

The Plume Also Rises: Trajectories of Pheromone Plumes Issuing from Point Sources in an Orchard Canopy at Night

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Abstract The trajectories of pheromone plumes in canopied habitats, such as orchards, have been little studied. We documented the capture of male navel orangeworm moths, *Amyelois transitella*, in female-baited traps positioned at 5 levels, from ground level to the canopy top, at approximately 6 m above ground, in almond orchards. Males were captured in similar proportions at all levels, suggesting that they do not favor a particular height during ranging flight. A 3-D sonic anemometer was used to establish patterns of wind flow and temperature at 6 heights from 2.08 to 6.65 m in an almond orchard with a 5 m high canopy, every 3 h over 72 h. The horizontal velocity of wind flow was highest above the canopy, where its directionality also was the most consistent. During the time of *A. transitella* mating (0300–0600), there was a net vertical displacement upward. Vertical buoyancy combined with only minor reductions in the distance that plumes will travel in the lower compared to the upper canopy suggest that the optimal height for release of pheromone from high-release-rate sources, such as aerosol dispensers (“puffers”), that are deployed at low densities (e.g., 3 per ha.) would be at mid or low in the canopy, thereby facilitating dispersion of disruptant throughout the canopy. Optimal placement of aerosol

dispensers will vary with the behavioral ecology of the target pest; however, our results suggest that current protocols, which generally propose dispenser placement in the upper third of the canopy, should be reevaluated.

Keywords *Amyelois transitella* · Lepidoptera · Pyralidae · Mating disruption · Orientation · Odor plume

Finding resource-linked odor sources by heading upwind when the odor is sensed is a common strategy of many insects (Cardé and Willis 2008). The paths that wind-borne odor plumes take in daytime have been documented in open fields, savannahs, and forests by following the paths of airborne “parachute” seeds (Aylor et al. 1976), smoke puffs (David et al. 1982; Brady et al. 1989), and neutrally buoyant balloons and smoke puffs (Elkinton et al. 1987). These studies have shown that plumes have trajectories that can be essentially straight for relatively short distances but, more typically, change course with shifts in wind direction and velocity, such that the path viewed from above would appear to “snake” through the environment (Elkinton et al. 1987; Brady et al. 1989). Plumes also can flow around objects such as shrubs (Murlis et al. 1992); all of these trajectories appear to have far less vertical than horizontal movement. Flow through tree canopies is mainly in the horizontal plane (Shaw 1982; Oke 2003). During daytime, plumes in forest habitats, however, can rise when they encounter sunlit patches with upward convective currents (Fares et al. 1983; Elkinton et al. 1987; Thistle et al. 2004). Dispersal of wind-borne odor plumes at night is less well understood. Schal (1982) documented that in a tropical rain forest at night, odor plumes rise slowly, due to warm convective currents and a temperature lapse (decreasing temperature with height), although the prevalence of this phenomenon in other habitats has not been documented.

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Flight presumed to be in search of an odor plume is termed ranging flight. Its direction with respect to wind flow may be tailored to maximize the chance of encountering the plume. What evidence exists, however, suggests that male moths fly at random with respect to the direction of instantaneous wind flow (reviewed by Cardé et al. 2012). However, there is evidence that in an orchard environment male codling moths, *Cydia pomonella*, are more apt to find sources of synthetic pheromone placed near the top of a tree canopy rather than in mid or low positions (Sibbett et al. 1993). Similarly, in parkland environments, males of the oak processionary moth, *Thaumetopoea processionea*, are caught more often in pheromone traps placed in the upper canopy (Williams et al. 2013); a pattern of forest-dwelling moths generally preferring pheromone traps placed high in a forest canopy has been documented by Williams et al. (2013). These distributions of catch suggest that the ranging flight of males either favors flight near the top of the tree canopy or that sources placed near the top of the canopy are more readily followed because the plumes originating there are not fragmented by passing through foliage. It is not known where in the canopy female codling moths perch and call (emit pheromone); indeed, relatively little is known about where female moths of any species position themselves for calling.

To assess the three-dimensional flow of wind, and therefore the potential trajectory of odor plumes, we used a 3-D sonic anemometer to document the flow of wind in an almond orchard. We also determined the captures of male navel orangeworm moths, *Amyelois transitella* (Lepidoptera: Pyralidae), in female-baited traps positioned at five heights, from ground level to the top of the canopy. The aims of this study were to determine the natural patterns of odor flow in an almond orchard environment, and to assess how vertical displacement of pheromone plumes influences the ability of male *A. transitella* to find pheromone sources at various canopy heights. Understanding the vertical dynamics of plume dispersal may provide insights into strategies for selecting optimal calling sites by females, and the optimal height of flight by ranging males for interception of the pheromone plume. Such information also may suggest effective deployment strategies for release of synthetic pheromone from dispensers used to interfere with mating of moth pests of orchard crops (Cardé and Minks 1995). This may be particularly relevant when disruptant is emitted from sparsely placed, high release rate dispensers, such as atomizing puffers (Shorey and Gerber 1995). Mating disruption of *A. transitella* is effective (Burks et al. 2005, 2006; Higbee and Burks 2008), with protocols calling for the placement of puffer dispensers in the top third of the tree canopy (Burks and Brandl 2004; Zalom et al. 2012). Such guidelines replicate those for other moth pests of orchard crops, including oriental fruit moth, *Grapholita molesta* (Bentley et al. 2012) and codling moth (Caprile et al. 2011).

Methods and Materials

Male Trapping Experiments Trapping experiments were conducted over a 9-week period in each of 3 years (2009–2011) from early June to late August. Experiments were located in one almond orchard in 2009 (35° 32' 50.018" N, 119° 38' 59.166" W,) and three almond orchards in 2010 and 2011 (35° 29' 43.628" N, 119° 42' 10.663" W; 35° 29' 18.231" N, 119° 40' 2.771" W; 35° 29' 17.751" N, 119° 41' 38.652" W), located approximately 20 km west of Shafter, California. During this period, the almond trees were in full leaf, and the almonds were going through the process of hull split. Navel orangeworm eggs are most commonly laid on new crop nuts after the initiation of hull split (Zalom et al. 2012). Orchards ranged in age from 10 to 12 yr-old, were square in shape (each side ca. 0.4 km, or ca. 64.8 ha), and uniformly planted, such that the distance between rows of trees was 7.3 m and, between trees within rows, 6.4 m. The mean height to the top of the canopy in all orchards was 6 m, and the space between the tree canopies was 1–2 m between rows and <1 m within rows.

