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Natural parasitism of lepidopteran eggs by *Trichogramma* species (Hymenoptera: Trichogrammatidae) in agricultural crops in Minas Gerais, Brazil

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Abstract

The genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) includes insect egg parasitoids that are widely used throughout the world as control agents of pest insects. The aim of this study was to identify the species of *Trichogramma* naturally associated with the eggs of lepidopteran pests of the following agricultural and horticultural crops: collards, *Brassica oleracea* L. (Brassicales: Brassicaceae); papaya, *Carica papaya* L. (Capparales: Caricaceae); tomato, *Lycopersicon esculentum* Mill. (Solanales: Solanaceae); cassava, *Manihot esculenta* Crantz (Malpighiales: Euphorbiaceae); banana, *Musa* sp. L. (Zingiberales: Musaceae); passion fruit, *Passiflora* sp. Degener (Malpighiales: Passifloraceae); sugarcane, *Saccharum* sp. L. (Poales: Poaceae); and corn (maize), *Zea mays* L. (Poales: Poaceae); and an invasive species (Sodom's apple milkweed, *Calotropis procera* Aiton; Gentianales: Apocynaceae) in the semiarid region of Minas Gerais, Brazil. We report natural parasitism by *Trichogramma* in eggs of *Agraulis vanillae vanillae* (L.) (Lepidoptera: Nymphalidae), *Antichloris eriphia* F. (Lepidoptera: Arctiidae), *Danaus* sp. (L.) (Lepidoptera: Nymphalidae), *Diatraea saccharalis* F. (Lepidoptera: Crambidae), *Erinnyis ello* L. (Lepidopera: Sphingidae), and *Protambulyx strigilis* L. (Lepidopera: Sphingidae). In total, 2,242 specimens of *Trichogramma* were obtained, belonging to the species *T. pretiosum* Riley, *T. manicobai* Brun, Moraes & Soares, *T. marandobai* Brun, Moraes & Soares, and *T. galloi* Zucchi. These species of *Trichogramma* may be candidates for biological control programs of lepidopteran pests in the semiarid region of Minas Gerais and in other semiarid regions.

Key Words: egg parasitoid; biological control; identification; agroecosystem

Resumen

El género *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) incluye insectos parasitoides de huevos que son ampliamente utilizados en el mundo como agentes de control de plagas. El objetivo de este estudio fue identificar las especies de *Trichogramma* naturalmente asociadas con los huevos de plagas de lepidópteros en diversos cultivos: collards, *Brassica oleracea* L. (Brassicales: Brassicaceae); papaya, *Carica papaya* L. (Capparales: Caricaceae); tomate, *Lycopersicon esculentum* Mill. (Solanales: Solanaceae); yuca, *Manihot esculenta* Crantz (Malpighiales: Euphorbiaceae); plátano, *Musa* sp. L. (Zingiberales: Musaceae); maracuyá, *Passiflora* sp. Degener (Malpighiales: Passifloraceae); caña de azúcar, *Saccharum* sp. L. (Poales: Poaceae); y maiz, *Zea mays* L. (Poales: Poaceae); y una especie invasora (manzana de Sodoma, *Calotropis procera* Aiton; Gentianales: Apocynaceae) en la región semiárida de Minas Gerais, Brasil. Se observó parasitismo natural por *Trichogramma* en huevos de *Agraulis vanillae vanillae* (L.) (Lepidoptera: Nymphalidae), *Antichloris eriphia* F. (Lepidoptera: Arctiidae), *Danaus* sp. (L.) (Lepidoptera: Nymphalidae), *Diatraea saccharalis* F. (Lepidoptera: Crambidae), *Erinnyis ello* L. (Lepidopera: Sphingidae) y *Protambulyx strigilis* L. (Lepidopera: Sphingidae). Se obtuvo un total de 2 242 especímenes de *Trichogramma* pertenecientes a las especies de *T. pretiosum* Riley, *T. manicobai* Brun, Moraes & Soares, *T. marandobai* Brun, Moraes & Soares, y *T. galloi* Zucchi. Estas especies de *Trichogramma* podrían ser útiles en los programas de control biológico de plagas de lepidópteros en la región semiárida de Minas Gerais y en otras regiones semiáridas.

