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## SUSCEPTIBILITY OF SWEET ORANGE (*Citrus sinensis* L. Osbeck) AND TAHITIACID LIME (*Citrus latifolia* Yu. Tanaka) TO FRUIT FLY INFESTATION (Diptera: Tephritidae) UNDER CONTROLLED AND FIELD CONDITIONS

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### ABSTRACT

Within the scope of international trade in fresh fruits, Brazil stands out as an exporter of Tahiti acid lime (LAT) to the European Union. In Brazil, the phytosanitary certification process falls under the responsibility of the Ministry of Agriculture and Livestock (MAPA) and the State Agricultural Defense Agencies. In order to meet the requirements of European Community Directives 523/2019 and 2021/2285/EC, this study presents field data (adult monitoring) and laboratory data (forced infestation). Over 45 weeks in 2020, adults were collected on four farms—Aros Agrícola, Reboúças, Agropecuária Gavião, and ITACITRUS—using McPhail traps and food attractants. Females were identified by a specialist taxonomist. The MAD index (flies/trap/day) expressed population density, and the Faunistic Index expressed species diversity of fruit flies (FF) on each farm. Forced infestation was carried out at the Entomology Laboratory of Embrapa Cassava and Fruits (2023/2024), using *Anastrepha obliqua*, in three sweet orange varieties—‘Valencia’, ‘Natal’, and ‘Pera’—and Tahiti acid lime, with 10 replicates per variety, including a positive control with mango and an absolute negative control (fruit only). The MAD index for the Reboúças, Aros Agrícola, Agropecuária Gavião, and ITACITRUS farms was 7.44, 6.16, 2.28, and 0.69, respectively. The prevalent species were *A. fraterculus*, *A. obliqua*, *A. pseudoparallela*, *A. distincta*, *A. consobrina*, *A. sororcula*, *A. dissimilis*, *A. quiinae*, *A. zenilidae*, and *Anastrepha* sp. In the faunistic analysis, *A. fraterculus* was the dominant species (52.67%), followed by *A. obliqua* (22.32%) and *A. pseudoparallela* (9.82%). Regarding frequency, species were classified as Constant (*A. fraterculus*), Common (*A. obliqua*, *A. pseudoparallela*, *A. distincta*), and the remaining species as Rare. Symptoms of punctures and/or oviposition on the ‘Pera’ variety (28.8%) were higher than on the ‘Valencia’, ‘Natal’, and LAT varieties, with 11.9%, 8.3%, and 7.1%, respectively. No fruit showed larval development or subsequent adult emergence. The positive control (mango) showed symptoms in 96.7% of the fruits, with 349 pupae and 100% pupal viability. The genus *Anastrepha* was prevalent in the evaluated areas. The MAD index indicated low population density of fruit flies. Considering MAD values below 1.0, only the ITACITRUS farm would be able to export Tahiti acid lime fruits without phytosanitary treatments. The population characterization of fruit flies in orchards, as well as the assessment of host preference, enables official agencies to develop pest control strategies and to ensure phytosanitary certification at origin, an instrument that enables citrus exportation, thereby maintaining economic agreements within the Southern Common Market (Mercosur), generating foreign exchange for Brazil and ensuring thousands of jobs in the service sector linked to citrus production in Bahia.

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## INTRODUCTION

Ranking fifth worldwide—behind only Mexico, Turkey, South Africa, and Egypt—Brazil exported the equivalent of 106.7 thousand tons of Tahiti Acid Lime (LAT) in the first half of 2025, representing an increase of 18.17% compared to the same period of the previous year.

