Fruit flies or vinegar flies? Bait preferences of commonly occurring New Zealand drosophilids

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"a spoonful of honey will catch more flies than a gallon of vinegar"
Benjamin Franklin

Introduction

Drosophilid flies oviposit and breed in a wide range of fermenting substances, leading to a number of common names being applied to this group. Unwin (1907) described *Drosophila* (*funebris*) as 'vinegar flies', whereas other early 20th Century authors used 'pomace fly', pomace being the solid remains (seeds, skin, seeds) of fruit after pressing (e.g. Barrows 1907; see Green 2002). The title of 'fruit fly' grew to prominence in the mid-1900s as *D. melanogaster* became the organism of choice for genetic research. Many drosophilids, however, occur in habitats other than fermenting fruit, such as leaf-mines, rotting cacti, decomposing vegetation and even live crabs, so the moniker of 'fruit fly' is not always appropriate (Carson & Wheeler 1968; Law et al. 1997; Green 2002; Pfeiler & Markow 2011).

Clearly, 'fruit fly' could be interpreted to imply that drosophilids cause damage to fruit (crops) and has led to confusion with species that are actually pests of fruit, such as the Mediterranean fruit fly (*Ceratitis capitate*) and the Queensland fruit fly (*Bactrocera tryoni*), both in the family Tephritidae. The situation was further complicated by the rise of *Drosophila suzukii*; an actual pest of fruit crops and therefore a legitimate 'fruit fly'. *Drosophila suzukii* was for a short time given the rather unhelpful moniker of 'cherry vinegar fly', although things appear to have settled on 'spotted wing Drosophila'.

The attraction of drosophilids to fermenting substances and volatiles has been studied for over a century (e.g. Barrows 1907), with rotting fruit, fungi, fermenting cactus, malt preparations, vinegar, and numerous alcoholic beverages employed in the collection of wild *Drosophila* (Lakovaara et al. 1969; Chambers 1988; Hodge 1996; Hodge et al. 2010; Basoalto et al. 2013). In an applied setting, several baits (e.g., apple cider vinegar, red wine, yeast) and individual compounds associated with fermentation (e.g. ethanol, methanol, acetic acid, ethyl acetate) have recently been tested for their ability to lure and trap *Drosophila suzukii* (Basoalto et al. 2013; Iglesias et al. 2014; Kleiber et al. 2014).

Hodge et al. (2017) recorded seven species of adult drosophilids near Christchurch using 20 different types of bait. Comparisons of the effectiveness and specificity of the different baits was problematic, however, as the baits were not replicated equally and were not all used in the same sampling locations and on the same dates. Previous work demonstrated that even examining the same traps on different days might result in different *Drosophila* species being collected (Hodge & Arthur, 1996).

The current study aimed to rectify this weakness in the prior investigation and systematically compare drosophilids captured with different baits. As vinegar and wine baits are often proposed for monitoring *D. suzukii*, the catches on oft-used vegetable baits (e.g. banana, orange and mushroom) have been compared with those on three types of vinegar (white, malt and cider) and two types of wine (merlot and pinot noir). Previous studies have examined the influence of trap height on the abundance and diversity of drosophilid captures (e.g. Shorrocks 1975; Tanabe 2002; Roque et al. 2013). Therefore, in one collecting episode, flies were collected from baited traps set at intervals from 0 m to 2 m above the ground.

Methods

Sampling

Plastic containers (60 mm high; 40 mm diameter) with yellow plastic lids were used as trapping vessels. Nine holes (4 mm diameter) were drilled into the plastic lids to allow entry of flies. A layer of tissue paper was placed in the bottom of each pot, to which the baits were added.

Flies were collected each day for three consecutive days after the traps were initially placed out. Identifications of specimens were made by reference to Harrison (1959), Baechli et al. (2004), and Bock (1976, 1982).

Season #1

Initial sampling was performed in a garden in the coastal suburb of New Brighton 9 km to the east of Christchurch city centre (43.519˚S, 172.729˚E). Five different bait treatments were used: ~20g banana slice, ~20g orange piece, 5 ml malt vinegar, 5 ml white vinegar, 5 ml apple cider vinegar. Pots with 5ml of water added to the tissue were used as a control treatment to assess the general attractiveness of the yellow-lidded pots to these flies. During the collecting phase the pots were placed on their sides on the ground, approximately 20 cm apart, under shrubs and among plants bordering a central lawn.

