Host Status of Blueberry to Invasive Tephritid Fruit Flies in Hawaii

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ABSTRACT Forced infestation studies were conducted to determine whether northern or southern highbush blueberries, *Vaccinium corymbosum* L., are hosts for the invasive tephritid fruit flies in Hawaii. Fruit were exposed to gravid female flies of *Bactrocera dorsalis* Hendel (oriental fruit fly), *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly), or *Bactrocera cucurbitae* Coquillet (melon fly) in screen cages outdoors for 6 h and then held on sand in the laboratory for 2 wk for pupal and adult emergence. The number of puparia, number of puparia per gram, and percentage of adult emergence on 'Bluecrop' blueberry were significantly higher for *B. dorsalis* and *C. capitata* than *B. cucurbitae*; *B. dorsalis*, *C. capitata*, and *B. cucurbitae* produced an average of 1.06, 0.60, and 0.09 pupae per g fruit and had 50.8, 54.1, and 12.7% adult emergence, respectively. 'Berkeley' blueberries produced an average of only 0.06, 0.02, and 0.0 pupae per g fruit for *B. dorsalis*, *C. capitata*, and *B. cucurbitae*, respectively. Similarly, six blueberry cultivars were harvested weekly for 10 wk, exposed to *Bactrocera latifrons* (Hendel) in cages, and held for pupal and adult emergence on either sand or artificial diet. In total, 2,677 blueberries were exposed to 2,681 *B. latifrons* and held on sand, and no pupariation or adult emergence was observed. Small numbers of *B. latifrons* puparia and adults emerged from the artificial diet treatment in all cultivars. Results from rearing on sand and diet indicate that blueberry is an acceptable oviposition host for *B. latifrons* but not an adequate developmental host. These data suggest blueberry is potentially a good host for *B. dorsalis* and *C. capitata*, and an adequate host for *Bactrocera cucurbitae*, but that there may be significant variation in resistance among cultivars. Blueberry seems to be a nonhost for *B. latifrons*.

KEY WORDS nonhost, *Vaccinium* spp., Mediterranean fruit fly, oriental fruit fly, melon fly

Southern highbush blueberry, *Vaccinium corymbosum* L. (Ericaceae), is a new potentially high-value niche crop for Hawaii (Zee et al. 2006, Hummer et al. 2007). Hawaii-grown blueberries are ripe at times U.S. mainland berries are unavailable and could be used to supply local markets or shipped to the U.S mainland to compete with Central and South America blueberries. Four species of tephritid fruit flies prevent the export of fresh fruits from Hawaii due to a federal quarantine. The host status of blueberry to the three main fruit fly pests—Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); oriental fruit fly, *Bactrocera dorsalis* Hendel; and melon fly, *Bactrocera cucurbitae* Coquillet— is poorly documented. A fourth pest tephritid, *Bactrocera latifrons* (Hendel) (sometimes called the "Malaysian or solanaceous fruit fly"), normally attacks plants in the Solanaceae and Cucurbitaceae (Vargas and Nishida 1985, Liquido et al. 1994, McQuate et al. 2007) and has not been tested. Because blueberry is a new crop in Hawaii, previous surveys of fruit fly hosts in the state did not include blueberry (Vargas and Nishida 1985, Liquido et al. 1991). A laboratory host status determination study was conducted for blueberry with *C. capitata, B. dorsalis, B. cucurbitae,* and *B. latifrons* to determine whether this crop is potentially susceptible to infestation.

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Materials and Methods

Laboratory cage infestation experiments were conducted to determine the host status of blueberry to *C. capitata, B. dorsalis, B. cucurbitae,* and *B. latifrons.* Commercial varieties of northern or southern highbush blueberries were used depending on availability. All tests used laboratory flies obtained from colonies maintained at USDA–ARS laboratory in Honolulu, HI, that were reared on standard diets for each species (Vargas 1989, Vargas et al. 1990). In the first experiment, northern highbush blueberries, *V. corymbosum* 'Bluecrop' or 'Berkeley', purchased at a local supermarket were exposed to gravid female *B. dorsalis, C. capitata,* and *B. cucurbitae* in no-choice tests. Bluecrop berries were grown in British Colombia, and the Berkeley berries were grown in Chile. For each fruit fly species, 50 fruit spread out in a single layer were exposed to 50 gravid female flies in 25- by 25- by 25-cm screen cages outdoors for 6 h. After the 6-h exposure, fruit were removed from each cage and placed in a 3.8-liter plastic bucket with a screened lid and sand and held at 20–25°C and a photoperiod of 14:10 (L:D) h

