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Chapter

Use of Parasitoids as a Biocontrol Agent in the Neotropical Region: Challenges and Potential

Yelitza C. Colmenarez, Natália Corniani, Simone Mundstock Jahnke, Marcus Vinicius Sampaio and Carlos Vásquez

Abstract

The agricultural production in the Neotropical region is highly affected by the attack of pests and diseases. Due to the overuse of pesticides, sustainable methods of control are in demand, such as biological control. Integrated Pest Management (IPM) considered the use of Biological control as a method to suppress the population of pests in several field agricultural systems and in protected crops systems. Biological control is generally appreciated today as an important component of IPM, and the demand for it is likely to spread as the IPM programs develop worldwide. The tropics present an important region for the application of biological control. The Neotropical region is characterized by its rich biodiversity, resulting in a wide range of natural enemies of pests represented by parasitoids, predators, and pathogens. Parasitoids are the natural enemies most used around the world for biological control. In this chapter, we present biological control programs using parasitoids established in the Neotropical region to control key pests of economic importance. Agricultural practices that maintain and enhance the action of natural enemies in crops will be reviewed, as the challenges and potential for the establishment of Biological Control programs using parasitoids in the Neotropical region.

Keywords: integrated pest management, sustainable agriculture, biological control, parasitoids, agricultural pests

1. Introduction

The agricultural production in the Neotropical region is highly affected by the attack of pests and diseases. Due to the overuse of pesticides, sustainable pest control methods are in demand, within the context of Integrated Pest Management (IPM) [1]. Biological control is a tool used in Integrated Pest Management (IPM) for several field agricultural systems and in protected crops systems [2]. Biological control, the use of living organisms as pest control agents, has enjoyed varying popularity in the past, but today is well established as an important component of IPM. Biological control is the most environmentally safe and economically
profitable pest management method, when considering all the different factors together and its benefits to them.

The Neotropical region is characterized by its rich biodiversity, providing the opportunity to use a wide range of natural enemies of pests represented by parasitoids, predators, and pathogens [3, 4].

Dozens of species of insect predators and parasitoids are reared worldwide, and in some instances, these programs have been shown to be economically competitive with alternative methods of control. According to [5] in 2010, no less than 230 species of invertebrate natural enemies—originating from 10 taxonomic groups—were used in pest management worldwide. From this, within the arthropods, 52.2% were represented by parasitoids of the Hymenoptera group. Parasitoids can be used as biological control agents against insect pests in agro-ecosystems. Today, parasitoids are the most used natural enemies for classical biocontrol around the world, and many success cases have been reported in many countries of the Neotropical Region [2].

The term parasitoid defines the behavior of host use that exists only in insects (Table 1). Most of the parasitoids hosts are other insects and the parasitoids could be the same size as the host. Parasitoids can develop on or within their host, and parasitoids larvae kill their hosts to complete their life cycle from egg to adult and only need to feed on a single host to reach adulthood. The adult form has a free life. For killing their hosts, the parasitoids are the most effective natural enemies for pest biological control [2].

As an important aspect, the host imposes certain restrictions on the development of the parasitoids. In addition to this, the physiology and behavior of the host while it lives are in benefit of the parasitoid that develops, and when necessary, it can control them. As a result, the parasitoid has the opportunity to regulate host physiology [6]. Several parasitoids exhibit predatory adult behavior [7], but this does not alter the evolutionary interrelationships between the developing parasitoid and the host. Thus, the developmental duration of a parasitoid as carnivorous or saprophyte is continuous in some species, while Trichogramma rapidly kills the host and feeds on the preserved tissues [8]. Another interesting case is represented by the endoparasitoid from the Braconidae family, Microplitis croceipes (Cresson), which completes its development and emerges leaving the living host although reproductively dead [9].

There are different types of parasitoids:

**Primary parasitoid**: Species that develops on nonparasitized hosts.

**Hyperparasitoid** (or secondary parasitoid): Parasitoid that develops in another parasitoid (it is a parasitoid of a parasitoid). There may be several levels of hyperparasitism.

**Endoparasitoid**: Parasitoid that develops inside the body of the host. The endoparasitoid can be solitary (when a single larva completes its development in a given host) or gregarious (when several larvae develop to maturity in a single host).

