

Pupal parasitoids for the biological control of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae)

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Submetido em 15/03/2018

Aceito para publicação em 23/10/2018

Resumo

Parasitoides pupais para o controle biológico da *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae). *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), popularmente conhecida como broca da cana-de-açúcar, é a maior praga da cultura de cana de açúcar e é responsável por consideráveis danos econômicos. O manejo dessa praga é complexo devido ao alojamento no interior do colmo; quando imatura, cria galerias que enfraquecem as plantas, reduz a biomassa e a qualidade do suco da cana, e perdas na produtividade agrícola. O uso excessivo de inseticidas químicos propicia resistência dessas pragas, danos ao meio ambiente, à fauna e à flora e alto custo. Dessa forma, um componente imprescindível do manejo de pragas é o controle biológico. Todas as espécies animais e vegetais apresentam inimigos naturais (parasitoides, predadores, entomopatógenos) que atacam diversos estágios de desenvolvimento. Parasitoides representam uma importante ferramenta no controle biológico de pragas lepidópteras. O conhecimento da atividade do parasitoide pupal é importante para o desenvolvimento de estratégias de controle biológico para essa praga, a qual ocasiona perdas significativas. Portanto, este artigo usa dados publicados para destacar a importância e a eficácia dos parasitoides contra a pupa da broca da cana-de-açúcar.

Palavras-chave: Broca da cana-de-açúcar; Controle biológico; Controle de pragas; Parasitismo; Pupa

Abstract

Diatraea saccharalis (Fabricius, 1794) (Lepidoptera: Crambidae), popularly known as the sugarcane borer, is the greatest pest of sugarcane crops, and is responsible for considerable economic damages. This pest is difficult to manage, because it lives inside the culm when immature, creates galleries that weaken the plants, reduces the biomass and the quality of sugarcane juice, and lowers agricultural productivity. The excessive use of chemical insecticides leads to resistance in these pests and damages the environment, fauna, and flora, and these products are expensive. Thus, an indispensable component of pest management is biological control. All animal and plant species have natural enemies (parasitoids, predators, and entomopathogens) that affect different stages of development. Parasitoids represent an important tool in the biological control of lepidopteran pests.



Knowledge of pupal parasitoid activity is important for the development of biological control strategies for this pest, which causes significant losses. Therefore, this paper uses published data to highlight the importance and effectiveness of parasitoids against sugarcane borer pupae.

Key words: Biological control; Parasitism; Pest control; Pupa; Sugarcane borer

Introduction

Diatraea saccharalis (Fabr.) (Lepidoptera: Crambidae) is an insect found in North and South America (CRUZ et al., 2011), and is considered the most common pest of sugarcane, maize, and sorghum (CRUZ et al., 2011; DINARDO-MIRANDA et al., 2012; SVEDESE et al., 2013). The direct effects of this insect occur through the development of galleries by larvae inside the stem of the plant, resulting in a loss of sugarcane biomass, and reduced quality of the plant juice. The apical meristem is usually damaged, which manifests as a yellowing-to-whitening, as well as atrophy of the internode, side shoots, and aerial rooting, which causes the plant to die, and the disease is also known as “dead heart” (GALLO et al., 2002; DINARDO-MIRANDA et al., 2012; SHOWLER; REAGAN, 2012). The indirect losses are considerable, since the tunnels made by the sugarcane borer allow colonization by phytopathogenic microorganisms, causing so-called “red rot,” which reduces sucrose in stalks via its conversion into glucose and fructose; this consequently leads to decreased substrate for alcohol production (DINARDO-MIRANDA et al., 2012; ROSSATO et al., 2013; SANTOS et al., 2015).

Because of the habits of this pest, and the extensive cultivation of target crops, control using conventional methods, such as chemical insecticides, is associated with conventional, economic, and environmental obstacles. Therefore, there is a need for frequent applications of synthetic insecticides to reduce risks and economic losses. This causes adverse effects on the environment and human health, and induces abiotic changes (MOSCARDI; SOUZA, 2002). Other factors include the elimination of beneficial arthropods, the resurgence of pests, and the selection of resistant individuals (MENEZES, 2005). The control of *D. saccharalis* is currently ineffective because the insects are inside the stem of the plant, where they are protected

from topically-applied insecticides (ANTIGO et al., 2013).

