

FUNDAMENTALS OF BIOLOGICAL CONTROL OF PESTS

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Abstract: Biological control is the use of non-chemical and environmentally friendly methods of controlling insect pests and diseases by the action of natural control agents. In recent decades, the increase use of biological control is due to its safety, species specific and long-term action on the target pests. Unlike chemical method, which kills non target species, cause detrimental health effects to human beings and pollute environment. This paper is designed to cover major aspects of biological control of pests with emphasis on different strategies employed for any successful control program in farms, green houses, garden and forests. These includes, Conservation biological control, importation other wise called Classical biological control; involves the introduction of non-native biocontrol agent into a new area or country for permanent and long-term pests control. In addition, Augmentation biological control is the supplemental release of natural enemy either by inundation or inoculation. The former involves release of large number of natural enemies for immediate reduction of pest populations, the latter, involves release of small amount at an interval throughout the pest period. However, the most common biological control agents used in the pest control are Predators, parasitoid and microbial antagonists with emphasis on entomopathogenic nematodes, fungi, *Bacillus thuringiensis* (B.t) and Baculovirus. They are manipulated and applied for various integrated pest control programs in Agriculture. Unfortunately, the primary factors affecting adoption of biological control are efficacy, predictability and high cost.

Key words: biological control, biocontrol strategies, natural enemies/biocontrol agents.

Introduction

Biological control is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of natural enemies. It relies on predation, parasitism, herbivory, or other natural mechanisms, but typically also involves an active human management role (J. Brodeur et al. 2013). According to S. H. Dreistadt, 2007 Biological control is the beneficial action of predators, parasites, pathogens, and competitors in controlling pests and their damage. Biocontrol provided by these living organisms (collectively called natural enemies) is especially important for reducing the numbers of pest insects and mites. Biological control efforts against plants and insects have different histories, with insect biological control being used for much of its first century largely against crop pests. Only in the 1990s did insect biological control against environmental pests develop as an independent goal (Van Driesche, 2008).

Biological control has been actively practiced for more than 100 years and the history of biocontrol, its failures and successes, has been extensively reviewed. Interest in biological control has increased over recent decades for many reasons (Bailey et al., 2009). First, a greater appreciation for environmental stewardship among regulators, growers, and the public has promoted

development of more sustainable farming practices. Second, a number of arthropod pests have developed

resistance to one or more pesticides leaving growers to search for alternative management strategies (McCaffery, 1998). Finally, consumers increasingly demand products that are grown in a sustainable manner and are free of insecticide residue (Dabbert et al., 2004). Despite this, growers have been slow to adopt biological control as part of their pest management program. For example, biological control is practiced in just 5% of the estimated 741,290 acres of greenhouses world-wide (van Lenteren, 2000). The primary factors affecting adoption of biological control are efficacy, predictability, and cost (Van Driesche and Heinz, 2004).

Basically there are three types of biological control strategies applied in pests control programs. These are **Importation** (sometimes called classical biological control), **Augmentation** and **Conservation**. Classical biocontrol is defined; as the intentional introduction of an exotic (nonnative), usually co-evolved biological control agent for permanent establishment and long-term pest control (Van Driesche, 2008). This is usually done by government authorities. In many instances the complex of natural enemies associated with a pest may be inadequate, a situation that can occur when a pest is accidentally introduced into a new

geographic area, without its associated natural enemies. In recent years, classical biological control has come under increasing scrutiny for its nontarget effects (Coryand Myers, 2000). On the other hand, augmentation involves; the supplemental release of natural enemies, boosting the naturally occurring population. Relatively few natural enemies may be released at a critical time of the season (inoculative release) or millions may be released (inundative release). An example of inoculative release occurs in greenhouse production of several crops. The conservation of existing natural enemies in an environment is the third method of biological pest control. Natural enemies are already adapted to the habitat and to the target pest, and their conservation can be simple and cost-effective, through vegetation manipulation, Classical Biological Control can provide control of both primary and secondary pests, while reducing the likelihood of pest outbreaks and resurgences (Naranjo and Ellsworth, 2009, Godfray et al., 2010).

