicz et al. 2019; 2021

(Table 1 – continued next page)

(Table 1 – continuation)

Pathogens	Cited studies
Diabrotica virgifera virgifera	
Diabrotica virgifera virgifera virus 1 (DvvV1)	Liu et al. 2017a
Diabrotica virgifera virgifera virus 2 (DvvV2)	Liu et al. 2017b
Diabrotica virgifera virgifera virus 3 (DvvV3)	Liu et al. 2017c
Nudivirus (DvvNV)	Liu et al. 2021
Bacillus pumilus Meyer and Gottheil, 1901	Disi et al. 2018
Bacillus thuringiensis	Herrnstadt & Soares 1989; Moellenbeck et al. 2001; Al Deeb & Wilde 2005; Siegfried et al. 2005; Meissle et al. 2011
Chromobacterium subtsugae Martin et al., 2007	Martin et al. 2007
Pseudomonas aeruginosa	Hamilton 1968
Pseudomonas chlororaphis (Guignard and Sauvageau 1894) Bergey et al. 1930	Jaffuel et al. 2019
Pseudomonas protegens Flügge, 1886	Jaffuel et al. 2019
Wolbachia sp. (group A)	Giordano et al. 1997; Clark et al. 2001; Degrugillier et al. 1991
Beauveria bassiana	Brooks & Raun 1965; Branson et al. 1975; Oloumi-Sadeghi & Levine 1989; Mulock & Chandler 2000; Mulock & Chandler 2001a, 2001b; Bruck & Lewis 2002; Toepfer & Kuhlmann 2004; Humber & Hansen 2005; Alvarez-Zagoya & Perez-Dominguez 2006; Pilz et al. 2008; Rudeen et al. 2013; Hoffmann et al. 2014; Cagáň et al. 2019
Beauveria brongniartii	Cagáň et al. 2019
Clonostachys rosea (Link) Schroers, 1999	Modic 2007; Modic et al. 2008
Fusarium oxysporum (Schlecht. Emend. Snyder & Hansen)	Oloumi-Sadegh & Levine 1989
Metarhizium anisopliae	Toepfer & Kuhlmann 2004; Pilz et al. 2007; Pilz et al. 2008; Meissle et al. 2009; Rudeen et al. 2013; Babendreier et al. 2015; Cagáň et al. 2019
Metarhizium brunneum	Hoffmann et al. 2014; Zottele et al. 2021
Metarhizium pemphigi	Toshova et al. 2022
Paecilomyces lilacinus (Thom) Samson, 1974	Modic 2007; Modic et al. 2008
Eugregarines	Jackson 1986; Brooks & Jackson 1990
Heterorhabditis bacteriophora	Toepfer et al. 2005, 2009, 2010; Kurtz et al. 2007, 2009; Ehlers et al. 2008; Hiltpold et al. 2010; Hoffmann et al. 2014; Babendreier et al. 2015; Jaffuel et al. 2019; Geisert et al. 2018; Toth et al. 2020
Heterorhabditis megidis	Kurtz et al. 2007; Hiltpold et al. 2010; Toepfer et al. 2008, 2010; Geisert et al. 2018
Steinernema arenarium	Van der Burgt et al. 1998; Toepfer et al. 2005
Steinernema abassi	Toepfer et al. 2005
Steinernema bicornutum	Toepfer et al. 2005
Steinernema carpocapsae	Munson & Helms 1970; Jackson & Brooks 1995; Nickle et al. 1994, Journey & Oslie 2000; Toepfer et al. 2005; Geisert et al. 2018
Steinernema diaprepesi Nguyen & Duncan	Geisert et al. 2018
Steinernema feltiae	Van der Burgt et al. 1998; Toepfer et al. 2005; 2008, 2010; Kurtz et al. 2007, 2009; Hiltpold et al. 2010; Hoffmann et al. 2014; Geisert et al. 2018; Jaffuel et al. 2019
Steinernema glaseri	Toepfer et al. 2005
Steinernema kraussei	Toepfer et al. 2005
Steinernema rarum Doucet	Geisert et al. 2018
Steinernema riobrave Cabanillas, Poinar & Raulston	Geisert et al. 2018

Conclusion

Our review summarizes the pathogens of three important beetle pests of agricultural plants used for human and animal food. Biological control approaches are increasingly being developed as environmentally friendly alternatives to chemical insecticides. Among bacteria, fungi, and nematodes, there are effective species for control, and the development of biopreparations based on these bioagents are innovative, promising, and environmentally friendly practices that will be further developed.

