



Protein feeding of Queensland fruit fly *Bactrocera tryoni* and cucumber fly *Zeugodacus cucumis* (Diptera: Tephritidae) on non-host vegetation: effect of plant species and bait height

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Abstract

Perimeter-baiting of non-crop vegetation using toxic protein baits was developed overseas as a technique for control of melon fly, *Zeugodacus* (*Zeugodacus*) *cucurbitae* (Coquillett) (formerly *Bactrocera* (*Zeugodacus*) *cucurbitae*), and evidence suggests that this technique may also be effective in Australia for control of local fruit fly species in vegetable crops. Using field cage trials and laboratory reared flies, primary data were generated to support this approach by testing fruit flies' feeding response to protein when applied to eight plant species (forage sorghum, grain sorghum, sweet corn, sugarcane, eggplant, cassava, lilly pilly and orange jessamine) and applied at three heights (1, 1.5 and 2 m). When compared across the plants, Queensland fruit fly, *Bactrocera tryoni* (Froggatt), most commonly fed on protein bait applied to sugarcane and cassava, whereas more cucumber fly, *Zeugodacus* (*Austrodacus*) *cucumis* (French) (formerly *Bactrocera* (*Austrodacus*) *cucumis*), fed on bait applied to sweet corn and forage sorghum. When protein bait was applied at different heights, *B. tryoni* responded most to bait placed in the upper part of the plants (2 m), whereas *Z. cucumis* preferred bait placed lower on the plants (1 and 1.5 m). These results have implications for optimal placement of protein bait for best practice control of fruit flies in vegetable crops and suggest that the two species exhibit different foraging behaviours.

Key words crop borders, foraging behaviour, fruit fly management, protein baiting.

INTRODUCTION

Fruiting vegetable crops in Queensland are attacked by two key fruit fly species, Queensland fruit fly, *Bactrocera tryoni* (Froggatt), and cucumber fly, *Zeugodacus* (*Austrodacus*) *cucumis* (French) (formerly *Bactrocera* (*Austrodacus*) *cucumis*, Virgilio *et al.* 2015). *B. tryoni* has a wide host range which includes a number of solanaceous vegetable crops such as capsicum, chilli, tomatoes and eggplants (Hancock *et al.* 2000). Although native to coastal Queensland, it is now widely established in eastern Australia (Drew *et al.* 1982). More recently, there have been increasingly frequent incursions into the Fruit Fly Exclusion Zone in the southern states of Australia (Dominiak *et al.* 2015), and climatic warming may further threaten horticultural industries in these areas (Sutherst *et al.* 2000). *Z. cucumis* primarily attacks cucurbits and tomatoes; its distribution ranges from northern New South Wales up to the Northern Territory and Torres Strait Islands (Drew *et al.* 1982; Hancock *et al.* 2000). Management of both species in vegetable crops has relied almost exclusively on organophosphate cover sprays. Recent restrictions in the use of dimethoate and fenthion, following review by the Australian Pesticides and Veterinary Medicines Authority, have made fruit fly control more difficult and alternative management techniques are required (Clarke *et al.* 2011).

Protein baiting is an effective method for control of *B. tryoni*, commonly practiced in tree crops. A yeast-based protein bait plus toxicant is applied to the foliage of host trees, where the adult fruit flies naturally feed and forage (Drew & Yuval 2000). The efficacy of protein baiting for control of *B. tryoni* and *Z. cucumis* in vegetable crops has not been evaluated. However, evidence from management of other vegetable-infesting fruit fly species overseas suggests that protein baiting may be more effective when applied to vegetation on the perimeter of a vegetable crop, rather than to the crop itself. Nishida and Bess (1957) observed that the melon fly, *Z. cucurbitae* (Coquillett), which is closely related to *Z. cucumis* (Nakahara & Muraji 2008), spends much of its time roosting and feeding in non-host vegetation adjacent to the crop, only entering the crop to oviposit. As a consequence, perimeter baiting of non-crop vegetation was developed for control of this species in Hawaii (Nishida *et al.* 1957; Prokopy *et al.* 2003; McQuate & Vargas 2007). A number of other cucurbit-infesting species have also been shown to roost in non-host vegetation on the crop perimeter, including *Dacus cilatus* (Loew), *Dacus demmerezi* (Bezzi) and *Myiopardalis pardalina* (Bigot) (Abdullah *et al.* 2007; Deguine *et al.* 2012, 2015).

