

Relative flight responses of *Rhagoletis indifferens* as influenced by crowding, sex, and resources

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Abstract

Although flight is believed to be the primary mechanism for dispersal in the Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), an orchard pest of both sweet (*Prunus avium* L.) and sour (*Prunus cerasus* L.) (Rosaceae) cherry crops, the movement of these flies between host patches is difficult to quantify in the field. A tethered flight mill system was used in the laboratory to examine the flight behaviour of sexually mature flies exposed to different levels of conspecific contact and resource availability. A complete $2 \times 2 \times 3$ factorial design compared the relative influence of the factors 'context' (crowded, isolated), 'sex' (female, male), and 'resources' (low = food only; medium = food + leaf; high = food + leaf + cherries) on flight performance measures including distance flown, net trial time, and stopping patterns. Rather than using a minimum time or distance to determine trial length, flight observations were continued for each fly until a behavioural protocol based on stopping time was met. In this protocol each successful trial was composed of three consecutive flight intervals and included a minimum of three stops lasting a combined total of 5 min. Of the 160 flies tested, 86.9% flew <500 m on the flight mill. Individuals from both sexes were capable of maximum flights in the same order of magnitude, ca. 3 km on the flight mill. Distance flown was significantly influenced by 'context' such that crowded individuals flew >1.5-fold farther than isolated individuals. Sex influenced the frequency and duration of stops made, with females stopping more often and longer than males. Although females and males in high resource treatments had the shortest net trial times, the factor 'resources' did not produce any highly significant main effects, but did generate significant interaction terms with the factors 'context' and 'sex', suggesting that past experience with 'resources' modifies individual flight behaviour. We have shown for the first time using a tethered flight mill system that *R. indifferens* flight behaviour is context dependent and sensitive to adult crowding. The implications of this study for improved field experiments on dispersal are discussed.

Introduction

The movement of individuals into new habitats represents a critical process in population dynamics. However, long-distance dispersal that contributes individuals to colonization and gene-flow events is difficult to record and parameterize in nature (Sutherland et al., 2000; Higgins et al., 2003; Muller-Landau et al., 2003). Gaps in our knowledge on the basic biology, perceptual range, and dispersal ability of species also hamper field experimentation

and modeling efforts (Lima & Zollner, 1996). It has been argued that the renewed interest in dispersal and movement can be attributed to the lack of such information for many species causing difficulty in the advancement of population dynamics and spatial ecology (Cain et al., 2003). This is particularly true for pest species where an improved knowledge of life history and dispersal could enhance management strategies.

Tephritid flies present a particular challenge for pest managers because fly movement between host patches can be difficult to quantify in the field (Fletcher, 1989). Studies on various *Rhagoletis* species have demonstrated that fruit ripeness (Smith, 1984; Mayer et al., 2000; Kuenen & Silk, 2001), infestation levels (Roitberg et al., 1984, 1990; Papaj

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et al., 1989), and habitat/host structure (Roitberg & Prokopy, 1982; Messina, 1990; Green et al., 1994; Rull & Prokopy, 2001) influence their movements between host trees within patches. However, the evidence for *Rhagoletis* spp. movements between patches is quite scarce, despite the persistence of these pests in managed crop systems. Field studies have suggested that most individuals of *Rhagoletis cerasi* (Boller et al., 1980; Kneifl et al., 1997), *Rhagoletis pomonella* (Phipps & Dirks, 1933; Maxwell & Parsons, 1968; Neilson, 1971; Prokopy et al., 2003), *Rhagoletis indifferens* (Jones & Wallace, 1955), and the non-pest species *Rhagoletis basiola* (Roitberg & Mangel, 1997) move <1 km when released near suitable hosts, although field movement of ca. 3 km was recorded by Boller et al. (1980) for *R. cerasi* males. Field experiments on interpatch movement are typically limited to the release of males because females may damage a commercial cherry crop (Boller et al., 1980; Prokopy et al., 2003). However, understanding the movement of both sexes is necessary for improved modelling and pest management, particularly for determining whether both sexes engage in long-distance dispersal or if only gravid females disperse (Hughes & Dorn, 2002).

