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Spatio-Temporal Abundance of The Mexican Fruit Fly *Anastrepha ludens* (Loew, 1873) with The Huanglongbing

Jesús Armando VARGAS-TOVAR[1a] Carolina DELGADO-LUNA[2] Ricardo ÁLVAREZ-RAMOS[3] Vidal ZAVALA-ZAPATA[4] Ausencio AZUARA-DOMÍNGUEZ[1b*]

[1]Tecnológico Nacional de México, Instituto Tecnológico de Cd. Victoria, Tamaulipas, MEXICO

[2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro Experimental Río Bravo, Tamaulipas, MEXICO

[3]Comité Estatal de Sanidad Vegetal de Tamaulipas, Tamaulipas, MEXICO

[4]Universidad Autónoma Agraria Antonio Narro, Departamento de Parasitología Agrícola, Coahuila, MEXICO

e-mails: [1a]armandoobiol@gmail.com, [2]delgadolunac29@gmail.com, [3]rialra@yahoo.com, [4]vidal.convergens@gmail.com, [1b]azuarad@gmail.com

ORCID IDs: [1a]0009-0000-2343-714X, [2]0009-0004-8276-4051, [3]0009-0009-0834-7985, [4]0000-0001-8786-3308, [1b]0000-0002-1180-1538

*Corresponding author

ABSTRACT

Citrus production faces significant phytosanitary challenges caused by *Anastrepha ludens* (Loew, 1873) and the bacterium *Candidatus* Liberibacter asiaticus (Jagoueix, Bové, and Garnier, 1994), transmitted by *Diaphorina citri* (Kuwayama, 1908), which causes Huanglongbing (HLB) disease. HLB-infected fruits are poorly colored (reverse ripening, yellow/green), deformed, small, and have reduced acidity and total soluble solids. These fruit characteristics may influence the abundance of *A. ludens*, since fruits are used as feeding, oviposition, and refuge sites. This study associated the abundance of *A. ludens* in commercial citrus orchards (with different numbers of HLB-infected trees) in the municipalities of González, Güémez, Hidalgo, Llera de Canales, Padilla, and Victoria, Tamaulipas, Mexico. The association between *A. ludens* abundance and the number of trees with HLB was analyzed using Multiple Correspondence Analysis (MCA). MCA revealed a higher frequency of *A. ludens* adults in Güémez, Padilla, and Victoria, where HLB incidence was highest (highest number of trees). In contrast, the lowest abundance of *A. ludens* adults was recorded in González, where HLB was virtually absent (lowest number of trees). It is recommended that the municipalities of Güémez, Padilla, and Victoria be prioritized in integrated management plans for *A. ludens* and HLB to mitigate the impact on citrus production.

Keywords: Behavior, Distribution, Insects, Population, Yellow dragon.

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INTRODUCTION

Citrus fruits, which include several species, are cultivated in more than 140 countries, with an annual production of 183 million tons (Azuara-Domínguez, Vargas-Madriz, Álvarez-Ramos, & Vargas-Tovar, 2025). In Mexico, production is 8.9 million tons valued at MX$52.2 million pesos, with Valencia oranges accounting for 54% of the total, lemons 37%, grapefruit 5%, and mandarins 4% (SIAP, 2024). However, this productivity faces constant threats from a wide variety of pests and disease (SENASICA, 2020).

In the citrus region of Tamaulipas, Mexico, citrus crops are mainly affected by Mexican fruit fly *Anastrepha ludens* (Loew, 1873) (Diptera: Tephritidae) and the Asian citrus psyllid *Diaphorina citri* (Kuwayama, 1908) (Hemiptera: Liviidae) (Zapata, Osorio, Silva, & Segura, 2022). *A. ludens* is considered an important pest because females lay eggs inside the fruits, and the larval instars feed on the pulp (Vargas-Tovar, Delgado-Luna, Zavala-Zapata, & Azuara-Domínguez, 2025a). Meanwhile, *D. citri* is a primary pest as it is a vector of the bacterium *Candidatus* Liberibacter asiaticus (*C*Las) (Jagoueix, Bové, & Garnier, 1994); main agent of Huanglongbing (HLB) disease (Zavala-Zapata et al., 2024). HLB is recognized as the most devastating disease in the citrus industry (Villar-Luna, Santos-Cervantes, Rodríguez-Negrete, Méndez-Lozano, & Leyva-López, 2024). In several Asian countries, it caused the complete disappearance of plantations or was factor in shifting them to other production areas (da Graça & Korsten, 2004; López, Ortega, Pérez, & Villanueva, 2022).

