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EAG-Measurement of pheromone concentrations in apple orchards treated for mating disruption of *Cydia pomonella*

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Abstract: Mating disruption in apple orchards may be greatly improved by improving our knowledge on the distribution of the pheromone concentration in the orchard. We have measured pheromone concentrations with our EAG-system, which offers short measurement cycles and fast readout. An absolute calibration had been provided by simultaneous air sampling experiments. We have investigated the pheromone density profile at the upwind edge of a pheromone-treated orchard, the change of pheromone density with treee height, and the concentrations resulting from different dispenser types in apple orchards in Southern Sweden.

Key words: mating disruption, pheromone concentration, field EAG measurements, *Cydia pomonella*

Introduction

Measurements of airborne pheromone concentrations can make an important contribution to the further development of the mating disruption technique. Investigations of wind effects, plant-surface interactions and concentration profiles in vertical and horizontal dimensions all require the acquisition of multiple measurements during time intervals which can be considered short in comparison to the time course of changes in temperature or wind.

Methods using air sampling and subsequent chemical analysis (*e.g.* Flint et al. 1993) offer absolute average concentration values, but they are not fast enough to yield a sufficient number of measurements in the required time.

Field EAG measurements of pheromone concentrations yield reproducible concentration values on a relative scale. The field EAG measurement system developed in Kaiserslautern has been used to measure pheromone concentrations in vineyards (Milli 1990; Sauer 1991; Karg 1992; Karg et al. 1990, 1995; Koch et al. 1992, 1995; Färbert 1995; Karg & Sauer 1995), in cotton fields (Cardé et al. 1993; Färbert & Koch 1993; Färbert 1995; Färbert et al. 1996), in pea fields (Bengtsson et al. 1994) and in apple orchards (Milli 1993; Karg et al. 1994; Suckling et al. 1994; Milli et al. 1996). The newer models of our system make use of a signal superposition technique to suppress the influence of plant odors and other non-pheromonal airborne stimuli on the pheromone concentration measurements.

Methods

The field EAG system has been described in detail by Färbert et al. (1996a, b). Briefly, it consists of an excised antenna of *Cydia pomonella* (for measurements of airborne codlemone) placed in a special antenna holder. The holder is attached to the bottom of a vertical tube in which a steady current of air (14 ml/s) is maintained using a suction pump. A charcoal filter is placed at the tube upper entrance to remove all stimulating odors from the incoming air.

Three calibration sources (glass syringes, containing a vial with a pheromone-oil mixture; Sauer 1991) are connected to the tube in such a way that activation of the syringe piston generates an air puff (0.25 ml, 0.6 s duration) with defined pheromone content, which is injected into the main airstream. The antennal responses to activation of the calibration syringes with pheromone concentrations in three decade steps are used to construct a dose-response curve characterizing the properties of the antenna.

When the charcoal filter is removed from the tube, outside air reaches the antenna and produces a rise in the EAG signal similar to a step function. The height of this step is caused by background odors as well as pheromones, and cannot be used as a measure for pheromone concentration. While the filter remains open, additional calibration pulses are released. The additional response of the EAG signal to the superimposed calibration puffs is used to measure the airborne pheromone. The calculation of the pheromone concentration is based on the dose response curve as measured continuously, and a basic model of the mixing of the different airstreams and their effective pheromone concentrations at the antenna.

The EAG measurement system including pumps, calibration syringes and associated step motor drives is mounted on a compact probe which is fully remote controlled and can be operated on a pole up to 5.5 m high. Wind velocity and direction are recorded in 40 ms intervals by two vector anemometers, one mounted on the EAG probe, the other at 5.7 m height.

Results

The relative pheromone concentration units used in the following figures are defined as follows: a concentration of 10^{-6} relative units is the concentration present in the headspace of a calibration syringe containing a vial with 10^{6} parts of paraffin oil (Merck No.7161) and 1 part of pheromone. In calibration measurements involving air sampling and subsequent GC analysis (Bäckman, 1997), the equivalent absolute concentration was determined as: 1.0 x 10^{-6} relative units or 1.85 * 0.3 ng/m^3.