Rhagoletis indifferens Curran (Diptera: Tephritidae) is a tephritid species that primarily exploits sweet (*Prunus avium* L.) and sour (*Prunus cerasus* L.) (Rosaceae) cherries that are grown commercially and in backyards as tree fruits in the Pacific Northwest. The phenology of this pest is well documented (Frick et al., 1954; Brown & AliNiazee, 1977; AliNiazee, 1978; Jones et al., 1991). Larvae from infested cherries drop to the soil beneath the tree, overwinter as pupae, and emerge as adults the following spring and summer to exploit the new cherry crop. With the relative stability of this system, i.e., perennial hosts and pests that overwinter beneath host trees, there appears to be little impetus for adults to move long distances. Fly density within host trees varies seasonally (Yee, 2002), but whether flies respond to high fly density situations by leaving an orchard or backyard is unknown. Messina (1989) examined the distribution of larvae in sweet cherries, created by the oviposition decisions of foraging females, and did not find a positive relationship between larval abundance and fruit density at any spatial scale or level of fly infestation. Thus, factors such as adult fly density and host resource availability that may contribute to an individual fly choosing to leave a host patch or orchard are poorly understood and difficult to quantify in the field. Knowledge of the flight responses of *R. indifferens* would greatly improve our understanding of its ecology and management.

There are many possible ways to investigate flight in insects and tethered flight mill systems have been used successfully to improve our understanding of flight physiology,

energy requirements, flight mechanics, flight endurance, and flight behaviour (examples include Roitberg et al., 1984; Roitberg, 1988; Sappington & Showers, 1993; Yee & Anderson, 1995; Blackmer et al., 2004; Sun, 2005). Flight mills are often used to measure maximum flight capability such that insects are either flown to exhaustion or flown over an extended period of hours or days to derive this maximum response (Roitberg, 1988; Yee & Anderson, 1995; Schumacher et al., 1997). However, flight mills can also be used to measure the effects of past experience on movement proclivity (Roitberg et al., 1984). In these experiments, insects are exposed to different conditions and then tested for their flight response using the flight mill. No difference between treatments suggests that no behavioural response, or plasticity of flight propensity, is occurring. Thus, differences in flight response under these circumstances provide a relative measure of behavioural response to the treatment (experience) provided. In this article we describe the relative flight responses of both male and female *R. indifferens* to a complete factorial comparison of the factors 'context' (crowded, isolated), 'sex' (female, male), and 'resources' (low, medium, high), as measured with a tethered flight mill system. Our main objective was to examine the contribution of these factors to the flight behaviour of sexually mature flies and to consider the implications for the spread of this pest.

Assuming that prior experience will influence flight behaviour as measured on a tethered flight mill, we predicted three possible main effects and three factor interactions: main effects: (i) flies in isolation should have longer flights than crowded individuals because isolated flies have no opportunity to reproduce in their current situation and so should disperse in search of new habitat (i.e., make long flights on the mill); (ii) treatments with 'high resource' levels should generate the shortest flights because flies experience additional cues locally that are important to reproduction when both fruits and leaves are available; (iii) although females are generally larger than males, we do not expect a 'sex' effect for distance flown on the flight mill given that wing length and wing aspect ratios for a similar species, *R. pomonella*, do not show significant differences between the sexes (Sivinski & Dodson, 1992) suggesting similar flight capabilities between the sexes; and interactions: (i) females should respond differently to resources than males because fruits provide different fitness acquisition opportunities (i.e., oviposition in females vs. mating opportunities in males) that should result in significant 'resource'*'sex' interactions. We expect that females should fly farther in the 'low' and 'medium resource' treatments than in the 'high resource' treatments because females require fruits for oviposition. Males should fly farther in the 'low resource' treatments only because males gain

access to mates on both leaves and cherries (Yee, 2002) making the 'medium' and 'high resource' treatments functionally equivalent for males; (ii) both females and males should respond in a similar manner to isolation or to extreme crowding, thus we do not expect 'context'*'sex' interactions; and (iii) flies should respond similarly to resource availability regardless of crowding, therefore we also expect no 'resource'*'context' interactions.

Materials and methods

Source insects

Pupae were obtained by collecting infested sweet cherries (*P. avium*) near Lillooet, British Columbia, Canada, in 2004 and by allowing the mature larvae to drop from the fruits spread on plastic mesh suspended over sand trays. The pupae were gathered into glass jars containing vermiculite and stored at 5 ± 1 °C. In May 2005, the pupae were placed into Petri dishes to emerge at 24 ± 1 °C. Emergence took ca. 30 days.

