*Physiological Entomology* (1989) **14**, 1–12

# Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behaviour

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ABSTRACT. Peak-to-trough electroantennogram amplitudes (bursts), caused by the individual filaments of a plume of female pheromone, diminish as high-emission-rate sources are approached by male *Grapholita molesta*, and this reduction is correlated with in-flight arrestment (ceasing to advance upwind). These findings are consistent with the hypothesis that one cause of in-flight arrestment in response to high-concentration point sources is the attenuation of the peak-to-trough amplitudes close to the source. High burst frequency, high pheromone flux, or low levels of continuous neuronal activity all are less well correlated with arrestment. Rather, arrestment appears due to a reduction of chemosensory input to the CNS during flight up the plume, even though the actual molecular concentration continues to increase. In a laboratory wind tunnel, upwind flight initiation by more than 20% of males was elicited only by pheromone source concentrations evoking significant fluctuations in EAG amplitudes at downwind release points. The burst frequencies that evoked high levels of upwind flight initiation ranged from a mean of 0.4–2.2 bursts/s. Because a previous study revealed that flying male *G. molesta* change their course angle within 0.15 s of losing or contacting pheromone, these EAG burst frequencies indicate that during flight in a pheromone plume, many manoeuvres are probably made in response to contact with individual plume filaments. Thus, upwind flight tracks may be shaped by hundreds of steering reactions in response to encounters with individual pheromone filaments and pockets of clean air. Field-recorded EAGs reveal that burst amplitudes diminish from 3 to 30 m downwind of the source, whereas burst frequencies do not, averaging *c.* 1/s at 3, 10 and 30 m downwind.

**Key words.** Pheromone plume, pheromone fluctuations, upwind flight, oriental fruit moth, *Grapholita molesta,* electroantennogram.

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## Introduction

Recent behavioural evidence from three moth species suggests that fluctuating, rather than continuous, pheromone stimulation is necessary for sustained upwind flight by males to pheromone sources (Kennedy *et al.*, 1980, 1981; Willis & Baker, 1984; Baker *et al.*, 1985; A. R. Ludlow, C. T. David and J. S. Kennedy, unpublished). Kennedy (1983) hypothesized that the amplitude of stimulation, from peak to trough, from filaments in a plume (Wright, 1958) must be sufficiently high to evoke and sustain upwind flight. Baker (1985, 1986) additionally hypothesized that the arrestment in flight (cessation of upwind flight) sometimes observed close to a source with high emission rate may be due to the pheromone stimulation becoming effectively fused and therefore attenuated.

These hypotheses require that the moth's sensory system adequately registers the fine structure of the plume (the ups and downs in concentration occurring within the plume envelope; Murlis & Jones, 1981; Murlis, 1986), and that elements in the central nervous system (CNS) respond to such fluctuating input by generating upwind flight behaviour. Investigations to test these hypotheses require measurement of the fine structure of a plume, as registered by the moths' receptors (Baker, 1985, 1986).

Murlis & Jones (1981) and Murlis (1986) had previously simulated a pheromone plume with ionized air and used an ion detector to gain information about the plume's physical structure only. Baker (1985, 1986) pointed out that the actual fine structure possibly registered by moths would have both physical and biochemical limits. The receptors of the male, far from being idealized ion detectors, have limits in their speed of response, including their ability to degrade pheromone biochemically and transport it away from the dendrites (Kaissling, 1974). These limits might significantly affect the receptors' ability to send sufficiently fluctuating signals to the CNS. Thus the animals' own receptors, plus the pheromone blends known to evoke or cause cessation of upwind flight, should be used to look for correlations between behaviour and fluctuations within plumes (Baker, 1985, 1986).

Miller & Roelofs (1978) first used the EAG to measure the cross-sectional, time-averaged diameter of a plume of redbanded leafroller (*Argyrotaenia velutinana*) pheromone, but their purpose was not to use the EAG to measure fine structure. They used concentrations higher than necessary for full behavioural response in order to get maximum depolarization as they moved the preparation rapidly laterally across the plume. Conner *et al.* (1980) used an EAG to measure the fluctuations in pheromone concentration from female *Utethesia ornatrix* which rhythmically extrude their ovipositors when calling, causing pulses which were registered on the male's antenna from 60 cm downwind in low windspeeds. They did not correlate the fluctuations with behaviour, or examine the fine scale fluctuations of the pheromone plume caused by air turbulence.

In an initial study using EAGs to measure fluctuations due to plume structure, Baker *et al.* (1985) found that lack of upwind flight in a uniform cloud was correlated with a continuous high level of EAG activity. When the same high level of EAG activity was made to fluctuate to lower levels as well by pulsing the cloud, significant levels of upwind flight occurred. The present paper reports further studies using this EAG system for the oriental fruit moth *Grapholita molesta*, to compare fluctuations in plume filament with fluctuations in antennal output at different distances from the source and with different plume concentrations and wind speeds. We also report EAGs recorded in the field, in which we measured changes in the plume structure at 3, 10 and 30 m from the source.

## Materials and Methods

Adult males were reared as previously described (Baker & Cardé, 1979) and used when 1–2 days old for the EAG recordings. The mobile EAG preparations for both the wind tunnel and the field were alike except that in the laboratory the preparation was placed on a rolling platform on tracks (Fig. 1), and in the field it was attached to a step ladder (Fig. 2).

