

Pheromone puff trajectory and upwind flight of male gypsy moths in a forest

J. S. ELKINTON, C. SCHAL,* T. ONO† and R. T. CARDÉ

Department of Entomology, University of Massachusetts, Amherst, U.S.A.

ABSTRACT. Pheromone released from a point source beneath a forest canopy usually follows a non-linear trajectory as demonstrated by the paths of small, neutrally-buoyant, helium-filled balloons or puffs of smoke. Mark-recapture experiments show that few male gypsy moths (*Lymantria dispar* L.) follow a pheromone plume over distances greater than 80 m even though they can easily detect pheromone at that distance as indicated by wingfanning assay. The directional consistency of successive puffs of pheromone appears more important than the linearity of their trajectories in enabling males to locate a pheromone source.

Key words. Pheromone, plume, puff trajectory, pheromone dispersion, anemotaxis, threshold, *Lymantria dispar*, gypsy moth.

Introduction

David *et al.* (1982, 1983) have demonstrated that the long axis of a pheromone plume is often aligned across both the instantaneous and mean wind directions. Previously the pheromone plume had been visualized as a meandering cloud whose axis was parallel to the wind direction (Fig. 1A). Furthermore, David *et al.* presented evidence that the individual puffs of pheromone-laden air that constitute a pheromone plume follow linear trajectories for a distance of at least 20 m after leaving a point source (Fig. 1B). Consequently, an insect that flies upwind upon detection of pheromone will fly

* Present address: Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey 08903, U.S.A.

† Present address: Biology Laboratory, Kinjo Gakuin University, Omori, Moriyamaku, Nagoya 463, Japan.

Correspondence: Dr J. S. Elkinton, Department of Entomology, University of Massachusetts, Amherst, MA 01003, U.S.A.

towards the source, but not necessarily along the centre line of the plume. However, their experiments were conducted *c.* 1.5 m above a large field of short grass. In this study we monitored the trajectory of puffs of smoke and neutrally-buoyant balloons under more natural conditions for gypsy moth (*Lymantria dispar* L.) pheromone communication beneath a forest canopy. We also determined what proportion of male gypsy moths were able to locate a pheromone source following their release into a pheromone plume at varying distances downwind under a variety of wind conditions.

Materials and Methods

Experiments were conducted on the Prescott Peninsula of the Quabbin Reservoir Forest, Massachusetts, in August and September of 1982 and 1983 in a pine (*Pinus resinosa*) plantation with a closed canopy, and devoid of understorey vegetation. The trajectory of individual

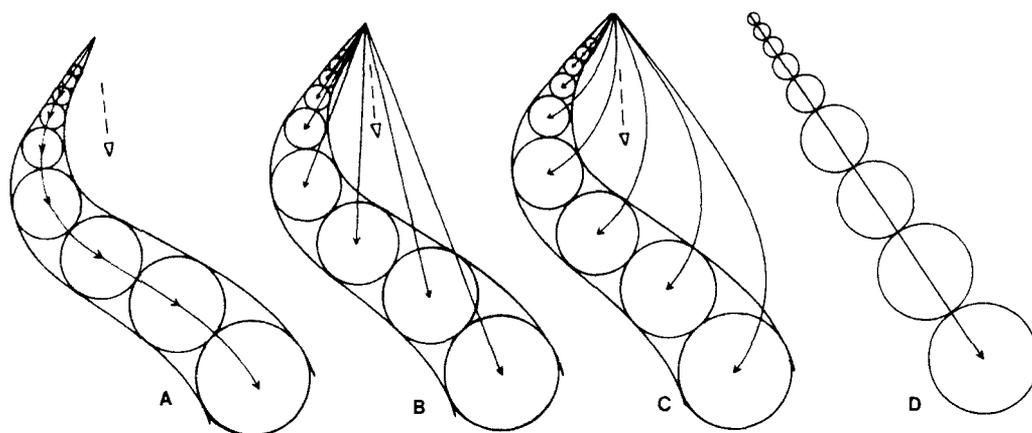


FIG. 1. Schematic representation adapted from David *et al.* (1982) of the trajectory of individual puffs of which a pheromone plume is comprised: (A) the trajectory envisioned by many people prior to the publication of David *et al.*; (B) the trajectory as described by David *et al.* for experiments conducted above an open field; (C) the trajectory that most nearly describes those observed in this experiment beneath a forest canopy; (D) the trajectory characteristic of the plume during short intervals when the wind direction does not vary.

