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Calligrapha (*Zygogramma*) *signatipennis* (Stal, 1859) (Coleoptera: Chrysomelidae) and its Potential for Biological Control of *Tithonia tubaeformis* (Jacq.) Cass., 1825) (Asterales: Asteraceae)

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ABSTRACT

The objective of this research was to identify the insects associated with damage to acahual plants. During August and September 2024, sampling was carried out in Tepalcingo, Morelos, and Iguala de la Independencia, Guerrero, Mexico, on acahual plants (*Tithonia tubaeformis*) with signs of defoliation. Plant structures and whole plants were collected, as well as adult insects associated with the damage, which were preserved in 70% ethanol. Weed identification was carried out through morphological comparisons with CONABIO; insect identification was performed through morphological comparison and stereoscopic microscopy. For this purpose, one specimen per collection site was mounted separately on sample slides, where they were coated with graphite, for observation with a JEOL IT300-LV SEM stereomicroscope, equipped with a Quantax EDS detector: Xflash 6|30. The morphological characteristics of the plants allowed the identification of the species *Tithonia tubaeformis* (Asteraceae), and regarding the insect, *Calligrapha* (*Zygogramma*) *signatipennis* (Coleoptera: Chrysomelidae) was identified, whose damage to *Tithonia tubaeformis* was moderate to severe in some plants. This damage was specific to this species, suggesting that *Calligrapha* (*Zygogramma*) *signatipennis* could have potential as a biological control agent for the management of *Tithonia tubaeformis*. It is worth noting that this species has a greater feeding preference for acahual plants that are in the vegetative stage, where it can cause good control of this weed.

Keywords: Defoliation, Weed management, SEM analysis, Chrysomelidae, Insect-plant interaction

INTRODUCTION

Traditionally, the use of synthetic pesticides has been the predominant method for controlling insects, diseases caused by fungi or bacteria, and weeds (Hernández-Rosas et al., 2020; Tariq et al., 2020; Nchu, 2024). However, the intensive use of chemical pesticides generates persistent negative impacts on ecosystems and represents a significant risk to the health of humans, animals and non-target organisms in agroecosystems (Chaudhary et al., 2024). Furthermore, its continuous application has contributed to the development of resistance in various species. A clear example of this phenomenon in insects is the case of the fall armyworm (*Spodoptera frugiperda*, J.E. Smith) (Lepidoptera: Noctuidae), which by 2021 already showed resistance to 33 active ingredients in different regions of the world (Cerna-Chávez et al., 2022). Similarly, in weeds, by 2022, 350 cases of glyphosate resistance had been documented globally, including multiple resistance in 23 species distributed in 17 countries, where the families that have developed the greatest resistance to the largest number of herbicides are: Poaceae, Asteraceae, Brassicaceae, Cyperaceae and Amaranthaceae (Heap, 2022; Arispe-Vázquez et al., 2023).

Faced with this problem, biological pest control is emerging as a sustainable and ecologically responsible alternative. Biological pest control is a tool in pest management that uses natural enemies, whether native or introduced, to reduce populations of harmful organisms such as pathogenic fungi, bacteria, nematodes, insects, weeds, and others (Eilenberg et al., 2001; Pal and Gardener, 2006; Hoddle, 2024). In the specific case of biological weed control, various insect families have proven effective, among the most successfully used are: Curculionidae, Cerambycidae, Chrysomelidae, Buprestidae, (Coleoptera); Nymphalidae, Tortricidae (Lepidoptera); Agromyzidae, (Diptera) (Kumari et al., 2022). However, the Chrysomelidae family is a recognized source of weed biocontrol agents (Winston et al., 2014; Reddy et al., 2021), highlighting *Zygogramma signatipennis* (Stal) and *Zygogramma piceicollis* (Stal) (Simelane et al., 2011).

Acahual can sometimes be perceived as weeds due to their high development rate in the vegetative stage. This increases competition for nutrients and water with crops, resulting in decreased crop yields. Therefore, managing this weed is important as part of efforts to produce food through sustainable, low-environmental-impact management (SNVM, 2011; Soto et al., 2011). For this reason, proper management of this weed is essential as part of efforts to balance agricultural production (Soto et al., 2011; SNVM, 2011). Therefore, the objective of this work was to identify the insects associated with damage to acahual plants.

MATERIALS AND METHODS

During August and September 2024, sampling was carried out in Tepalcingo, Morelos, México (18.620151, -98.863248) and Iguala de la Independencia, Guerrero, México (18.343085, -99.503235; 18.348239, -99.502788) (Fig. 1). Sampling was directed at acahual plants that showed defoliation; complete plants were collected from 1.1 to 1.7 m in height.