An intact male was held in a narrow glass tube with head and antennae protruding through a small hole at the end. The tube was

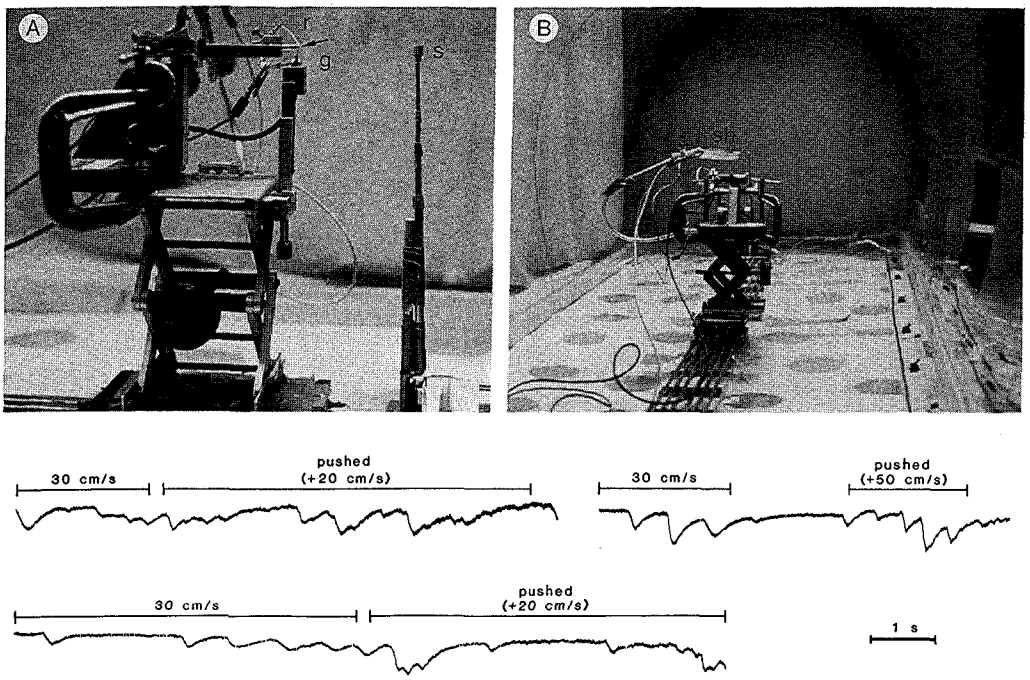
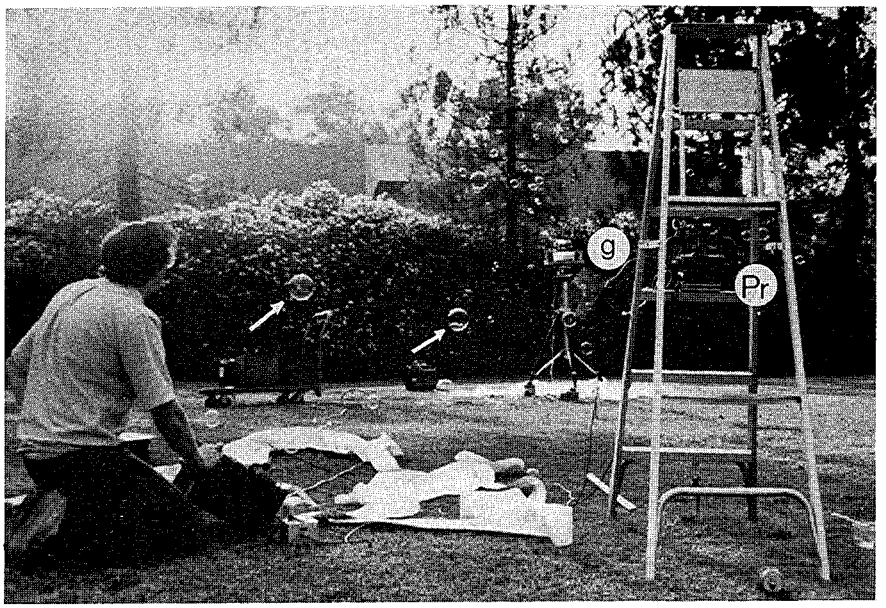



FIG. 1. The mobile EAG preparation. (A) Male moth is restrained in horizontal glass tube, and head and antennae protrude from small hole to right (arrow). Rubber septum (s) is 10 cm to right (upwind) of antenna. The white teflon saline tube housing the recording electrode (r) is positioned at 45° from upper left of head. The ground electrode (g) is positioned vertically from below to impale thorax. (B) The preparation is pushed up and down tunnel on tracks. Long shielded cables connect electrodes to the amplifier (not shown) and a grounded copper screen shield (sh) is positioned above moth. (A, B, Lower) EAG recordings of plume structure at airspeed of 30 cm/s (stationary) and then when the preparation was pushed up the tunnel at 50 cm/s (short push) or 20 cm/s (long push) to vary the moth's airspeed, resulting in different depolarization time-courses.

then mounted on a micromanipulator with the head and antennae upwind (Fig. 1). A sharpened silver/silver chloride electrode was manipulated to impale the thorax from below, within the glass constraining tube. A silver/silver chloride recording electrode was housed in a 3 cm-long piece of 1 mm (i.d.) teflon tubing filled with Ringer's solution (Roelofs, 1985). After the tip of one antenna was snipped off, the recording electrode was brought into position to touch the antennal tip. A shielded cable (5 m) housing both the ground and recording electrodes carried the signal to the pre-amplifier, which was connected to a Gould model 220 Brush recorder. For most recordings the chart speed was 5 mm/s, but for recordings under different windspeeds and airspeeds, it was 25 mm/s.

