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SHORT COMMUNICATION

Tendency for upwind movement in the sibling fruit fly species, *Bactrocera tryoni* and *B. neohumeralis* and their hybrids (Diptera: Tephritidae): influence of time of day, sex and airborne pheromone

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Introduction

Bactrocera tryoni (Froggatt) and *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae) are sympatric sibling species and the most significant insect pests to horticulture in Australia (Drew, 1989). The Australian distribution of *B. neohumeralis* is contained entirely within that of *B. tryoni* and the species infest the same wide range of host fruits (Drew, 1989). The species are identified by the colour of their humeral calli: yellow in *B. tryoni* and brown in *B. neohumeralis*.

Hybridization can be forced in the laboratory by caging males of one species with females of the other. Hybrid flies are almost always intermediate in appearance, possessing calli that have both yellow and brown regions. Such intermediate forms are found at low frequencies in the field, but these may be the result of intraspecific variation of the two typical forms (Wolda, 1967; Gibbs, 1968).

Mating is governed by a circadian rhythm and is influenced by light intensity (Smith, 1979). *Bactrocera tryoni* mates at dusk under dim lighting conditions (Tychsen & Fletcher, 1971). In this species, mating is preceded by the formation of a flying swarm of males which settles as a compact cluster on the upwind sides of a tree. Females enter this settled swarm singly while males frequently release pheromone and defend individual leaf territories, engaging in lekking behaviour (Tychsen, 1977). Mating in *B. neohumeralis* occurs over a period of approximately 7 h centred on the middle of the day, and bright lighting conditions are optimal. Males sporadically emit pheromone during this period, but flying swarms or leks have never been recorded (Gee, 1969; Tychsen, 1977). Hybrids have a distribution of mating times that is intermediate between those of the parent species, but which overlaps significantly with both (Gibbs, 1968).

Pheromone release by *Bactrocera* males is achieved in a series of steps termed 'stridulation' (Monro, 1953) or 'spraying' (Kuba & Sokei, 1988). The pheromone is exuded from an internal abdominal gland via the anus and transferred with the hind tarsi to specialized setae that are only found on the wings of males. The pheromone is made airborne by rapid beating of the wings against abdominal bristles that are, again, uniquely male traits. The pheromone is detectable by the human nose and has a sweet, vaguely acidic smell (Bellas & Fletcher, 1979). The pheromones of *B. tryoni* and *B. neohumeralis* are indistinguishable by analysis and identification of their major components (Bellas & Fletcher, 1979), but differential responsiveness to (as yet unknown) minor constituents could be a cause of pre-mating reproductive isolation.

Three roles have been proposed for the male pheromones of tephritids: (i) attraction of females to males; (ii) stimulation of females resulting in increased readiness to mate; and (iii) mutual attraction of males to cause lek-formation (review by Fletcher, 1987). Evidence for roles (i) and (ii) in the biology of *B. tryoni* has been documented repeatedly (e.g. Fletcher, 1968; Giannakakis & Fletcher, 1978). However, this evidence pertains only to effects observed over short distances (a few cm) that would be relevant to recipients at close quarters (i.e. on the same or adjacent leaf). Role (iii) has been discussed by Tychsen (1977) but there has been no investigation of the ability of the pheromone of either *B. tryoni* or *B. neohumeralis* to attract either females or males over longer distances and correspondingly lower concentrations (e.g. from the other side of a tree canopy or from an adjacent tree).

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In *Bactrocera*, locomotion occurs predominantly by trivial flights and walks between leaves and twigs, and movement towards a source of odour appears to be consistent with mechanoreceptive anemotaxis in a series of steps (Fletcher, 1989; Aluja & Prokopy, 1992; Meats & Hartland, 1999; Meats & Osborne, 2000). Such behaviour is most appropriate when odour is received intermittently and winds are both light and variable in speed and direction (Brady *et al.*, 1989; Griffiths *et al.*, 1995).

In the current study, tests were performed on both males and females of each of the parent species and their hybrids and were conducted both in the middle of the day under bright light and at the time of dusk under dim light. The particular purpose of these investigations was to determine if *B. tryoni*'s pheromone influenced the inducement of upwind anemotaxis when it was at a concentration pertinent to detection at distances significantly greater than the short distances over which it has previously been shown to elicit mating responses.