parcels of air was determined by tracking puffs of mineral-oil smoke (initial puff size *c.* 0.5 m diameter) generated with a 1 s release from a smoke generator (Model 84-11, Fire Safety Products, Minneapolis, Minn.) and 7 cm diameter helium-filled balloons weighted so that they were neutrally buoyant. An observer followed *c.* 2 m behind a smoke puff for as long it was discernible, or followed the balloon until it collided with a tree or the ground. At 5 s intervals the position of the balloon or smoke puff was marked by a stake on the ground below it. The direction and distance of each stake from the central release point were then measured and transcribed onto polar graph paper.

The male flight experiments were conducted in August 1982. During each trial, thirty laboratory-reared, 1–2-day-old male gypsy moths were placed individually in cylindrical wire mesh cages mounted on vertical 61 × 61 cm wiremesh racks (Elkinton *et al.*, 1984) 1.6 m above ground at distances of 20, 40, 80 and 120 m from a pheromone source. Males were marked on the wings with spots of indelible ink in a pattern unique for each trial, rack and distance. The pheromone source was a cotton wick with 100 µg of (+)-disparlure on top of a wooden stake at a height of 1.6 m. A 20 cm diameter black cylinder made of stove pipe was placed around the stake from a height of 15 cm below the pheromone wick down to ground level. Another

section of stove pipe was suspended vertically 15 cm above the wick to a height of 3.3 m. The stove pipe served as a vertical silhouette that enhanced the attraction of males over a distance of a few metres from the source and the 30 cm gap between the two sections of pipe allowed the pheromone to disperse freely in all directions.

The pheromone was put in place when the anemometer indicated a wind direction blowing directly towards the males. Observers at each rack noted the occurrence of wing fanning, a sensitive indicator of the presence of pheromone (Elkinton *et al.*, 1984). When at least 30% of the moths at all four distances initiated wing fanning, all cages were opened. Males approaching within 2 m of the source were captured in insect nets and time of capture (within 15 s) was recorded. The identity of the mark, and thus the site of release, was established subsequently. The capture of moths continued for 40 min after release of the males.

For the puff trajectory trials, horizontal wind speed and direction were measured at 1 s intervals with an ultrasonic anemometer (Model SA-200, Kaijo Denki Co., Tokyo, Japan). This instrument was capable of measuring wind speed and direction at speeds below 30 cm s⁻¹ which are common beneath a dense forest canopy. Digital output from the anemometer was recorded on video cassette and subsequently transcribed onto a computer file. For the male

flight experiment, wind speed and direction from the anemometer were recorded at 10 s intervals on a CR-21 datalogger (Campbell Scientific Inc., Logan, Utah). The anemometer was positioned at a height of 2 m at the centre of the grid for the balloon and smoke trajectory experiments, and c. 5 m south of grid centre for the male flight experiments.

Results

The trajectories of smoke puffs and balloons were often highly non-linear, even over distances of less than 10 m (Fig. 2). The mean differences between the upwind direction at the time of release and the moment at which the balloon or smoke puff reached a distance of 20 m from the source were respectively 58° and 35° (Table 1), and the means of total change in direction of the puff over the interval were 106° and 158°. At higher wind speeds the tracks of the smoke puffs and balloons were straighter. There

TABLE 1. Mean differences (\pm SD) between the instantaneous directions of a smoke puff or balloon at 5, 10 and 20 m from the source and the direction taken at the instant of release, and the mean (\pm SD) total change in direction of the puff between the source and 5, 10 and 20 m; $n=26$ trials with balloons, 20 with smoke puffs.

