THE ROLE OF FOLIAGE IN DIFFERENTIAL LANDING OF THE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE) ON ODORIFEROUS AND ODOURLESS FRUIT MODELS

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Summary

Experiments testing the landing response of mature Queensland fruit flies, *Bactrocera tryoni*, on fruit models used odourless and odoriferous models with iso-amyl acetate or cuelure (4-(3-oxobutyl) phenyl acetate) as baits. Models were either surrounded or not, by artificial foliage. Adjacent foliage increased the landing rate of *B. tryoni* on odourless models by up to three fold. Foliage also played a role in allowing flies to discriminate at close range between odourless and odoriferous fruit models. Very local odour gradients (within 50 mm) can be used as cues to the direction of the source of an odour. Flies discriminated between closely placed visually identical models that differed only with respect to the presence or absence of an odour source. In the case of a fruit odour (iso-amyl acetate) only females did this and only to a slight (but significant) extent and only when aided by the presence of surrounding foliage that provided a platform for discrimination prior to a short approach flight. In the case of the male attractant, cuelure, male flies discriminated strongly between models on the basis of odour source and could do so regardless of whether the approach flight was short (from adjacent foliage) or long (when adjacent foliage was absent). Females appeared to be largely repelled by cuelure and only a few landed on the baited and unbaited models.

Keywords: *Bactrocera,* Tephritidae, discrimination, host finding, fruit models, cuelure, amyl acetate.

INTRODUCTION

Compared to certain moths, the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) has a limited ability to find an odour source. However, it is capable of a limited form of upwind anemotaxis that could bring a fly within close range of an odour target (Meats and Hartland 1999). There is evidence that the finding of an odoriferous object at close range usually involves an increased rate of movement within a tree canopy. This is not oriented towards the odour source but results in a process of trial and error with success being confirmed by contact chemical stimulation or by locally higher odour concentration (Eisemann and Rice 1985; Fletcher and Prokopy 1991; Dalby-Ball and Meats 2000 a, b). Visually guided trial and error would explain the success of odourless sticky traps that mimic the shapes and sizes (but not odour) of leaves and fruit (see review by Katsoyannos 1989). The importance of locally high concentration gradients has not been examined in the environment of a tree canopy.

Odour plumes in light winds in treed habitats usually become chaotic within a few centimetres of their source and hence would give no cues as to the direction of the source (Griffiths and Brady 1995). Fruit flies may be able to use local diffusion gradients as cues to the location of the odour. In such circumstances it is likely that they would also be close enough to see the source of the odour. Hence, during the final stage in locating an odoriferous resource they may rely solely on visual cues although odour may provoke the response to such cues (Bernays and Chapman 1994). This is not always the case, however, as Meats and Osborne (2000), for example, found that the wild tobacco fly, *Bactrocera cacuminata* (Hering) could move up local odour gradients both with and without visual cues.

Leaves and other elements of a tree canopy may obscure the view of foragers and reduce finding rate. Drummond *et al.* (1984) determined that sticky unbaited red spheres trapped several times more apple maggot flies, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), if fruit and foliage was cleared to a distance of 250 or 500 mm. However, this was not the case if clearance was to 1 m, suggesting that the benefits of clearer exposure can be offset by longer distance. Cloyd and Sadof (2000) found that parasitism of the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), was less in denser plant canopies. A counter example is provided by Weisser (1995) of a braconid parasitoid that was more successful in attacking aphid colonies if the surrounding leaves were not removed. Odour was not a cue in the first of these examples, but we do not know whether or not it had a role in the others. The use of a local odour gradient is suggested if flies discriminate between adjacent odoriferous and odourless models. This paper investigates whether *B. tryoni* can discriminate between odoriferous and

odourless fruit models and whether discrimination is assisted by the presence of nearby foliage at a distance of 50 or 400 mm.

