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Artículos

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Complejo de perforadores: *Ecdytolopha fabivora* (Meyrick), *Cydia deshaisiana* (Lucas) y *Cydia pomonella* (L.) en cultivo de frijol criollo (*Phaseolus lunatus* L.) y canavalia (*Canavalia ensiformis* L.) en México

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Abstract: Backyard crops of creole bean (*Phaseolus lunatus* L.) and canavalia (*Canavalia ensiformis* L.) were monitored in the town of Cerro Gordo, municipality of Emiliano Zapata, State of Veracruz, from September 2015 to July 2016. Symptoms of hyperplasia were found in stems and pods, caused by a complex of borers from the Tortricidae family. The morphological identification of the adult specimens by characteristics of genitalia and chaetotaxy of the larvae, as well as molecular biology identification, showed that the specimens found in beans belong to two species, *Cydia deshaisiana* (Lucas) and *Ecdytolopha fabivora* (Meyrick). Those found in canavalia belonged to *C. deshaisiana* and *Cydia pomonella* (L.), being the first report of *C. pomonella* in *C. ensiformis*. The sequences were published in the GenBank, under accession codes KX357712 (*E. fabivora*) and MN972449 (*C. deshaisiana*), presenting a coincidence of 96% at genus level, due to the lack of sequences of this species in the data base. Sequence obtained from

C. pomonella specimens were published with accession code MN972450, and presented a 92% similarity with the mentioned species.

Keywords: Fabaceae, Molecular identification, Morphology, Tortricids.

Resumen: Se monitorearon cultivos de traspatio de frijol criollo (*Phaseolus lunatus* L.) y canavalia (*Canavalia ensiformis* L.) en la localidad de Cerro Gordo, municipio de Emiliano Zapata, estado de Veracruz, entre septiembre de 2015 y julio de 2016. Se encontraron síntomas de hiperplasia en tallos y vainas causados por un complejo de perforadores de la familia Tortricidae (Lepidoptera). La identificación morfológica de la genitalia de los ejemplares adultos y quetotaxia de las larvas, así como la identificación por biología molecular de los especímenes encontrados en frijol, demostraron que pertenecen a dos especies, *Cydia deshaisiana* (Lucas) y *Ecdytolopha fabivora* (Meyrick) mientras que los presentes en canavalia correspondieron a *C. deshaisiana* y *Cydia pomonella* (L.) siendo éste el primer reporte de *C. pomonella* en *C. ensiformis*. Las secuencias fueron publicadas en el GenBank bajo los códigos de acceso KX357712 (*E. fabivora*) y MN972449 (*C. deshaisiana*), presentando una coincidencia de 96% a nivel de género, debido a que no existen secuencias de éstas especie en la base de datos. Las secuencias obtenidas de ejemplares de *C. pomonella* fueron publicadas bajo el código MN972450 y presentaron un porcentaje de similitud de 92% con dicha especie.

Palabras clave: Fabaceae, Identificación molecular, Morfología, Tortrícido.

INTRODUCTION

Beans are one of Mexico's basic crops. They are produced practically in almost all regions of the country, soil, and climate conditions. This crop has the second place in importance due to the area sown nationally; 1,676,230 hectares were sown in 2017, with a production of 1,183,868 tons (SIAP, 2018). Mexico, considered one of the centers of origin of beans, is one of the main producing countries. There are about 70 native bean varieties, which are distributed in seven main groups: black, yellow, white, purple, bay, mottled, and pinto, among which *Phaseolus lunatus* L. is found.

The bean crop cultivation is traditional in Mexico, since it represents an important source of employment and income for the peasant economy, and for many years it has been a main source of food, as well as a guarantee of food security, via self-consumption. While in the diet, it represents the main and only source of proteins for broad layers of the Mexican population. This crop is affected by droughts, early frosts, excess of rain out of time and the attack of pests and diseases. These are important factors when we consider that in the last years, 70% of the production was obtained from non-irrigated areas (Clarke, 1972; Alvarado, 2012; Manríquez, 2012).