Factorial design

We employed a complete $2 \times 2 \times 3$ factorial design using the factors 'context', 'sex', and 'resources'. Context, or the social interactions an individual fly was exposed to, was tested at two extremes: (i) crowded – an individual test fly was housed with two females and two males such that the test fly experienced near constant visual and/or physical contact with other individuals, and all individuals were assumed to be mated; or (ii) isolated – an individual, unmated test fly was housed alone for the duration of the experiment. Sex was explicitly considered by testing both females and males at each level of 'context' and 'resources'. The factor 'resources' was tested at three levels: (i) low – flies were only provided with food and water in their containers; (ii) medium – flies were provided with food and water and received a *P. avium* leaf on the fourth day of the experiment; or (iii) high – flies were provided with food and water and received both a *P. avium* leaf and two fruits on the fourth day. This design results in 12 treatments and allows for the interaction between factors to be explicitly considered. All flies were held in their treatments for 7 days, at which point the test fly was removed and individually tested on a tethered flight mill system.

Insect handling prior to testing

The day of emergence was considered as day 0 of an experimental trial. Flies were removed from Petri dishes as they emerged and held in individual 14 ml polypropylene tubes (Falcon®, Becton Dickinson Labware, Franklin Lakes, NJ, USA) for handling. Once enclosed, the flies were

sexed and randomized into treatments. Flies were tapped from the holding tubes into treatment containers, 850 ml plastic food-storage container (Plastipak Industries Inc., La Prairie, Quebec, Canada) closed with a 20×22 cm piece of floating row cover fabric (Stokes, Buffalo, NY, USA) held in place by an elastic band. Each container was provided with a food strip (yeast extract and sucrose on wetted filter paper) and a water wick made from a wetted cotton dental roll. Neither food nor water was limiting in any treatment during this experiment. Containers were wrapped with a paper towel to create a cone-shaped cover that blocked all but a small triangular window on the container. The towel prevented visual contact between adjacent containers, but permitted fly observation. Containers were labelled and held on shelves at L14:D10 and ca. 24 ± 1 °C. Daily handling of containers was limited to misting with water to prevent desiccation, except on day 4.

On day 4 additional resources were provided to the 'medium' and 'high resource' treatments. The 'low resource' treatments did not receive additional resources on day 4, but food was not tested as a limiting factor. 'Medium resource' treatments were provided with a fresh *P. avium* leaf in a centrifuge tube filled with water and sealed with hot glue. Leaves were of approximately the same size, taken from the same sweet cherry tree, and maintained adequate quality for 3 days. 'High resource' treatments received a leaf as described, but also two sweet cherry fruits suspended by their stems from the rim of the container using a metal/paper 'twist tie' as a hook. Every effort was made to ensure the cherries were uninfested at the time they were introduced into the treatment containers. Some variation in fruit ripeness, from yellow/green to red, occurred over the course of the experiment, but there was no apparent trend in our data to suggest this variation influenced our results.

On day 7, the test fly was removed from each container and placed into a handling tube in preparation for tethering. For the 'isolated' treatments, only one fly was held in each container and it constituted the test fly. For the 'crowded' treatments, three flies of the same sex resided in each container and represented the pool from which the test fly would be drawn. All three flies were removed from the container into separate tubes and a test fly was chosen using a random number table to reduce any bias created by unequal catchability from the container.

Equipment specifications

The tethered flight mill system was similar to the type described in Roitberg et al. (1984) with the following differences. The balance arm, a 32-cm balsa wood stick notched in the centre to accommodate a 4×0.15 cm double-pointed nail, was held in place by adjustable rare

earth magnets so as to minimize friction. An adhesive label with a pin hole in the center placed over the lower magnet surface ensured the nail remained centered during mill operation and did not ‘walk’ out of alignment. A small horizontal notch in the attachment end of the wooden balance arm was made to facilitate the alignment of the fly tether. A metallic flag on the opposite side of the balance arm triggered the computerized infra-red counting mechanism on the flight mill.

Two flight mills were housed in an enclosure that blocked external light sources, minimizing interference with the photo receptors and preventing external stimulation of the fly. Light was provided by two shielded fluorescent tubes (Supersaver Cool White 34 W, Sylvania, Mississauga, Ontario, Canada) positioned 60 cm above the benchtop. The enclosure used black and white materials to prevent color stimulation. A white paper mat with thick black lines drawn 12 cm apart was placed beneath the flight mills to enable flies to detect movement across a surface. The flight mill and computer equipment were checked daily for optimum performance prior to testing flies.

