

Biological Control of Coleopteran Pests

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Abstract

Coleopteran pests having the modified forewings which serves as protective covers for the membranous hind wings and commonly called as beetle. Most of them are important pest of agriculture, forestry, fruit trees and stored grain while only a few species are considered to be beneficial. Man has probably been plagued by insect pests ever since he began to grow crops and rear animals. Worsened by the ability of these pests to emerge and re-emerge, man, his crops, livestock as well as his socio-economic status stand on the threshold of destruction. Challenged by this danger and frustrated by the demerits of chemical pest control, man began to explore the use of identified natural enemies as agents of bio control to manage potentially damaging population below levels that they can cause economic injury. Biologically we can control the coleopteran pests with the help of several predators, parasitoids and entomopathogens, ants, mites and mammals. Tree creepers, chickadees, nuthatches, flycatchers, swallows, tanagers, finches, thrushes, jays, wrens, kinglets, and warblers are the predatory birds that feed on the different species of beetles. *Diabrotica* sp., *Solenopsis geminate* and *Pheidole megacephala* are the predatory ants that removed 80% of exposed *Diabrotica* eggs. *Iponemus* sp., *Pyemotes giganticus* and *Pymotes dryas* are the predatory mites that are used in controlling the pine and bark beetles. Similarly, entomopathogens are also play an important role in controlling the coleopteran pests and it includes fungi, viruses, bacteria and nematodes.

Keywords: Beetles, Entomopathogens, predator, bacteria, fungi, virus

1. Introduction

The name Coleoptera, derived from the Greek words “*koleos*” meaning sheath and “*ptera*” meaning wings given to the group by Aristotle, refers to the modified front wings which serve as protective covers for the membranous hind wings. Coleopterans also called beetles and beetles are the flying insects belonging to the order ‘Coleoptera’ which are represented by 3, 60,000 species and 178 families worldwide and constitute the largest order of insects. It includes 40% of all insects and nearly 30% of all animal species. It means, about one third of the described animal species may be beetles. Thus, it is considered to be the largest collection of living organisms and believed to be in existence since 270 million years ago. Beetle family is very diverse and wide. Found in almost every part of the world, they are the most common insects thriving in a varied range of habitats except polar and marine regions. The food habits also differ in each and every species, depending on their habitat. Some feed on plants and leaves, whereas some are carnivorous; they catch insects or invertebrates to eat. There are also other types like beetles feeding on fungus, pollens, crustacean and other insects. Most of them are important pest of

agriculture, forestry, fruit trees and horticulture, while only a few species are considered to be beneficial.

2. Classification

Coleoptera is divided into four sub-orders; Adephaga, Archostemata, Polyphaga and Myxophaga. But only two of these, Adephaga and Polyphaga include common families.

2.1. Adephaga

It is the first sub-order and carnivorous in nature. The most abundant families in this order are the Carabidae and Cicindelidae which includes the ground beetle and tiger beetle, respectively. The carabidae live on the soil surface, ground or ant nests and plants; they feed on insects, spiders, or mollusks. Most adults have phototaxis and hip glands of adults can release defensive substances such as formic acid or *p*-benzoquinone. Some of them are phytophagous, damaging cereals, strawberries and potatoes.

2.2. Archostemata

It is the second sub-order which lives under bark. It is the smallest sub-order placed in the Coleoptera order that has only five families with less than fifty identified species.



The family included in this sub-order are Crowsoniellidae, micromalthidae, Ommatidae, Cupedidae and Jurodidae.

2.3. Polyphaga

It is the largest sub-order, containing 85% of known species, many of them are phytophagous. It consists mainly of the families Scarabaeidae, Curculionidae, Elateridae, Bruchidae, Chrysomelidae, Tenebrionidae, Cerambycidae, Buprestidae, Coccinellidae and Canthridae. The major damage caused by members of these families is as follows: some Scarabaeidae and Chrysomelidae are critical pests in agriculture and forestry; some Elateridae live under the soil and are important pests of crops; some Buprestidae and Cerambycidae eat wood and cause the loss of precious timber.

2.4. Myxophaga

Myxophaga sub-order is the second smallest group of Coleoptera order, first being Archostemata. This sub-order consists of just four families comprising 65 species of tiny beetles. The beetles of this sub-order are either semi-aquatic or aquatic in nature and associated with hygropetric habitats, drift material, or interstitial habitats among sand grains.

3. Economic Importance

Many beetles are regarded as major pests of agricultural plants and stored products. They attack all parts of living plants as well as processed fibers, grains, and wood products. Scavengers and wood boring beetles are useful as decomposers and recyclers of organic nutrients. Predatory species, such as lady beetles, are important biological control agents of aphids and scale insects. The major damage caused by members of these families is as follows: some Scarabaeidae and Chrysomelidae are critical pests in agriculture and forestry; some Elateridae live under the soil and are important pests of crops; some Buprestidae and Cerambycidae eat wood and cause the loss of precious timber.

In addition, some Bruchidae endanger the growth of the leguminous plants. Longhorn beetles are the most dangerous pest, they are phytophagous and most of them damage woody plants, including pine, cypress, willow, elm, citrus, apples, peaches and tea. Some coleopterans damage crops such as cotton, wheat, corn, sorghum, sugarcane and hemp, while others damage wood, construction, housing and furniture. They are important pest in forestry fields, crop cultivation and construction timber. In 1957, people found that the longhorn beetles destroyed lead sheets of telephone cables, resulting in the exposure of internal wires. They are also harmful to timber and dry goods. Books stored in bookstore were invaded by a variety of long horn beetle larvae and then dug a tunnel through five books. Therefore, the damage done by long horn beetle is very severe, especially for fruit trees and forests, and they are an extremely serious threat to human possessions.