Traps using virgin females as a pheromone source were used to monitor male attraction and were placed at varying heights in the canopy. A single, newly eclosed virgin female was sealed in a mesh bag, which was hung from the top of a slightly modified (Kuenen et al. 2005) orange wing trap with glue liners (Suterra, Bend, OR, USA), and then placed in the field within 24 h (as described by Burks and Brandl 2004). Moths were reared from a laboratory colony originally obtained in 1966 from the University of California, Berkeley, and maintained on a wheat bran diet (Tebbetts et al. 1978). Traps were checked, and females were replaced weekly, and liners removed and replaced if they contained moths or were dirty. Old liners were taken to the laboratory to confirm field counts and moth identification. Observations during the course of the study suggested that survival of virgin females used as lures changed during the course of each trapping season. During late June, when ambient temperatures were lower, the majority of females survived until they were due to be replaced. However, during July and August, all females died by the end of the 7-day period, probably due to the higher temperatures during these months. From these observations, it was not clear whether mortality varied with trap height and, therefore, whether female calling duration also may have varied among heights.

In 2009, wing traps were placed in 10 trees, 30.5 m apart, across one orchard. Within each tree, a trap was located in the upper canopy, mid-canopy, lower canopy, on the trunk, or on the ground, respectively ca. 5.2, 3.6, 1.7, 0.8, and 0 m above ground level. A second experiment with the same tree spacing was conducted in parallel, placing only one wing trap at a certain height in one tree, with 10 replicates for each position, for a total of 50 trees. In 2010 and 2011, the design of the first

experiment was used (five traps per tree) and a total of 9 replicates were divided among three orchards. The distance between replicate trees was ca. 198 m.

Orchard Characteristics for Anemometer Recordings

Anemometer recordings were made between 13 and 16 July 2010 in a 7-yr-old almond orchard, ca. 10 km due east of Shafter, California. The orchard was approximately triangular, its borders measuring ca. 685 m (north) \times 874 m (east) \times 1,241 m (southwest), and was located ca. 130 m above sea level on level terrain. Rows of trees ran in an approximate north to south orientation. Trunks of trees within a row were ca. 6.4 m apart, and between rows trunks ca. 7.3 m apart. The top of the canopy extended to ca. 5 m, and the gap between tree canopies was <1 m within rows and ca. 2 m between rows (see Supplementary Fig. 1).

Sonic Anemometer Recordings Orthogonal wind components and air temperature were measured in the almond orchard using a 3-D sonic anemometer (CSAT-3, Campbell Scientific, Logan, UT, USA). This anemometer measures wind speed and the speed of sound on three non-orthogonal axes and, using these measurements, calculates orthogonal wind speed and sonic temperature. It has a resolution of $1 \text{ mm}\cdot\text{s}^{-1}$ root mean square. Instantaneous wind velocity measurements were sampled in $\text{m}\cdot\text{s}^{-1}$ at a rate of 60 Hz, and digital outputs were recorded on a laptop computer (Cardé et al. 2012). All measurements were made at a point that was central within the orchard ($35^{\circ} 29' 50''\text{N}$, $119^{\circ} 09' 19''\text{W}$), at least 250 m from any edge. The anemometer was mounted on a BlueSky® AL1 aluminum tripod (BlueSky® Mast Inc., Odessa, FL, USA), which was leveled by using a built in bubble level. The anemometer was attached to the tripod by a pole mount side arm, so that the recording region was 1.13 m horizontally away from the tripod pole. The tripod was erected at the midpoint between two rows of trees, and the side arm was directed to run parallel with the rows, so that the anemometer was equidistant from trees in both rows and could be faced either southward or northward. When oriented to the south, the anemometer was located in a straight line between the two closest trunks from neighboring rows, placing it closer to the canopy; when oriented to the north, the anemometer was located equidistant from 4 trees, placing it further away from the canopy. The anemometer height could be raised by adding 0.91 m sections to the tripod, allowing recordings to be made at six distances above ground level of the orchard: 2.08, 2.99, 3.91, 4.82, 5.74 and 6.65 m. Readings made at 4.82 m were approximately at the top of the canopy (see Supplementary Fig. 1). Ground surface temperatures were recorded periodically from 0530 to 2100, using an Extech infrared thermometer (model 42529, Extech Instruments Corp., Waltham, MA, USA)

Five-minute recordings of orthogonal wind components and air temperature were taken at each anemometer height

every 3 h. Recordings were made over three contiguous 24 h periods, commencing at 1200 on 13 July 2010 through to 0900 on 16 July 2010 (one data file from 2.08 m at 0900 on July 14 was corrupt and not included in the analyses). Sunrise was at ca. 0551 and sunset at 2012 PDT. Male *A. transitella* exhibit maximum flight activity in the last 3 h of scotophase (Coffelt et al. 1979), which in the context of these experiments would be between 0300 and 0600. Therefore, where necessary for brevity, data presented in figures are limited to those recordings made between 0000 and 0900. Equivalent figures containing all time points are available online as [supplementary material](#). For statistical analyses, data from all time points were used; each 24 h period was defined as a one-day block. Therefore, 3 days blocks were recorded, each comprising recordings made at 8 time points. The recording region of the anemometer was oriented to face northward for recordings made on day block one and southward on day blocks two and three.

Data outputs from the anemometer were used to calculate the mean temperature at each time point at each height. In addition, the orthogonal wind component data were used to calculate a variety of other variables at each time point and height over the 5-min records, including: 1) mean wind direction, to give a measure of the directionality of the wind; 2) circular standard deviation (SD) of mean wind direction, to give a measure of fluctuation around the mean wind direction; 3) maximal fetch [calculated as mean wind velocity ($\text{m}\cdot\text{s}^{-1}$) multiplied by the number of seconds of the recording], to give a measure of the maximum distance a plume would travel; 4) vertical air displacement [calculated as the mean wind velocity ($\text{m}\cdot\text{s}^{-1}$) in the vertical plane multiplied by the number of seconds of the recording], giving a measure of the vertical deviation a plume would make; 5) the percentage of time that wind blew at different vertical vectors, to give a measure of the amount of time the body of air in the orchard was rising or falling; and 6) mean wind direction in the vertical plane, to give a measure of the mean directionality of the wind in the vertical plane. Before calculating these variables, all orthogonal data were subjected to signal conditioning, to remove noise created from output records in which values were below the sensitivity of the anemometer. All outputs that were $<1 \text{ mm}\cdot\text{s}^{-1}$, which is the resolution level of the anemometer, were reset to zero.