In the following graphs, the measurement data points carry two different indicators for the confidence interval: the thin lined bars with caps indicate the confidence interval for each individual measurement, caused by noise in the antenna, and the uncertainties in determining the amplitudes of EAG responses to each stimulation. The thick black bars without cap respresent the confidence interval due to the averaging of several measurement values. If the signal fluctuates strongly, *e.g.* due to wind influence, the thick bar may become larger than the individual measurement error.

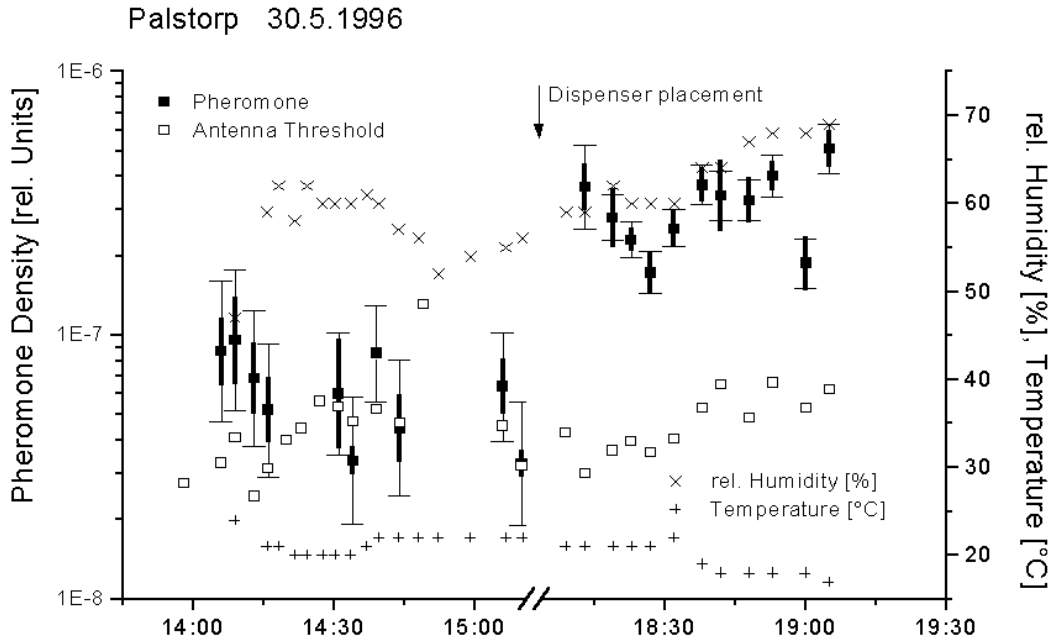

Figure 1 Pheromone concentration before and after dispenser placement

Background and dispenser installation

Figure 1 shows results from the installation of the dispensers on May 30, 1996 in an orchard at Pålstorp, 50 km north of Malmö. Between 13:45 and 15:00, the pheromone concentration was measured in the orchard. There were no dispensers exept a small number of Shin-Etsu dispensers from last year‘s treatment that had been left on the trees. These may have given rise to the small amount of pheromone concentration which is above the threshold concentration (white squares) at the beginning of the measurement. Later on, the threshold rose steadily, and thus occluded the detection of pheromone signals of such low densities. At 17:00, all dispensers had been placed in the orchard. The concentration values measured now were clearly above threshold and showed a steady increase, after a short decline. We attribute these changes in concentration to the changes in wind speed.

Effects of dispenser type and density

The measurements presented in Figure 2 were taken on June 11, 1996 in the same orchard at Pålstorp, on a transect parallel to the rows. The orchard had been treated differently in three sections: in the first section, Ecopom (Isagro) dispensers loaded with 250 mg of codlemone had been applied at a density of 400/ha. In the middle section, Ecopom-dispensers loaded with 250 mg of (codlemone) and 250 mg of (E,E)-8,10-dodecadienyl acetate (codlemone acetate) had been applied at a density of 400/ha; in the third section, the dispensers of the middle section had been applied at a density of 1000/ha. The results show a clear dependence of the recorded signal on the type and density of pheromone dispensers: in the first section, we recorded only concentration values near threshold, whereas in the middle section, there were much stronger concentration signals. In the third section, the concentration values are between 3 and 4 times higher than in section 2.

