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New Records of *Anastrepha grandis* (Diptera, Tephritidae) and *Neosilba zadolicha* (Diptera, Lonchaeidae) in Cucurbitaceae Species in Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. Author FBB provided the collections and the laboratory activities. Author LHCB helped the field collections. Author LJG identified the Lonchaeidae specimens. Author AR designed the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This is the first report of natural infestation of fruit flies *Anastrepha grandis* Macquart (Tephritidae) and *Neosilba zadolicha* McAlpine & Steyskal on bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] and mogango (*Cucurbita pepo* L.). Six cultivars of Cucurbitaceae were susceptible to infestation by *A. grandis* and their larvae reach pupation and adult emergence. The average weight of pupae was similar between zucchini, squash 'Menina Brasileira' and mogango. Orange pumpkin and 'Atlas' butternut showed the lowest average weight of pupae and their sampled pupae showed similarity of weight by Morisita index. The pupal viability ranged from 57.5% to 95.8%.

Keywords: Tephritidae; Lonchaeidae; Anastrepha grandis; Neosilba zadolicha; Cucurbita.

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1. INTRODUCTION

The Cucurbitaceae is a distinct family without any close relatives, which includes many vegetables, such as cucumber, melon, watermelon, squash, pumpkin, and gourds. *Cucurbita pepo* L. include pumpkin, gourd, acorn squash, marrow, summer squash, and zucchini. *Cucurbita maxima* L. types are called winter squash, and *Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poir] is squash or pumpkin [1] that are cultivated worldwide. Phytosanitary issues are among the main factors that compromise the Brazilian production of cucurbits [2].

Among more than 230 described *Anastrepha* species (Diptera: Tephritidae) [3], there are seven species of economic importance: *A. fraterculus* (Wied.)*, A. grandis* Macquart*, A. ludens, A. obliqua, A. serpentina, A. striata*, and *A. suspensa* [4]. Most species of *Anastrepha* that are of no economic importance, and even a few with such importance (i.e., *A. grandis* and *A. striata* Schiner), are either monophagous or stenophagous and have long adult life spans [5].

The South American cucurbit fly (SACF), *A. grandis* is a pest of various native and introduced species of Cucurbitaceae in many areas of South America [6], even spreading into Panama (Central America) [7]. SACF is a quarantine pest for many countries from different continents, because represent phytosanitary risk to be spread in other regions by fresh vegetable trade.

Immature fruits of cucurbits apparently are preferred, but mature or nearly mature fruits of some varieties are occasionally infested by *A. grandis* [8]. Generally, in all fruit samples infested by *Ceratitis capitata* (Wied.) or *Anastrepha* spp. in the state of São Paulo, Brazil, specimens of *Neosilba* (Lonchaeidae) are recovered [9]. From 1985-2001, specimens (1,004) of Lonchaeidae were intercepted from plants in U.S. ports of entry [10]. The family comprises eight genera and 561 species; some have shown economic damage in many crops [11,12]. *Neosilba* comprises 40 species in the Neotropical region [11], of which 10 are polyphagous species.

SACF is registered in ten Brazilian states, which the majority is located in central, southeast, and southern regions [13]. *Anastrepha grandis* is very common in eastern side of the state of São Paulo. In that region, immature stages are recovered from squash fruits, while *A. grandis* adults are captured in McPhail traps, in many fruit crops. Data from fruit infestation and respective hosts are scarce in the literature. This study's aim is to register the natural and artificial infestation data of fruit flies in species of Cucurbitaceae.

2. MATERIALS AND METHODS

2.1 Fruit Fly Occurrence

The fruits were collected from unsprayed plants from June 2015 to January 2016. Five developed fruits of bottle gourd *Lagenaria siceraria* (Mol.) Standl. were picked up from unsprayed plants in the municipality of Serra Negra (SP; 22°38'9.94"S; 46°41'53.36"O; 957m), with average weight of 2,694 g per fruit. Three fruits of mogango, *Cucurbita pepo* L., with average weight of 1,270 g, were picked up in commercial plantation situated in the municipality of Monte Alegre do Sul (SP; 22°43.460'S; 46°48.305'O; 702 m). Fruits were brought to the Laboratory of Economic Entomology of Instituto Biológico (Campinas, SP, Brazil) and they were placed individually in fruit-holding boxes containing vermiculite at the bottom. About 30 days later, the substrate was sieved and the Tephritoidea puparia were transferred to a glass cage (6,000 cm^3) to allow emergence. The material was kept at 25 ±2ºC and 70 ± 10% of relative humidity for 25 days to obtain adults. Adults were fed with a mixture of sugar and yeast extract (3:1) and water. For identification purposes, adults were transferred to labeled vials with 70% alcohol. *Anastrepha* females were identified based on key identifications for female [14,15,16], and specimens of Lonchaeidae were classified according to McAlpine & Steyskal [17,18]. Voucher specimens of Lonchaeidae were deposited at Museu de Biodiversidade of Federal University of Grande Dourados (MS).