Similarly, natural enemies of insect pests, (biological control agents) include the following; predators, parasitoids, and pathogens. Biological control agents of plant diseases are most often referred to as antagonists, where as Biological control agents of weeds include herbivores and plant pathogens. **Predators** are mainly free-living species that directly consume a large number of preys during their whole lifetime. A **parasite** is an organism that lives and feeds in or on a larger host. Insect parasites (more precisely called parasitoids) are smaller than their host and develop inside, or attach to the outside, of the host's body (S. H. Dreistadt, 2007). Pathogenic micro-organisms include bacteria, fungi, and viruses. They kill or debilitate their host and are relatively host-specific. Populations of some aphids, caterpillars, mites, and other invertebrates are sometimes drastically reduced by naturally occurring pathogens, usually under conditions such as prolonged high humidity or dense pest populations (S. H. Dreistadt, 2007).

Development of Biological Control

In 16th century Chinese were the first to use natural enemies to control insect pests were nests of the ant *Oecophylla smaragdina* were sold near Canton in the third century, for use in control of citrus insect pest such as *Tesseratoma papillosa*- Lepidoptera. Date growers in Yemen went to North Africa to collect colonies of predaceous ants which they colonized in date groves to control various pests (R. G. and T. S. Bellows, Jr. 1996). Interest in biological control has increased over recent decades for many reasons (Bailey et al., 2009). First, a greater appreciation for environmental stewardship among regulators, growers, and the public has promoted development of more sustainable farming practices

(Kogan, 1998). Second, a number of arthropod pests have developed resistance to one or more pesticides leaving growers to search for alternative management strategies. Finally, consumers increasingly demand products that are grown in a sustainable manner and are free of insecticide residue (Dabbert et al., 2004).

Following the Green Revolution in the second half of the 20th Century, biological control has resurfaced with renewed force in recent years, especially by the adoption of integrated pest management (IPM) programs. These programs were implemented as a consequence of the indiscriminate use of agrochemicals, which led to a number of problems, such as insect and mite resistance to insecticides and acaricides, as well as environmental contamination (Parra et al. 2002, Guillon 2008).

Unfortunately, growers have been slow to adopt biological control as part of their pest management program. For example, biological control is practiced in just 5% of the estimated 741,290 acres of greenhouses worldwide. The primary factors affecting adoption of biological control are efficacy, predictability, and cost (Parrella et al., 1992; Van Driesche and Heinz, 2004). However, for biological control to be fully utilized, it is essential for the user to have insects available whenever they are required, in order to use them in inoculative (classical biological control), inundative (applied biological control), or seasonal inoculative releases (biological control in protected crops) (van Lenteren 2000) to control the target pest.

Biological Control Strategies

As mentioned early in my introduction, there are 3 basics strategies in biological control of pests, these are; Classical Biological Control (Importation), Augmentation and Conservation. The aim is to highlight the concept of each strategy as it's used in integrated pest control programs.

Classical Biological Control (Importation)

Classical biological control is the importation of pest natural enemies from other countries, to a new locale where they do not occur naturally. It is the international introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long term pest control (Pickrell 2004). The goal of classical biological control is to find useful natural enemies, introduce them into the area of the target pest, and permanently establish them so that they will provide continuing pest control with little or no additional human intervention. The search for natural enemies in other countries is often referred to as foreign exploration. The process of importation involves;

- Determining the origin of the introduced pest

- Collecting appropriate natural enemies associated with the pest or closely related species.
- Selected natural enemies are then passed through a rigorous assessment, testing and **quarantine** process, to ensure that they will work and that no unwanted organisms (such as hyper parasitoids) are introduced.
- Mass production and release of selected natural enemies.
- Follow-up studies are conducted to determine if the natural enemy becomes successfully established at the site of release, and to assess the long-term benefit of its presence (<http://edis.ifas.ufl.edu>, 2013).