It has been suggested that *B. tryoni* has a close relationship with the host tree, which serves not only as a larval food source but may also be a site for other adult activities including protein feeding (Drew & Lloyd 1987). However, Ero *et al.* (2011), observing the behaviour of *B. tryoni* in a nectarine orchard, recorded feeding only rarely, suggesting that the fruit flies may

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have been foraging elsewhere for protein in this instance. The relationship between *B. tryoni* and the host plant is still not well understood, particularly in low-growing crops. Balagawi *et al.* (2014) recorded higher numbers of male *B. tryoni* in traps placed in vegetation bordering a strawberry crop than traps within the crop, particularly early in the season; while from the same study, Gu (2010) found that rates of infestation were correspondingly higher in fruit near the border than within the block. Furthermore, Balagawi *et al.* (2014) demonstrated that *B. tryoni* rested preferentially on tree-type structures rather than on a plant with a low, sprawling habit such as strawberry. Missenden (2014) subsequently demonstrated perimeter protein baiting as an effective approach for control of *B. tryoni* in strawberry. Together, these observations suggest that low-growing crops may not provide optimal sites for *B. tryoni* roosting and feeding, and that such activities may take place outside the crop.

For perimeter protein baiting to be applied in vegetable crops, it is necessary to determine what non-host vegetation may best serve as the border planting. A variety of non-host plants have been evaluated for use in perimeter baiting for the exotic *Z. cucurbitae*, including corn/maize (*Zea mays*), castor bean (*Ricinus communis*), sorghum (*Sorghum* sp.), sugarcane (*Saccharum officinarum*), panax (*Polyscias guilfoylei*), tiger's claw (*Erythrina variegata*), guava (*Psidium guajava*) and cassava (*Manihot esculenta*), and grower planting recommendations have been made based on differential response to these plants (Nishida & Bess 1957; Prokopy *et al.* 2003; McQuate & Vargas 2007; McQuate 2011). Thus, the first part of our study compared protein feeding of *B. tryoni* and *Z. cucumis* on eight different plant species. Five of these species were selected as they had previously been evaluated as perimeter plantings for *Z. cucurbitae*: forage sorghum-Sudan grass hybrid (*S. bicolor* \times *S. bicolor* var. *sudanese*), grain sorghum (*S. bicolor*), sweet corn (*Z. mays* var. *rugosa*), sugarcane (*S. officinarum*) and cassava (*M. esculenta*). Both forage sorghum and grain sorghum were included due to their differing structures, grain sorghum being shorter and producing less foliage than forage sorghum. Two perennials, lilly pilly (*Syzygium smithii*) and orange jessamine (*Murraya paniculata*), were selected as they are used as windbreaks or border hedges, respectively. Finally, eggplant (*Solanum melongena*) was selected due to its broad, sheltering leaf structure, and because it could be grown as a crop amongst the other vegetables.

As previous work has also demonstrated that the height of protein bait application has a significant influence on the foraging behaviour of *B. tryoni* (Balagawi *et al.* 2012), for the second part of our study, we compared feeding of *B. tryoni* and *Z. cucumis* on baits applied at three different heights. While Balagawi *et al.* (2012) demonstrated a preference for *B. tryoni* protein foraging in the mid to upper canopy of tree crops, how this might relate to potential border plantings is unknown. Additionally, as *Z. cucumis* has specialised on fruits of the Cucurbitaceae, and cucurbits generally have a vining/creeping habit, it is possible that this latter fruit fly species is more likely to forage close to the ground.

MATERIALS AND METHODS

Fruit flies

Fruit flies, *B. tryoni* and *Z. cucumis*, were obtained from colonies maintained by the Market Access research group at the Department of Agriculture and Fisheries (DAF) (Brisbane, QLD). All fruit flies were mixed sex adults, between 3 and 7 days post emergence. Fruit flies were provided with sugar and water *ad libitum* but did not receive protein.

