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An unusual food plant for *Cydia pomonella* (Linnaeus) (Lepidoptera, Tortricidae) in Mexico

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ABSTRACT. An unusual food plant for *Cydia pomonella* (Linnaeus) (Lepidoptera, Tortricidae) in Mexico. Larvae of *Cydia pomonella* (Linnaeus, 1758) were discovered on floral cones of *Magnolia schiedeana* (Schltdl, 1864) near the natural reserve of La Martinica, Veracruz, México. *Magnolia* represents an unusual host for this moth species, which is known throughout the world as the "codling moth", a serious pest of fruits of Rosaceae, especially apples. The larvae were identified using taxonomic keys, and identification was corroborated using molecular markers. Further sampling resulted in no additional larvae, hence, the observation was probably that of an ovipositional error by the female, and *M. schiedeana* is not at risk of attack by this important moth pest.

KEYWORDS. Alternative host; codling moth; Insecta; unusual behavior.

As currently defined, the genus *Cydia* includes approximately 200 described species worldwide (Horak 2006), some of which are serious pests of fruit and legumes. Hosts are primarily in Rosaceae, but some species of *Cydia* are specialists on Pinaceae, Fabaceae, Fagaceae, Salicaceae, and a few other families of vascular plants (Brown *et al.* 2008). Among the most notorious pests of pome and stone fruit are *C. pomonella* (Linnaeus, 1758), (Chen & Dorn 2009) and *Cydia lobarzewskii* Nowicki, 1860; among the most important pests of Pinaceae are *Cydia toreuta* Grote, 1873 and *Cydia ingens* Heinrich,1926 (Coulson & Witter 1990; Bédard *et al*. 2002) and important pests of Fagaceae include *Cydia fagiglandana* Zeller, 1841 and *Cydia splendana* Hubner, 1799 (Speranza 1999; Pedrazzoli *et al.* 2012).

Despite their specialized feeding habits, which often are categorized as oligophagous (Pérez-Contreras 1999), variations in ovipositional patterns are frequently observed in Lepidoptera. These variations are considered "ovipositional mistakes" by some authors (e.g., Thompson & Pellmyr 1991; Stefanescu *et al*. 2006; Brown, pers. comm.). This may refer to two main phenomena: (1) oviposition on a host outside the normal range of preferred hosts, and (2) oviposition on a site or substrate that is inappropriate in terms of larval development and/or survival.

Data on oviposition patterns have been used to help understand the evolution of plant-insect relationships (Thompson & Pellmyr 1991; Janz 2002). These relationships are subjected to a wide range of enviromental factors that are undergoing constant transformations. The response capacity of female Lepidoptera to changes in host availability and changes in habitat structure have ocasionally resulted in permanent host shifts and therefore new plant sanitary problems. Because there are reports of *C. pomonella* from hosts outside its "normal range," the possibility of a permanent host shift is always present. *Cydia pomonella* is a cosmopolitan species and one of the most succsesful pests worldwide (Thaler *et al*. 2008). It is believed to be native to southeastern Europe and southwestern Asia, along with its primary host, apple (*Malus domestica,* Borkh,1803) (Shel´Deshova 1967; Chapman 1973).

The purpose of this paper is to present the first record of *Magnolia schiedeana* as a host plant for *Cydia pomonella*, with the latter identified using morphological and molecular characters.

MATERIAL AND METHODS

The study was conducted in the Natural Reserve of La Martinica in Veracruz, Mexico, at 19°35' N, 95°56' W, at 1600 m above mean sea level, in a location referred to as SINACVER (Sitios de Patrimonio Natural y Cultural de Veracruz). Larvae of Lepidoptera were discovered feeding within flower buds of *M. schiedeana*. They were collected and taken into the laboratory where they were killed and preserved. The larvae were identified using taxonomic keys for Lepidoptera of MacKay (1956), Weisman (1986) and Stehr (1987), along with setal maps of the head capsule and full body and crochets of the third to sixth and tenth abdominal segment.