The Netherlands was the main importer and distributor of the fruit within the European Union. Characteristics such as size, quality, and flavor have made Brazilian lime a benchmark in the United Kingdom (ABRAFRUTAS, 2025). At the national level, LAT is cultivated on properties smaller than 25 ha, typically dominated by family labor. In Bahia, the size of these properties is even smaller, ranging from 3 to 10 ha. Citrus production is a relevant activity, as it is present in

14,766 rural households, placing the state in first position in the North and Northeast regions in the ranking of establishments cultivating oranges, limes, and mandarins (IBGE, 2022). Most orange production in the state of Bahia is destined for supplying the industrial park in the state of Sergipe, which processes concentrated and frozen juice. However, citrus growers producing fruits with superior organoleptic and cosmetic quality access markets that provide better remuneration. Thus, the export of fresh fruit represents an important market niche to be maintained and expanded, corroborated by data indicating a 38% increase in the area planted with LAT in Bahia, which already exceeds 6,000 hectares (VIDAL, 2021).

Comparing the performance of Bahia's LAT production—ranked third nationally—between 2023 and 2024, an increase of 0.41% was observed (ABRAFRUTAS, 2025b), confirming this activity as a profitable venture for the production chain. This trend has persisted over the last decade, as evidenced by the most recent sector figures in 2025: exports began the year with a volume of 17 thousand tons in January, reaching a peak of 27 thousand tons in July 2025 (ABRAFRUTAS, 2025). Since 2019, an important commercial window for Brazilian fruit production has opened with the signing of the agreement between the Southern Common Market (MERCOSUR) and the European Union (EU), as some products received immediate tariff exemptions, while others underwent tariff reduction (gradual removal of duties), such as limes, lemons, watermelons, and melons (CEPEA, 2026).

However, it was expected that certain conditions would be established by the importing bloc, given the need to mitigate risks associated with potentially damaging pests. Phytosanitary measures may therefore be applied and are legally supported by the International Standard for Phytosanitary Measures (ISPM) No. 5 (FAO, 2009), which lists different methods, including phytosanitary certification, fumigation, hydrothermal treatment, cold treatment, and irradiation. International trade associated with potential biological invasions—when the traded commodities are fruits, grains, and propagative materials (seeds, roots, tubers, seedlings)—grants importing countries the prerogative to establish strict phytosanitary criteria to safeguard their territories from exotic and quarantine pests (FAO, 2009).

Stancioli and Sugayama (2015) define risk, in plant health defense, as the interaction between the probability of a given hazard occurring (introduction of a pest into a pest-free area). In other words, risk is the product of the probability of an undesirable event occurring versus the resulting impacts (economic, social, environmental). Accordingly, the EU published the following regulations to support trade with MERCOSUR: Directive 2000/29/EC; Delegated Regulation (EU) 2019/829; and Implementing Directive (EU) 523/2019. The latter introduced the most restrictive conditions for the entry of citrus fruits due to the risk of fruit fly dissemination (Tephritidae, non-European), specifically targeting *Anastrepha fraterculus* and *Anastrepha obliqua*, species prevalent in Brazil.

Considered major pests of global fruit production, the most important fruit fly species in Brazil belong to the genus *Anastrepha* and *Ceratitis capitata*, causing direct damage to fruit production, as larvae feed on the pulp, rendering fruits unsuitable for fresh consumption or industrial processing, and indirect damage through quarantine barriers imposed by importing countries (PARANHOS *et al.*, 2004). The objective of this study was to analyze the phytosanitary risk associated with fruit fly infestation in sweet orange and Tahiti acid lime fruits, aiming to support the maintenance of phytosanitary certification and compliance with European Union requirements for citrus export.

## MATERIALS AND METHODS

**Characterization of the Fruit-Growing Landscape:** Between February 13 and 14, 2020, four fruit-growing landscapes were mapped: two in the Agreste de Alagoinhas and Northern Coast territory (LN) (Agropecuária Gavião Farm and Itacitrus Farm), one in

the Recôncavo Baiano (RB) (Rebouças Farm), and one in the Baixo Sul (BS) region (Peralba Farm – Aros Agrícola). A drone (DJI Mavic Pro) was used to conduct aerial surveys of the four rural enterprises.