In citrus trees, *C*Las bacteria are heterogeneously distributed in the bark phloem, midribs of leaves, roots, flowers, and fruit (Ding, Duan, Paul, Brlansky, & Hartung, 2015). In *C*Las infected fruits, Huanglongbing (HLB) disease causes color inversion during ripening, increased peel thickness and reduction in size, weight, acidity, and total soluble solids content (Sajid et al., 2022; Bouvet, Hochmaier, & Burdyn, 2023). The impact of HLB-induced fruit physicochemical characteristics on *A. ludens* populations has not been investigated under field conditions. Therefore, in this article, we aimed to associate the spatiotemporal distribution of HLB with the frequency of *A. ludens* adults in commercial citrus (*Citrus* spp.) orchards in the citrus-growing region of Tamaulipas, Mexico.

MATERIAL AND METHODS

Study area

The study was conducted from January 2018 to December 2021 in commercial citrus orchards of Mexican lime (*Citrus x aurantifolia* (Christm.) Swingle, 1818), mandarin (*Citrus reticulata* Blanco, 1837), Valencia orange (*Citrus x sinensis* (L.) Osbeck, 1765), and red grapefruit (*Citrus x paradisi* var. 'Ruby Red', MacFad., 1895) distributed in the municipalities of González, Güémez, Hidalgo, Llera de Canales, Padilla, and Victoria, Tamaulipas, Mexico (Fig. 1). González covers 2,111 ha, Güémez 9,555 ha, Hidalgo 6,735 ha, Llera de Canales 5,657 ha, Padilla 12,843 ha, and Victoria 3,464 ha. All areas were farmed using insecticides, fertilization, and a rolling or micro-sprinkler irrigation system. The associated climatic conditions vary, due to the heterogeneity of the municipalities, so there are temperatures that range between 12°C and 26°C, precipitation between 400 and 1,200 mm, and relative humidity levels of 1% to 97% (INEGI, 2010).

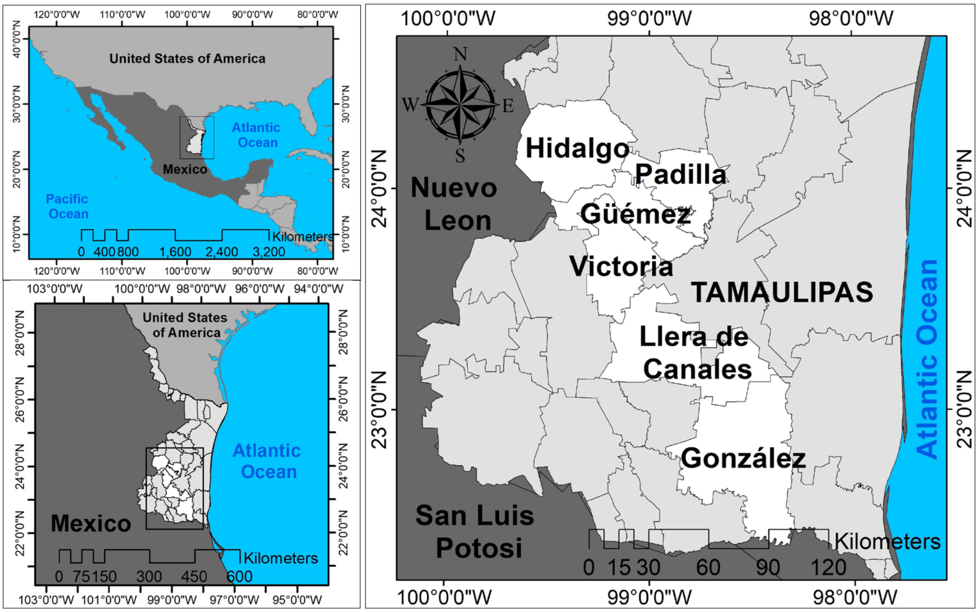

Figure 1. Geographic location of the municipalities of González, Güémez, Hidalgo, Llera de Canales, Padilla and Victoria, in the state of Tamaulipas, Mexico.