Evaluation of plant species

Eight plant species were selected for evaluation: forage sorghum-Sudan grass hybrid, grain sorghum, sweet corn, sugarcane, eggplant, cassava, lilly pilly and orange jessamine. The majority were grown in 300 mm pots from seed, seedlings or cuttings and were at a fully grown stage at the time of the trial. The lilly pilly and orange jessamine plants were obtained as small shrubs and were therefore not mature plants; for trials, they were placed on stands (ca. 30 cm high) to give extra height, an approach also used by Balagawi *et al.* (2014) for *B. tryoni* cage foraging trials. For trials with *B. tryoni*, the eggplant and grain sorghum plants were also placed on stands, as it was observed in preliminary tests that this species did not respond well to baits placed below 1 m. The plant heights varied according to species and individual specimens: on average, the shortest were eggplant (1.0–1.1 m) and orange jessamine (1.0–1.4 m), and the tallest were sweet corn, sugarcane and cassava (2.0–2.4 m). Each plant species was represented by a group of two or three plants dependent on the size of individual plants.

Protein baits were used as a standardised method to allow a relative assessment of the number of fruit flies on each plant. Yeast hydrolysate enzymatic (MP Biomedicals, LLC, Solon, OH, USA) was applied to clear plastic discs (55 mm diameter), and one disc clipped onto one of the plants in each group. For trials with *Z. cucumis*, protein baits were placed at 1 m, the maximum height of the shortest plant. For trials with *B. tryoni*, protein baits were placed at 1.5 m, with the shortest plants on stands.

Trials were performed within four large, metal frame, netted cages (3 m \times 3 m base, 2.5 m high) within a shade house at the DAF Redlands Research Facility (Cleveland, QLD). One plant species was placed in a corner of each cage, with four different plant species per cage. Sugar and water were placed on the floor in the centre of the cage. Approximately, 300 *B. tryoni* or *Z. cucumis* were released and left for a minimum of 30 min to acclimate. Protein baits were then placed on the plants and the experiment commenced. Counts of fruit flies on each protein bait were made at 30 min intervals for a total of 2 hours. Trials were performed between 30 October and 11 November 2013 (*Z. cucumis*) and between 14 November and 5 December 2013 (*B. tryoni*). Trials took place between the hours of 12.30 pm and 3.45 pm.

Eight replicates were performed for each fruit fly species, with each replicate spread across two cages. A resolvable incomplete block design was used to allocate the plant species to the cages. The data were analysed using a generalised linear mixed

model assuming a Poisson distribution and log link function, with plant species and assessment time as fixed factors. Pairwise comparisons of means were performed using Fisher's protected 95% least significant difference (LSD). Statistical analyses were performed in GenStat for Windows 16th Edition (VSN International 2013).

Evaluation of bait height

Cassava and forage sorghum were selected for use in the tests; these two plant species were preferred as roosting sites by *B. tryoni* and *Z. cucumis*, respectively (Results). Three cassava or three sorghum plants were placed in a field cage, one plant in each of three corners. Three protein baits were clipped to each plant at a height of 1, 1.5 and 2 m, giving three baits at each height per cage. Sugar and water were placed on the floor in the centre of the cage. Approximately, 300 *B. tryoni* or *Z. cucumis* were released into each cage and left for 30 min to acclimate before protein baits were placed on the plants. Counts of fruit flies on each protein bait were made at 30 min intervals for a total of 2 hours. Trials were performed between 18 November and 21 November 2013 (*Z. cucumis*) and between 6 December and 16 December 2013 (*B. tryoni*) between the hours of 1.00 pm and 6.00 pm.

Three replicates were performed for each fruit fly species and each plant species. Trials with each fruit fly species and plant species were conducted and analysed separately. The mean number of fruit flies on protein baits at each height per cage replicate was calculated and data analysed using a repeated measures analysis of variance (ANOVA) with bait height and assessment time as treatment factors. A square root transformation for mean counts was required to improve the assumptions underlying the ANOVA for *B. tryoni* on forage sorghum. Pairwise comparisons of means were performed using Fisher's protected 95% LSD.

RESULTS

Evaluation of plant species

There was a significant effect of plant species on response of *B. tryoni* ($F_{(7, 212.8)}=4.64$; $P<0.001$), with most fruit flies found on grain sorghum, forage sorghum, sugarcane and cassava (Fig. 1). There was also a significant effect of assessment time ($F_{(3, 208.4)}=30.74$; $P<0.001$), with mean counts decreasing over time. However, there was no interaction between plant species and assessment time ($F_{(21, 208.4)}=0.41$; $P=0.991$), indicating that response over time was similar for each plant species. There was also a significant effect of plant species on response of *Z. cucumis* ($F_{(7, 222.6)}=13.14$; $P<0.001$), with the most fruit flies found on sweet corn and forage sorghum (Fig. 2). There was no effect of assessment time ($F_{(3, 209.5)}=0.83$; $P=0.477$) and no interaction between plant species and assessment time ($F_{(21, 209.5)}=0.36$; $P=0.996$).