Distance from source (m)		Difference from direction taken at release	Total change in direction
5	Balloon	23 \pm 35°	27 \pm 42°
	Smoke	20 \pm 28°	28 \pm 49°
10	Balloon	38 \pm 33°	62 \pm 71°
	Smoke	28 \pm 28°	64 \pm 84°
20	Balloon	58 \pm 92°	106 \pm 100°
	Smoke	35 \pm 33°	158 \pm 149°

was a negative correlation between wind speed and the total change in puff direction ($r=-0.37, -0.35, -0.52; P<0.01$) at 5, 10 and 20 m respectively. Mean wind speed during the 1 min interval following release was 0.66 m s⁻¹ for smoke puffs and 0.55 m s⁻¹ for balloons.

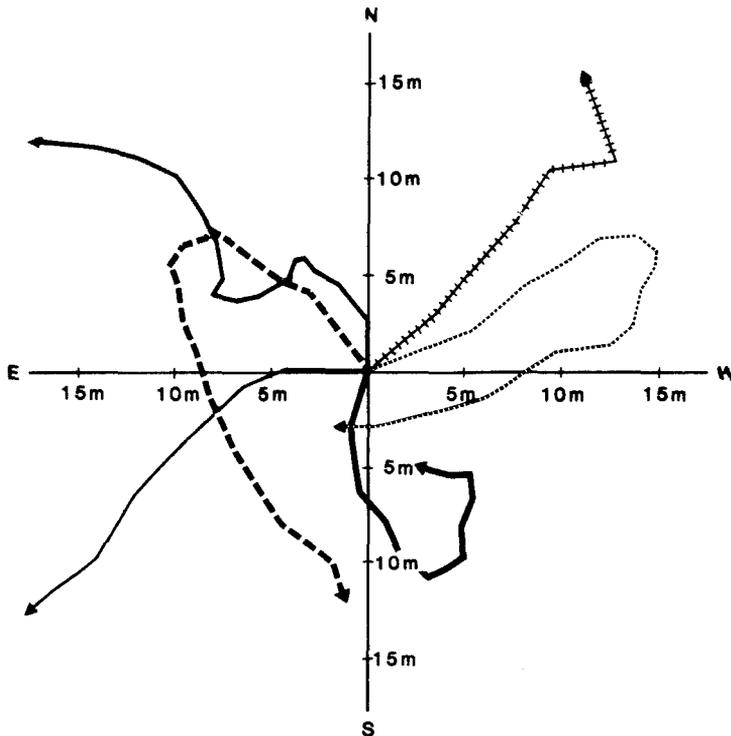


FIG. 2. Five typical trajectories in the horizontal plane of small helium-filled, neutrally-buoyant balloons and released at a height of 2 m within a (*Pinus resinosa*) stand. Each point on the trajectory represents the position of the balloon at 5 s intervals.

TABLE 2. Correlations between instantaneous puff direction 15, 30 and 60 s after release and (1) the initial puff direction at the moment of release, and (2) the instantaneous wind direction recorded simultaneously at the source. Number of tracks plotted was 46, 46 and 26 at the respective times.

	Pearson's correlation coefficient*		
	15 s	30 s	60 s
Initial puff direction	0.84	0.73 [†]	0.59 [†]
Simultaneous wind direction at source	0.97	0.97	0.88

* All correlations significant at $P < 0.01$.

[†] Correlation with simultaneous wind direction at source is significantly higher ($P < 0.05$; z transformation, t -test; Sokal & Rohlf, 1981, p. 589) than with initial puff direction.

The correlation between the initial direction of the puff and its subsequent direction declined from 0.84 to 0.59 between 15 and 60 s after release (Table 2). There was a significantly higher correlation (t -test, Fisher's z transformation; Sokal & Rohlf, 1981, p. 589) between the puff direction at 30 and 60 s after release and the wind direction measured simultaneously at the source, than with the initial puff direction. This implies that a large change in direction of the

puff was usually accompanied by a nearly simultaneous and similar change in direction at the source and elsewhere over an area spanning at least 20 m. In other words, Fig. 1C more nearly describes the trajectory of a pheromone plume than Fig. 1B.

