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# Assessment of Navel Oranges, Clementine Tangerines, and Rutaceous Fruits as Hosts of *Bactrocera cucurbitae* and *Bactrocera latifrons* (Diptera: Tephritidae)

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**ABSTRACT:** Export of *Citrus* spp. fruits may require risk mitigation measures if grown in areas with established tephritid fruit fly (Diptera: Tephritidae) populations capable of infesting the fruits. The host status of *Citrus* spp. fruits is unclear for two tephritid fruit fly species whose geographic ranges have expanded in recent years: melon fly, *Bactrocera cucurbitae* (Coquillett), and *Bactrocera latifrons* (Hendel). In no choice cage infestation studies, *B. latifrons* oviposited into intact and punctured Washington navel oranges (*Citrus sinensis* [L.] Osbeck) and Clementine tangerines (*C. reticulata* L. var. Clementine), but eggs rarely developed to the adult stage. *B. cucurbitae* readily infested intact and punctured tangerines, and to a lesser extent punctured oranges, but did not infest intact oranges. Limited cage infestation and only a single literature report of field *Citrus* spp. infestation suggest that risk mitigation of *Citrus* spp. for *B. latifrons* is not needed. Risk mitigation options of *Citrus* spp. for *B. cucurbitae,* including heat and cold treatments and systems approaches, are discussed.

**KEYWORDS:** citrus, host status, *Bactrocera cucurbitae*, *Bactrocera latifrons*, melon fly, risk mitigation

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## Introduction

*Citrus* spp. (Rutaceae family) are believed to be native to tropical and subtropical regions of Southeast Asia, but are now widely cultivated throughout the tropics and subtropics.[1] Throughout their geographic range of distribution, *Citrus* spp. fruits can be subject to infestation by a range of different tephritid (Diptera: Tephritidae) fruit fly species. Although some tephritid fruit fly species, like Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and Oriental fruit fly, *Bactrocera dorsalis* (Hendel), can be major pests of *Citrus* spp.,[2,3] the host status of *Citrus* spp. is less clear for other tephritid fruit fly species, such as melon fly, *Bactrocera cucurbitae* (Coquillett), and *Bactrocera latifrons* (Hendel). Both these species have expanded their ranges far beyond Southeast Asia into some Pacific Islands[4,5] and into the African continent.[6,7] If *Citrus* spp. are hosts of these fruit fly species, then regulatory procedures would need to be developed in countries of citrus production where these fruit fly species are present in order to minimize the risk of introducing these fruit flies during export of fruits to areas where they are not established. A pest risk assessment (PRA), aimed to examine plant pest risks associated with the movement into the continental United States of *Citrus* spp. fruits (*Citrus paradisi* Macfaiden, *Citrus limon* [L.] Burm. f., *Citrus aurantiifolia* [Christmann] Swingle, *C. sinensis* [L.] Osbeck, *Citrus grandis* [L.] Osbeck, and *C. nobilis* Lour. var. *deliciosa* [Ten.] Swingle) grown in Hawaii, concluded that *B. dorsalis, B. cucurbitae*, and

*C. capitata* are high-risk pests of citrus fruits, and that specific phytosanitary measures were strongly recommended to achieve quarantine security mandated by USDA-APHIS-PPQ.[8] The Hawaii Department of Agriculture (HDOA) petitioned the USDA-Animal and Plant Health Inspection Service (APHIS) to approve a cold treatment schedule (≤0.99°C, for 17 days, or ≤1.38°C for 20 days) as a quarantine treatment to mitigate fruit fly infestation in *C. sinensis*.

Further data on the infestability of citrus by *B. cucurbitae* and *B. latifrons* are, however, needed to better establish the host status of *Citrus* spp. Here, we (1) present results of laboratory trials that assess the infestability of two citrus species (Washington navel oranges, *C. sinensis* (L.) Osbeck, and Clementine tangerines, *C. reticulata* L. var. Clementine) by *B. cucurbitae* and *B. latifrons*, and (2) summarize references in published literature to infestation of fruits belonging to the plant family Rutaceae by *B. cucurbitae* and *B. latifrons*. Potential regulatory procedures are discussed to mitigate the risk of introduction of fruit fly pests exported from areas with established *B. cucurbitae* and/or *B. latifrons* populations.