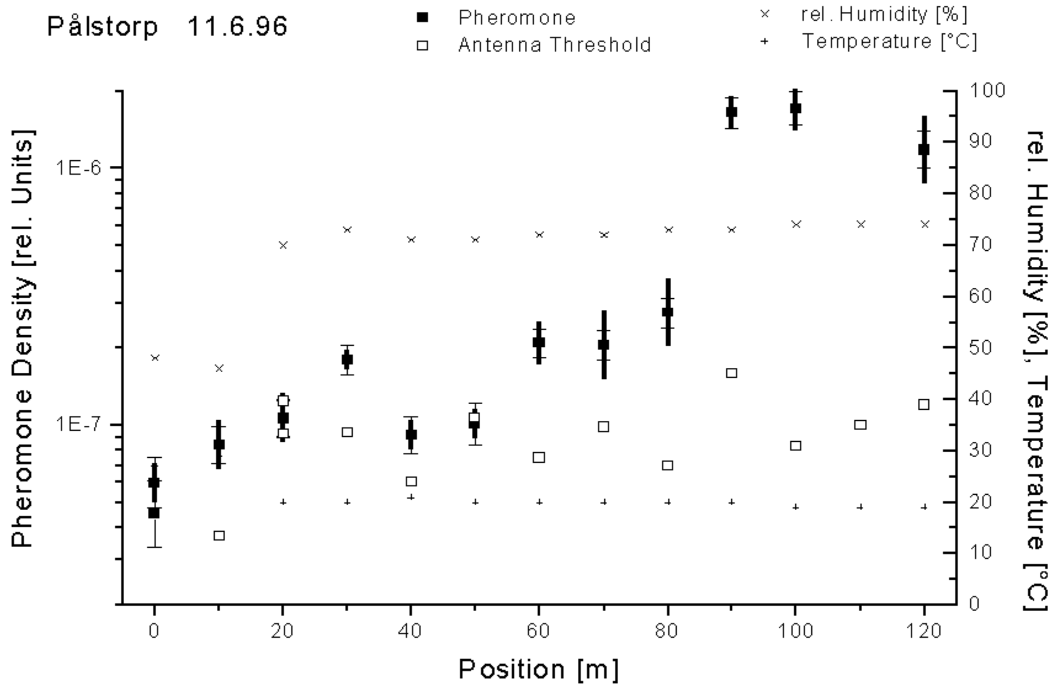

Figure 2. Pheromone concentration in 3 sections of the Pålstorp orchard. Borders between zones of different treatments are at 40 and 80 m.