**Field Monitoring of Adult Fruit Flies (FF):** Data were collected over 45 weeks (315 days) in 2020 at citrus-exporting farms: Agropecuária Gavião and Nossa Senhora do Bom Sucesso – ITACITRUS (Northern Coast), Aros Agrícola (Baixo Sul), and Rebouças (Recôncavo Baiano). Ten McPhail-type traps (Fig. 03) were installed in each sampled orchard, spaced 20 meters apart, along the perimeter of blocks containing 2,000 plants, baited with 7% sugarcane molasses (SourceDourada® brand). Two of these traps were installed in each “home orchard” on each property.



**Fig. 1. Installation of McPhail-type traps, replacement of food attractant, and preparation of fruit fly samples collected from the traps at Rebouças Farm, Cruz das Almas, 2020. (Source: Stenilson Nascimento)**

The MAD index (flies/trap/day) was used to express the population density of the target insect. The collected material was sent to the Department of Entomology and Acarology at USP/ESALQ and to ADAB/CETAB for species-level identification through eversion of the female genitalia. Faunistic indices were expressed based on the parameters of Dominance, Frequency, and Constancy to characterize the degree of prevalence of each *Anastrepha* species within the sampled universe.

**Forced Infestation Tests under Laboratory Conditions:** The activities began with a pre-test conducted over a three-week period, using guava fruits (*Psidium guajava*) as a reference control. After evaluation, a low infestation rate was observed, leading to the use of mango fruits (*Mangifera indica*), a preferred host of *A. obliqua*. Forced infestation assays for the fruit fly *A. obliqua* were conducted using three sweet orange varieties (*Citrus sinensis* L. Osbeck): ‘Valencia’, ‘Natal’, and ‘Pera’, as well as Tahiti acid lime (*C. latifolia* Yu. Tanaka), with 10 replicates per variety. The experimental design included a positive control with mango and an absolute negative control (fruit only). The assays were carried out at the Entomology Laboratory of Embrapa Cassava and Fruits, located in Cruz das Almas, Bahia, Brazil, between 2023 and 2024, in a climate-controlled room ( $26 \pm 2$  °C, 60% RH, and a 12 h/12 h photophase). In screened cages (Fig. 04), three fruits of each citrus

variety/species were placed and exposed to 10 pairs of sexually mature *A. obliqua* for a period of 72 hours. After exposure, the fruits were removed from the cages and individually placed in plastic containers containing vermiculite.

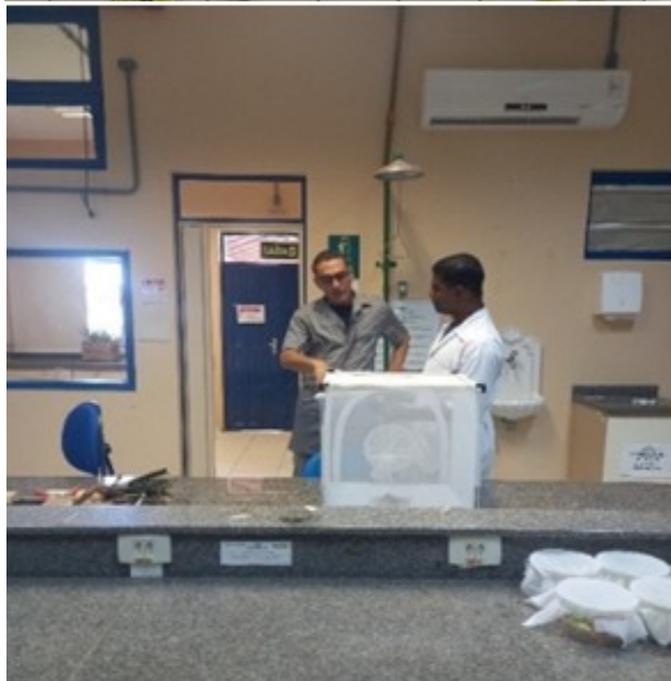


Fig. 2. Cages with individualized treatments for forced infestation assays of *Anastrepha obliqua* on sweet orange and Tahiti acid lime fruits, EMBRAPA, Cruz das Almas, 2024. (Source: Vanderson Rodrigues and Suely Brito)