Tethering and flight mill testing

Flies were tethered as follows: test flies were immobilized by chilling the holding tube in an ice water bath. Each fly was transferred onto the surface of a covered ice pack, and the end of a 1-cm piece of fine horse hair was dipped in model paint (1169 Mat Calvary Yellow, Testors Canada, Ontario, Canada) and centered on the thorax of the fly. The tether was placed such that it rested flat on the thorax and extended back between the wings of the fly, centred relative to the vertical axis of the fly’s body. Flies remained immobilized on the ice pack for 2–4 min to allow the paint to set. An Elmer’s glue stick (Elmer’s Products Inc., Columbus, OH, USA) was applied to the balance arm, the latter being held stationary by a platform blocking the arm’s movement. The tether was touched to the glue, and slight adjustments to the angle of the fly relative to the balance arm were made to create an optimum attachment (Figure 1). Attachment to the flight mill took 10–55 s to achieve depending on how quickly the correct flight angle was obtained.

The following behavioural protocol was developed using >30 flies (not included in the analysis) to ensure that we could capture useful data without setting a distance or time limit on the trial: Once the test fly was attached, the blocking platform was removed from the balance arm and as the fly warmed up it was able to move and initiate flight spontaneously. This usually occurred within 2.5 min of being removed from the ice pack. A start was considered successful if a fly made at least three complete revolutions



Figure 1 Optimum tether attachment of *Rhagoletis indifferens* on an electronic flight mill. Actual size of the fly is 0.5 cm.

prior to its first stop, and flies were allowed to fly and stop at will during the trial. The flight mill system recorded distance, time, and speed for each fly. An observer further recorded the number of stops, stopping time, and administered the tarsal release stimulus. Following a stop lasting 2 min (first interval), stainless steel forceps were touched lightly to the tarsi and quickly removed to stimulate flight (Roitberg et al., 1984). The fly was allowed to fly and stop at will until another 2 min stop was recorded (second interval) and a second tarsal release was then administered. The fly was monitored until it made a final 1 min stop, ending each trial (third interval). Therefore, each successful trial was composed of three consecutive flight intervals and included a minimum of three stops lasting a combined total of 5 min. Flies were not included in the analysis if they did not meet the complete protocol: for example, by taking >3.5 min to fly spontaneously, not responding to the tarsal release, if a proper flight angle could not be obtained before spontaneous flight, or whenever obvious signs of tethering complications (i.e., improper wing beat patterns or twisting of the tether) were observed.

Data and statistical analysis

We measured the distance flown (m), flight speed (m s^{-1}), net trial time (gross trial time – all stopping time = actual flying time in seconds), net total number of stops (gross number of stops – three required stops), and net stopping time (gross stopping time in seconds – 300 s for required stops). We also measured for each interval: time to 2 min stop, net number of stops, net stopping time, and net interval time. All results are shown as mean \pm SD.

The analysis followed a complete factorial design and was conducted using JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA). The factors ‘context’, ‘sex’, and ‘resources’ were examined for main effects while the interaction terms between these factors were considered for context-dependent influence (Quinn & Keough, 2002).

Table 1 Percentage of *Rhagoletis indifferens* females (n = 71) and males (n = 89) that flew <500 m or >500 m by treatment. The full factorial analysis of these data is provided in Tables 2 and 3

'Context'*'resources'	Percentage of females		Percentage of males	
	<500 m	>500 m	<500 m	>500 m
Isolated*low	15.5	1.4	22.5	3.4
Isolated*medium	19.7	2.8	15.7	1.1
Isolated*high	16.9	0.0	15.7	2.2
Crowded*low	14.1	4.2	7.9	5.6
Crowded*medium	10.0	1.4	13.5	1.1
Crowded*high	11.3	2.8	11.2	0.0
Total percentage	87.3	12.7	86.5	13.5

Results

Observations were made on 160 flies (71 females and 89 males) using the tethered flight mill system and protocol as described. The proportion of flies traveling less than 500 m on the flight mill was similar for both sexes, averaging 86.9% (Table 1). Overall, females flew faster on the flight mill than males, averaging $0.27 \pm 0.008 \text{ m s}^{-1}$ while males averaged $0.22 \pm 0.007 \text{ m s}^{-1}$ ($F = 53.4$, d.f. = 1, $P < 0.0001$). The maximum flight speed observed was 0.60 m s^{-1} for females and 0.48 m s^{-1} for males. The maximum distance recorded by any individual female was 3.7 km flown in 304.8 min (net trial time; female-crowded-medium resources treatment). This individual reached a maximum speed of 0.52 m s^{-1} and made 15 stops totalling 10 min during this trial. In contrast, the next closest distance flown by a female was only 1.98 km, suggesting that this maximum was a rare observation. The maximum distance flown by an individual male was 2.9 km flown in 138.6 min (net trial time; male-crowded 'medium resources' treatment). This male reached a maximum flight speed of 0.44 m s^{-1} and made five stops, totaling 5.8 min, whereas the next closest distance flown by a male was 2.8 km.