In the 1990s, it was reported that species *Echinocnemus squameus* (Coleoptera: Anthribidae) from the sub-order

Rhyncophora (now in family Curculionidae) seriously damaged rapeseed, wheat and cotton crops and is one of the major pests in paddy fields (Tan, 1992). The adults ruins leaves and the larvae ruins roots, causing serious hazards. Some individuals of Anthribidae are poisonous to the rice weevil, *Sitophilus granaries* (Coleoptera: Curculionidae) is another agricultural pest that damage a wide variety of crops ranging from cotton, hemp and cereals to sugar beet, melon, corn, peanut, soybean, sunflower, sorghum, tobacco and seedlings of fruit trees. The adults feed on the cotyledons, shoots and leaves of cotton and other newly sprouted seedlings. These insects often cluster together while they eat the leaves and bite tops off of stems; this clustering results in a circle or semi-circle of notches on one leaf.

4. Biological Control of Coleopteran Pests

Biological control may be defined as the action of predators, parasitoids, pathogens and antagonists or competitor populations to suppress a pest population, making it less abundant and less damaging than it would otherwise be (Eilenberg et al., 2001). Biological control of coleopteran pests is a method that uses an organism to deal with pests. It includes means such as the use of predators, parasitoids and pathogens (it includes bacteria, fungi, viruses and nematodes) that can reduce coleopteran pest populations. Its greatest advantage is that it does not pollute the environment.

4.1. Predators

Predators are characterized as follows:

- They are usually larger than their prey;
- They may be predaceous in either the immature stage, the adult stage, or both;
- They eat many prey during the course of their life;
- They are generally fairly mobile so that they can find or catch their prey; and
- Most of the species tend to have a fairly broad host range.

The main predators of coleopteran insects are birds, ants, mites, predatory insects and mammals.

4.1.1. Birds

Birds belong to the class Aves. Birds are one of the most important biological control agents. Birds in 11 families have been recorded as preying on beetles, primarily during the short period of beetle emergence, flight, and attack on trees. However, since any mortality agent that acts on the pre-ovipositing female beetle is proportionally more important than one which acts on any of the earlier brood stages, the "in-flight" mortality affected by these birds is probably significant (Blackman, 1931).

4.1.1.1. Woodpackers

Woodpeckers belong to the family picidae and are thought to be one of the most important biotic mortality agents of bark beetles (Dahsten, 1982). They have been most studied as predators of species of *Dendroctonus*, especially of *D.*



rufipennis (Kirby) (spruce beetle), *D. brevicornis* (LeConte) (western pine beetle), *D. ponderosae* (Hopkins) (mountain pine beetle) and *D. frontalis* (Zimmermann) (southern pine beetle). Woodpeckers, by their mode of searching for bark beetle and other subcortical prey on infested trees, create the most conspicuous evidence of predation. All the outer bark and sometimes even the inner bark may be stripped from infested portions of bark beetle infested trees. Besides consuming bark beetle brood, woodpeckers also indirectly kill the insects by dislodging them from the tree and reducing the survival rate of insects remaining on the tree (desiccation, increased parasitism and predation) (Otvos, 1979).

A number of recommendations have been made with regard to enhancement of woodpecker populations, the most common being the provision of adequate numbers of snags suitable as nesting sites (Evans et al., 1979). Snag management may be the cheapest option, because it involves the modification of existing forestry practices and use of existing natural nesting sites.

Woodpecker food supplies may be enhanced by girdling some trees to increase populations of secondary stem insects, by culturing and dispersing other insects attractive to woodpeckers (Koplin, 1972), and by "high-stumping" during cutting operations to provide additional feeding sites (termites and carpenter ants).

4.1.1.2. Red-winged blackbird

The Red-winged blackbird, *Agelaius phoeniceus* L. (Passeriformes: Icteridae) frequently preys on corn rootworm beetles, *Diabrotica longicornis* (Coleoptera: chrysomelidae) in maize in Canada (Bllinger and Caslick, 1985).

4.1.1.3. Perching birds

These birds Included tree creepers, chickadees, nuthatches, flycatchers, swallows, tanagers, finches, thrushes, jays, wrens, kinglets, and warblers (Baldwin, 1968; Dahlsten, 1982; Otvos, 1969; Stallcup, 1963). Theobald in 1911 listed flycatchers, finches, starlings, several different warblers and tits as being predatory on vine weevil in the UK. Being general insectivores, these birds probably also consume insect predators and parasitoids of bark beetles (Otvos, 1979) and other beetles. This impact, which has not been assessed, must be balanced against the bird's consumption of bark beetles. Thus, at this stage the total impact of bird predation is not known.

4.1.2. Predatory ants

Ants are important predators of arthropods in many habitats and geographical regions, helping to control insect pest such as *Diabrotica* sp. (Paulson and Akre, 1992). In field experiment Costa Rica, *Solenopsis geminate* (Fabricius) and *Pheidole megacephala* (Hymenoptera: Formicidae) removed 80% of exposed *Diabrotica* eggs within 3 days (Risch, 1981). As common predators in agricultural settings worldwide, ants are quick to exploit coleopteran prey. In Cuba, the myrmicine ants, *Pheidole megacephala* (Fabricius) and *Tetramorium guinense* (Nylander) have been used as biological control agents against

banana weevils (Castenieras and Ponse, 1991).

Myopopone, *Pheidole*, *Pochycondyla*, and *Monomorium* established their colonies inside corms or pseudostems of banana plants, while *Anoplolepis gracilipes*, *Camponotus* (*Tanaemyrmex*) sp., *Diacamma rugosum*, *Odontomachus rixosus*, and *Pseudolasius* sp. colonies were found in pseudostem leaf sheaths or in leaf trash at the base of mats. *Myopopone castanea* was directly observed attacking and removing banana weevil larvae from pseudostem and corm galleries.