Statistical Analyses Total trap catch per trap was analyzed using a one-factor randomized block model univariate ANOVA, with trap height as a factor and tree number as a random factor. Temperature, mean fetch and mean vertical displacement were analyzed using a series of two-factor randomized block model univariate ANOVAs, with anemometer height and time of day as factors and day block as the random blocking factor (Doncaster and Davey 2007).

All data were analyzed by parametric tests. Data were examined for normality using frequency histograms and

normality tests. Those variables that were not normally distributed were subjected to a range of transformations and re-examined to ascertain whether any of the transformations resulted in the variable being normally distributed. As a result, mean trap catch was square root transformed, fetch was log transformed, and vertical air displacement was reflected and log transformed. Where data were transformed for analyses, back-transformed means are shown in figures. All statistical analyses were conducted using SPSS v.19.

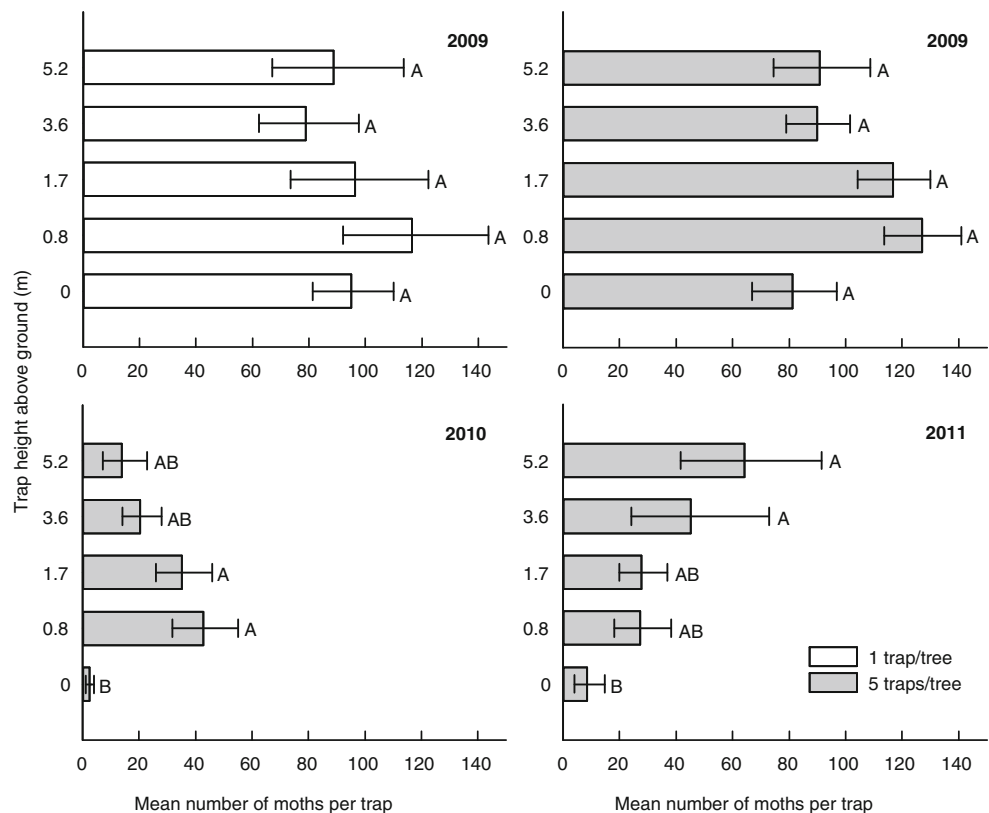
Analyses of circular data were conducted using Oriana 3 software v3.21, including calculation of: 1) mean wind direction [a mean vector length (*r*-value) of one indicates that all the data points recorded are toward the same direction, and zero signifies that the data points are dispersed evenly]; and 2) mean circular SD (a high mean circular SD indicates that a sample is highly dispersed) (Batschelet 1981). To investigate whether there were differences in the mean wind direction at different anemometer heights, a series of 15 pairwise comparisons were made between the circular SD values for the six different recording heights, at each time point on each day block. When comparing two heights, if the circular SDs did not overlap, the mean directions at those two heights were considered to be different. A total count was produced for the number of differences between heights at each time point on each day block (ranging between zero and 15), and these were compared by two *chi-square* analyses, comparing differences between day block and time of day. Variations in the

magnitude of the circular SD recorded at different anemometer heights were compared at each time point using a series of Kruskal-Wallis tests. Mean wind direction in the vertical plane was analyzed to investigate whether the mean vectors across all the recordings were more commonly in either an upward or downward direction. If a vector was above zero degrees (horizontal) in the vertical plane, it was scored as moving in an upward wind direction, and if below, a downward direction. These two values were then totaled and compared using a *chi-square* test. Whether the wind was blowing upward or downward for a longer percentage of time was compared for different anemometer heights, times of day, and day blocks. For each recording, if the wind was rising for more than 50 % of time it was scored with a one, and if falling more than 50 % of the time, a zero. Responses were then compared using a generalized linear model using a binomial distribution and logit link function.

Results

Trap Catch When traps were not in competition on the same tree, capture levels were roughly equivalent at all heights (Fig. 1). In 3 years of comparisons of traps in competition from ground level to the upper canopy in the same tree, the findings were variable. In 2009, there were no differences in

Fig. 1 Mean numbers of male *Amyelois transitella* moths caught in virgin female-baited wing traps (\pm SE) positioned in almond trees within an orchard for three different years (2009, *N*=10 trees; 2010/11, *N*=9 trees). Traps were located at ground level and at four different heights above ground level within the trees' canopies. There were two configurations of traps, either one trap per tree or traps at all 5 heights in the same tree. The total catch for each trap was recorded over a 9-week period in July and August of each year. Within each plot, bars with different letters are different at *P*<0.01 (Tukey's tests). Mean number of moths was square root transformed for analysis



capture levels at any of the tested heights. In 2010, there were differences in mean trap catch between different trap positions ($F_{4,32}=6.66$, $P=0.001$), with the highest mean trap catch at 0.8 m above the ground, and the lowest in the traps placed on the ground. In 2011, capture levels were highest at 5.2 m but, again, there were few differences among the other heights. These comparisons suggested that males likely engage in ranging flight at heights throughout the canopy and that trap captures likely reflect the height at which they first encounter the pheromone plume. It does appear, however, that the ranging flight near ground level (below the canopy) is less frequent.