Growing interest in the sustainability of the agroecosystem, and the use of natural enemies in the control of insect pests favors the implementation of programs such as integrated pest management (IPM). This emphasizes the long-term mitigation of these insects through various control methods, using, for example, cultural, mechanical, physical, chemical, and biological techniques (MACHTINGER et al., 2015; DITOMASO et al., 2017), based on economic, ecological, and social parameters. Therefore, IPM requires knowledge of the crops, insect pests, and population density of these insects and their natural enemies. In addition, it requires the determination of control methods and careful selection and use of techniques that are more effective at reducing pest populations to levels that do not lead to economic damage (ZANETTI et al., 2014; MACHTINGER et al., 2015).

Accordingly, biological control programs can be an integral component of the management of invasive species, and have great potential for success and return on investment when IPM systems are used (DITOMASO et al., 2017). This has become increasingly common due to international interest in agricultural production oriented towards sustainable development, which favors the conservation and rational use of natural enemies. The control of insect pests using natural enemies has proved to be effective and accessible (BARBOSA et al., 2008); this biological control method is becoming increasingly popular. The sugarcane borer is protected by various intrinsic defense mechanisms, and its development is not hampered while inside the stem of the plant (GALLO et al., 2002). However, the plants have no effective protection directly related to attack by natural enemies. In this way, several strategies associated with IPM have been successfully used to control these pests; one

strategy is the use of parasitoids (PARRA et al., 2002), which are special types of natural enemies introduced against pests. Most parasitoids belong to the order Diptera or Hymenoptera, with some representatives of the orders Coleoptera and Lepidoptera (PENNACCHIO; STRAND, 2006).

Pupal parasitoids play an important role in reducing the populations of insect herbivores and represent the main group of natural enemies in agricultural systems. Many species of pupal parasitoids have been successfully used in biological control programs (VINSON; IWANTSCH, 1980; PENNACCHIO; STRAND, 2006; ROSSI et al., 2014), especially the Hymenoptera parasitoids (TAVARES et al., 2013). Furthermore, knowledge about bioecology is necessary for the success of biological control methods (GUEVARA; WIENDL, 1980). Understanding the characteristics of parasitoids is essential for the development of techniques for use and improvement in mass rearing in the laboratory and possible releases in the field. Thus, the present study aimed to analyze the main pupal parasitoids as natural enemies of *D. saccharalis* and their interactions, highlighting publications and satisfactory results obtained in biological control programs against the sugarcane borer.

Development

Diatraea saccharalis (Fabr., 1794) (Lepidoptera: Crambidae)

The order Lepidoptera is of great relevance in agriculture (SALIM et al., 2016). Among the pest insects of great economic importance, *D. saccharalis* is the most important one in the sugarcane (GALLO et al., 2002; DINARDO-MIRANDA et al., 2012) and maize and sorghum (CRUZ et al., 2011) crops. Lepidopterans belonging to the superfamily Pyraloidea, and the family Crambidae, probably originated from Central and South America and are very small moths (GALLO et al., 2002).

The sugarcane borer undergoes holometabolous development; a pupal stage between larval and adult development is characteristic of this type of development, during which metamorphosis occurs (GALLO et al.,

2002; CRUZ-LANDIM, 2009). Numerous efforts have been made to reduce infestations of this pest.

Biological cycle

The sugarcane borer has a biological cycle of 53 to 60 days, with three to four generations per year depending on the geographical region, and in some cases, five generations, depending on weather conditions. Adult moths have pale yellow anterior wings and whitish hind wings (GALLO et al., 2002). They have nocturnal habits, and remain hidden in the morning (CAPINERA, 2016). The female posture is preferentially on the dorsal side of the leaf after mating (GALLO et al., 2002). The eggs are flattened with an oval shape, forming an imbricated cluster of overlapping eggs, similar to fish scales. Clusters contain 2–50 eggs, which have a white color initially, acquire an orange tint over time, and turn black prior to hatching. This stage lasts around 4–6 days. Average fecundity is approximately 700 eggs when the pest is fed on sugarcane and corn crops (CAPINERA, 2016).