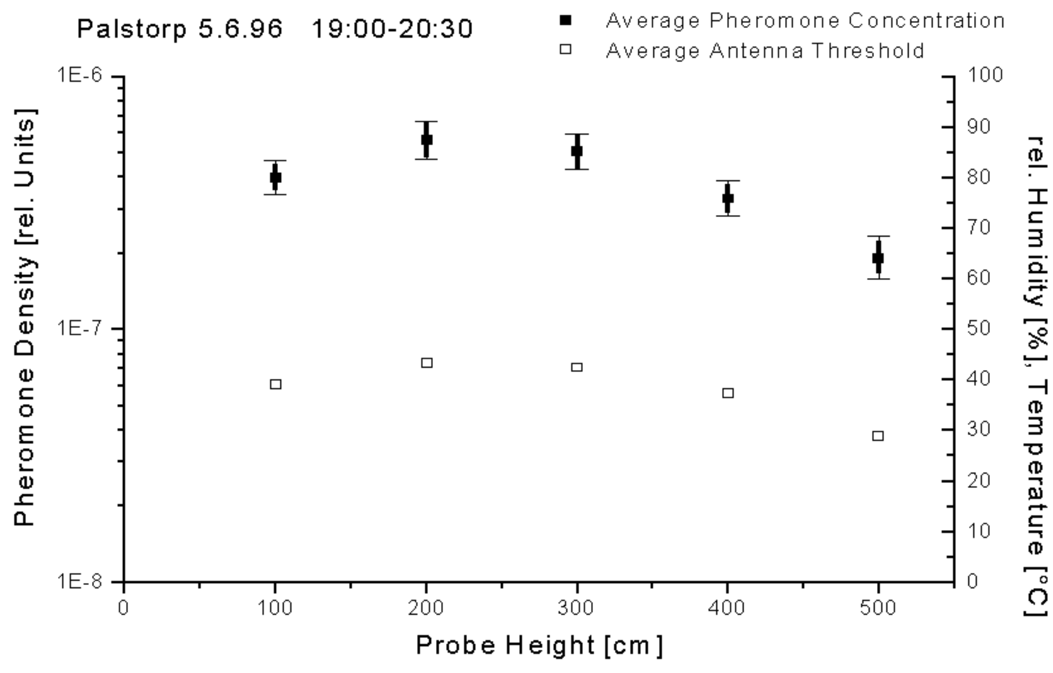

Figure 3. Averaged pheromone concentration plotted versus height of the measurement probe.

Height profile

Figure 3 shows pheromone concentrations measured at different probe heights in the third section of the Pålstorp orchard. The concentration increases slightly from the values found at 1 m probe height to a flat maximum about 2 m probe height; this is the height at which the dispensers were fixed in the trees. The concentration then decreases slightly at a probe height of 3 m and decays further at probe height of 4 m and 5 m. The height of the canopy was around 3.50 m. These height profiles were measured on different occasions. They always showed a similar pattern.

Concentration profile at an upwind orchard edge

The following measurement was made to asess the pheromone distribution at the upwind edge of an apple orchard. The measurements were made at Fjelje, an orchard 20 km north of Malmö. The tree spacing was 1.5 m, the distance between rows was 2 m and the canopy height was 2.20 m. The concentration measurements were taken along a transect parallel to the tree rows. The wind direction was parallel to the transect; the measurement was performed starting at the upwind edge of the orchard (0 m).

Figure 4 shows the concentration values found along the transect: At 5 m upwind from the orchard, there is no pheromone signal detectable above treshold. At the orchard edge, the pheromone signal fluctuates strongly and is barely above threshold. As the measurements proceed along the transect into the orchard, the pheromone concentration rises further. A stable level of the pheromone concentation is only reached after proceeding 20 m from the upwind edge into the orchard.

When looking at the wind speeds recorded at the different positions along the transect, it can be seen vey clearly that the wind speed at 1 m probe height decreases markedly while the probe moves further into the orchard, wheras the windspeed at the top of the measurement pole remains the same. This illustrates how the wind is attenuated by the foliage further inside the orchard.

Discussion

While EAG measurements in apple orchards have been reported repeatedly (Milli 1993; Karg et al. 1994; Suckling et al. 1994; Milli et al. 1996), the measurements described here are the first which have a concentration scale that can be traced to absolute concentrations gained by air sampling and GC analysis. The details of these parallel measurements can be found in Koch et al (1997) and Bäckman (1997). Further details on the orchards and the dispenser applications can be found in Witzgall et al. (1999).

Some nonlinear interactions between plant volatile background and pheromone reactions in the EAG signal had been reported by Rumbo et al (1995) as disturbing quantitative measurements of pheromone concentrations with the field EAG in *Cydia pomonella*. Since in our evaluation system, the superposition of external field pheromone, plant volatiles and additional pheromone calibration stimuli was modeled adequately, the remaining nonlinearities were small compared to other measurement errors. Therefore, the concentration values recorded were not significantly affected by the replacement of an antenna or by changes in the concentration of background volatiles.

