[Idesia (Arica)]

versión On-line ISSN 0718-3429

Idesia vol.35 no.2 Arica jun. 2017 Epub 13-Mayo-2017

http://dx.doi.org/10.4067/S0718-34292017005000015

Parasitoids of *Neoleucinodes elegantalis* Gueneé (Lepidoptera: Crambidae) in Ecuador

Parasitoides de Neoleucinodes elegantalis *Geneé (Lepidóptera: Cambidae) en Ecuador*

Michelle Noboa[1], Ana Díaz[2], Wilson Vásquez[3], William Viera[1*]

[1] Programa de Fruticultura, INIAP, Av. Interoceánica km 14, Tumbaco, Ecuador.
[2] Centro de Investigación La Selva, CORPOICA, km 7 vía Las Palmas, Antioquia, Colombia.
[3] Facultad de Ingeniería y ciencias Agropecuarias, UDLA, Av. Granados, Quito, Ecuador. * Corresponding author: William.viera@iniap.gob.ec

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ABSTRACT

Copidosoma sp. (Hymenoptera: Encyrtidae), *Lyxophaga* sp. (Diptera: Tachinidae), *Bracon* sp. (Hymenoptera: Braconidae) and *Chelonus* sp. (Hymenoptera: Braconidae) are reported for the first time infecting pupae of the fruit borer *Neoleucinodes elegan-talis* (Gueneé) (Lepidoptera: Crambidae) from *Solanum quitoense* and *Solanum betaceum* in Ecuador. Determining the natural enemies in Ecuador useful in sustainable biological control strategies for integrated management of *N. elegantalis* in fruit crops.

Key words: Encyrtidae, Braconidae, Tachinidae, Hymenoptera, fruit borer, Solanaceae.

RESUMEN

Copidosoma *sp. (Hymenoptera: Encyrtidae),* Lyxophaga *sp. (Diptera: Tachinidae),* Bracon *sp. (Hymenoptera: Braconidae) y* Chelonus *sp. (Hymenoptera: Braconidae) son reportados por primera vez infectando pupas del barrenador del fruto* Neoleucinodes elegantalis *(Gueneé) (Lepidoptera: Crambidae) de* Solanum quitoense *and* Solanum betaceum *en Ecuador. Identificándose enemigos naturales* *en Ecuador para incorporarlos en estrategias de control biológico sustentable para un manejo integrado de* N. elegantalis *en cultivos frutícolas.*

Palabras clave: *Encyrtidae, Braconidae, Tachinidae, Hymenoptera, perforador del fruto, Solanaceae.*

Introduction

Neoleucinodes elegantalis (Guneeé) (Lepidoptera: Crambidae) is a moth native to Neotropical regions of South America (Capps, 1948), where it is an important pest of Solanaceae crops like: *Solanum lycopersicum, Solanum melongena, Solanum betaceum, Solanum quitoense, Solanum capsicum* (Díaz & Brochero, 2012). In Ecuador, this pest is present wherever naranjilla (S. *quitoense)* is cultivated. The larvae damage the fruit pulp directly boring in to fruit and feeding on the mesocarp, producing galleries of approximately 3 cm inside the developing berries. The objective of this research was to identify parasitoids of *N. elegantalis* in two Solanaceae hosts (S. *quitoense* and *S. betaceum)* in Ecuador to determine the incidence of parasitism and understand the degree of host specificity among these Solanaceae fruit crops, where this pest and the parasitoid are present.

Materials and Methods

Study sites: Sampling was carried out in seven representative localities, in six provinces, where *S. quitoense* is grown (Figure 1). In each of these sites, samples of *S. betaceum* infected with *N. elegantalis* were also collected. These zones are described by the Holdridge system (Holdridge, 1967) and corresponded to premontane humid forest (bh-PM), very humid premontane forest (bmh-PM); tropical humid forest (bh-T) and premontane pluvial forest (pb-PM) (Table 1).