Historically, the first attempt by man at classical biological control of an arthropod pest was a spectacular success. The cottony cushion scale (*Icerya purchasi* Maskell) program in California over the period 1877-1879 was the first scientifically and institutionally backed biological control program. Foreign exploration by Arthur Koebele resulted in the importation and release of two natural enemies, the vedalia beetle (*Rodolia cardinalis* [Mulsant]) and a parasitic fly (*Cryptochaetum iceryae* [Williston]) from Australia for cottony cushion scale control in California. The combined impact of these two natural enemies drove cottony cushion scale densities to almost undetectable levels and by saving the young citrus industry from imminent destruction, put California on an economic trajectory towards unprecedented wealth and prosperity (M.S. Hoddle, 2003).

In recent years, classical biological control has come under increasing scrutiny for its nontarget effects (Cory and Myers, 2000; Hawkins and Marino, 1997; Howarth, 1991). However, there are many examples of successful biological control (Bellows, 2001), and the need for biological control is increasing (Cory and Myers, 2000). Lastly, Classical biological control is a powerful tool for suppression of invasive plants and insects in natural ecosystems. It will play an increasingly important part in ecological restoration because; it provides a means to permanently suppress invaders over large landscapes without long-term resource commitments and hence is sustainable. As such, it merits use against many invasive plants and insects that are environmental pests in sensitive landscapes (Morin et al., 2009).

Augmentation Biological Control

Augmentation is the periodic release of a natural enemy that does not occur naturally in sufficient numbers to keep a pest below damaging levels. It's also defined as the release of additional numbers of a natural enemy when too few are present to control a pest

effectively (van Lenteren 2000). The practice of augmentation is based on the knowledge or assumption that in some situations there are not adequate numbers or species of natural enemies to provide optimal biological control, but that the numbers can be increased (and control improved) by releases (<http://entomology.ifas.ufl.edu>). This relies on an ability to mass-produce large numbers of the natural enemy in a laboratory or by companies to produce and sell them. There are two general approaches to augmentation: **inundative releases** and **inoculative releases**.

Inundative Releases

Inundation involves releasing large numbers of natural enemies for immediate reduction of a damaging or near damaging pest population. It is a *corrective* measure; the expected outcome is immediate pest control. The inundative approach is achieved by flooding the crop with multiple releases of insectary-reared natural enemies. The released insects control pests present at the time, but there is little expectation that later generations will persist at sufficient levels to provide control (<http://www.entomology.wisc.edu>). In practice, releases are often repeated if pest populations were not all present in a susceptible stage during the previous application, if new pests disperse into the crop, or if the crop is long lived, increasing the length of time it could become infested (Eilenberg *et al.*, 2001).

Moreover, Inundative release of natural enemies is undertaken; causing effects similar to the use of conventional insecticides, as there is a knockdown effect of the target host population. Therefore, it may be used in the field and in greenhouse as seasonal release (Cohen 2004, Schneider 2009). However, because of the nature of natural enemy activity, and the cost of purchasing them, this approach using predaceous and parasitic insects is recommended only in certain situations, such as the mass release of the egg parasite *Trichogramma* for controlling the eggs of various types of moths. *Trichogramma evenescen* Westwood, *T. brassicae* Bezdenko, *T. cacoeciae* Marchal, and *T. dendrolimi* Matsumura are sold in Europe to control lepidopterans in greenhouses, *Ostrinia nubilalis* Hubner in corn, and other Lepidopterans in orchards (van Lenteren 2003). The utilization of some microbial insecticides (such as those containing *Bacillus thuringiensis*) is also inundation.

Inoculative Releases

Inoculation on the other hand; involves releasing small numbers of natural enemies at prescribed intervals throughout the pest period, starting when the pest population is very low. The natural enemies are expected to reproduce themselves to provide more long-term

control. However, the expected outcome of inoculative releases is to keep the pest at low numbers, never allowing it to approach an economic injury level; therefore, it is more of a preventive measure (<http://www.entomology.wisc.edu>).