The simultaneous change in trajectory was visually apparent with smoke puffs. By the time the smoke puff had reached a distance of 30 m or more from the source it had usually grown into a large cloud at least 5 m in diameter and enveloped several trees simultaneously. Nevertheless the entire puff would change direction at the same time. Thus the observed changes in trajectory did not seem to be caused by deflection from individual trees. David *et al.* (1983) reported a similar, nearly simultaneous change of parallel wind vectors across an array of wind direction indicators. However, in their experiment the change in wind direction moved across the array at the same speed as individual puffs and thus did not overtake and change the trajectory of previously released puffs.

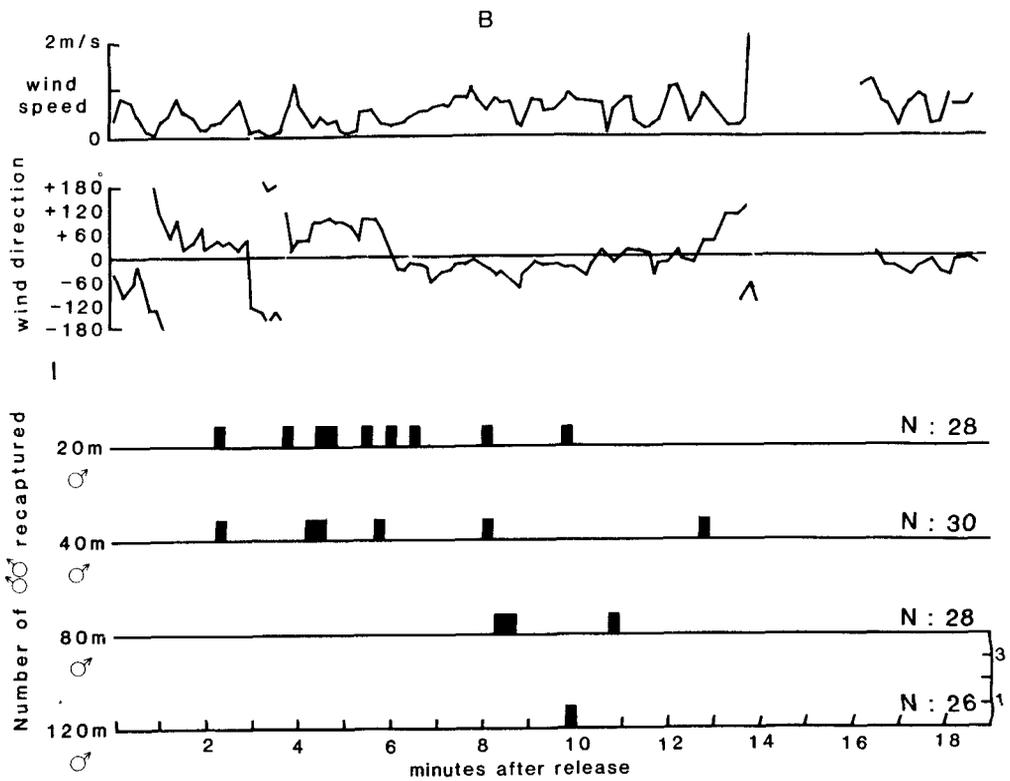
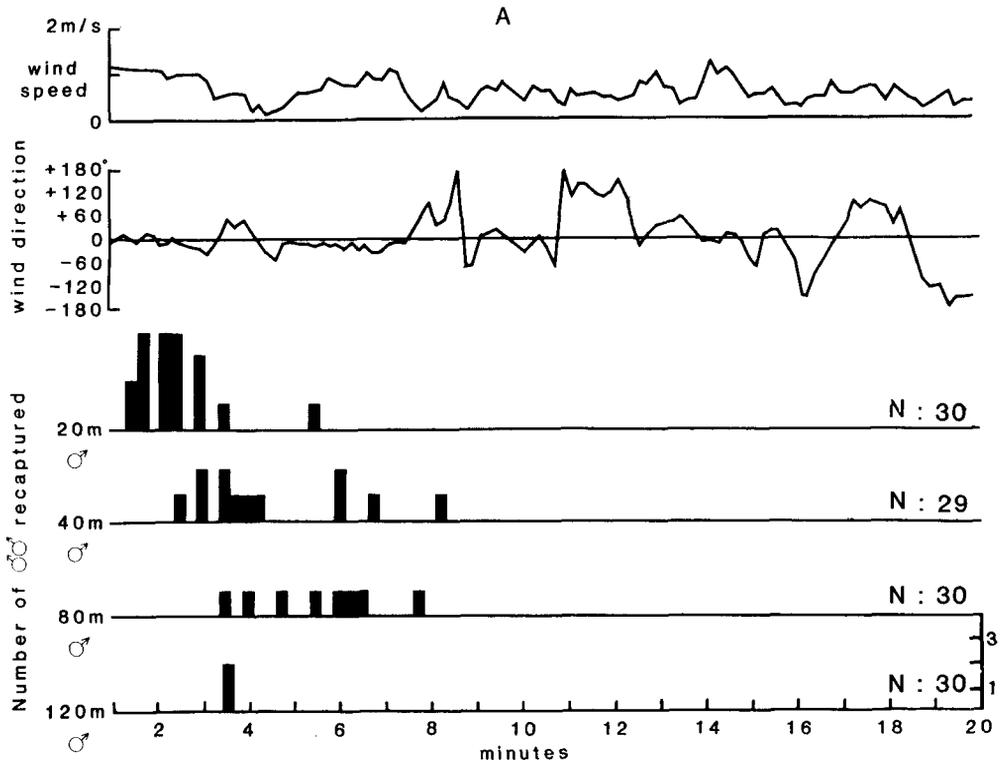
Of the males that flew out of the wire mesh cages, the percentage that arrived at the source over the subsequent 40 min interval declined from 45% at 20 m to 8% at 120 m (Table 3). There was considerable variability between trials in the proportion of moths arriving. On trials when the wind direction blew in the