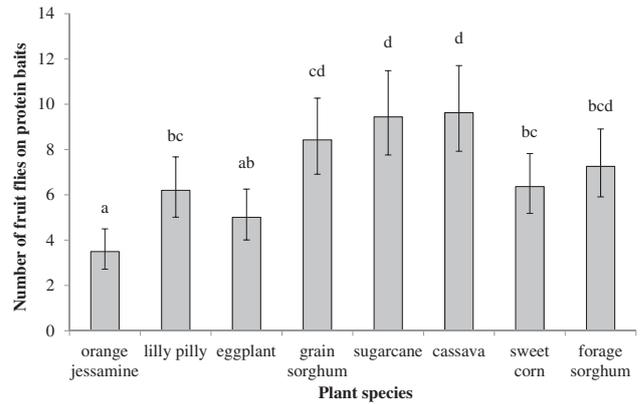


Fig. 1. Mean (back-transformed mean \pm 1 standard error) number of *B. tryoni* recorded on protein baits on eight plant species, across all sampling times. Means with a letter in common are not significantly different ($P>0.05$); letter values relate to the transformed data.

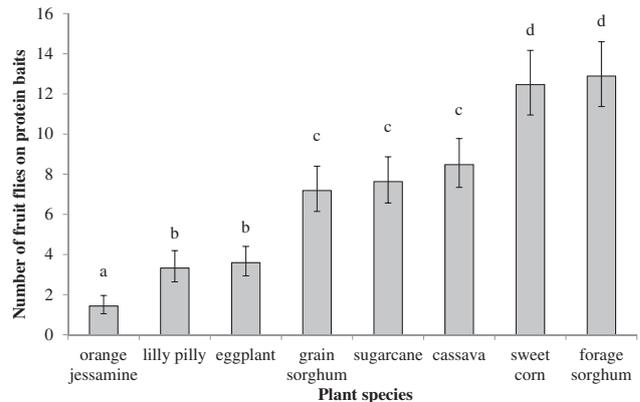


Fig. 2. Mean (back-transformed mean \pm 1 standard error) number of *Z. cucumis* recorded on protein baits on eight plant species, across all sampling times. Means with a letter in common are not significantly different ($P>0.05$); letter values relate to the transformed data.

Evaluation of bait height

There was no significant effect of protein bait height on the response of *B. tryoni* when protein bait was applied to cassava ($F_{(2, 4)}=5.32$; $P=0.075$). There was a significant effect of time ($F_{(3, 18)}=21.57$; $P<0.001$), with numbers of fruit flies at the protein baits declining over time. There was also a significant interaction between height and time ($F_{(6, 18)}=5.37$; $P=0.009$), from which we can infer that the mean counts at each height did not show a similar pattern at each assessment. Pairwise comparisons between heights within a time found significantly more fruit flies on 2 m protein baits compared to 1.5 or 1 m protein baits at the 30 and 60 min assessments, but no significant differences between heights at 90 or 120 min (Fig. 3).

There was a significant effect of bait height on the response of *B. tryoni* when protein bait was applied to forage sorghum

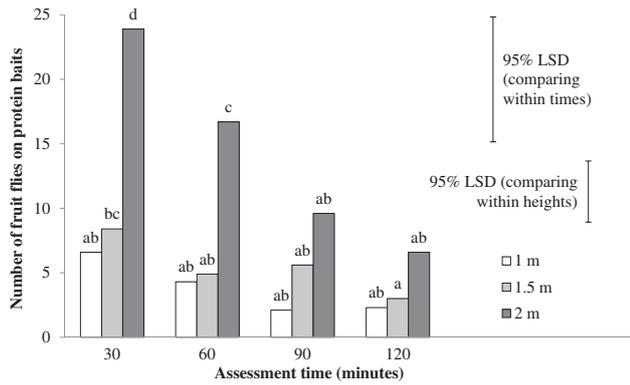


Fig. 3. Interaction means resulting from a repeated measures ANOVA for *B. tryoni* on protein baits at three different heights on cassava. Means with a letter in common are not significantly different ($P > 0.05$).