Collections were made once each month between January 2014 and December 2014. On each sampling event there were four replicates of each bait treatment, arranged in four spatial ‘blocks’ around the garden. One replicate of each treatment was placed in each block.

Season #2

During May-June 2017 sampling took place at the BHU/ Future Farming Centre (FFC) organic farm at Lincoln, approximately 18 km south east of Christchurch (43.648˚S, 172.457˚E). Collecting pots were placed on the ground under shrubs and trees on field boundaries. Collecting was performed on three occasions, using six baits: ~20g banana piece, 20g mushroom piece, 5 ml white vinegar, 5 ml apple cider vinegar, 5 ml pinot noir, 5 ml merlot (Table 3). Wines were New World supermarket ‘cleanskin’ versions; fresh supplies were necessary for each sampling episode. Five replicates of each bait were used on each occasion.

Season #3

During April 2018 sampling took place on three occasions at the BHU/FFC. Five bait treatments were used on each occasion: ~20g banana piece, 20g mushroom piece, 5 ml apple cider vinegar, 5 ml pinot noir (as described above) and 'blank' pots containing only moistened tissue.

For this trial, the plastic collecting containers were glued onto five 2.1 m wooden stakes, so that when the end of the stake was sunk into the soil, the traps were at heights of 0, 0.5, 1.0, 1.5 and 2 m. On each sampling occasion, no bait treatment was duplicated on any one stake, so there was one trap of each bait treatment at each height, arranged in a Latin-square design.

For those species collected with a total of 10 or more individuals, the effects of bait type and trap height were assessed for their effects on fly catches using a generalized linear model, treating the count data as Poisson distributed, with a log-link function and allowing the dispersion parameter to be estimated during the modelling process (Genstat v17, VSNI, UK).

Results

Season #1

A total of 155 drosophilids were collected, belonging to four species: *D. immigrans* Sturtevant 1921, *D. pseudoobscura* Frolova 1929, *D. simulans* Sturtevant 1919, and *Scaptodrosophila enigma* (Malloch 1927) (Table 1). All of these species have previously been recorded from New Brighton (Hodge et al. 2017).

No flies were collected in the water-baited control pots, so data from these traps were not considered further. However, the remaining data were still highly zero-inflated, with no flies being collected in 179 (75%) of the 240 samples (5 baits × 12 months × 4 replicates). Therefore, simple chi-square tests were used to examine whether the distribution of total fly counts among the baits was significantly different to that expected by chance.

All four species of fly exhibited statistically significant associations with bait type ($\chi^2 > 30$, 4 df, $P < 0.001$). Seventy eight percent of the individuals of the most abundant species, *D. immigrans*, were caught using banana bait, which also captured 68% of *D. pseudoobscura* and 52% of *D. simulans*. Of the species captured, the orange bait appeared to be most attractive to *D. simulans*, capturing 45% of the individuals of this species (Table 1).

Collectively, the vinegar baits recorded all four species of fly, although only *S. enigma* exhibited a strong preference for vinegar, with 37 of 38 (97%) specimens obtained on the vinegar-baited traps. The catches of *S. enigma* were lower on white vinegar than the other vinegar baits (Table 1).

Table 1. Drosophilid flies captured at New Brighton in 2014 using ground-lying traps with different baits: (BA-banana; OR-orange; MV-malt vinegar; WV-white vinegar; ACV-apple cider vinegar). Values are totals collected in 48 traps of each bait.

	BA	**OR**	**MV**	**WV**	**ACV**	**Total**
D. immigrans	42	9	2	-	1	54
D. pseudoobscura	13	4	-	1	1	19
D. simulans	23	20	1	-	-	44
S. enigma	1	-	20	3	14	38
Total	75	33	23	4	16	155

Regarding temporal patterns in abundance, *D. pseudoobscura* was collected over most of the year, albeit in low numbers (Table 2). In contrast to this result, Lambert & McLea (1983) used banana baits to sample *D. pseudoobscura* in North Island and did not collect any specimens during the winter months. *Drosophila immigrans* showed higher abundance in the summer months (November-February) than winter, and *D. simulans* was also more common in summer and autumn (January-April). *Scaptodrosophila enigma* was recorded from mid-winter to early summer and thus showed a very different seasonal pattern in abundance compared with the other species (Table 2).