The separation of inoculation from inundation is clear. A release with the expectation that the released organism will control the target after multiplication is inoculation. In glasshouses, the early release of parasitoids and predators, often with alternative food sources, is inoculation biological control. Examples of this are the releases of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and other natural enemies, now commonly practiced in glasshouses (Eilenberg et al., 2000; van Lenteren, 2000). The number of insects released is insufficient to control the pest insects, and success depends on the ability of the released organisms to multiply and reduce the target population. At the end of the season, the glasshouse is emptied, and no permanent establishment of the biological control organisms is achieved. When the next generation of plants is grown in the glasshouse, the predators and parasitoids should be released again. This type of release or application to control pest insects is strongly dependent on population regulation and density dependent processes.

Conservation Biological Control

Conservation biological control is defined as modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effect of pests. Habitat manipulation often involves increasing the species diversity and structural complexity of agro ecosystems. Habitat manipulation approaches provide natural enemies with resources such as nectar, pollen, physical refugia, alternative prey and alternative hosts and operate to reduce pest densities via an enhancement of natural enemies (<http://www.rkmp.co.in>).

However, although conservation biological control often increases natural enemy abundance, reduced pest abundance or increased yield has rarely been demonstrated (Johnson et al., 2008). For example, flowering strips and other shelter habitats, as conservation biological control tactics, increase predation, parasitism, or yield in some cases but not others (Pfiffner and Wyss, 2004 and Griffiths et al., 2008). In addition to natural enemies, conservation biological control tactics, such as habitat manipulation, attract and sustain a diverse suite of herbivores, detritivores, and plant provided foods (Landis et al., 2000; Frank and Shrewsbury, 2004).

Research has been done on myriad arthropod pests, including species with high levels of insecticide resistance such as *Chilo suppressalis* (Lepidoptera:

Crambidae) and *Helicoverpa armigera* -Lepidoptera: Noctuidae (Cory S. Straub et al, 2007). As an example of conservation biological control, alternative habitats for natural enemies are provided, in the form of 'beetle-banks' in Britain or 'sown seed strips' in Switzerland in cereal crops. These practices are highly successful and are among the few documented uptakes of a biological control option in temperate open-field arable agriculture (Landis et al., 2000).

Biological Control Agents

Biological control agents or natural enemies are organisms such as Insects or plants disease that are use to control pest species. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens. Biological control agents of plant diseases are most often referred to as antagonists. Biological control agents of weeds include herbivores and plant pathogens.

Predators

Predatory insects are beneficial because they feed directly on other insects like aphids. Common predatory insects include lacewings, ladybugs, and praying mantids. Ladybugs or lady beetles (*Coleomegilla maculata*) have been recognized by many cultures for their predatory behaviors for centuries. Adults of these insect predators are some of the most widely recognized insects in the United States. As early as the late 1800s, lady beetles were being used in biological control programs in the United States (<http://edis.ifas.ufl.edu>). Adult and larvae feed on large numbers of small, soft-bodied insects such as aphids but they will also eat other small, soft-bodied insect larvae, insect eggs, and mites (www.gardeningsolutions.ifas.ufl.edu). Example of predatory insect commonly found in garden is Green lacewing (*Chrysoperla carnea* Stephens) the adult, primarily feed on nectar and other fluids, but some species also consumes a few small insects. The larvae sometimes called aphid's lions; are voracious predators capable of feeding on small caterpillars and beetles, as well as aphids and other insects. They possess excellent searching qualities, exhibit high dispersal ability. Predatory stink bug, *Euthyrhynchus floridanus* (Linnaeus), is considered a beneficial insect because most of its prey consists of plant-damaging bugs, beetles, and caterpillars. Nymphs of the Florida predatory stink bug, *Euthyrhynchus floridanus* (Linnaeus) feeding on an earwig (Anthony Ditro, 2013).