2.2 Artificial Infestation

Fruits of zucchini cv. 'Caserta' (*C. pepo*), squash cv. 'Menina Brasileira' [*Cucurbita moschata* (Duch. ex Lam.) Duch. Ex Poir], `Atlas´ butternut (*C. moschata*), mogango, orange pumpkin (*Cucurbita maxima* Duch. ex Lam.) and bottle gourd *L. siceraria* were exposed to *A. grandis* infestation during approximately 10 hours in cage (40 x 40 x100 cm), containing up to 150 couples of SACF sexually matures. After infestation, all fruits were submitted to the same above process to obtain pupae and respective adults. After infestation, fruits were submitted to the same

above process to obtain pupae and adult infestation. Immature and mature stages of *A. grandis* are illustrated on Fig. 1(a, b, c, d, f).

Pupal weights of *A. grandis* obtained in six cucurbits were compared using the Morisita index, to determine host similarity among the cultivars. Data were submitted to PAST program [19].

3. RESULTS AND DISCUSSION

3.1 Fruit Fly Occurrence

All fruits of bottle gourd and mogango were naturally infested by *A. grandis.* In total, 283 puparia of SACF were recovered from bottle gourd, which corresponding to 21.01 puparia per Kg of fruit. In mogango, we obtained 362 puparia of SACF and 71.26 puparia per Kg of fruit. SACF has infested cucurbits since young immature fruits. *Anastrepha grandis* is strongly related to *Cucurbita* genus [20,21], including many botanical species of economic importance.

The mature fruits of mogango and bottle gourd (Fig. 1h) present hard shells, but *A. grandis* female is able to oviposit and the third larva is capable of crossing the modified plant tissue to pupate in the soil. At the end of larval stage, the bottle gourd showed dark spots on peel (Fig. 1h) and modified pulp by larva feeding (Fig. 1i).

Co-infestation of *A. grandis* and *Neosilba zadolicha* McAlpine & Steyskal occurred in both cucurbits. From bottle gourd fruits, 24 adults of *Neosilba* emerged. The identification of *N. zadolicha* was based on 10 males of *Neosilba*.

A polyphagous species, *N. zadolicha* infests hosts of Anacardiaceae, Annonaceae, Chrysobalaneae, Combretaceae, Convolvulaceae, Cucurbitaceae, Ebenaceae, Fabaceae, Lauraceae, Loganiaceae, Loranthaceae, Malpighiaceae, Mimosaceae, Moraceae, Musaceae, Myrtaceae, Olacaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae, Sapotaceae, and Solanaceae [12,22, 23,24,25].