<table>
<thead>
<tr>
<th>Specialized in choice of host</th>
<th>Tend to be smaller than the host</th>
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<tr>
<td>Only the female searches for the host to lay eggs</td>
<td>Parasitoids develop on only one host individual during the immature stages</td>
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<tr>
<td>Eggs are usually laid in, on, or near the host</td>
<td>The immature stages remain on or in the host and almost always kill the host</td>
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Table 1. **Characteristics of parasitoids.**
**Ectoparasitoid**: Species that develops outside the body of the host (they feed by inserting the buccal parts through the integument of the host). Like the endoparasitoids, there are solitary and gregarious endoparasitoids.

**Multiple parasitism**: Situation in which more than one species of parasitoids occur within or on a single host. In many cases, only one individual survives, others succumb. In rare cases, as species of *Trichogramma* (parasitoids of lepidoptera eggs), more than one species can complete its development in the egg.

**Superparasitism**: In this case, several individuals of a species of parasitoids can develop in a host. When superparasitism occurs with solitary endoparasites, mutual destruction of the physiological suppression of larvae or surplus eggs may result in survival of a dominant individual. In some cases, however, the host dies prematurely, before the surplus is eliminated, and all die.

**Adelphoparasitism**: Also called autoparasitism, case in which a parasitoid species is a parasite of itself. For example, in *Coccophagus scutellaris* (Dalman), the male is obligatory parasitoid of the female.

**Kleptoparasitism**: In this case, a parasitoid preferentially attacks hosts that are already parasitized by other species. The kleptoparasitoid is not a hyperparasitoid. A kleptoparasitoid depends on another parasitoid to increase its reproductive success. It can act by opportunities, that is, using the oviposition holes or search trails made by another parasitoid species to lead it to the host.

**Heteromes**: The male and female are parasitoids of different hosts.

**Polyembryony**: The adult places a single egg per host, which later divides into many cells, each one developing independently. Several embryos are formed from a parasitized egg. It is common in Encyrtidae and Braconidae. The parasitic nematode *Ageniaspis citricola* of the citrus miner, *Phyllocnistis citrella*, produces 2–10 individuals per parasitized egg.

There are parasitoids of eggs, larvae (or nymphs), pupae, and adults.

Different types of biological control can be found: natural, conservation, inoculative (=classical), and augmentative biological control.

a. Natural biological control is the reduction of pest organisms by their natural enemies. It takes place in all of the world’s ecosystems without any human intervention and, in economic terms, is one of the biggest contributions of biological control to agriculture and sustainable ecosystems [10].


c. Inoculative biological control, the natural enemies are collected in an area where they are present (usually the area of origin of the pest) and then released in new areas where the pest was accidentally introduced. The aim is that the offspring of the released natural enemies increases the populations in significant way in order to cause the suppression of pest populations during many subsequent years. This type of biological control has been used most frequently against introduced pests, which are presumed to have arrived in a new area without their natural enemies.

d. Classical biological control, it was the first type of biological control practiced widely [12]. This type of biological control implies the introduction of a natural enemy, which is from an exotic origin to control a pest, which usually is exotic. There are very successful cases as a result of the establishment of a classical biological control Program worldwide.
e. In augmentative biological control, natural enemies are mass reared in bio-factories for release in large numbers in order to obtain an immediate control of pests. In some areas of agriculture, such as fruit orchards, maize, cotton, sugarcane, soybean, vineyards, and greenhouses, it has been considered to be an environmentally and economically sound successful alternative to chemical pest control [13].

In some countries, natural, conservation, and inoculative biological control are generally implemented using public funding, whereas augmentative biological control is often a commercial activity due to the need mass production and large scale regular releases of natural enemies.

Inoculative biological control is predicted to be applied on 350 million hectares worldwide [14], and over the last 120 years, 165 pest species have been brought under long-term control [15]. According to [15], 170 species of invertebrate natural enemies are mass produced and sold for release in augmentative biological control of approximately 100 pest species on about 0.4% of land under cultivation.

