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Natural Field Infestation of *Mangifera casturi* and *Mangifera lalijiwa* by Oriental Fruit Fly, *Bactrocera dorsalis* (Diptera: Tephritidae)

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ABSTRACT: Mango, *Mangifera indica* (Anacardiaceae), is a crop cultivated pantropically. There are, however, many other *Mangifera* spp ("mango relatives") which have much more restricted distributions and are poorly known but have potential to produce mango-like fruits in areas where mangoes do not grow well or could be tapped in mango breeding programs. Because of the restricted distribution of many of the *Mangifera* spp, there has also been limited data collected on susceptibility of their fruits to infestation by tephritid fruit flies which is important to know for concerns both for quality of production and for quarantine security of fruit exports. Here, we report on natural field infestation by the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), of two mango relatives native to Indonesia: *Mangifera casturi* and *Mangifera lalijiwa*. Rates of infestation of fruits of these two *Mangifera* spp by tephritid fruit flies have not previously been reported.

KEYWORDS: Mango, *Mangifera* spp, *Mangifera casturi*, *Mangifera lalijiwa*, *Bactrocera dorsalis*, mango relative

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Introduction

The mango, *Mangifera indica* L. (Anacardiaceae), is the best known and most widely cultivated species in the genus *Mangifera*. It is native to India and Burma and has been cultivated in India for more than 4000 years but has also been widely distributed so that it is now pantropically cultivated.[1,2] Many people are not aware that there are many other species within the genus *Mangifera* that, although having much more restricted distributions, also have excellent fruits which may be of comparable or even superior quality relative to the mango.[2] A total of 69 species of *Mangifera* have been described,[2] of which edible fruit is produced by at least 26 species beyond mango.[3] This genus is strictly Asian in origin and, with the exception of the pantropical cultivation of mango, occurs in tropical parts of Asia: India, Burma, Sri Lanka, Thailand, Indochina, South Tropical China, Malaysia, Indonesia, Papua New Guinea, the Philippines, the Solomon Islands, and a few species in the Pacific Islands.[2] The nature and quality of fruits vary among *Mangifera* spp, and there is also variation in adaptability to different climates, with some species growing well in areas where mangoes cannot be grown satisfactorily, such as in an ever-humid climate, without a prolonged dry season.[4]

The diversity among *Mangifera* spp has generated interest in testing various species for suitability for fruit production in areas beyond Asia as well as consideration for use as rootstocks and for breeding with mango. The Center for Tropical Plant Conservation of the Fairchild Tropical Botanic Garden in Coral Gables, FL, USA, has a project focused on identification, collection, and propagation of "wild mangoes," which includes *Mangifera caesia* Jack, *Mangifera casturi* Kosterm., *Mangifera foetida* Lour., *Mangifera griffithii* Hooker f., *Mangifera lalijiwa* Kosterm., *Mangifera laurina* Bl., *Mangifera odorata* Griff., *Mangifera pajang* Kosterm., *Mangifera pentandra* Hooker f., and *Mangifera zeylanica* (Bl.) Hooker f. Their objective is both to contribute to the long-term conservation of these species and to use them for breeding with mango.[5] Two of these wild mango species (*M casturi* and *M lalijiwa)* have been introduced to Hawaii by a local nursery as trees with potential to produce mango-like fruits in wet climates without having anthracnose problems commonly found with mango flowers and fruits.

Tephritid fruit flies are major pests of many tropical fruits, and *Mangifera* spp are not an exception. Twelve *Mangifera* spp have been listed for which infestation data by *Bactrocera* spp (Diptera: Tephritidae) have been recorded (*Mangifera altissima* Blanco, *M caesia, Mangifera caloneura* Kurz, *M foetida, M griffithii, M indica, M laurina, Mangifera longipetiolata* King, *Mangifera minor* Bl., *M odorata, M pajang*, and *M zeylanica*).[6,7] A total of 46 fruit fly species have been listed for which *M indica* has been documented to be a host, although some records require confirmation.[8] The five fruit fly genera included are as follows: *Anastrepha* (8 species), *Bactrocera* (29), *Ceratitis* (7), *Dirioxa* (1), and *Toxotrypana* (1). There are also at least two species of *Dacus* (*Dacus bivittatus* and *Dacus ciliatus*) for which there are records that mango is a host (*D bivittatus*[9,10] and *D ciliatus*[11]). Because of lesser commercial development and more restricted geographic ranges of other *Mangifera* spp, there is much less published regarding infestation of other *Mangifera*

spp by tephritid fruit flies. As an example, a comprehensive publication on fruit flies of economic importance only lists infestation of one other *Mangifera* sp (infestation of *M foetida* by *Bactrocera tau* [Walker]).[8]