A specimen previously identified as *C. pomonella* from the entomological collection of LATEX (Laboratory of High Technology of Xalapa) was used as a positive control. The essay consisted of two repetitions with the larvae in study and the control larvae. DNA was extracted using the direct method. The first abdominal segment of the larvae was cut with a sterile blade. After a rinsing with distilled water, the segment was placed in a sterile 0.5 mL eppendorf tube, with 100 µL of ultra-pure water for PCR reactions. Tissue was macerated using a sterile pellet in a Qiagen Tissue Lyser tissue disruptor. The upper fluid was withdrawn with a 100 µL pipet and inserted in a clean 0.5 mL eppendorf tube. The resulting material was used as a DNA source.

Specific cpo-D (5´AGCTCTTTTACTTCTTTTATCATT 3´) and cpo-I (5´ATAGGATCACCTCCACCA 3´) primers were used to amplify 112–116 bp of the COI gene. Amplification was performed based on the method of Barcenas *et al*., (2005), with the following modifications: in a 25 µL reaction volume containing 2.5 µL of $MgCl_2$ 25 mM, 2.5 µL of buffer 10X, 0.625 µL of dNTPs 25 mM, 2.0 µL of each primer (10 Pm/µL of cpo-D primer and 10 pM/µL of cpo-I primer), 0.3 µL of Taq DNA polymerase 5U/µL, 13.075 µL of PCR water and 2 µL of template DNA.

The PCR temperature program was as follows: 10 cycles of 94°C for 5 min, 94°C for 1 min, 50°C for 30 seconds, and 72°C for 1 min, followed by 10 cycles of 94°C for 1 min, 55°C for 30 sec and 72°C for 1 min, and finally 10 cycles of 94°C for 1 min, 60°C for 30 sec, and 72°C for 1 min, with a final extension of 72°C for 5 min, programmed in a thermocycler Eppendorf Master Cycler.

The amplification was analyzed in a Bio Rad electrophoresis chamber using 1.8% agarose gel with 0.5 TBE buffer at 90 volts for 1 hour. Gel was stained with ethidium bromide for 20 min and revealed in a photo documenter.

RESULTS

Twelve caterpillars were found feeding on flower buds of *Magnolia schiedeana* (Fig. 1). The larvae were identified as *Cydia pomonella* and their setal maps are presented in Figs. 2 and 3. The PCR technique corroborated the identification.

Morphology. The head is yellow-brown, often with a darker brown pattern; the prothoracic and anal shields have dark speckling; and the spines and integument are distinct. The anal comb is absent. The head is hypognathous, with the adfrontals reaching or nearly reaching the epicranial notch, and the epicranial suture is short. There are six stemmata present in an uneven distribution, with the sixth in a more nearby position than the fourth (Fig. 4). The prespiracular group of the prothorax has three setae (Fig. 2). Setae L1 and L2 on abdominal segments 3 to 6 are near and ventral to the spiracle. L1 and L2 on abdominal segment 9 are usually on the same pinaculum, separate from L3, although in some specimens all three setae can be in the same pinaculum. Abdominal segment 9 has D1 and SD1 on same pinaculum (Figs. 2–3).

Molecular markers. The results from the PCR with the specific primers designed to amplify fragments of the COI gene confirmed that the larvae found on *M. schiedeana* is *C. pomonella,* the gel with the amplification product, with fragments at 115 bps of the COI gene, which according to Barcenas *et al*. (2005) corroborates a positive result (Fig. 5).