In the wind tunnel, a 30×30 cm piece of copper screening was required to shield the antennae from background electrical noise, but in the field this was unnecessary. The shield was positioned horizontally *c.* 5 cm above the male (Fig. 1) and created no visible disturbance to wind flow, as checked by $TiCl_4$ smoke. Electrical interference emitted by the fan at the upwind end of the tunnel could not be shielded completely without interfering with the structure of the plume. Consequently, as the preparation was pushed up the tunnel, the electrical noise from the fan increased, but not enough to affect the accuracy of the recordings. The depolarization threshold we chose for these studies was 0.2 mV, after preliminary experiments showed us that this level of stimulation from pheromone filaments in the plume could be discerned above the drift and fluctuations of the baseline. All measurements of plume structure were periodically (*c.* every 2–3 min) interspersed with a 1 ml, 30 ms puff



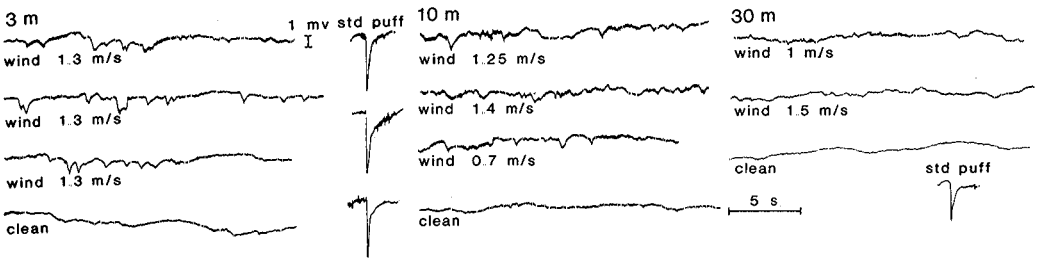




FIG. 2. Recording EAG responses in the field, 10 m from the source. Ladder at right supports the preparation (Pr) while bubbles (arrows) released from bubble generator (g) indicate windspeed and presence of pheromone. (Lower) Some typical EAG responses to plume filaments striking the antennae at 3, 10 and 30 m from the source.

from a standard pasteur pipette pheromone cartridge, delivered directly onto the antenna from *c.* 2 cm away in clean wind. Changes in antennal response to these standard puffs were carefully monitored throughout all experiments.

For experiments with varying pheromone dosages in the wind tunnel, we used a series of rubber septa containing 0.036, 0.36, 36 and 1000 $\mu$g of the three-component pheromone blend 5.9%(*E*)-8-dodecenyl acetate plus 3%(*Z*)-8-dodecenyl alcohol (Cardé *et al.*, 1979) in (*Z*)-8-dodecenyl acetate (Roelofs *et al.*, 1969). These dosages refer to the (*Z*)-8-dodecenyl acetate, as quantified by GC (Willis & Baker, 1988), and were chosen to elicit a full range of levels of upwind flight and arrestment (Baker *et al.*, 1981; Linn & Roelofs, 1983). All septa were placed, loaded end up, on a small pole at the same height as the antennae in the EAG preparation (Fig. 1). Because fine structure is affected by the physical effects of turbulence from solid structures such as the septa, care was taken to choose septa that were as similar as possible in shape and size with no obvious physical imperfections or other differences. Care was also taken to place each septum in exactly the same attitude (straight up) on the pole. Numerous trials with $TiCl_4$ smoke allowed the antenna to be positioned as close as possible to the centre of the plume at all distances from the source.

For the experiment on dosage and distance, wind was constant at 70 cm/s with a temperature of 23°C. The EAG preparation was placed so that the antenna was 3 m downwind of the pole and, beginning with the lowest dosage, all septa were placed in turn on the pole for *c.* 40 s. The pole was rinsed with acetone, the preparation moved up the tunnel to 1 m, and the dosage series repeated again from lowest to highest. This procedure was repeated at 0.3 m and 0.1 m, but there were many times when the preparation ceased to respond before a complete series of dosages and distances would be recorded on the same antenna. For the antennae on which we tested a complete series, the reduction in responsiveness from beginning to end was only *c.* 25%, as measured by the amplitude of the response to the standard puff.

Wind and airspeed experiments in the tunnel were performed with the 1000 $\mu$g source. The antenna was positioned 3 m downwind of the source and wind was generated at 10, 30, 50, 80 and 100 cm/s, then 10 cm/s again for each antenna. For measuring the effects of the moth's airspeed at a constant windspeed, the windspeed was set at 30 cm/s. The preparation was then pushed upwind in the plume on the tracks at 20 cm/s or at 50 cm/s to attain 50 and 80 cm/s airspeeds. The pushing speeds were measured by pressing the event recorder button on the Brush recorder to mark the chart when the antenna had traversed 1 m, marked from 2.7 to 1.7 m downwind of the source, with a head start from 3 m.

In the field, the preparation was clamped to a step-ladder, at a height of 1.3 m above the short grass in an open area on campus (Fig. 2). The 1000 $\mu$g source was used because preliminary experiments indicated that it was very difficult to detect pheromone from the 36 $\mu$g source at 30 m. The source was placed on a wire suspended 30 cm below a motor-driven bubble generator (David *et al.*, 1983). The bubbles emerged from wire loops rotating through detergent solution and were blown straight up by a small motor-driven fan so that no extra velocity or directional bias was imparted to them. Power for the Brush recorder and bubble machine was supplied by a Honda portable generator.