Here, we report on a survey to assess whether *M casturi* and *M lalijiwa* are naturally infested in the field by tephritid fruit flies. There has been no record to date of tephritid fruit fly infestation in fruits of these two *Mangifera* spp, so we took advantage of an opportune time when both species were fruiting, which may not be all that common because of irregular bearing, especially with *M casturi*.[5]

Materials and Methods

Field site

The field research was conducted from July 2 to 30, 2015 at Kabuganaan Farm located in the vicinity of Kurtistown on the Island of Hawaii, Hawaii, USA (Universal Transverse Mercator grid [USGS 2001]: Easting 0284328, Northing 2165904 m, Zone 05 Q) and was at 277-m elevation. Relative positions of the *M casturi* and *M lalijiwa* trees in the orchard at Kabuganaan farm and the relative position of Kurtistown on the Island of Hawaii are presented in Figure 1. A Davis Instruments Vantage Pro2 Weather Station (Hayward, CA, USA) was deployed at Kabuganaan farm for the collection of temperature, relative humidity, and rainfall data. Over the course of the study, temperature averaged 23.7°C ± 0.06°C (SEM), relative humidity averaged 86.8% ± 0.16% (SEM), and there was a total of 56-mm rain.

Fruit tree species

Two mango relatives were tested in this field trial, *M casturi* and *M lalijiwa*. The former species name is listed by The Plant List as an accepted name, whereas the latter species name is listed as an unresolved name. Fruits of *M casturi* (referred to as "kastooree" in its native area) were of the "Kasturi" form, where the fruit skin is "smooth, glossy green with dark spots which multiply and at maturity make the fruit completely or partly black." The pulp is dark orange and very sweet. *Mangifera casturi* is rather common in South Kalimantan, Indonesia, around Banjarmasin and in the Martapura District, but is also found in Central and East Kalimantan. It is not known in the wild, being only found under cultivation. Fruits of *M lalijiwa*, larger than those of *M casturi*, are green when immature and turn yellowish at maturity. They are locally called "Mangga ubi" (West Java), "laleejeewo" (East Java), or "tabar" (Madura Island) in their native areas. The pulp is pale yellow with a sweet acid taste. *Mangifera lalijiwa* is present in Java, Madura, Bali, and probably in Sumatra as well. It is cultivated and likely very rare in the wild.[2] Relative external and internal appearances of mature fruits of these two species are presented in Figure 2A and B, respectively.

Traps

Tephritid fruit fly detection traps were deployed from July 2 to 30, 2015, using yellow bottom Multilure traps from Better World Manufacturing (Fresno, CA, USA) baited with a protein bait solution: 8% Solulys (Roquette America, Inc., Geneva, IL, USA), 4% Borax 20 Mule Team (Scottsdale, AZ, USA), and 88% water. Each trap contained 300 mL of protein bait solution. Traps were serviced weekly (July 9, 16, 23, and 30). Fresh bait was used on July 2, 9, and 23. Water was added on July 16 to make the fluid volume to 300 mL. A total of 4 protein-baited traps were deployed, 2 each in the *M casturi* and the *M lalijiwa* trees. Traps were placed 2 to 3 m above the ground, were surrounded by foliage, and were placed near developing fruits when possible. The 2 traps per tree were placed on opposite sides of each tree, with trap position shifted each week.