Materials and methods

Cultures

Flies of *B. tryoni* and *B. neohumeralis* were taken from cultures that had been maintained in the laboratory at the University of Sydney for at least ten generations. The F_3 hybrid flies used were descended from flies that were the result of forced interbreeding between these two stocks.

Flies remained in a controlled environment of 25 ± 1°C and 65 ± 5% relative humidity at all times, including during wind-tunnel tests. The 24 h lighting cycle had a photophase (which included the final hour of dusk light) of 13 h duration. Other environmental conditions and culture techniques have been described by Bateman (1967).

Insects used for tests

All flies were sexually mature when tested two to three weeks after puparial eclosion. Mated females were tested at four weeks after eclosion, having been continuously exposed to mature males.

Experimental design

The factorial arrangement of the treatments is given in table 1. Midday tests were conducted during the 8th hour of the photophase, and dusk tests were conducted during the 13th hour.

Responses to the presence and absence of wind alone were tested for males and females at midday and at dusk in all species. Responses to pheromone in the presence of wind were tested for males and females of both *B. tryoni* and *B. neohumeralis* at both midday and dusk. Because the number of hybrids available was severely limited, they could not be included in midday tests of response to the pheromone. (No response was indicated in preliminary tests.) In addition to unmated females, mated females of *B. tryoni* were included in tests of responsiveness to pheromone and wind so as to provide an index of a nil response: any response to pheromone is inhibited after mating, and mated females rarely mate again (Fletcher & Giannakakis, 1973).

Wind tunnel

The wind tunnel was a rectangular box constructed from sheets of transparent acrylic plastic. Its total internal dimensions were 1800 × 800 × 100 mm (l × w × h). The observation area (700×800 mm) was located in the centre of the wind tunnel and was delimited on each side by banks of plastic tubes (205 mm long, 5 mm in diameter) that created a laminar air flow. Screens of 2 mm gauge square wire mesh were placed against these tubes to prevent flies from entering. A 100 mm grid visible beneath the observation area divided into seven equal rows, with row 7 being at the upwind end. Air was drawn through five round holes (30 mm in diameter) at the upwind end of the tunnel by the means of a domestic vacuum cleaner located at the downwind end. After passing through the tunnel,

Table 1. Factorial design of treatments (each consisting of 4 replicates of 10 flies) to which the different groups (*Bactrocera neohumeralis*, *B. tryoni* and F_3 hybrids) were subjected. The response of hybrids to pheromone at midday was not tested as too few individuals were available.

		Pheromone absent		Pheromone present	
		Wind absent	Wind present	Wind absent	Wind present
Midday	Male	*neohumeralis* *tryoni* hybrids	*neohumeralis* *tryoni* hybrids		*neohumeralis* *tryoni*
	Unmated female	*neohumeralis* *tryoni* hybrids	*neohumeralis* *tryoni* hybrids		*neohumeralis* *tryoni*
	Mated female	*tryoni*	*tryoni*		*tryoni*
Dusk	Male	*neohumeralis* *tryoni* hybrids	*neohumeralis* *tryoni* hybrids	*tryoni*	*neohumeralis* *tryoni* hybrids
	Unmated female	*neohumeralis* *tryoni* hybrids	*neohumeralis* *tryoni* hybrids	*tryoni*	*neohumeralis* *tryoni* hybrids
	Mated female	*tryoni*	*tryoni*		*tryoni*

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contaminated air was expelled from the room via a fume cupboard. The upwind chamber was of sufficient size (345 × 800 mm) to allow for the turbulent mixing of the airborne pheromone to be administered to the flies, while the downwind chamber of equal size acted to draw the air evenly through the tunnel. The rate of airflow through the wind tunnel was 0.23 m s^{-1} (0.84 km h^{-1}). Further details are supplied by Meats & Hartland (1999).