TABLE 3. Mean (\pm SD) proportion of thirty male gypsy moths leaving wire mesh cages at 20, 40, 80 and 120 m from a pheromone source after initiation of pheromone-induced wing-fanning response. The average proportion (\pm SD) of those leaving that arrived at the source within a 40 min period and the average minimum (\pm SD) and median (\pm SD) time of transits; $n = 23$ replications.

Distance from source (m)	Proportion leaving cages	Proportion leaving that arrived at source	Average median time of transit (min)	Average minimum time of transit (min)
20	0.89 \pm 0.14 ^{a*}	0.45 \pm 0.20 ^a	3.9 \pm 2.6 ^a	1.7 \pm 1.3 ^a
40	0.85 \pm 0.13 ^{ab}	0.27 \pm 0.14 ^b	5.7 \pm 3.9 ^b	3.8 \pm 2.7 ^{bd}
80	0.81 \pm 0.16 ^{bc}	0.17 \pm 0.15 ^c	7.0 \pm 3.8 ^b	5.0 \pm 3.3 ^c
120	0.65 \pm 0.27 ^c	0.08 \pm 0.07 ^d	9.7 \pm 3.7 ^c	8.9 \pm 4.5 ^d

Values followed by the same lower-case letter are not significantly different by pairwise comparisons, Wilcoxon's signed rank test at an experimentwise $P < 0.05$ via Sidak's inequality (Sokal & Rohlf, 1981, p. 242).

FIG. 3. Number of male gypsy moths recaptured with insect nets within 2 m of a pheromone source at 15 s intervals following simultaneous releases of thirty males from wiremesh cages at 20, 40, 80 and 120 m downwind. Releases occurred when at least 30% of the males at all distances initiated wing fanning, indicating that they had detected pheromone. Wind speed and direction were recorded at 10 s intervals with an ultrasonic anemometer located near the grid centre. A wind direction of 0° occurs when the wind is blowing directly from the source toward the moth cages. N is the number of males that left the cage.



general direction of the moths for a few minutes after release, substantial proportions arrived from all except 120 m (Fig. 3A). When the wind direction deviated widely from the initial direction toward the racks, few males came in from any distance (Fig. 3B).