MATERIALS AND METHODS

Study site and flies

All experiments were conducted within the main grounds of the University of Sydney in a field cage that had a hooped frame with an area of 4.0 x 3.1 m and a roof rising from 2 m at the sides to 2.2 m at the centre. Shade cloth covered the cage allowing air to circulate while reducing incident light by approximately 50% (simulating light levels in a tree canopy of moderate density). Experiments were done at times when ambient temperature exceeded 25°C and wind speed inside the cage ranged from being undetectable to about 0.5 ms^{-1}.

Flies were from a culture that was 20-25 generations old and reared at 25°C according to the method of Bateman (1967). Flies were mature and between 20 and 35 days old when used in the experiments.

Fruit models

Bright yellow-green tennis balls (65 mm diameter) were used as fruit models. Tennis balls were chosen as their spherical shape and size is similar to that of many orchard fruit and because bright green and yellow have been found to be the most attractive colours to *B. tryoni* (Hill and Hooper 1984). Each was covered in polybutane (Tangletrap®, Tanglefoot Company, Grand Rapids, Michigan, USA) which is a sticky, transparent, odourless substance to which flies adhere upon contact. Three holes (10 mm diameter) were drilled in the upper hemisphere of each fruit model and each hole was fitted with a lidded plastic vial.

Test substances and foliage

The vials were charged with 1 mL of iso-amyl acetate or 1 mL of cuelure. For odourless models, 1 mL of water was used. Iso-amyl acetate has a fruity odour and was found to increase random searching activity and egg laying by *B. tryoni* (Dalby-Ball and Meats 2000b). Cuelure acts as an attractant of mature male flies and can cause upwind anemotaxis by *B. tryoni* in a series of short flights and walks (Meats and Hartland 1999). If deprived of males, mature virgin female flies (but not mated ones) of certain species can respond positively to male lures over short distances at dusk apparently reacting as if to male pheromone (Fitt 1981). During daylight hours, cuelure probably acts as a repellent to *B. tryoni* (Hill 1986).

Strands of odourless green nylon artificial foliage, similar to ivy, were used in the experiments.

Experimental arrangements

Three experiments were conducted. Experiment 1 was designed to test the hypothesis that the densities of flies landing on the fruit models would be greater where models were surrounded by foliage than where it was absent. For this experiment, all models were odourless. Inside the field cage, eight square wire grids (400 x 400 mm with mesh size 50 mm) were arranged with a separation distance of 600 mm in a 2 x 4 array. Four models were hung from each grid so that they were 1.5 m above the ground in a 2 x 2 array with a separation distance of 50 mm. Four of the grids had eight strands of artificial foliage hanging from their edges so that about 90 leaves surrounded the models in a girdle extending approximately 200 mm above and below them. The nearest leaves came within 100 mm of the models. For models on grids without leaves, the nearest leaf came no closer than 850 mm. Foliage was arranged systematically so that a grid with foliage in one row was opposite a grid without foliage in the other row.

Eight trials were done, four on one day, four on the next, all between the times of 10.30 and 16.00. With each new trial, each grid was hung in a new position and the foliage was transferred to another grid so that after all eight trials each grid had occupied all eight positions and was surrounded by foliage in 50% of the trials.

Experiments 2 and 3 were designed to test the ability of flies to differentiate between odoriferous and odourless models and to examine if the presence of foliage increased ability to differentiate between models. The experimental arrangements for the second and third experiments were identical except for the use iso-amyl acetate in the second experiment, and cuelure in the third experiment.

The basic arrangement was the same as for experiment one, with three changes to the design. Firstly, two of models on each grid were odoriferous (at opposite corners) and two were not. Secondly, four of the eight grids had models separated by 400 mm while the remaining grids had models separated by 50 mm. The last difference was that half the trials were conducted with foliage around all the grids and remaining trials were done without foliage, that is, there was no choice available between 'with foliage' and 'without foliage' arrangements.

When foliage was used in a trial, it was hung so the

distance between a model and the nearest leaves was 100 mm. This was true regardless of the distance between models.   When no foliage was used the minimum flight path between the roof or sides of the field cage and the models was 600 mm.