($F_{(2, 4)} = 14.19$; $P = 0.015$), with significantly more fruit flies on 2 m protein baits than lower baits (Fig. 4). There was also a significant effect of time ($F_{(3, 18)} = 10.79$; $P = 0.002$), with the number of fruit flies on protein baits decreasing over time. However, there was no significant interaction between protein bait height and assessment time ($F_{(6, 18)} = 0.54$; $P = 0.712$), suggesting a similar pattern of response over time.

There was a significant effect of protein bait height on the response of *Z. cucumis* when protein bait was applied to cassava ($F_{(2, 4)} = 18.11$; $P = 0.010$), with significantly fewer fruit flies on 2 m protein baits compared with 1 and 1.5 m protein baits (Fig. 5). There was also a significant effect of assessment time ($F_{(3, 18)} = 14.22$; $P = 0.002$), with mean counts decreasing over time. However, there was no significant interaction between protein bait height and assessment time ($F_{(6, 18)} = 1.67$; $P = 0.240$), suggesting a similar pattern of response over time. When forage sorghum was used as the test plant, there was no significant effect of plant height on *Z. cucumis* protein foraging ($F_{(2, 4)} = 1.42$; $P = 0.341$) (Fig. 5). There was also no significant

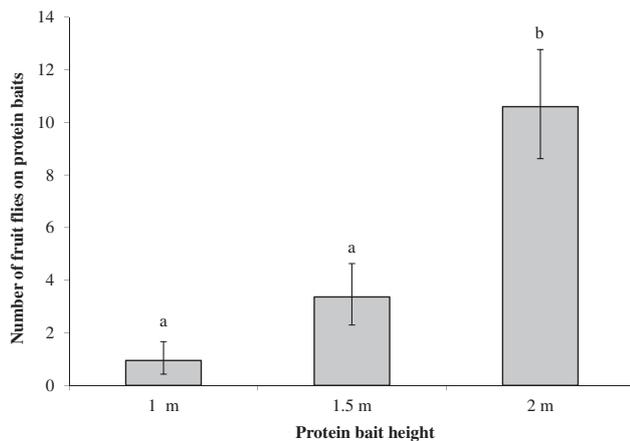


Fig. 4. Mean (back-transformed) number of *B. tryoni* recorded on protein baits placed at three heights on forage sorghum, across all sampling times. Error bars represent 95% least significant difference (LSD). Means with a letter in common are not significantly different ($P > 0.05$); letter values relate to the transformed data.

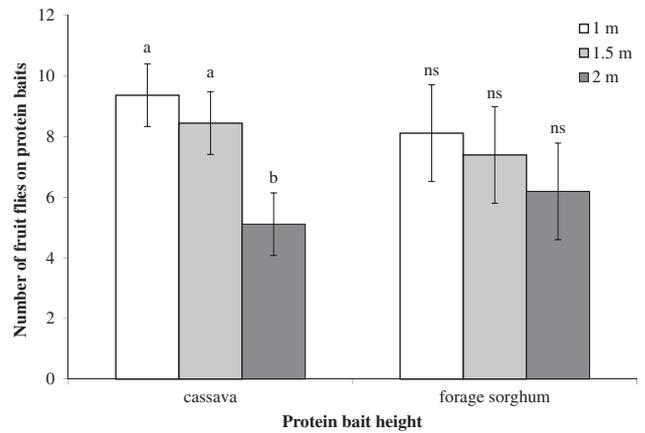


Fig. 5. Mean number of *Z. cucumis* recorded on protein baits placed at three heights on cassava or forage sorghum, across all sampling times. Error bars represent 95% least significant difference (LSD). For each plant species, means with a letter in common are not significantly different ($P > 0.05$). ns, not significant ($P > 0.05$).

effect of time ($F_{(3, 18)} = 2.11$; $P = 0.166$), and no significant interaction between the two factors ($F_{(6, 18)} = 0.40$; $P = 0.800$).