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References

Adang, M.J., Crickmore N., Jurat-Fuentes J.L. (2014): Chapter Two—Diversity of *Bacillus thuringiensis* Crystal Toxins and Mechanism of Action. In: Dhadialla, T.S, Gill S.S., (eds.), Advances in Insect Physiology 47: 39-87.

Al Deeb, M.A., Wilde, G.E. (2005): Effect of *Bt* corn expressing the Cry3Bb1 toxin on western corn rootworm (Coleoptera: Chrysomelidae) biology. Journal of

the Kansas Entomological Society 78: 142-152.
Alvarez-Zagoya, R., Perez-Dominguez, J.F. (2006): Enemigos naturals de *Diabrotica virgifera* y *D. balteata* en Durango y Jalisco, XXIX Congreso Nacional de Control Biologico, Manzanillo, Colima, Mexico. pp. 212-216.
Babendreier, D., Jeanneret, P., Pilz, C., Toepfer, S. (2015): Non-target effects of insecticides, entomopathogenic fungi and nematodes applied against western corn rootworm larvae in maize. Journal of Applied Entomology 139: 457-467.
Bača, F. (1994): New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* LeConte (Coleoptera, Chrysomelidae). Zastita Bilja 45: 125-131
Bai, B.B., Worth, R.A., Johnson, K.J.R., Brown, G. (2002): Detection survey and population monitoring of cereal leaf beetle in Oregon, 2001. Proceedings of 61st Annual Pacific Northwest Insect Management Conference, Portland, Oregon
Bamisile, B.S., Akutse, K.S., Siddiqui, J.A., Xu, Y. (2021): Model application of entomopathogenic fungi as alternatives to chemical pesticides: prospects, challenges, and insights for next-generation sustainable agriculture. Frontiers in Plant Science 12: 741804.
Barbulescu, A., Voinescu, I., Sadagorschi, D., Penescu, A., Popov, C. Vasilescu, S. (2001): Cruieser 350 FS-A new product for maize and sunflower seed treatment against *Tanymecus dilaticollis* Gyll. Romanian Agricultural Research 15: 77-87.
Barčić, J.I., Bažok, R., Maceljski, M. (2003): Research on the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) in Croatia (1994-2003). Entomologia Croatica 7 (1-2): 63-83.
Bažok, R., Lemić, D., Chiarini, F., Furlan, L. (2021): Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe: Current status and sustainable pest management. Insects 12: 195.
Boriani, M., Agosti, M., Kiss, J., Edwards, C.R. (2006): Sustainable management of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in infested areas: experiences in Italy, Hungary and the USA. Bulletin OEPP/EPPO 36: 531-537.
Branson, T.F., Guss, P.L., Krysan, J.L., Sutter, G.R. (1975): Corn rootworms: laboratory rearing and manipulation, ARS-NC-28, Agricultural Research Service, US Department of Agriculture.
Brookes, G., Barfoot, P. (2015): Environmental impacts of genetically modified (GM) crop use 1996–2013: Impacts on pesticide use and carbon emissions. GM Crops and Food - Biotechnology in Agriculture and the Food Chain 6(2): 103-133.
Brooks, D.L., Raun, E.S. (1965): Entomogenous fungi of corn insects in Iowa. Journal of Insect Pathology 7: 7981.
Brooks, W.M., Jackson, J.J. (1990): Eugregarines: current status as pathogens, illustrated in corn rootworms. Department of Entomology, University of Adelaide. Proceedings of the Vth International Colloquium of Invertebrate Pathology and Microbial Control, Adelaide, Australia: 512-515.
Bruck, D.J., Lewis, L.C. (2002): Whorl and pollen-shed stage application of *Beauveria bassiana* for suppression of adult Western corn rootworm. Entomologia Experimentalis et Applicata 103: 161169.
Cagáň, L., Števo, J., Gašparovič, K. Matušikova, S. (2019): Mortality of the Western corn rootworm, *Diabrotica virgifera virgifera* larvae caused by entomopathogenic fungi. Journal of Central European Agriculture 20 (2): 678-685.
Čamprag, D., Sekulic, R. (2002): Kukuruzna pipa (*Tanymecus dilaticollis* Gyll.). Design Studio Stanisic, Backa Palanka, Serbia, Yugoslavia, 138 pp. (In Serbian)
Casagrande, R.A., Ruesink, W.G., Haynes, D.L. (1977): The behaviour and survival of adult cereal leaf beetles. Annals of the Entomological Society of America 70: 19-30.
Chiang, H.C. (1973): Bionomics of the northern and western corn rootworms. Annual Review of Entomology 18: 47-72.
Clark, T.L., Meinke, L.J., Skoda, S.R., Foster, J.E. (2001): Occurrence of *Wolbachia* in selected Diabroticite (Coleoptera: Chrysomelidae) Beetles. Annals of the Entomological Society of America 94: 877-885.
Cristea, M., Cabulea, I., Sarca, T. (2004): Maize. Monographic study. Romanian Academy Publishing-house 1: 589-626.
Degrugillier, M.E., Degrugillier, S.S., Jackson, J.J. (1991): Nonoccluded, cytoplasmic virus particles and *Rickettsia*-like organisms in testes and spermathecae of *Diabrotica virgifera*. Journal of Invertebrate Pathology 57: 50-58.
Dematheis, F., Kurtz, B., Vidal, S., Smalla, K. (2012): Microbial communities associated with the larval gut and eggs of the western corn rootworm. PLoS One 7: e44685.
Disi, J.O., Kloepper, J.W., Fadamiro, H.Y. (2018): Seed treatment of maize with *Bacillus pumilus* strain INR-7 affects host location and feeding by Western corn rootworm, *Diabrotica virgifera virgifera*. Journal of Pest Science 91: 515–522.
Dosdall, L., Cárcamo, H., Olfert, O., Meers, S., Hartley, S., Gavloski, J. (2011): Insect invasions of agro-ecosystems in the western Canadian prairies: case histories, patterns, and implications for ecosystem functioning. Biological Invasions 13: 1135-1149.