According to Capinera (2016), the eggs hatch at the same time, or at least, within a few hours. The larvae feed almost immediately after hatching. The number of instars varies, with three to ten recorded; the average is five to six. The larvae are pale yellow with a pale brown head. They complete larval development at around 40 days when the pupal stage begins (GALLO et al., 2002).

Pupa

Pupation occurs inside the stalks, in a tunnel created by the larvae, which has been cleaned and expanded before the pupal stage, with a thin layer of plant tissue for rupture during the emergence of adults (GALLO et al., 2002; CAPINERA, 2016). The pupae are elongated, thin, and obtecta (body appendices are closely connected to the body and enable pupal differentiation), showing little movement (FERRER; SALAZAR, 1977; BOTELHO; MACEDO, 2002; GALLO et al., 2002). They are around 16–20 mm in length, with a focus on prominent tubercles in the distal segments (GALLO et al., 2002; CAPINERA, 2016). The pupal tegument is fairly sclerotized, and protects against desiccation and

predation. The first abdominal and thoracic segments are visible only dorsally (RAFAEL et al., 2012).

The genital pore is located in the terminal portion of the abdomen, and can be used for sexual differentiation when viewed under a stereoscopic microscope. The wings can be visualized in the pupae in the mesoventral region, reaching the fourth abdominal segment when they are open (FERRER; SALAZAR, 1977; BOTELHO; MACEDO, 2002; GALLO et al., 2002). This phase lasts 9-14 days; however, this period may extend for 22 days in cold climates (GALLO et al., 2002; CAPINERA, 2016). The newly formed pupae are white, but they turn brown over a few hours. In general, individuals that will originate females are heavier and larger than males (FERRER; SALAZAR, 1977; BOTELHO; MACEDO, 2002; GALLO et al., 2002). In a study by Melo and Parra (1988), temperature was found to influence pupal development, where temperatures between 20 and 30°C favored heavy female pupae, whereas development was affected by temperatures above 30°C.

Biological control

IPM programs are based on different methods and incorporate a diverse set of techniques to reduce insect pest populations, implementing combinations of strategies that maximize pest control and minimize environmental, health, and economic risks. For successful IPM, a number of steps should be considered before the initiation of a biological control program with pupal parasitoids, for example, cyclical elements of pest detection and identification, the use of established thresholds, monitoring of abundance, the application of methods more suited to management, and the evaluation of results (MACHTINGER et al., 2015).

According to Menezes (2003), the use of biological control began in the third century, when the Chinese used predation by ants (*Oecophylla smaragdina*) to control citrus plague. However, biological control only began to gain momentum in research on its implementation in agricultural ecosystems in the 20th century (FALEIRO et al., 2011). Biological control involves a natural process, where natural enemies regulate the abundance of plants and animals, consequently, causing biotic mortality

(GALLO et al., 2002; PARRA et al., 2002). All plant and animal species have natural enemies that attack them during various life stages (PARRA et al., 2002), which can be used to limit or reduce pest populations (MAHR et al., 2008). De Bach (1968) was the first to use the term biological control, defined as “the action of parasitoids, predators, and pathogens in maintaining the density of another organism at a lower level than would normally occur in their absences.”

Biological control is very important in IPM programs, mainly for integrated production to be directed towards sustainable agriculture (BARBOSA et al., 2008), and it represents a part of any IPM program. Since natural enemies are the main cause of natural mortality in the agroecosystem, they help to manage pests, along with other methods, such as plant resistance to insects and physical and behavioral (pheromonal) methods, with the possibility of integration with chemical methods (PARRA et al., 2002). As an alternative, the use of biocontrol agents, including parasitoids, predators, nematodes, and microorganisms (PENNACCHIO; STRAND, 2006; SALIM et al., 2016), has stimulated several breeding studies *in vitro*.

Biological control with parasitoids

Parasitoids are a group of natural enemies of great relevance to the biological control of the class Insecta (FAVERO et al., 2013). These individuals are important due to their diversity and levels of parasitism achieved in hosts (TAVARES et al., 2012). Natural enemies commonly used in biological control programs include parasitoids of the order Hymenoptera, especially the families Aphelinidae, Braconidae, Encyrtidae, Ichneumonidae, Pteromalidae, Trichogrammatidae, and Eulophidae and, to a lesser extent, Diptera, especially Tachinidae (VAN DRIESCHE; BELLOWS, 1996; GALLO et al., 2002), and Coleoptera, Lepidoptera, Trichoptera, Neuroptera, and Strepsiptera as well (PENNACCHIO; STRAND, 2006).