4.1.3. Mites

Mites constitute a very diverse group of organisms, occupying a wide variety of habitats. Species in about 60 families have been recorded as associates of bark beetles and other beetles; many of these are probably incidental associations, since many mite species occupy the bark habitat of healthy trees. Mites that feed on bark beetle brood have been variously designated as parasitic (Kinn, 1971; Lindquist, 1969), predacious (Dahsten, 1982; Moser, 1975), or both (Mills, 1983).

4.1.3.1. *Iponemus* sp.

This species of mites belong to the family Tarsonemidae and order Acarina. Species of *Ips* (Lindquist, 1969), probably phoretic on all scolytids and atleast one tenebrionid beetle associate of bark beetles (Moser, 1981). *Iponemus* sp. feed and effective only on eggs of pine beetle (Moser et al., 1981).

4.1.3.2. *Pyemotes giganticus*

All life stages except the adult of bark beetles are preyed upon by this mite and feed on eggs, larvae and pupae. This mite contains venom which kills the host even though no feeding may occur (Moser et al., 1981). The impact of mite predation on bark beetle populations has been assessed only rarely. Estimation of mortality due to mite predation range from less than 1% to more than 90% (Lindquist, 1969). *P. giganticus* from western North American bark beetles as a predator of the southern pine beetle. The mite readily rode the southern pine beetle and other bark beetles.

4.1.3.3. *Pyemotes dryas*

Moser and co-workers in 1981 assessed *Pyemotes dryas* (Vitzthum) from Poland as a predator of the southern pine beetle. Although this species is phoretic on a wide range of European bark beetles that attack conifers, it was found not to be phoretic on the southern pine beetle or six other associated beetles. This mite readily consumed brood of the southern pine beetle, but to be useful as a biological control agent.

4.1.3.4. *Pyemotes scolyti*

Pyemotes scolyti (Oudemans) using alternate hosts, has been suggested as a possible biological control agent for bark beetles (Beaver, 1967; Mills, 1983; Weiser, 1963).

4.1.3.5. *Hypoaspis* sp. and *Tetrapolypus rhynchophori*

The mite species, *Hypoaspis* sp. and *Tetrapolypus rhynchophori* Ewing (Pymotidae) have also been recorded infesting the adult

beetles of Red palm weevil (*Rhynchophorus ferrugineus*) in coconut, India (Tamil-Nadu) (Peter, 1989).

4.1.4. Predatory insects

4.1.4.1. Checkered beetles (Coleoptera: Cleridae)

The checkered beetles or clerids prey both as adults and as larvae on the adults and brood of various bark beetle species. Adult clerids are attracted by pheromones emitted by bark beetles during the attack phase (Dahsten, 1982), and so (Aggregate on trees with abundant prey). Estimates range from less than 1% in the mountain pine beetle (Schmid, 1970) to 4% in the spruce beetle (Deyrup, 1975). Clerid larvae are general predators in the bark of infested trees, and are capable of mining through the bark to find their prey. *Thanasimus formicarius* (Linnaeus), a clerid from Germany released against the southern pine beetle in the eastern United States in 1882 and 1883 failed to get established (Hopkins, 1899; Dowden, 1962).

4.1.4.2. Trogositid Beetles (Coleoptera: Trogositidae)

Trogositids prey both as adults and as larvae on the adults and brood of various bark beetle species, as well as on other bark and wood-inhabiting insects. Adult trogositids are attracted by certain bark beetle pheromones and host tree volatiles (Bedard et al., 1969), enabling them to find high prey densities (numerical response by aggregation). Larval trogositids are voracious feeders capable of tunnelling through intact bark. Cannibalism tends to regulate their numbers in infested trees (Struble, 1942).

4.1.4.3. Rhizophagid beetles (Coleoptera: Rhizophagidae)

The genus *Rhizophagus* is holarctic, most often associated with various bark beetle species. *Rhizophagus grandis* (Gyllenhal) is the most studied rhizophagid in Europe; it is considered to be an important predator of *Dendroctonus micans* (Kugelman) (Gohm et al., 1954; Karunakar et al., 1999). These predator releases were the only ones ever made in Canada against any bark beetle species.

4.1.4.4. Long-legged flies (Diptera: Dolichopodidae)

Many species of the genus *Medetera* has been reported as predators of bark beetles in North America, Europe and Asia (Kolomietz and Bogdanova, 1980; Dahsten, 1982; Mills, 1983). Adult flies of *Medetera* vary feed on small live insects on the surface of the bark of trees (De Leon, 1935). Fly larvae feed on eggs and larvae of bark beetle. All life stages of *Medetera* are preyed upon by other organisms. Larvae of *Medetera* sp., predacious on *Dendroctonus micans* in Russia, brought into the United States in 1978 for tests against *Dendroctonus* spp. died in quarantine (Coulson, 1981).

4.1.4.5. Lonchaeid flies (Diptera: Lonchaeidae)

Bedard in 1938 considered *Lonchae corticis* Taylor to be the most important predator of the Douglas-fir beetle, *D. pseudotsugae*, since he found *L. corticis* to be more abundant than *Medetera*, while Kline and Rudinsky (Kline

and Rudinsky, 1964) consider *L. corticis* larvae to be more scavengers than predators in Douglas-fir beetle galleries. *Lonchaea furnissi* McAlpine, the most studied North American species, associated with the Douglasfir beetle, was found to be a scavenger (Johnsey et al., 1965). In the European and Asian literature various species of *Lonchaea* are considered to be predatory on bark beetle broods. Larvae of *Lonchaea* spp., predacious on Scolytidae (general), *D. micans* and on *Blastophagus piniperda* L. Scolytidae) in Russia, which were brought into the United States in 1978 and 1979 for tests against *Dendroctonus* spp. either were dead on arrival or died in quarantine (Coulson, 1981).

4.1.4.6. Robber flies (Diptera: Asilidae)

Robber flies have on occasion been observed to capture and consume flying bark beetles (Mills, 1983) estimated a predation rate of 1% by *Laphria gilm* L. on an emerging population of mountain pine beetles and suggested that numbers of this predator be supplemented.