## Materials and Methods

**Insect colonies.** *B. latifrons* and *B. cucurbitae* flies used in experiments were obtained from laboratory colonies at the USDA ARS Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center in Hilo, HI. The *B. latifrons* colony has been maintained for over 20 years (over ~208 generations), and the *B. cucurbitae* colony has been maintained for over 36 years (over ~478 generations) with infrequent infusion of wild flies. Fruit flies used in our tests were kept in an insectary at 24–27°C, 65–70% RH, and a photoperiod of 12:12 (L:D) hours. Adults were fed water and a diet of sugar cubes and a "protein cake" consisting of three parts of sucrose, one part of protein yeast hydrolysate (Enzymatic; United States Biochemical Corporation, Cleveland, OH), and 0.5 part of torula yeast (Lake States Division, Rhinelander Paper Co., Rhinelander, WI) from the time of emergence from puparia until noon, the day before the experiment, at which time, cohorts of 50 gravid females were placed with a water wick and two sugar cubes (no "protein cake"), in 26.5 × 26.5 × 26.5 cm cubical screened cages. When fruits were added (see below), the water wick remained in the cage, but the two sugar cubes were removed. Adult flies were approximately 16–18 days old at the start of the experiments.

**Bioassays.** Bioassays were conducted from 24 October, 2012, to 2 August, 2013. Fruits used were randomly selected from unblemished fruits available at a local grocery store. For each bioassay, a thoroughly rinsed single harvest-mature fruit (either navel orange or tangerine) was weighed and then placed in each of the eight cages. The fruit in four of the cages was undamaged (intact), while the fruit in the other four cages was randomly punctured 50 times using a 1.0 mm diameter probe, with probes penetrating to a depth of 1.0 cm. An undamaged control fruit, known to be a good host of the fruit fly species being tested, was placed in another cage. This group of nine concurrent cage tests is hereafter referred to as a "trial." Trials were conducted separately for *B. latifrons* and *B. cucurbitae*. For *B. latifrons*, the control fruit was either eggplant (*Solanum melongena* L.), Anaheim pepper (*Capsicum annuum* L. var. Anaheim), or papaya (*Carica papaya* L.). For *B. cucurbitae*, the control fruit was papaya. Fruits were introduced into the cages with 50 gravid female flies at 9:00 am and removed after 24 hours. Holding conditions during the time of fruit exposure were 24–27°C, 65–70% RH, and a photoperiod of 12:12 (L:D) hours. Following fruit fly exposure, fruits were transferred to 5 L screen-topped HI-PLAS buckets (Highland Plastics, Inc., Mira Loma, CA), which held a 300 mL layer of sand on the bottom to serve as a pupariation medium. After 2 weeks, sand from the buckets was sieved, and fruits cut open to recover all pupariating larvae and pupae, which were then transferred to 7.0 cm (diameter) × 7.5 cm screened-top cups with 20 mL sand and held for adult emergence. Numbers of pupae recovered and the number of emerged adults were recorded for each fruit. For the Clementine tangerine trials, data are reported only for those bioassays where at least 10 flies were recovered from the associated control fruit.

**Statistical analyses.** Separate statistical analyses were performed for each fruit species (including results of both fruit fly species) with their respective controls. For each fruit species, significance of differences of pupal and adult recoveries per kilogram fruit among intact, punctured, and control fruits for both fruit fly species was tested using analysis of variance (ANOVA) on trial averages, after square root transformation (sqrt[catch + 0.5]). The data for the ANOVA came from the average *B. latifrons* recovery from intact test fruits for each trial, the average *B. latifrons* recovery from puncturerd test fruits for each trial, the average *B. latifrons* recovery from the control fruit from each trial, the average *B. cucurbitae* recovery from intact test fruits for each trial, the average *B. cucurbitae* recovery from punctured test fruits for each trial, and the average *B. cucurbitae* recovery from the control fruit from each trial. Separate ANOVAs were performed for pupal recovery per kilogram fruit for navel oranges and for tangerines, and for adult recovery per kilogram fruit for navel oranges and for tangerines. Tukey's honest significant difference (HSD) was used to test for mean separation. Statistical analyses were performed using JMP 10.0.0.[9] Untransformed data are presented in the summary charts.