Season # 2

At the BHU site in 2017, 131 drosophilids were collected belonging to three species, all of which were primarily caught using banana baits: *D. simulans* (86 specimens; 86% on banana); *D. immigrans* (28; 86% on banana); *D. pseudoobscura* (17; 82% on banana; Table 3). No flies were captured on the mushroom baits, a few specimens of all species were captured on the wine baits and six *D. simulans* were captured on the vinegar baits (Table 3). All three species were collected on all three sampling occasions, confirming that these species are present during the colder months of the year.

Table 2. Numbers of drosophilids collected at ground level at New Brighton, NZ, over the course of 2014 using a suite of baits. Values are totals collected on 20 traps for each month.

	D. immigrans	***D. pseudoobscura***	***D. simulans***	***S. enigma***
Jan	2	3	5	0
Feb	3	1	12	0
Mar	0	1	9	0
Apr	1	1	11	0
May	0	0	3	0
Jun	1	0	1	0
Jul	0	3	0	2
Aug	2	1	2	22
Sep	0	1	0	3
Oct	0	1	1	8
Nov	36	5	0	3
Dec	9	2	0	0
	54	**19**	**44**	**38**

Season # 3

At the BHU site in 2018, 696 drosophilids were collected belonging to five species. No drosophilids were captured in the unbaited ‘blank’ traps. As in 2017, banana bait caught the majority (86%) of specimens. *Drosophila simulans* (71% of specimens) and *D. immigrans* (22% of specimens) were the most common species (Table 4). Thirty-six specimens of *D. busckii* were recorded, 86% of which were collected on mushroom baits. Five specimens of *S. enigma* were collected, on three different baits; the first time I have recorded this species from this location.

All three species that were recorded with 10 or more specimens showed a significant difference among bait types: *D. simulans* and *D. immigrans* preferring banana bait and *D. busckii* showing a significant association with the mushroom bait (GLM, $P < 0.001$ for all species).

Drosophila simulans ($P = 0.178$) and *D. immigrans* ($P = 0.289$) did not show statistically significant differences in the number of specimens recorded at the different trap heights (Figure 1). However, no *D. busckii* were collected in the traps at ground level, and so *D. busckii* catches tended to be greater the higher the traps were off the ground ($P = 0.008$).

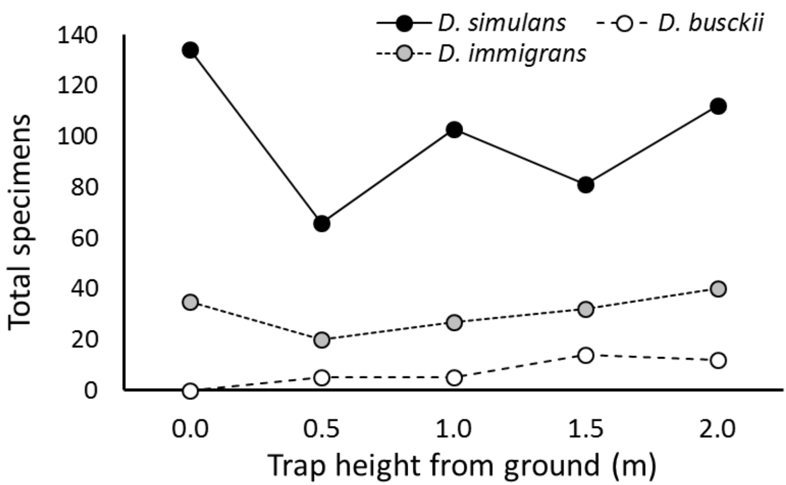
Table 3. Captures of *Drosophila* at the BHU/FFC in 2017 using six different baits: Banana (Ba), Mushroom (Mu), white vinegar (WV), apple cider vinegar (ACV), pinot noir (PN) and merlot (ME). Values are totals collected in five traps of each bait on each date.

Dates	**Species**	**Ba**	**Mu**	**WV**	**ACV**	**PN**	**ME**
13 Apr-16 Apr	*D. immigrans*	4	-	-	-	-	-
	D. pseudoobscura	3	-	-	-	-	-
	D. simulans	34	-	-	1	-	-
1 May-5 May	*D. immigrans*	3	-	-	-	2	-
	D. pseudoobscura	7	-	-	-	-	-
	D. simulans	24	-	-	-	-	1
2 Jun-8 Jun	*D. immigrans*	17	-	-	1	1	-
	D. pseudoobscura	4	-	-	-	-	3
	D. simulans	16	-	1	4	2	3
	Total	112	0	1	6	5	7