Draganova, S., Takov, D., Toshova, T.B. (2012): Fungal pathogens on *Tanymecus* (*Episomecus*) *dilaticollis* (Coleoptera: Curculionidae) and bioassays with some *Beauveria bassiana* isolates. Acta Zoologica Bulgarica 64: 289-294.
Dysart, R.J., Maltby, H.L., Brunson, M.H. (1973): Larval parasites of *Oulema melanopus* in Europe and their colonization in the United States. Entomophaga 18: 133-167.
Ehlers, R.-U., Hiltpold, I., Kuhlmann, U., Toepfer, S. (2008): Field results on the use of *Heterorhabditis bacteriophora* against the invasive maize pest *Diabrotica virgifera virgifera*. Insect Pathogens and Insect Parasitic Nematodes, IOBC/WPRS Bulletin 31: 332-335.
Fall, E.H., Wesseler, J.H.H. (2008): Market analyses for the potential use of biological control products in high value maize systems (WP 2 Task 3). Environmental Economics and Natural Resources. No. D02.15
Fătu, A.C., Dinu, M.M., Ţane, C.M., Cojanu, D., Ciornei, C., Andrei, A.M. (2019): Susceptibiliy of *Calliteara pudibunda* L. on *Beauveria bassiana* (Bals) Vuill. Romanian strains. Romanian Journal for Plant Protection 12: 91-92.
Fătu, A.-C., Georgescu, E., Iamandei, M., Mateescu, M., Vlad, I.A. (2023): Evaluation of *Beauveria bassiana* and *Beauveria pseudobassiana* against *Tanymecus dilaticollis*. Romanian Agricultural Research 40: in press.
Ferracini, C., Blandino, M., Rigamonti, I.E., Jucker, C., Busato, E., Saladini, M.A., Reyneri, A., Alma, A. (2021): Chemical-based strategies to control the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Crop Protection 139: 105306.
Furlan, L., Chiarini, F., Contiero, B., Benvegnù, I., Horgan, F.G., Kos, T., Lemić, D., Bažok, R. (2022): Risk assessment and area-wide crop rotation to keep Western corn rootworm below damage thresholds and avoid insecticide use in European maize production. Insects 13: 415.
Gage, S.H. (1974): Ecological investigations on the cereal leaf beetle, *Oulema melanopus* (L.), and the principal larval parasite, *Tetrastichus julis* (Walker). PhD. Thesis, Michigan State University.
Gallo, J. (2007): Parasites on *Oulema* (*Lema*) *lichenis* Voet, 1826. pp. 446-448. In: Pimentel, P.D. (ed.), Encyclopedia of Pest Management, Volume II (1st Edition). CRC Press.
Geisert, R.W., Cheruiyot, D.J., Hibbard, B.E., Shapiro-Ilan, D.I., Shelby, K.S., Coudron, T.A. (2018): Comparative assessment of four Steinernematidae and three Heterorhabditidae species for infectivity of larval *Diabrotica virgifera virgifera*. Journal of Economic Entomology 111 (2): 542-548.
Georgescu, E. (2023): *Tanymecus dilaticollis* (maize leaf weevil). In: CABI Compendium. Wallingford, UK: CAB International. https://doi.org/10.1079/cabicompendium.54122
Georgescu, E., Fătu, C., Cana, L. (2022): Research concerning the effectiveness of the entomopathogenic fungi for controlling the maize leaf weevil (*Tanymecus dilaticollis* Gyll) in the greenhouse conditions. Annals of the University of Craiova – Agriculture, Montanology, Cadastre Series 52 (1): 149-160.
Georgis, R., Kaya, H.K., Gaugler, R. (1991): Effect of steinernematid and heterorhahditid nematodes (Rhahditida: Steinernematidae and Heterorhahditidae) on nontarget arthropods. Environmental Entomology 20: 815-822.
Gerginov, L. (1989): Insect pests of maize in Bulgaria and their control. Acta Phytopathologica et Entomologica Hungarica 24: 81-84.
Giordano, R., Jackson, J.J., Robertson, H.M. (1997): 'The Role of Wolbachia Bacteria in Reproductive Incompatibilities and Hybrid Zones of *Diabrotica* beetles and *Gryllus* crickets', Proceedings of the National Academy of Sciences of the United States of America 94: 11439-11444.
Grala, B., Mrówczyński, M., Banaszak, H., Kaniuczak, Z. (1991): Opłacalność chemicznego zwalczania skrzypionek (*Lema* spp.) występujących na zbożach [Profitability of chemical control of cereal leaf beetles (*Lema* spp.)]. Materiały XXXI Sesji Naukowej Instytutu Ochrony Roślin 1: 161-168 [in Polish].
Hajek, A.E., St. Leger, R.J. (1994): Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 39: 293-322.
Hamilton, E.W. (1968): *Pseudomonas aeruginosa* in species of *Diabrotica*. Journal of Invertebrate Pathology 12: 188-191.
Haynes, D.L. (1973): Population management of the cereal leaf beetle. pp. 232–240. In: Geier, P.W., Clark, L.R., Anderson, D.J., Nix, H.A. (eds.), Proceedings of the 14th International Congress of Entomology, Canberra, Australia, 22–30 August 1972. Canberra, Australia: Ecological Society of Australia.
Haynes, D.L., Gage, S.H. (1981): The cereal leaf beetle in North America. Annual Review of Entomology 26: 259-287.
Helgesen, R.G., Haynes, D.L. (1972): Population dynamics of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae): a model for age specific mortality. Canadian Entomologist 104: 797-814.
Herrnstadt, C., Soares, G.C. (1989): Cotton boll weevil, alfalfa weevil, and corn rootworm via contact with a strain of *Bacillus thuringiensis,* United States Patent, US 4797 276.
Heyer, W. (1977): Biologie und Schadwirkung der Getreidehähnchen *Lema* (*Oulema* spp.) in der industriemäßigen Getreideproduktion. Nachrichtenblatt fur den Pflanzenschutz in der DDR 31: 167-169.
Hilterhaus, V. (1965): Biologish-okogische Untersuchungen an Blattafern der Fattungen *Lema* und Gastroidea (Chrysomelidae: Coleoptera) (Ein Beitrag zur Agarokologie). Zeitschrift fuer Angewandte Zoologie 52: 257-295.
Hiltpold, I., Toepfer, S., Kuhlmann, U., Turlings T.C.J. (2010): How maize root volatiles affect the efficacy of entomopathogenic nematodes in controlling the