In this chapter, we present biological control programs established in the Neotropical region to control pests of economic importance such as *Diatraea saccharalis* (Lepidoptera: Crambidae), *Diaphorina citri* (Hemiptera: Psyllidae), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae), and *Aulacaspis yasumatsui* (Hemiptera: Diaspididae). Agricultural practices that maintain and enhance the action of natural enemies in crops will be reviewed, as the challenges and potential for the establishment of Biological Control programs using parasitoids in the Neotropical region.

2. Examples of successful biocontrol programs with parasitoids in the Neotropical Region

2.1 *Diatraea saccharalis* (Lepidoptera: Crambidae)

The sugarcane borer, *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), is considered the most important sugarcane pest in the western hemisphere [16]. It is an insect pest of great economic importance in the sugarcane crop, due to the serious damages caused by its attack, which contributes to significant reductions in productivity and industrial use [17].

In Brazil, a very large program has been operating for 40 years to control the sugar cane borer with the larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a braconid originally from Japan [18]. This is the most efficient biological program in Brazil, and it is among the best in the world. *C. flavipes* is released using inundative application in more than 3.3 million ha each year [19] (Figure 1). Another natural enemy used for this pest is the egg parasitoid *Trichogramma galloi*. In 2010, *T. galloi* was also used on 500,000 ha of sugarcane to control the eggs of the sugar cane borer [20].

In Colombia, sugar cane is the second most valuable crop. More than half of the surface area of the Cauca River Valley is planted to sugarcane, and the sugar cane borer has long been the principal pest causing high annual losses [21].

Efforts to improve biological control of *Diatraea* in Colombia began in the early 1970s with releases of *Trichogramma* spp. parasitoids (Hymenoptera: }
Trichogrammatidae), followed by *Cotesia flavipes* that proved unsuited to conditions in the Cauca River Valley [22, 23]. Despite the introduction of several different geographic strains of *Cotesia flavipes* and repeated mass releases, few parasitoid cocoons were found in surveys, and it is not considered to be permanently established in the region [21].

Later, it was reported that only *Trichogramma exiguum* Pinto & Platner was recovered from eggs of three primary *Diatraea* species and the augmented species, *Trichogramma pretiosum* Riley, was not [23]. The development of integrated pest management (IPM) programs that incorporated the economic impact of the pests, their population dynamics, improved sampling procedures, and alternative methods of control such as use of the native egg parasitoids *T. exiguum* contributed to improved management of stem borers and reductions in their economic impact in Colombia [24].

### 2.2 *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae)

The hibiscus mealybug (HMB), *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), was first detected in the Caribbean on the island of Grenada in 1993 [25], after which it rapidly spread through countries in the Caribbean, becoming one of the most important pest species [26].

Following the limited success of physical and chemical measures to control the pest populations, regional biological control programs for *M. hirsutus* were initiated in 1996. These have been joint efforts involving national programs with assistance from regional and international organizations like FAO, CARDI, CABI Bioscience, MCA, the USDA Animal and Plant Health Inspection Service (APHIS), and INRA [27].

Two parasitoids have been introduced and released against HMB, and these are *Anagyrus kamali* Moursi imported from China and Hawaii and *Gyranusoidea indica* Shafee, Alam, and Agarwal (Hymenoptera: Encyrtidae) imported from Egypt [28]. A third parasitoid, *A. dactylopii*, was also considered but was not introduced [26]. On some Caribbean islands, inundative releases of *Cryptolaemus montrouzieri* Mulsant were employed to provide supplemental control of heavy mealybug populations until parasitoids could be established [28].
The biological control program against this pest has been a tremendous success and may open the doors for further cooperative projects in that domain, and some of the experiences gained may be directly applied to finding solutions to problems caused by future invasive pests.

2.3 *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae)

The citrus blackfly (CBF) *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae) is assumed to be a native of Southern Asia [2]. The first report of *A. woglumi* in the Western Hemisphere was made by Ashby in Jamaica in 1915 [29]. From here, the citrus blackfly invaded other countries and now is widely distributed in the Western Hemisphere.

Damage caused by the CBF results from feeding, particularly on new growth of the host plants and from excretion of honeydew on which sooty-mold fungus develops. Badly infested leaves wither and drop-off as a result of injury caused by extraction of the cell sap. Such defoliation weakens the tree, interferes with its normal development and fruiting, and makes it unsightly [30].