Parasitoids

The term parasite is frequently used for insects that parasitize other insects. Parasites are usually much

smaller than their host and have a shorter life cycle than their host. Usually they do not kill their host. Examples include tapeworms and ticks (<http://insects.about.com>). While a **parasitoid** is an organism that spends a significant portion of its life history attached to or within a single host organism in a relationship that is in essence parasitic. However, unlike a true parasite, it ultimately sterilizes or kills, and sometimes consumes, the host. When the parasitoid completes its life cycle, it becomes a free-living insect, no longer dependent on the host (Consoli, 2010). Parasitoids may comprise up to 25% of all insects (*Parasitoids*, Nick Mills, University of California, and Berkeley). Most parasitoids belong to the *Hymenoptera* or *Diptera* orders. Parasitoid attacked different life stages of pest, i.e. egg parasitoid.

Similarly, Egg parasitoids, by definition, are parasitoids that both attack and complete their development within a host eggs. They may be either solitary or gregarious, but in all cases prevent the host egg from hatching and use only a single host individual to complete their development. This distinguishes true egg parasitoids from other guilds of parasitic Hymenoptera such as egg-pre-pupal parasitoids that attack the host egg but delay development to kill the host just before pupation and egg predators that consume multiple eggs within an ovisac or egg pod (van Lenteren 2003). Example of egg parasitoid is *Trichogramma Spp.* It's used to control Corn borer (*Ostrinia spp*) in the farm, especially in developed countries like China and USA.

Dennise Medina and Arellys Rodriguez, 2010 categorized parasitoids with respect to its effect on host as; **Idiobiont** parasitoids are those that prevent further development of the host after initially immobilizing it, and, almost without exception, develop outside the host. **Koinobiont** parasitoids allow the host to continue its development while feeding upon it, and may parasitize any host life stage. In turn, Koinobiont can be subdivided further into **endoparasitoids**; which develop inside body of the host, and **ecto- parasitoids**; which develop outside the host body, though the parasitoids frequently are attached or embedded in the host's tissues.

Pathogens

Pathogens are microorganisms including certain bacteria, fungi, nematodes, protozoa, and viruses that can infect and kill the host. Populations of some aphids, caterpillars, mites, and other invertebrate are sometimes drastically reduced by naturally occurring pathogens, usually under conditions such as prolonged high humidity or dense pest populations. In addition to naturally occurring disease outbreaks, some beneficial pathogens are commercially available as biological or microbial pesticides. These include *Bacillus thuringiensis* or B.t, entomopathogenic nematodes, and granulosis viruses (S. H. Dreistadt, 2007). The application of microorganisms

for control of insect pests was proposed by notable early pioneers in invertebrate pathology such as Agostino Bassi, Louis Pasteur, and Elie Metchnikoff (L. A. Lacey et al., 2001).

Naturally occurring entomopathogens are important regulatory factors in insect populations. Many species are employed as biological control agents of insect pests in row and glasshouse crops, orchards, ornamentals, range, turf and lawn, stored products, and forestry and for abatement of pest and vector insects of veterinary and medical importance. The advantages of use of microbial control agents are numerous. These include safety for humans and other nontarget organisms, reduction of pesticide residues in food, preservation of other natural enemies, and increased biodiversity in managed ecosystems (R.R. Sharma, et al., 2009).

Similarly, microbial control agents can be effective and serve as alternatives to broad-spectrum chemical insecticides. However, their increased utilization will require-, increased pathogen virulence and speed of kill improved pathogen performance under challenging environmental conditions (cool weather, dry conditions, etc.); greater efficiency in their production; improvements in formulation that enable ease of application, increased environmental persistence, and longer shelf life; better understanding of how they will fit into integrated systems and their interaction with the environment and other integrated pest management (IPM) components; greater appreciation of their environmental advantages; and acceptance by growers and the general public (L. A. Lacey et al., 2001).