western corn rootworm? Chemoecology 20: 155-162.

Hitchcox, M., Klaus, M., Campbell, C., Gould, M., Miller, S. (2000): Cereal leaf beetle survey and biological control activities in Washington State 2000. 2000 Entomology Project Report – WSDA Pub. 044. Laboratory Services Division, Pest Program, Washington State Department of Agriculture, Olympia, Washington.

Hitchcox, M.E., Klaus, M.W., Gould, M C., Pike, K., Miller, S. (2002): Cereal leaf beetle survey and biocontrol activities in Washington State, 2001. WSDA Pub, 077 (N/8/02), Washington State Department of Agriculture, Plant Protection Division, Pest Program 3/13.

Hoffmann, A.M.,French, B.W., Jaronski, S.T., Gassmann, A.J. (2014): Effects of Entomopathogens on Mortality of Western Corn Rootworm (Coleoptera: Chrysomelidae) and Fitness Costs of Resistance to Cry3Bb1 Maize. Journal of Economic Entomology 107 (1): 352–360.

Humber, R.A., Hansen, K.S. (2005): *Beuaveria* and allied fungi. USDA-ARS Plant Protection Research Unit, US Plant, Soil & Nutrition Laboratory, Ithaca, NY, USA

ISAAA. (2017): Global status of commercialized biotech/GM Crops in 2017: Biotech crop adoption surges as economic benefits accumulate in 22 years. ISAAA Brief : 53.