CBF has several natural enemies. According to the many authors’ reports, the most effective agents for controlling CBF are the parasitic wasps, *Encarsia perplexa* Huang & Polaszek (Hymenoptera: Aphelinidae) Polaszek (= *E. opulenta* Silvestri, Misidentified), and *Amitus hesperidum* Silvestri (Hymenoptera: Platygastridae) [31]. In Central America and Caribbean islands, the biocontrol of CBF is an important success case. In all the infested countries, the introduction of these biological control agents resulted in rapid and effective control of the CBF [32, 33].

In the 1990s, there was a resurgence of CBF as a serious problem in several countries in the Caribbean including Dominica, Guyana, French Guyana, St Kitts and Nevis, and Trinidad and Tobago [34]. In response to the threat posed by the pest, a classical biological control program was set up [26, 35] and the introduction of *A. hesperidum* in combination with *E. perplexa* appeared to be the best strategy for management of the pest [34].

2.4 *Diaphorina citri* (Hemiptera: Lividae)

*Diaphorina citri* (Kuwayama) is a global pest of citrus that transmits the bacteria *Candidatus liberibacter* spp. associated with the disease Huanglongbing (HLB) or greening. HLB is widespread in almost all citrus-producing regions except the Mediterranean [36]. Infection of groves results in complete loss of productive capacity within 4 years, and young citrus trees never produce [37].

According to [38] cited by [36], natural enemies play an essential role in regulating the population of *D. citri* in the field, and the elimination of these control agents by intensive use of insecticides can increase the spread of the disease.

Two parasitoid species associated with the citrus psyllid are *Tamarixia radiata* (Hymenoptera: Eulophidae), an idiobiont ectoparasitoid, and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), an endoparasitoid [36]. *T. radiata* is believed to be more efficient than *D. aligarhensis* in controlling *D. citri* [36]. The wasp has provided excellent control of the psyllid on Reunion Island and good results on the islands of Guadeloupe and Puerto Rico [39, 40], Mexico [41], and Brazil [42], although its performance in Florida has been mediocre [38].

*T. radiata* has been reported in Brazil, Puerto Rico, Venezuela, Argentina, Colombia, Cuba, Mexico, and in the state of Texas, USA, without the need for a previous introduction [36].
2.5 Paracoccus marginatus (Hemiptera: Pseudococcidae)

Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Pseudococcidae), popularly known as papaya mealybug (PMB), is a small hemipteran found to attack several genera of host plants and causes damage to many economically important ornamental and crop plants [43]. The PMB is native to Mexico and/or Central and North America [44, 45] and was first described in 1992 [46]. Since its first description, PMB has spread to several Caribbean islands and Central and South America countries [44, 45, 47].

PMB infestations are typically observed as clusters of cotton-like masses on the above-ground portion of host plants and cause damages to various parts including the leaves, stems, flowers, and fruits. The insect sucks the sap by inserting its stylets into the epidermis of the leaf, fruit, and stem. While feeding, it injects a toxic substance into the leaves resulting in curling, crinkling, rosetting, twisting, and general leaf distortion. Heavy infestations are capable of rendering fruit inedible due to the buildup of thick white wax [48, 49].

Mealybugs are difficult to control because they live in protected areas such as cracks, crevices and under the bark of their host plants. Besides that, most of the stages are covered with thick waxy that difficult its control with conventional insecticides [48, 49].

Biological control was identified as a key component in the PMB integrated management [50]. In addition to predators, several parasitoids may attack PMB. A total of 22 natural enemies either occurring naturally or introduced were reported on PMB in different countries, and Acerophagus papayae Noyes and Schauff (Encyritidea) is considered as one of the most efficient parasitoids for the suppression of PMB in its native range [51] (Figure 2).

2.6 Phyllocnistis citrella (Lepidoptera: Gracillariidae)

The citrus leafminer (CLM), Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae), is a pest native of Eastern and Southern Asia that since 1993 invaded all citrus-growing regions in America and the Mediterranean basin. The female moth lays its eggs on developing leaves, and the larvae form serpentine mines on the upper and lower surfaces of the leaves, sometimes even on the fruit. The CLM attacks and causes problems mainly in young trees, nurseries, and

Figure 2.
a) Acerophagus papayae during parasitism process and b) Acerophagus papayae with its empty mummy with exit hole (Photos: Y elitza Colmenarez).
overgraftings [52]. Damages caused by the CLM include loss of photosynthetic capacity and stunting and malformation of leaves. In addition to the direct damage, the larval mining may facilitate the incidence of the citrus canker disease caused by *Xanthomonas axonopodis pv. citri*. Loss of access to international markets due to phytosanitary controls is a major economic impact related to leafminer damage [49].