In all tests which required pheromone, air was drawn through a 'source' cage (250 × 250 × 380 mm) placed over the tunnel's upwind inlets. This cage contained 400 males of *B. tryoni* which were taken from the mature laboratory stocks or from mature stocks produced by a mass-rearing facility (see Acknowledgement). Tests which required pheromone in the absence of wind were prepared by drawing pheromone-laden air through the tunnel for 30 s (equivalent to approximately ten changes of air). Pheromone release by mature males of *B. tryoni* occurs spontaneously at dusk (Barton Browne, 1957). In cases where pheromone was required during the day, pheromone-producing males were conditioned to a diel cycle which lagged 5 h behind that of the test flies. Thus, 'midday' for the test flies was made to coincide with 'dusk' for pheromone-producing males that were provided with a dusk photon flux density of 0.3 μE m^{-2} s^{-1} by using a shadecloth filter.

The daytime photon flux density (70 ± 5 μE m^{-2} s^{-1}) was provided by a metal halide lamp suspended 1 m above the tunnel, while dusk light (0.3 μE m^{-2} s^{-1}) was furnished by an incandescent globe suspended from the same height. For simplicity's sake, the transition from the day to the dusk lighting levels was instantaneous as this has no significant effect on flies' propensity to mate under laboratory conditions (Barton Browne, 1957; Tychsen & Fletcher, 1971).

Estimation of pheromone release

The frequency of pheromone release (termed stridulation) was observed in a small cage (150 × 130 × 250 mm) containing 50 males of *B. tryoni* and an identical cage containing 50 males of *B. neohumeralis*. The number of flies stridulating in each of six consecutive 5-min periods was recorded. Observations were made at midday (with recording commencing 15 min before) and at dusk for both species.

Further data on *B. tryoni* at dusk were obtained by observing 400 males in a cage of the same size as that used as a source cage for the wind tunnel (250 × 250 × 380 mm). Firstly, the durations of individual stridulation events were recorded (n = 162). Observations for these data took place over two dusk periods. The cage was scrutinized until a fly was observed to start stridulating whereupon that fly was observed exclusively until it stopped stridulating. Secondly, an estimate was made of the number of stridulations occurring at any one moment. For convenience, the cage was scanned by eye once in a 9-s period (one third of the cage per 3 s) so that no part of the cage was observed for more than one second. This process was repeated 97 times over two dusk periods.

Observations in the wind tunnel

No fly was used more than once. Each test was replicated four times. (The replicated treatments and controls are set out in table 1.) A replicate test comprised the simultaneous insertion of 10 flies at row 1 of the wind tunnel followed by the recording of their row positions after 0, 1, 5, 10, 15 and 20 min. As insertion marked the commencement of a test, all flies were located in row 1 at time 0. Each replicate test was performed on a different day due to the short period over which pheromone release, or any other dusk activity, can occur.

As flies were not able to move downwind from the insertion point, the tests measured upwind displacements only. The tunnel was considered too short for the insertion point to be in the middle row (row 4) as trials indicated that random movement in windless conditions could lead to displacements of three to four rows in 5–10 min. An ability to detect displacements of seven rows (700 mm) was desirable if flies were making non-random movements associated with upwind anemotaxis.

Analysis

Data were subjected to analysis of variance followed by comparison of means using the Student-Newman-Keuls multiple range test. All data passed Cochran's test for homoscedasticity and were not transformed.

Results

Stridulation and pheromone release in the observation cages

Comparison of the small observation cages which each contained 50 males demonstrated a clear behavioural difference between the species. No stridulation occurred in the *B. tryoni* cage at midday and no stridulation occurred in the *B. neohumeralis* cage at dusk. In observations of *B. tryoni* at dusk, several individuals stridulated in each of the six periods, giving a mean of 5.1 ± 0.9 (S.E.) per period. The pheromone was routinely smelled by the observers. Stridulation in *B. neohumeralis* was infrequent even at the peak mating time for the species (midday) and occurred in only three of the six observation periods. The mean number of *B. neohumeralis* males stridulating per 5-min period was 1.2 ± 0.7.

Observation at dusk of the larger cage containing 400 *B. tryoni* males enabled stridulation in the source cage used in the wind-tunnel tests to be estimated. Stridulatory activity per fly was greater in this larger group. Some males appeared to stridulate regardless of the presence of neighbouring flies, some appeared to stridulate at other males, and others attempted to mount other males while stridulating. The airborne pheromone was again strongly detectable to the observers. The mean duration of 162 stridulations was 5.38 s (S.D. = 5.1; mode = 2; max. = 31). The mean number of flies observed stridulating in 98 scans of the cage was 6.27 (S.D. = 2.8; mode = 5; max. = 14).