Jackson, J.J. (1986): Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata* howardi. pp. 25-47. In: Krysan, J.L., Miller T.A. (eds.), Methods for the Study of Pest Diabrotica, New York: Springer Verlag.

Jackson, J.J., Brooks, M.A. (1995): Parasitism of western corn rootworm larvae and pupae by *Steinernema carpocapsae*. Journal of Nematology 1: 15-20.

Jaffuel, G., Imperiali, N., Shelby, K., Campos-Herrera, R., Geisert, R., Maurhofer, M., Loper, J., Keel, C., Turlings, T.C.J., Hibbard, B.E. (2019): Protecting maize from rootworm damage with the combined application of arbuscular mycorrhizal fungi, *Pseudomonas bacteria* and entomopathogenic nematodes. Scientific Reports 9: 3127.

Journey, A.M., Oslie, K.R. (2000): Biological control of the western corn rootworm (Coleoptera: Chrysomelidae) using the enthomopathogenic nematode *Steinernema carpocapsae*. Environmental Entomology 29(4): 822-831.

Kaniunczak, Z. (1988): Is cereal cultivation in south-eastern Poland threatened by *Oulema* spp.? Ochrona Roślin 33: 9-55.

Kher, S.V. (2014): Sustainable management of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae), a new invasive insect pest of cereal crops in western Canada. Ph.D. Thesis, University of Alberta, Department of Agricultural, Food and Nutritional Science 1-304.

Kher, S.V., Cárcamo, H.A., Dosdall, L.M., Goettle, M. (2012): Laboratory bioassay of *Beauveria bassiana* strain GHA (BotaniGardTM) efficacy on cereal leaf beetle (*Oulema melanopus*) and non-target effects on its parasitoid *Tetrastichus julis*. Proceedings of the 60th Entomological Society of Alberta Annual Meeting, Alberta, November 4-7.

Kirkov, K. (1967): *Tanymecus dilaticollis* – its biology and control. Rastenievadni nauki 6: 45-50. (In Bulgarian, English summary)

Kostov, K. (2001): Breeding wheat lines for host-plant resistance to cereal leaf beetle by using the cross mutation method. Bulgarian Journal of Agricultural Science 7: 7-14.

Krasteva, H., Panajotova, M., Tonev, T., Karadzhova, Y., Milanova, S., Nikolov, P., Dimitrova, A., Stefcheva, M., Ventsislavov, V., Chavdarov, L., Velichkov, A. (2006): Good plant protection practice in maize crops. Ministry of Agriculture and Food, Sofia 2, 015, 1: 69-77.

Kreft, A., Skrzypek, H., Kazimierczak, W., Skrzypek, T. (2013): Susceptibility of *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) larvae to entomopathogenic nematodes (Rhabditida) rural development 2013. The Sixth International Scientific Conference Proceedings, Aleksandras Stulginskis University Akademija, Kaunas district, Lithuania 6 (2): 146-148.

Krysan, J.L. (1986): Introduction: Biology, distribution, and identification of pest *Diabrotica*. pp. 1-23. In: Krysan, J.L., Miller, T.A., (eds.), Methods for the Study of Pest Diabrotica, 1st Edition. Springer: New York, NY, USA.

Kuhlmann, U., Van der Burgt, W.A.C.M. (1998): Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in central Europe. Biocontrol News Information 19 (2): 59-68.

Kurtz, B., Toepfer, S., Ehlers, R.U., Kuhlmann, U. (2007): Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. Journal of Applied Entomology 131(6): 420-425.

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

Lacey, L.A., Georgis, R. (2012): Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. Journal of Nematology 44(2): 218-225.

Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S. (2015): Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology 132: 1–41.

Laznik, Ž., Štrukekj, M., Trdan, S. (2010a): Activity of entomopathogenic nematodes (Rhabditida) against cereal leaf beetle (*Oulema melanopus* [L.], Coleoptera, Chrysomelidae) adults under laboratory conditions. Acta Agriculturae Slovenica 95 (2): 141-148.

Laznik, Ž., Tóth, T., Lakatos, T., Vidrih, M., Trdan, S. (2010b): *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) adults are susceptible to entomopathogenic nematodes (Rhabditida) attack: Results from a laboratory study. Journal of Plant Diseases and Protection 117: 30-32.

Laznik, Ž., Vidrih, M., Vučajnk, F., Trdan, S. (2012): Is foliar application of entomopathogenic nematodes (Rhabditida) an effective alternative to thiametoxam in controlling cereal leaf beetle (*Oulema melanopus* L.) on winter wheat? Journal of Food, Agriculture and Environment 10 (2): 716-719.

