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New associations and host status: Infestability of kiwifruit by the fruit fly species *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, and *Ceratitis capitata* (Diptera: Tephritidae)

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ABSTRACT

We conducted no-choice cage and field infestation studies to determine if the fruit of kiwifruit (*Actinidia chinensis* var. *deliciosa*, 'Hayward' [green-fleshed], and *Actinidia chinensis* var. *chinensis*, 'Zesy002' [gold-fleshed]) are hosts for three invasive tephritid fruit fly species that may enter New Zealand or other kiwifruit growing areas. For cage studies, punctured and unpunctured (intact) fruits of green and gold kiwifruit were exposed to gravid female flies of *Bactrocera dorsalis* (Hendel) (Oriental fruit fly), *Zeugodacus cucurbitae* (Coquillet) (melon fly), or *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly), in screen cages outdoors for 24 h, and then held on sand in the laboratory for three weeks for pupal development and adult emergence. Unpunctured green kiwifruit produced an average of 1.3, 0.0 and 48.8 puparia per kg of fruit for *B. dorsalis*, *Z. cucurbitae* and *C. capitata*, respectively. Unpunctured gold kiwifruit produced an average of 54.7, 6.1 and 0.0 puparia per kg of fruit for *B. dorsalis*, *Z. cucurbitae* and *C. capitata*, respectively. For comparison, unpunctured papaya, a preferred host for all three species, produced 492–795 puparia per kg of fruit across all species. These results indicate that kiwifruit is a poor ovipositional host for *B. dorsalis, Z. cucurbitae* and *C. capitata*. When kiwifruit were punctured to facilitate oviposition, the number of puparia per kg fruit increased significantly compared with those on unpunctured fruit for *B. dorsalis,* but not for *Z. cucurbitae* or *C. capitata*, indicating that kiwifruit is a poor developmental host for these two species. For all fruit fly species, the average weight of individual puparia recovered from green and gold kiwifruit was roughly 50% of the weight of puparia recovered from papaya. Field infestation of kiwifruit suspended from papaya trees resulted in no infestation of green kiwifruit and very low infestation of gold kiwifruit by *B. dorsalis*, and no infestation in either cultivar by *Z. cucurbitae* under natural conditions. Overall, kiwifruit is a poor host for *B. dorsalis*, *Z. cucurbitae* and *C. capitata*. This information will help inform decisions about quarantine restrictions and potential crop loss in the event of incursions of these fruit flies into New Zealand or other kiwifruit producing countries.

1. Introduction

World trade and travel have resulted in ever-increasing arrivals of alien insect pests. Most alien pests fail to establish because of small founder populations (Liebhold and Tobin, 2008) or habitat resistance (Follett, 2017). The detection of alien insect pests of quarantine significance can result in a regulatory response, often before the insect has established or spread. Knowledge of the host status of available plants, particularly for crops representing new host associations, during a pest incursion may help to inform decisions about a regulatory response and quarantine restrictions.

The arrival of new quarantine pests and diseases is a significant issue for New Zealand horticulture because of potential disruption to trade. New Zealand has proposed to develop pre-approved quarantine treatments that would enable continued market access from affected areas should a pest fruit fly (Diptera: Tephritidae) arrive and establish in an agriculturally important region. New Zealand's main exported fruit crops are kiwifruit (*Actinidia chinensis*) and apple (*Malus pumila*). During the past 30 years, New Zealand has had nine incursions of pest fruit flies, including Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (one incursion), Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (one incursion), and Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (five incursions) (Anon., 2016). All fruit fly incursions were successfully eradicated as a result of a biosecurity response following detection.

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New Zealand has identified these three fruit fly species, and melon fly, *Zeugodacus (Bactrocera) cucurbitae* (Coquillett), as target species for research into quarantine treatment options such as cold, irradiation, and systems approaches.

Three of the polyphagous invasive fruit fly species of greatest concern to New Zealand are established in Hawaii, *B. dorsalis*, *Z. cucurbitae*, and *C. capitata*, and therefore Hawaii was selected as a location to develop pre-approved quarantine treatments. *B. dorsalis* and *Z. cucurbitae* are important economic pests of a wide variety of fruits and vegetables throughout southern Asia and several Pacific Islands (White and Elson-Harris, 1992; CABI, 1997). *C. capitata* has spread from its origin in Africa to Mediterranean Europe and many parts of Central and South America (CABI, 1997), and is among the most destructive horticultural pests of more than 100 species of fruits, such as citrus (*Citrus* spp.), apples, pears (*Pyrus communis*), mangos (*Mangifera indica*), guavas (*Psidium guajava*) and peaches (*Prunus persica*). Tephritid fruit flies feed as larvae on the pulp of fruits, causing direct damage and facilitating infection with fruit diseases. Also, female fly oviposition holes can cause scarring, which lowers fruit quality.

Kiwifruit are native to north-central China (Morton, 1987). Cultivation spread from China in the early 20th century to New Zealand, where they were planted commercially for the first time. The plant genus *Actinidia* contains about 60 species. The most commonly available commercial cultivar from New Zealand is the green-fleshed, fuzzy skin 'Hayward' (*Actinidia chinensis* var. *deliciosa*). The newer gold-fleshed, smooth skinned cultivar 'Zesy002' (*Actinidia chinensis* var. *chinensis*) is becoming increasingly available.