The EAG was set up beginning 3 m downwind of the source and moved back successively to 10 and 30 m. The presence of bubbles passing by the ladder indicated that pheromone might be contacting the antenna. The bubbles were also used to measure the windspeed at the time of EAG responses. An observer pressed the event recorder marker button as a bubble passed the beginning and end of a 1 m stick placed on the ground just upwind of the antenna. Thus a record was kept of the windspeed on the same chart paper that also recorded EAGs. Between recording at each distance, puffs from the standard 0.36 $\mu$g cartridge were delivered directly onto the antenna from 2 cm away as in the wind tunnel.

## Results

In the wind tunnel at a given distance from the source, both the amplitude and the frequency of depolarizations (burst amplitude and frequency) from pheromone filaments in the plume increased with the emission rate of the source (Fig. 3). Higher emission rates resulted in a greater proportion of bursts occurring as multiples, which we defined as depolarizations occurring before the response level from a preceding burst had returned two-thirds of the way to the baseline. The greatest occurrence of multiple bursts was at 0.3 m from the source, where 23%, 34%, 41% and 68% of the bursts were multiple with the 0.36, 3.6, 36 and 1000 $\mu$g sources, respectively. At 3, 1 and 0.3 m, the percentages of bursts that were multiples ranged 16–23% for the 0.36 $\mu$g source, 22–34% for the 3.6 $\mu$g source, 41–45% for the 36 $\mu$g source, and 46–68% for the 1000 $\mu$g source. At all emission rates, higher frequencies than the averages depicted in Fig. 3 were routinely encountered during selected 1 s spans. For instance, we recorded numerous instances of 4 bursts/s with the 1000 $\mu$g and 36 $\mu$g sources at all distances, and 3 bursts/s with the 3.6 $\mu$g source.

At the higher emission rates, multiple bursts occur because of the rate-limiting step of repolarization combined with higher burst frequency. This is exemplified by the recovery interval from single puffs with our standard cartridge compared to those from single bursts with plume filaments (Table 1). After a depolarization with the standard puff (Fig. 2), 50% recovery was achieved by 0.26 s, and 67%

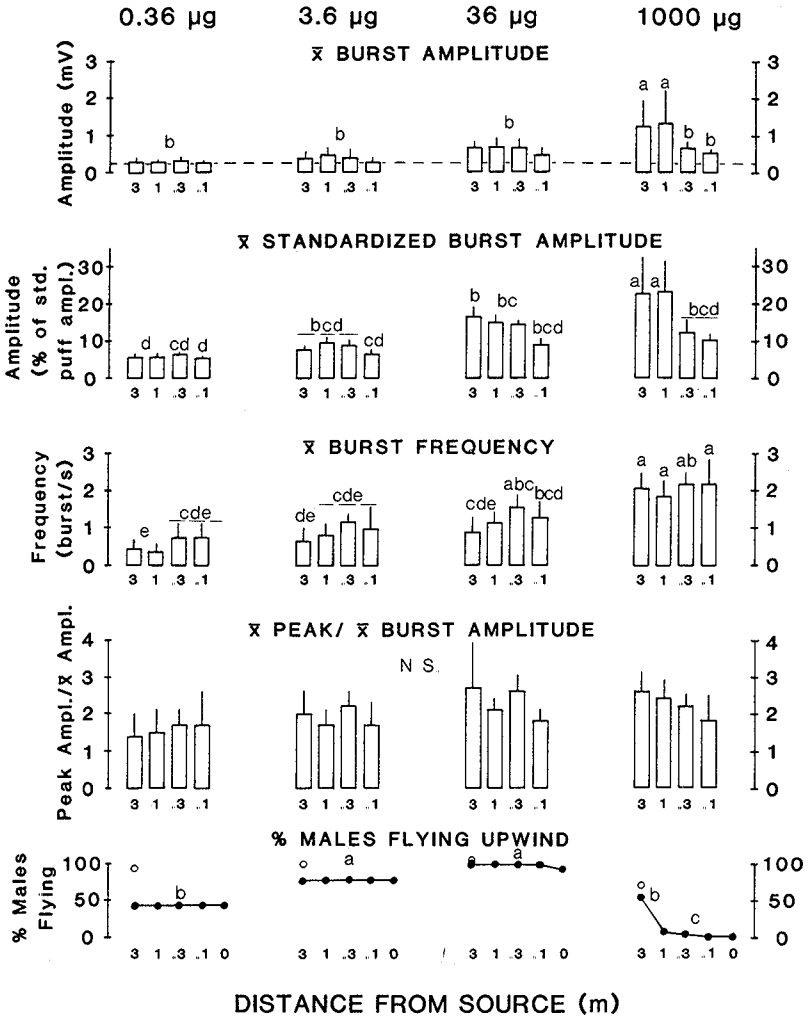



FIG. 3. Characteristics of EAG responses of antennae in plumes of various concentrations at different distances from the source. $n=3$ antennae with a 20 s sampling period for each distance and concentration. Values in each of the first four rows having no letters in common are significantly different according to Duncan's Multiple Range Test ($P<0.05$), and those in the last row according to Ryan's $\chi^2$ test with adjusted significance levels.

recovery by 0.46 s. This would mean that on average, 100% of such high-amplitude bursts should be multiples if the burst frequency exceeded a little more than 2/s. The 50% and 67% recovery intervals for single bursts in the 0.36, 3.6 and 36 $\mu$g plumes at 1 and 3 m from the source were nearly identical to those of the standard, *c.* 0.25 s and 0.40 s, respectively. However, bursts in the 1000 $\mu$g plume took less time to recover to 50% and 67%, indicating that the higher frequency of bursts may not have allowed the neuronal activity to return to its resting level. In other words, even without the highly apparent effect of multiple bursts, perhaps the true baseline was now never really approached, which would contribute to pushing the system to its capacity. At high frequencies and concentrations, even when we were not measuring multiple bursts our peak-to-trough measurements would depend more on the biochemical events that determine quick onset and offset of neuronal activity