Leibee, G.L., Horn, D.J. (1979): Influence of tillage on survivorship of cereal leaf beetle and its larval parasites, *Tetrastichus julis* and *Lemophagus curtus*. Environmental Entomology 8: 485-486.

Levine, E., Oloumi-Sadeghi, H. (1991): Management of diabroticite rootworms in corn. Annual Review of Entomology 36: 229–255.

Lipa, J.J. (1967): Studies on gregarines (Gregarinomorpha) of arthropods in Poland. Acta Protozoologica 5: 97-179.

Liu, S., Chen, Y., Sappington, T.W., Bonning, B.C. (2017a): Genome sequence of the first coleopteran iflavirus isolated from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Genome Announcements 5(6): e01530-16.

Liu, S., Chen, Y., Sappington, T.W., Bonning, B.C. (2017b): Genome sequence of *Diabrotica virgifera virgifera virus 2*, a novel small RNA virus of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Genome Announcements 5 (20): e00366-17.

Liu, S., Chen, Y., Sappington, T.W., Bonning, B.C. (2017c): Genome Sequence of a Novel Positive-Sense, Single-Stranded RNA Virus Isolated from Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte. Genome Announcements 5 (20): e00365-17.

Liu, S., Sappington, T.W., Coates, B.S., Bonning, B.C. (2021): Nudivirus sequences identified from the southern and western corn rootworms (Coleoptera: Chrysomelidae). Viruses 13: 269.

Lopes, R.B., Alves, S.B. (2005): Effect of *Gregarina sp*. parasitism on the susceptibility of *Blattella germanica* to some control agents. Journal of Invertebrate Pathology 88 (3): 261-264.

Machowicz-Stefaniak, Z., Miczulski, B. (1985): Tests to determine the pathogenicity of the fungus *Alternaria alternata* Keissler to the larvae of the blue cereal leaf beetle. Roczniki Nauk Rolniczych 15: 151-157 (Polish with English summary).

Martin, P.A.W., Hirose, E., Aldrich, J.R. (2007): Toxicity of *Chromobacterium subtsugae* to southern green stink bug (Heteroptera: Pentatomidae) and corn rootworm (Coleoptera: Chrysomelidae). Journal of Economic Entomology 100: 680-684.

Mazurkiewicz, A., Jakubowska, M., Tumialis, D., Skrzecz, I., Roik, K., Pezowicz, E., Gross, A. (2019): Laboratory bioassay of selected entomopathogenic nematodes as mortality factors of *Oulema melanopus* (Coleoptera: Chrysomelidae). Journal of Entomological Science 54(4): 390.

Mazurkiewicz, A., Jakubowska, M., Tumialis, D., Bocianowski, J., Roik, K. (2021): Foliar application of entomopathogenic nematodes against cereal leaf beetle *Oulema melanopus* L. (Coleoptera: Chrysomelidae) on wheat. Agronomy 11: 1662.

Meissle, M., Pilz, C., Romeis, J. (2009): Susceptibility of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to the entomopathogenic fungus *Metarhizium anisopliae* when feeding on *Bacillus thuringiensis* Cry3Bb1-Expressing Maize. Applied and Environmental Microbiology 75 (12): 3937-3943.

Meissle, M., Romeis, J., Bigler, F. (2011): *Bt* maize and integrated pest management - a European perspective. Pest Management Science 67 (9): 1049-1058.

Meissle, M., Knecht, S., Waldburger, M., Romeis, J. (2012): Sensitivity of the cereal leaf beetle *Oulema melanopus* (Coleoptera: Chrysomelidae) to Bt maize-expressed Cry3Bb1 and Cry1Ab. Arthropod-Plant Interactions 6: 203-211.

Meyling, N.V., Thorup-Kristensen, K., Eilenberg, J. (2011): Below- and aboveground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. Biological Control 59: 180-186.

Modic, S. (2007): Bionomics and spreading of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte, Coleoptera, Chrysomelidae) in Slovenia. Unpublished M.Sc. thesis, Ljubljana Plant Protection Institute, Slovenia.

Modic, S., Schoers, H.J., Urek, G. (2008): Survival of eggs of *D. v. virgifera* during winter and isolation of their fungal enemies. Conference on Western Corn Rootworm: Research and Long Term Management Needs for the European Maize Production, 25-29 May 2008, Göttingen, Germany, (Poster).