Palabras Clave: parasitoides de huevos; control biológico de plagas; identificación; agroecosistemas

The genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) has the largest number of species in the family Trichogrammatidae. Among its 210 species (Pinto 2006), 41 have been reported in South America and 26 in Brazil (Zucchi et al. 2010). Many species of *Trichogramma* are used worldwide for the biological control of lepidopteran pest species (Smith 1996; Mills 2010) through inundative releases into millions of hectares of a wide variety of crops (Li 1994; Parra & Zucchi 2004; Pizzol et al. 2012).

Some favorable characteristics of *Trichogramma* species have led to the widespread use of this genus in integrated pest management programs. These characteristics include the ease of rearing on alternative hosts (Parra 2010a; Díaz et al. 2012); parasitism of the pest's egg stage and before the pest can damage crops (Ulrichs & Mewis 2004; Gardner et al. 2011); highly aggressive parasitism of various species of lepidopteran pests (Botelho 1997); and a wide geographic distribution (Pinto 2006).

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The key to the success of *Trichogramma*-based biological control is to use adequate native species, if possible, to reduce the population of specific pests (Hassan 1994), as native parasitoids are well adapted to their natural environments. In light of this premise, population surveys of pest insects and their natural enemies provide information about the local fauna and help prevent a leading cause of failure of biological control programs, namely, lack of knowledge of existing populations (Hassan 1994; Smith 1996; Pinto 1999).

The taxonomy of *Trichogramma* is fundamentally important for the maintenance of these natural enemies in agricultural areas and for the establishment of integrated pest management programs that use these wasps as agents of biological control. Few studies on the native species of *Trichogramma* in the State of Minas Gerais, Brazil, have been published (Murta et al. 2008; Zanuncio et al. 2009; Macedo-Reis et al. 2013). The northern region of Minas Gerais has a semiarid climate and a diverse range of agricultural crops, and the composition of its native fauna is not completely known.

The objective of this work was to identify the species of *Trichogramma* that are naturally associated with eggs of pest lepidopterans on 8 agricultural crop species and 1 invasive species in the semiarid region of Minas Gerais, Brazil. The 9 species were as follows: collards, *Brassica oleracea* L. (Brassicales: Brassicaceae); papaya, *Carica papaya* L. (Capparales: Caricaceae); tomato, *Lycopersicon esculentum* Mill. (Solanales: Solanaceae); cassava, *Manihot esculenta* Crantz (Malpighiales: Euphorbiaceae); banana, *Musa* sp. L. (Zingiberales: Musaceae); passion fruit, *Passiflora* sp. Degener (Malpighiales: Passifloraceae); sugarcane, *Saccharum* sp. L. (Poales: Poaceae); and corn (maize), *Zea mays* L. (Poales: Poaceae); and an invasive species (Sodom's apple milkweed, *Calotropis procera* Aiton [Gentianales: Apocynaceae]). The overall aim of this study was to provide information for the development of pest management initiatives in the region.

Materials and Methods

Random qualitative collections were made in the municipalities of Jaíba, Janaúba, Nova Porteirinha, and Porteirinha, located in the semiarid region of the state of Minas Gerais, Brazil. The vegetation at these locations consists of transition areas between the Caatinga and Cerrado biomes. The vegetation observed in this ecotone is a typical Dry Forest (Drummond et al. 2005), also known as Deciduous Seasonal Forest. Dry Forests are characterized by a predominantly deciduous stratum and are observed in areas with 2 well-defined seasons, namely, a rainy season and an extended dry season (Veloso et al. 1991).