**Literature review.** References to infestation of fruits in the family Rutaceae by *B. cucurbitae* and *B. latifrons* were taken from host status summaries on host plants presented in various state, national and international host listings, as well as in scientific publications indexed in searchable databases, such as Agricola, CAB Abstracts, and Scopus. Host data also were obtained from pest interceptions reported by U.S. Federal and State governments.

Host data were classified as "field infestation data," "laboratory infestation data," or as "listing only" if no supporting data were provided. For field and laboratory infestation data, a summary was prepared detailing the number of fruits collected, from where the fruits were collected, the condition of the fruits, and the level of infestation found, when this information was available.

## Results

In the navel orange trials, there was a significant difference among treatments in pupal recovery per kilogram fruit ($F = 72.1$; df = 6, 104; $P < 0.0001$), and in adult recovery per kilogram fruit ($F = 19.7$; df = 6, 104; $P < 0.0001$). Pupal recovery per kilogram fruit, for both fruit fly species, was significantly greater for control fruits than for any other treatment groups. *B. cucurbitae* pupal recovery per kilogram fruit was significantly greater for punctured fruits than for intact fruits, where there was no recovery from any tested fruit. *B. cucurbitae* pupal recovery per kilogram fruit from punctured fruits was also greater than *B. latifrons* pupal recovery per kilogram fruit for either punctured or intact treatment fruits (Table 1). There was no significant difference among *B. latifrons* pupal recovery per kilogram fruit in intact or punctured fruits. Relative magnitudes of adult per kilogram recovery among the different treatment groups were comparable with the pupae per kilogram fruit recoveries, but the difference in *B. latifrons* recovery per kilogram fruit between intact control fruits and punctured navel oranges was not significantly different from the *B. cucurbitae* recovery from punctured navel oranges (Table 1).

In the Clementine tangerine trials, there was significant difference among treatments in pupal recovery per kilogram fruit ($F = 17.1$; df = 5, 66; $P < 0.0001$) and in adult recovery per kilogram fruit ($F = 13.5$; df = 5, 66; $P < 0.0001$). *B. cucurbitae* pupal recoveries per kilogram fruit from control fruits and from both intact and punctured treatment fruits were not significantly different, but were significantly greater than *B. latifrons* recovery from both intact and punctured treatment fruits. *B. latifrons* pupal recoveries per kilogram fruit from control fruits were significantly greater than *B. latifrons* recovery per kilogram fruit from both intact and punctured treatment fruits. Relative magnitudes of adult per kilogram recovery among the different treatment groups were comparable with the pupae per kilogram fruit recoveries (Table 1).

***Bactrocera latifrons.***

*Bioassays.*

Navel oranges. Out of a total of 16 trials, one trial with intact fruits and nine trials with punctured fruits had infested fruits. Considering actual fruit numbers, one of the 64 intact navel oranges and ten of the 64 punctured navel oranges were infested, whereas 100% of the control fruits (nine of the nine eggplants and seven of the seven papayas) were infested. Overall recovery from the intact and punctured navel oranges averaged 0.36 pupae/kg fruit and 5.51 pupae/kg fruit, respectively, whereas 454.1 pupae/kg fruit (eggplant) and 333.1 pupae/kg fruit (papaya) were recovered from the nine control eggplants and the seven control papayas (Table 1).

Clementine tangerines. Out of a total of 20 trials, at least 10 adult flies were recovered from control Anaheim peppers in 12 trials (Table 1). Of those 12 trials, one had infestation in intact fruits and two had infestation in punctured fruits. Considering actual fruit numbers, one of the 48 intact Clementine tangerines and two of the 48 punctured Clementine tangerines were infested, whereas 100% of the Anaheim peppers were infested. Overall recovery from the intact and punctured tangerines averaged 0.51 pupae/kg fruit and 0.39 pupae/kg fruit, respectively, whereas 378.7 pupae/kg fruit were recovered from the 12 control peppers (Table 1).

**Literature review.** There are only two reports of field infestation of fruits of the plant family Rutaceae by *B. latifrons*, no reports of laboratory infestations, and ten "listing only" references (Table 2). One field infestation report is a citrus species, lime, *Citrus aurantiifolia* [Christm.] Swingle; the other is a non-citrus species, mock orange, *Murraya paniculata* [L.] Jack. Both these infestations came from extensive fruit collections in Malaysia and Thailand.[11] In both cases, *B. latifrons* was recovered from only one collection. The publication, however, did not report the total number of fruits included in the collection or the total number of collections made. The "listing only" references come from two *Citrus* spp.