Anemometer and Temperature Recordings During the course of the recordings, the average mean, maximum and minimum temperatures recorded by the anemometer were 28.7 ± 1.0 , 36.9 ± 0.2 , and 21.0 ± 1.8 °C, respectively; there was no rainfall. The values recorded by the nearest weather station to the anemometer site, 9.8 km away at Bakersfield's Meadows Field Airport ($35^{\circ} 26' 11''\text{N}$, $119^{\circ} 3' 27''\text{W}$), were similar, with average mean, maximum, and minimum temperatures of 30.0 ± 1.1 , 37.5 ± 0.6 , and 22.8 ± 1.4 °C, respectively, and no rain (source: Wunderground.com). The average mean, maximum, and minimum temperatures recorded at this station for the duration of the trap catch experiments were 27.6 ± 0.3 , 35.4 ± 0.3 , and 19.7 ± 0.3 °C, respectively, again with no rain. These data suggest that, for these meteorological variables, conditions were similar during both the trap catch experiment and anemometer recordings. During the course of these observations, mean temperature varied with height ($F_{5,10}=18.41$, $P<0.001$), time of day ($F_{7,14}=42.78$, $P<0.001$), and day block ($F_{2,14}=18.55$, $P<0.001$; Fig. 1). There was an interaction between time of day and day block ($F_{14,69}=37.17$, $P<0.001$), but no interaction between height and time of day ($F_{35,69}=1.45$, $P=0.10$) or height and day block ($F_{10,69}=0.823$, $P=0.61$).

During daytime, the temperatures were similar at all heights measured, rising throughout the daylight hours until near sunset at 1800; thereafter, temperatures decreased until sunrise at 0600 (Fig. 2). At nighttime, and particularly between 0000 and 0600, air temperatures were stratified by height, with the lowest temperatures around 2 m above ground, and the highest above the canopy; the temperature with height differential ranged up to ca. 3 °C. Ground surface (bare soil) temperatures during nighttime, however, were higher than air temperatures. For example, on 15 July at 0600, soil surface temperatures were 21–23 °C (about 1–3 °C higher than air temperature near 2 m above ground), and on 16 July at 0530, 26–27 °C (also about 2–3 °C above the temperature at 2 m).

Wind direction generally was consistent at different heights at each time point and day block, but was least consistent at 0000 (Fig. 3). Furthermore, wind direction at different

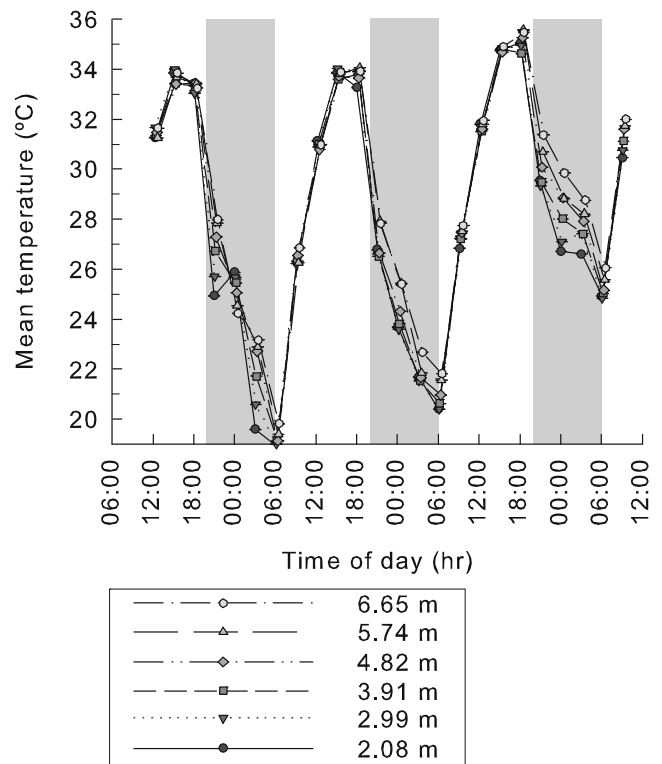
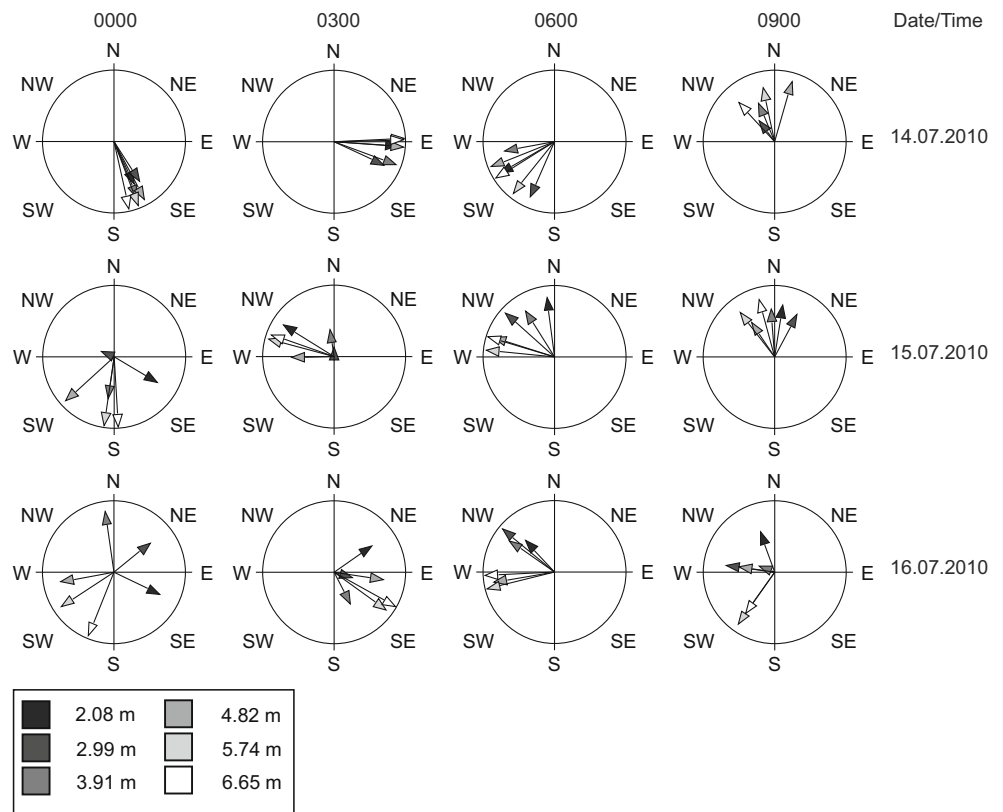