Each fruit and vermiculite set was sieved at 15- to 21-day intervals to obtain pupae. The pupae and larvae were counted and transferred to jars with vermiculite to obtain adults. After adult emergence, the flies were counted and sexed using a Leica MZ12 stereomicroscope. Parameters analyzed: number of fruits with puncture and/or oviposition symptoms, number of pupae and adults obtained.

**Pupal Viability (PV):** The pupal viability index, expressed as a percentage, was calculated using the formula proposed by Castro Portilla (2002):

$$PV (\%) = \frac{\text{number of emerged adults} \times 100}{\text{number of pupae}}$$

**Faunistic Analysis:** Data obtained from biweekly collections of *Anastrepha* spp. adults were used for faunistic analysis to characterize and delimit each of the evaluated communities. The faunistic analysis of fruit fly species was performed based on Silveira Neto *et al.* (1976). Species dominance is calculated as relative frequency, i.e., the proportion of individuals of a given species in relation to the total number of fruit flies captured during the analyzed period and location. The basic formula is:

$$D(\%) = \left( \frac{x \text{ species}}{Y \text{ total species}} \right) \times 100$$

where D is the proportion of individuals of a given species (x) relative to the total number of captured species (Y).

Frequency indicates the proportion of individuals of a species relative to the total number of individuals in the sample and was calculated using the formula:

$$P_i = \frac{n_i}{N}$$

where  $n_i$  is the number of individuals of species  $i$  and N is the total number of individuals in the sample.

Constancy corresponds to the percentage of samples in which a given species was present and was calculated as:

$$C = \frac{p \times 100}{N}$$

where p is the number of samples containing the species and N is the total number of samples.

Species classification according to constancy:

- Constant species (w): present in more than 50% of samples
- Accessory species (y): present in 25–50% of samples
- Accidental species (z): present in less than 25% of samples

**MAD Index:** The Fly/Trap/Day index (MAD) was calculated using the formula (number of flies captured / number of traps installed / number of days between collections) (Araújo & Zucchi, 2003):

$$MAD = \frac{N}{A \times D}$$

where MAD = flies/trap/day; N = total number of flies captured; A = number of traps evaluated; D = interval (days) between collections.

## RESULTS AND DISCUSSION

**Fruit-Growing Landscapes:** Some striking characteristics of the fruit-growing landscapes (Fig. 05) at each studied location were recorded:

- (i) In the Northern Coast (LN), the citrus-cultivated area in the two orchards is ten times larger than that of the other two orchards in the RecôncavoBaiano (RB) and Baixo Sul (BS);
- (ii) In the LN, citrus-cultivated areas form a large and continuous area with the presence of the same host, whether planted with Tahiti acid lime or sweet orange;
- (iii) Orchards in the LN exhibit more technified management, including irrigation systems, nutrition programs based on soil analysis results, weed control, and pest monitoring conducted by inspectors;
- (iv) In the LN, the evaluated areas are located approximately 2.5 km away from domestic orchards that harbor several hosts for the genus *Anastrepha*;
- (v) The Peralba Farm orchard, in its surroundings, is adjacent to a dense Atlantic Forest area and several domestic orchards, resulting in greater diversity of fruit fly occurrence;
- (vi) Rebouças Farm is surrounded by small domestic orchards averaging approximately 3.0 ha.