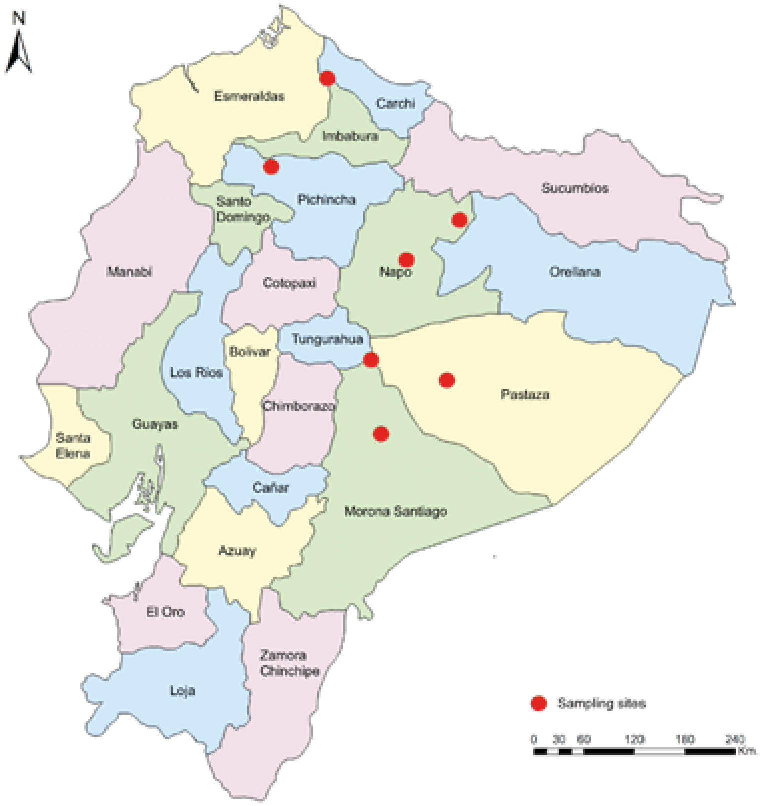

Figure 1. Sampling sites in six provinces of Ecuador.

Table 1. Sites for sampling of fruit infected by fruit borer *(N. elegantalis)* in Ecuador.

Province	Life zone*	Altitude (masl)	Coordenates
Pichincha	bmh-PM	681	S 0º 00'83" W 78º 53'40"
Napo	bh-PM	1,600	S 0º 21'57" W 77º 48'69"
Tungurahua	bmh-PM	1,242	S 1º 24'94" W 78º 12'12"
Napo	bh-T	615	S 0º 53'76' W 77º 46'16"
Carchi	bh-PM	814	N 0º 50'24" W 78º 22'32"
Morona Santiago	bp-PM	1,123	S 1º 27'38" W 78º 08'35"
Pastaza	bp-PM	1,016	S 1º 40'24" W 77º 57'80"

Collection of samples and insect rearing: During all 2014 and the first trimester of 2015, fruits infected with *N. elegantalis* were collected from *S. quitoense* and *S. betaceum.* Fruits were placed in growth chambers with controlled environmental conditions (temperature of 22 ± 1°C, 55% relative humidity and a photoperiod of 12 hours of light) to allow the emergence of adults of both *N. elegantalis* and the other possible parasitoids. Fruits were left inside the chambers until adults emergedand then they were counted. Collected specimens were mounted using entomology pins and stored following standard entomological procedures. Microhomoptera specimens were preserved in entomological vials with alcohol at 70%; while Dipterans and major Hymenoptera specimens were mounted in white cardboard triangles (Diaz & Brochero 2012).

Counting specimens: Parasitoids were counted per individuals of *N. elegantalis* and are presented as proportions of parasitoids infecting *N. elegantalis* individuals.

Taxonomic identification: Taxonomic identifications were carried out by specialists for each family using taxonomic key guides. Felipe Vivallo from the Natural History

Museum of Quinta da Boa Vista in Río de Janeiro (Brazil) identified samples of the Order Hymenoptera, Family Braconidae; Irene Ávila from Corpoica (Colombia) for the Order Hymenoptera, Family Encyrtidae; and Nancy Carrejo from University of Valle (Colombia) for the Order Diptera, Family Tachinidae. Identifications were done just until the insect gender because to reach species level molecular analyzes are needed.

Results and Discussion

Four genera of parasitoids *(Copidosoma* sp., *Lyxophaga* sp., *Bracon* sp. and *Chelonus* sp.) were associated with populations of *N. elegantalis* collected from the two Solanaceae hosts in the different provinces of Ecuador (Table 2).