Table 1. Mean number (± SEM) of *C. capitata*, *B. cucurbitae*, and *B. dorsalis* developing at 2 wk after forced infestation of two cultivars of blueberries and holding on sand in the laboratory

Species	Replicates	Total no. fruit	Total no. gravid flies	Reared from host[a]			
				Fruit wt (g)	No. puparia	No. puparia/ g fruit	% adult emergence[b]
Bluecrop							
C. capitata	12	600	600	81.3 (1.7)a	48.2 (11.2)b	0.60 (0.14)b	54.1 (6.0)a
B. cucurbitae	12	600	600	79.9 (2.4)a	7.2 (3.8)c	0.09 (0.04)c	12.7 (7.6)b
B. dorsalis	12	600	600	80.1 (2.4)a	84.8 (9.3)a	1.06 (0.11)a	50.8 (4.0)a
Berkeley							
C. capitata	8	400	400	67.1 (4.9)a	1.4 (1.2)a	0.02 (0.01)a	60.0 (40.0)a
B. cucurbitae	8	400	400	67.6 (4.0)a	0.0 (0.0)a	0.0 (0.0)a	
B. dorsalis	8	400	400	66.5 (4.0)a	3.9 (2.0)a	0.06 (0.03)a	30.0 (20.0)a

Data are means (± SEM) of replicates where 50 fruit were exposed to 50 gravid fruit flies in cages.
[a] Means within a column followed by the same letter are not significantly different by a Tukey's test ($P > 0.05$).
[b] Data were arcsine transformed before analysis.

in the laboratory for larval development and pupariation. After 2 wk, fruit and sand were inspected for puparia. Puparia were transferred to 120-ml plastic cups for adult emergence. Two or three replicate cages were run on each of six different days, and data were analyzed as a completely randomized design. Ripe papayas, *Carica papaya* L. 'Rainbow', a preferred host for all three fruit fly species, were exposed similarly in a separate test to demonstrate the suitability of the host testing methods and adult female fly competence.

Data on fruit fly infestability were subjected to two-way analysis of variance (ANOVA), with fruit fly species and cultivar as main effects; because there was a significant interaction effect, each cultivar was analyzed separately. For each cultivar, data for each fruit fly species on the number of puparia, the number of puparia per gram of fruit weight, and arcsine transformed percentage of adult emergence were subjected to one-way ANOVA, and means separations were done using a Tukey's test ($P > 0.05$) (SAS Institute 2002). Data on papaya infestability were analyzed similarly.

In the second experiment, six southern highbush blueberry cultivars ('Biloxi', 'Misty', 'Sharp Blue', 'Sapphire', 'Emerald', and 'Jewel') grown at Mealani Experiment Station, Waimea, HI, (elevation, 908 m) were harvested weekly for 10 wk to obtain yield data and exposed the day after harvest to *B. latifrons* in cages. On each date for each cultivar, 45–50 fruit spread out in a single layer in 3.8-liter plastic tubs were exposed to 50 gravid female flies in 25- by 25- by 25-cm screen cages outdoors for 24 h. (A smaller number of Jewel fruit were tested due to limited availability.) After exposure, fruit were divided evenly into two groups and held in the laboratory in 3.8-liter plastic tubs on either sand (300 g) or with artificial diet (Vargas and Nishida 1985) for 2 wk at 21°C under a photoperiod of 14:10 (L:D) h for fruit fly emergence. Holding fruit on artificial diet was included to examine whether blueberries might be an acceptable oviposition substrate but not an adequate rearing host. In total, 5,354 blueberries were exposed to 5,362 *B. latifrons.*

Two preferred hosts, cherry tomato, *Lycopersicon esculentum cerasiforme* L. ('Dunal') and pepper, *Capsicum annum* L. ('Anaheim'), were exposed to *B. latifrons* in similar tests, but with fewer fruit and using a 6-h exposure to demonstrate the suitability of the host testing methods for this species and adult female fly competence. For peppers and tomatoes, 10–12 replicates were run on three different days by using a completely randomized design. Data for each host on the number of puparia per gram of fruit weight and arcsine transformed percentage of adult emergence were subjected to one-way ANOVA. Differences are reported using a *t*-test ($P > 0.05$) (SAS Institute 2002).