Fig. 2 Mean temperature (\pm SE) recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken over 5-min periods at six different heights above ground level, every 3 h, from July 13–16, 2010. Grey-shaded areas indicate scotophase

anemometer heights was not randomly distributed among day blocks ($\chi^2=16.09$, $d.f. = 2$, $P<0.001$), with day blocks two and three (when the anemometer was facing south and closer to the canopy) possessing more differences than day block one. There was also a non-random distribution among times of day ($\chi^2=64.92$, $d.f. = 7$, $P<0.001$), with 2100 and 0000 having the most differences in wind direction among anemometer heights (Table 1), suggesting that fluctuations in wind direction with height are more likely to occur in the four hours after sunset. In comparisons of the magnitude of circular SD at different anemometer heights and different time points, there were differences at 0600 (K-W $\chi^2=13.87$, $N=18$, $P=0.02$), 1800 (K-W $\chi^2=11.76$, $N=18$, $P=0.04$), 2100 (K-W $\chi^2=13.12$, $N=18$, $P=0.02$), and 0000 (K-W $\chi^2=14.01$, $N=18$, $P=0.02$), but not at any of the other time points. In all cases, magnitude of circular SDs decreased with increasing height, with the exception of values recorded at the lowest anemometer height (Fig. 4), suggesting that fluctuations in wind direction were greater at lower heights at these time points. A breakdown of the circular SDs into 30 sec intervals at the 0300 time point gives a measure of the intensity of fluctuations in wind direction at each height over the 5-min time course and, therefore, a proxy of the turbulence of air flow (Fig. 5). Notably, there was a dramatic reduction in circular SDs measured above the top of the canopy compared

Fig. 3 Mean wind direction recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken over 5-min periods at six different heights above ground level, every 3 h, from July 13–16, 2010 (only results for 0000–0900 are shown, for other time points see Supplementary Fig. 2). Arrows indicate the mean wind direction, and the length of each arrow represents the mean vector length (*r*-value) of the corresponding mean direction



to those measured below the top of the canopy, and peak fluctuations appeared to occur at heights in line with the central portion of the canopy (2.99 m).

Mean wind velocities measured over the recording period ranged from 0.05 to 2.01 m·s⁻¹. Translated into maximal fetch, this means the distance a plume could have traveled over the 5 min recording period ranged between ca. 15–600 m (Fig. 6). Mean maximal fetch varied with anemometer height ($F_{5,10}=22.27, P<0.001$), time of day ($F_{7,14}=7.69, P=0.001$), and day block ($F_{2,18}=4.64, P=0.02$). There was an interaction between anemometer height and day block ($F_{10,69}=2.63, P=0.01$), and time of day and day block ($F_{14,69}=6.15, P<$

0.001), but not between anemometer height and time of day ($F_{35,69}=1.02, P=0.46$). There were no differences in mean vertical displacement among anemometer height ($F_{5,10}=1.14, P=0.40$), time of day ($F_{7,14}=0.26, P=0.96$), or day block ($F_{2,16}=2.29, P=0.13$; Fig. 7). There was, however, an interaction between time of day and day block ($F_{14,69}=4.16, P<0.001$).

In total, for all the recordings made, the mean vertical wind direction was not randomly distributed ($\chi^2=71.34, d.f. = 1, P<0.001$), being above horizontal in 122 recordings and below horizontal in 21 (Fig. 8). However, whether the wind blew toward an above horizontal or below

Table 1 The total numbers of differences in mean wind direction, resulting from pairwise comparisons of wind direction recorded at different heights above ground level in an almond orchard. Measurements of wind direction were taken over 5 min periods at six different heights, every 3 h, between July 13–16, 2010. Each cell in the table contains the

total count of the number of differences in mean wind direction in pairwise comparisons between the six different heights at that time point on that day block; therefore the maximum count for each cell is 15. Mean wind direction was considered to be different between two heights if the circular SD of the mean wind directions did not overlap

Day block	Time of day								Total
	0000	0300	0600	0900	1200	1500	1800	2100	
1	0	0	0	0	0	1	0	0	1
2	6	0	3	0	0	0	0	8	17
3	10	0	2	2	0	0	0	4	18
Total	16	0	5	2	0	1	0	12	36

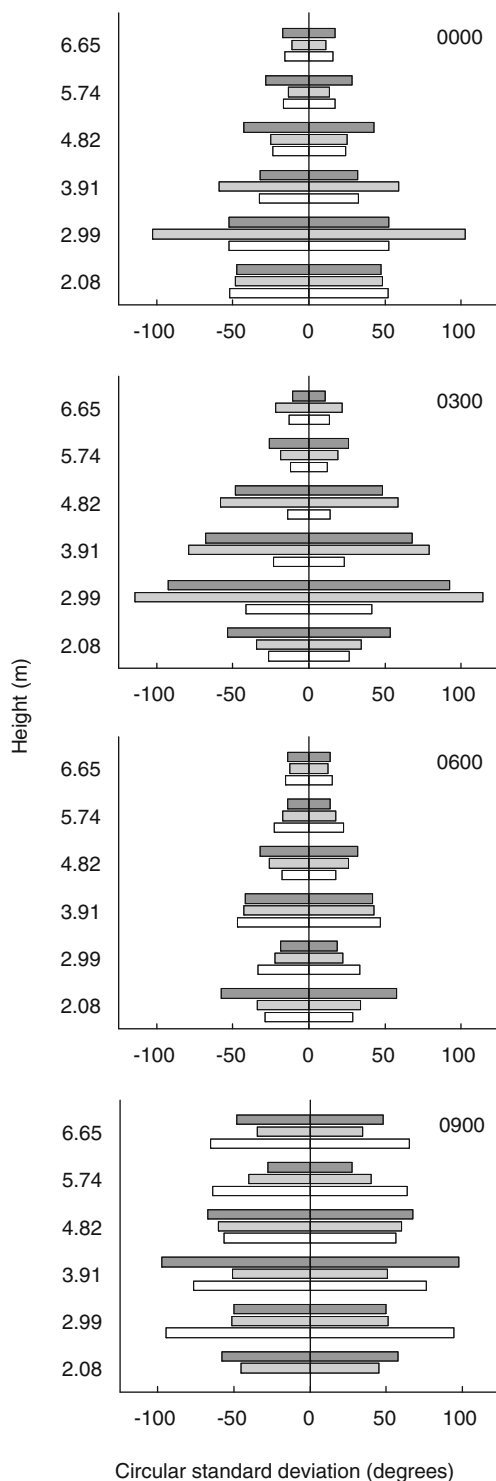


Fig. 4 Circular standard deviation of the mean wind direction, in degrees, recorded over a 5-minute period within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken at six different heights above ground level, every 3 h, from July 13–16, 2010 (only results for 0000–0900 are shown, for other time points see Supplementary Fig. 3). For each measurement, mean wind direction is adjusted to zero degrees to allow for direct comparison of standard deviations. White bars represent measurements taken on day block 1, light grey day block 2 and dark grey day block 3