Table 2. Parasitoids found in *N. elegantalis* associated with the two Solanaceae host plant species.

Parasitoids		Percentage of parasitism (%) [a]	Number of individuals paracitized (n=15)	Province	Host plant
Hymenoptera: Encyrtidae	*Copidosoma* sp.	27.5	4	Pichincha	*Solanum quitoense*
		35.0	5		*Solanum betaceum*
		70.2	10	Napo	*Solanum quitoense*
		31.3	5	Tungurahua	
Hymenoptera: Braconidae	*Bracon* sp.	5.8	1	Pichincha	*Solanum betaceum*
		8.2	1		*Solanum quitoense*
		26.6	4	Tungurahua	*Solanum quitoense*
		10.3	2	Napo	
	Chelonus sp.	12.5	12	Carchi	*Solanum quitoense*
		81.2	3	Carchi	
		18.3	1	Napo	
Diptera: Tachinidae	*Lixophaga* sp.	8.2	2	M. Santiago	*Solanum quitoense*
		9.8	1	Carchi	
		6.3	1	Tungurahua	
		4.3	6	Pastaza	*Solanum betaceum*

Copidosoma sp. (Hymenoptera: *Encyrtidae)* (Figure 3A), was found in pre-pupae of *N. elegantalis* in both *S. quitoense* and *S betaceum* in Pichincha and in *S. quitoense* only in Napo and Tungurahua provinces. Overall parasitism of 41.0% was observed (Table 2), with an average of 123 adult parasitoids recovered per larva of *N. elegantalis.* The highest percentage of parasitism (70.2%) was found in infected fruit of *S. quitoense* in Napo. Parasitism of larvae of *S. betaceum* was found in Pichincha only (Table 2). Symptoms of parasitism were visible in the final states of larvae. Initially the larvae were cream colored, but later changed to gray-brown when *Copidosoma* was in the imago/adult stage (Figure 4 A, B). This micro-hymenopteran has polyembrionic reproduction and can give rise to progeny of the same sex (Ode *et al.,* 2004). In this study, we found that this parasitoid has a wide geographical distribution (Pichincha, Tungurahua and Napo), increasing its potential value for the establishment of a biological control program.