Bioassay

Fruits were collected weekly from July 2 to 30, 2015. Each week, 10 random ripe intact (undamaged) fruits were harvested from the *M casturi* tree and 10 random ripe undamaged fruits were also collected from the ground underneath the *M casturi* tree. Concurrently, 10 random ripe intact (undamaged) fruits were harvested from the *M lalijiwa* tree. Ground fruits, though, were not as readily available under the *M lalijiwa* tree. Only 3 undamaged ground fruits were collected from the *M lalijiwa* tree in the third week. No undamaged ground fruits were available at the collection times for the other 3 weeks. All fruits were brought back to the Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center in Hilo, HI, for processing for assessment of infestation by tephritid fruit flies. The fruits were weighed and notes were taken on external skin characteristics (blemishes). All collected fruits were held individually without sand in double-stacked 4-L Hi-Plas buckets (Highland Plastics, Inc., Mira Loma, CA, USA). The top bucket had screened top and screened bottom holes that permitted the draining of fluids from the fruit to prevent drowning of any infesting tephritid fruit fly larvae (Figure 3). A HOBO Pro v2 data logger (Onset Computer Corporation, Bourne, MA, USA) was deployed in the fruit holding room. Temperature over the course of the holding period fell within the range of 25°C to 27°C, whereas relative humidity ranged from 80% to 82%. Two weeks after fruits were first placed in the holding buckets, pupae were recovered from the holding buckets and fruits were cut open to recover all larvae remaining inside the fruit. The larvae and pupae were transferred to 9.0 cm (diameter) × 4.5 cm screened-top Hi-Plas cups (Highland Plastics, Inc.) with 20-mL sand per container for use as a pupariation medium and held for adult emergence. The total numbers of dead larvae, dead pupae, and adult flies recovered from each fruit were recorded. Species and sex of the adult flies were identified. Dead pupae