Moellenbeck, D.J., Peters, M.L., Bing, J.W., Rouse, J.R., Higgins, L.S., Sims L., Nevshemal, T., Marshall, L., Ellis, R.T., Bystrak, P.G., Lang B.A., Stewart, J.L., Kouba, K., Sondag, V., Gustafson, V., Nour, K., Xu, D.P., Swenson, J., Zhang, J., Czapla, T., Schwab, G., Jayne, S., Stockhoff, B.A., Narva, K., Schnepf, H.E., Stelman, S.J., Poutre, C., Koziel, M., Duck, N. (2001): Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nature Biotechnology 19: 668-672.

Moeser, J., Hibbard B.E. (2005): A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (Le Conte) in the New and Old World – nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in

Europe? pp. 41-65. In: Vidal, S., Kuhlmann, U., Edwards C.R. (eds.), Western corn rootworm – ecology and management. CABI Publishing, Wallingford, United Kingdom.

Mulock, B.S., Chandler, L. (2000): Field-cage studies of *Beauveria bassiana* (Hyphomycetes: Moniliaceae) for the suppression of adult western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Biocontrol Science and Technology 10: 51-60

Mulock, B.S., Chandler, L.D. (2001a): *Beauveria bassiana* transmission in western corn rootworm (Coleoptera: Chrysomelidae). Canadian Entomologist 133: 105-107.

Mulock, B.S., Chandler, L.D. (2001b): Effect of *Beauveria bassiana* on the fecundity of western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Biological Control 22: 16-21.

Munson, J.D., Helms, T.J. (1970): Field evaluation of a nematode (DD-136) for control of corn rootworm larvae. Proceedings of the Entomological Society of America, North Central Branch 25: 97–99.

Nickle, W.R., Connick, Jr., W.J., Cantelo, W.W. (1994): Effects of pesta-pelletized *Steinernema carpocapsae* (All) on western corn rootworms and Colorado potato beetles. Journal of Nematology 26: 249–250.

Oloumi-Sadeghi, H., Levine, E. (1989): Controlling fungi that colonize eggs of the western corn rootworm in the laboratory. Entomologica Experimentalis et Applicata 50: 271-279.

Paschke, J.D. (1965): Infection of the cereal leaf beetle, *Oulema* melanopa (Linnaeus) by *Beauveria* bassiana (Balsamol) Vuillemin. Journal of Invertebrate Pathology 7 (1): 101-102.

Paulian, F., Popov, C., Dinu-Pana, M. **(**1969): The corn leaf weevil (*Tanymecus dillaticolis* Gyll.) in Romania and its control. Contemporary Agriculture 5-6: 643-652.

Pilz, C., Wegensteiner, R., Keller, S. (2007): Selection of entomopathogenic fungi for the control of the Western Corn Rootworm *Diabrotica virgifera virgifera*. Journal of Applied Entomology 131(6): 426-431.

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

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

Popov, P. (1969): Studies on *Tanymecus dilaticollis* Gyll. (Curculionidae, Coleoptera) in Bulgaria. Rastenievadni nauki 6: 111–123. (In Bulgarian, English summary).

Popov, C., Raranciuc, S., Spiridon, C., Vasilescu, S., Cana, L. (2007): Phytosanitary state of the cereals, leguminous for grains, forage and technical crops from Romania. Problems of Plant Protection 35(1): 1-24.

Raizada, U. (1976): A preliminary report on the fungi infesting thrips (Thysanoptera: Thripidae). Entomon 1: 155-158.

Rudeen, M.L., Jaronski, S., Petzold-Maxwell, J.L, Gassman, A.J. (2013): Entomopathogenic fungi in cornfields and their potential to manage larval western corn rootworm *Diabrotica virgifera virgifera*. Journal of Invertebrate Pathology 114(3): 329–332.

Sáringer, G., Takács, A. (1994): Biology and control of *Tanymecus dilaticollis* Gyll. (Col., Curculionidae). Acta Phytopatologica et Entomologica Hungarica 29: 173–185.

Siegfried, B.D., Vaughn, T.T., Spencer, T. (2005): Baseline susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) toCry3Bb1 *Bacillus thuringiensis* toxin. Journal of Economic Entomology 98: 1320–1324.

Stilmant, D. (1995): Population dynamics of cereal leaf beetles, *Oulema melanopus* L. and *O. lichenis* Voet (Coleoptera: Chrysomelidae), on wheat fields in southern Belgium. Belgian Journal of Zoology 125: 199-205.

Sutter, G.R. (1969): Treatment of Corn Rootworm Larvae and Adults with *Bacillus thuringiensis* and *B. popilliae*. Journal of Economic Entomology 62(3): 756–757.