horizontal vector for a larger percentage of each recording did not differ between anemometer height (Wald $\chi^2=7.49$, $d.f. = 5$, $P=0.19$) or time of day (Wald $\chi^2=9.02$, $d.f. = 7$, $P=0.25$), but did differ between day block (Wald $\chi^2=21.91$, $d.f. = 2$, $P<0.001$). A generally upward movement of plumes was corroborated by observations of plume flow from point sources of TiCl_4 positioned throughout the canopy; these most often had either upward or horizontal trajectories (Suppl. Fig. 5).

Discussion

Mate finding by male moths has been characterized as “race to find the female” (Greenfield 1981). Male ranging flight should be expected to favor strategies that maximize the possibility of encountering a female’s pheromone plume quickly and with energetic efficiency. This question has been considered mainly with respect to direction of wind flow in the horizontal plane (reviewed by Cardé et al. 2012). In habitats, such as forests and orchards, where females might call over heights extending over many meters, females conceivably could position themselves at heights that provide maximum reach of the plume. Given that plumes can be shredded as they pass through dense foliage, calling from near the top of a dense canopy could prove advantageous to securing a mate.

We have, however, little direct information on the heights that female moths select for calling. In the flightless strain of the gypsy moth, *Lymantria dispar*, a forest denizen, most females pupate on tree trunks and, following eclosion, call very nearby their eclosion site (Doane 1968; Cardé and Hagaman 1984). Female calling height and substrate are, therefore, largely set by the larva’s choice of a pupation site, which can range from the duff on the forest floor (in which case the female will crawl to a vertical object and ascend it) to the top of the canopy. Male gypsy moths appear to shuttle between two mate-location strategies: mainly horizontal flight (Elkinton and Cardé 1983) and tree-oriented vertical flight (Cardé and Hagaman 1984). Because gypsy moths mate during daytime, direct observations of flight tracks and female calling strategies are readily obtained.

In *A. transitella*, as with nearly all moths that occur in a habitat with a vertical expanse, we have no idea whether calling females perch at preferred heights in the canopy or not. The captures of males at traps positioned at various heights in almond trees (Fig. 1), however, suggest that males engage in ranging flight with roughly equal frequency throughout the canopy, and that calling females also are likely distributed throughout the canopy. This distribution contrasts with that found for *C. pomonella* which, based on preferential capture in pheromone traps near the top of apple (Weissling and Knight 1995) and walnut trees (Sibbett et al. 1993),

Fig. 5 Circular standard deviation of the mean wind direction, in degrees, recorded every 30 sec, over a 5-min period, at 0300 PST within (<5 m) and above (>5 m) the canopy of an almond orchard. For each 30 sec time point, only the positive standard deviation is represented. Black lines represent measurements taken on day block 1, dark grey day block 2 and light grey day block 3

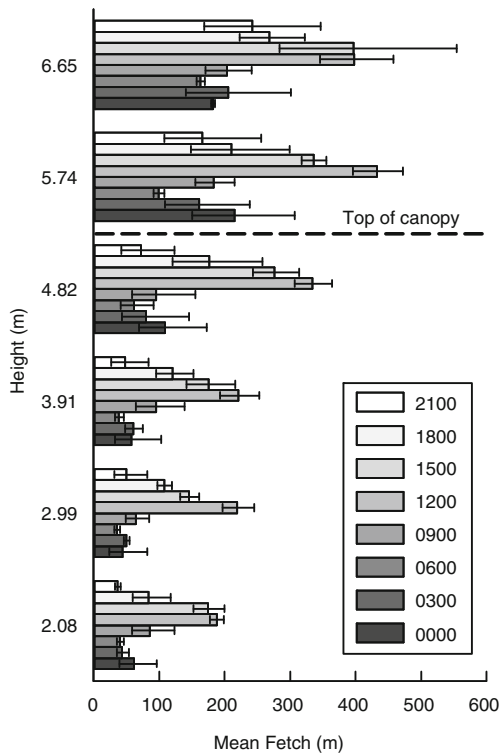
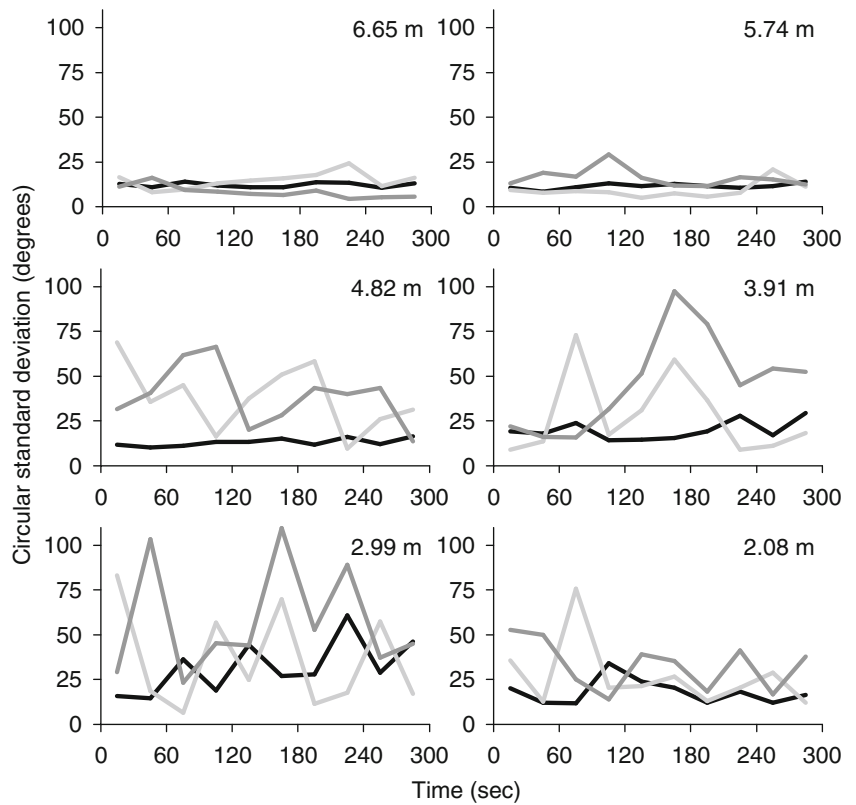