Trials with iso-amyl acetate were done first, eight with foliage and eight without. Trials took place over four days with four trials per day (foliage trials alternating with non-foliage trials). A similar series was then done with cuelure as the volatile substance.

Experimental procedure
In each trial approximately 2250 mature flies, (M/F = 50:50), were released into the field cage. The grids with the models ($\pm$ foliage) were then hung in the cage. The cage door was shut and the experimental period of one hour started.

Models were exposed to the flies for 1 h per trial so that the number of flies caught on any one model did not exceed 15. A pilot studied revealed the number of subsequent flies landing on a sticky fruit model was influenced by the number of flies previously stuck and that the threshold number was around 15 previous flies. The pilot study showed no fruit model had over 12 flies landing on it within 1 h hence this was the observation time chosen.

In experiments 2 and 3 vials containing either iso-amyl acetate or cuelure were opened before each trial. At the end of an hour, the models and the remaining untrapped flies were removed from the field cage. Tweezers were used to remove caught flies from the fruit models.   Thirty minutes after the end of one trial another was started.

Analysis
For experiment 1, data were analysed by a four factor nested ANOVA. Factors were Days (2), Trials (8), Foliage ($\pm$) and Sex (M/F). Days and Trials were considered random factors, the others fixed.

Experiments 2 and 3 were each analysed with a five factor Analysis of Variance (ANOVA) testing for the effect of: Trials (8), Foliage ($\pm$), Bait ($\pm$), Distance between baited and unbaited models (50 or 400 mm) and Sex (M/F). Trial was considered a random factor the others were fixed. Where Days or Experiments are included as factors in the analysis they were considered as random factors. Before analysis, a Cochran's test was performed to determine whether variances were homogeneous.   Non-homogeneous data were transformed to natural logarithms to homogenise the variances.

So that replicates were independently sampled, only the number of flies that landed on either baited or unbaited models from each group of four were used in the analyses. As there were two unbaited models in each group the number of female flies that landed on an unbaited model was taken from one and the number of males from the other unbaited model; the same was done for counts of flies on baited models. Thus for each combination of Bait ($\pm$), Distance (5 or 400 mm) and Sex (M/F) the number of flies (M or F) on two replicate fruit models per trial were used in the analysis. Therefore counts of flies from n = 16 replicate fruit models were analysed for each treatment combination because eight trials were performed with foliage and eight without foliage for each of the two test substances.
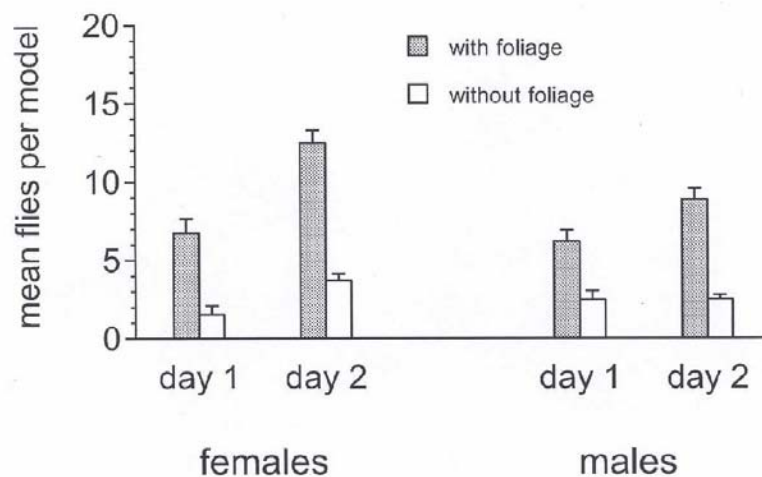

Figure 1. Number (mean $\pm$ SE) of female and male flies landing on fruit models either surrounded or not surrounded by foliage. Models with and without foliage were presented simultaneously (flies having 'choice').