Table 4. Captures of *Drosophila* in April 2018 at the BHU/FFC, Lincoln, using four different baits: Banana (Ba), Mushroom (Mu), apple cider vinegar (ACV), and pinot noir (PN). Values given are the totals obtained in 15 traps of each bait (see Methods for details)

	Ba	Mu	ACV	PN	Total
D. busckii	2	31	1	2	36
D. immigrans	136	5	2	11	154
D. pseudoobscura	4	1	0	0	5
D. simulans	453	22	8	13	496
S. enigma	2	0	1	2	5
Total	597	59	12	28	696

Figure 1. Captures of *Drosophila* in April 2018 at the BHU/Future Farming Centre, in traps set at five heights above the ground Values given are the totals obtained in 12 traps at each heights (3 reps of 4 baits) (see Methods for details; Table 4)



Discussion

In addition to the previous study at these locations (Hodge et al. 2017), other studies in New Zealand have also reported drosophilid bait preferences. For example, Chambers (1988) found that *D. melanogaster/D. simulans* were over 10 times more abundant in traps baited with beer than in traps baited with fruit, but two other cosmopolitan species, *D. busckii* or *D. hydei*, were not collected on the beer bait at all.

Drosophila funebris and *D. hydei* were recorded at these locations by Hodge et al. (2017) but not found during these surveys. *Drosophila busckii* is commonly found in mushroom-baited traps and has been collected from fungi in other New Zealand surveys (Hodge et al. 2010, 2017; Osawa et al. 2011). Although no *D. busckii* were collected during the 2017 BHU survey, this species showed a strong preference for the mushroom bait in the 2018 BHU collections. That no drosophilids at all were found in the mushroom-baited traps during the 2017 BHU survey was unexpected, although the traps did contain several other dipteran families, such as Sphaeroceridae, Phoridae and Muscidae (Hodge et al. 2000).

Drosophila busckii was the only species identified as showing a significant relationship between the number of records and trap height. Because this species is associated with fungi it was predicted to be more common near the ground where naturally-occurring fungi were more abundant. The relationship with trap height for this species requires further study to examine its repeatability. Also the use of naturally-occurring fungi on the ground and at different heights on tree trunks by *D. busckii* may shed further light on this phenomenon.

Compared with other studies (e.g. 8 m – Roque et al. 2013; 10 m - Shorrocks 1975; 16.5 m - Tanabe 2002), the maximum trap height used in this survey was (deliberately) low (2 m), so that traps could be emptied without the need of ladders, climbing equipment, ‘cherry-pickers’ etc. To gain a fuller understanding of how the assemblage of drosophilids, and other Diptera, and their associated predators and parasitoids, is vertically stratified within forests or urban sites then clearly more work is required using a greater range of collecting heights.

Scaptodrosophila enigma is an Australian species, described as ‘urban’ by Bock (1982), and first highlighted as being present in New Zealand by Parsons (1980). The species spread through Australia quite rapidly, probably because it can tolerate cold temperatures (-1^{o}C) and is attracted to a wide range of fermenting fruit and mushrooms (Parsons 1980). Bock (1976) also indicated that the species could be collected on a number of fruit baits and be reared in the laboratory using a standard corn meal diet, and so *S. enigma* is likely to find plentiful breeding resources in New Zealand. The specimens Parsons (1980) discussed were collected from rainforests near Colville on the Coromandel, and the species has increased its distribution fairly rapidly, as least as far south as Canterbury in the intervening years. Although *S. enigma* can be collected using baited traps, other *Scaptodrosophila* tend not to occur at baits and can be collected only by sweeping vegetation (Bock 1982).

From a methodological aspect, the results of this study illustrate once again that with entomological field sampling, what you see often depends to a large extent on how you look. Because different drosophilid species respond differently to different baits, the structure of the drosophilid assemblage *perceived* to occur at a particular location at a particular time will be highly dependent upon which bait or baits are used. Overall, banana bait produced the most specimens and the most species in all surveys, and was the preferred bait of *D. immigrans*, *D. pseudoobscura* and *D. simulans*. However, *S. enigma* was found predominantly on vinegar baits at the New Brighton site, and *D. busckii* predominantly occurred on mushroom baits during the 2018 survey. Thus, the patterns of bait preference observed in these surveys suggest that *D. immigrans, D. pseudoobscura* and *D. simulans* could be considered as ‘fruit flies’ or exhibiting ‘generalist’ resource use, whereas *D. busckii* is primarily a fungus fly and *S. enigma* might just be considered a ‘vinegar fly’!

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