Another Fabaceae with agro-ecological importance is the canavalia (*Canavalia ensiformis* L.), since its uses range from green manure, cover crop, fodder for livestock, biological control of weeds and some insects, and intercropped in various crops for its ability to fix nitrogen. Its cultivation is not considered economically important, because it has alkaloids that are toxic when consumed in large quantities, so it is a backyard crop (Duke, 1981; Buckles et al., 1998; Caamal-Maldonado et al.,2001; Ayala-Sánchez et al*.,* 2009).

Both, bean and canavalia crops are vulnerable to various types of insect pests, which cause disease or damage to both foliage and fruits. The most

important pests of these crops are due to lepidopterans, which present patterns of oviposition that vary according to each taxon, including genera such as *Cydia* Hübner that has approximately 200 species described worldwide (Horak, 2006), most of them considered pests of fruits of the families Rosaceae, Fabaceae, Pinaceae, Fagaceae, Salicaceae and other families of vascular plants (Brown et al., 2008). They feed on plant tissues such as foliage, pods, cones and seedlings (Thompson & Pellmyr, 1991; Stefanescu et al., 2006; Brown, personal communication). One of the most important species of the family Tortricidae is the Pod borer worm *Ecdytolopha fabivora* (Meyrick). In the larval stage, it destroys the seeds, makes holes in the pods, and its excrement forms agglomerates that cause considerable damage in the crop, therefore producing yield decreasing that affects their commercialization (Cano-Ortíz, 1998; González, 2003; Barcenas et al., 2005).

For this reason, the objective of this study was to identify the borers complex of the Tortricidae family associated with backyard orchards of beans and canavalia in Emiliano Zapata, Veracruz, with the purpose of establishing a precedent in the dispersion and epidemiology of these insect species.

Material and methods

Monitoring took place in the town of Cerro Gordo (19° 26′ 00″ N, 96° 42′ 00″ W; 582 masl), municipality of Emiliano Zapata, State of Veracruz, Mexico.

Collection of specimens

From September 2015 to July 2016, pods stems of plants damaged by larvae were collected every 14 days. Both were collected from approximately 180 bean plants and 80 canavalia plants with symptoms and signs. For collecting, pruning shears were used and the material was placed in paper bags to transport it to the laboratory (Fig. 1).

Fig. 1.a. Hyperplasia on main stem. b. Symptoms in pods.

Morphological identification

Collected pods and stems were processed in the Research Center in Applied Mycology. For this, they were dissected in order to look for the presence of insects inside. Larvae in several instars were found and preserved in 70% ethanol for identification through morphological characteristics, using taxonomic keys, and observing setal maps (MacKay, 1956; Weisman, 1986; Stehr, 1987).

Thirty bean pods and twenty canavalia pods with signs of damage were placed in plastic boxes with perforated lid of 20 x 40 cm on a layer of soil and plant litter at room temperature (27 ± 2 °C), and sealed with Parafilm for maturation of larvae into adults. Once the adult specimens emerged, identification was made by extraction and assembly of genitalia, with the support of the Carl Zeiss Stemi 1000 stereoscopic microscope and the Carl Zeiss Axiostar brightfield compound microscope. Finally, the rest of both adult and larval specimens were preserved in 70% ethanol.

Molecular identification

Total DNA extraction was performed following the methodology proposed by Saldamando & Márquez (2012). A larval segment of each sample previously preserved in 70% ethanol was used. A piece of larva was placed on sterile filter paper, where it was completely ground and then cut, placed in an Eppendorf tube with 400 µl of CTAB Buffer (2x) and incubated at 65 °C for 30 min. Finally, it was centrifuged at 11,000 g for 3 minutes, and the filter paper was removed from the tube, in order to be resuspended in nuclease-free water. Subsequently, the mitochondrial gene Cytochrome Oxidase Subunit I (COI) was amplificated by PCR using two pairs of initiators: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and C1-J1718 (5'-GGAGGATTTGGAAATTGATTAGTGCC-3') and C1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC-3') (Simon et al., 1994). The conditions for the first pair of initiators were 94 °C for 60 s, 4 cycles at 94 °C for 30 s, 45 °C for 90 s and 72 °C for 60 s, followed by 35 cycles at 94 °C for 30 s, 51 °C for 90 s and 72 °C for 60 s, ending with an extension at 72 °C for 5 min. For the second, 94 °C for 60 s followed by 4 cycles at 94 °C for 30 s, 45 °C for 90 s and 72 °C for 60 s. 35 cycles at 94 °C for 30 s, 51 °C for 90 s and 72 °C for 60 s, ending with an extension at 72 °C for 5 min. Finally, the samples were analyzed on a 1.5% agarose gel with ethidium bromide (10 mg / uL) (Sambrook & Russell, 2001) and visualized in a GELDOC EZ UV light photodocumenter, Bio Rad® brand, using Image Lab software.