RESULTS

Effect of foliage

Models with adjacent foliage were presented simultaneously with ones without foliage (experiment 1). The densities of female and male flies landing on fruit models surrounded by foliage were significantly greater than that on fruit models without foliage (Figure 1). This result was consistently significant in all eight trials over the two days, however, an interaction between Day x Foliage occurred because the effect of foliage was slightly more pronounced on day 2 than day 1 (F=9.79; df, 1,1; P<0.05).

For experiments two and three, (pertaining to iso-amyl acetate and cuelure respectively) separate replicate trials were run alternately either with or without adjacent foliage. The flies, therefore, had no choice between foliage ane non-foliage situations and trials were run until similar total numbers were captured. Thus we cannot discern the effect of foliage on overall landing rate but we can tell whether or not flies can discern between models depending on odour and whether any discernment depends upon the presence or absence of adjacent foliage.

Interaction of iso-amyl acetate and foliage

When foliage was present around fruit models, female flies landed in significantly greater densities on models baited with iso-amyl acetate than on unbaited models. Discrimination occurred even when models were as close as 50mm (Figure 2). In the absence of foliage flies did not discriminate between odoriferous and odourless models (Foliage x Bait: F=14.15; df, 1,1; P<0.01).

The distance separating baited and unbaited models did not affect the ability of female flies to discriminate. Unlike female flies, male flies landed in similar densities on fruit models baited with iso-amyl acetate and models without bait. This occurred irrespective of the presence/absence of foliage (Sex x Bait, F=6.37; df, 1,1; P<0.05).

Interaction of cuelure and foliage

Very few female flies landed on any of the fruit models in any trial with cuelure and no significant difference between number of female flies landing on models with and without cuelure was detected (Figure 3). In contrast, male flies landed on baited models in significantly greater densities than on unbaited models (interaction Sex x Bait, F=6.37; df, 1,1; P<0.05.
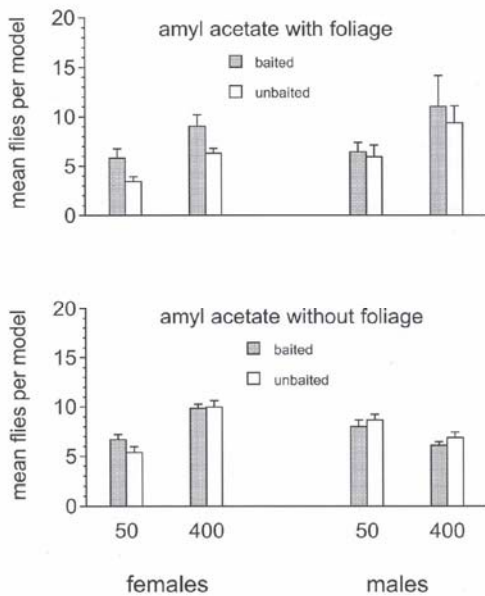

Figure 2. Number (mean ± SE) of female and male flies landing on fruit models when flies had a choice between models that were baited with iso-amyl acetate or not. Baited models were separated from unbaited ones by either 50 mm or 400 mm. Separate replicate trials with adjacent foliage (upper graph) or without (lower graph) were run alternately (therefore flies had no choice between foliage ane non-foliage situations and trials were run until similar total numbers were captured).
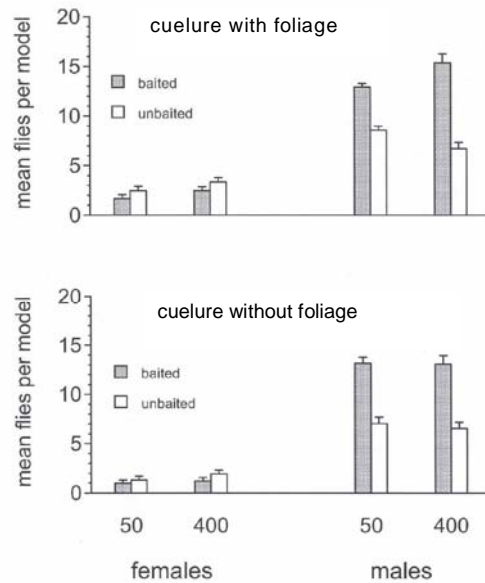

Figure 3. As for Figure 2 but baited models had cuelure.