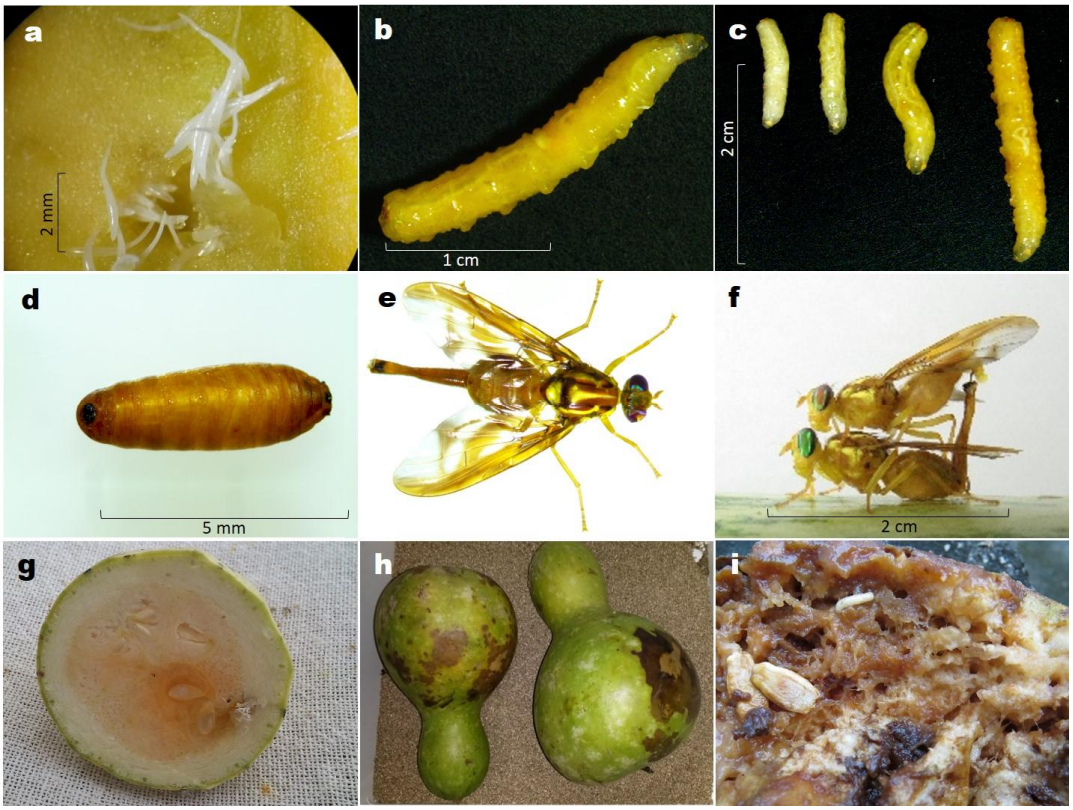

Fig. 1. Egg masses (a), larvae (b, c), pupa (d), female (e) and copula (f) of *A. grandis* and, symptons in squash (g) and bottle gourd (h, i) (Photos: F. B. Baldo)

It is likely that the soft plant tissue, previously infested by SACF, facilitates further infestation by *N. zadolicha* [26], although some hosts can be infested exclusively by lonchaeids [9]. *Neosilba zadolicha* has been registered in the state of São Paulo in other cultivar of *C. moschata, C. maxima,* and "Tetsukabuto" interspecific hybrid (*C. maxima* x *C. moschata*) [12].

Bottle gourd (*L. siceraria*) is originally from Africa [27], while *Cucurbita* genus is native to the Americas, with a natural distribution from the US to Argentina [28]. Both Tephritoid species have demonstrated a plasticity in terms of cucurbit host range [12,19], and they have been captured all year long.

3.2 Artificial Infestation

The six cucurbits were susceptible to *A. grandis* infestation, under laboratory conditions; and they provided nutritional conditions to that species to complete the life cycle (Table 1). The weight of puparia was highest and similar between squash and mogango, but it was lowest in orange pumpkin and 'Atlas' butternut.

The highest pupal viability was obtained in bottle gourd and the lowest in orange pumpkin (Table 1). The value of this parameter is partially related to the weight of puparia. Although we do not measure egg-adult duration, zucchini provided the shortest life cycle.

Zucchini, squash, mogango, and orange pumpkin produced 35, 58, 163, and 736 puparia of *A. grandis* per fruit or, 111.5, 179.2, 143.0, and 223.3 puparia per kilogram of fruit, respectively. Considering that the *A. grandis* fecundity can reach 538.6 per female [20], the potential risk of SACF spread increases substantially, because one puncture can store up to 110 eggs [29]. From ten punctures of SACF in orange pumpkin, it was obtained an average of 35.3 (9 - 86) eggs per puncture, under cage conditions. In laboratory, from 32 egg masses of *A. grandis* in zucchini cv. Caserta (Fig. 1g), we obtained an average of 51.4 eggs per laying (17 – 96).

Depending on the host, the average period from egg to adult is 49 days [2]. Considering the duration of development cycle and pre-oviposition period, a new generation of *A. grandis* will occurred 83 days after the first oviposition, under mild temperatures.

Based on Morisita index, the weights of puparia of *A. grandis* in ´Atlas´ butternut and orange pumpkin were similar and provided a special group. Another group similar in terms of weight of puparia was performed by zucchini cv. 'Caserta', squash 'Menina Brasileira' and mogango (Fig. 2).

´Atlas´ butternut is exported from the western regions of the state of São Paulo, which is growing under status of low-prevalence areas of *A. grandis*. Due to long life cycle, natural infestation of *A. grandis* may not be detected when packing and shipping processes. Considering the *A. grandis* fecundity can reach 538.6 per female [2], the potential risk of SACF spread increase substantially because only one puncture can show up to 110 eggs [29]. Although in our conditions, SACF is not an invasive species, the transportation of infested fruits can facilitate the establishment of the pest into new areas, where many wild and cultivated cucurbit species are available.