Estimation of pheromone concentration in the wind tunnel

Tychsen (1977) reported the distance at which females of *B. tryoni* display a mating response in nature as a few centimetres. The puffs of pheromone 'spray' photographed by Kuba & Sokei (1988) have a radius of approximately 15 mm after travelling approximately 30 mm in still air. This represents dispersal of the instantaneous output from a single male through a cross-sectional area of 15.9 cm^2. In the experiments described here, the volatiles from a mean of

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6.27 flies were dispersed uniformly through a cross-sectional area of 800 cm^2, equivalent to 127.6 cm^2 from one insect. Even assuming that the pheromone vapour disperses more rapidly than the 'spray' photographed by Kuba & Sokei (1988), it is likely that the concentration of pheromone used in the experiments described here is similar to that at a distance much greater than 30 mm under natural conditions and hence more relevant to a response involving the initial movement towards stridulating males.

Observations in the wind tunnel

The number of flies in the upwind part of the tunnel did not change significantly over the course of the 10 min, 15 min and 20 min observations within any test. Data from the 10 min observation have been arbitrarily selected for presentation (fig. 1).

Response to pheromone

No significant difference was detected among the treatments in which pheromone was present and the treatments in which pheromone was absent. This was true for both the midday and the dusk periods for *B. tryoni*, *B. neohumeralis* and hybrids.

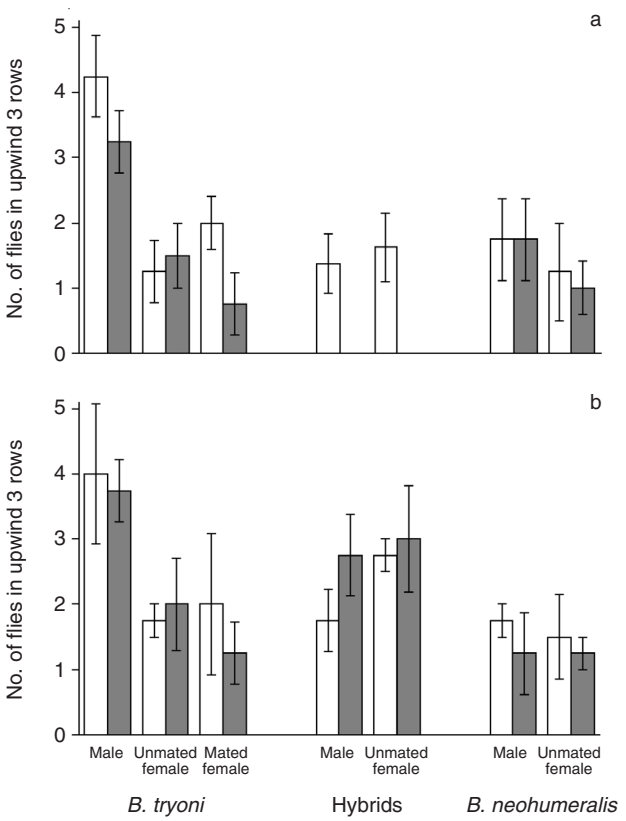

Fig. 1. Mean number of *Bactrocera* flies out of 10 (± S.E., n = 4) found in the upwind rows 5, 6, and 7 of the wind tunnel (after 10 min) in the presence (■) or absence (□) of pheromone at midday (a) and dusk (b). The response of hybrids to pheromone at midday was not tested as too few individuals were available. All values are derived from tests done in the presence of wind, but, with the exception of those which demonstrate the upwind response of *B. tryoni* males (see text), these do not differ significantly in paired comparisons from values derived from tests done in the absence of wind ($P > 0.05$).

Responses to wind

Analysis of variance detected a significant interaction between the factors 'species', 'wind', and 'sex'. This is due to the greater number of *B. tryoni* males found in the upwind part of the tunnel when wind was present, both at midday and at dusk (S-N-K test for *B. tryoni*, $P < 0.05$). This greater upwind response of *B. tryoni* males is also presented in fig. 1. No other significant difference in response to wind was found among males, unmated females, and mated females within or among any of the species. Indeed, the S-N-K test which incorporated all three 'species' had insufficient power to distinguish between the number of *B. tryoni* males and the number of hybrid females found in the upwind part of the tunnel. It was noted, however, that the hybrid females were unusually active at all times and that, in both the presence and absence of wind, similar numbers moved to rows 5, 6 and 7.