***Bactrocera cucurbitae.***

*Bioassays.*

Navel oranges. Out of a total of 21 trials, no trials with intact fruits and 17 trials with punctured fruits had infested fruits. Considering actual fruit numbers, zero of the 84 intact navel oranges and 44 of the 84 punctured navel oranges were infested, whereas 100% of the control papayas were infested. Overall recovery from the intact and punctured navel oranges averaged 0.0 pupae/kg fruit and 84.5 pupae/kg fruit, respectively, whereas 466.7 pupae/kg fruit were recovered from the control fruits (papaya).

Clementine tangerines. Out of a total of 13 trials, at least 10 adult flies were recovered from control papayas in 12 trials. In these 12 trials, eight trials with intact fruits and eleven trials with punctured fruits had infested fruits. Considering actual fruit numbers, 15 of the 48 intact Clementine tangerines and 28 of the 48 punctured tangerines were infested, whereas 100% of the control papayas were infested. Overall recovery from the intact and punctured Clementine tangerines averaged 340.8 and 240.1 pupae/kg fruit, respectively, whereas 310.2 pupae/kg fruit were recovered from the control fruit (papaya).

**Literature review.** There are ten reports of field infestation (covering six species), three reports of laboratory infestation (covering three separate species), and 115 "listing only" reports (covering 13 species) of infestation of fruits of the plant family Rutaceae by *B. cucurbitae* (Table 3). Five of the six plant species for which field infestations are reported are of *Citrus* spp., while all the three reported laboratory infestations are of *Citrus* spp. Of the "listing only" references, nine are of *Citrus* spp. In *Citrus* spp. field infestation studies, adults of *B. cucurbitae* were recovered from citron, *C. medica* L.; Kaffir lime, *C. hystrix* DC; pummelo, *C. maxima* (Burm.) Merr.; tangerine, *C. reticulata* Blanco; and, sweet orange,

**Table 1.** Infestation of intact and punctured Washington navel oranges and Clementine tangerines relative to papayas, eggplant, and Anaheim peppers following 24 hours exposure of individual fruits to 50 gravid female *B. cucurbitae* or *B. latifrons*.

| FRUIT FLY SPECIES | FRUIT ID | FRUIT STATUS | NO. TRIALS | NO. TRIALS WITH INFESTATION | TOTAL NO. FRUITS | FRUIT WEIGHT (kg) | NO. INFESTED FRUITS (%) | WEIGHT INFESTED FRUITS (kg) | AVG. NO. PUPAE RECOVERED PER kg FRUIT | AVG. NO. ADULTS RECOVERED PER kg FRUIT | AVG. % EMERGENCE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *B. cucurbitae* | Clementine | Intact | 12 | 8 | 48 | 2.78 | 15 (31.2) | 0.84 | $340.8^{b}$ | $284.7^{a}$ | 74.2 |
| *B. cucurbitae* | Clementine | Punctured | 12 | 11 | 48 | 2.92 | 28 (58.3) | 1.59 | $240.1^{a,b}$ | $168.2^{a}$ | 69.8 |
| *B. cucurbitae* | Papaya | Control | 12 | 12 | 12 | 6.48 | 12 (100.0) | 6.48 | $310.2^{a,b}$ | $256.5^{a}$ | 80.4 |
| *B. latifrons* | Clementine | Intact | 12 | 1 | 48 | 5.69 | 1 (2.1) | 0.16 | $0.51^{c}$ | $0.51^{b}$ | 100.0 |
| *B. latifrons* | Clementine | Punctured | 12 | 2 | 48 | 5.77 | 2 (4.2) | 0.32 | $0.39^{c}$ | $0.39^{b}$ | 100.0 |
| *B. latifrons* | Anaheim pepper | Control | 12 | 12 | 12 | 0.82 | 12 (100.0) | 0.82 | $378.7^{a}$ | $285.2^{a}$ | 69.7 |
| *B. cucurbitae* | Navel orange | Intact | 21 | 0 | 84 | 26.28 | 0 (0.0) | 0.00 | $0.0^{c}$ | $0.0^{c}$ | – |
| *B. cucurbitae* | Navel orange | Punctured | 21 | 17 | 84 | 25.85 | 44 (52.4) | 13.72 | $84.5^{b}$ | $24.8^{b}$ | 17.4 |
| *B. cucurbitae* | Papaya | Control | 21 | 21 | 21 | 10.59 | 21 (100.0) | 10.59 | $466.7^{a}$ | $307.3^{a}$ | 69.1 |
| *B. latifrons* | Navel orange | Intact | 16 | 1 | 64 | 17.98 | 1 (1.6) | 0.26 | $0.36^{c}$ | $0.060^{c}$ | 16.7 |
| *B. latifrons* | Navel orange | Punctured | 16 | 9 | 64 | 18.40 | 10 (15.6) | 2.96 | $5.51^{c}$ | $2.06^{c}$ | 37.6 |
| *B. latifrons* | Eggplant | Control | 9 | 9 | 9 | 1.44 | 9 (100.0) | 1.44 | $454.1^{a}$ | $388.7^{a,b}$ | 85.8 |
| *B. latifrons* | Papaya | Control | 7 | 7 | 7 | 2.94 | 7 (100.0) | 2.94 | $333.1^{a}$ | $275.8^{a,b}$ | 83.7 |