The amplified fragments were purified with Promega's Wizard® SV Gel and PCR Clean-Up System kit, and sequenced with the Sanger method (GA 3130 Applied Biosystem®) at the National Phytosanitary Reference Center of SENASICA. The sequences were compared and uploaded in the gene bank of the NCBI GenBank.

Results

Collection of specimens

In the dissected bean pods, 139 larvae were found in total of which 34 corresponded to the species *Cydia deshaisiana* (Lucas) and 105 to the species *E. fabivora.* In the pods for the maturation of larvae, 20 adults of *C. deshaisiana* and 16 adults of *E. fabivora* were obtained, respectively. In the case of canavalia, 155 larvae of which 98 corresponded to the *C. deshaisiana* and 57 to *Cydia pomonella* (L.) were found, and 17 and 12 adults were developed from each species found, respectively.

Morphological identification

The three species found presented all the taxonomic characters related to tortricids such as: T1 segment of the larval thorax with three prespiracular setae, the seta L1 is closer to L2 than to L3. None of them presented anal comb (Hinton, 1946).

The morphological characteristics of the genitals of adults of the three species collected, as well as the setals maps of the larvae corresponded, in the case of bean to *E. fabivora* (Figs. 2, 3, 8a) and *C. deshaisiana* (Figs. 4, 5, 8b), and for canavalia to *C. deshaisiana* (Figs. 4, 5) and *C. pomonella* (Figs. 6, 7, 8c).

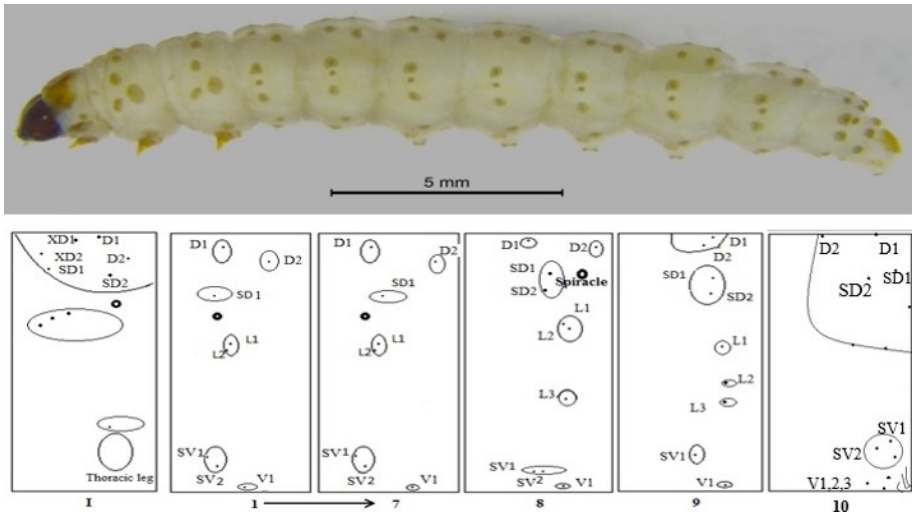

Fig. 2. Larval morphology of *Ecdytolopha fabivora* and setal map.
I: distribution of setae in prothorax; 1-10: setae in abdominal segments from 1 to 7, 8, 9 and 10.

Fig. 3. Genitalia of *Ecdytolopha fabivora*. a. Male. b. Female.

Fig. 4. Larval morphology of *Cydia deshaisiana* and setal map.
I, distribution of setae in the preespiracular and prothorax group; 1-9: setae in abdominal segments from 1 to 7, 8 and 9.