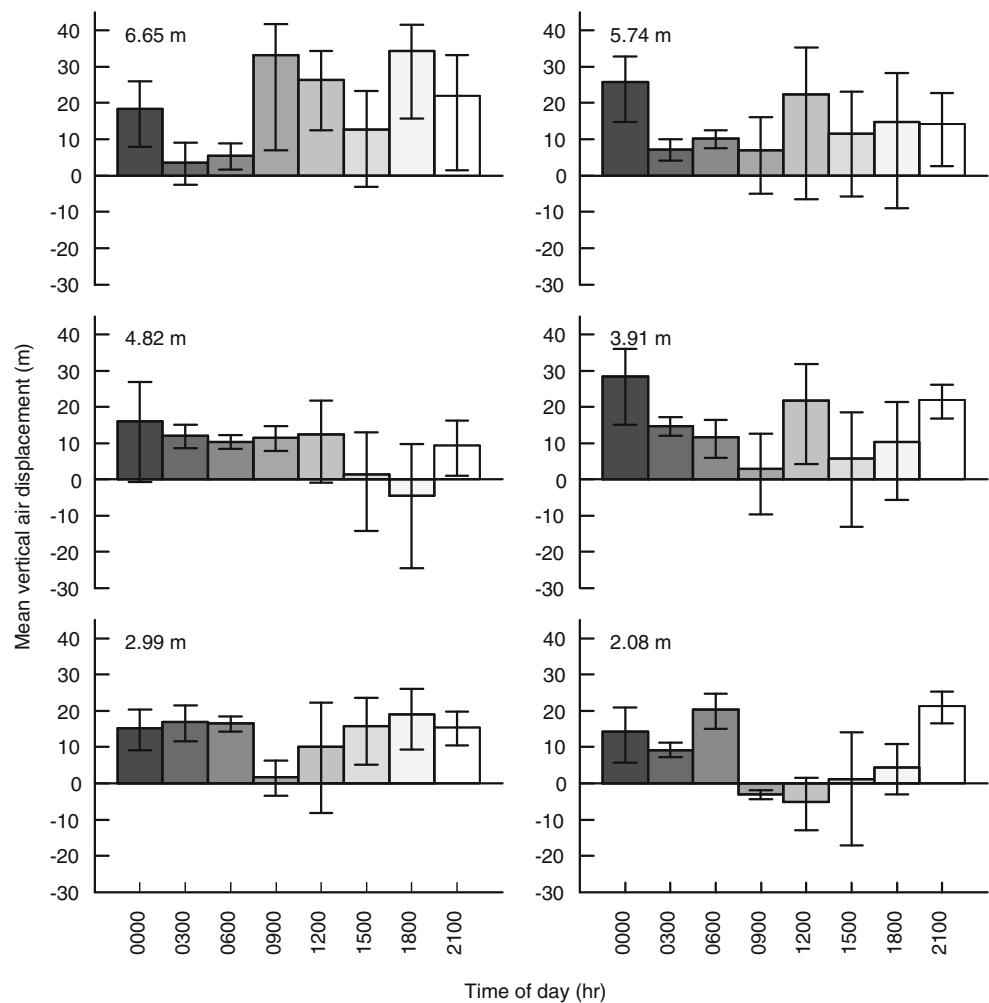
Fig. 6 Mean maximal fetch (m), over a 5-min period (\pm SE) recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken at six different heights above ground level every 3 h from July 13–16, 2010 ($N=3$). Results shown in bars above the dotted line were recorded above the canopy of the orchard. Mean maximal fetch was log transformed for analysis

suggests that males favor ranging flight near the canopy top. A confounding aspect of this interpretation is that when a pheromone source is near the top of the canopy, the plume’s integrity and greater downwind projection should also favor captures at this height.

The anemometer recordings were taken in midsummer during the second flight period of *A. transitella*; at this time, almond trees are fully leaved and temperatures are at their yearly peak. Our overall findings, at the time of *A. transitella* mating (3:00 to 6:00; Coffelt et al. 1979), were: 1) wind flow velocities were highest above the canopy, and attenuated at heights in line with the center of the canopy; 2) there was a net flow of air upward through the canopy and above the canopy, although for a much smaller proportion of time air movement had a net downward flow; 3) temperatures increased from 2.08 to 6.65 m, but were highest on the soil surface; and 4) variability in wind direction or meander was greatest at heights in line with the center of the canopy where wind speed is attenuated. The latter correlation generally matches the observations of Brady et al. (1989) in Zimbabwe where in open woodland with a shrubby understory the rate at which wind changed direction was negatively correlated with wind speed.

Our measurements of vertical movement of the plume are similar to the observations of Schal (1982) in a Costa Rican rainforest during the dry season. There, a lapse rate of several degrees between ground level and ca. 1 m imparted a

Fig. 7 Mean vertical air displacement (m), over a 5-min period (\pm SE) recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. Positive values equate to a net rise in air movement and a negative value, a net fall. Measurements were taken at six different heights above ground level, every 3 h from July 13–16, 2010 ($N=3$). Results shown in bars above the dotted line were recorded above the canopy of the orchard. Mean air displacement was reflected and then log transformed for analysis