The collections were made by visual inspection for eggs or egg masses of pest lepidopterans on 9 plant species. The plants and pest species were as follows: collards – *Ascia monuste orseis* (Latreille) (Lepidoptera: Pieridae); Sodom's apple – *Danaus* sp. (L.) (Lepidoptera: Nymphalidae); papaya – *Protambulyx strigilis* L. (Lepidoptera: Sphingidae); cassava – *Erinnyis ello* L. (Lepidoptera: Sphingidae); banana – *Antichloris eriphia* F. (Lepidoptera: Arctiidae); passion fruit – *Agraulis vanillae vanillae* (L.) (Lepidoptera: Nymphalidae) and *Dione juno juno* (Cramer) (Lepidoptera: Nymphalidae); sugarcane – *Diatraea saccharalis* F. (Lepidoptera: Crambidae); tomato – *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae); and corn – *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae). The collections were made between Dec 2007 and Oct 2008. The agricultural crop species to be sampled were chosen according to their economic importance to the region and their availability during the sampling period.

The selected plants were inspected for a period of 20 min; when lepidopteran eggs or egg masses were observed, they were collected along with part of the inspected plant. The collected eggs or egg masses of these lepidopterans were stored in labeled paper bags and transported to the Entomology Laboratory at the Montes Claros State University (UNIMONTES) campus in the city of Janaúba, Minas Gerais.

The collected eggs or egg masses of pest insects were quantified and each individual egg mass was considered to be an egg unit. These eggs were stored in glass containers covered with plastic wrap and were maintained in the laboratory under room conditions. Observations were made on a daily basis until the lepidopteran larvae or the parasitoid wasps had emerged. The percentage of parasitism of each host insect species was calculated as the ratio of parasitized eggs or egg masses to the total number of collected eggs or egg masses (number of parasitized eggs × 100 / total number either of collected individually laid eggs or of collected egg masses).

The emerged parasitoids were counted and sexed, and the males were preserved in 70% ethanol for species identification. The females were preserved in 90% ethanol. Species identification of *Trichogramma* is based on the morphological characteristics of males. Therefore, the collected females were identified only if they had emerged together with males from the same host egg. In these situations, all individuals were assumed to belong to the same species. The identification to species was not performed when only females emerged.

The specimens of *Trichogramma* were mounted in Hoyer's medium on microscope slides (Querino & Zucchi 2002, 2011). Species identification was based on characteristics of the genitalia, antennae, and wings of males, using an illustrated identification key for *Trichogramma* species of Brazil (Querino & Zucchi 2005). The collected specimens were deposited in the collection of the Entomology Laboratory at UNIMONTES.

Results

In total, 2,242 specimens of *Trichogramma* (1,712 females and 530 males) were obtained from eggs of lepidopterans collected on the above mentioned agricultural crops and on the 1 invasive species in Minas Gerais State (Table 1). Four species of the parasitoid wasps were identified, i.e., *T. pretiosum* Riley; *T. manicobai* Brun, Moraes & Soares; *T. marandobai* Brun, Moraes & Soares; and *T. galloi* Zucchi. The species *T. pretiosum* was obtained from the eggs of all sampled species of Lepidoptera that were found to be hosts to parasitoids, with the exception of *A. eriphia* on banana in which case the parasitoid species found could not be identified.

Natural parasitism by *Trichogramma* was observed in the lepidopteran eggs collected on 6 plant species in the sampled agricultural ecosystems (Table 1), namely, passion fruit, banana, Sodom's apple, sugarcane, cassava, and papaya. Eggs of *A. vanillae vanillae*, *A. eriphia*, *Danaus* sp., *D. saccharalis* (egg masses), *E. ello*, and *P. strigilis*, respectively, were collected from these plant species. In contrast, no eggs of *A. monuste orseis* and *T. absoluta* or egg masses of *D. juno juno* and *S. frugiperda* were found on collards, tomato, passion fruit, and corn, respectively.