Discussion

The non-linear trajectory of pheromone puffs in the forest suggests that moths flying upwind in response to pheromone often will not be headed directly towards the source. However, the *mean* puff direction is more likely to be towards the source, so that directional errors will tend to cancel one another and net movement towards the source will still occur. Of greater importance, however, is the existence of short intervals in which the wind direction as measured at the source does not vary, resulting in plume segments in which the long axis of the plume, the upwind direction and the direction toward the source are coincident. Moths that fly upwind upon encountering these segments move towards the source. During these intervals the trajectory of each puff is, indeed, linear as David *et al.* have suggested, but in this situation all three models (Fig. 1A, B, C) of the plume would appear identical. In other words Fig. 1D would be characteristic of the instantaneous plume for short intervals of time regardless of which of the models is correct.

The differences between the three models became apparent and our findings differ from those of David *et al.* (1982, 1983) when the wind direction, as recorded at the source, changes. When this occurs in the forest the trajectory of each individual puff also changes (Fig. 1C) and moths flying upwind in response to pheromone would no longer be flying directly towards the source. Furthermore, when the wind direction changes, the resulting plume is aligned across the instantaneous wind direction (Fig. 1B, C) as David *et al.* (1982) have observed. When that occurs, upwind flight takes the moths out of the plume and little progress towards the source ensues, even in their experimental conditions in which puff trajectory remained linear (Fig. 1B). Thus we believe that substantial movement by a moth in the direction of the source only occurs when the long axis of the plume and the wind direction are parallel (Fig. 1D). It is the consis-

tency of wind direction over short intervals of time and not so much the linearity of pheromone puff trajectory that enables moths to locate a source.

David *et al.* (1982, 1983) found that substantial movement towards the source was made during the crosswind casting flight that occurs when a moth flies out of the plume into clean air. They suggested that although such casts are directed with equal probability away from as towards the source, casts towards the source are more likely to encounter new puffs of pheromone. In our opinion the difference in probability of pheromone encounter between opposite legs of a cast would only be significant near the source and thus crosswind casts would probably contribute little to the net progress toward the source from distances in excess of 10 m. Indeed, we rarely observed casting behaviour in our experiments, except within 1–2 m of the source. It appeared that moths that flew out of the pheromone plume engaged primarily in tree-oriented vertical flight (Elkinton & Cardé, 1983). This behaviour is presumably a visually guided response to nearby trees, but such visual cues were not present in the experiments of David *et al.* (1982, 1983). In practice, however, it is difficult to always distinguish between tree oriented and casting flights, especially without a simultaneous record of both moth flight tracks and pheromone plume tracers. Observations of this type are needed to determine the frequency of casting flight behaviour in the forest situation.

In this study the most non-linear of the puff trajectories were associated with low wind speeds. Wind speed, however, affects pheromone communication in a number of ways. For instance, as wind speed increases, the maximum distance of pheromone detection decreases because of greater dilution of the pheromone and higher turbulence intensities, resulting in more rapid expansion of each successive puff (Elkinton & Cardé, 1984). On the other hand, at lower wind speeds the optomotor tracking capability of moths is probably reduced (Cardé, 1984). However, gypsy moths in a wind tunnel are capable of visually guided anemotaxis in wind speeds as low as 10 cm s⁻¹ (Cardé & Willis, unpubl.). In the forest we rarely recorded wind speeds as low as 10 cm s⁻¹ and never for more than a few seconds. We believe that the most important effect of increased wind speed on pheromone communication is the decrease in

the meander of wind direction (Slade, 1968, p. 50) resulting in longer tracks of successive puffs travelling in the same direction. Thus for gypsy moth and perhaps for most organisms the *effective* distance of communication and the numbers attracted to the source are likely to increase with wind speed at the lower end of the range of wind speeds under which upwind flight occurs.