Before development of a quarantine treatment, the host status of kiwifruit for various fruit flies must be determined. The literature on kiwifruit infestation by *Bactrocera dorsalis*, *Zeugodacus cucurbitae* and *Ceratitis capitata* is limited. The objective of this study was to 1) determine the potential susceptibility of kiwifruit (cultivars 'Hayward' and 'Zesy002') to infestation by *B. dorsalis*, *Z. cucurbitae*, and *C. capitata*; and 2) summarize references in published literature to infestation of kiwifruit by the three fruit fly species. Potential regulatory procedures to mitigate the risk of introduction of fruit fly pests in fruits imported from areas with established populations, or to move fruit out of quarantined areas during active invasions, are discussed.

2. Methods

2.1. Insects

Ceratitis capitata, *Zeugodacus cucurbitae*, and *Bactrocera dorsalis* were obtained from colonies maintained at the USDA-ARS, Daniel K. Inouye U. S. Pacific Basin Agricultural Research Center in Hilo, Hawaii. These fruit fly colonies have been maintained for 20–30 years (~200–400 generations) with intermittent infusion of wild flies (Vargas and Carey, 1990). Fruit flies used in our tests were maintained in an insectary at 24-27° C, 65–70% RH, and a photoperiod of 12:12 (L:D) hours. Approximately 500 adult flies were allowed to emerge inside cubical screen cages (30 × 30 × 30 cm). Fruit flies were supplied with a 3:1 mixture of sucrose and USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, Ohio, USA) as a food source, and water ad libitum. Adult flies were approximately 12 days old at the time of testing and females were reproductively mature and actively laying eggs.

2.2. Screen cage tests

Insect exposures to fruit were conducted at the USDA-ARS, U.S. Pacific Basin Agricultural Research Center in Hilo, Hawaii. Trials were conducted within the time period of 11 October – 6 November 2017. This laboratory is located on the windward side of the Big Island of Hawaii at an elevation of 100 m.

All experiments were conducted outdoors under semi-natural conditions using screened cages placed on wooden shelves under a roof with sky lighting. Tests were conducted with green and gold cultivars of kiwifruit and papaya (a preferred host; Vargas and Carey, 1990) simultaneously. Kiwifruit ('Hayward' and 'Zesy002' cultivars) were harvested in New Zealand in April–June 2017 and shipped to the USDA-ARS, U.S. Pacific Basin Agricultural Research Center in Hilo, Hawaii, where they were held in cold storage rooms at 1–2 °C. Ripe papayas ('Rainbow') were obtained from a local packing house and ripened at room temperature to the fully ripe maturity stage. Each "trial" consisted of single fruit exposures to 25 gravid fruit flies in 18 separate test cages–three fruit fly species x three cultivars (gold and green kiwifruit, papaya) x two puncture treatments (punctured, unpunctured [intact])–conducted concurrently. Fruits were introduced into cages with female flies between 11:30 and 1:00 h, and removed after 24 h. For punctured fruit, a probe 1.0 mm in diameter was used to haphazardly puncture fruit 25 times to a depth of 1.0 cm. Unpunctured fruit had skin intact and undamaged. Each 30 × 30 × 30 cm cage contained a single fruit (either intact or punctured), with water ad libitum and two sugar cubes. The 24-h exposures of kiwifruit and papayas to fruit flies were replicated six times over a 2-week period with fresh adult flies on each day and one replicate (18 cages) per day. The average temperature at the location during the experiments was 24.7 °C (range 19.5–31 °C), the average relative humidity was 74% (range 69–79%), and average daylight was 11.5 h.

Following fruit fly exposure, fruits were transferred to laboratory rearing rooms and into 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 pupation medium. Holding conditions were 24–27 °C, 65–70% RH, and a photoperiod of 12:12 (L:D) hours. At two weeks post-exposure, kiwifruit and papaya fruits were processed by sieving sand from the bucket to recover puparia (the thickened, hardened larval skin in which the pupa is formed in higher Diptera), and at three weeks post exposure sand from the bucket was sieved again and fruits were dissected to recover any additional puparia and remaining larvae. Puparia and late instar larvae ('poppers') were transferred to 7.0 cm (diameter) x 7.5 (height) 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 kiwifruit and papaya fruit. For all fruit samples, 25 individual pupae were randomly selected and weighed; when < 25 pupae emerged from a fruit, all available pupae were weighed.

2.3. Field test

Kiwifruit are not grown in Hawaii, and therefore New Zealand fruit from the same shipment used in the screen cage tests were used in a field test. Although nearly one year in storage at 1–2 °C, the kiwifruit were still firm and highly edible. Gold- and green-fleshed kiwifruit were placed in papaya fields to test for natural infestation. Studies were conducted during February–April 2018 in several 5- to 10-acre commercial papaya ('Rainbow') fields located on Hawaii Island near the Kapoho area in lower Puna, which is the main papaya production area in Hawaii. Typically only *Bactrocera* dorsalis and *Zeugodacus cucurbitae*, and not *Ceratitis capitata*, infest papayas in this area (Vargas et al., 1995). To test for potential fruit infestation, kiwifruit were placed inside wire baskets made from 2.5-cm Hex Mesh chicken wire fencing and suspended from the trunks of papaya trees at approximately 1.5 m height for one week. Ripe papayas in wire baskets were suspended at the same time as controls to demonstrate fruit fly activity. The papaya treatment contained one fruit per basket, and the gold and green kiwifruit treatments contained four fruits per basket to standardize the weight of fruit per basket at approximately 400–500 g. Preliminary tests in the laboratory demonstrated that papaya fruit inside the wire mesh baskets were easily accessed by fruit flies (*B. dorsalis* and *Z. cucurbitae*), and infestation rates were similar in papaya fruit with and without wire mesh baskets. After one week in the field, the fruit(s) from each basket were brought back to the laboratory and transferred to plastic buckets