The complete factorial design of this experiment allows for the examination of the main effects (i.e., the effect of each factor independent of the others), and the interactions between factors (i.e., how the effects of one factor depends on the level of one or more additional factors), to be considered for each variable measured (Quinn & Keough, 2002). Both the distance flown and the related variable of net trial time had significant main effects attributed to the factor 'context' because crowded individuals flew farther, and thus longer, than individuals that had experienced the 'isolated' treatment (Table 2). Crowded flies traveled >1.5-fold the distance of isolated flies

($F = 4.02$, d.f. = 1, $P = 0.05$), which is contrary to what we had predicted. The significant 'resource'*'sex' interaction term for the variable net trial time, as predicted, indicates that females and males are responding to the availability of resources in different ways, but not in the manner we expected. While females and males respond similarly after having experienced the 'high resources' treatment (cherry, leaf, and food), females flew the longest when they had experienced the 'medium resource' treatment (leaf and food) and the least when from the 'low resources' treatment (food only). Males, however, responded as predicted and showed decreased net trial times as their exposure to resources increased, such that the longest times were for males that had experienced the 'low resource' treatments.

One might expect that flies prone to active dispersal and long-distance movements would display substantial periods of continuous flight on the flight mill under the idealized conditions of the laboratory. Our results show a significant main effect of the factor 'sex' for both the net number of stops and the net stopping time demonstrating a discontinuous nature of the flight pattern (Table 2). On average, females stopped twice as often as males ('sex': $F = 6.86$, d.f. = 1, $P = 0.01$) and this behaviour was significantly influenced by a 'context'*'sex' treatment interaction such that crowded females stopped nearly three times more often than isolated females or isolated males, while crowded males actually stopped less often than isolated males ($F = 5.12$, d.f. = 1, $P = 0.02$). A similar pattern was noted for the net stopping time during the trial, with females stopping for longer periods than males. Again a significant 'context'*'sex' interaction was noted for stopping time ($F = 3.80$, d.f. = 1, $P = 0.05$). Thus, despite the faster average speed of females on the flight mill as noted earlier in the results, both males and females had similar distances and net trial times (Table 2).

Further examination of the flight mill results as three consecutive flight intervals reveals some consistent trends in the data (Table 3). The first flight interval is longer than either of the second or third intervals. The factor 'context' produces a consistent effect during the flight mill trial because on average crowded individuals fly longer than isolated individuals in each interval, but the factors 'resources' and 'sex' contribute differentially to the results in each interval. Our results suggest that the significant differences in flight behavior were captured within the first two intervals on the flight mill, with the third interval contributing little additional data. Conspicuously absent from our results are any significant main effects attributed to the factor 'resources'. Although the first interval suggests that the shortest distances are flown by flies having experienced access to the most resources prior to testing (high treatment), the factor 'resources' only approaches significance

Table 2 Flight mill results from a complete factorial analysis of the factors ‘context’ (C, crowded; I, isolated), ‘resources’ (H, high; M, medium; L, low), and ‘sex’ (f, female; m, male) for the variables distance flown, net trial time, net number of stops, and net stopping time of *Rhagoletis indifferens*