TABLE 1. The rates (± SD) of EAG depolarization (onset) and repolarization (return) in pheromone plumes of various concentrations and at different distances from the source in the wind tunnel. The onset rates and the time for 50%, 67% and 90% return to baseline ($T_{50\%}$, $T_{67\%}$, $T_{90\%}$) for the five largest single bursts in a 20s interval (no multiple bursts) were measured using three different antennae. Means within each column having no letters in common are significantly different according to Duncan's multiple range test ($P<0.05$).

| Distance | Source concentration | Burst amplitude (mV) | Onset slope (mV/s) | $T_{50\%}$ return (s) | $T_{67\%}$ return (s) | $T_{90\%}$ return (s) |
|---|---|---|---|---|---|---|
| 3 m | 0.36 $\mu$g | $0.35^{c}$ ±0.27 | $2.6^{b}$ ±1.70 | $0.24^{a}$ ±0.11 | $0.37^{ab}$ ±0.20 | $0.50^{ab}$ ±0.27 |
| | 3.6 $\mu$g | $0.62^{c}$ ±0.40 | $4.1^{b}$ ±0.80 | $0.26^{a}$ ±0.04 | $0.43^{a}$ ±0.06 | $0.73^{a}$ ±0.03 |
| | 36 $\mu$g | $1.58^{abc}$ ±0.54 | $11.6^{ab}$ ±1.90 | $0.26^{a}$ ±0.07 | $0.39^{ab}$ ±0.11 | $0.80^{a}$ ±0.28 |
| | 1000 $\mu$g | $2.47^{a}$ ±1.67 | $19.1^{a}$ ±10.8 | $0.16^{b}$ ±0.01 | $0.25^{c}$ ±0.04 | $0.39^{b}$ ±0.05 |
| 1 m | 0.36 $\mu$g | $0.33^{c}$ ±0.23 | $2.9^{b}$ ±2.4 | $0.27^{a}$ ±0.03 | $0.39^{ab}$ ±0.04 | $0.53^{ab}$ ±0.06 |
| | 3.6 $\mu$g | $0.68^{c}$ ±0.34 | $5.3^{b}$ ±3.4 | $0.25^{a}$ ±0.07 | $0.38^{ab}$ ±0.12 | $0.06^{ab}$ ±0.18 |
| | 36 $\mu$g | $1.16^{bc}$ ±0.45 | $10.0^{ab}$ ±3.10 | $0.21^{ab}$ ±0.01 | $0.33^{b}$ ±0.04 | $0.63^{ab}$ ±0.15 |
| | 1000 $\mu$g | $1.94^{ab}$ ±0.74 | $21.3^{a}$ ±14.3 | $0.12^{b}$ ±0.01 | $0.23^{c}$ ±0.02 | $0.41^{b}$ ±0.05 |
| 2 cm | Standard puff | 3.93 ±1.22 | 31.6 ±4.2 | 0.26 ±0.10 | 0.46 ±0.13 | 1.44 ±0.47 |

shaping the tip of each peak rather than the slower processes shaping the base. This is supported again by the shorter 90% recovery interval after the 1000 $\mu$g-evoked single bursts compared to the 90% recovery intervals with the lower concentrations. The higher amplitudes with the 1000 $\mu$g source should have resulted in longer 90% recovery intervals than with bursts at the lower emission rates, perhaps approaching the levels of the bursts in response to the standard puffs; yet the rates were shorter (Table 1).

The higher frequencies and multiple bursts nearer to the 1000 $\mu$g and 36 $\mu$g sources were associated with reductions in peak-to-trough burst amplitude (Fig. 3). These amplitudes diminished by *c.* 30% from 0.3 to 0.1 m at the 36 $\mu$g dosage, and by *c.* 50% from 1 to 0.3 m (and 0.1 m) at the 1000 $\mu$g dosage (Fig. 3). This would mean that for a male approaching a 1000 $\mu$g source, antennal neuronal output caused by plume filaments would diminish by 50% as he flew from 1 m to 30 cm downwind of the source, if such output were directly related to the peak-to-trough DC potentials that we measured by the EAG technique. The diminution of burst amplitude as the highest concentration sources were approached again would appear to be due to a clipping of the slower portion of the recovery phase of each burst, effectively resetting the baseline to a higher level without a corresponding boost in the amplitude of each peak.

Although there had been previous studies of the proportions of males taking flight and reaching the source (Baker *et al.*, 1981; Linn & Roelofs, 1983), we performed a small behavioural experiment under temperature and windspeed conditions identical to those in this EAG dosage study. At 0.036, 0.36 and 3.6 $\mu$g dosages, increasing proportions of males flew upwind in the plume with no arrestment whatsoever (Fig. 3; the 0.036 $\mu$g proportions are not shown, but eight of thirty-six males, or 22%, flew upwind in the plume and all eight reached the source). Significant EAGs of increasing amplitude and frequency were recorded at these three increased dosages at 3 m, and also at closer distances. On one of the antennae we measured EAGs with the 0.036 $\mu$g dosage at the males' take-off point 3 m from the source; the frequency averaged 0.37 bursts/s (cf. 0.1/s for the highest clean-air control) and the mean standarized amplitude was 6.3% of the standard puff (cf. 5.1% for the clean-air control, which is equal to the threshold level of 0.2 mV).