The first attempt to control CLM in the tropics was using broad spectrum insecticides, but chemical control appeared to be a costly and short-term solution ([53, 54] in [52]).

According to [52], native parasitoids, in some environments, have been able to control CLM population, that is, *Galeopsomyia fausta* Lasalle, in Mexico, Central, and South America. In USA and Brazil, for example, the effort was also made in Classical Biological Control programs with the introduction of exotic parasitoids. In USA, an endoparasitic wasp of Asian origin, *Ageniaspis citricola* Logviniskaya (Hymenoptera: Eulophidae) was imported from Australia and released in Florida during 1994 and 1995 [55]. The population of *Ageniaspis citricola* quickly established and dispersed throughout the state, reaching parasitism levels near 100% in some areas [56].

In Barbados, *Ageniaspis citricola* was introduced in 2003–2004 from Florida and was released in the orchards around the island. Initial releases were conducted without taking into account the need of pruning to ensure new leaves and the presence of early larval stages (Figure 3). The biological control program was successful after the corrections of the initial problems. Currently, it is proving an excellent control of the pest. Another local parasitoid was found and identified as *Cirrospilus* sp. (Head of the Entomology Department Barbados Ministry of Agriculture Mr. Ian Gibbs).

Many native CLM parasitoids were identified in citrus groves in Brazil [57]. However, based on the parasitism potential of *Ageniaspis citricola* and the successful biological control of CLM achieved in other countries, it was decided to introduce this parasitoid in 1998 [58] (Figure 4). *Ageniaspis citricola* was soon established in several Brazilian states and it became the most common CLM parasitoid [59, 60].

2.7 *Aulacaspis yasumatsui* (Hemiptera: Diaspididae)

Cycads, commonly called “sago palms”, are highly desired by landscapers and homeowners because they are long-lived, require low maintenance, and are resistant to most pests [61]. However, many cycad species are facing extinction.
in the wild due to insect pests [62]. The cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), is one of the serious pest of cycads [63]. CAS was first described from species collected in Thailand [64]. The first report of CAS on cycads outside Thailand was in 1996 in Florida where it infested ornamental plants [65]; this pest was also reported in Mexico [66], Guam [67], the Cayman Islands, Puerto Rico, the Vieques Islands, and the Hawaiian Islands in the Americas [64]. This pest produces dense populations on the leaves, fruits, and trunk, resulting in premature death of leaves which can reduce plant longevity, as well as reducing its ornamental value. Many plants throughout Florida and the Caribbean have died as a result of this pest [61].

Chemical control can be expensive and provide inconsistent results [68]. Classical biological control of CAS began in 1998 when a parasitic wasp, *Coccobius fulvus* (Compere and Annecke), and a predatory beetle, *Cybocephalus nipponicus* Endrödy-Younga, were imported from Thailand and released in Florida [69]. In Barbados, both species were responsible for the suppression of the population of the pest in an effective way (Head of the Entomology Department Barbados Ministry of Agriculture Mr. Ian Gibbs). In addition, 16 species of predatory lady beetles (Coccinellidae) have been found on scale-infested plants in South Florida. However, nearly all these predatory natural enemies appear to be ineffective at providing satisfactory control. Therefore, research continues to examine natural enemies that may contribute to the overall biological control of the scale [68]. Besides, an alternative approach is to enforce strict quarantine measures in countries where CAS has not yet been introduced by prohibiting the importation of cycad plants from infested countries [64].

3. The increase of parasitoid action through conservative biological control

Conservative biological control is based on the preservation and/or modification of the environment through anthropic interventions to maintain and increase the survival of natural enemies in agroecosystems, in addition to improving their performance in natural pest control.