4.1.4.7. Snake flies (Neuroptera: Inocellidae and Raphidiidae)

Snakefly larvae and adults have frequently been reported as predators or associates of bark beetles, their role in bark beetle ecology is poorly documented. Wichmann in 1957 considered snakefly larvae to be general predators on the bark surface of infested trees, and thought they may do more harm than good by consuming eggs of clerids and dolichopodids, the larvae of which are better predators of bark beetles.

4.1.4.8. Flower bugs (Hemiptera: Anthocoridae)

These bugs generally are quite small they can live within the bark beetle galleries. Both the nymphs and adults feed on all stages of bark beetles, using their piercing mouthparts to suck the juices from their prey. The abundance of anthocorids has been assessed only on trees attacked by southern pine beetle, where one species was found to be the most abundant predator (Lint and Stephen, 1983) (Table 1).

4.1.4.9. Pirate bug (Hemiptera: Anthocoridae)

The warehouse pirate bug, *Xylocoris flavipes* attacked most immature stages of beetles (Jay et al., 1968). The Red rust flour beetles were suppressed by warehouse pirate bugs in a warehouse (Press et al., 1975).

4.1.5. Mammals

It includes Insectivora, Rodentia, Chiroptera-shrews, rodents and bats. Miscellaneous small mammals consume bark beetles, particularly in overwintering sites at the bases of infested trees (Mills, 1983). Three types of insectivorous mammals (hedgehogs, moles and shrews) fed on *Otio rhynchussulcatus*. He stated that hedgehogs were prolific predators and on some occasions their diets were composed entirely of vine weevil adults. Moles were also recorded as predators of *O. sulcatus* (Breakey, 1959), but they tend to prefer moister areas than vine weevil. In the USA, Smith in 1932 observed predation by skunks and deer mice.



Table 1: List of predatory insect used against the coleopteran pest

Predatory insect	Order	Family	Host	References
Checkered Beetles, <i>Thanasimus formicarius</i>	Coleoptera	Cleridae	Bark beetle, <i>Dendroctonus</i> sp. Pine beetle Spruce beetle, <i>Dendroctonus ruffipenis</i> Shot hole borer of tea, <i>Xyleborus fornicatus</i>	(Dahsten, 1982) (Schmid, 1970) (Dyer et al., 1975) (Austin, 1956)
Trogositid Beetles, <i>Temnochila chlorodia</i>	Coleoptera	Trogositidae	Bark beetle, <i>Dendroctonus</i> sp.	(Berryman, 1967)
Rhizophagid beetles, <i>Rhizophagus grandis</i>	Coleoptera	Rhizophagidae	Great spruce bark beetle, <i>D. micans</i>	(Kobakhidze et al., 1973)
<i>Paratheresia</i> sp.	Diptera	Tachinidae	Larvae of red palm weevil	(Clausen, 1978)
Robber fly	Diptera:	Asilidae	Bark beetle, <i>Dendroctonus</i> sp.	(Mills, 1983)
Lonchaeid Flies, <i>Lonchaea corticis</i>	Diptera:	Lonchaeidae	Spruce beetle, <i>D. pseudotsugae</i> , <i>D. micans</i> and on <i>Blastophagus piniperda</i>	(Kline and Rudinsky, 1964)
Snakefly	Neuroptera	Inocellidae and Raphidiida	Bark beetle, <i>Dendroctonus</i> sp.	(Wichmann, 1967)
Earwig, <i>Chelisoches morio</i>	Dermaptera	Forficulidae	Red palm weevil, <i>Rhynchophorus ferrugineus</i> eggs	Abraham et al., 1973

4.2. Parasitoids

4.2.1. Hymenoptera: Braconidae-Braconid wasps

The family Braconidae contains the greatest number of genera reported to be parasitoids of bark beetles (Bushing, 1965). The genera *Ropalophorus*, *Cosmophorus* and *Cryptoxilos* contain species which are endoparasitoids of adult bark beetles (Bushing, 1965; Mills, 1983). The remaining genera are all ectoparasitoids of bark beetle larvae and occasionally of pupae.

4.2.1.1. *Dendrosoter protuberans* and *Ecphyllus sileseacus* (Braconidae)

The family Braconidae contains the greatest number of genera reported to be parasitoids of bark beetles (Bushing, 1965). Two braconids, *Dendrosoter protuberans* (Nees) and *Ecphyllus sileseacus* (Ratzeburg), have been brought into North America against the smaller European elm bark beetle, *Scoietyus multistriatus* (Marshall). *D. protuberans* was obtained from France in 1965 and released against *S. multistriatus*, starting in 1966 (Hosteler and Btewer, 1976; Schroder, 1974).

4.2.1.2. *Spathius benefactor* (Braconidae)

A native North American braconid, *Spathius benefactor* Matthews (*S. canadensis* Ashmead if reared from elm by Peacock in 1975 parasitizes the introduced elm bark beetle.

4.2.2. Hymenoptera: Pteromalidae-Pteromalid Wasps

The family Pteromalidae contains the second-greatest number

of genera reported to be parasitoids of bark beetles Bushing, 1965. The genera *Tomicobia* and *Karpinskiella* are strictly endoparasitoids of adult bark beetles (Beda, 1965; Furniss and Carolin, 1977).

4.2.2.1. *Cheipachus quadrum*

Supposedly of European origin, has been in North America at least since 1888 (Ashmead, 1988), yet adults of this species were imported into the United States in 1964 for release against *S. multistriatus* (Schroder, 1974).

4.2.2.2. *Rhopalicus tutela*

It is the only other pteromalid which has been imported and released against bark beetles.

4.2.3. Parasitic wasp

Parasitic wasps that attack stored grain beetles tend to be host specific, but there are several species that will attack more than one beetle species. e.g. *Theocolax elegans* will attack all of the stored grain weevils and the lesser grain borer.