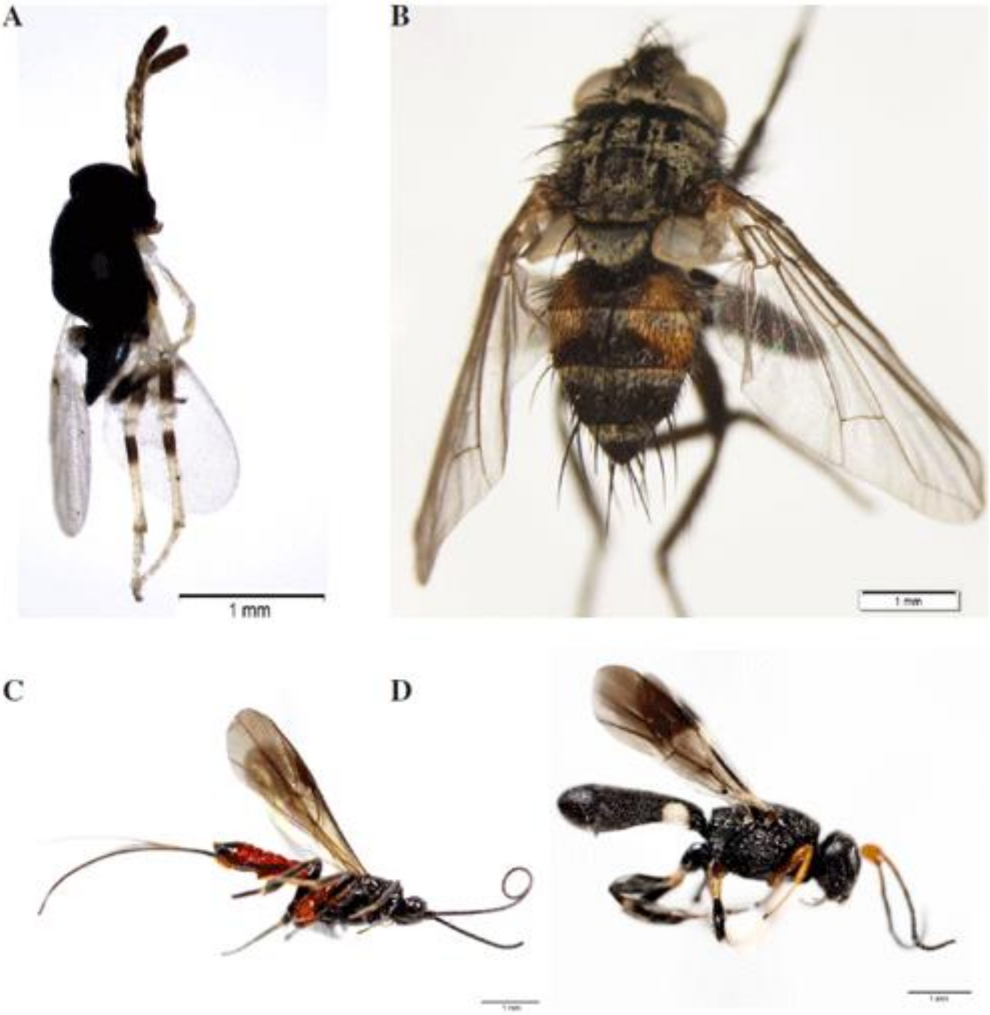

Figure. 3. A. *Copidosoma* sp. (Hymenoptera: Encyrtidae) B. *Lyxophaga* sp. (Diptera: Tachinidae) C. *Bracon* sp. (Hymenoptera: Braconidae) D. *Chelonus* sp. (Hymenoptera: Braconidae).

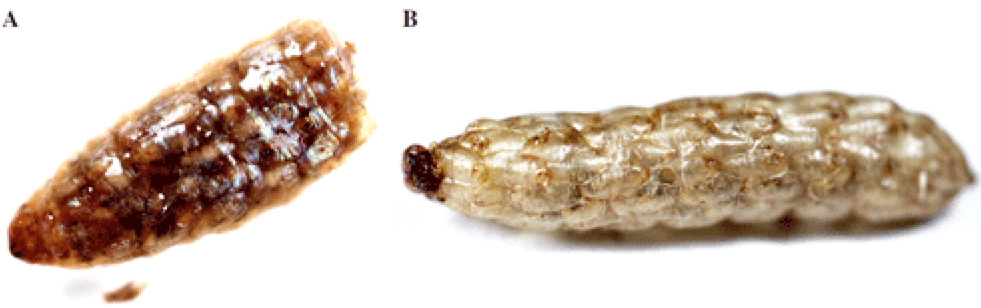

Figure 4. A. Copidosoma adults within N. elegantalis larva, B. Empty larva of N. elegantalis at the final of Copidosoma sp. pa-rasitism process.

The genus *Bracon* (Figure 3C), belonging to the subfamily Braconinae, are idiobiont ectoparasitoids of larvae of Lepidoptera and Coleoptera (Quicke, 1997). Generally, all members are synovigenic, meaning that the parasitoid can produce eggs throughout the adult stage. *Bracon* species are either solitary or gregarious parasitioids that, prior to oviposition, paralyze the host by injecting a poison that is synergized by association with viral endosymbionts (Quicke, 1997). In the field, it was observed that *Bracon* females oviposited its larva into *N. elegantalis,* inside the fruit of *S. quitoense* (Figure 2), through antennal patters on the surface of the infected fruit. Detection of *N. elegantalis* larvae by the parasitoid is achieved by antennal modifications is known as "basins of percussion". However, in other species, vibrations made by *N. elegantalis* may be perceived by the female parasitoid through extensions of the subgenitals organs that are located in the posterior tibia (Díaz & Brochero, 2012). The female parasitoid introduces its elongated and sclerotized ovipositor through the epidermis of the fruit to place its eggs inside or over the larvae host (Fernández & Sharkey, 2006). In this study parasitism by the genus *Bracon* reached an average of 12.7% of the pupa of *N. elegantalis* infected fruit from both host plants (Table 2).

Figure 2. *Bracon* sp. feeling the movements of *N. elegantalis* larvae with her antennas.