Tabashnik, B.E., Gould, F. (2012): Delaying corn rootworm resistance to Bt corn. Journal of Economic Entomology 105: 767–776.

Takov, D., Draganova, S., Toshova, T. (2013): Gregarine and *Beauveria bassiana* infections of the grey corn weevil *Tanymecus dilaticollis* (Coleoptera: Curculionidae). Acta Phytopatologica et Entomologica Hungarica 48: 309–319.

Toader, M., Georgescu, E., Ionescu A.M. (2017): Testing of some insecticides allowed in organic farming against *Tanymecus dilaticollis* attack of maize crops. Scientific Papers. Series A. Agronomy 60: 419-424.

Toader, M., Georgescu, E., Ionescu, A.M., Şonea, C. (2020): Test of some insecticides for *Tanymecus dilaticollis* Gyll. control, in organic agriculture conditions. Romanian Biotechnological Letters 25(6): 2070-2078.

Toepfer, S., Kuhlmann, U. (2004): Survey for natural enemies of the invasive alien chrysomelid, *Diabrotica virgifera virgifera*, in Central Europe. BioControl 49: 385–395.

Toepfer, S., Gueldenzoph, C., Ehlers, R.-U., Kuhlmann, U. (2005): Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. Bulletin of Entomological Research 95 (5): 473–482.

Toepfer, S., Peters, A., Ehlers, R.-U., Kuhlmann, U. (2008): Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. Journal of Applied Entomology 132: 337–348.

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

Toepfer, S., Kurtz, B., Kuhlmann, U. (2010): Influence of soil on the efficacy of entomopathogenic nematodes in reducing *Diabrotica virgifera virgifera* in maize. Journal of Pest Science 83(3): 257–264.

Toshova, T.B., Velchev, D.I., Abaev, V.D., Subchev, M.A., Atanasova, D.Y., Tóth, M. (2017): Detection and monitoring of *Diabrotica virgifera virgifera* LeConte, 1858 (Coleoptera: Chrysomelidae) by KLP+ traps with dual (pheromone and floral) lures in Bulgaria. Acta Zoologica Bulgarica, Supplement 9: 247–254.

Toshova, T., Velchev, D., Pilarska, D., Todorov, I., Draganova S., Holuša, J., Takov, D. (2021): Effect of bioinsecticides on the grey maize weevil *Tanymecus dilaticollis*. Plant Protection Science 57 (3): 240-247.

Toshova, T., Velchev, D., Barta, M., Takov, D., Todorov, I., Pilarska, D., Tóth, M., Berkov, S., Nikolova, M. (2022): Insecticide activity of Greek oregano essential oil and entomopathogenic fungus *Metarhizium pemphigi* against *Diabrotica virgifera virgifera* LeConte. Cereal Research Communications, 50: 1045–1054.

Toth, S., Szalai, M., Kiss, J., Toepfer, S. (2020): Missing temporal effects of soil insecticides and entomopathogenic nematodes in reducing the maize pest *Diabrotica virgifera virgifera*. Journal of Pest Science 93: 767–781.

Van der Burgt, W.A.C.M., Budai, C.S., Lucskai, A., Fodor, A. (1998): Parasitism of western corn rootworm adults by *Steinernema* spp. and *Heterorhabditis* spp. Proceedings of the Society for Experimental and Applied Entomology 9: 165–170.

Voinescu, I. (1985): Maize seed treatment with carbamic insecticides, effective method of *T. dilaticollis* Gyll control. Problems of Plant Protection 13 (2):151-156.

Wesseler, J., Fall, El H. (2010): Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe – the "no control" Scenario. Journal of Applied Entomology 134 (5): 385–394.

Yıldırım, H. (2021): The first record of *Vairimorpha hostounsky* sp. nov. Infection in the blue shieldbug, *Zicrona caerulea* Linnaeus, 1758 (Hemiptera: Pentatomidae). Microbial Pathogenesis 155: 104883.

Yin, Y., Flasinski, S., Moar, W., Bowen, D., Chay, C., Milligan, J., Kouadio, J.L., Pan, A., Werner, B., Buckman, K., Zhang, J., Mueller, G., Preftakes, C., Hibbard, B.E., Price, P., Roberts, J. (2020): A new *Bacillus thuringiensis* protein for Western corn rootworm control. PLoS ONE 15 (11): e0242791.

Zottele, M., Mayerhofer, J., Embleton, H., Wechselberger, K., Enkerli, J., Strasser, H. (2021): Biological *Diabrotica* management and monitoring of *Metarhizium* Diversity in Austrian maize fields following mass application of the entomopathogen *Metarhizium brunneum*. Applied Sciences 11 (20): 9445.