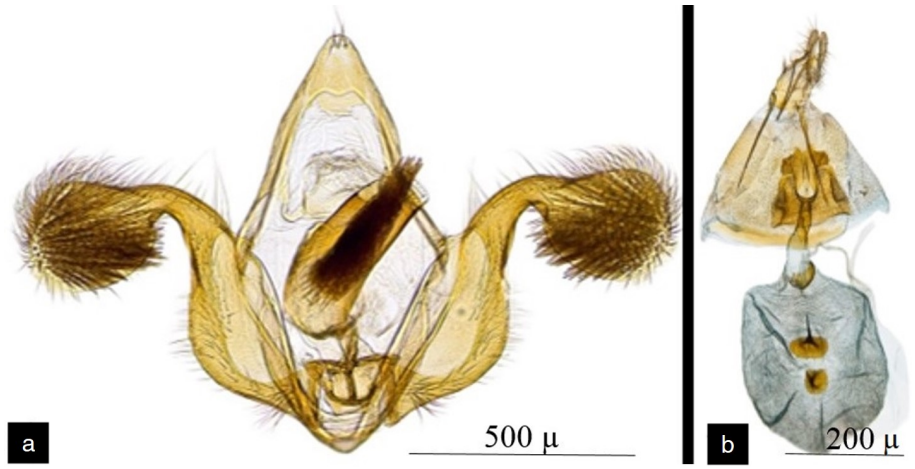

Fig. 5. Genitalia of *Cydia deshaisiana*. a. Male. b. Female.

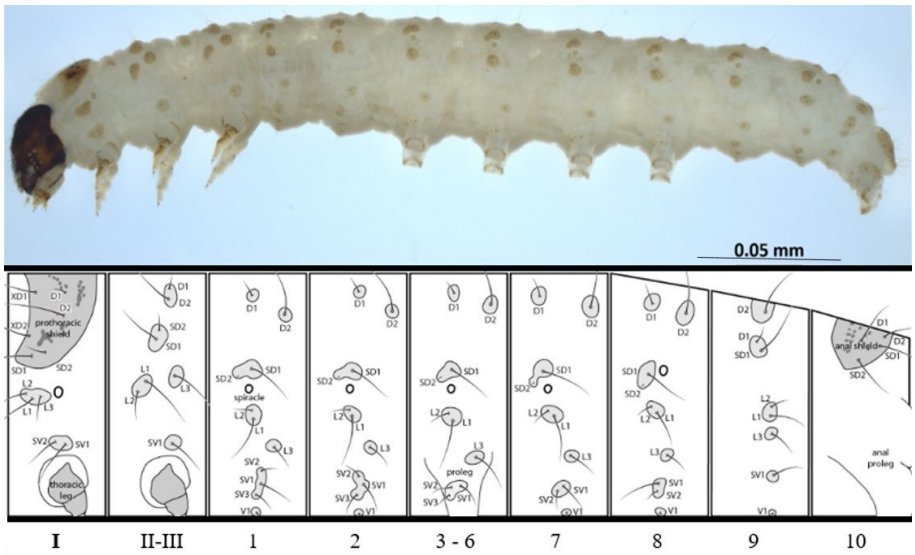

Fig. 6. Larval morphology and setal map of *Cydia pomonella* (from Gilligan & Epstein, 2014).

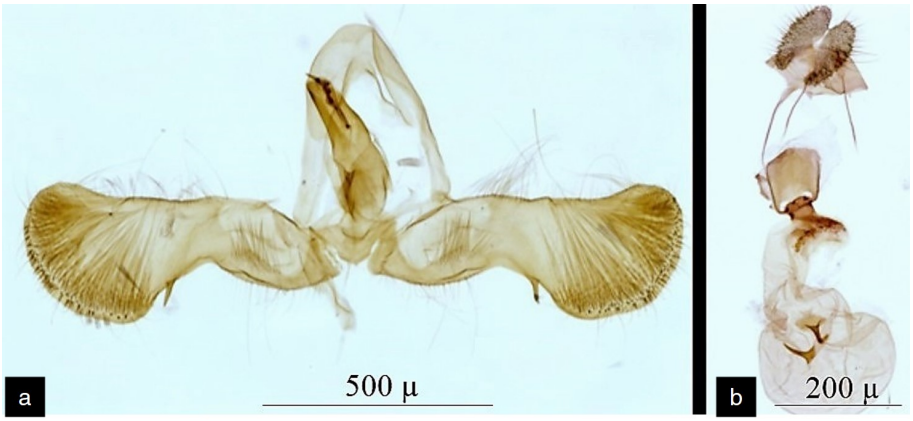

Fig. 7. Genitalia of *Cydia pomonella*. a. Male. b. Female.