Chelonus sp. (Figure 3D) was also found as larvae-pupae parasitoid of *N. elegantalis* (Díaz & Brochero, 2012), which means that the parasitoid begins its colonization in the larval stage of *N. elegantalis* and emerges as adult from the pupal state, thus interrupting the life cycle of the insect pest. This species belongs to the subfamily Cheloninae (Shaw, 1983). They are solitary koinobiont endoparasitoids of eggs and larvae of Lepidoptera, especially of borers or tunneling insects of stalks, buds or fruits such as of the superfamilies Tortricoidea and Pyraloidea (Shenefelt, 1973). Some species attack exposed eggs of their host, while other species with long ovipositor attack hosts in cryptic or hidden locations (Shaw, 1983). This parasitoid showed an average parasitism of 37.3% in larvae of *N. elegantalis* collected from fruit of *S. quitoense* in the provinces of Carchi and Napo (Table 2).

The Dipteran parasitoid *Lyxophaga* sp. (Diptera: Tachinidae) (Figure 3B) were recovered from *N. elegantalis* in rearing cages. The average parasitism was 7.1% (Table 2). This parasitoid was found only attacking *N. elegantalis* larvae collected from *S. quitoense* in the provinces of Carchi, Tungurahua and Pastaza. Díaz and Brochero (2012) observed that the female of this parasitoid locates its host in infected fruit still on the plant and attracted by the odor of the excrement of *N. elegantalis.* It is likely that *Lyxophaga* sp. places its eggs in the excrement so that neonate larvae can enter the fruit through feeding galleries to attack the larva of *N. elegantalis* (Díaz & Brochero, 2012). This genus has been widely used for biological control as the parasitoids can be multiplied in large quantities in rearing facilities for mass release in the field (Carrejo *et al.,* 2013).

Natural enemies of *N. elegantalis* are diverse, abundant and prevalent in some crops (Díaz & Brochero, 2012). The knowledge about these parasitoids is still scarce. However, this paper represents the beginning of the study of the interactions between these four parasitoids and *N. elegantalis.* Knowledge on the ecology of each parasitoid would help to develop biological control strategies that can be incorporated into an integrated pest management program. Biological control could also be enhanced by mass rearing parasitoids in the laboratory for massive release (Wang *et al.,* 2014).

Altieri (1999) stated that diverse agro-ecosytems provide many potential resources to natural enemies of crop pests. These include on or in the hosts themselves, prey of shortage pests, food (pollen and nectar) for the adult parasitoids and predators, shelters for hibernation, nesting, and maintenance of acceptable populations of the pest for extended periods, to ensure the continued survival of these beneficial insects. Thus, an adequate management of the habitat (Simpson *et al.,* 2011) such as the manipulation of weeds (Asteraceae, Poaceae and Cyperaceae) with flowers as sources of food or refuge for these parasitoids (Viera *et al.,* 2015) is important to facilitate or enhance biological control of pest species.

Conclusions

The diversity and wide geographic distribution of parasitoids of *N. elegantalis* shows their potential for biological control of the *N. elegantalis* attacking *S. quitoense* (naranjilla) and *S. betaceum* (tree tomato) crops in Ecuador. The greatest diversity of parasitoid species infecting *N. elegantalis* was found on *S. quitoense,* while micro-hymenopteran *Copidosoma* sp. was the most common parasitoid found on *S. betaceum. Copidosoma* sp. are potentially suitable for mass rearing and inundative releases because of its wide distribution in Ecuador's different climatic zones. In addition, the implementation of this biological control strategy would reduce pesticide use in naranjilla crops.

Acknowledgments

The authors want to thank to the Fondo Regional de Tecnología Agropecuario (FONTAGRO) that through the Colombian Corporation of Agricultural Research (CORPOICA) financed this research by the project: "Fundamentals for the development of biological control strategies of the fruit borer *Neoleucinodes elegantalis* in exotic Andean Solanceae fruits". In addition, we would like to thank to Dr. Saulo Soria (Prometeo) who collaborated with this research and Dr. Charles W. Barnes of the Instituto Nacional de Investigaciones Agropecuarias for editorial comments and suggestions.

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Fecha de Recepción: 3 Noviembre, 2016. Fecha de Aceptación: 5 Abril, 2017.