Gallo et al. (2002) defined a parasitoid as “an organism that kills the host and requires only one individual to complete development.” They usually have higher specificity than predators (VAN

DRIESCHE; BELLOWS, 1996). By killing the host, parasitoids regulate the population of hosts of different orders, and this has been practiced in numerous biological control programs (QUICKE, 2014). It is important to identify and conserve these insects for IPM implementation, and to reduce dependence on chemical insecticides. However, the use of natural enemies in biological control programs depends on the availability of host species, and on suitable characteristics for the development and maintenance of these parasitoids (TAVARES et al., 2012).

Hymenoptera: pupal parasitoids

Hymenoptera corresponds to the second most diverse and beneficial order of insects. Parasitic wasps are considered the most important group of natural enemies of pest insects (MAHR et al., 2008). Sources estimate that the hymenopteran wasps used against insect pests in agroecosystems comprise up to 20% of all insect species (BONET, 2009). There are about 320,000 species, representing almost 75% of the estimated parasitoid species (BONET, 2009). Few records of pupal parasitoids are available in the literature for *D. saccharalis*. Desneux et al. (2010) reported the same for *Tuta absoluta*. This shows the need for future research to further explore various parasitoids against the sugarcane borer. However, studies investigating pupal parasitoids of *D. saccharalis* systematically use the order Hymenoptera. In general, the borers are attacked by various natural enemies, including hymenopteran insects (DUALE, 2005).

These parasitoids regulate the complex dynamics of food webs, thus interfering with trophic chains and the overall stability of agroecosystems (BARBOSA; CASTELLANOS, 2005; VAN VEEN et al., 2008). In addition to the maintenance of populations of herbivorous species, the hymenopteran parasitoids present host specificity and act as bioindicators of ecosystem health and diversity (ANDERSON et al., 2011). Numerous lepidopterans cause losses in agricultural production, which prompt studies on parasitoids of this group in biological pest control programs (SOUZA et al., 2006).

The injection of certain chemicals by the females to halt host development and to preserve tissue is one of the strategies used by idiobiont pupal parasitoids to exploit hosts. To facilitate the digestion of pupal tissue, enzymes are also released corresponding to the available nutritional resources inside the host. (PENNACCHIO; STRAND, 2006). The pupae of holometabolous insects undergo physicochemical changes during their structuring and synthesis of new tissue. Muscle tissue, alimentary tract, and salivary glands degenerate. Degeneration caused by tissue histolysis results in the release of a significant amount of nutrients that will be used for the synthesis of new pupal structures. In addition to structural changes, there are also changes in the chemical composition of the pupa, reducing the concentration of nutrients, such as carbohydrates, and host proteins throughout metamorphosis (PANNIZI; PARRA, 2012).

The use of these organisms can reduce the application of agrochemicals that damage the environment and harm beneficial organisms (BERNARDI et al., 2010). In addition, it is important to determine the potential of a species as a biological control agent through both functional and numerical responses. The functional response evaluates parasitoid search efficiency in addition to understanding host-parasitoid interactions, and the numeric response determines the increase in the natural enemy population as a function of host density (MAHMOUDI et al., 2010).

Knowledge of these processes will enable strategies to be stepped up and the efficiency of such agents to be improved. However, we need to enhance our knowledge of the physiology and metabolism of the parasite and its interaction with the host. To develop an effective biological control program, taxonomic studies, as well as knowledge of the bioecological aspects of predators and parasitoids, physiology and metabolism, and parasite-host dynamics is essential (GARIEPY et al., 2008).

Eulophidae

The family Eulophidae Westwood, 1829 is biologically large and diverse, consisting of small parasitic wasps (usually 0.4-6.0 mm) belonging to the

superfamily Chalcidoidea and represented by more than 5,000 species described in 443 genera (BAUR, 2015; NOYES, 2015). Individuals are generally associated with insect hosts or related arthropods (MAHR et al., 2008), and show greater variety than any other parasitic wasp family. Most species are described as primary or secondary parasitoids of Lepidoptera, Coleoptera, Diptera, and Hymenoptera, and a wide variety of arthropods. The eulophids are of great economic importance, and many species are useful in biological control programs of pests worldwide (YEFREMOVA, 2007; TALEBI et al., 2011; BAUR, 2015; NOYES, 2015).