**Fig. 3. Aerial survey conducted by ADAB using a DRONE in four orchards across three regions of Bahia: Baixo Sul, RecôncavoBaiano, and Northern Coast, March 2020**

Among the four evaluated sites, the farms located in the Northern Coast (LN) had the largest citrus-planted areas. AgropecuáriaGavião Farm, which cultivated citrus (sweet orange and Tahiti acid lime), was located near a rural settlement whose surrounding smallholdings contained non-commercial hosts for fruit flies (cashew, mango, guava, acerola) and small citrus plantings, which likely contributed to the migration of fruit flies from these areas into the evaluated orchard. ITACITRUS Farm, with approximately 700 ha of a continuous Tahiti acid lime cultivation belt, was geographically isolated from other orchards; moreover, its surroundings included some properties with cattle pastures. Under this scenario, there was low pressure from visiting fruit flies in the evaluated orchard, resulting in the lowest population density of *Anastrepha*. In the Recôncavo region, Rebouças Farm cultivated only Tahiti acid lime and was surrounded by the BR-101 highway, small rural properties, and a packing house providing citrus fruit processing services. In the Baixo Sul region, Peralba Farm (Aros Agrícola) cultivated only Tahiti acid lime and was surrounded by Atlantic Forest fragments and isolated from other citrus orchards, constituting the orchard with the highest population pressure and diversity of fruit flies. Studying ecotones between forests and orchards in Mato Grosso do Sul, Uchoa *et al.* (2023) identified that natural environments present greater stability and vegetation heterogeneity, expressing high species richness and greater evenness, given the many habitats and niches available to fruit fly species. Wild hosts play a predominant role in shaping fruit fly populations, as they enable the multiplication of fruit flies; however, this condition alone is not sufficient to maintain fruit flies within native vegetation domains, since they tend to migrate to orchards in search of oviposition sites (Sugayama&Malavasi, 2000).

**Table 1. Fruit fly species of the genus *Anastrepha* captured in McPhail traps installed in citrus orchards at AgropecuáriaGavião Farm, Nossa Senhora do Bom Sucesso Farm (ITACITRUS), Rebouças Farm, and Aros Agrícola Farm, and identified by the Entomology Laboratories of ESALQ/USP and CETAB/ADAB, 2022**

Fruit fly species	No. of individuals
<i>Anastrepha fraterculus</i>	59
<i>Anastrepha obliqua</i>	25
<i>Anastrepha pseudoparallela</i>	11
<i>Anastrepha distincta</i>	7
<i>Anastrepha consobrina</i>	3
<i>Anastrepha sororcula</i>	3
<i>Anastrepha dissimilis</i>	1
<i>Anastrepha quiinae</i>	1
<i>Anastrepha zenildae</i>	1
<i>Anastrepha sp.</i>	1
<b>Grand total</b>	<b>112</b>

**Field monitoring of adult fruit flies:** At the Rebouças, Aros Agrícola, AgropecuáriaGavião, and ITACITRUS farms, the calculated MAD indices were 7.44, 6.16, 2.28, and 0.69, respectively. With MAD values below 1.0, only ITACITRUS Farm would be able to export fruit without the need to apply any population control strategy for fruit flies. From a total of 112 individuals, ten fruit fly morphospecies of the genus *Anastrepha* were captured, nine of which were identified at the species level: *A. fraterculus*, *A. obliqua*, *A. pseudoparallela*, *A. distincta*, *A. consobrina*, *A. sororcula*, *A. dissimilis*, *A. quiinae*, *A. zenildae*, and *Anastrepha sp.* (Table 1). According to the faunistic analysis, *Anastrephafraterculus* was the dominant species (52.67%), followed by *A. obliqua* (22.32%) and *A. pseudoparallela* (9.82%). Based on the frequency parameter, the species were classified as Constant (*A. fraterculus*), Common (*A. obliqua*, *A. pseudoparallela*, *A. distincta*), and there maining species as Rare. Although several *Anastrepha* species were observed in the evaluated orchards, only two species (*A. Fraterculus* and *A. obliqua*) accounted 69,64% of all fruitfly individuals captured in the traps, as they were the most frequent. This pattern characterizes the high dominance of the former species and is consistent with similar findings reported by Aluja *et al.* (1996) and by studies conducted in Brazil (Nascimento & Zucchi, 1981), Mexico (Malo *et al.*, 1987), and Costa Rica (Soto-Manitiú & Jirón, 1989). When comparing the occurrence of *A. Fraterculus* among the evaluated orchards, Aros Agrícola Farm recorded the highest number of captured specimens (Table 02), most likely due to the geolocation of its orchard near areas with Atlantic Forest fragments. This scenario favors fruitfly populations by providing a diversity of host plants, as it represents a relatively undisturbed ecosystem, that is, an areathat has not undergone significant alterations in its original composition.