For a given angular velocity (change in direction per unit time), higher wind speeds will transport a puff over a greater distance and the resulting track will be more linear. This may explain, in part, why we observed more linear trajectories at higher wind speeds and why David *et al.* (1982), who were tracking smoke puffs at mean wind speeds 2–3 times greater than those reported here, observed nearly linear trajectories, whereas we observed highly non-linear trajectories over a comparable distance. However, if we compare the changes in direction that we recorded at 5 or 10 m from the source (Table 1), with that observed by David *et al.* (1982) at 20 m from the source, it appears that we were recording higher angular velocities as well. Furthermore, we suspect that as long as there is at least some wind over open ground, one would rarely observe the highly convoluted trajectories (changes in direction exceeding 180° over an interval <30 s) that we frequently observed (Fig. 2) under light wind conditions beneath a forest canopy. When wind speeds above the canopy are low, wind direction below the canopy may be completely uncoupled from that above (Oliver, 1975). The sudden changes in direction that we recorded in these conditions may be due to changes in the pressure gradient across the stand due to transient convections arising from clearings at the edge of the stand (J. Murlis, personal communication) or within the canopy of the stand (Oliver, 1975). Alternatively, they may arise from the penetration of gusts of wind from the ambient flow above the canopy (Smith *et al.*, 1972). The apparent differences in angular puff velocity that we recorded compared with those of David *et al.* (1982) may be explained by the results of Allen (1968) who computed turbulent power spectra from wind speed measurements taken at various heights within and above a Japanese larch plantation. He found that below the canopy the turbulence is concentrated at frequencies of 0.04–0.10 cps. These correspond to gusts of wind and, in our results, to major changes in puff direction occur-

ring on the average of every 10–25 s. Above the forest canopy (which is presumably more similar to the experimental conditions of David *et al.*) a larger proportion of the turbulent energy was concentrated at both lower and higher frequencies. The higher frequencies correspond to turbulence occurring at a scale too small to cause major changes in the trajectory of a soap bubble, balloon or puff of smoke. Compared with over open ground, turbulence beneath a forest canopy is more concentrated in the frequency range that results in more violent plume meander and changes in puff trajectory within a time frame of 5–30 s. At lower wind speed parcels of air have less momentum and smaller changes in pressure gradients are required to induce substantial changes in direction (J. Murlis, personal communication).

Our findings have implications for field tests of synthetic attractants. Higher release rates or more attractive blends may elicit response at greater distances downwind but may not result in increased trap catch. For instance, the number of male gypsy moths captured in traps was elevated only 16% when the release rate of pheromone was raised by increasing the dose of (+)-disparlure applied to a cotton wick from 50 to 500 µg, whereas a change from 5 to 50 µg increased catch 104% (Cardé *et al.*, 1977). Such results are usually interpreted as evidence of deterrence by high release rates as the responding insect enters the region of high concentration near the source. There are direct observations supporting such arrestment of upwind progress in *Grapholitha molesta* (Busck) (Cardé *et al.*, 1975; Kuenen & Baker, 1982) and the gypsy moth (Charlton *et al.*, 1987). However, our study suggests another important factor. Elevation of pheromone emission rates can result in detection by males at greater distances, but few of these moths would be able to locate the source. Certainly in our experiments most of the males initiated wing fanning at distances of 80 and 120 m from the source, but few of them flew to the site of pheromone release over a 40 min interval.

Our results also have significance for the evolution of pheromone release rates, and thresholds for pheromone perception. Early studies have been interpreted as demonstrating that male moths could detect and orient to pheromone over distances of several km (e.g. Collins & Potts, 1932; Bossert & Wilson, 1963).

Our data suggest that it is unlikely that moths could follow a pheromone plume over such distances. Female gypsy moths release pheromone at rates substantially lower than the synthetic lures used in this study (Charlton & Cardé, 1982). Thus, with the gypsy moth, higher rates of pheromone release or lower response thresholds might not substantially increase the effective distance of attraction.