***Trichospilus diatraeae* (Cherian & Margabandhu, 1942) (Eulophidae, Eulophinae)**

The pupal endoparasitoid, *Trichospilus diatraeae*, parasitizes and develops in numerous hosts. This parasitoid has been studied since 1942 in the host *D. venosata*, a borer of grass stems in South India. Since then, it has been one of the most studied pupal parasitoids of *D. saccharalis* (BOUCEK, 1976; PARON; BERTI-FILHO, 2000). *T. diatraeae* is characterized as a gregarious pupal parasitoid, with a preference for insects of the order Lepidoptera (BOUCEK, 1976). In Brazil, knowledge of the potential of this insect as a biological agent is limited (FAVERO, 2009; GRANCE, 2010). Little information is available on this parasitoid and its hosts (FAVERO, 2009). The results summarized in the literature (FAVERO, 2009; CHICHERA et al., 2012; RODRIGUES et al., 2013; VARGAS et al., 2013; 2014; CALADO et al., 2014; GLAESER et al., 2014) indicate a high level of parasitism in *D. saccharalis* pupae, demonstrating its potential as a parasitoid agent that can be used in biological control programs.

The parasitism of *D. saccharalis* and *Tenebrio molitor* pupae by *T. diatraeae* was compared by Favero (2009), who showed that the biological characteristics of this natural enemy are more favorable in *D. saccharalis* larvae compared with *T. molitor*. Both exhibit a high rate of parasitism; however, in *D. saccharalis*, pupal parasitism was 100%. The search and parasitism of *T. diatraeae* in the pupal host *D. saccharalis* was analyzed

by Chichera et al. (2012) and Vargas et al. (2013). Chichera et al. (2012) performed *in vitro* studies and also simulated natural conditions of parasitism, showing a greater efficacy of this parasitoid in parasitism and emergence in pupae of *D. saccharalis* inside sugarcane stalks. The pupae of the sugarcane borer were not infested following the joint release of *T. diatraeae* and *P. elaeisis*. Additional studies are necessary to understand the interactions of parasitoids in the control of lepidopteran pest populations.

The parasitism capacity of *T. diatraeae* was evaluated by Vargas et al. (2013) by simulating *in vitro* the natural conditions of parasitism of sugarcane borer in stalks. Three conditions were tested in that study, all of which involved the stalk, pupa only, pupa and larva, and pupa together with fecal material. Overall, the host was successfully identified, but the study highlighted the high level of search activity in the presence of the larva. Volatile compounds released by plants attacked by herbivores help parasitic insects to locate their hosts (FONTANA et al., 2011). The pupae allocated with larvae had a higher level of parasitism; therefore, the authors suggested that the parasitoid is able to differentiate between components released by natural enemies in the presence of larvae, which could be close to the pupal period, in contrast to the presence of fecal material, which reduced parasitism by *T. diatraeae* (VARGAS et al., 2013).

The development of *T. diatraeae* in pupae at certain temperatures was analyzed in a study by Rodrigues et al. (2013), who observed a high percentage of parasitism. However, favorable temperatures were found to be between 16 and 28°C, indicating that the upper thermal limit oscillates between 28 and 31°C. Calado et al. (2014) evaluated the biological characteristics of the parasitoid, and observed satisfactory reproductive development, good suitability, and acceptability in sugarcane borer pupae.

The female density of *T. diatraeae* per pupa was analyzed by Vargas et al. (2014), who found a maximum percentage of parasitism and emergence for all densities, showing that parasitism is not affected by the density of the parasitoid. However, densities of 14 to 21 per pupa of *D. saccharalis* larvae were more favorable for parasitoid

establishment under laboratory conditions. *T. diatraeae* exhibited good biological characteristics as a potential agent in the control of sugarcane borer pupae. Pastori et al. (2013) evaluated the dispersion of *T. diatraeae* females in commercial sugarcane plantations, and showed that females parasitized pupae of the sugarcane borer. However, pupal parasitism by *D. saccharalis* decreased with distance from the release point. Several factors can interfere with the reduced demand of the host contained in the traps, such as the presence of other insects, alternative hosts, groups of predators in the experimental area, and the number of parasitoids released for parasitism to be satisfactory.