**Table 2. Number of fruit fly specimens of the genus *Anastrepha* captured in McPhail traps in citrus orchards at Agropecuária Gavião Farm, Nossa Senhora do Bom Sucesso Farm (ITACITRUS), Rebouças Farm, and Aros Agrícola Farm. Data compiled in 2024**

Fruit fly species	Rebouças Farm	Gavião Farm	Aros Farm	Itacitrus Farm	CNPMF
<i>Anastrepha fraterculus</i>	6	11	42	0	0
<i>Anastrepha obliqua</i>	7	1	11	0	6
<i>Anastrepha pseudoparallela</i>	0	11	0	0	0
<i>Anastrepha distincta</i>	0	0	4	3	0
<i>Anastrepha consobrina</i>	0	3	0	0	0
<i>Anastrepha sororcula</i>	0	2	0	1	0
<i>Anastrepha dissimilis</i>	0	0	1	0	0
<i>Anastrepha quiinae</i>	0	0	1	0	0
<i>Anastrepha zenildae</i>	1	0	0	0	0
<i>Anastrepha sp.</i>	0	0	1	0	0
<b>TOTAL</b>	<b>14</b>	<b>28</b>	<b>60</b>	<b>4</b>	<b>6</b>

These data are corroborated by evaluations carried out by Calvo *et al.* (2022) in citrus orchards in Uruguay under different landscape conditions, considering both food availability (native and exotic fruits) and management practices (commercial and non-commercial environments). Those authors observed the predominance of *Ceratitidis capitata* in introduced fruits, whereas *Anastrepha fraterculus* predominated in native fruits. Considering exotic hosts of commercial interest, pear trees (*Pyrus communis* cv. William's) showed the highest infestation levels by *A. fraterculus*, and to a lesser extent citrus fruits, such as grapefruit (*Citrus paradisi* Macfadyen cv. Duncan and cv. Ruby) and Afourer mandarin (*C. clementina* cv. Afourer). These findings indicate that, despite the constant presence of *A. fraterculus* in traps installed in citrus orchards in Bahia, its preferred hosts are other plant species, which may occur in orchard ecotones and native vegetation areas. Regarding the association between fruit flies and their hosts, Zucchi (2000) relates *A. fraterculus* to the botanical families Anacardiaceae, Annonaceae, Malpighiaceae, Myrtaceae, Passifloraceae, Rutaceae, Sapotaceae, among others. Within the Rutaceae, the author cites the following species: sour orange, navel orange, sweet orange, Japanese orange, Valencia orange, and lime. For *A. obliqua*, occurrence is reported only in sour orange and sweet orange fruits. Therefore, Tahiti acid lime fruits are not considered preferred hosts for *Anastrepha* species, which substantially reduces the quarantine risk associated with fruit flies in shipments exported to the European Union.

