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Identification, Parasitoids, and Population Dynamics of a Blackberry Leafroller (Lepidoptera: Tortricidae) from Michoacán, Mexico

Ignacio López¹, Samuel Pineda², José Isaac Figueroa², José Antonio Sánchez³, Ana Mabel Martínez², Roger N. Williams⁴, and Ángel Rebollar-Alviter¹

Abstract. The blackberry, Rubus sp., crop in the state of Michoacán, Mexico is the second-most important crop after avocado, Persea americana Mill., in relation to value of production and employment. In this study was identified a blackberry leafroller, its parasitoids, and population dynamics in two commercial orchards at Michoacán, Mexico, during the growing seasons of 2007 and 2008. Collected rolled leaves containing larvae and pupae where maintained in a laboratory until leafroller adults and their parasitoids emerged. Population dynamics of moths were determined using wing traps containing the sex pheromone of Argyrotaenia citrana (Fernald) (Lepidoptera: Tortricidae). Numbers of males caught peaked in September and November 2007 at the two locations. After these months, numbers gradually decreased to almost zero during the dry season in 2008. Moths that emerged were identified as Argyrotaenia montezumae (Walsingham) (Lepidoptera: Tortricidae). Of the A. montezumae larvae collected in the field, 38% were parasitized by Apanteles near aristoteliea Viereck (Hymenoptera: Braconidae), 9% by Colpoclypeus michoacanensis Sánchez and Figueroa (Hymenoptera: Eulophidae), and 3% by unidentified specimens of the family Ichneumonidae. Males of A. montezumae were attracted to the sex pheromone of A. citrana. The greatest number of moths trapped during the growing season coincided with the periods of intense vegetative growth and harvest.

Introduction

The blackberry, *Rubus* sp., crop in Mexico is relatively new compared with conventional crops. The first blackberry cultivars were established in the early 1980s, introduced mainly from the United States of America. Since then, the area planted to this crop has significantly increased in the states of Michoacán, Jalisco, Guanajuato, Hidalgo, and Mexico because of demand in national and international markets (Sánchez 2008). Currently, Michoacán is the leading producer and exporter of blackberries in the world; 97% of production in Mexico is on an area of 16,000 ha (SIAP 2012).

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With the increase in the area of blackberry cultivation in the state of Michoacán, phytosanitary problems have increased, causing serious damage to the crop during the growing season. The blackberry leafroller was detected and identified in 1997 as *Argyrotaenia* sp. Stephens 1852 (Lepidoptera: Tortricidae) (unpublished data). In the USA, the most important species of leafrollers on blackberry and other small fruit crops are the orange tortrix, *A. citrana* (Fernald), and the oblique banded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), which also have been reported in Europe and New Zealand (Fisher 1991). Despite the economic importance of leafrollers on small fruit crops, no information has been published in relation to the species of blackberry leafroller in Michoacán, Mexico, its parasitoids, and population dynamics in relation to crop development. The objectives of this study were to identify the species of blackberry leafroller, its parasitoids, and its population dynamics at two locations producing blackberries in the state of Michoacán, Mexico, during the growing seasons of 2007 and 2008.

Materials and Methods

Blackberry Leafroller and its Parasitoids. Larvae of different instars of *Argyrotaenia* sp. were collected starting on 10 October 2007 from rolled leaves of 'Tupy' blackberry plants. Collection was from transects distributed in zigzag sampling schemes in 2-ha commercial plantings near the town of Huatarillo, municipality of Peribán, Michoacán. Shoots approximately 5-7 cm long containing rolled leaves infested with larvae were collected. Blackberry shoots with rolled leaves were placed in ventilated plastic boxes (32 x 22 x 8 cm) and incubated at laboratory conditions (22 \pm 2°C) until pupation. To prevent dehydration of leaves, a moistened cotton ball was used to cover the base of each shoot and replaced every 2 days to prevent fungal growth. Larvae in plastic boxes were checked at 24-hour intervals to collect emerged parasitoids that were preserved in 70% ethanol. These data were used to estimate natural parasitism in the field.