Trapping of adults of *A. ludens*

Adult *A. ludens* were trapped every seven days using Multilure® traps (Better World Manufacturing, Inc., Fresno, CA) baited with three pellets of hydrolyzed protein plus 250 mL of water to attract adults to the trap (SENASICA, 2017). The traps were placed ¾ of the way up the tree, facing east, with a separation of a one kilometer between each trap. These traps were installed according to the *Technical Manual for Field Operations of the National Campaign against Fruit Flies* and were therefore subject to strategic changes during each year of the assessment (Table 1). The trapped adults were preserved in bottles with 70% alcohol and transported to the State Committee of Plant Health of Tamaulipas (CESAVETAM in Spanish) laboratory for taxonomic determination.

Table 1. Number of Multilure traps in each municipality for each year of trapping.

Municipality	Number of Multilure traps per year				Total
	2018	2019	2020	2021	
González	18	18	17	17	70
Güémez	301	291	223	243	1058
Hidalgo	183	71	29	46	329
Llera de Canales	172	171	123	129	595
Padilla	373	380	284	308	1345
Victoria	159	87	79	83	408
Total	1206	1018	755	826	3805

Taxonomic determination of adults of *A. ludens*

Adult *A. ludens* were identified every eight days using a binocular stereoscopic microscope (model DZ. 1805, Euromex®), fine-tipped forceps (for manipulation), reference specimens (in alcohol), and the taxonomic keys published in the *Fruit Fly Identification Guide* (NOM-023-FITO-1995) (SENASICA, 2018).

Registration of plant material positive to '*Candidatus* Liberibacter asiaticus'

The identification of plant material positive for the bacterium *C*Las (causal agent of Huanglongbing, HLB) was carried out by CESAVETAM-certified technical personnel using two methods. The first method, visual inspection, was conducted every eight days in all citrus orchards during each year of monitoring. Initial symptoms of infected trees were taken as reference, including diffuse chlorosis in tissue and foliage, diffuse asymmetric mottling, yellow leaf shoots, defoliation, and rib thickening (Robles-González et al., 2013). Notably, the symptoms of HLB are consistent wherever the disease occurs (Bové, 2006).

The second method consisted of collecting nine samples of plant material from each orchard and sending them to the National Plant Quarantine and Sanitation Station in Querétaro for analysis using the Real-Time Polymerase Chain Reaction technique (q-PCR). The selection of the nine samples was standardized across all orchards in each municipality; plant material was collected from one symptomatic tree, and plant material from the eight asymptomatic trees surrounding the tree. The q-PCR is accredited by the General Directorate of Plant Health - National Service for Health, Safety, and Agri-Food Quality (DGSV-SENASICA in Spanish) and meets the standards established for such analyses (NOM-079-FITO-2002). The q-PCR method employed markers amplifying the 16S rDNA gene (OI1/OI2c) (Jagoueix et al., 1994) and protein gene-based markers (operon-B, A2/J5) (Hocquellet, Toorawa, Bové, & Garnier, 1999). A total of 135 trees positive to *C*Las (Huanglongbing) were recorded (Table 2).

Table 2. Number of *C*Las-positive cases in each municipality for each year of monitoring.

Municipality	Number of *C*Las-positive plants per year				Total
	2018	2019	2020	2021	
González	0	1	0	0	1
Güémez	1	1	14	0	16
Hidalgo	0	0	2	0	2
Llera de Canales	27	2	4	0	33
Padilla	1	3	8	36	48
Victoria	5	27	3	0	35
Total	34	34	31	36	135

Statistical analysis

The analysis of the association between the frequency of *A. ludens* adults, the incidence of Huanglongbing (HLB), the municipality, and the evaluation period was conducted using Multiple Correspondence Analysis (MCA) (Greenacre, 2008). The number of *A. ludens* adults was categorized into six groups (A1, A2, A3, A4, A5, and A6), with intervals of 100, based on the methodology used by Vargas-Tovar, Delgado-Luna, Zavala-Zapata, & Azuara-Domínguez (2024a). The incidence of HLB was divided into three categories: ABS (absent), LOW (less than 10), and HIG (greater than 10), based on the number of *C*Las-positive plant samples each year; the classification criteria were their own. The municipalities were coded as GO, GU, HI, LL, PA, and VI, corresponding to the six evaluated municipalities, and the evaluation periods were divided into four years: 2018, 2019, 2020, and 2021.