Acknowledgments

We are indebted to Dr C. Schwalbe of USDA, APHIS, for supplying laboratory reared gypsy moth pupae, and to the National Science Foundation (PCM-79-12014) for the ultrasonic anemometer. We thank R. Charlton, M. McManus and M. Sabelis for reviewing the manuscript. We are greatly indebted to J. Murlis, C. David and J. S. Kennedy for helpful discussion and reviews.

References

- Allen, L.H. (1968) Turbulence and wind speed spectra within a Japanese larch plantation. *Journal of Applied Meteorology*, **7**, 73–78.
- Bossert, W.H. & Wilson, E.O. (1963) The analysis of olfactory communication among animals. *Journal of Theoretical Biology*, **5**, 443–469.
- Cardé, R.T. (1984) Chemo-orientation in flying insects. *Chemical Ecology of Insects* (ed. by W. J. Bell and R. T. Cardé), pp. 111–124. Chapman and Hall, London.
- Cardé, R.T., Baker, T.C. & Roelofs, W.L. (1975) Ethological function of components of a sex attractant system for Oriental fruit moth males. *Grapholitha molesta* (Lepidoptera: Tortricidae). *Journal of Chemical Ecology*, **4**, 475–491.
- Cardé, R.T., Doane, C.C., Baker, T.C., Iwaki, S. & Marumo, S. (1977) Attractancy of optically active pheromone for male gypsy moths. *Environmental Entomology*, **6**, 768–772.
- Charlton, R.E. & Cardé, R.T. (1982) Rate and diel periodicity of pheromone emission from female gypsy moths (*Lymantria dispar*) determined with a glass-adsorption collection system. *Journal of Insect Physiology*, **28**, 423–430.
- Charlton, R.E., Kanno, H., Collins, R.D. & Cardé, R.T. (1987) Influence of pheromone concentration and ambient temperature on flight parameters of the gypsy moth, *Lymantria dispar*, in a sustained-flight wind tunnel. *Physiological Entomology*, in press.
- Collins, C.W. & Potts, S.F. (1932) Attractants for the flying gypsy moths as an aid in locating new infestations. *U.S.D.A. Technical Bulletin* **336**, 43pp.
- David, C.T., Kennedy, J.S., Ludlow, A.R., Perry, J.N. & Wall, C. (1982) A reappraisal of insect flight towards a distant, point source of wind-borne odor. *Journal of Chemical Ecology*, **9**, 1207–1215.
- David, C.T., Kennedy, J.S. & Ludlow, A.R. (1983) Finding of a sex pheromone source by gypsy moths released in the field. *Nature*, **303**, 804–806.
- Elkinton, J.S. & Cardé, R.T. (1983) Appetitive flight behavior of male gypsy moths (Lepidoptera: Lymantriidae). *Environmental Entomology*, **12**, 1702–1707.
- Elkinton, J.S. & Cardé, R.T. (1984) Odor dispersion. *Chemical Ecology of Insects* (ed. by W. J. Bell and R. T. Cardé), pp. 73–91. Chapman and Hall, London.
- Elkinton, J.S., Cardé, R.T. & Mason, C.J. (1984) Evaluation of time-average dispersion models for estimating pheromone concentration in a deciduous forest. *Journal of Chemical Ecology*, **10**, 1081–1108.
- Kuenen, L.P.S. & Baker, T.C. (1982) The effects of pheromone concentration on the flight behaviour of the oriental fruit moth *Grapholitha molesta*. *Physiological Entomology*, **7**, 423–434.
- Oliver, H.R. (1975) Ventilation in a forest. *Agricultural Meteorology*, **14**, 347–355.
- Slade, D.H. (1968) *Meteorology and Atomic Energy*. U.S. Atomic Energy Commission, Oak Ridge, Tenn.
- Smith, F.B., Carson, D.J. & Oliver, H.R. (1972) Mean wind direction shear through a forest canopy. *Boundary-Layer Meteorology*, **3**, 178–190.
- Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*, 2nd edn. W. H. Freeman, San Francisco.

Accepted 2 April 1987