The largest number of *Trichogramma* species was obtained from eggs of *E. ello* on cassava (Table 1). We observed *T. pretiosum*, *T. manicobai*, and *T. marandobai* in association with this host. Two species, *T. galloi* and *T. pretiosum*, parasitized the same egg mass of *D. saccharalis* on sugarcane. The lepidopteran *A. vanillae vanillae* was parasitized by *T. pretiosum* on passion fruit. On papaya, eggs of *P. strigilis* were parasitized by *T. pretiosum* and *T. manicobai*. Both of these *Trichogramma* species parasitized eggs of *Danaus* sp. on Sodom's apple milkweed. This is the first report of parasitism by *T. manicobai* of eggs of *Danaus* sp. and *P. strigilis*. *Trichogramma* specimens that emerged from eggs of *A. eriphia* collected on banana were not identified because of the absence of males.

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Table 1. Plant species sampled for assessment of parasitism by *Trichogramma* wasps in eggs of pest lepidopterans in the semiarid region of the state of Minas Gerais, Brazil, during Dec 2007 to Oct 2008.

			No. of eggs		*Trichogramma*				
Host plant	Host insect	No. of collections	Collected	Parasitized	Species	Total no.	Females	Males	No. of individuals per egg
Brassica oleracea (collards)	*Ascia monuste orseis*	—	—	—	—	—	—	—	—
Calotropis procera (Sodom's apple)	*Danaus* sp.	17	17	6	*T. pretiosum*	8	7	1	5.33
					T. manicobai	8	7	1	
					Trichogramma sp.[a]	16	14	2	
Carica papaya (papaya)	*Protambulyx strigilis*	24	70	16	*T. pretiosum*	82	66	16	13.38
					T. manicobai	121	109	12	
					Trichogramma sp.[a]	11	11	0	
Lycopersicon esculentum (tomato)	*Tuta absoluta*	—	—	—	—	—	—	—	—
Manihot esculenta (cassava)	*Erinnyis ello*	22	85	37	*T. pretiosum*	21	16	5	17.08
					T. manicobai	93	88	5	
					T. marandobai	465	398	67	
					Trichogramma sp.[a]	53	49	4	
Musa sp. (banana)	*Antichloris eriphia*	22	16	1	*Trichogramma* sp.[a]	7	7	0	7.00
Passiflora sp. (passion fruit)	*Agraulis vanillae vanillae*	10	31	9	*T. pretiosum*	47	37	10	6.78
					Trichogramma sp.[a]	14	12	2	
	Dione juno juno	—	—	—	—	—	—	—	—
Saccharum sp. (sugarcane)	*Diatraea saccharalis*	18	52	52	*T. pretiosum*	26	19	7	24.92[b]
					T. galloi	1,016	715	301	
					T. galloi, *T. pretiosum*	223	130	93	
					Trichogramma sp.[a]	31	27	4	
Zea mays (corn)	*Spodoptera frugiperda*	—	—	—	—	—	—	—	—
Total	**—**	**113**	**271**	**121**	**—**	**2,242**	**1,712**	**530**	**—**

[a] *Trichogramma* sp. specimens were females or damaged parasitoids.
[b] Number of individuals of *Trichogramma* per egg mass of *Diatraea saccharalis*.

Table 2. Percentage of natural parasitism of pest lepidopteran eggs by *Trichogramma* wasps in the semiarid region of the state of Minas Gerais, Brazil, during Dec 2007 to Oct 2008.

		Eggs or egg masses		
Host plant	Host insect	Total no.	Parasitized	% Parasitism
Calotropis procera (Sodom's apple)	*Danaus* sp.	17	6	35.3
Carica papaya (papaya)	*Protambulyx strigilis*	70	16	22.9
Manihot esculenta (cassava)	*Erinnyis ello*	85	37	43.5
Musa sp. (banana)	*Antichloris eriphia*	16	1	6.3
Passiflora sp. (passion fruit)	*Agraulis vanillae vanillae*	31	9	29.0
Saccharum sp. (sugarcane)	*Diatraea saccharalis*	52	52	100.0

The number of parasitoid adults that emerged per egg of the host insects ranged from 5.33 to 17.08 (Table 1). An average of 24.92 parasitoids emerged per egg mass of *D. saccharalis*. The average number of *Trichogramma* adults obtained from eggs of *D. saccharalis* was assessed per egg mass. On average, 11 eggs of *D. saccharalis* were counted per egg mass of this host pest.