Since the analysis combined more than two categorical variables, the X^2 value was not applicable, as it is only valid for two-way tables (Vargas-Tovar, Venegas-Barrera, Lázaro-Dzul, Zavala-Zapata, & Azuara-Domínguez, 2025b). Associations between the categories were visualized on a two-dimensional graph, which shows the canonical positions of the categories (Gotelli, 2001). Categories positioned closer together on the graph indicate a strong association, while those farther apart indicate a weaker association (Le Roux & Rouanet, 2005). The center of the graph (coordinate 0.0) represents the mean of all variables. The variation contributed by each variable to the canonical space was calculated by dividing the total X^2 of the variable by the total X^2 of all variables, multiplied by 100 (Vargas-Tovar et al., 2025b). Additionally, a one-way PERMANOVA test was performed to assess differences between categories; the test used 9,999 permutations, and the Bray-Curtis distance. PERMANOVA is a variant of ANOVA, which compares multidimensional distance within and between groups (Anderson, 2017).

RESULTS

The PERMANOVA indicated that capture frequencies differed among municipality categories ($F = 3.027$, $P = 0.002$), number of adults ($F = 23.89$, $P = 0.0001$), and study period ($F = 2.401$, $P = 0.0298$), whereas capture frequencies among HLB incidence were similar ($F = 1.804$, $P = 0.123$). The MCA revealed that the municipality variable contributed 32.43% of the X^2 test value, followed by the number of adults (31.18%), the study period (20.37%), and HLB incidence (16%) (Fig. 2). The canonical position of the category representing zero adults (A1) indicated a strong association with the municipality of González (GO). In contrast, during 2018, abundances ranging from 1 to 500 adults (A2, A3, A4, A5, A6) were more frequent in the municipalities of Güémez (GU), Padilla (PA), and Victoria (VI) when HLB incidence was high (HIG). On the other hand, during 2019 and 2020, the canonical positions of the municipalities of Llera de Canales (LL) and Hidalgo (HI) showed a negative association with categories of high adult abundance; however, the canonical position of Llera de Canales (LL) indicated a strong association with low HLB incidence (LOW) during 2019 and 2020. In contrast, during 2021, the canonical position of Hidalgo (HI) showed a strong association with the absence of HLB (ABS).

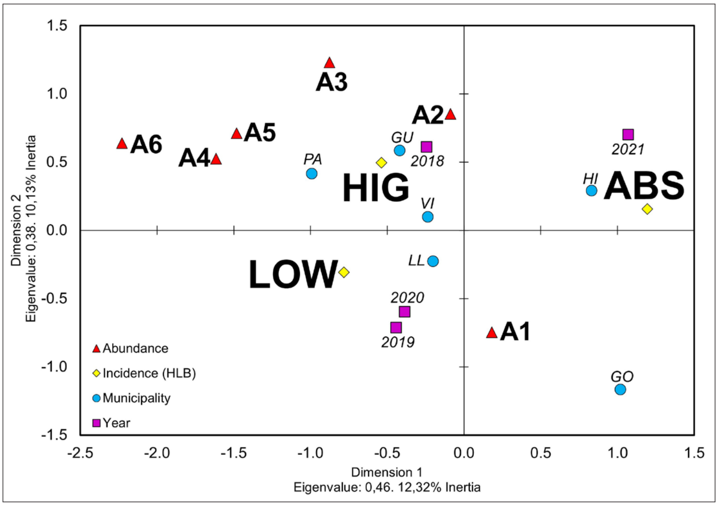

Figure 2. Multiple Correspondence Analysis for the frequency of *A. ludens* adults in relation to Huanglongbing incidence, municipality, and study period. Where: A1 = 0 adults, A2 = 1-100 adults, A3 = 101-200 adults, A4 = 201-300 adults, A5 = 301-400 adults and A6 = 401-500 adults; ABS = Absence of HLB, LOW = Low level of HLB and HIG = High level of HLB; GO = González, GU = Güémez, HI = Hidalgo, LL = Llera de Canales, PA = Padilla and VI = Victoria.