In two of the four replicate tests involving males of *B. tryoni* within the treatment with wind alone, an unusual behaviour was observed. In each case, up to eight of the ten males moved upwind to row 7 to form a stable, closely-packed group (approximately 100 mm in diameter) on the upwind screen where they commenced stridulating. These groups persisted for the duration of both the tests in which they formed (20 min) and were never composed of fewer than five flies.

Discussion

It is clear that males of *B. tryoni* have a greater tendency to move upwind both at midday and at dusk than any of the other flies tested. The groups of males of *B. tryoni* which moved and settled in concert and formed on two occasions may be analogues of the swarms known for this species under natural conditions. This speculation is supported by the fact that, on both occasions, these groups formed at dusk. As swarms were observed in only two of the possible eight dusk tests and similar numbers of flies were found upwind in each of these and in the midday tests, it is likely that upwind movement is only partially attributable to the swarming activity.

Tychsen (1977) reported that the male swarms of *B. tryoni* invariably settled on the upwind side of tree canopies. A tendency for upwind movement could produce this effect if an additional behaviour discouraged flies from leaving a canopy. Tychsen (1977) speculated that the dense swarms sometimes seen on a single tree would require the attraction of males from surrounding canopies. It is unlikely that any such attraction would be mediated by the pheromone during the flying-swarm stage, as males are apparently able to emit it only while standing (Monro, 1953; Fletcher, 1969; Kuba & Sokei, 1988). It is therefore likely that release can commence only after flying swarms have settled. Swarm formation may also rely on visual cues or pheromonal cues other than those presented.

Upwind anemotaxis in response to the pheromone of *B. tryoni* presented in laminar air flow did not occur in *B. tryoni*, *B. neohumeralis* or hybrids under any of the conditions applied. It has, however, been recorded in *B. tryoni* in response to an artificial lure (Meats & Hartland, 1999) and, in conditions similar to ours, to pheromones and artificial

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lures in several other tephritid species (Jones *et al.*, 1981; Landolt *et al.*, 1985; Meats & Osborne, 2000).

The lack of any observed response to pheromone under the current limited test conditions offers no support for the proposition that the pheromone serves to attract either males or females over distances likely to be appropriate to group formation within a tree. It may be argued that the flies were not presented with a fluctuating dose of pheromone and that, as is the case for certain moths (e.g. see Murlis *et al.*, 2000), a response should only be expected if filaments of higher concentration are present within the plume. However, this was not requisite for the positive responses documented in *B. tryoni* and *Bactrocera cacuminata* (Hering) (Diptera: Tephritidae) to artificial lures (Meats & Hartland, 1999; Meats & Osborne, 2000). It is also clear that stridulation in *B. tryoni* and *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) involves very rapid wing vibration that produces a homogenous pheromone cloud that is unlike the filamentous plumes that are usual for moths (Monro, 1953; Kuba & Sokei, 1988).

The pheromone may be responsible for the attraction of females to males over distances intermediate between those presented here and those that cause the mating response. At similar distances, it may also cause male aggregation. It could mediate in these behaviours either by acting as an arrestant (inducing flies to remain in the vicinity of the pheromone's source) or by causing flies to move up its concentration gradient. Either mechanism could explain lek formation in *B. cucurbitae*, a species in which the flying swarms (which precede lekking in *B. tryoni*) do not form (Kuba & Koyama, 1985).

The contrast between the strategies of dusk and day mating with respect to *B. tryoni* and *B. neohumeralis* has been discussed by Gee (1969). Lekking in *B. tryoni* may maximize the number of mating encounters which occur over the short period of dusk during which mating is possible. The mating strategy of *B. neohumeralis* may be an opportunistic one in which leks play no role (Gee, 1969). The pheromone may serve predominantly as an indicator of male quality or possess aphrodisiacal properties for females at very close range. Field observations designed to examine these theories are yet to be conducted, but some supportive evidence is found in the work of Fitt (1981) which demonstrated that females of another day-mating species, *Bactrocera tenuifascia* (May) (Diptera: Tephritidae), were stimulated to mate on extremely close encounters with males, but were not attracted to males over short distances.

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