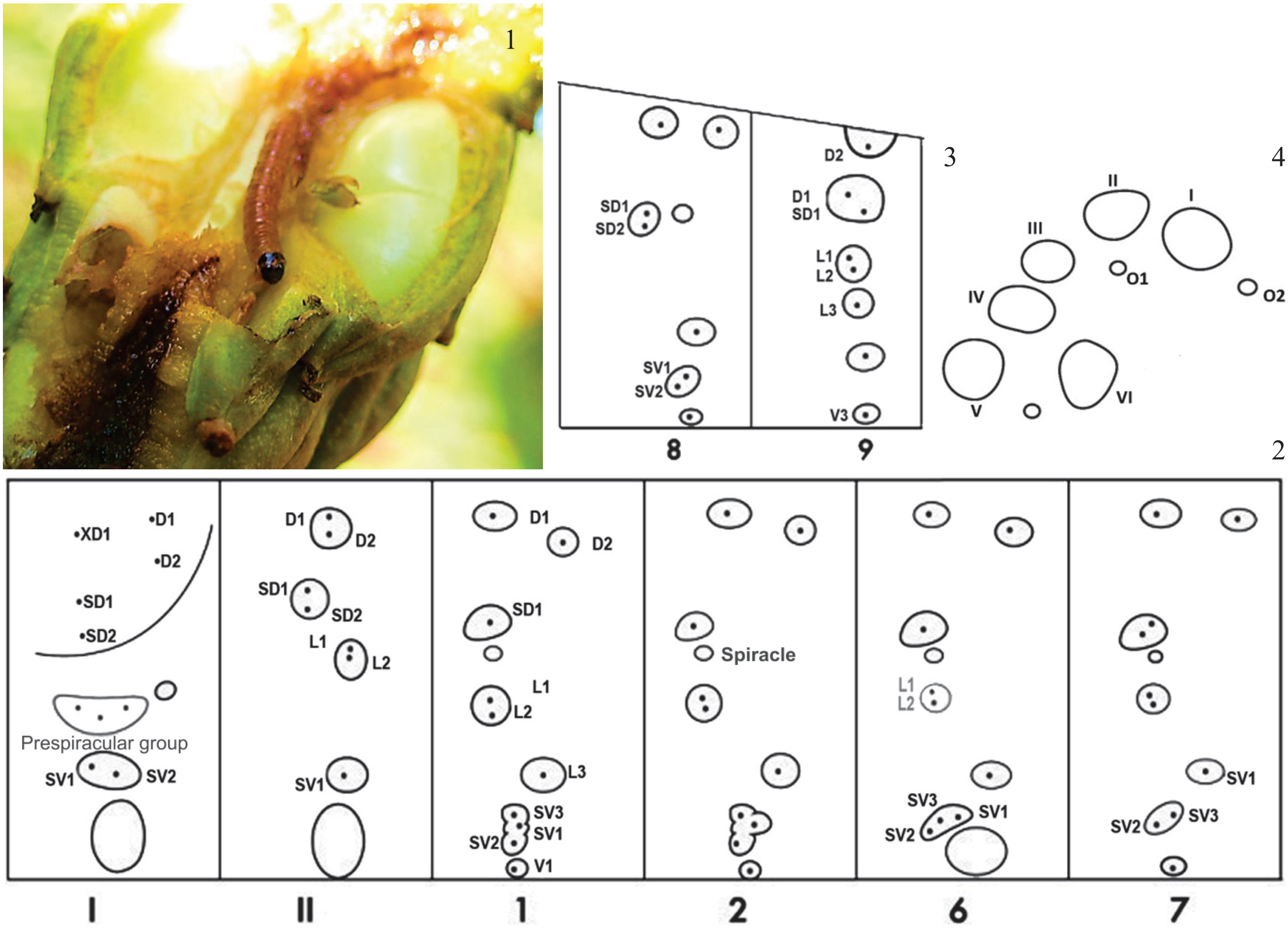
DISCUSSION

The study site supports one of the few remaining patches of cloud forest in the state of Veracruz, Mexico. The integrity of this ecosystem is at high risk due to human population density in the area and its surroundings, where there is a rapidly increasing exploitation of the forest's natural resources (CONABIO 2010). The expansion of human communities and the constant modification of La Martinica are associated with the growth of the neighboring county of Banderilla and the increase in the areas devoted to cattle grazing

The loss of the forest causes an ever-increasing pressure on *C. pomonella* populations (possibly established in the area because nearby agricultural practices combine apple and plum growing with short term cycle plants like maize and bean), reducing host availability and altering the topographical structure and architecture (through deforestation and changing of land tenure), both of which may trigger the selection of alternate hosts, such as *M. schiedeana* and other ornamentals which may be more acceptable for this pest. Variation in ovipositional pattern has been observed in some agricultural pests when the main host is unavailable (Tabashnik 1983). In a study of oviposition by *Iphiclides podalirius* Linnaeus, (1758), Stefanescu *et al*. (2006) reported that some females deposit eggs on unusual plants (outside the normal host range), and that this behavior can be attributed to a combination of factors, including low specificity to prefered hosts and a low number (or density) of prefered hosts owing to suburban areas in which the species main hosts were lacking. Studies have also shown that Lepidoptera are highly susceptible to transformations in topographic conditions and are often guided towards hosts by plant height and structure (Singer 2003; Reudler-Talsma *et al*. 2008). When the appropriate cues are not encountered, alteration in their reproductive habits (mating and oviposition) may occur (Severns 2008).