Emerged adults of blackberry leafroller from field-collected larvae were identified to family at the Laboratorio de Entomología (LE) of Instituto de Investigaciones Agropecuarias y Forestales (IIAF) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH). To identify the species, six adults were sent simultaneously to the Systematic Entomology Laboratory, Department of Agriculture, USA; the Society of Lepidopterology Hispano Luso in Madrid, Spain; and Direccion General de Sanidad Vegetal, Mexico. The specimens were deposited in the Collection of the National Museum of Natural History, USA, and in the Natural Museum of History in Madrid, Spain.

Parasitoids that emerged from blackberry leafroller larvae were identified at LE-IIAF-UMSNH and sent for confirmation to the LE of the Centro Interdisciplinario y de Investigación para el Desarrollo Integral Regional of the Instituto Politécnico Nacional (CIIDIR-IPN), Oaxaca, Mexico. All adult parasitoids were deposited in the collection of parasitic Hymenoptera of IIAF-UMSNH.

Monitoring Adult Males. Population dynamics of adult male blackberry leafroller were evaluated from mid-July 2007 until the end of April 2008 in plots near the towns of Atapan, municipality of Los Reyes (19° 39' 8.7474", -102 25' 46.275" West), and Huatarillo, municipality of Periban (19° 34' 1.9554", -102° 27' 33.843"). Three or four wing traps (one trap per hectare) with the sex pheromone of *A. citrana* (Pherocon Trap®, Trece Inc., Salinas, CA) were placed at each location to cover an

area of 3-4 ha on 14 September. The number of males captured was recorded every 10 or 20 days after wing traps were placed in the orchards. The pheromone dispenser was replaced every 30 or 45 days four times during the study at each location. The lures were changed on 28 October and 28 December 2007 and 1 March 2008 at Atapan, and 28 October, 28 November, and 28 December 2007 and 23 February 2008 at Huatarillo. From the numbers of males captured per trap on each date of evaluation, an average count was calculated and plotted to show the population dynamics of males at each location. In addition, to understand the relationship between average counts of captured moths and the amount of rolled leaves (to indicate the presence of larvae and/or pupae) on each sampling date, the number of rolled leaves in 3-m lengths of plant rows were recorded on the same dates as moth catches in pheromone traps. Four sections of plant rows within a radius of 10 m of the trap were randomly selected at each date of evaluation to record the rolled leaves. Data obtained from the traps were averaged to obtain a value for each sampling date at each location. The average catch and number of rolled leaves on each date and location were used for Pearson correlation analysis (SAS 9.3).

Results and Discussion

Identification of Blackberry Leafroller and its Parasitoids. Adults of the blackberry leafroller that emerged from field-collected larvae at Huatarillo, Michoacán were identified as *Argyrotaenia montezumae* (Walsingham) (Lepidoptera: Tortricidae). Of the 105 larvae collected, 38% were parasitized by a species of *Apanteles* near *aristoteliae* Viereck (Hymenoptera: Braconidae), 9% by the new species *Colpoclypeus michoacanensis* Sánchez and Figueroa (Hymenoptera: Eulophidae), and 3% by unidentified specimens of the Ichneumonidae family.

The genus *Argyrotaenia* Stephens 1852 comprises about 88 species distributed from Canada to Argentina, 13 of which are reported in Mexico (Brown and Cramer 1999, Razowski and Becker 2000, Betancourt and Scatoni 2002). Larvae of *A. monezumae* were collected only from leaves at the stage of active growth of the crop. Previously, Obraztsov (1961) reported the presence of *A. montezumae* in the states of Guerrero, Mexico, Puebla, and Veracruz, but did not cite the host. Later, Palmer and Pullen (1995) recorded the species on the shrub verbenas, *Lantana* spp. in Central Mexico (Aguascalientes, Guanajuato, Querétaro, Hidalgo, Puebla, México, Morelos, and Distrito Federal), and more recently, Aguilera-Peña et al. (2008) reported *A. motezumae* in peach (*Prunus persica* L.) orchards of Chihuahua, Mexico, Michoacán, and Zacatecas states attracted to the *A. citrana* pheromone without damage to the crop. The insect has also been reported in Arizona and New Mexico in the USA (www.boldsystems.org, http://mothphotographersgroup.msstate.edu, http://www.tortricidae.com).