Interactions between species in an ecological system not only act on the species populations involved but also regulate the operation of complex networks.
These networks describe interactions between individuals or species and evaluate emerging system properties such as stability, resilience, or efficiency of energy transfer [70].

To reach success in conservative biological control, it is necessary to know the structure and function of each trophic level of a network in the environment, and so it can be managed in a way that the desirable species can be conserved in the system [71]. A basic point in this proposal is the diversification of vegetation in the cultivated area which favors natural enemies due to the availability and abundance of pollen and nectar that can meet their nutritional needs [72]. Offering refuge areas and appropriate microclimate, as well as alternative prey and host for development, are also important [73].

Habitat enhancement for natural enemies has been researched around the world through “habitat management” that has become a subdiscipline of pest management in many ecological studies centers [74].

About parasitoids, specifically, the studies have focused mainly on two axes:

(1) The action of wild or native vegetation adjacent to the crop in the diversity (richness and abundance) of parasitoids and

(2) and the increase of the parasitism with the diversification of the vegetation in polycultures or use of flower strip.

The first axis, the influence of wild vegetation on the diversity and abundance of parasitoids in cultivated areas, was evaluated in different regions and crops.

An example is the work of [75] who identified and compared diversity of parasitoid assemblages in an irrigated rice crop under organic management (OR) and in a nearby protected area (Wildlife Refuge Banhado dos Pachecos—BPWR) in the south of Brazil. Specimens were collected with Malaise and Moericke traps. As expected, the authors found a greater parasitoid diversity at the BPWR than at the OR. But the interesting thing is that the Platygastridae and Braconidae families, important natural enemies of agricultural pests, were the ones that had the highest number of morphospecies shared between the areas.

Thus, it is possible to infer that the legal reserve area of wild vegetation may be serving as a natural repository of parasitoid hymenoptera on organic crops if the distance between the areas is adequate.

To deepen the knowledge in this respect, another work was developed to evaluate the contribution of the presence of fragments of natural vegetation near rice-growing areas and the influence of different management of the crop on the abundance and diversity of families of hymenopteran parasitoids through distance gradients [76]. The work took place one rice crop with organic management (OM) and another one with conventional management (CM), in RS, Brazil, during two crop seasons. The parasitoids were collected with Malaise trap arranged at different distances in relation to the native vegetation surrounding the rice crop in both places. The most abundant families were Platygastridae, Mymaridae, Encyrtidae, Eulophidae, and Trichogrammatidae. Parasitoid average abundance was significantly higher on OM only in the second season. This may be due to the use of nonselective (neurotoxic) insecticides (neonicotinoids and pyrethroids) applied to the crop in 2014–2015 different from the first season analyzed, when growth regulators insecticides were used (buprofezin and benzylurea; farmer personal communication Mr. Denis).

There was a negative correlation between distance from native vegetation and parasitoid abundance in CM areas (Figure 5).

This result suggests the importance of this area in the presence of parasitoids in the crop. The role of these forest fragments in maintaining the richness and abundance of parasitoids has been described among others, by [77]. Interestingly, in the area of organic management, the distance gradient of the refuge did not significantly affect the abundance of parasitoids. The authors attribute this point to the
fact that in the OM area, there is a wild rice levee vegetation, including the presence of flowering species that increases the richness of parasitoids. The levees are created to produce a controlled flood environment in rice fields, and it has a variety of wild vegetation that growing in them. Therefore, the abundance may not be so dependent on the presence from preserved areas. Levees can serve as corridors extending the distances traveled by parasitoids [74].

The role of levee vegetation in the diversity of parasitoids was also tested in rice fields, comparing area [78]. One of them the wild vegetation from the levees was cut (C) monthly since the beginning of the planting period until the harvest, and in the other, the wild vegetation was not cut (NC). The average number of collected individuals by trap and sampling occasion was significantly larger in the NC area (42.5 ± 12.9) than in C area (23.9 ± 7.2; H = 7.0687; p < 0.05). The NC subarea has greater species richness than subarea C. This is demonstrated by the rarefaction curve which plotted the estimated number of morphospecies in relation to the number of individuals sampled (Figure 6). In the graph, the cutoff point (around 1200 individuals) shows that the richness curve observed in area C was below the 95% confidence limit of the curves in the NC subarea, which had a larger parasitoid assemblage.