CONCLUSIONS AND DISCUSSION

Given the results, the present study demonstrates that the abundance of adult *Anastrepha ludens* varies between municipalities due to the frequency of trees with Huanglongbing (HLB) with which they are associated. The presence of adult *A. ludens* was more frequent in municipalities with a higher incidence of HLB than in those where HLB was almost absent. The response patterns of *A. ludens* suggest that it may benefit from the presence of HLB. However, it is not certain whether the larval instars are developing inside the diseased fruits (Vargas-Tovar, Delgado-Luna, Álvarez-Ramos, Guardiola-Alcocer, & Azuara-Domínguez, 2024b). Nevertheless, HLB causes fruit greening (Bové, 2006), a color that is considered attractive to males and precopulatory females (García-Ramírez, Cibrián-Tovar, Arzufí-Barrera, López-Collado, & Soto-Hernández, 2004). Therefore, if it is confirmed that the larval instars of *A. ludens* are developing inside fruits affected by HLB, the importance of *A. ludens* is highlighted because its tolerance can be used as a potential study model to mitigate the impact of HLB. Given the above, it is feasible that in future studies the inspection of fruits affected by HLB will be carried out, considering fruits with circular yellow or necrotic spots, visual characteristics that aid in the detection of larval fruits (Chino-Cantor et al., 2025).

On the other hand, although *A. ludens* preference for green fruits may be suggest that females may using HLB-infected fruit as oviposition sites, in tephritids, oviposition site selection is influenced by visual cues (shape and size) and chemical signals (fruit volatiles), which help locate optimal hosts (Prokopy & Roitberg, 1984; Fitt, 1990; Jang & Light, 1991; Aluja, Díaz-Fleischer, Papaj, Lagunes, & Sivinski, 2001; Aluja et al., 2016; Robacker &

Fraser, 2002a, 2002b). Previous studies analyzing *A. ludens* abundance in natural conditions primarily used temperature and relative humidity as predictive variables (Tucuch-Cauich, Chi-Que, & Orona-Castro, 2008; Vanoye-Eligio, Barrientos-Lozano, Pérez-Castañeda, Gaona-García, & Lara-Villalon, 2015a; Vanoye-Eligio, Pérez-Castañeda, Gaona-García, Lara-Villalón, & Barrientos-Lozano, 2015b; Vanoye-Eligio, Vázquez-Sauceda, Chacón-Hernández, Mora-Ravelo, & Rocandio-Rodríguez, 2018). These studies found positive correlations between environmental characteristics and *A. ludens* abundance. However, the presence of *A. ludens* begins with locating host patches, which depends on host availability and oviposition resources (Aluja & Prokopy, 1992; Aluja & Mangan, 2008; Prokopy, Papaj, Hendrichs, & Wong, 1992; Prokopy, Hsu, & Vargas, 1993; Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Hernández, Flores, Villaseñor, Muñoz, & Pérez, 2015). The intercropping of citrus varieties also sustains and increases *A. ludens* populations (Baker, Stone, & Plummer, 1944; Aluja, 1994; Robacker & Fraser, 2002a; Thomas, 2003; Birke et al., 2006; Padrón-Chávez & Rocha-Peña, 2009; Vanoye-Eligio, Barrientos-Lozano, Mora-Olivo, Sánchez-Ramos, & Chacón-Hernández, 2016). Therefore, high densities of *A. ludens* are more closely related to fruit availability than climatic conditions (Celedonio-Hurtado, Aluja, & Liedo, 1995). This study specifically used fruit as the primary predictive variable, excluding environmental characteristics.

The study areas were defined based on citrus-producing regions cultivated with the highest proportion of cultivated citrus, with Hidalgo, Güémez, Llera de Canales, Padilla and Victoria being the highest producing regions (Vanoye-Eligio et al., 2015a). The selection of these areas allows for the identification of precise responses regarding the abundance of *A. ludens* influenced by the dispersal *C*Las, given that mango trees are also cultivated in the southern regions of Tamaulipas (Vanoye-Eligio et al., 2023). Another preferred host for *A*. *ludens* (Weems, Heppner, Steck, Fasulo, & Nation, 2025). Furthermore, the Valencia orange is cultivated at least 30,000 hectares of the 46,000 hectares designated for citrus cultivation (SIAP, 2021; CESAVETAM, 2024). This variety is most susceptible to HLB disease (Mendoza-Pérez et al., 2024).

Finally, our study established itself as one of the first reports on the effect of HLB on the abundance of *A. ludens* adults. This knowledge can lead to a more effective integrated pest management strategy, given that although it would be thought that *A*. *ludens* is absent in regions where HLB is present, the presence of HLB does not mitigate *A*. *ludens* populations. Therefore, it is recommended that integrated management plans targeting *A. ludens* and HLB be prioritized in the municipalities of Güémez, Padilla, and Victoria, Tamaulipas.

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