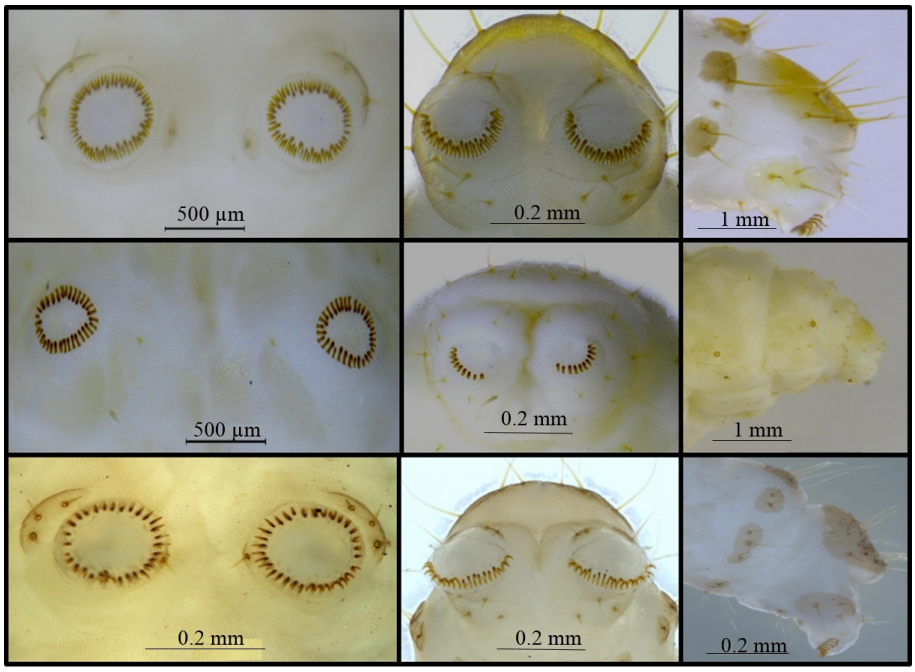

Fig. 8. Distribution of setae in prolegs of the third abdominal segment, anal segment crochets and lateral setae of the 9th abdominal segment. a. *Ecdytolopha fabivora*. b. *Cydia deshaisiana*. c. *Cydia pomonella*.

Molecular identification

Fragments of approximately 700 bp were observed. Regarding C. deshaisiana, it showed a similarity of 96% with the KF402517 at the genus level, since there are no sequences of this species in the database. The specimens of C. pomonella had a 92% similarity with the accession HM874287. The sequences were published in GenBank with accession codes KX35772 (*E. fabivora*), MN972449 (*C. deshaisiana*) and MN972450 (*C. pomonella*).

Discussion

The Tortricidae family includes pests in a wide variety of agricultural, horticultural and forestry crops. However, dicotyledons are the most common hosts (Brown & Adamski, 2003; Brown, 2006; Gilligan & Epstein, 2014). Although it has a cosmopolitan distribution, the family is better adapted in temperate, tropical and subtropical regions (Meijerman & Ulenberg, 2000; Valera-Fuentes et al., 2009). There are 687 species reported worldwide included in 164 genera that are considered pests, which makes this family one of the most economically important among the lepidopterans (Zhang, 1994; Horak, 2006; Lincango, 2015).