**Forced Infestation Tests Under Laboratory Conditions:** Symptoms of punctures or oviposition were more frequent in the 'Pera' variety (28.8%) than in the 'Valencia', 'Natal', and Tahiti acid lime varieties, with 11.9%, 8.3%, and 7.1%, respectively. No fruit showed larval development or adult emergence, a positive condition when assessing the quarantine risk of fresh fruits as potential pathways for pest dissemination. This result may be associated with the chemical composition of the fruit peel. Schneider (1968) and the University of California (1984) characterize citrus fruits by their three distinct layers: the outermost portion, the flavedo or exocarp, composed of the cuticle, epidermis, and hypodermis containing oil glands; the middle portion of the peel, known as the albedo or mesocarp; and the endocarp or pulp, which constitutes the edible part of the fruit. The flavedo region contains oil glands whose number and size vary according to citrus species and variety. Batish *et al.* (2008) describe essential oils as secondary plant metabolites composed of monoterpenes, sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones, which determine characteristic odors and aromas and are produced for protection against insects, mites, and pathogens. According to Anwar *et al.* (2016) and Bhattacharya (2016), essential oils present in citrus fruits may exhibit antibacterial, antifungal, antioxidant, preservative, and insecticidal activities, particularly those from bitter orange (*Citrus aurantium* L.). The positive control (mango) showed symptoms in 96.7% of the fruits, with 349 pupae and 100% pupal viability. These data demonstrate that fruit flies were sexually active and that mango fruit is used as a preferred host for their reproduction. However, for citrus fruits, a low puncture rate—on average 82.8% lower than that observed in mango—and the absence of pupal and adult development were recorded.

These results are scientifically supported by the observations of Raga and Galdino (2017), who demonstrated that the essential oils of true lemons and Tahiti acid lime (*Citrus latifolia* Yu. Tanaka) are more toxic to fruit fly eggs and larvae than those of other citrus species of economic importance. Thus, when eggs are laid within oil glands, the survival of first-instar fruit fly larvae is compromised. Under controlled laboratory conditions, forced infestation of Tahiti acid lime and Sicilian lemon resulted in egg and larval mortality of *Anastrepha fraterculus*. According to Raga and Galdino (2017), for most fruit fly species there is high egg and larval mortality when attacking citrus fruits (Greany *et al.*, 1983). Eggs and larvae of *A. fraterculus* and *Ceratitidis capitata* are exposed to toxic compounds present in the flavedo of citrus species. Moreover, host preference should be considered in the establishment of phytosanitary restrictions. Dias *et al.* (2017), in a comparative study on the oviposition of *Anastrepha*

*fraterculus* and *Ceratitidis capitata* on citrus fruits (orange 'Navelina', mandarin 'Clemenules', and lemon 'Siciliano'), reported that the number of eggs laid on orange and mandarin fruits did not differ significantly between the two fruit fly species, whereas no oviposition occurred on lemon fruits. *A. fraterculus* preferred to oviposit on mandarin fruits, while *C. capitata* showed a preference for orange fruits. Regarding quarantine risk associated with international traffic, in a study conducted at inspection points (airports and ports) in southern Italy, Pace *et al.* (2022) observed an alarming number of organisms potentially harmful to local crops or posing threats to the European Union. These were associated with international passenger baggage, as travelers were often unaware of phytosanitary threats and therefore transported plant materials for food or decoration. When compared to the transit of large commercial consignments of plant products (such as mango, banana, grape, Tahiti acid lime, among others), this category represents a lower risk of pest dissemination, since such products entering Europe require phytosanitary certification, undergo post-harvest treatments, and are endorsed by National Plant Protection Organizations (NPPOs)—in Brazil's case, the Ministry of Agriculture and Livestock (MAPA).

## FINAL CONSIDERATIONS

The present study contributes to strengthening the technical and scientific basis that guides Brazilian agricultural defense in the context of international citrus trade. In line with the principles of the International Plant Protection Convention (IPPC), applicable phytosanitary measures must be proportional to risk, technically justified, and transparent. Thus, the data generated support the actions of MAPA and state-level authorities in negotiating and, when necessary, adjusting the requirements imposed by the European Union, promoting phytosanitary security with regulatory rationality.

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