4.2.3.1. *Anisopteromalus calandrea* and *Lariophagus distinguendus*

This is true of the parasitic wasps and used against stored grain weevils.

4.2.3.2. *Cephalonomia waterstoni*

Cephalonomia waterstoni, which attacks the rusty grain beetle only attack a single species. These parasitoids are typically



small (1 to 2 mm), and do not feed on the grain. They will normally die within 5 to 10 days if no beetles are present in the grain. These parasitoids are found naturally in the grain, which suggests that after they are released they may continue to suppress pests for many years (Arbogast and Mullen, 1990). Because the adult wasps are external to the grain, they can be easily removed using normal grain-cleaning processes.

4.2.4. Diptera: Phoridae-hump-backed flies

The only non-hymenopterous parasitoid of bark beetles mentioned in the literature is the fly *Megaselia aletiae*

(Comstock) (Phoridae), which infests adults of the fir engraver, *Scolytus wntralis* LeConte. Parasitism apparently occurs during flight or attack, and parasitized females excavate normal galleries but lay no eggs. 1–2% of parent females were parasitized, a parasitism rate that was considered to be of minor importance (Ashraf and Berryman, 1969; Berryman, 1967) (Table 2).

4.3. Application of pathogenic microorganisms to prevent coleopteran pests

It includes the entomopathogenic bacteria, fungi, viruses and

Table 2: List of parasitoids used against different coleopteran pests

Parasitoid	Target pest	host	References
<i>Lariophagus distinguendus</i> (Larval parasitoid)	Rice weevil, <i>Sitophilus oryzae</i> , Maize weevil, <i>S. zeamais</i> Granary beetle, <i>S. granarius</i>	Rice and maize Stored grain pest	(Ryoo et al., 1991) (Brower et al., 1996)
<i>Scleroderma guani</i> (Larval parasitoid)	Small and large longicorn species of beetles	Mulberry	(Press and Mullen, 1992)
<i>Anisopteromalus calandrae</i>	Rice weevil, <i>S. oryzae</i> and Maize weevil, <i>S. zeamais</i>	Rice and Maize	(Choo et al., 2001)
<i>Cephalonomia waterstoni</i>	Rusty grain beetle, <i>Cryptolestes ferrugineus</i>	Wheat	(Flinn et al., 1996)
<i>Theocolax elegans</i>	Lesser grain borer, <i>Rhizopertha dominica</i>	Wheat grain	(Flinn et al., 1996)

nematodes.

4.3.1. Entomopathogenic bacteria

Despite the great bacterial diversity, those causing an infectious disease to insects bear a poor diversity if compared to other groups as protozoa and fungi. About 90 bacterial species cause infectious disease in insects, but only few of them have a great potential as biological control agents (Cloutier and Cloutier, 1992). Bacteria produce epizootics when there is high host density (stored product, insect colonies, fresh water populations subjected to particular physical or chemical conditions); in other circumstances epizootics are rare or are not detected (Castillo and Ponce, 1991).

4.3.1.1. *Bacillus thuriangiensis*

Bacillus thuriangiensis preparations make up the largest output of microbial pesticides in the world and are widely applied to control pests in agriculture, forestry, storage and medicine (Sun et al., 2003; Yu, 1990; Zhang and Deng, 1998). Eighty years ago, research was focused on finding strains toxic to Lepidoptera and Diptera pests. In 1983, Krieg and co-workers discovered the first *B. thuriangiensis* strain active against Coleopteran pests. Since then, new strains have been continuously discovered and much research on these strains has been carried out. One of the most harmful Coleopteran pests is *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) which cause damage to potato and tomato crops in several regions of America and Europe. This insect has developed resistance against a variety of chemical pesticides.

There are also a number of Coleopteran pests that cause serious damages to forest and reserve materials, such as grain, food and forage. There is important to conduct research on *B. thuriangiensis* preparations to control Coleopteran pests.

• Crystal protein against Coleopteran pests

The current nomenclature distinguishes 174 holotype sequences that are grouped into 55 *cry* and 2 *cyt* families. A total of 47 toxins were tested against 39 species of Coleoptera in 190 bioassays. About 80% of bioassays pertained to four *cry* families: *cry3* (32%; 26.7% for *cry3Aa* alone), *cry8* (24%), *cry1* (18.2%; 9.1% for *cry1B* alone), and *cry34/35* (8%, alone or in combination). The broadest range of toxins was tested against *Diabrotica* sp. (23 toxins) and *L. decemlineata* (16 toxins). The only toxin was tested against a broad range of test species was *Cry3Aa* (23 species, from which 60% were susceptible) (Van Frankenhuyzen, 2009).

• *Cry3A* insecticidal crystal protein

B. thuriangiensis subsp. *tenebrionis* strain BI256-82 has a single insecticidal crystal protein gene, *cry3A*, which encodes a 73.1 kDa protein. Under the effect of spore protease, the insecticidal crystal genes from *Bacillus thuringiensis* against coleopteran pest protein is degraded, forming a parasporal crystals, the crystals are solubilized in the midgut, resulting in active and toxic insecticidal peptides of 55 kDa (Krieg, 1983) (Table 3).

• *Cry3B* insecticidal crystal protein

B. thuriangiensis subsp. *tolworthi*, H9 strain EG2838 possess

Table 3: Several important insecticidal crystal proteins against coleopterans were described [68] as follows: Insecticidal crystal genes from *Bacillus thuringiensis* against coleopteran pest

cry gene	Bt strain	Target pest	References
cry3Aa	Bt subsp. <i>Tenebrionis</i> strain B1256-82	<i>L. decemlineata</i> , <i>C. scripta</i> , <i>Callosobruchus maculatus</i> , <i>Diabrotica balteata</i>	(Sekar, 1987)
cry3Bb	Bt EG4961	<i>L. decemlineata</i> , <i>Dandroctonus undecempunctata</i>	(Donavan et al., 1992)
cry3Ca	Bt <i>kurstaki</i> Btl109P	<i>L. decemlineata</i>	(Lambert et al., 1992)

the insecticidal crystal protein genes cry3B which encodes 74.2 kDa proteins. A mixture of the spores and crystals of these strains have exhibited toxicity against *L. decemlineata* larvae. The spore crystal mixture of EG4961 has demonstrated specific toxic activity against *Diabrotica undecempunctata howardi* Barber (Coleoptera: Chrysomelidae) larvae (Dowden, 1962).