However, evaluating only egg parasitoids of plant hopper, [79] shows that these communities in rice fields are independent of the availability of noncrop habitats, providing additional nectar resources and retreat areas. The authors note that in contrast to temperate host parasitoid systems, rice hopper parasitoids seem to be very well adapted to the spatial and temporal heterogeneity because they have evolved in a rice monoculture system that offers sufficient resources that occur on traditional smallholder farms of Southeast Asia. Thus, other factors such as specific environmental and climatic conditions should be considered in the evaluation and implementation of conservative biological control.

Botanical species with high floral abundance can influence the attractiveness of floral visitors and natural enemies, since plants that offer more resources should be visited more frequently [80]. Therefore, the selection of plants with these characteristics is a relevant point.
Nutritional quality of plants with flowers or extrafloral nectaries in the performance of the parasitoids has been studied in the laboratory and in the field, like the work of [81] who showed that the fertility and longevity of *Dolichogenidae tasmanica* (Cameron) (Hym., Braconidae) were increased in the treatments, in which there were flowers of *Lobularia maritima* (L.). [82], which also observed that adults of *Trichogramma carverae* Oatman & Pinto (Hym., Trichogrammatidae) had an increased survival rate with consequent increase in parasitism of *Epiphyas postvittana* (Walker) (Lep., Tortricidae) eggs, when confined with alyssum flowers.

In this way, we arrive at the second axis that focuses on the diversification of the vegetation in polyculture and the use of flower strips through habitat management. This kind of study can be exemplified by works such as [83], who concluded that tomato (*Solanum lycopersicum* L.) cultivated in polyculture with coriander (*Coriandrum sativum* L.) (Apiaceae), marigold (*Tagetes minuta* L.), and sorghum (*Sorghum bicolor* L.) presented lower losses due to pest attack. On the other hand, [84] stated that the wheat and pea consortium reduces the presence of pests not by increasing the number of natural enemies but by complicating the search of host plant for pests.

The distance between flowers is also an indirect factor that results in more attractiveness of beneficial insects; therefore, floral visitors should be more likely to visit flowers that are closer to each other, minimizing the energy spent in their activities [85]. In this sense, there are suggestions of flower arrangements between cultures such as the technical bulletin of Embrapa Agrobiologia [86] (Figure 7).

For example, the distance of the buckwheat flower strips grown between wheat (*Triticum aestivum* L. Poaceae) affected significantly the rate of the aphid *Metopolophium dirhodum* Walker (Hem., Aphididae) parasitism by the parasitoid *Aphidius rhopalosiphi* De Stefani-Peres (Hym., Aphidiidae), with exponential decline with increasing distance from flower strips [87].

Unfortunately, we do not have many examples of this kind of study in the American continent, but several countries in Europe have been studying the influence of flowers on survival, density, and pest control ability by parasitoids and predators. We can cite the work of [88], of the National Institute of Agricultural
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Sciences (INCA) in Havana, Cuba who evaluated phytophagous insects and natural enemies in tomato-maize a polyculture and concluded that tomato-corn polyculture has a dissuasive influence on the development of pest populations in tomato crop besides enabling colonization by pest natural enemies.

In a work carried out at the Agricultural Research Company of Minas Gerais (EPAMIG), Brazil, the authors also evaluated the effect of the use of Tagetes erecta L. and Calopogonium mucunoide (Benth) on the occurrence of pest and natural enemies in crop of roses. The occurrence of Praon volucre (Hym., Braconidae) and Pimpla croceiventris (Cresson) (Hym., Ichneumonidae) was observed. The authors concluded that plant diversification contributed to a reduction in the occurrence of pests in this system [89].

Several interlayer designs of alders (L. maritima) were evaluated with organic lettuce for the control of aphids in ARS experimental fields in Salinas, California [90]. The authors also did studies with other plants, such as yellow mustard, and commented that these flowering plants attract syrphids and parasitoids that feed on pollen and flower nectar.

It is expected that this kind of study will increase in the Neotropical region in the next years with the increase in demand for alternative technologies of control and pest management.

Thus, we can reinforce the idea that multiple crop species grown in a single land increase biodiversity and encourage the presence of parasitoids. Plantation of multiple crops exploits different environmental niches, enhancing the total productivity per unit of land and expanding natural biological control.