This occurred in all experiments where foliage was present and in six of the eight experiments where foliage was absent. Males discriminated between baited and unbaited fruit models when the models were 50 mm and 400 mm apart. Despite the fact that unbaited models caught fewer male flies than baited ones, they still caught significantly more males than females (P<0.01).

DISCUSSION

Effect of foliage

Fruit models surrounded by foliage had three times the density of flies landing on them than on models without foliage. Foliage may provide a greater visual target to which flies can orientate from a relatively far distance. Large visual cues such as clusters of foliage may be used to locate resources, as it is unlikely that flies can see individual fruit from distances further than one metre (Wehner 1981; Drummond *et al.* 1984; Harris *et. al.* 1993). Once on the foliage, flies may be attracted to a fruit model by either visual or chemical cues. Alternatively, the foliage may bring the flies close to the fruit model and then random flights may increase the probability of landing on it.

Interaction of iso-amyl acetate and foliage

Female flies landed in greater densities on fruit models baited with iso-amyl acetate than on unbaited fruit models but only when the fruit models were surrounded by foliage. This suggests that foliage does play a role in the ability of flies to discriminate between odoriferous and odourless models.

Foliage may provide a platform close to the fruit on which flies can be stationary, perhaps enabling them to assess more accurately the direction and concentration gradient of a volatile as it is emitted from the resource. Meats and Hartland (1999) showed that odour-mediated upwind anemotaxis of *B. tryoni* can occur in light winds by means of a series of short flights or walks. This is consistent with the 'mechanoreceptive' or 'aim and shoot' method (Bursell 1987) whereby wind direction (and by our inference, odour direction) is assessed only when the fly is stationary. It is also possible that some species can simply move up an odour gradient without the extra cue of wind (see Meats and Osborne 2000), but this would be more accurate if it were possible to pause along the way. If either of these methods applied to our flies, discrimination of directional information from a volatile substance would have been greatest when a fly was stationary close to the source. Male flies landed in similar densities on models baited with iso-amyl acetate and unbaited models. This occurred whether foliage was present or absent. It may be that iso-amyl acetate is of no significance to male *B. tryoni* although it is known that it increases activity in females (Dalby-Ball and Meats 2000a).

Interaction of cuelure and foliage

Male flies were able to distinguish between baited and unbaited fruit models when foliage is present and on six out of eight occasions when foliage was absent. This suggests that cuelure can be located either at a distance before taking flight from the sides of the field cage or that it is located whilst on an approaching flight without the aid of a close 'inspection platform' provided by nearby leaves. Meats and Hartland (1999) found no evidence that flies changed direction when flying into a plume of cuelure odour but the odour immediately around the models may have been at a higher concentration and may have caused a landing response or could have made landing more likely.

The very low densities of female flies landing on both baited and unbaited models in the experiments with cuelure, suggest that this substance may act as a repellent to mated females even at a distance of 400 mm. This finding is consistent with that of Hill (1986) who showed that male lures (including cuelure) deter the capture of mated conspecific females on sticky or in protein baited traps.

Application to monitoring and control of fruit flies

The finding that nearby foliage significantly increases the number of *B. tryoni* caught on sticky models counters the results for *R. pomonella* by Drummond *et al*. (1984) who recommended for this species that fruit and foliage within 250 mm of traps should be removed. Thus no general rule can be applied and each tephritid species must be assessed independently. The augmentative effect of foliage in the case of *B. tryoni* is probably also true of odoriferous traps although it is not possible to verify this with the present data as odoriferous models with and without foliage were not tested simultaneously. In the field, flies will have a choice between areas of different foliage density and the latter may influence trapping rates or discovery rates of fruit.

The deterrent effect of cuelure on mated females would require the traps that target females be located greater than 400 mm from male targeted traps.

ACKNOWLEDGMENTS

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