- Cry3C insecticidal crystal protein

The parasporal crystal of *B. thuriengiensis* subsp. *gelleriae*, strain BTS137J, is diamond-shaped, with a molecular weight of 129.4 kDa. The active fragment of 72 kDa has shown toxic activity against *L. decemlineata*.

- Cry3D insecticidal crystal protein

The cry3D protein of *B. thuriengiensis* subsp. *kurstaki*, H3ab strain BT1109P, encoded by its corresponding gene, has higher toxicity against *L. decemlineata* than other cry3 proteins. Its LD₅₀ value is 0.7 mg ml⁻¹; this LD₅₀ value was obtained by measuring the trypsin hydrolysate activity of cry3D by the leaf dipping bioassay (Lambert et al., 1992).

- Application

Several commercial bio-insecticides, such as M-one™, M-trak™, Trident™ and Foil™, have been produced in the United States of America. These bio-insecticides, based on *san diego* and *tenebrionis* subsp. are designed for the control of leaf-feeding flea beetle species including the Colorado potato beetle (*L. decemlineata*), cottonwood leaf beetle (*C. scripta*), Elm leaf beetle *Pyrrhalta luteola* Muller (Coleoptera: Chrysomelidae) and willow leaf beetle *Plagioder versicolora* Laicharting (Coleoptera: Chrysomelidae) among others.

Among the *B. thuriengiensis* strains isolated in China, the C-001-006 and ES-017 strains exhibited different toxicity levels against *Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Anomala corpulenta* larvae. A new strain of *B. thuriengiensis*, HBF-1, isolated from Hebei province, China has high insecticidal activity against *A. exoleta* (Coleoptera: Scarabaeidae) and *A. corpulenta* larvae and the mortality on first and second instars larvae of the cinnamon beetle treated with the strain was 100%. This isolate is the first discovered and reported to have specific insecticidal activity against beetle larvae in China (Table 4).

Table 4: List *Bacillus thuriengiensis* based commercial products for the prevention of coleopteran pests

Company	Product
Mycogen	M-one
Sandoz	Trident
Abbott	Ditera
Novo Biokontrol	Novodor
Ecogen	Foil

4.3.1.2. *Bacillus papillae*

It is an effective method of preventing Japanese beetle (*Popilliae japonica*) larvae; it was found a little later than *B. thuriengiensis*. In the 1930s, the strain was first isolated by Dutky from Japanese grubs that were infected by milky disease. *B. popilliae* is a pathogen of the golden tortoise beetle larvae. It is highly efficient and environmentally friendly States microbial insecticide promoted in the United States since 1939.

B. popilliae and the infected larvae can move in a large area before death increasing the infection probability of other beetle larvae. *B. popilliae* can maintain viability for several years the soil and drought conditions. The advantages of using commercial preparations of *B. popilliae* are its narrow host range (they are effective only against Japanese beetles) and their complete safety for humans and other vertebrates.

4.3.2. Entomopathogenic fungi

Among the biological control agents, the fungi play an important role for insect control. Many pathogenic fungi are natural control agents of many insect pests and other arthropods frequently cause epizooties and significantly reduce host populations. Although entomopathogenic fungi have long been recognized to exhibit a strong potential in controlling insect pests, attempts to manipulate fungi as real control agents began in the late ninetieth century with little or moderate success. There are more than 50 different fungal products registered, with nine able to control coleopterans (Yu, 2000).

In the last 20 years, many countries have carried out extensive research to examine the use of insect pathogenic fungi. Fungal insecticides mainly include *Beauveria bassiana* and *Metarhizium anisopliae* (Table 5).



Table 5: List of important fungal species against different coleopteran pests

Fungi	Target pest	References
	Boll weevil, <i>Anthonomus grandis</i>	(Wright, 1993)
	Rice water weevil, <i>Lissorhoptrus oryzophilus</i>	(Rice and Cogburn, 1993)
	Red rust flour beetle, <i>Tribolium castaneum</i>	
	Citrus root weevil, <i>Artipus floridanus</i>	(Padin et al., 2002)
	Lesser grain borer, <i>Rhizopertha domonica</i>	(Lord, 2005)
	Scarab beetle	(Yokoyama, 1998)
<i>B. brongniartii</i>	Scarab beetle, <i>Ectinohoplia rufipes</i>	(Choo et al., 2001)
<i>Metarhizium anisopliae</i>	Rice weevil, <i>Sitophilus oryzae</i>	(Batta, 2003)
	Colorado potato beetle	
	Scarab beetle, <i>Ectinohoplia rufipes</i>	(Fujiie, 1993)
	Red Palm weevil, <i>Rhynchophorus ferrugineus</i>	(Prior and Aruma, 1985)

4.3.2.1. *Beauveria bassiana*

The genus *Beauveria* has shown virulence against insects from order Coleoptera such as beetle larvae (Pu and Li, 1996). *Beauveria* belongs to the category of fungal agents, which is the most widely used category. After more than 20 years of extensive experiments and research, its target range has been greatly expanded. It has been used to kill borers, fruit tree pests and soil insects, among others (Gan et al., 2007). In the USA, attempts were made as early as 1962 by Nutrilite products to obtain registration for *B. bassiana* as Biotrol™, but these attempts were unsuccessful. A wettable formulation of *B. bassiana* conidia, based on isolate ARSEF 252, was developed by Abbott Laboratories and used in pilot tests against the Colorado potato beetle during the 1980s.