In the semiarid region of Minas Gerais, the percentage of natural parasitism of individual eggs of species that lay each egg individually ranged from 6.3% for *A. eriphia* on banana to 43.5% for *E. ello* on cassava (Table 2). However, in the same region, 100% of the egg masses of *D. saccharalis* on sugarcane were parasitized (Table 2).

Discussion

Parasitism by *Trichogramma* was observed in fields planted with fruit and vegetable crops in Minas Gerais State, as had been observed in other studies in *Eucalyptus*-growing areas (Zanuncio et al. 2009; Macedo-Reis et al. 2013). Natural parasitism of pest Lepidoptera eggs by *Trichogramma* species was verified in 6 of 9 of the plant species in the sampled agricultural ecosystems. This result shows the importance of *Trichogramma* species in the natural biological control of Lepidoptera pests (Zanuncio et al. 2009). In addition, the mass rearing and the use of *Trichogramma* for the biocontrol of host-pests began in Brazil over 30 yr ago with excellent results for several crops (Parra 2010b).

Trichogramma pretiosum emerged from the eggs of the largest number of pest lepidopteran species. This result can be explained by the generalist behavior of *T. pretiosum* (Li 1994), which is widely distributed in all countries of South America (Zucchi et al. 2010) and has been reported in association with over 240 host species (Pinto 1999). Among the species of lepidopterans reported here, *A. vanillae vanillae*, *Danaus* sp., *D. saccharalis*, and *E. ello* had already been reported as hosts of *T. pretiosum*; however, this is the first report of *T. pretiosum* as a parasitoid of *P. strigilis*.

A substantial number of *Trichogramma* species was obtained from eggs of *E. ello* on cassava. In addition, *T. atopovirilia* Oatman & Platner and *T. demoraesi* Nagaraja had already been reported in association with *E. ello* in Brazil (Zucchi et al. 2010). The parasitism of *P. strigilis* and *Danaus* sp. eggs by *T. manicobai* was unknown before this study. Prior to this study, the lepidopteran *E. ello* was the only reported host of *T. manicobai* (Zucchi et al. 2010).

The emergence of *T. pretiosum* and *T. galloi* from the same egg mass of *D. saccharalis* in sugarcane was verified. The species *T. galloi* is the most widely used species in this agricultural crop, and is released over about 300,000 ha of sugarcane each year in order to control the sugarcane borer (Parra 2010b). In addition to these 2 species, other species of *Trichogramma* have already been associated with *D. saccharalis*, namely, *T. dissimilis* Zucchi, *T. distinctum* Zucchi, and *T. jalmirezi* Zucchi (Zucchi & Monteiro 1997). *Trichogramma pretiosum* and *T. galloi* show great potential for use in biological control on several crops in Brazil, and their ease of rearing in the laboratory enhances their potential for even wider use as natural enemies (Parra & Zucchi 2004).

The variation in number of *Trichogramma* specimens obtained per egg or egg mass of pest lepidopterans, as well as the varying percentages of parasitism, may be related to the specific morphological characteristics of each host egg (Sa & Parra 1994), the size and age of the host eggs (Beserra et al. 2002; Brotodjojo & Walter 2006), and the quality of the eggs for parasitoid development (Roriz et al. 2006). Parasitism rates by *Trichogramma* parasitoids, which are usually recorded in the field, may often underestimate the total mortality induced in the host populations (Tabone et al. 2010).

The natural parasitism of eggs of lepidopterans by *Trichogramma* species in various highly profitable agricultural and horticultural crops and the substantial percentages of biological control achieved against some species of pest lepidopterans in northern Minas Gerais provide important information about the interactions between natural enemies and host insects and plants in this region. In addition, the natural occurrence of *Trichogramma* suggests that these parasitoids may be well adapted to this environment. Consequently, the species of this genus may be considered as candidates for inclusion in integrated pest management programs especially in arid and semiarid tropical and subtropical regions.

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