The most abundant parasitoids emerged from larvae of *A. montezumae* collected in the field were *C. michoanensis* and *A.* near *aristoteliae*. In the genus *Colpoclypeus* only the species *Colpoclypeus florus* (Walker) (Hymenopera: Eulophidae), a gregarious ectoparasitoid of larvae of family Tortricidae (Brunner 1996, Brunner et al. 2001), has been described. In Europe, this species is considered the main biological control agent in various fruit crops, causing as much as 95% parasitism (Evenhuis 1974, Gruys and Vaal 1984). *Colpoclypeus florus* was introduced to Ontario, Canada, from France and Italy to control the leafrollers.

Ancylis comptana fragariea (Walsh and Riley) and Argyrotaenia velutinana (Walker) (Lepidoptera: Tortricidae) in 1966 and 1967, respectively (Brunner 1996). The same author was the first to report *C. florus* attacking the leafroller *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae) in apple (*Malus* sp.) orchards in Washington State. The discovery of the new species *C. michoacanensis* reported here (Sánchez-García et al. 2011) represents an extension of the geographical distribution of the genera as well as a new host record.

The genus *Apanteles* Foerster includes about 1,000 species distributed in America, 13 of which are in Mexico (Whitfield 1997, Yu et al. 2005). Most *Apanteles* species are solitary parasitoids of larvae of microlepidoptera, but also occur as gregarious parasitoids on macrolepidoptera (Whitfield 1997). The specimens of *Apanteles* found in this study were very near to the *aristoteliae* species; if confirmed, this would represent the first record of the species in Mexico. *Apanteles aristoteliae* is a promising agent for biological control of *Argyrotaenia* spp. The parasitoid parasitized 20 and 33% of larvae of *A. citrana* and the orange tortrix moth, *A. franciscana* (Walsingham), respectively, in apple orchards in California (Walker and Welter 2004). Similar percentage of natural parasitism was found in this study in orchards at Huatarillo, Peribán, Michoacán, Mexico.

Population Dynamics of A. montezumae and Relationship with Rolled Leaves. Adult male A. montezumae were attracted by the sex pheromone of A. citrana, which allowed determination of population dynamics in two commercial blackberry orchards in Michoacán, Mexico. Numbers of males caught peaked in September and November 2007 at Atapan and Huatarillo (Figs. 1, 2). After these months, numbers gradually decreased to almost zero during the dry season in 2008. Besides the presence of the insect during crop development, damage to leaves remained low during the period of sampling, especially at Atapan where adult males were captured until April 2008, but with few or no larvae on leaves, in contrast to Huatarillo where more damage was detected on leaves during crop development. Pearson correlation analysis did not show a significant correlation between the number of adults caught and the presence of rolled leaves at either locality (r = 0.54, P = 0.13 for Atapan; r = 0.32, P = 0.37 for Huatarillo). The tendency did not change when the data from both sampling sites were pooled for analysis (r = 0.28, P = 0.14). As noted in Figs. 1 and 2, the dynamics of A. montezumae were similar at both sampling areas. Peak catches were related to an intense vegetative flush, which occurred after bud break during the months of August and September, and in the harvest period in late October and November. Studies by Rufus and Smucker (1980) using sex pheromones to assess flight activity of A. citrana and C. rosaceana on red raspberry (Rubus idaeus L.) in the Willamette Valley of Oregon indicated that activity of C. rosaceana started in the first week of June, with peak captures between 15 June and 15 July. A year later, the peaks were during the first week of June and early September. Argyrotaenia citrana flight activity began in mid-May and June, indicating June was the critical month, resulting in the first infestation of larvae during the 2 years of study. A second peak was in late August and mid-September. According to Fisher (1991), the number of adult A. citrana peaked during April and mid-May, the first generation after the winter when infestations began on shoots and fruits. Data from our research showed a partial coincidence to the dynamics reported for A. citrana on raspberry in the Willamette Valley, Oregon, especially the second peak (Rufus and Smucker 1980). In Oregon, the harvest of raspberries begins in the third week of June and ends in late July. In contrast, in Michoacán State the blackberry crop is

planned to be harvested from October to December, with a second period from February to May the following year. This kind of harvesting ensures more young shoots, flowers, and fruit are available between mid-August to late October and early November.