Results

In the first experiment, the fruit fly species by cultivar interaction effect was highly significant for the number of puparia and the number of puparia per gram of fruit ($P < 0.001$); therefore, the two cultivars were analyzed separately. For Bluecrop berries, ANOVA on the effect of fruit fly species was significant for the number of puparia ($F_{2,35} = 19.9$; $P < 0.0001$), the number of puparia per gram of fruit ($F_{2,35} = 20.1$; $P < 0.0001$), and the percentage of adult emergence ($F_{2,32} = 17.4$; $P < 0.0001$). The number of puparia and number of puparia per gram of fruit were significantly higher for *B. dorsalis* than *C. capitata,* and both were significantly higher than *B. cucurbitae* ($P < 0.05$; Tukey's test) (Table 1); the percentage of adult emergence was significantly higher in *C. capitata* and *B. dorsalis* than *B. cucurbitae.* The number of puparia and number of puparia per gram of fruit developing from Berkeley berries was significantly lower than for Bluecrop berries ($P < 0.0001$). For Berkeley berries, ANOVA on the effect of fruit fly species was not significant for the number of puparia, the number of puparia per gram of fruit, and the percentage of adult emergence from blueberries ($P > 0.10$) (Table 1).

For the preferred host papaya (fully ripe), ANOVA on the effect of fruit fly species was not significant for the number of puparia ($P = 0.16$), number of puparia per gram of fruit ($P = 0.21$), and percentage of adult emergence ($P = 0.75$) (Table 2). The large number of

Table 2. Mean number (± SEM) of *C. capitata*, *B. cucurbitae*, and *B. dorsalis* developing at 2 wk after forced infestation of papayas and holding on sand in the laboratory

Species	Replicates	Total no. fruit	Total no. gravid flies	Reared from host[a]			
				Fruit wt (g)	No. puparia	No. puparia/ g fruit	% adult emergence[b]
C. capitata	5	5	250	314.4 (13.1)a	190.2 (91.4)a	0.66 (0.35)a	61.5 (9.0)a
B. cucurbitae	5	5	250	327.9 (24.5)a	546.6 (150.6)a	1.71 (0.52)a	61.2 (7.7)a
B. dorsalis	5	5	250	314.9 (24.4)a	305.4 (125.2)a	0.92 (0.34)a	68.1 (5.9)a

Data are means (± SEM) of replicates where one fruit was exposed to 50 gravid fruit flies in cages.
[a] Means within a column followed by the same letter are not significantly different by a Tukey's test ($P > 0.05$).
[b] Data were arcsine transformed before analysis.

flies reared from papaya demonstrates the suitability of the host testing methods for blueberries and adult female fly competence for the three fruit fly species. The number of puparia per gram of fruit was similar between papaya and Bluecrop blueberry for *C. capitata* and *B. dorsalis* (Tables 1 and 2); however, the number of puparia per gram of fruit for *B. cucurbitae* was significantly lower on Bluecrop blueberry compared with papaya.

In the second experiment, 2,677 blueberries exposed to 2681 *B. latifrons* and held on sand produced no puparia or adults (Table 3). One live larva was collected from a Misty blueberry 2 wk after infestation, but it did not develop to the pupal stage. A relatively small number of puparia and adults emerged from infested blueberries of all cultivars held on artificial diet after exposure to fruit flies (Table 3). This indicates that supplemental diet was necessary for larvae in blueberries to complete development. Tomato and pepper were acceptable hosts for *B. latifrons* under no-choice conditions (Table 4). Pepper produced significantly more puparia per gram of fruit than tomato ($F_{1,20} = 21.7$; $P < 0.001$) but had significantly lower percent adult emergence. These results with preferred hosts of *B. latifrons* demonstrate the suitability of the host-testing methods for blueberry and adult female fly competence.