This unusual behavior (e.g., ovipositon on less than optimal hosts) is difficult to estimate because oviposition does not depend on one factor alone. The other issue is how females chose alternate hosts. Establishing exaclty what leads a female to an alternate host plant is difficult. It may be a combination of factors (Barenbaum 1983; Thompson & Pellmyr 1991).

Some of the specimens of *C. pomonella* discovered on *M. schiedeana* were in the last instars, what may support the hypothesis that female Lepidoptera choose oviposition sites where larvae will most likely develop and survive, although this is not always the case (Thompson & Pellmyr 1991; Pérez-Contreras 1999). Similarities in chemical compounds between typical and unusual hosts have been studied because chemical reception is an important factor in host selection. Hence the female may be attracted to a new host with compounds similar to that of their typical hosts (Becerra 1997; Becerra & Venable 1999). Wearing & Hutchins (1970) proposed that ovipositional behavior in *C. pomonella* is induced by á-farmasene isomers present in



Figs. 1–4. *Cydia pomonella*. 1. Damage of *Cydia pomonella* on *Magnolia schiedeana*. 2. Setal map of segments I, II and 1–7. 3. Setal distribution in abdominal segments 8–9. 4. Stemmata distribution in the head.

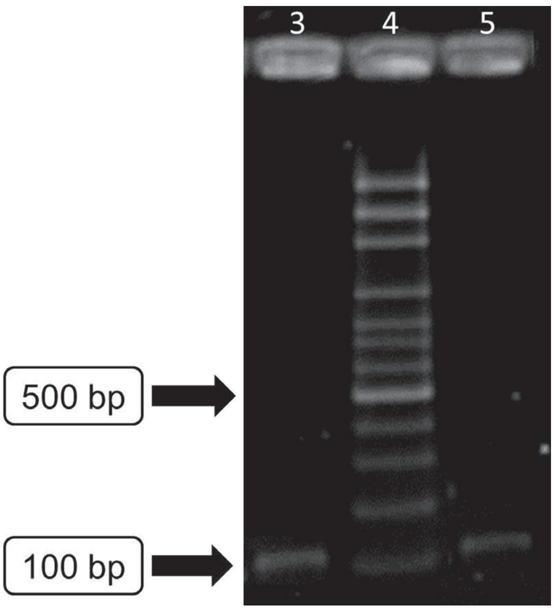


Fig. 5. PCR products in 1.8% agarose gel stained with ethidium bromide. (3) Positive control of *Cydia pomonella* at 115 bps. (4) 100 bp Ladder. (5) *Cydia pomonella* of study at 115 bps.

the host fruit. Larvae that hatch on leaves or branches are also attracted to the fruit by the same compound. Presence of this compound in *M. schiedeana* could have been investigated, but it was not possible to carry out chemical analyses of floral buds of this unusual host.

Despite somewhat specific feeding habits, *C. pomonella* has been reported on hosts outside Rosaceae, including *Castanea sativa* (Fagaceae), *Citrus* sp. (Rutaceae), *Macadamia* sp. (Proteaceae), and *Juglans regia* (Juglandaceae). Whether unusual host plants are misinterpretations of chemical compounds by females or acts of desperation (absence of primary hosts) leading the female to oviposit on something that has the appropriate leaf texture or plant architecture has been little studied in Tortricidae (Brown 2013, pers. comm.).

When an alternate host plant is widely available and larvae are able to survive and complete their life cycle, these oviposition "mistakes" can lead to permanent host shifts, and *C. pomonella* may represent such an example. Outside the Rosaceae, *C. pomonella* has adapted at some point to walnut (*Juglans regia*), and it is currently an important pest of this plant in South America (Chile and Argentina) (Chapman 1973; Fernández *et al.* 2010).

Erratic oviposition may on some occasions have selective advantage, where females with the capacity to choose hosts outside their normal range will have larger chances of survival (Futuyma 1983). Females that lay eggs on more suitable host plants will have a larger number of descendants than females that oviposit on inappropriate host plants in terms of larvae survival and growth (Rausher 1979). The evolutionary process of herbivorous insects is full of beneficial accidents; the term "ovipositional errors" does not imply events to be of no transcendence in plant-insect relationships.

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