**Notes:** Numbers of pupae or adults recovered per kilogram fruit followed by the same letter are not significantly different at the $\alpha = 0.05$ level (analyses run separately for lementine fruits [with respective controls] and for navel oranges [with respective controls]).

**Table 2.** Summary of fruits from the plant family Rutaceae, which have been reported to be infested by *Bactrocera latifrons*.

| SCIENTIFIC NAME | COMMON NAME | GRIN NO. | INFESTATION RECORD | REFERENCE CITATIONS AND INFESTATION SUMMARIES |
|---|---|---|---|---|
| *Citrus* spp. | Citrus species | 312282 | Listing Only | 5,10 |
| *Citrus aurantiifolia* (Christm.) Swingle | Lime | 10683 | Field Infestation | From fruit collections in Peninsular Malaysia (1986 to 1988) and in East Malaysia (Sabah and Sarawak) and Thailand (1990 to 1994), *B. latifrons* was recovered from 1 sample. No infestation rate data given.[11] |
| *Citrus limon* (L.) Burm. f. | Lemon | 10732 | Listing Only | 5,10,12,13 |
| *Citrus sinensis* (L.) Osbeck | Sweet orange | 10782 | Listing Only | 5,10,12,13 |
| *Murraya paniculata* (L.) Jack | Mock orange | 24704 | Field Infestation | From fruit collections in Peninsular Malaysia (1986 to 1988) and in East Malaysia (Sabah and Sarawak) and Thailand (1990 to 1994), *B. latifrons* was recovered from 1 sample. No infestation rate data given.[11] |

**Notes:** Included, for each plant species, is a reference to the taxonomy of the plant species (as provided by the USDA-ARS Germplasm Repository Information Network [GRIN]), the citation of the references from which the infestation data were recovered, along with an indication as to whether the references were based on field data, laboratory data or were "listing only." In cases where there were laboratory or field infestation data, succinct summaries of the infestation data are also provided.

*C. sinensis* (L.) Osbeck. Infestation rates of *B. cucurbitae* in these aforementioned *Citrus* spp. were low (Table 3). For the non-citrus rutaceous fruit for which field infestation was reported, ie, limeberry, *Triphasia trifolia* [Burm. f.] P. Wilson, the infestation rate by *B. cucurbitae* was also low. In laboratory infestation studies, *B. cucurbitae* larvae developed through pupation in sour orange, *Citrus aurantium*, and in tangerine, *C. reticulata*, but failed to develop through to pupation in lemon, *C. limon*.

## Discussion

***Bactrocera latifrons.*** There has only been one report of field infestation of *B. latifrons* in *Citrus* spp., and that is from only one sample of *C. aurantiifolia* (lime) collected in Southeast Asia;[11] the number of collections conducted during the field host determination survey and the number of fruit samples during each collection were not specified. However, the field host survey conducted in Southeast Asia met the highest reliability of the presence of a pest in an area as defined by the ISPM 8: Determination of Pest Status in an Area,[61] as the survey team included several fruit fly specialists, including the taxonomic expert for the genus *Bactrocera* (R. A. Drew). By logical extension, the field infestation record of *B. latifrons* in lime[11] is reliable.