In this study we found, in the creole bean cultivation, stems with hyperplasia and perforated pods caused by the species *E. fabivora* and *C. deshaisiana*, the first being reported as an important pest of beans in South America (Clarke, 1958; Brown, 2006). *Ecdytolopha fabivora* is widespread in Central America, in countries such as Costa Rica, El Salvador, Panama, Brazil, Colombia, Ecuador, Peru and Venezuela (CABI, 2007). Meanwhile, in Mexico there are already reports of its presence but there is no formal document that describes and indicates its primary host; therefore, this study is the first to report the species as causing damage to creole beans (*P. lunatus*), in addition to the correct identification by morphological and molecular tests of the three species found.

Cydia deshaisiana is native to Mexico and affects *Sebastiania pavoniana* (Müll.Arg.) Müll.Arg., known as jumping beans, precisely because of the movement of the seed caused by this plague. It is found in the states of Sinaloa and Sonora, which are known as the "Mexican capital of the jumping beans of the world" (Powell & Opler, 2009). In this study, it was found affecting two new hosts, creole bean and canavalia.

Clarke (1958) mentioned *E. fabivora* as an important pest for bean cultivation. He reported that larvae cause considerable damage when drilling stems and pods and cause atrophy of host plants and a reduction in yield. Similarly, Stanly & Sanchez (1990) indicated that the plague could generate a large population and cause damage, since it is capable of completing three generations *per* crop cycle.

It should be noted that larvae of *C. pomonella* were found affecting *C. ensiformis* plants, which is considered as a new host species, since there are no previous reports in this cultivar. The light brown apple moth, as it is known, is distributed in Mexico in the states of Hidalgo, Oaxaca, Queretaro, Tamaulipas and Veracruz. Worldwide, it is widely distributed in the main reception areas, such as Pomaceas (*Malus domestica* Borkh and *Pyrus communis* L.). Although it is reported to affect legumes (*Glicine max* L., *Vicia faba* L.), citrus fruits (*Citrus sinensis* Osbeck), stone fruits (*Prunus persica* (L.) Batsch), and walnut (*Juglans regia* L.), it went from being a secondary host to a primary one (Brown, personal communication; Fernández-Górgola et al., 2010).

The three species found in beans and canavalia belong to the Tortricidae family, which undoubtedly represent a constant problem

and danger in the various crops in the area, and a serious problem in the production of the cultivar attacked, and of course for small rural producers. It is important to mention that the oviposition patterns of Tortricidae females have been studied as ecological indicators of insect-plant interactions (Thompson & Pellmyr, 1991). These depend on environmental factors and availability of resources (Andrade, 1998) as observed in Peru where crops in a normal sowing cycle showed greater affectation by *E. fabivora* (Castillo, 2010).

Another important aspect in dispersion and survival is that the larvae employ a wide range of feeding strategies, from roots and seeds to flowers or even the leaf litter of the plant, although the latter is unusual (Horak & Brown, 1991; Powell et al., 1998; Cepeda & Cubillos, 2011). The great adaptation and plasticity of this family could result in the adoption of a new host, as in the case of *C. pomonella* found in *Magnolia schiedeana* (Schltdl) (Salinas Castro et al., 2014). The larvae of the well-known light brown apple moth were found in floral cones of *M. schiedeana* near the natural reserve of La Martinica, Veracruz, México. Magnolia represents an unusual host for this species of moth, since it is a serious pest of the fruits of the Rosaceae, especially apples.

It is considered necessary to maintain a constant monitoring in the area and its surroundings in order to prevent an accelerated population growth of these pests, because there is a great diversity of cultivars that can be new secondary hosts and even become new primary hosts. Moreover, the climatic conditions required for their development are ideal; species such as *Pachyrhizus erosus* (L.) Urb. and other Fabaceae are cultivated in the area provided the region's producers plant it with the main objective of nourishing the soil.

As previously stated, bean crop cultivation in this town of great importance, since there are small producers that sow in backyard or in small extensions of land and are threatened by the presence of this pest; if it is detected in areas surrounding the area, crops already established in the region could suffer great damage, as it can also attack alternative crops such as *P. erosus*, or *Mucuna* spp., where the damage could reach almost 100%, if not controlled in time. In the case of beans in particular, the damage documented in this work was approximately 72%. For this reason, it is necessary to continue with a more extensive work on the risks that this pest and its possible hosts would represent for the crops of the region.

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