Main effects	d.f.	Level	Distance flown (m) Mean ± SD	Net trial time (s) Mean ± SD	Net number of stops Mean ± SD	Net stop time (s) Mean ± SD
Factor						
Context	1	C	416.6 ± 72.2 ^a	1854.7 ± 324.2 ^a	5.4 ± 1.0	199.4 ± 36.9
		I	233.1 ± 61.1	1061.9 ± 274.4	3.5 ± 0.9	160.1 ± 31.2
Resources	2	H	197.8 ± 85.2	861.6 ± 382.8	3.6 ± 1.2	145.5 ± 43.5
		M	332.9 ± 82.8	1383.2 ± 371.9	4.6 ± 1.2	176.7 ± 42.3
		L	376.6 ± 77.1	1815.7 ± 346.3	4.6 ± 1.1	198.1 ± 39.4
Sex	1	f	275.5 ± 70.0	1156.1 ± 314.3	5.9 ± 1.0 ^b	227.6 ± 35.8 ^a
		m	335.3 ± 63.5	1574.6 ± 285.3	3.1 ± 0.9	135.5 ± 32.5
Interactions						
	d.f.	Levels	Least mean ² ± SD	Least mean ² ± SD	Least mean ² ± SD	Least mean ² ± SD
Context*resources	2	C*H	186.1 ± 129.8	886.2 ± 583.0	4.10 ± 1.8	146.2 ± 66.3
		C*M	435.9 ± 128.9	1767.5 ± 578.8	7.22 ± 1.8	247.3 ± 65.8
		C*L	601.4 ± 116.1	2791.1 ± 521.7	5.98 ± 1.6	234.0 ± 59.3
		I*H	206.7 ± 110.5	846.9 ± 496.3	3.35 ± 1.5	147.2 ± 56.5
		I*M	297.9 ± 104.3	1151.8 ± 468.4	3.52 ± 1.5	158.6 ± 53.3
		I*L	177.9 ± 101.9	870.8 ± 457.8	3.17 ± 1.4	152.8 ± 52.1
Context*sex	1	C*f	351.4 ± 105.6	1547.3 ± 474.5	9.04 ± 1.5 ^a	313.2 ± 54.0 ^a
		C*m	464.2 ± 98.5	2082.5 ± 442.6	2.49 ± 1.4	105.1 ± 50.3
		I*f	202.5 ± 92.4	785.0 ± 415.2	3.52 ± 1.3	162.7 ± 47.2
Resources*sex	2	I*m	232.5 ± 80.1	1128.0 ± 360.0	3.16 ± 1.1	143.1 ± 41.0
		H*f	179.2 ± 124.1	838.3 ± 557.3 ^a	5.45 ± 1.7	209.2 ± 63.4
		H*m	213.6 ± 116.0	894.7 ± 520.9	2.00 ± 1.6	84.2 ± 59.2
		M*f	420.4 ± 122.9	1755.3 ± 552.3	8.92 ± 1.7	323.7 ± 62.8
		M*m	283.4 ± 109.9	1163.9 ± 493.6	1.81 ± 1.5	82.3 ± 56.1
		L*f	231.3 ± 116.1	904.9 ± 521.7	4.48 ± 1.6	181.0 ± 59.3
		L*m	548.0 ± 101.9	2757.1 ± 457.8	4.67 ± 1.4	205.8 ± 52.1

P-values of the F-test between factor means: ^aP<0.05; ^bP<0.01.

during the first and second time intervals on the flight mill ($F = 2.92$, d.f. = 2, $P = 0.057$) (Table 3). Our results suggest that flight response is conditional to the previous experience of the individual and that the factors of ‘context’ and ‘sex’ more directly influence that flight response than does the factor ‘resources’.

Discussion

Environmental factors, such as conspecific crowding and access to resources, may influence an individual’s propensity to leave a given location, and this flight response may differ between females and males. We have demonstrated for the first time, using a tethered flight mill system, that past experience with environmental factors alters flight response in *R. indifferens*. Although flight mill values cannot be translated directly to the field because of the artificial nature of tethered flight, the lack of normal cues (olfactory or visual), and the optimum flight conditions of constant temperature and no wind, our

results suggest that female and male *R. indifferens* are capable of flights of the same order of magnitude, but differ in the factors that influence this flight behaviour. Most flies tested (86.9%) made short flights covering <500 m, which is a distance that would be akin to within patch movements in the field and is similar to values from field studies on cherry-infesting *Rhagoletis* spp. (*R. cerasi*: Haisch, 1974; Boller et al., 1980; Kneifl et al., 1997; *R. indifferens*: Jones & Wallace, 1955). Of the factors we tested for influence on flight behaviour, ‘context’ was the only one to be significant as a main effect for the distance-flown variable. The factor ‘sex’ had significant main effects on the flight speed and stopping patterns of female and male flies, but had no main effects for the distance flown or net trial time variables. Surprisingly, the factor ‘resources’ had no significant main effects, or even interaction terms with ‘sex’ or ‘context’, when flight distance was considered.

Our results consistently showed across intervals that crowded individuals made longer flights than isolated individuals (Table 3), contrary to our prediction, although

Table 3 Results from a complete factorial analysis of the factors 'context' (C, crowded; I, isolated), 'resources' (H, high; M, medium; L, low), and 'sex' (f, female; m, male) for time spent flying by *Rhagoletis indifferens* on flight mills: net first interval time, net second interval time, and net third interval time