The use of *T. diatraeae* in *D. saccharalis* was also studied by Glaeser et al. (2014) to determine whether the establishment of parasitoid on the alternative host *T. molitor* would affect its biological characteristics when later reared on the pupae of *D. saccharalis*, and showed that the biological quality of this parasitoid was not compromised. According to Dias et al. (2008), the alternative host presents advantages, such as reduced costs, improved parasitoid development in the laboratory, and efficient mass rearing.

***Tetrastichus howardi* (Olliff., 1893) (Eulophidae, Tetrastichinae)**

This species is distributed in a large part of Australia, from the northern Australia to China and to western Pakistan. This species has also been recorded in Ghana, Nigeria, and South Africa (LA SALLE; POLASZEK, 2007). *Tetrastichus howardi* is a gregarious pupal parasitoid reported to be a primary parasitoid or facultative hyperparasitoid, which attacks pest populations of Lepidoptera species of important crops (BAITHA, et al., 2004; PRASAD et al., 2007). In 1997, this pupal parasitoid was detected in Matanzas, a province of Cuba, in rice crops and later in sugarcane. Álvarez et al. (1998) reported it as *Aprostocetus* (*Aprostocetus* Westwood) (Hymenoptera: Eulophidae, Tetrastichinae), and it was reclassified in 1999 by LaSalle as *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae, Tetrastichinae).

In a sample of parasitism in *D. saccharalis* by *T. howardi*, the parasitism of borer pupae was 15.3%

(ALVAREZ et al., 2003). A similar result was obtained by Félix et al. (2005) who concluded that the combination of the larval parasitoid *Lixophaga diatraeae* and pupal parasitoid *T. howardi* may be a better option for the management of the sugarcane borer. Recent studies have indicated that *T. howardi* is a promising biological agent in the pupal control of *D. saccharalis* (VARGAS et al., 2011; CRUZ et al., 2011; COSTA et al., 2014). Studies of this natural enemy using larvae and pupae were also carried out by Vargas et al. (2011), who concluded that *T. howardi* was parasitic at both stages. However, additional studies are needed to improve methods of mass rearing and to examine their combinations with other parasitoids. Cruz et al. (2011) parasitized pupae of the sugarcane borer with parasitoid females of *T. howardi* and concluded that the natural enemy studied may be an option for the control of key pests in crops such as sugarcane, corn, and soybean. Figueiredo et al. (2011) investigated *T. howardi* parasitism in pupae of the sugarcane borer, and considered the parasitoid an additional option for IPM against *D. saccharalis*.

The relationship between pupa age (24, 48, 72, 96 and 120 h) and parasitism by three *T. howardi* females reared in the laboratory was evaluated by Costa et al. (2014), who observed a change in the life cycle of the parasitoid. However, parasitism, emergence, and progeny were not influenced by the age of the hosts, while the life cycle of the parasitoid was shorter in pupae at 24 h of age and longer in pupae at 120 h of age. In general, pupae of the borer at 24 and 120 h exhibited good *T. howardi* multiplication. Favero et al. (2015) performed tests at different temperatures (16, 19, 22, 25, 28, and 31°C) to determine the effect on the development of this natural enemy in the *D. saccharalis* host. The authors found that *T. howardi* exhibited better performance, fecundity, and longevity between 19 and 28°C, indicating good plasticity of development, and highlighting its use as a differential biocontrol agent for the multiplication of the sugarcane borer in the laboratory.

Large-scale parasitoid multiplication is essential for the implementation of biological control programs. Accordingly, the biological characteristics of *T. howardi* parasitizing all stages of *D. saccharalis* were studied, and the results suggested that the pupal stage of the host

is the most suitable for parasitism and development of this natural enemy (PEREIRA et al., 2015). Piñeyro et al. (2015) evaluated whether the multiplication of *T. howardi* in three generations in the pupae of the alternative host *Bombyx mori* would compromise the biological characteristics of this parasitoid when parasitizing the host *D. saccharalis*, and they found that reproductive performance was not affected when rearing in pupae of the sugarcane borer.