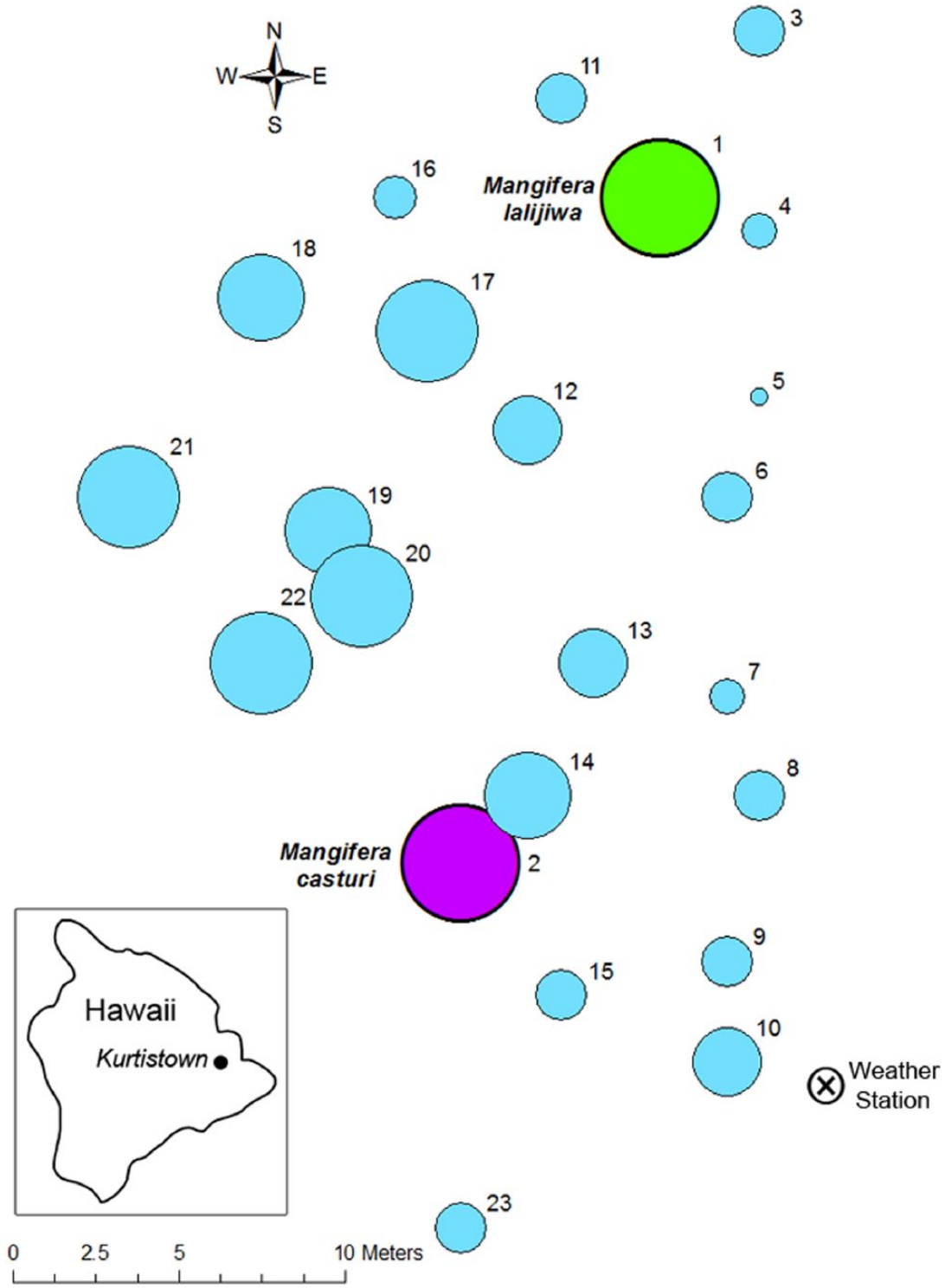

Figure 1. Relative positions of the *Mangifera lalijiwa* (no. 1) and the *Mangifera casturi* (no. 2) trees in the orchard at Kabuganaan Farm and the relative position of Kurtistown on the Island of Hawaii. Other fruit trees present in the orchard are as follows: 3—Tahitian lime (*Citrus latifolia* (Yu. Tanaka) Tanaka; 4—pommelo (*Citrus maxima* (Burm.) Merr. cv. "*Halawa*"); 5—navel orange (*Citrus sinensis* (L.) Osbeck cv. "*Cara* Cara"); 6—tangelo (*Citrus paradisi* Macfad. × *Citrus reticulata* Blanco cv. "*Minneola*"); 7—orange (*Citrus sinensis* (L.) Osbeck cv. "*Valencia*"); 8—navel orange (*Citrus sinensis* (L.) Osbeck cv. "*Fisher*"); 9—tangerine (*C reticulata* Blanco cv. "*Honey*"); 10—lemon (*Citrus limon* (L.) Burm. f. cv. "*Variegated Pink Eureka*"); 11—pommelo (*C maxima* (Burm.) Merr. cv. "*Chandler*"); 12—starfruit (*Averrhoa carambola* L. cv. "*Kari*"); 13—white sapote (*Casimiroa edulis* La Llave & Lex. cv. "*Suebelle*"); 14—guava (*Psidium guajava* L. cv. "*White Indonesian*"); 15—wampi (*Clausena lansium* (Lour.) Skeels); 16—longan (*Dimocarpus longan* Lour. cv. "*Biew Kiew*"); 17—rambutan *(Nephelium lappaceum* L. cv. "*Jitlee*"); 18 abiu (*Pouteria caimito* (Ruiz & Pav.) Radlk. cv. "*Gray*"); 19—rambutan *(Nephelium lappaceum* L. cv. "*R-9*"); 20—longan (*Dimocarpus longan* Lour. cv. "*Kohala*"); 21—mango (seedling tree grown from seed from a *Mangifera indica* L. cv. "*Carabao*" fruit); 22—lychee (*Litchi chinensis* Sonn. cv. "*Kaimana*"); and 23—rollinia ("*Rollinia mucosa*" (Jacq.) Baill.). The size of the circle representing each tree is an indication of the relative cross-sectional area of the canopy of each tree species. Only *Clausena lansium, M casturi*, and *M lalijiwa* were fruiting at the time of the study. Map prepared by C.D.S. using ArcGIS.[12]