Entomopathogenic Nematodes

Nematodes are simple roundworms, colorless, unsegmented, and lacking appendages. They may be free-living, predaceous, or parasitic. Many of the parasitic species cause important diseases of plants, animals, and humans. Other species are beneficial in attacking insect pests, mostly sterilizing or otherwise debilitating their hosts (Stuart, R. J. et al., 2006). Entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* have been used to suppress populations of insects pest in a variety of agro ecosystems, and in several cases their positive effects on crop yield have been shown (Mráček, 2002; Georgis et al., 2006).

However, entomopathogenic nematodes (EPN) are mass produced for use as biopesticides using *in vivo* or *in vitro* methods (Shapiro-Ilan, et al., 2010). *In vivo* production (culture in live insect hosts) requires a low level of technology, has low startup costs, and resulting nematode quality is generally high, yet cost efficiency is low. *In vivo* production may be improved through innovations in mechanization and streamlining. A novel alternative approach to *in vivo* methodology is

production and application of nematodes in infected host cadavers; the cadavers (with nematodes developing inside) are distributed directly to the target site and pest suppression is subsequently achieved by the infective juveniles that emerge. *In vitro* solid culture, i.e., growing the nematodes on crumbled polyurethane foam, offers an intermediate level of technology and costs. *In vitro* liquid culture is the most cost efficient production method but requires the largest startup capital. Liquid culture may be improved through progress in media development, nematode recovery, and bioreactor design.

Furthermore, entomopathogenic nematodes are extraordinarily lethal to many important insect pests, yet are safe for plants and animals. This high degree of safety means that unlike chemicals, or even *B.t.*, nematode applications do not require masks or other safety equipment; and re-entry time, residues, groundwater contamination, chemical trespass, and pollinators are not issues. Most biological control agents require days or weeks to kill, yet nematodes, working with their symbiotic bacteria, can kill insects within 24-48 hours. Dozens of different insect pests are susceptible to infection, yet no adverse effects have been shown against beneficial insects or other non targets in field studies (Akhurst and Smith, 2002).

Lastly, it has been reported that the beneficial entomopathogenic nematodes including *Steinernema carpocapsae* and *Heterorhabditis indica* were effective in infecting and killing soil-dwelling larval (Yan et al., 2013) and possibly pupal stages of the striped flea beetle, *Phyllotreta striolata* and helping to reduce the emergence of future generation adult flea beetles from pupae. Similarly, the efficacy of EPNs against the cucumber fly, *Dacus ciliatus* Loew (Diptera: Tephritidae) using high virulence native strain of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) and a commercial strain of *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae). The efficacy of *S. carpocapsae* and *H. bacteriophora* was higher at 25 and 30°C than at 19°C. The results indicated that *S. carpocapsae* had the best potential as a biocontrol agent of *D. ciliatus*, based on its higher virulence and better ability to locate the fly larvae within infected fruits (Shokoofeh Kamali, et al. 2013).

Entomopathogenic Fungi

Entomopathogenic fungi act as parasites of insects – these fungi can kill, or seriously disable insect pests. Fungal entomopathogens are important regulators of insect populations with considerable potential as mycopesticides. Only recently, however, have fungal entomopathogens been shown to occur as endophytes, both naturally and in response to various inoculation methods. The ecological function of endophytic fungal remains largely unknown, but some studies have

implicated them in plant growth, herbivore resistance, and disease resistance. It is estimated that 750 to over 800 (Thackar, 2002) fungal species from more than 90 genera have been described as pathogenic against different insect species. But only a dozen of entomopathogenic fungus species are available for pest management at grower level (Amir Cheraghi, et al, 2013).

Similarly, the biological plant protection with entomopathogenic fungi has key role in sustainable pest management program. Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in environment, and increased biodiversity in human managed ecosystems (Lacey et al., 2001). Fungal biocontrol agents have unique mode of infection. In contrast to bacteria and viruses, they do not need to be ingested and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding mesh like eggs (Ujian and Shahzad, 2007; Anand and Tiwary, 2009) and pupae of insects (Nguyen et al., 2007; Anand et al., 2008).