DISCUSSION

Trials found significant differences in the number of *B. tryoni* and *Z. cucumis* feeding on protein bait placed on eight different plant species. *B. tryoni* were found in greatest numbers on grain sorghum, forage sorghum, sugarcane and cassava, whereas more *Z. cucumis* were recorded from sweet corn and forage sorghum. Corn (maize) and sorghum have traditionally been used in perimeter baiting for *Z. cucurbitae* (Nishida & Bess 1957; Prokopy et al. 2003) and several trials have found corn to be a preferred roosting site for this species (McQuate & Vargas 2007; McQuate 2011; Atiama-Nurbel et al. 2012). The findings from the current study suggest that *Z. cucumis* may behave similarly. Greer (2001) evaluated application of toxic bait to various plants grown alongside strawberries, for management of *B. tryoni*. Results generally supported the efficacy of perimeter baiting, but the authors did not directly compare the different types of plants (lupin, corn, a mix of ornamentals, windbreak plants and fringing plants of natural scrub). More recently, Missenden (2014) used sorghum as a perimeter planting for bait application as part of a systems approach to manage *B. tryoni* in strawberries. In the current study, sorghum (both grain and forage) was not statistically different to sugarcane and cassava, although the latter two species attracted greater absolute numbers of fruit flies than the sorghums (Fig. 1). Field trials are needed to determine if sorghum plantings are as effective as sugarcane and cassava, or if these latter two species should be used in place of sorghum.

The shorter plants (orange jessamine, lilly pilly and eggplant) were less preferred by both fruit fly species, despite the fact that *Z. cucumis* responded optimally to lower placed baits. It should be noted that orange jessamine and lilly pilly have the capacity to grow much larger and denser, and therefore, results may differ for established plants in the field. However, both these plants are

perennial trees and could only be recommended to growers for permanent field borders.

Very few studies have examined what attributes of non-host plants make them preferred roosting sites for fruit flies. A number of studies have suggested that *Z. cucurbitae* is attracted to plants which provide a source of food as well as shelter, in the form of extra-floral nectaries, pollen, bird faeces or honeydew produced by infestations of homopteran insects (Nishida & Bess 1957; McQuate & Vargas 2007; McQuate 2011; Atiama-Nurbel *et al.* 2012). Deguine *et al.* (2012) found that the number of fruit flies associated with corn borders was influenced by the vegetative quality of the plants and the presence of food. Balagawi *et al.* (2014) concluded that *B. tryoni* preferred a plant with an open, branched canopy compared with a dense, closed canopy: this may explain the high response of *B. tryoni* to cassava in the current trial. However, Raghu *et al.* (2004) found that the monophagous *B. cacuminata* (Hering) was positively associated with dense foliage, suggesting that this provided a favourable microclimate as well as shelter from predators. McQuate and Vargas (2007) suggested that leaf area is an important factor, with dense plantings of a border plant favoured over narrow borders. The microhabitat (temperature, shade and humidity) is also likely to differ between individual plants of one species as well as between species, and to impact on their suitability as roosting hosts.

B. tryoni and *Z. cucumis* exhibited contrasting responses to protein bait height. The response of *B. tryoni* was consistent with previous research showing that this fruit fly responds optimally to protein baits placed in the mid to upper canopy of trees (Balagawi *et al.* 2012). Likewise Lloyd (2005) recorded more *B. tryoni* feeding on protein baited boards placed at 1 or 1.8 m than those at 0.3 m. This has important practical implications, as label instructions for two commonly used commercial bait products (Naturalure™ and Fruit Fly Lure™) do not specify height of bait application. The effect of protein bait height on response of *Z. cucumis* has not been investigated previously. However, results of the current study suggest that it may behave similarly to *Z. cucurbitae*, which was more attracted to food baited or cue-lure baited traps placed close to the ground (Holbrook & Fujimoto 1969; Jiji *et al.* 2009).

Results from these small-scale trials give some indications regarding optimal placement of protein bait when applied to perimeter vegetation for best practice control of *B. tryoni* and *Z. cucumis* in a vegetable crop. However, the limitations of the trial should be recognised: trials took place in field cages under artificial conditions, and plants grown in pots are not usually typical of the field grown situation. For example, roots are restricted, and there are restrictions on plant densities, which may provide a different microclimate and shelter suitability than field grown plants. Similarly, the longevity and coverage of protein bait applied directly to the plants under field conditions would also be affected by leaf structure and density. Furthermore, the fruit flies were of a limited age range and similar physiological status (unmated, sugar fed and protein deprived). Raghu *et al.* (2004) found that the response of *B. cacuminata* to microclimate (temperature, humidity and light intensity) in a host plant varied according to the sex of the flies as well as the type of activity (resting or ovipositing). Similarly, Atiama-Nurbel *et al.* (2012)

found that the proportion of *Z. cucurbitae* and *D. demmerezi* roosting in the lower, middle and upper zones of non-host vegetation differed not only between plant species, as in the current trial, but also between fruit flies of different sex and maturity. Field trials are required to validate results in the field and further explore the potential of perimeter baiting for management of fruit flies in vegetable crops.

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