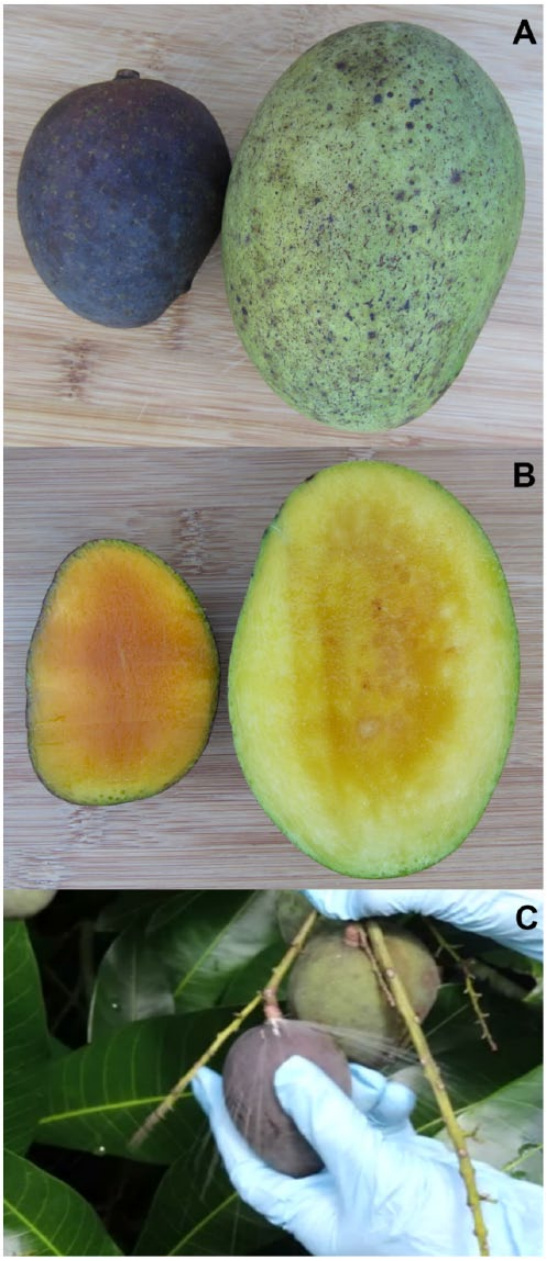

Figure 2. (A) Comparative appearance of mature, undamaged fruits: *Mangifera casturi* (left) and *Mangifera lalijiwa* (right). (B) Comparative appearance of pulp of mature fruits: *M casturi* (left) and *M lalijiwa* (right). (C) Spray of latex at the point of stem attachment in mature, undamaged *M casturi* fruit when the fruit is detached from the stem (frame from video [see Video 1]). Photos by G.T.M.

were identified to species based on counts of numbers of lobes in the prothoracic spiracles.[8]

Statistical analyses

Averages were calculated for weights of fruits collected and numbers of fruit flies recovered per kg fruit and per kg infested fruit. Percentage infestation was calculated based on total number of fruits collected. Oriental fruit fly, *Bactrocera dorsalis* (Hendel), catch per trap per day was calculated each week for traps in the *M casturi* and *M lalijiwa* trees.

Figure 3. Plastic buckets used for holding fruits for assessment of infestation by tephritid fruit flies. The picture on the left shows the screened bottom holes on the top bucket that permitted the draining of fluids from the fruit to prevent drowning of any infesting tephritid fruit fly larvae. The picture on the right shows the screen on the lid of the top bucket and the buckets as stacked together. Photo by G.T.M.

Results

Trapping

Bactrocera dorsalis was caught every week in traps placed in each tree species. Trap catch averaged 0.61 ± 0.88 (SEM) flies/trap/d (range: 0.14-1.14) in the *M casturi* tree and 1.82 ± 4.38 (SEM) flies/trap/d in the *M lalijiwa* tree (range: 1.57-2.14). One female melon fly (*Bactrocera cucurbitae* [Coquillett]) was also recovered from a trap in the *M lalijiwa* tree on 23 July (week 3).

External skin characteristics (blemishes) of collected fruits

For *M casturi*, no skin blemishes were noted on any of the 40 tree fruits or the 40 ground fruits collected. One tree fruit with a crack was found and that was held in addition to the 10 blemish-free fruits collected each week. For *M lalijiwa*, 10 blemish-free tree fruits were collected each week for the first 3 weeks, but only 6 blemish-free tree fruits were available for collection in the fourth week. In addition to these fruits, 13 tree fruits were collected over the course of the study that had cracks in the skin. Only 5 *M lalijiwa* ground fruits were collected over the course of the study, 3 of which were blemish-free, whereas 2 had cracks in the skin.