No choice infestation studies reported here showed that laboratory *B. latifrons* adults can oviposit in both intact and punctured navel oranges, *C. sinensis*, but the eggs rarely develop to the adult stage. Successful adult emergence was found with both intact and punctured fruits, but only one adult fly was recovered from intact oranges (0.060 adult flies/kg fruit), while 10 flies were recovered from punctured oranges (2.06 adult flies/kg fruit), compared to an average adult recovery of 275.8 and 388.7 adults/kg fruit in control papaya and control eggplant, respectively. There is no confirmation in the literature that citrus species such as oranges and tangerines can be natural hosts for *B. latifrons*.[62] However, it should be noted that it can be difficult to find citrus orchards (where fruit sampling could be done to assess infestation by tephritid fruit flies) where a well-established *B. latifrons* population is present, because *B. latifrons* field populations can be best represented in pastures or recently disturbed fallow lands having wild solanaceous plants.[5,63] The field recovery of *B. latifrons* from *C. aurantiifolia* in Southeast Asia,[11] combined with the observation that laboratory populations of *B. latifrons* in Hawaii can oviposit in both intact and punctured navel oranges and that the eggs can rarely develop successfully to the adult stage (Table 1), suggest that further research, especially research involving wild (field) *B. latifrons* populations interacting with intact fruits in the field, is needed to determine the host suitability of oranges and tangerines grown in Hawaii to *B. latifrons* following the guidelines specified in RSPM 30: Guidelines for the determination and designation of host status of a fruit or vegetable for fruit flies (Diptera: Tephritidae).[64]

***Bactrocera cucurbitae.*** There have been reports of field infestation of *B. cucurbitae* in multiple *Citrus* spp., including *Citrus hystrix*, *C. maxima*, and *C. medica*, as well as in *C. reticulata* and *C. sinensis* (Table 3). In the no choice infestation studies reported here, significantly more *B. cucurbitae* adults than *B. latifrons* adults were recovered from both Clementine tangerines and navel oranges. Both the literature reports and the results of the no choice infestation trials support a conclusion that *Citrus* spp., in general, are better hosts for *B. cucurbitae* than for *B. latifrons*. Comparing the results of no choice infestation trials of Clementine tangerine with those of navel orange by *B. cucurbitae*, it is interesting to note that there was no significant difference in infestation rate for intact versus punctured fruits in the relatively thin-skinned

**Table 3.** Summary of fruits from the plant family Rutaceae, which have been reported to be infested by *Bactrocera cucurbitae.*