Main effects	d.f.	Level	Net first interval time (s) Mean \pm SD	Net second interval time (s) Mean \pm SD	Net third interval time (s) Mean \pm SD
Factor					
Context	1	C	1169.1 \pm 261.0	544.4 \pm 120.3 ^b	138.5 \pm 34.7
		I	839.1 \pm 220.9	166.5 \pm 101.8	76.1 \pm 29.4
Resources	2	H	393.8 \pm 308.2 ^a	321.8 \pm 142.0 ^a	143.1 \pm 41.0
		M	1227.7 \pm 299.4	113.1 \pm 138.0	79.4 \pm 39.8
		L	1221.5 \pm 278.8	504.3 \pm 128.5	88.2 \pm 37.1
Sex	1	f	932.9 \pm 253.0	170.1 \pm 116.6 ^b	76.7 \pm 33.7
		m	1009.0 \pm 229.7	443.9 \pm 105.9	121.9 \pm 30.6
Interactions					
Context*resources	2	Least mean ² \pm SD		Least mean ² \pm SD	
		C*H	324.5 \pm 469.3	331.1 \pm 216.3 ^c	223.4 \pm 62.4
		C*M	1659.3 \pm 466.0	55.1 \pm 214.7	64.5 \pm 62.0
		C*L	1539.7 \pm 420.0	1116.5 \pm 193.5	128.7 \pm 55.9
		I*H	441.0 \pm 399.6	320.8 \pm 184.1	85.9 \pm 53.2
		I*M	991.8 \pm 377.1	129.2 \pm 173.8	87.3 \pm 50.2
		I*L	858.3 \pm 368.6	23.1 \pm 169.8	40.6 \pm 49.0
Context*sex	1	C*f	1154.8 \pm 382.0	256.1 \pm 176.1	189.2 \pm 3.7
		C*m	1194.2 \pm 356.4	742.7 \pm 164.2	185.3 \pm 3.4
		I*f	746.4 \pm 334.3	46.7 \pm 154.0	184.7 \pm 3.2
		I*m	780.9 \pm 289.9	238.0 \pm 133.6	190.0 \pm 2.8
Resources*sex	2	H*f	365.6 \pm 448.7	292.9 \pm 206.8	182.7 \pm 4.3
		H*m	399.9 \pm 419.4	359.0 \pm 193.3	185.3 \pm 4.0
		M*f	1764.6 \pm 444.6	21.6 \pm 204.9	194.4 \pm 4.3
		M*m	886.5 \pm 394.4	162.7 \pm 183.1	188.2 \pm 3.8
		L*f	721.6 \pm 420.0	144.1 \pm 193.5	183.8 \pm 4.0
		L*m	1676.3 \pm 368.6	949.3 \pm 169.8	189.4 \pm 3.5

P-values of the F-test between factor means: ^aP<0.06; ^bP<0.05; ^cP<0.01.

only the second interval is significant for main effects of 'context'. In our experiment, the level of crowding experienced by the individual test fly is thought to be quite extreme as there is frequent visual and physical contact between flies in these treatments, similar to or in excess of what would be expected in a heavily infested field situation (SE Senger, pers. obs.). Not surprisingly, individuals move away from high conspecific contact (AliNiasee, 1974; Prokopy & Roitberg, 2001). However, the lack of a strong response to the 'isolated' treatments may be an artifact of our study design if individuals are prone to disperse from unfavorable conditions prior to sexual maturity. Age-dependent flight responses using tethered flight mill systems have been demonstrated in other insects including codling moth, whose peak flight distances for mated females occurred on day 2 and for virgin females on day 3 of testing (Schumacher et al., 1997), and oriental fruit moth, whose peak flight distances were recorded at age 5 days (Hughes & Dorn, 2002). Our design tested flies at

sexual maturity (ca. 7 days) and thus could not detect an age-dependent response should one exist. The missing treatment to distinguish the impact of mating from that of crowding would be mated individuals that did not experience a high degree of crowding, however, achieving a consistent treatment for this proved challenging in practice and we dropped this treatment from the experiment. In our opinion, the observed response to 'crowding' suggests that flies make short flights, of the magnitude of leaving a tree, in response to local competition. Flight mills can exaggerate the length of short flights because the fly can only terminate a flight by stopping its wings, as opposed to flight under natural conditions wherein objects may stimulate landing behaviours or simple encounters with objects may terminate a flight. Therefore, the flight mills represent a conservative test of flight distance because they exaggerate short flights, making it harder to see a difference should one exist. Thus the observed effect of 'crowding' on flight response is probably real.