Infestation

Infestation by *B dorsalis* was found in ripe fruits of both *Mangifera* spp (Table 1). However, infestation of mature undamaged fruits on trees was only found with *M lalijiwa* fruits where infestation was found in all 4 fruit collections, with a total of 6 of 36 fruits infested (16.7%). Infestation of the mature undamaged on-tree *M lalijiwa* fruits averaged 12.5 ± 8.0 (SEM) pupae/kg fruit and 77.1 ± 4.3 (SEM) pupae/kg infested

Table 1. Natural field infestation by *Bactrocera dorsalis* of both undamaged and cracked mature *Mangifera casturi* and *Mangifera lalijiwa* fruits harvested from the tree or collected from the ground during July 2015 in the vicinity of Kurtistown, Hawaii, USA.

FRUIT SPECIES	FRUIT LOCATION	FRUIT STATUS	TOTAL NO. OF COLLECTIONS	TOTAL NO. OF FRUITS COLLECTED	TOTAL FRUIT WEIGHT, KG	TOTAL NO. OF INFESTED FRUITS	TOTAL WEIGHT OF INFESTED FRUITS, KG	NO. OF COLLECTIONS WITH *BACTROCERA DORSALIS* INFESTATION	AVG. NO. OF PUPAE/ KG FRUIT	AVG. NO. OF PUPAE/KG INFESTED FRUIT	AVG. NO. OF ADULTS/ KG FRUIT	AVG. NO. OF ADULTS/KG INFESTED FRUIT
Mangifera casturi	On tree	Undamaged	4	40	4.08	0	0.0	0	0.0	0.0	0	0
M casturi	On tree	Cracked	1	1	0.13	1	0.13	1	85.9	85.9	1	7.81
M casturi	On ground	Undamaged	4	40	4.30	3	0.33	2	2.09	27.2	0.70	9.07
Mangifera lalijiwa	On tree	Undamaged	4	36	10.4	6	1.69	4	12.5	77.1	1.63	10.1
M lalijiwa	On tree	Cracked	3	13	3.93	9	2.83	3	107.9	149.8	62.6	86.9
M lalijiwa	On ground	Undamaged	1	3	0.76	0	0.0	0	0.0	0.0	0	0
M lalijiwa	On ground	Cracked	1	2	0.61	0	0.0	0	0.0	0.0	0	0

fruit. Infestation rate of mature on-tree *M lalijiwa* fruits increased in fruits which had cracks (107.9 ± 51.3 [SEM] pupae/kg fruit and 149.8 ± 13.8 [SEM] pupae/kg infested fruit). A picture of an infested, cracked fruit taken from the *M lalijiwa* tree is shown in Figure 4. It had been infested with 35 *B dorsalis* larvae from which 18 male and 8 female adults emerged. The infestation in this fruit is included in the *M lalijiwa* "on-tree" "cracked" fruit data presented in Table 1. No infestation was found in the mature *M lalijiwa* fruits collected from the ground, either from the 3 undamaged fruits or from the 2 cracked fruits. Although no infestation was found in mature undamaged on-tree *M casturi* fruits, infestation was found in 1 mature on-tree *M casturi* fruit with a crack (85.9 pupae/kg fruit) and in 3 of 40 (7.5%) mature undamaged fruits collected from the ground (2.09 ± 1.30 [SEM] pupae/kg fruit and 27.2 ± 0.5 [SEM] pupae/kg infested fruit). A characteristic of *M casturi* fruits that may have prevented successful infestation by *B dorsalis* is the presence of a pressurized latex. When the fruit is harvested from the tree, there is typically a spray of latex from the fruit when the fruit is detached from the stem (Figure 2C and Video 1). No infestation was found in the mature intact on-tree fruits but was found under conditions where that latex pressure was released in a cracked on-tree fruit and in intact fruits recovered from the ground.