| SCIENTIFIC NAME | COMMON NAME | GRIN NO. | INFESTATION RECORD | REFERENCE CITATIONS AND INFESTATION SUMMARIES |
|---|---|---|---|---|
| *Aegle marmelos* (L.) Corrêa | Bael | 1560 | Listing Only | 14 |
| *Casimiroa edulis* La Llave & Lex | White sapote | 9292 | Listing Only | 13,15–20 |
| *Citrus aurantium* L. | Sour orange | 10684 | Laboratory Infestation | Using 1st instar larvae obtained from eggs oviposited on bottle gourd (*Lagenaria vulgaris*), 49 of 100 1st instar larvae (49%) raised on orange pupated, with an average time to pupation of 8.9 days. In a separate test, 94 of 100 1st instar larvae (94%) were found to feed on pieces of orange.[21] |
| | | | Listing Only | 13,15,17–20 |
| *Citrus deliciosa* Ten. | Italian tangerine | 314340 | Listing Only | 18 |
| *Citrus hystrix* DC. | Kaffir lime | 10714 | Field Infestation | In 1992, *B. cucurbitae* was recovered from 2 samples of *Citrus hystrix* [Thailand, Malaysia, Southern India]. Infestation rate data not given. *B. cucurbitae* individuals identified by R.A.I. Drew and D.L. Hancock.[11] |
| *Citrus limon* (L.) Burm. f. | Lemon | 10732 | Laboratory Infestation | Using 1st instar larvae obtained from eggs oviposited on bottle gourd (*Lagenaria vulgaris*), 49 of 100 1st instar larvae (49%) were found to feed on lemon. No larvae (0 of 100) completed growth to the point of pupation, but survived longer (4–6 days) than 1st instar larvae fed on diets of water alone or 2.5% agar gel.[21] |
| | | | Listing Only | 13,15,17–20,22,23 |
| *Citrus maxima* (Burm.) Merr. | Pummelo | 10744 | Field Infestation | *B. cucurbitae* adults were recovered from infested *C. maxima* fruits, randomly collected on Penang Island, West Malaysia. No infestation rate data given.[24] |
| | | | Listing Only | 4,13,15,19,22,25–29 |
| *Citrus medica* L. | Citron | 10745 | Field Infestation | Fallen and marketable sized *C. medica* fruits were harvested over a seven week period in September—October, 1975 in Hissar, India. Fruits were cut open to check for fruit fly incidence. *B. cucurbitae* was recovered in 6 of 7 weekly collections (85.7%), with an average weekly infestation rate of 28.0%. Overall, 13 of 52 collected fruits were infested by *B. cucurbitae*.[30] |
| *Citrus myrtifolia* Raf. | Myrtle-leaf orange | 10756 | Listing Only | 18 |
| *Citrus paradisi* Macfad. | Grapefruit | 10772 | Listing Only | 13,15,17–20,22,23 |
| *Citrus reticulata* Blanco | Tangerine | 10778 | Field Infestation | One adult *B. cucurbitae* and 259 adult *B. dorsalis* were recovered from 10 tangerine fruits collected from the Punahou area of Honolulu (Oahu, Hawaii) in April, 1947.[31] |
| | | | Field Infestation | *B. cucurbitae* individuals (adults?) were recovered from *C. reticulata* fruits collected between 2005–2007 in Benin, with infestation rate falling in the range of 1–25 *B. cucurbitae* per kg fruit. No data presented on the number of fruits collected, the weight of fruits collected or the percentage infestation of collected fruits.[6] |
| | | | Laboratory Infestation | In captivity, female melon flies laid eggs on cut fruits of *C. reticulata*. The eggs hatched out and the development of the larvae proceeded to continue normally through pupation. No data presented on methods used for the lab infestation or the resulting infestation rate.[32] |
| | | | Listing Only | 13–15,18,19,22,27,33–36 |

**Table 3.** (*Continued*).

| SCIENTIFIC NAME | COMMON NAME | GRIN NO. | INFESTATION RECORD | REFERENCE CITATIONS AND INFESTATION SUMMARIES |
|---|---|---|---|---|
| *Citrus sinensis* (L.) Osbeck | Sweet orange | 10782 | Field Infestation | Adult *B. cucurbitae* have been reared from oranges, but these fruits do not serve regularly as *B. cucurbitae* hosts. "Only in rare instances does the melon fly attack them, and then only slightly." No infestation rate data presented.[37] |
| | | | Field Infestation | In 1910, a few oranges provided by a farmer from Kaimuki (Oahu, Hawaii) were placed in a breeding jar from which mostly *Drosophila* spp. were recovered, but also one adult melon fly.[38] |
| | | | Field Infestation | About 10% of orange fruits recovered in the vicinity of the University of Agriculture in Faisalabad, Pakistan, were infested by *B. cucurbitae*.[39] |
| | | | Field Infestation | *B. cucurbitae* individuals (adults?) were recovered from *C. sinensis* fruits collected between 2005–2007 in Benin and in Burkina Faso, with infestation rate falling in the range of 1–25 *B. cucurbitae* per kg fruit. No data presented on the number of fruits collected, the weight of fruits collected or the percentage infestation of collected fruits.[6] |
| | | | Listing Only | 4,13–15,17–20,22,23,25–28, 31–36,40–48 |
| *Citrus* spp. | Citrus species | 312282 | Listing Only | 13,14,16,17,20–22,31,34,35,42,49–59 |
| *Citrus vulgaris* Risso | | 102860 | Listing Only | 18 |
| *Clausena lansium* (Lour.) Skeels | Wampi | 10811 | Listing Only | 13,15–20 |
| *Triphasia trifolia* (Burm. f.) P. Wilson | Limeberry | 40476 | Field Infestation | 13 of 29 samples (44.8%) of *T. trifolia* fruits made in Rota, Marianas Islands, between 1959–1963, were infested by *B. cucurbitae* and/or *B. dorsalis*. A total of seven *B. cucurbitae* adults were recovered from a total of 13,729 fruits.[60] |
| | | | Listing Only | 4,13,18 |

**Notes:** Included, for each plant species, is a reference to the taxonomy of the plant species (as provided by the USDA-ARS Germplasm Repository Information Network [GRIN]), the citation of the references from which the infestation data were recovered, along with an indication whether the references were based on field data, laboratory data, or were "listing only." In cases where there were laboratory or field infestation data, succinct summaries of the infestation data are also provided.