Discussion

B. dorsalis, *C. capitata*, and *B. cucurbitae* successfully infested Bluecrop blueberry under no-choice conditions in cages. *B. dorsalis* and *C. capitata* produced significantly more puparia per gram of fruit than *B. cucurbitae. B. dorsalis* and *C. capitata* also had a significantly higher rate of adult emergence than *B. cucurbitae.* The number of puparia produced per gram of fruit was comparable between Bluecrop blueberry and ripe papaya for *B. dorsalis* and *C. capitata.* This confirms that blueberry can be infested by these fruit flies under the conditions used in these experiments and that blueberry can be a suitable host for development. Berkeley blueberry, however, produced low numbers of *B. dorsalis* and *C. capitata* puparia and no *B. cucurbitae* puparia, indicating that there may be considerable cultivar to cultivar variation in susceptibility to these fruit flies. The Berkeley blueberries used in our study were apparently acceptable for oviposition but a poor host for larval development (i.e., possible antibiosis resistance) (Follett and Hennessey

Table 3. Number of *B. latifrons* developing at 2 wk after forced-infestation of blueberries and holding on sand or artificial diet in the laboratory

Cultivar	Replicates	Total no. fruit	Total no. gravid flies	Total fruit wt (g)	Reared from host	
					No. puparia or larvae	No. adults[a]
On sand						
Biloxi	10	492	492	922.6	0	0
Misty	10	483	483	924.0	1[b]	0
Sharpblue	10	500	500	975.2	0	0
Sapphire	10	480	480	892.3	0	0
Jewel	8	267	271	620.6	0	0
Emerald	10	455	455	1137.9	0	0
Total		2,677	2,681	5,472.6	1	0
On diet						
Biloxi	10	493	492	922.6	6	4
Misty	10	482	483	923.9	15	10
Sharpblue	10	500	500	975.1	17	16
Sapphire	10	480	480	892.2	14	7
Jewel	8	267	271	620.5	40	30
Emerald	10	455	455	1137.8	14	9
Total		2,677	2,681	5,472.1	106	76

[a] Number of adults reared from collected puparia or larvae.
[b] One live larva was found that did not pupariate successfully.

Table 4. Mean number (± SEM) of *B. latifrons* developing at 2 wk after forced infestation of tomatoes or peppers and holding on sand in the laboratory

Host	Replicates	Total no. fruit	Total no. gravid flies	Reared from host[a]			
				Fruit wt (g)	No. puparia	No. puparia/ g fruit	% adult emergence[b]
Tomato	10	203	500	168.7 (1.9)	59.3 (13.1)	0.14 (0.02)b	79.8 (4.1)a
Pepper	12	27	600	152.7 (11.5)	11.5 (4.6)	0.72 (0.11)a	56.7 (4.6)b

Data are means (± SE) for each replicate cage of fruit, with two to three fruit per cage for peppers and 13–35 fruit per cage for tomatoes exposed to 50 gravid *B. latifrons.*

[a] Means within a column followed by a different letter are significantly different by a *t*-test ($P < 0.01$).

[b] Data were arcsine transformed before analysis.

2007). Because the fruit were store-bought, we were not certain of the age or condition (e.g., possible presence of insecticide residues) of the Berkeley berries, and uncontrolled factors may have altered their true susceptibility. If antibiosis resistance traits are present, they could be exploited during cultivar development in the future.

Whether blueberry is a natural host for *B. dorsalis, C. capitata,* and *B. cucurbitae* in Hawaii is not known because blueberry is not yet widely grown. *B. dorsalis, C. capitata,* and *B. cucurbitae* have been trapped as part of an areawide fruit fly suppression program in the Waimea area of Hawaii island (Vargas et al. 2008), near the site of the experimental blueberry planting that supplied berries for the *B. latifrons* study; Zee et al. (2006) reported no obvious fruit fly damage in ripe blueberries harvested at this site during their cultivar evaluation trials that applied no insecticides. This suggests that blueberry may not be a preferred host for *B. dorsalis, C. capitata,* or *B. cucurbitae,* but more detailed field infestation studies are needed as blueberry plantings expand in Hawaii.