Moreover, Fungal biological control agents have demonstrated efficacy against a wide range of insect pests including Spodoptera species (Purwar and Sachan, 2005; Lin et al., 2007; Amer et al., 2008). Application of entomopathogenic fungi against termites has the minimum negative impact on the environment. There have been a number of studies evaluating the efficacy of the hypocrealean Hyphomycete *Beauveria bassiana* (Bals.) *vuillemin*, against subterranean termites. Similarly, *Ascomycete, Metarhizium anisopliae* (Metsch) *Sorokin*, present in the soil also acting as a causal agent for green muscardine of insects, is an important pathogen for the biological control of pests. Sharma R. R., et al., 2009 reported several post harvest diseases of fruits and vegetables can now be controlled by microbial antagonists. The basic approaches are; the use of microorganisms which already exist on the produce itself, which can be promoted and managed, or those that can be artificially introduced against postharvest pathogens. Different microbial antagonists like *Debaryomyces hansenii* Lodder & Krejer-van Rij, *Cryptococcus laurentii* Kufferath & Skinner, *Bacillus subtilis* (Ehrenberg) Cohn, and *Trichoderma harzianum* Rifai, are being used.

Lastly, the use of symbiotic fungal endophytes as biological control agents against fungal pathogens in cereals was first described in timothy (*Phleum pratense* L.) where plants containing the *Epichloa typhina* endophyte (E+) were less susceptible to disease by the fungus *Cladosporium phlei* (C.T. Greg.) G.A. de Vries, 1952 than E-plants (Karen A. O'Hanlon, et al., 2012). Elsewhere, Tian et al. (2008) carried out a study to investigate the effect of *Neotyphodium lolii* against 10 pathogens, including five *fusarium* species, on detached leaves and/or living plants of perennial ryegrass. Overall,

the endophyte appeared to have inhibitory effects against pathogens on leaves and intact plants, however, the extent of inhibition varied with the specific pathogen tested.

Bacillus thuringiensis (B.t)

Bacillus thuringiensis (B.t) is a spore forming bacterium that produces crystals protein (cry proteins), which are toxic to many species of insects. *Bacillus thuringiensis*, or simply *B.t*, is a naturally occurring soil bacterium that, when sprayed on plants, is toxic to certain pest insects. For years, farmers and home gardeners have used *B.t* as a microbial spray pesticide to control caterpillars, certain types of beetles, as well as mosquitoes and black flies (<http://www.bt.ucsd.edu>).

However, *B.t* occurs commonly in soils, and most insecticidal strains have been isolated from soil samples. Bacterial insecticides must be eaten by target insects to be effective; they are not contact poisons. Insecticidal products composed of a single *Bacillus* species or subspecies may be active against an entire order of insects, or they may be effective against only one or a few species. For example, products containing *Bacillus thuringiensis* var. *kurstaki* kill the caterpillar stage of a wide array of butterflies and moths. In contrast, *Bacillus popilliae* var. *popilliae* (milky disease) kills Japanese beetle larvae but is not effective against the closely related annual white grubs (masked chafers) that infest lawns in much of the Midwest. Indeed, commercial insecticides derived from this bacterium have a long history of successful use in the biocontrol of insect pests (Bravo et al., 2011; Federici, 2005; al., 2011; Sanchis, 2011), in agriculture (Navon, 2000; Sanchis and Bourguet, 2008; Sauka and Benintende, 2008) and forestry (van Frankenhuyzen, 2000), and disease vectors (Becker, 2000). *B.t* Cry toxins constitute the active ingredient in the most widely used biological insecticides and insect-resistant transgenic crops (Cannon, 2000; Ferré et al., 2008; James, 2010; Shelton et al., 2002).