Although ‘resources’ as we defined them in this experiment did not produce significant main effects on flight response, the factorial design reveals a ‘resource’*‘sex’ interaction for net trial time such that gender-specific effects of ‘resources’ are apparent (Table 2). Both females and males that experienced the ‘high resources’ treatment (food, leaf, and cherries) made short flights when tested on the mill. Thus the presence of fruit can arrest both sexes, and this implies a role for chemical or visual manipulation of the pest population. However, females flew the longest when they had experienced the ‘medium resources’ treatment (food and leaf) and the least when from the ‘low resources’ treatment (food only), in contrast to males whose flight times decreased with increasing resources as predicted. Why are females not responding to the situation of ‘low resource’ with the expected behaviour of flying away? Field observations of adult females suggest that they spend little time in trees without fruits (Yee, 2002). Thus, the strong reaction to the ‘medium treatment’ (leaf and food) may reflect this behaviour of leaving an area without hosts in which to oviposit. We predicted that the ‘low resources’ treatment should elicit a stronger response than the ‘medium treatment’, but instead a negative effect, or weaker response, was observed. This result is perplexing at first glance. Negative effects on flight behaviour following food deprivation have been demonstrated in the migratory moth *Agrotis ipsilon* where both larval and adult diet stress had a negative (not positive) effect on flight performance using a tethered flight mill system (Sappington & Showers, 1993). Similarly, efforts to induce a ‘sense of malaise’ in the tephritids *Ceratitis capitata*, *R. pomonella*, and *Rhagoletis mendax* by food deprivation failed to produce significant changes in ovipositional response to less-preferred hosts (Prokopy et al., 1993), again a negative behaviour response. While *R. indifferens* individuals were not food deprived in this experiment, the ‘low resources’ treatment contained no host cues. Our results suggest that females require cues from host trees (presence/absence of fruits) to engage in longer flights. Papaj (2000) suggests that ‘a specialist faced with a shortage of hosts can either disperse to find hosts or wait out the period of scarcity’. Our perplexing result may reflect the life history of this species, even if overwintering beneath native *Prunus* spp., females of which would emerge to find host plants without fruits, as opposed to emerging to find no host cues at all. This latter circumstance would occur if backyard or commercial cherry trees were cut down, leaving flies to emerge with no host cues. We are currently examining this response by females to determine if it is a physiological response to the lack of host cues (i.e., failure to mature eggs) or a behavioural response to the treatment (i.e., the individual is ‘choosing’ not to fly) as ovarian dynamics

may affect dispersal drive and flight performance (Papaj, 2000).

Implications for improving field experiments on dispersal

Detailed field studies on between-patch movements are expensive and difficult to perform successfully, therefore, any information that can refine field questions is useful for advancing fruit fly ecology (Wiens, 2001; Peck et al., 2005). We have shown that the previous experience, or handling of insects for release, has the potential to impact the results of field dispersal studies, at least for *R. indifferens*. This is particularly true for females where their exposure to fruits, leaves, and/or food appears to influence flight behaviour less predictably than for males. The maximum flight distances recorded for *R. indifferens* on the flight mill correspond well to the field maximums for *R. cerasi* (Boller et al., 1980), and suggest that distances of not less than ca. 3 km should be considered when designing field tests of interpatch dispersal ability. Alternatively, field studies need to clearly state the maximum detection capability of the study design to allow for easier comparison of results that derive from different spatial scales. Peck et al. (2005) have suggested that individual tephritids with strong flight capabilities may be inadvertently caught in the first traps they encounter during mark-release experiments and thus skew field results to shorter distances. Our results suggest that *R. indifferens* displays discontinuous flight patterns (i.e., a propensity to stop often), behaviour that is likely exaggerated in the field where olfactory and visual cues would contribute to arrestment, and behaviour that would indeed increase the probability that individuals would encounter the traps set close to the release point. Further research into the propensity of *Rhagoletis* spp. to make repeated short flights to disperse over longer distances in the field are required, and the potential confounding effects of ‘near’ traps vs. the difficulty of detecting individuals in ‘far’ traps needs more consideration in dispersal studies. We are currently working on a mark-recapture design that varies trap deployment through space and time to determine if the current practice of setting traps out prior to the release of marked insects is skewing dispersal results to shorter distances.

Analyzing the distribution of insects in the field may not identify the causal mechanisms underlying that distribution (Harris & Myers, 1984) and conversely the study of insect flight behaviour does not necessarily lead to the identification of insect distribution in the field (Roitberg, 1988). Understanding how environmental cues modify movement behaviour is a prerequisite for the development of improved methods for trapping and managing insect pests (Dabaly Ball & Meats, 2000). Dispersal patterns remain a poorly understood element in the ecology and

management of most invertebrates despite decades of attempts to elucidate this key parameter (Bullock et al., 2002). Indirect measurements of flight propensity and potential distance can be a valuable means to generate expectations of parameters that remain elusive.

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