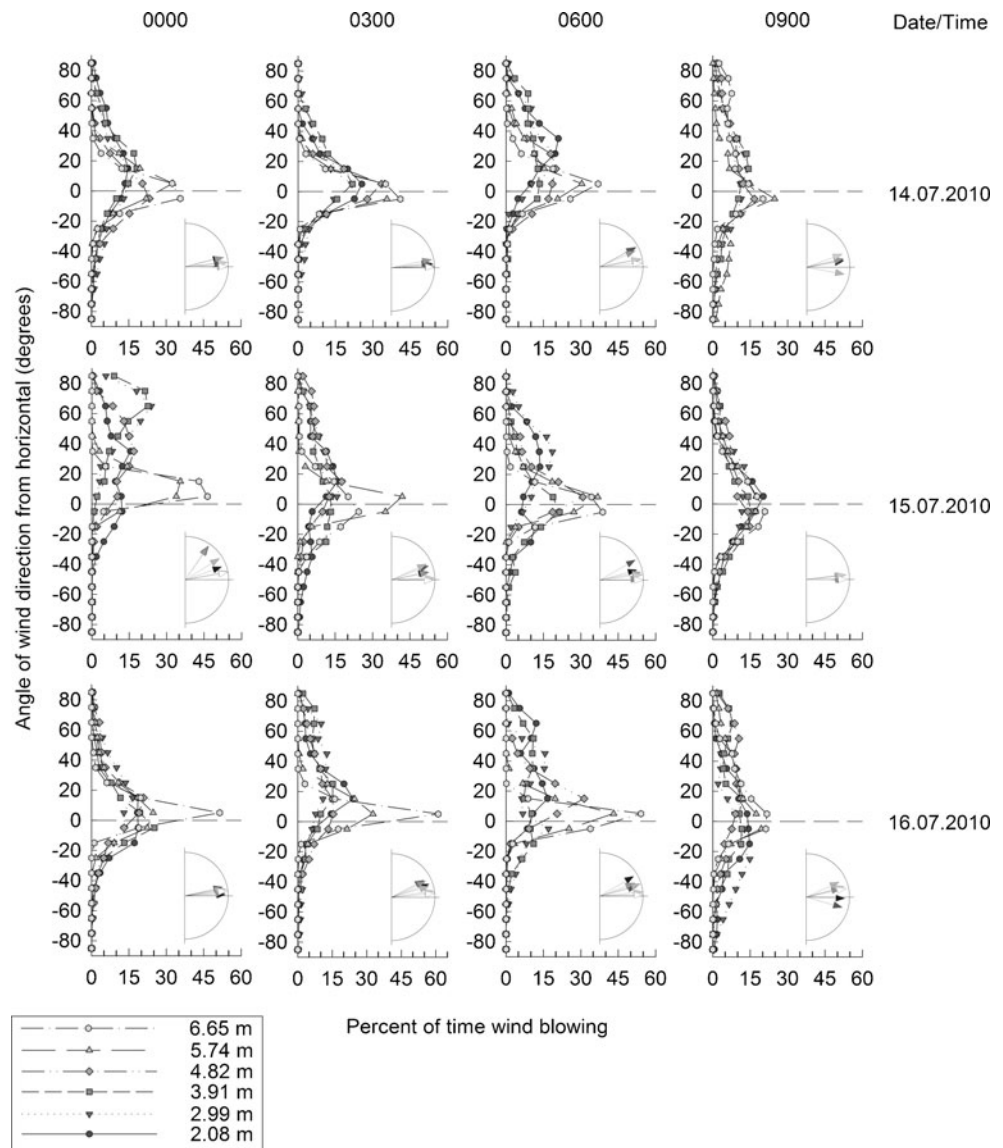
convective ascent to pheromone plumes, and males of several cockroach species adopted perching heights on trees generally higher than the calling sites used by conspecific females. This strategy should position cockroaches favorably for interception of rising pheromone plumes. In our observations, there was a similar lapse rate from 0300 to 0600 between ground surface temperature and 2 m, the lowest recording position for our sonic anemometer and its temperature sensor. At elevations from 2 m to above the canopy, however, the temperatures increased progressively. This should result in vertical mixing at these higher levels and, clearly, there were proportions of time when the net airflow was downward. Overall, however, net airflow was upward.

Daytime measurements of turbulent structure at 0.51 and 0.14 of canopy height in a similar, fully-leafed almond orchard (Baldochi and Hutchison 1987, 1988) verified that plant parts break down plumes that either originate from within the canopy or pass through it. This process attenuates the plume's filamentous structure, thereby potentially diminishing a moth's ability to orientate along the plume, while also reducing the plume's peak concentrations as it is carried downwind,

thereby diminishing its active space. Thus, the optimal calling position for *A. transitella* females in almond orchards may be near the top of the canopy, on the assumption that males would match their ranging flights to this height. Males also should be able to sample a wider swath of the orchard environment by flying just above the canopy top rather than through the tree foliage. Our comparisons of male capture at various heights, however, indicate that males must be ranging vertically throughout the canopy, and further suggests that calling females are distributed similarly.

The patterns of wind flow in this environment have implications for deployment of high-dose, point-source dispensers, such as aerosol sprayers ("puffers") used to disperse pheromone for mating disruption (Shorey and Gerber 1995). For *A. transitella*, these dispensers are typically placed in the top two thirds of the canopy of almond, pistachio, and fig trees at rates of about 5/ha, or about 20 m apart (e.g., Burks and Brandl 2004), and typically are set to release pheromone for the 12 h that encompass darkness. The height of placement has been predicated mainly on the assumption that mating might be more prevalent near the top of the canopy, and that the optimal

Fig. 8 The percentages of time that wind blew at different vertical vectors over a 5-min period within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken at six different heights above ground level, every 3 h from July 13–16, 2010. Positive angles equate to the wind blowing upward at that angle, negative angles downward and the hashed line horizontal. Within each plot, a semi-circle depicts the mean directional vector within the vertical plane for each measurement height at that time point on that day. The line dividing the semi-circle equates to a completely horizontal vector of zero degrees. The shade of the arrowheads indicates the measurement height, a black head representing 2.08 m, a white head 6.65 m, and increments of grey, the four heights in between (only results for 0000–0900 are shown, for other time points see Supplementary Fig. 4.)



dispersion of disruptant should be at this level. Because the net flow of air is upward, however, placement of dispensers only near the top of the canopy could render disruption near the lower reaches of the canopy less efficacious, with much of the disruptant released near the top of the canopy eventually rising above it and not contributing to disruption. Furthermore, because maximal fetch varies very little at different heights throughout the canopy, the height at which a dispenser is placed should have negligible effect on the coverage area of each dispenser. As high-dose dispensers are also used for mating disruption of orchard pests that mate at dusk and early evening (e.g., *C. pomonella*, Castroville and Cardé 1979) or in early afternoon (e.g., the oriental fruit moth, *G. molesta*, Baker and Cardé 1979), the vertical displacement of odor plumes is of importance at times other than 03:00–06:00. In

fact, *A. transitella* mating behavior (female calling and male attraction to calling females) shifts to earlier, warmer hours on cooler nights of spring and autumn (Landolt and Curtis 1982). One important caveat is that the measurements reported here were made at midsummer in fully-leaved almond orchards located in the Central Valley of California; whether they apply to springtime conditions (with comparatively little foliage and lower temperatures) or to other canopied environments, such as those on hill-sides, remains to be determined. However, the generally upward displacement of airflow (Fig. 8, Suppl. Fig. 4) from 15:00–06:00 found in the present study suggests that high-dose, low-density disruptant dispensers should be deployed at mid- or even low-canopy levels for all species that mate from late afternoon to dawn in orchards.

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