***Palmistichus elaeisis* (Delvare and LaSalle, 1993) (Eulophidae, Tetrastichinae)**

Palmistichus elaeisis is a pupal endoparasitoid studied by Bittencourt and Berti-Filho (2004a; 2004b), Chichera et al. (2012) and Candelária (2013). It has high potential as a biological control agent in *D. saccharalis* due to its development in the pupa host, permitting mass rearing in the laboratory. Bittencourt and Berti-Filho (2004a) found that some temperature conditions interfered with parasitoid development, suggesting that a suitable temperature for their rearing was 22°C. The upper thermal limit was 30°C in *D. saccharalis*, the biological cycle of the parasitoid was not complete above this temperature, even so, it has conditions to develop in this host. These results also highlight the need to evaluate regions for the possible release of parasitoids, because the temperature of some localities exceeds this limit. The development of immature stages of this eulophid was assessed by Bittencourt and Berti-Filho (2004b) in pupae of five lepidopteran species, including *D. saccharalis*. The results indicated that this parasitoid developed in five hosts tested and was not influenced by the number of instars, making it possible to use this endoparasitoid in mass rearing. This is consistent with the work of Bittencourt and Berti-Filho (1999), who showed non-preference for oviposition in the hosts *D. saccharalis*, *Anticarsia gemmatalis*, *Heliothis virescens*, and *Spodoptera frugiperda*.

The search and development capabilities of *P. elaeisis* were analyzed in terms of their suitability as a parasite of the sugarcane borer (CHICHERA et al., 2012). This parasitoid showed more favorable biological characteristics than *T. diatraeae*, which was also

evaluated *in vitro*. Two species were parasitized in the pupae of *D. saccharalis*, with *P. elaeisis* more effective than *T. diatraeae* in exploiting the host. Conversely, *T. diatraeae* proved to be more effective when parasitizing pupae in sugarcane internodes, simulating the natural conditions of parasitism, which is important when establishing populations of natural enemies in the field (CHICHERA et al., 2012). The quality of rearing is important for the effectiveness of the parasitoid in the field. Candelária (2013) evaluated the best age for pupae and the parasitoid, and determined the optimal density of *P. elaeisis* in pupae of *D. saccharalis*. According to Candelária (2013), the rearing of *P. elaeisis* is viable, being 27 parasitoids per host. According to this author, the age of the host resulted in changes in the progeny of the parasitoid, and the most satisfactory age group was 48–96 h for *P. elaeisis* and 72–96 h for *D. saccharalis*. However, the age of the parasitoid did not influence its life cycle.

Pupae of the sugarcane borer were exposed to two entomopathogenic fungi, *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metchnikoff), to determine whether these microorganisms would affect the biological characteristics of three species of eulophids, *P. elaeisis*, *T. howardi*, and *T. diatraeae*, developed in pupae of *D. saccharalis*. Furthermore Rossoni et al. (2016) found that the pupae parasitized by the three hymenopteran species exposed to fungi did not compromise the development of these parasitoids, suggesting that the use of these parasites was compatible with the two entomopathogenic fungi.

***Pediobius furvus* (Eulophidae, Entedoninae)**

Another parasitoid pupa of *D. saccharalis* is the hymenopteran *Pediobius furvus* (Gahan, 1928). This gregarious endoparasitoid parasitizes other species, such as *Busseola fusca* (Fuller, 1901) (Lep.: Noctuidae), *Chilo partellus* (Mayden; Kuhajda, 1885) (Lep.: Crambidae), *Sesamia calamistis* (Hampson, 1910) (Lep.: Noctuidae) (MOHYUDDIN; GREATHEAD, 1970; DUALE et al., 1997), and *Eoreuma loftini* Dyar (Lep.: Noctuidae) (SMITH et al., 1993).

Originally from Africa, this parasite has already been used successfully as a biocontrol agent in pupae of *D. saccharalis* in the southern United States. Tambasco (1997) conducted studies with this parasitoid and reported that the resistance of this parasitoid in sugarcane borer parasitization may be due to genetic variation in the populations of these parasitoids, with the possible existence of biotypes of greater efficacy in other regions of Africa.