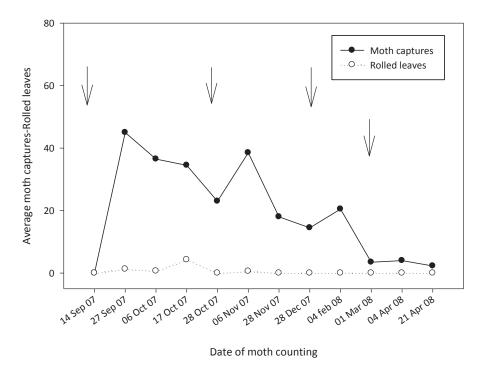


Fig. 1. Population dynamics of moths (males) of *Argyrotaenia montezumae* captured in sex pheromone traps of *Argyrotaenia citrana* and its relation with rolled leaves in a commercial blackberry orchard at Atapan, Los Reyes, Michoacán, Mexico. Solid circles represent numbers of trapped moths and open circles correspond to average rolled leaves per 3 linear meters. Arrows represent dates of pheromone replacement. Season 2007-2008.

As shown in Figs. 1 and 2, the few male *A. montezumae* in the first half of the year almost disappear at places monitored until April the next year (Atapan). Correlation analysis showed the number of rolled leaves was not directly related to the number of moths captured in traps, although on different sampling dates, rolled leaves were not observed but adults were captured in pheromone traps. It is likely that males from adjacent plots were attracted to monitored plots. Walker and Welter (2001) did not find a direct relationship between the number of larvae in rolled leaves and males captured in pheromone traps in apple orchards. For further studies of rolled leaves (presence of larvae and pupae at different stages of crop

development), larvae and pupae should be recorded to determine relationships that allow definition of action thresholds. Based on studies on the dynamics of raspberry leafrollers, Rufus and Smucker (1980) proposed a rudimentary threshold of 20 to 50 moths per trap per week during the peak of flight.

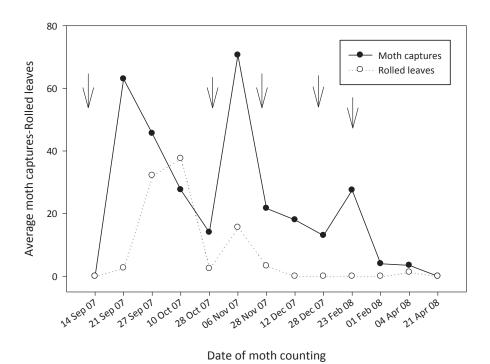


Fig. 2. Population dynamics of moths (males) of *Argyrotaenia montezumae* captured in pheromone traps of *Argyrotaenia citrana* and its relationship with rolled leaves in a blackberry orchard at Huatarillo, Periban, Michoacán, Mexico. Solid circles represent numbers of trapped moths and open circles correspond to average rolled leaves per 3 linear meters. Arrows represent dates of pheromone replacement. Season 2007-2008.

In conclusion, numbers of adult males peaked in September and November. It was interesting that, despite applications of different insecticides to control the insect, 50% parasitism by *A.* near *aristoteliae, C. michoacanensis*, and other unidentified ichneumonid parasitoids was found. Besides the observed biological control, it is important to highlight the effectiveness of the pheromone of *A. citrana* in monitoring *A. montezumae*. This tool for insect monitoring, along with parasitoids and application of biological insecticides, could contribute significantly to integrated management of blackberry leafroller in Michoacán, Mexico.

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