Clementine tangerines, while the infestation rate in the relatively thicker skinned navel oranges was significantly higher in punctured fruits than in intact fruits, where no infestation was observed. This suggests that fruit damage may be an important factor in *B. cucurbitae* infestation in navel oranges in the field. Overall, both published infestation data and the results of the no choice infestation trials reported here indicate that movement of both Clementine tangerines and navel oranges from Hawaii to the continental US will require appropriate risk mitigation measures.

**Risk mitigation options for tephritid fruit flies in oranges and tangerines.** Risk mitigation measures are not needed for *B. latifrons* because no field infestation by *B. latifrons* of citrus fruits like Clementine tangerines and navel oranges has been reported to date, but the laboratory studies reported here indicate that further field studies are needed, especially studies involving exposure of fruits on trees to gravid wild females. However, because both field and laboratory data show that *B. cucurbitae* can infest citrus fruits like Clementine tangerines and navel oranges, shipment of citrus fruits out from areas where *B. cucurbitae* is present will require risk mitigation measures. At present, there is one postharvest treatment that could currently be used, and several options are available for systems approaches. An irradiation quarantine treatment could be used based on research that established a 150 Gy minimum absorbed dose as a generic treatment dose for postharvest disinfestation of tephritid fruit flies in fruits and vegetables.[65,66]

Use of high-temperature forced-air is another possible tephritid fruit fly disinfestation treatment. A high-temperature forced-air disinfestation treatment using four temperature stages was developed that successfully disinfested color-break to half-ripe papaya (*Carica papaya* L.) of *C. capitata* (Wiedemann), *B. dorsalis* (Hendel), and *B. cucurbitae*, whether the fruit flies were introduced as eggs or as third instars.[67]

Alternatively, cold treatment is frequently used to control fruit flies in citrus.[68] A cold-based disinfestation treatment was shown to be effective for carambola (*Averrhoa carambola* L.) against *C. capitata* (Wiedemann), *B. dorsalis* (Hendel), and *B. cucurbitae* (Coquillett).[69] However, further research would be needed to demonstrate that the established temperature range would effectively disinfest tephritid fruit flies from citrus fruits. Assessment of whether established cold treatment schedules (for *C. capitata* or *Anastrepha ludens* [Loew]) would be effective for *Bactrocera zonata* (Saunders), the peach fruit fly, was recently tested in order to develop a cold treatment to safely permit shipment of oranges from Egypt (where *B. zonata* is established) to other localities where *B. zonata* is not present. In that research, *B. zonata* was found to be more cold tolerant than *C. capitata* but less cold tolerant than *A. ludens,* so treatment schedules previously developed for *A. ludens* were determined to provide quarantine security for oranges that might be infested by *B. zonata*.[70]

Finally, quarantine security could be sought through a systems approach incorporating a series of risk-reducing steps. Adequate quarantine security might not be achieved by each individual step, but could be achieved when multiple steps are applied sequentially. As an example, a systems approach could incorporate a cold treatment while using an alternative security measure, such as low prevalence,[71] or a pest-free production area.[72,73] In a recently established systems approach to permit the shipping of Sharwil avocados (a poor host of *B. dorsalis*) from Hawaii to the continental US,[74,75] a grower compliance agreement requires multiple risk-reducing steps including limiting the dates over which fruits can be picked, protecting picked fruits from exposure to *B. dorsalis* adults, monitoring *B. dorsalis* field population levels, and application of a protein bait spray if *B. dorsalis* trap catch, monitored weekly, exceeds a specified level.

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## Author Contributions

Conceived and designed the experiments: GTM, PAF, CDS. Analyzed the data: GTM, CDS. Wrote the first draft of the manuscript: GTM, NJL. Contributed to the writing of the manuscript: GTM, PAF, NJL, CDS. Agree with manuscript results and conclusions: GTM, PAF, NJL, CDS. Jointly developed the structure and arguments for the paper: GTM, PAF, NJL. Made critical revisions and approved final version: GTM, PAF, NJL, CDS. All authors reviewed and approved the final manuscript.

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