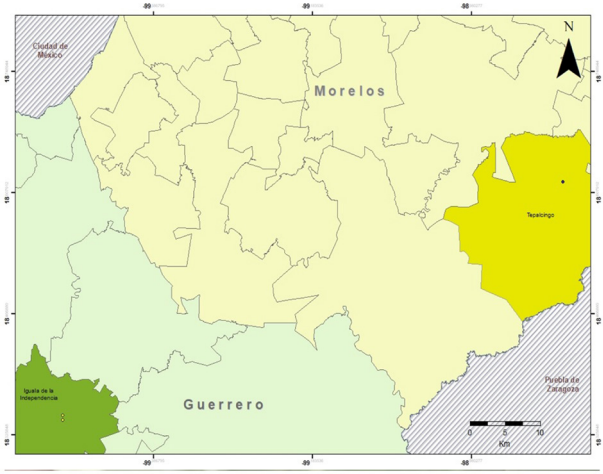

Figure 1. Geographic locations where *Tithonia tubaeformis* and *Calligrapha* (*Zygogramma*) *signatipennis* were collected.

The samples were placed in paper bags to prevent moisture accumulation. At the same time, adult insects associated with the damage observed on the plants were collected and preserved in 70% ethanol for subsequent identification using taxonomic keys. All samples were transferred to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuaria - Iguala Experimental Field (INIFAP - CEIGUA) for study. In a first stage, the taxonomic identification of the weed was carried out through morphological comparisons with CONABIO (CONABIO, 2025a) and Flora Mesomaricana (FM, 2025), and for insects using a Quasar Qm23 stereoscopic microscope and electron microscopy.

For detailed morphological analysis, specimens were mounted on aluminum sample holders using double-sided graphite adhesive tape. They were then coated with a conductive layer of graphite by thermal evaporation of carbon, using a Denton Vacuum

coater, Desk Carbon Accessory model. High-resolution observations and imaging were performed using a JEOL IT300-LV scanning electron microscope (SEM) equipped with a Quantax: Xflash 6|30 X-ray energy dispersive spectroscopy (EDS) detector (Bruker).

RESULTS AND DISCUSSION

***Tithonia tubaeformis* (Jacq.) Cass., 1825 (Asteraceae)**

Characteristics: Cylindrical stems; foliage with abundant trichomes, alternately arranged leaves, truncated base, crenate margin, pointed apex; inflorescence with a single flower head completely covered by trichomes.

***Calligrapha* (*Zygogramma*) *signatipennis* (Stal, 1859) (Coleoptera: Chrysomelidae**), according to Clark et al. (2024), the genus *Zygogramma* is considered a subgenus of *Calligrapha* (*Zygogramma)*, due to the high similarity between the species.

***Calligrapha* (*Zygogramma*) *signatipennis*:** Size approximately 6.51 to 7.80 mm, with an oval and convex body (Fig. 2a). Head black, with a slightly visible frontal groove; antennae clubbed; pronotum rectangular with sides curved in the apical third; the apical angles project toward the head; the pronotum has evenly distributed punctures, being deeper toward the sides (Fig. 2b). The scutellum is smooth and has a distinctive ogival shape. Elytra metallic green, 5.3-6.4 mm long and 2.1-2.7 mm wide, with irregularly distributed punctures (Fig. 2a). Symmetrically, the elytra present a sutural band from the base to the apex, with a large semicircular subapical projection, semicircular apical projection; subsutural band fused to the sutural band; ovoid discoidal marking; humeral marking projecting to the base(Fig. 2a). The tarsal claws are in a parallel position (Fig. 2c). The above description agrees with that reported by Benítez-García et al., (2017); Piña-Martínez (2022); Clark et al., (2024).

The damage of *Calligrapha* (*Zygogramma*) *signatipennis* in *Tithonia tubaeformis* was moderate to severe in the apical part of the plants (Fig. 3a-d) (40-50%), this damage was specific to this species, that is, it was not observed in other weeds (*Euphorbia* spp.) and crops (*Citrus* × *limon*), which even had the presence of *Calligrapha* (*Zygogramma*) *signatipennis* (Fig. 3e-i), which suggests that *Calligrapha* (*Zygogramma*) *signatipennis* could have potential as a biological control agent for the management of *Tithonia tubaeformis*, it should be noted that the damage increases when the plant is in the vegetative stage.