B. latifrons oviposited in blueberries and eggs hatched, but larvae died or rejected fruit. *B. latifrons* could complete development only if artificial diet was provided as an alternative or supplemental food source. The results from rearing on sand and artificial diet indicated that blueberry is an acceptable oviposition host for *B. latifrons* but not a suitable developmental host (Cowley et al. 1992, Follett and Hennessey 2007, NAPPO 2008).

The results from the preferred hosts (papaya, tomato, and pepper) used in our study suggested that the potential infestation pressure in laboratory cages was considerably higher than what fruit might experience in the field. For example, Vargas and Nishida (1985) reported rearing a high of 37.8 and 440 *B. latifrons* adults per kilogram of fruit from field collected tomato and pepper, respectively. The highest number of *B. latifrons* adults per kg fruit across replicates in our study using a high density of flies in small infestation cages and a short duration exposure was 3–5 times higher at 232 and 1,320 adults per kilogram of fruit for tomato and pepper, respectively.

The plant genus *Vaccinium* has the generic name "blueberry," which may be confused in the literature as being synonymous with commercial varieties of blueberry, including northern or southern high bush blueberry. The northern highbush blueberry is *V. corymbosum,* whereas the southern highbush blueberry has a complex ancestry, based largely on *V. corymbosum* with some genes from *V. darrowi* Camp. (Hummer et al. 2007). *Vaccinium* sp. is listed as a host for *C. capitata* (Liquido et al. 1991), but without a citation and with no reference to the species of *Vaccinium.* In Hawaii, two field studies have examined fruit fly infestation in a native plant in the genus, *Vaccinium reticulatum* Sm (ohelo); in one study, *B. dorsalis* was reared from ohelo berries (Maehler 1951), but in another study 36 collections of a total of 7,622 ohelo berries produced no *B. dorsalis* or *C. capitata* (Liquido et al. 1990). *B. latifrons* has not been reported from any *Vaccinium* sp. since its introduction to Hawaii (Vargas and Nishida 1985; Liquido et al. 1994; Harris et al. 2001, 2003). Numerous host lists for *B. dorsalis* and *B. cucurbitae* do not include commercial blueberry (e.g., Florida, Steck 2008). However, the absence of commercial species of blueberry from lists of tropical fruit fly hosts may simply reflect the distribution of blueberry—blueberry is naturally a temperate climate crop with a cold requirement, and therefore is not normally grown in tropical and subtropical climates, or tested against tropical or subtropical tephritid fruit flies such as the species used in our study. As commercial varieties of blueberry with low chilling requirements (e.g., southern highbush varieties) are introduced more widely to warm climates (Hummer et al. 2007, Bremer et al. 2008), the list of tephritid fruit flies attacking this crop will grow.

Our data suggest blueberry can be good host for *B. dorsalis* and *C. capitata* and an adequate host for *B. cucurbitae.* Blueberry is probably a nonhost for *B. latifrons.* Certain cultivars may show significant resistance to *B. dorsalis, C. capitata,* and *B. cucurbitae.* This information will aid in developing pest management recommendations for blueberry in Hawaii, and in the development of quarantine treatments for export. Numerous quarantine treatments have been developed in Hawaii against *B. dorsalis, C. capitata,* and *B. cucurbitae* to permit export of fruits and vegetables to the U.S. mainland and elsewhere. Irradiation or cold quarantine treatments are options for blueberries grown in Hawaii (Follett et al. 2009). In the United States, irradiation is already approved for control of all tephritid fruit flies irrespective of host (Follett and Griffin 2006), and therefore blueberries could be exported from Hawaii to the U.S. mainland by using irradiation without further research. Quarantine cold treatment development for blueberry should focus on control of *B. dorsalis*, *C. capitata*, and *B. cucurbitae* and can exclude *B. latifrons*, which seems not to be a pest.

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