However, Antagonistic bacteria such as *Bacillus licheniformis* (Govender et al., 2005), *Pseudomonas fluorescens* (Vivekananthan et al., 2004) and *Bacillus* spp. (Jager et al., 2001) or fungi such as *Trichoderma harzianum* or yeast such as *Saccharomyces cerevisiae* (Vivekananthan et al., 2004) and *Rhodotorula minuta* (Patinovera et al., 2005) have been found effective for the control of the anthracnose of mango under controlled research conditions. Another group of *B.t* isolates, including those from *Bacillus thuringiensis* var. *sandiego* and *Bacillus thuringiensis* var. *tenebrionis* are toxic to certain beetles. Within the order Coleoptera, species exhibit great differences in susceptibility to these isolates, presumably because of differences in the receptor sites in the gut wall of the insects where the bacterial toxins must attach (Weinzerl, R. and T. Henn, 2013).

Moreover, *B. thuringiensis* subsp. *israelensis* de Barjac (Bti) is used exclusively or in combination with other interventions for the control of larvae of dozens of species of medically important and pestiferous black flies and mosquitoes around the world (Lacey and Undeen, 1986; Skovmand et al., 2000). A prime example of the successful use of Bti occurred in the onchocerciasis control program in West Africa. High levels of resistance to the organophosphate insecticides that were originally employed for control of the Simulium vectors of onchocerciasis were threatening the future of the program (Lacey et al., 2001).

Baculoviruses

Baculoviruses are a large group of double-stranded DNA viruses (almost 1000 species have been described); the majority have been isolated from a few insect orders: Lepidoptera, Diptera, Hymenoptera and Coleoptera. Viral genome ranges in size from 80 to 200 kb. Individual baculoviruses usually have a narrow host range limited to a few closely related species. The most widely studied baculovirus is the *Autographa californica* Nucleopolyhedron-virus (AcMNPV). Baculovirus insecticides have been used in a wide range of situations from forests and fields to food stores and greenhouses (Kost et al., 2005).

Additionally, these viruses are excellent candidates for species-specific, narrow spectrum insecticidal applications. They have been shown to have no negative impacts on plants, mammals, birds, fish, or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall IPM program, or when an ecologically sensitive area is being treated (www.biocontrol.entomology.cornell.edu).

However, Baculoviruses have several advantages over conventional insecticides that make them highly acceptable control agents. Probably the most important is their specificity. They have narrow host ranges (sometimes limited to one or two species), and do not infect beneficial insects, making them very suitable for use in integrated control programs. Most also possess the capacity to persist in the environment, which can be utilized in the development of more ecologically based long-term control programs. At present, commercial production of baculoviruses has been carried out only in vivo, either by applying the virus against the host insect in the field and collecting diseased or dead larvae, or by producing the target insect in the laboratory on an artificial diet. The latter is the most commonly used method for producing baculoviruses in many countries but both methods have been used successfully for commercial production of the *Anticarsia gemmatalis* baculovirus (AgMNPV) in Brazil (Moscardi, 2007)

Similarly, the activity of baculoviruses against their natural hosts may be enhanced by introduction of insect-specific toxins or by interference with insect physiology (Inceoglu et al., 2001). Most of the research was devoted to the studies of arthropod toxin genes isolated from the scorpion or spiders (Inceoglu et al., 2007). The most potent insect-specific toxin gene used for construction of baculovirus recombinants was the gene coding for a toxin from scorpion *Androctonus australis*. The feeding damage caused by larvae infected with this modified baculovirus was reduced by about 60% in comparison to a wild type baculovirus (Inceoglu et al., 2001).

Conclusion

Biological control of pest is the use of pathogens, predator and parasitoid to kills pests by reducing their populations or eliminating them completely from our farms, garden and forest, their by increasing productivity and safety of the consumers and environments. In recent years, microbial antagonists are used for the control of pest and diseases. Typical example abounds on the *Bacillus thuringiensis* which are toxic to many species of insects. In addition, Entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* have been used to suppress populations of pest insects in a variety of agro ecosystems, and in several cases their positive effects on crop yield have been shown. Lastly, the advantages of use of microbial control agents include safety for humans and other no target organisms, reduction of pesticide residues in food, preservation of other natural enemies, and increased biodiversity in managed ecosystems.

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