Results from field experiments using the Abbott's wettable formulation against the Colorado potato beetle were reported, although these results differed from year to year. Although the larval populations of the first and second generations adults were lower than that found for adults from the fenvalerate treatment group.

In the former Soviet Union, commercial preparations of *B. bassiana* (Boverin™) were applied to more than 10,000 ha of farmland, mainly against the Colorado potato beetle. In Belgium, under greenhouse conditions, an 84% control of introduced larvae of black vine weevil was obtained after an application of 2×10^8 conidia of *B. brongniartii* per liter of peat (Table 6).

Table 6: Fungal-based commercial products against Coleoptera

Fungi	Product name	Target pests	Manufacturer or country
<i>Beauveria bassiana</i>	Boverin	<i>Leptinotarsa decemlineata</i>	The former Soviet Union
<i>B. bassiana</i>	Boverol	<i>L. decemlineata</i>	Czechoslovakia
<i>B. bassiana</i>	Bovero-sil	<i>L. decemlineata</i>	Czechoslovakia
<i>Metarhizium anisopliae</i>	BioPat	Coleopteran pests	America
<i>M. anisopliae</i>	BioBlas	Coleopteran pests	America
<i>M. anisopliae</i>	Biogreen	Coleopteran pests	Australia

4.3.2.2. *Metarhizium* spp.

The most common control agent is *Metarhizium anisopliae* var. *anisopliae* Sorokin. The soil-inhabiting larvae of scarab beetles are typically hosts of *Metarhizium* spp. and co-evolution has led to some isolates being specific to one or two genera of scarab.

• Application

In Australia, Biogreen™ and BioCane™ are the two registered myco-insecticides for coleoptera pests. Biogreen™ is a granular product consisting broken rice particles on which spores of *M. flavoviridae* grow. It has been used to control red-headed pasture cockchafer, *Adoryphorus couloni* (Coleoptera: Scarabaeidae), in turf and pasture in Victoria and Tasmania. Another rice granule formulation of *M. anisopliae* var. *anisopliae* isolate FI-1045 is highly virulent against the greyback canegrub, *Dermolepida albohirtum* (Coleoptera: Scarabaeidae), which is the worst single pest in Australia, causing losses over \$5 M per year. Extensive field trials over several seasons have shown that BioCane™ applied correctly at 33 kg ha^{-1} or 6.6×10^{13} conidia ha^{-1} leads to 50% to 60% control of that season's grubs.

In America, BioPat™ and Bioblast™ are two myco-insecticides registered for the control of Coleoptera. In China, spray spore suspensions of *M. anisopliae* control nearly 50% of rice water weevil population 7-9 days after application. When *M. anisopliae* was used to control the North China black Melolonthidae larvae by the Shanxi Institute of Zoology, larval mortality was increased as spore concentration increased. At spore concentrations of 2.1×10^4 , 1.9×10^5 , 2.2×10^7 , 2.0×10^7 and 1.8×10^8 cfu/ml, the mortality of larvae was 20%, 65%, 90%, 100% and 100%, respectively.

4.3.3. *Microsporidia*

First considered to be early diverging intracellular parasites



and most often grouped with the Protozoa, microsporidia now are considered to be fungi. Microsporidia, pathogenic protists related to the Fungi, are considered to be primary pathogens of many aquatic and terrestrial insect species and have important roles in insect population dynamics, managed insect disease, and biological control of insect pests.

Hosts are infected when spores are ingested and/or by transmission via the eggs. When ingested, spores germinate in a unique fashion: a polar tube that is coiled within the spore rapidly everts and punctures the host midgut cells, injecting the spore contents into the cell cytoplasm. Effects on the host are typically chronic; therefore, the use of microsporidia in biological control programs focuses on inoculative introductions, augmentative release, and conservation biology.

4.3.3.1. *Nosema scripta* (Microsporidia: Nosematidae)

It is a species of microsporidian parasite, is described from the cottonwood leaf beetle, *Chrysomela scripta* (Coleoptera: Chrysomelidae), in North America (Bauer, 1993). Both horizontal transmission and vertical transmission have been demonstrated for this microsporidium in *C. scripta* in the laboratory. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was susceptible to infection with this pathogen in cross-infectivity studies.

4.3.3.2. *N. epilachnae* and *N. varivestis*

N. epilachnae and *N. varivestis* are the North American species used as biological control

Against Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae) (Brooks et al., 1985) (Table 7).

Table 7: List of microsporidia used against different coleopteran pests

Microsporidia	Host	References
<i>Nosema whitei</i>	Red rust flour beetle, <i>Tribolium castaneum</i>	(Khan and Selman, 1988)
<i>N. scripta</i>	Cotton wood leaf beetle, <i>Chrysomela scripta</i> and <i>Leptinotarsa decemlineata</i> , bean leaf beetle, <i>Ceratoma trifurcata</i>	(Bauer, 1993)
<i>N. epilachnae</i> and <i>N. varivestis</i>	Mexican bean beetle, <i>Epilachna varivestis</i>	(Brooks, 1995)

4.3.4. Entomopathogenic nematodes

These nematodes search for their host insects based on the chemical stimulation of the host itself or of other substances. The smell of the excrement of the host or the carbon dioxide generated by its respiration may lure the nematodes in the infection period (Gaugler, 1981) (Table 8).