With the 36 $\mu$g source, on the other hand,

although the percentage of males flying upwind increased still further, 7% of them became arrested in the plume and thus never reached the source. At 1000 $\mu$g, only 53% of the males began flying upwind in the plume, and all of them became arrested before reaching the source. The mean closest approach to the source for arrested males was 3.5 cm and 215 cm, respectively, with the 36 and 1000 $\mu$g sources. Thus arrestment occurred in plumes in which EAG peak-to-trough amplitudes diminished significantly as the moth approached the source. Furthermore, arrestment occurred farther from the 1000 $\mu$g source than from the 36 $\mu$g source, attenuation of EAG amplitude showing the same pattern.

However, the absolute distance at which arrestment occurred with the 1000 $\mu$g source did not correspond with the distance at which EAG attenuation was significant. This raised the possibility that the higher airspeeds from the moths' own upwind movement could have generated increases in the frequency of contacting filaments and caused amplitude attenuation farther from the source than we measured with our stationary EAG.

To test whether increased airspeeds could decrease EAG amplitudes at *c.* 2 m from the source, we pushed the EAG preparation up the tunnel with the 1000 $\mu$g dosage at the source. The higher airspeeds resulted in a higher frequency of contact with filaments, but did not reduce the peak-to-trough amplitudes (Table 2). The slopes of the EAG onsets and recoveries increased significantly across the range of airspeeds generated in this matter, and may have contributed to the ability of the antenna to increase burst frequency detection without a reduction in amplitude (Fig. 1, Table 2). There was a proportionately greater increase in the onset slope than in the recovery slope, as airspeed increased.

Likewise, when the 'airspeed' of the preparation was increased by increasing the windspeed, the burst frequency again increased without a reduction in amplitude (Table 3, Fig. 4). The slopes of the EAG onset and recovery again increased with airspeed (Fig. 4), and again there was a proportionately greater increase in the slope of the onset than of the recovery (Table 3). It did appear that the slope of the recovery (at the steepest, 0.2–0.3 s) reached its maximum of 6 mV/s at 50 cm/s. The EAG response at the 30, 50 and 80 cm/s airspeeds, either by pushing the preparation or by increasing the windspeed, were quite similar. It is clear that repolarization is the rate-limiting step for the antenna to register more

TABLE 2. Characteristics of EAG responses at different male airspeeds at a constant windspeed of 30 cm/s. The EAG preparation was pushed up the tunnel from 2.7 to 1.7 m from the source to create the higher airspeeds. $n$=3 antennae, and 11, 8 and 10 measurements for the 30, 50 and 80 cm/s airspeeds, respectively. Means within each column having no letters in common are significantly different according to Duncan's multiple range test ($P$<0.05).

| Airspeed | Burst frequency (/s ±SD) | Burst amplitude (mV ±SD) | Slope onset (mV/s ±SD) | Slope return (mV/s ±SD) |
|---|---|---|---|---|
| 80 cm/s (pushed) | 3.8±1.3[a] | 1.09±0.39[a] | 20.0±10.3[a] | 6.5±1.7[a] |
| 50 cm/s (pushed) | 2.2±0.5[b] | 0.96±0.15[a] | 12.5±3.8[b] | 5.5±0.9[a] |
| 30 cm/s (stationary) | 1.6±0.40[b] | 1.06±0.40[a] | 8.5±4.1[b] | 4.0±0.9[b] |

TABLE 3. Characteristics of EAG responses from a stationary preparation at different windspeeds, 3 m from a 1000 $\mu$g point source in the wind tunnel. $n$=5 antennae, with 20 s sampling interval for each antenna at each windspeed. Means within each column having no letters in common are significantly different according to Duncan's multiple range test ($P$<0.05).

| Wind velocity (cm/s) | Burst frequency (/s ±SD) | Burst amplitude (mV ±SD) | Slope (mV/s ±SD), burst onset | Slope (mV/s ±SD), burst return |
|---|---|---|---|---|
| 100 | 2.6±0.4[a] | 1.0±0.1[a] | 31.0±3.1[a] | 5.8±0.7[a] |
| 80 | 2.9±0.3[a] | 1.0±0.2[a] | 23.5±2.6[b] | 5.3±0.5[a] |
| 50 | 2.8±0.3[a] | 0.9±0.9[a] | 15.0±2.8[c] | 6.1±0.8[a] |
| 30 | 2.0±0.4[b] | 1.1±0.2[a] | 7.5±1.3[d] | 4.7±0.2[b] |
| 10 | 0.6±0.2[c] | 1.0±1.0[a] | 2.9±0.2[e] | 1.9±0.2[c] |

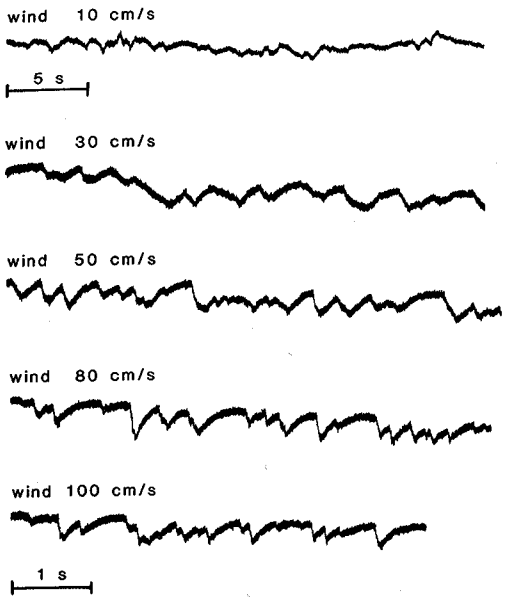



FIG. 4. EAG tracings from the same antenna exposed to the 1000 $\mu$g plume at different windspeeds. Note the increase in slope of the onset of depolarization as windspeed is increased (time-scale is 5 times slower for the 10 cm/s tracing).

pheromone filaments at accurate amplitudes; at the higher airspeeds it is 3–4 times slower than depolarization.