Ichneumonidae

The family Ichneumonidae is rich in species, with more than 60,000 distributed worldwide. The wasps are important parasitoids of the larvae and pupae of hosts from the orders Lepidoptera, Coleoptera, and Hymenoptera (GAULD, 1991; GUPTA, 1991). Most ichneumonids are parasitoids and their development can occur within (endoparasitoid) or outside (ectoparasitoid) the host, killing it. Koinobiont species specialize, and become tolerant to the host's chemical and immune defenses, maintaining their development after oviposition. However, the idiobionts are generalists and circumvent the immune mechanisms of the host, which they permanently paralyze or kill (SANTOS; QUICKE, 2011).

The pupal endoparasitoid *Xanthopimpla stemmator* (Ichneumonidae, Pimplinae) Thunberg 1822 was imported from Texas as a biological alternative to control the sugarcane borer. Hailemichael et al. (1994) demonstrated the potential of this species in the biological control of *D. saccharalis*, which was hosted by a high percentage of pupae, where the younger pupae showed a higher parasitism, proving their suitability, acceptance, and success.

Chalcididae

The family Chalcididae Latreille, 1817 is distributed worldwide, displaying particular diversity in tropical regions (GHONEIM, 2014). It has been described globally, consisting of 93 genera and 1,474 species (AGUIAR et al., 2013), and comprises parasitoid wasps of various holometabolous insects. Species of this family are characterized as primary parasitoids or facultative

or obligatory hyperparasitoids of larvae or pupae of Diptera and young pupae of Lepidoptera, although some species may also parasitize Hymenoptera, Coleoptera, Diptera, and Neuroptera. They can be ectoparasitoids or endoparasitoids, and most have idiobiont behavior, while some species are koinobionts; most are primary parasitoids, with some being gregarious (SALIM et al., 2016).

Conura acuta (Fabr., 1804) (Hymenoptera, Chalcidinae)

Conura (syn. *Spilochalcis*) is a genus mainly of the New World, with approximately 1,000 species distributed in the neotropics, which parasitize pupae of Lepidoptera, Diptera, Coleoptera, and Hymenoptera (HANSON; GAULD, 1995). The parasitism of *C. acuta*, among other parasitoids, was studied in Mexico by Vejar-Cota et al. (2005) in pupae of *D. saccharalis*. These authors revealed very little acceptance of this parasitoid by insect pests. However, acceptability was higher when the pupae were exposed in combination with larval feces, suggesting the presence of semiochemicals, such as kairomones, favoring search and acceptability. A similar result was observed by Hailemichael et al. (1994) in pupae of the sugarcane borer with *Xanthopimpla stemmator*. *C. acuta* develops in different biotypes, resulting in differential host preference (VEJAR-COTA et al., 2005). For example, this parasitoid is commonly found in Guyana (DELVARE; BOUČEK, 1992). Parasitism was found to decrease progressively with host age, which is similar to the results obtained for *X. stemmator* (HAILEMICHAEL et al., 1994).

Conclusion

D. saccharalis continues to be a major economic problem worldwide, despite the considerable attention given to this pest over the years. The pupal parasitoids studied are generally satisfactory agents for the control of populations of lepidopteran pests, such as *D. saccharalis*. The high levels of parasitism in pupae indicates the potential biological control of this insect pest (VARGAS et al., 2011; 2013; CALLADO et al., 2014).

Studies on parasitoids of pupae can identify effective alternatives to reduce populations of *D. saccharalis*, mainly in the sugarcane crop, in terms of cost reduction, minimization of agrochemical use, ease of management, and biological characteristics. However, the studies covered did not compare these parasitoids to chemical methods or determine their viability, which would be valuable information for biological control studies. The dissemination of materials such as booklets and videos, and providing short courses, explaining the importance of these biological control agents, can reinforce awareness about the abusive use of pesticides. In this way, it is possible to observe the importance of biological control through pupal parasitoids. However, further investigations on the efficacy of these parasitoids are still required for field releases, and studies on their interactions with other agroecological methods, as well.

Acknowledgements

We would like to thank the National Council for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) for their financial support, and the State University of Maringá. Dr. A. Leyva (USA) provided English editing of the manuscript.

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