Entomopathogenic nematodes in the family's Steinernematidae and Heterorhabditidae of the order Rhabdida are effective control agents of dozens of insect species in soil and cryptic habitats (Georgis and Gaugler, 1991; Kaya and Gaugler, 1993; Koppenhofer et al., 2000). In India two recorded nematode species are *Praecocilenchus ferruginophorus* and *P.*

Table 8: List of nematodes

Nematode species	Host	References
<i>Steinernema scarabaei</i>	White grubs, <i>Exomala orientalis</i>	(Stock and Koppenhofer, 2003)
<i>Heterorhabditis bacteriophora</i>	White grubs, Oriental beetle, <i>Exomala orientalis</i>	(Koppenhofer and Fuzy, 2003)
<i>Steinernema kushidai</i>	June beetle, <i>Polyphyla decemlineata</i> Popillia japonica	(Mamiya, 1989) (Artyukhovskiy, 1967)

rhabdidiophorus used against red palm weevil (*Rhynchophorus ferrugineus*) in coconut. *P. ferruginophorus* was recovered from *R. ferrugineus* in India (Rao and Reddy, 1980).

Sugarcane white grub, *Holotrichia serrata* was found susceptible to EPNs (Karunaker et al., 1999; Shankamarayan et al., 2006). It was biologically controlled by *Heterorhabditis indica* isolate from India and other countries (Stack et al., 2000). Research on biological control of white grubs in sugarcane using indigenous EPNs strain was carried out at SBI, Coimbatore under an international collaborative research project involving leading research laboratories from Ireland and Germany.

• Application

In China, a parasitic nematode was used to control *Actinidia chinensis*, which parasitizes *Ephedra equisetina*. The larval mortality reached 94.4% to 100% in 4-6 days after application. In the forest, each warm hole contained 10,000 infective nematodes, leading to greater than 90% control. *Steinernema* was used to control *Anomala germari*, which occurs on the poplar. Each hole was injected with 6,000 *Steinernema* and the mortality was about 82.1% (Liu, 1993). Banana plants were treated with a *S. carpocapsae* preparation to control the black weevil, and the larval mortality was between 73% and 90%, the mortality of pupa was between 68% and 92%, and the mortality of adult was between 25 and 80%.

Entomopathogenic nematodes are a new biological control factor with greater variation. More than 2,000 strains have been reported and experts dedicated to the transgenesis and molecular biology of nematodes hope to produce heat-resistant, anti-drought nematode strains in order to expand their production and application. Therefore, these will play a greater role in bio-ecological agricultural pest control systems.



4.3.5. Viruses

Viruses are considered excellent candidates for insect pest control, mostly because they are highly specific, as they show no negative effect on plant, domestic animal, birds, fish or non-target insect. Viruses, as many of their infections cause lethal diseases to susceptible individual and therefore they can be important biotic factors to keep insect pest population densities under control.

4.3.5.1. *Oryctes virus*

In 1966, Huger placed the *Oryctes virus* in a new genus and named it *Rhabdionvirus oryctes* like *Baculovirus oryctes* under *Nudibaculovirinae*. However, this pathogen has recently been removed from the *Baculoviridae* and placed under its own virus category-*Oryctes* (Evans and Shapiro, 1997). Further studies on the virus structure strengthened its inclusion under *Baculoviridae* and it was named *Baculovirus oryctes* (David, 1975; Payne, 1974).

The coconut is an important plantation crop in India. The rhinoceros beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) is a serious pest of coconut throughout India and Southeast Asia, causing an estimated 10% yield loss in the crop. Successful biological control of this pest could be achieved using the non-occluded *Oryctes virus* (syn. *Baculovirus oryctes* or *Oryctes baculovirus*) (Evans and Shapiro, 1997).

Oryctes virus also used as biological control against the other species of rhinoceros beetle like *Oryctes nasicornis* (Huger, 1966), *O. boa* (Julia and Mariau, 1976), *O. monoceros* (Purrini, 1989), Taro beetle, *Papuana uninodis* (Zelazny et al., 1998), Brown rhinoceros beetle *Xylotrupes Gideon* (Danger et al., 1994), Mango seed weevil, *Sternochetus mangiferae* (Shukla et al., 1984). Cashew stem and root Borer, *Ploceaderus obesus* and *P. ferrugineus*.

4.3.5.2. *Cytoplasmic polyhedrosis virus*

There is one record in the literature of viruses being recovered from palm weevils. In 1990 Gopinadhan and co-workers found a highly potent cytoplasmic polyhedrosis virus (CPV) specific to red palm weevil in Kerala, India. The virus infected all stages of the insect and laboratory infection of the late larval stages resulted in the development of malformed adults.

4.3.5.3. *Application of entomopathogenic virus*

A practical method of virus dissemination is through the release of virus-infected adults (Bedford, 1981). An inoculum is prepared by homogenizing 1 g infected larval tissue in 1 litre of phosphate buffer (0.05 M, pH 8.0) and 3% sucrose, which amounts to 31.6 LD₅₀ dose (Mohan et al., 1989). Healthy beetles collected from the field are allowed to wade through virus inoculums contained in a basin for 30 min. The beetles can also be inoculated with the sterile viral filtrate prepared as described by Zelazny and co workers in 1987.

After this swim treatment beetles are confined together

for 12 to 24 hrs, and released after dusk at the rate of 15 inoculated beetles per hectare (Mohan et al., 1989). This method eliminates the disadvantages of other methods of application, where a compost heap or a split coconut log heap is prepared artificially and inoculated with virus killed larval tissues (Bedford, 1981). The infected beetles disperse widely before death, spreading the disease directly into the wild population, and contaminating breeding sites which may contain larval broods and other beetles, including the palm crowns (Zelazny, 1976).

5. Conclusion

Researches and applications of biological control of coleopteran pests have made a great progress in recent decades. Major commercial products are based on *Bacillus thuringiensis*, *Paenibacillus popilliae*, *Metarhizium anisopliae* and *Baeuveria bassiana*. Entomopathogenic nematodes show good efficacy toward the soil inhabiting insect pests. Parasitoids and predators will be useful elements of a strategic approach to control the coleopteran pests. Overall, there is good evidence that accelerated exploration of biological control options may provide the advances in coleopteran pest management.

6. References

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