Using this insect as a biological control tool can be more economical and sustainable than herbicide sprays, and could be especially useful in crops where *Tithonia tubaeformis* is a problem. Biological agents contribute to the restoration of ecological balance by controlling weeds in a natural and less invasive way (SPREP, 2020; CU, 2025). In Mexico, *Calligrapha* (*Zygogramma*) *signatipennis* is mainly found in the states of Jalisco, Guanajuato, Querétaro, Mexico City, Puebla and Morelos (CONABIO, 2025b); however, in Guerrero and Morelos other species have been reported such as *Zygogramma lemur*, *Zygogramma piceicollis*, *Calligrapha argus*, *Calligrapha consputa*, *Calligrapha diversa*, *Calligrapha labyrinthica*, *Calligrapha multiguttata*, *Calligrapha notatipennis* and *Calligrapha serpentina* (Burgos-Solorio et

al., 2004). It is worth noting that for the state of Guerrero, *Calligrapha* (*Zygogramma*) *signatipennis* is reported for the first time feeding on *Tithonia tubaeformis*.

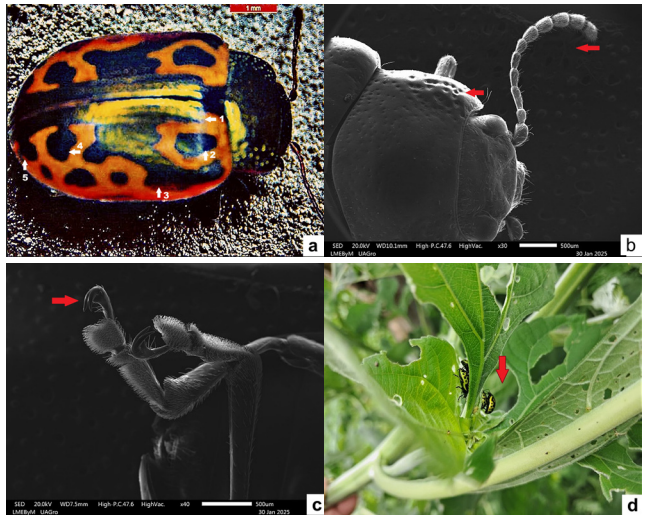

Figure 2. a) 1- Sutural band fused with the subsutural band, 2- discoidal marking, 3- humeral spot fused with the sutural band, 4- subapical projection of the sutural band, 5- semicircular apical projection. b) pronotum with evenly distributed pits, becoming deeper laterally, and clubbed antenna. c) tarsal claws. d) *Calligrapha* (*Zygogramma*) *signatipennis* feeding on *Tithonia tubaeformis* in the vegetative stage.

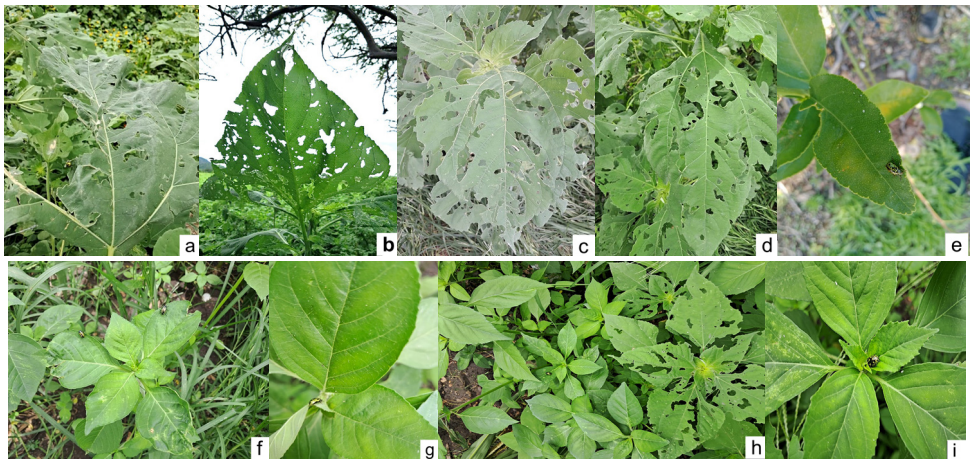

Figure 3. a-d) Damage caused by *Calligrapha* (*Zygogramma*) *signatipennis* on *Tithonia tubaeformis*. e) Presence of *Calligrapha* (*Zygogramma*) *signatipennis* in lemon crops. f-i) Weeds where the presence of *Calligrapha* (*Zygogramma*) *signatipennis* was detected, but with no evidence of visible damage, only *Tithonia tubaeformis* was damaged.

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