4. Major challenges for the implementation of biological control programs in the Neotropical region

The list below highlights the major challenges that farmers, extension officers, and researchers have found when implementing biological control programs in Latin America and the Caribbean region:
a. Understanding and familiarization: At the farmer’s level, it is important to ensure an efficient technology transfer method that facilitates the understanding of the technology and a high adoption at the field level. Experience working with farmers showed that once they understand and are familiar with the technology of application, way of action, and requirements of the bioproducts, they tend to use it much more. As an example of a good technology transfer methodology, the plant clinics established as part of the Plantwise program (www.plantwise.org) in the region can be mentioned. In this way, the extension officers/researchers provide information about sustainable methods of control, including the use of bioproducts, helping farmers to understand the way of action, and the best way/time for their application [1].

b. Integration of pests control methods: In order to change the current agricultural practices which depend heavily in the frequent application of pesticides, it is important to develop and transfer a package of sustainable production for the key crops. This will include the integration of methods of control, taking into account the crop phenology, time of the year when the pest attack most, and changes in climatic conditions per year that can favor the establishment and attack of new and current pests. In order to establish an effective Integrated Pest Management, it is important to highlight the need to study the selectivity of agrochemicals to natural enemies; this needs to be done case by case considering also local adaptations [1, 91].

c. Commercialization and availability of the bioproducts: Despite the high biodiversity the Neotropical region has and the potential for the use of Biological Control, the commercialization is a key factor to ensure the use of biological control agents at field level. Many potential/efficient biocontrol agents are not being commercialized as yet, among other reasons, in one hand due to problems when trying to mass produce them and in another hand due to their efficacy get compromised when integrating it with other methods of control. Therefore, more studies are needed to overcome these limitations, as well as the approximation and close work between scientific community and the companies that produce/commercialize the biocontrol products. Although some biological control agents are commercialized in countries in the region, it is very difficult for farmers to buy them in remote areas in some cases. Farmers in those locations can easily buy heavy toxic chemicals but their access to bioproducts seems to be very limited. Therefore, the distribution of these products in rural agricultural communities is also important to ensure their use [1, 91].

d. Government incentive: It is important that the government established strategies and incentives to ensure farmers, and in particular, small farmers located in very remote areas have the opportunity to get clear information about the bioproducts that are available in their region, understanding the way of actions, benefits, and minimal requirements to ensure their efficacy. In addition to this, clear information about the technology of application is crucial to ensure that the bioproducts show the level of efficacy expected. This can be achieved establishing a good and efficient national extension system [1].

5. Working group on parasitoids of the Neotropical region

In order to approximate scientists and share the information and results about the biological control programs carried out in the region, it is important to establish
The use of parasitoids in biological control programs has a big potential in the Neotropical region, which can be confirmed by the positive results obtained in the programs that are being implemented in the region. For example, the large areas under sugarcane cultivation in Brazil and to another extend in Colombia, using biological control of the sugarcane borer (Diatraea saccharalis) by the use of the larval parasitoid Cotesia flavipes and other species of parasitoids, make farmers and the agricultural community in general to perceive biological control as an efficient method that can be applied in large areas, increasing their interest in its use. In order to overcome the major challenges and constraints in parasitoids utilization as biological control agents in the region, it is necessary to grant farmers a better understanding of the benefits/requirements of these natural enemies. Helping farmers to become more familiar with the use of natural enemies, allowing an efficient integration of biological control with other control methods, could improve its application. It is worth mentioning that the commercialization and availability of the natural enemies are key factor to improve the adoption of biological control by farmers. Further studies are needed to develop and improve the mass production of some parasitoids species, which have been reported as efficient natural enemies of key pests.

Likewise, it is very important that the scientific community works together with extension officers to understand the major barriers experienced by farmers using biological control tactics, in order to present efficient, feasible, and cost effective solutions at field level. It is also important to reinforce the current platforms/networks where scientist, extension officers, students, industries, and practitioners could share results, experiences, and information to promote and incentive the use of biological control in a higher level in the region.

The implementation of biological control as part of an integrated pest management program could increase the sustainability of the agricultural production, reinforcing food security in the Neotropical region.
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