In the field, one unexpected result was the difficulty in getting the pheromone plume to contact the stationary antenna to register EAGs at all. Often the bubbles were in the right direction, but the plume was apparently either a little too high or too low. The point, however obvious it might have seemed before, was thus brought home with extra force, that to be able to move about rapidly as a flying moth does, is a tremendous advantage in locating and maintaining contact with the meandering odour plume. EAG bursts did not change very dramatically from 3 to 30 m downwind of the 1000 $\mu$g source (Table 4). The peak amplitude was greater at 3 m compared to 10 m and 30 m downwind, as was the mean amplitude at 3 m, but burst frequencies were not significantly different at these three distances. At first, it would appear surprising that the mean amplitude at 3 m was less than that in the wind tunnel. However, in the field the septum was placed just below the bubble generator, which had a fairly bulky frame and probably caused extra turbulence and shearing of the filaments. In addition, the wind in the field was not likely to be as laminar as the wind tunnel.

For analysis of the field EAG recordings, we chose the relatively infrequent 10 s intervals in which EAG bursts were registered virtually every second (no gaps of more than 2 s were allowed), so we were confident that the plume was not meandering due to shifts in wind direction creating gaps between the bursts that were not due to the fine structure of the plume. Still, we cannot be certain that there was no meandering, either vertically or laterally, so we can only conclude that the antennae of males should be able to register burst frequencies that are at least this high in the

TABLE 4. Characteristics of EAG responses in the field at different distances from a 1000 $\mu$g point source mounted on a bubble generator. Each sampling interval was 10 s of continuous activity (never more than 2 s without a depolarization) except for maximum values, which were the three highest amplitudes and frequencies from each antenna; $n$=23 sampling intervals, six males for the 3 m distance, twenty-two intervals, six males for the 10 m distance, and seven intervals, three males for the 30 m distance. Average windspeed was 1.44, 1.27 and 1.55 m/s for 3, 10 and 30 m recordings, respectively. Means within the same column having no letters in common are significantly different according to Duncan's multiple range test ($P$<0.05).

| Distance from source (m) | Amplitude (mV±SD) | | Amplitude (propor. of std) | | Frequency (bursts/s ±SD) | | Slope (mV/s ±SD) | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | Max. | $\bar{X}$ | Max. | $\bar{X}$ | Max. | Onset | Return |
| 3 | 0.56[a] ±0.13 | 1.12[a] ±0.31 | 0.11[a] ±0.04 | 0.21[a] ±0.07 | 1.06[a] ±0.30 | 2.91[a] ±0.67 | 6.7[a] ±2.3 | 2.8[a] ±1.0 |
| 10 | 0.45[b] ±0.09 | 0.80[b] ±0.17 | 0.07[b] ±0.03 | 0.13[b] ±0.04 | 0.93[a] ±0.27 | 2.23[a] ±0.43 | 3.91[b] ±0.95 | 2.15[b] ±0.98 |
| 30 | 0.36[c] ±0.10 | 0.60[c] ±0.17 | 0.07[b] ±0.03 | 0.12[b] ±0.04 | 1.06[a] ±0.49 | 2.86[a] ±0.90 | 3.93[b] ±1.52 | 1.91[b] ±0.42 |

field. Again, higher airspeed during upwind flight (often exceeding 2 m/s in the field; T. C. Baker and K. F. Haynes, unpublished) would increase the burst frequency somewhat to levels greater than the *c.* 1 bursts/s recorded here at these average airspeeds (windspeeds on the stationary preparation) of 1.3 m/s or so.

## Discussion

Upwind flight by male *G. molesta* was better correlated with EAG peak-to-trough burst amplitude than with burst frequency. Upwind flight was initiated in greater proportions of males as EAG amplitude increased, and it ceased under those treatments that were associated with decreases in amplitude. Burst frequency, on the other hand, did not diminish along with upwind flight behaviour. Nevertheless, the diminished EAG amplitudes in the arrestment zone should have been sufficiently high to continue to evoke upwind flight, because they were higher than those at lower dosages that had successfully activated resting males into performing sustained upwind flight. Thus, it appears that arrestment may occur when lower-amplitude receptor activity *follows* higher receptor activity.

The frequencies occurring in the arrestment region were no higher than those experienced by males in another study when they zigzagged upwind in and out of a side corridor of uniform pheromone (Willis & Baker, 1984). At two different concentrations of pheromone cloud males experienced frequencies of *c.* 3.5 bursts (contact with the cloud) per second, and significant percentages of males flew upwind without being arrested (Willis & Baker, 1984). In the present study, frequencies of only *c.* 2/s were correlated with 100% arrestment. Therefore, excessively high burst frequency alone does not explain arrestment in this study, but perhaps the indirect effect of burst frequency in contributing to reduced burst amplitude during multiple bursts does explain it.