Table 1. Fruit fly infestation observed in cucurbits in the State of São Paulo, Brazil

Fruit	No. of fruits	Average weight of fruit (g)	No. of puparia	Average weight of puparia (mg)*	No. of adults	Pupal viability (%)
Zucchini cv. 'Caserta'	24	315.82	845	34.5 (18.9-47.3)	486	57.5
Squash cv.'Menina Brasileira'	12	325.12	699	35.3 (19.9 – 48.5)	600	85.8
´Atlas´ Butternut	11	1,012.00	871	31.0 (24.8 – 41.1)	726	83.3
Mogango	1	1,140.00	163	35.6 (23.5 - 47.4)	107	65.6
Orange Pumpkin	3	3,296.00	2,208	31.9 (23.7 - 40.5)	1,242	56.2
Bottle gourd	1	2,450.00	48	40.00 (35.7 - 51.4)	46	95.8

**n = 55 puparia, except for bottle gourd (48)*

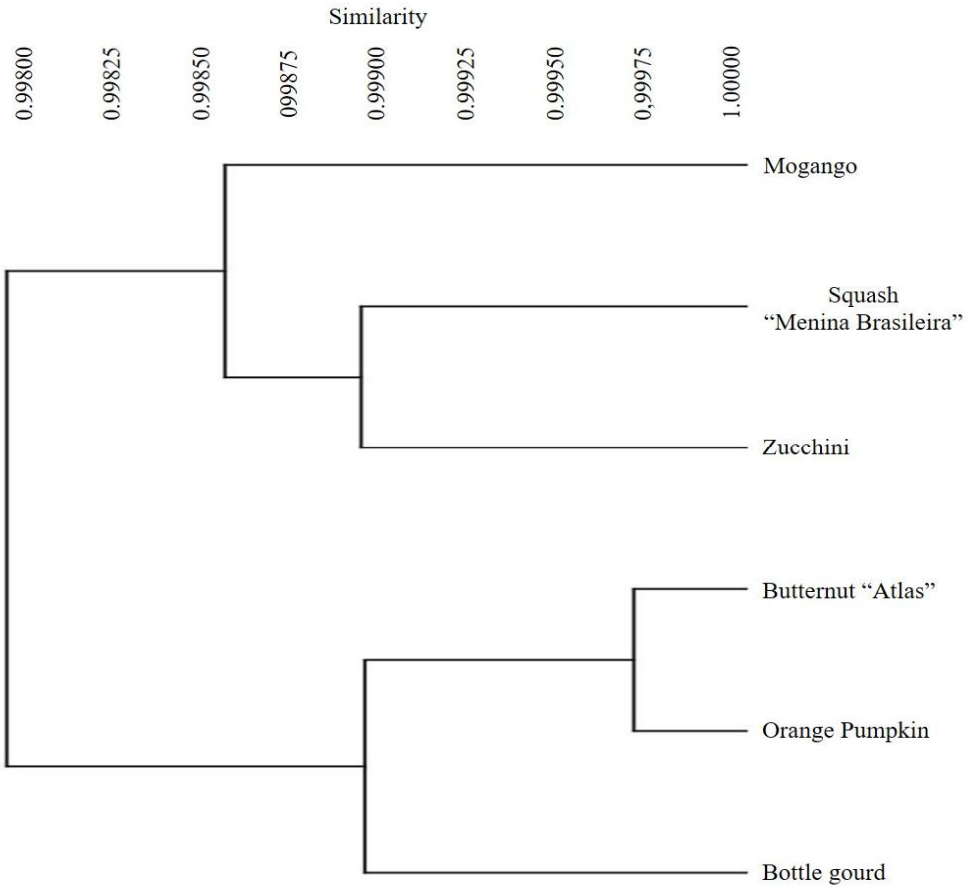

Fig. 2. Dendrogram based on the similarity of pupal weight of *Anastrepha grandis* among six cucurbits, using Morisita index

Squash, mogango, and orange pumpkin are popular among consumers in Brazil, and those vegetables are growing in fields and home gardens. Sometimes, where SACF is endemic, cucurbits has been cultivated without chemical control of *A. grandis.* Therefore, a considerable percentage of fruits is lost during the phases of growth, harvest, selection, transportation, and commercialization. As a result, the losses caused by the SACF are underestimated, and reinforce the need to improve vigilance systems to spread the insect to free areas. Data of host suitable and their biology are essentials to manage insect populations, especially quarantine pests.

4. CONCLUSION

This study demonstrated the potential damage of *A. grandis* in cucurbit crops and the risk of its spread to another regions and continents by infested fruits. The most fruits of *C. pepo, C. maxima, C. moschata* and hybrids in Brazil, is highly susceptible to *A. grandis* infestation. From just one oviposition, approximately 25 couples in average can emerge, which is favorable to establish the pest into new areas. Thus, integrated pest management and accurate inspection in packing houses should be improved for phytosanitary safe guards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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