Discussion

We provide here the first report of infestation of *M casturi* and *M lalijiwa* by *B dorsalis*. Because it was natural field infestation for both *Mangifera* spp, both species can be considered to be "suitable hosts" for *B dorsalis*.[13,14] The rate of infestation of undamaged *M lalijiwa* fruits by *B dorsalis* (1.63 flies/kg fruit) fell within the lower part of the range of infestation rates of mango by *B dorsalis* reported from the vicinity of Hilo, Hawaii from 1950 to 1962 (range: 0.78-103.68 flies/kg fruit).[6] Of the two *Mangifera* species, undamaged fruits of *M lalijiwa* were more readily infested by *B dorsalis*, with no undamaged *M casturi* on-tree fruits found to be infested. Infestation was, though, found in damaged fruits of both species. Although it might be expected that average infestation levels would be higher in ground fruits than in undamaged fruits on the tree, this was not found to be the case with *M lalijiwa* fruits sampled. It is thought, though, that the lack of infestation in the *M lalijiwa* ground fruits could be an artifact of low sample size (only 5 ground fruits were collected).

The overall list of suitable hosts of *B dorsalis* was most recently documented to be 478 plant taxa, belonging to 211 genera in 76 plant families.[13] This host list includes reports of infestation by *Bactrocera invadens* (Drew, Tsuruta, & White), *Bactrocera papaya* (Drew & Hancock), and *Bactrocera philippinensis* (Drew & Hancock), which had been considered to be separate species, but recently published research has concluded that these 3 species, actually, are all also *B dorsalis*.[15] This brings to 11 the total number of *Mangifera* spp which are documented to be suitable hosts of *B dorsalis* (and to 14 the total number of

Figure 4. Ripe, cracked, infested *M lalijiwa* fruit harvested from the tree. A total of 35 *Bactrocera dorsalis* larvae were present in this 0.499 kg fruit, from which 18 males and 8 females emerged. Photo by G.T.M.

Video 1. Spray of latex at the point of stem attachment in mature, undamaged *Mangifera casturi* fruit when the fruit was detached from the stem (video). Video by G.T.M. The video is uploaded online as Supplementary material.

Mangifera spp which are documented to be suitable hosts of *Bactrocera* spp). Given that some of the *Mangifera* spp are good hosts for *B dorsalis*, we expect that there are additional, yet undocumented, *B dorsalis* hosts among others of the 69 *Mangifera* spp identified by Kostermans and Bompard.[2] It is, however, challenging to document the host status of many of the *Mangifera* spp. *Mangifera* spp tend to occur as scattered individuals at very low densities in tropical lowland rainforests. These widely scattered trees can be quite tall such that tree crowns, where fruits are present, can be rather inaccessible, resulting in *Mangifera* spp being poorly represented even in herbarium collections. An additional problem is that it is common for fruiting to occur only at intervals of 3 to 8 years.[4] As an example, *M casturi* can vary considerably in regularity of bearing among different locales. A tree planted from seed in the Bogor (Indonesia) Botanical Gardens was bearing fruit after 10 years and, subsequently, regularly bore fruit twice a year.[2] Conversely, *M casturi* is reported to not flower consistently in South Florida.[5] The *M casturi* tree sampled at Kabuganaan Farm in this study has, thus far, only fruited one year (the year that this study was conducted) since it was planted out in 2002. The *M lalijiwa* tree was also planted out in 2002 but has borne fruit a number of years since 2002, although not every year. Although *M lalijiwa* is reported to not have anthracnose fungus problems when grown in the Waimanalo area of the Island of Oahu, Hawaii (http://www.frankiesnursery.com/), bearing seems to be enhanced in the Kurtistown area of Hawaii Island in years where it is drier during flowering (G.T.M—unpublished data [2002 - 2017]). Given that no infestation was recorded in mature *M casturi* fruits that were undamaged and still on the tree, there is a possibility that this fruit could be a conditional nonhost for *B dorsalis*. Further fruit collections, though, would be required to establish this. Its irregular bearing in Florida and Hawaii, however, could be problematic for the development of commercial production of *M casturi*.

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Author Contributions

CDS and GTM conceived and designed the experiments and analyzed the data. GTM wrote the first draft of the manuscript. CDS, GTM, and NJL contributed to the writing of the manuscript; agree with manuscript results and conclusions; and made critical revisions and approved final version. GTM and NJL jointly developed the structure and arguments for the paper. All authors reviewed and approved the final manuscript.

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