An assumption that such reduced EAG burst amplitudes are associated with reduced behaviour would require that the frequency of action potentials to the central nervous system be similarly reduced. Single-cell recordings that would provide the link between DC and action potentials have been very difficult to obtain for *G. molesta* due to the size of the moths. However, recent experiments with *G. molesta* revealed a very strong relationship between action potential frequency and peak-to-trough, but not peak-to-baseline, amplitude. They also demonstrated that repeated 20 ms puffs of pheromone at a rate of 2/s or greater caused reductions in both peak-to-trough DC amplitude and action potential frequency, especially if the antenna was chilled, reducing its ability to recover to baseline levels after each buff (Baker *et al.*, 1989). Figures from Kaissling (1986) also showed parallel reductions in both DC peak-to-trough amplitudes and in action potential frequency in *A. polyphemus* antennal neurons as the pulse rate of pheromone increased and baseline recovery was not possible. Thus, as far as can be discerned from the unanalysed recordings in the figures, the fast *tempo* of the arriving intermittent pulses was adequately registered in the tempo of action potential output, but the *strength* of each pulse was not (Kaissling, 1986). These data thus again appear to support our interpretation that DC peak-to-trough amplitude is associated with the occurrence of behaviour because it is highly correlated with action potential frequency. In the current experiments it is conceivable that as a moth approached a high-emitting source, the CNS would receive less action potential stimulation from the receptors even though the pheromone flux had increased.

If arrestment were due to decreased input to the CNS as pheromone concentration increased along the plume, the males' behaviour at arrestment should be similar to that while flying into a lower actual molecular concentration. In fact this is so, because at arrestment males abruptly (within 0.15 s) steer a more cross-wind course (while still zigzagging narrowly) and increase their airspeed after reducing it briefly on the first inter-reversal leg (0.15 s) (Willis & Baker, 1988). They also slowly increase their reversal frequency after several reversals, thus contributing to the widening of the zigzags. These behavioural changes are identical to those which occur when males lose contact with pheromone in a shifting wind-field and begin casting widely (Baker & Haynes, 1987), except that in the latter situation the brief airspeed reduction on the first leg was not significant. In non-shifting

wind, however, significant airspeed reduction does occur on the first leg following pheromone plume loss, in addition to all the other changes that are identical with arrestment (T. C. Baker, M. A. Willis and K. F. Haynes, unpublished).

The arrestment-correlated reduction in peak-to-trough amplitude as registered by EAG burst amplitudes is consistent with the hypothesis (Baker, 1985) that at arrestment by excessively high-concentration pheromone sources, the intermittent odour signal from the plume's filamentous structure becomes effectively fused and too uniform to stimulate upwind zigzagging flight. The present study indicates that the fusion does not need to be total as originally envisaged (Baker, 1985). Only a *relative* smoothing of the signal and reduction in amplitude is needed. Nevertheless, that hypothesis is consistent with the current finding that the smoothing occurred with excessively high concentrations which evoked both high burst frequencies and amplitudes. Here, the limit of receptor capacity could have been reached, causing multiple bursts at so great a frequency that they fail to summate to the levels that reflect the actual filament concentrations.

Yet another explanation for arrestment would be that the total pheromone flux (Elkinton & Cardé, 1984) became too high, causing total neuronal output to reach excessive levels that changed the behaviour from upwind flight to arrestment. This explanation would not need to invoke peak-to-trough burst amplitude, but rather would merely hypothesize that the time-averaged frequency of action potentials would become excessively high closer to the source and exceed some upper threshold to trigger an end to upwind flight. First, this explanation is not supported by the concurrent *reduction* of neuronal activity and behaviour observed closer to the source in the present study. Moreover, it ignores results of previous studies which demonstrated that total flux from a constant uniform cloud that had appeared to be excessive, then became effective if it were increased by superimposing on it the filaments from a point-source plume (Willis & Baker, 1984; Baker *et al.*, 1985). Thus we conclude that arrestment in excessively high-concentration plumes is best related to the reduction, through time, of burst amplitude experienced by males, and the corresponding reduction in action potential frequency that would impinge on the CNS.

For initiation of significant levels of upwind flight, the EAG as a monitor of the plume's fine structure comes close to detecting behaviourally significant levels of pheromone, even with our arbitrary 0.2 mV threshold. In the wind tunnel, significant EAGs were recorded in a 0.36 $\mu$g point-source plume from all three antennae from 3 m away, and more than 40% of the males took flight upwind in this plume from that distance. On one of three antennae we did measure significant EAGs at 3 m from a 0.036 $\mu$g point source, and 20% of the males took flight upwind to this treatment from that distance. We did not test lower dosages for upwind flight initation, but from other studies (Baker *et al.*, 1981; Linn & Roelofs, 1983) it seems unlikely that we would have been able to evoke upwind flight to a 10-fold-reduced point source.

Likewise in the field, we were able to measure significant EAGs at 30 m downwind from the 1000 $\mu$g septum, a distance at which males readily take flight upwind to this dosage (Baker & Roelofs, 1981; Linn *et al.*, 1987; T. C. Baker and K. F. Haynes, unpublished). On the other hand, we were unable to measure significant EAGs 30 m downwind from a 36 $\mu$g source, and this was difficult even at 10 m. We could get no males to fly upwind to the 36 $\mu$g source at 30 m (Baker & Haynes, unpublished), consistent with previous studies (Linn *et al.*, 1987; Baker & Roelofs, 1981), whereas males do fly upwind to this source from 10 m and 3 m (T. C. Baker and K. F. Haynes, unpublished; Baker & Roelofs, 1981; Linn & Roelofs, 1987). Thus we are confident that our EAG preparation came very close to being able to measure behavioural threshold bursts of pheromone.

## Acknowledgments

This research was supported by NSF Grant No. 8310980 (to T.C.B.). We thank Rick Vetter for rearing the insects, performing statistical analyses, drawing the figures, and other technical assistance, and Bob Esposito for technical assistance.

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Accepted 21 April 1988