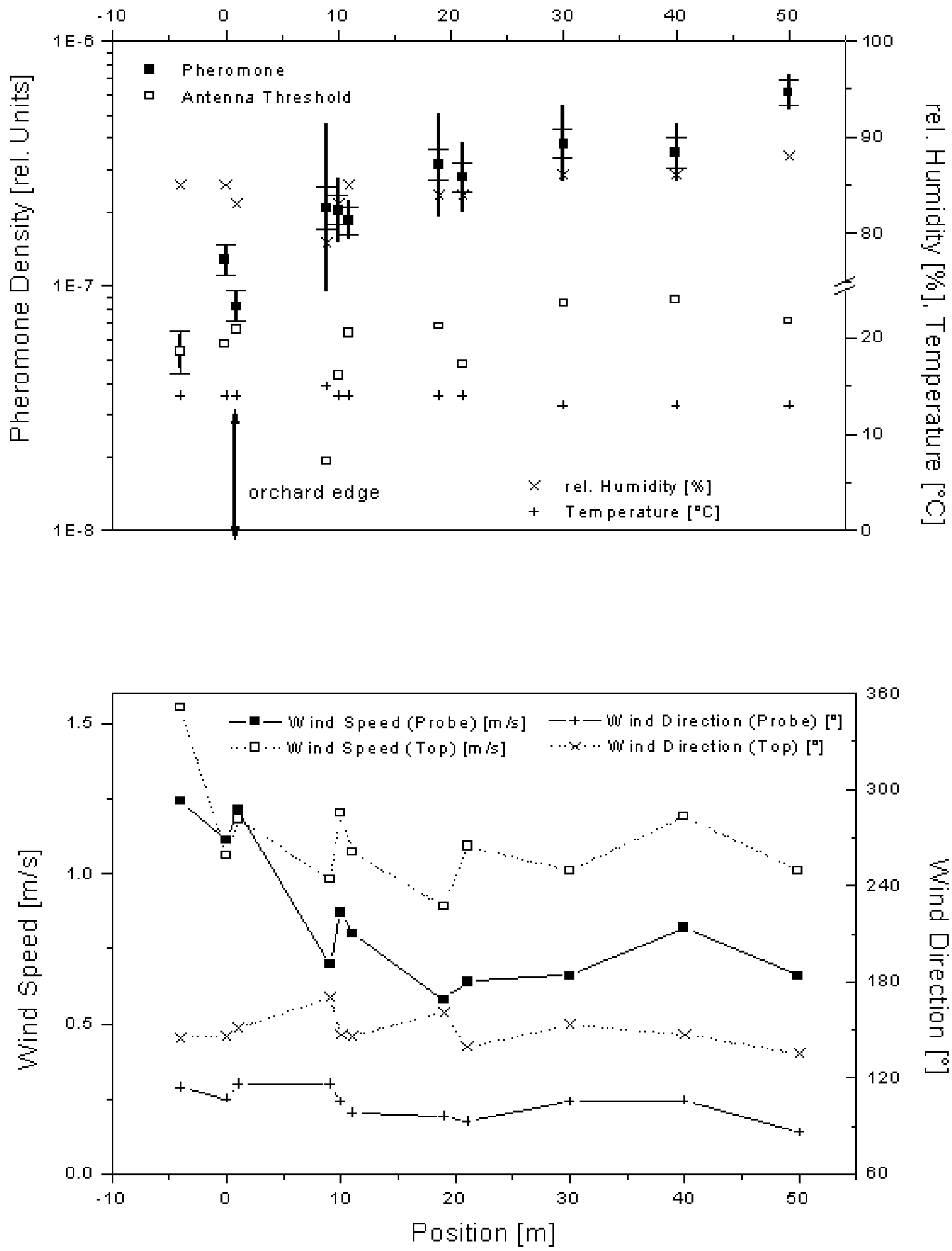

Figure 4. Pheromone concentration and wind data plotted versus distance from the upwind edge (0 m) of an orchard at Fjelje. Note the wide zone of pheromone depletion at the orchard edge.

While Karg et al. (1994) and Suckling et al. (1994) measured pheromones in orchards treated against *Epiphyas postvittana*, Milli (1993) and Milli et al. (1996) measured pheromone concentrations in apple orchards treated against *Cydia pomonella*. Our measurements of the pheromone distribution at the upwind orchard edge confirm the results of Milli et al., who had described an area of pheromone depletion in a zone of 15 m width at the upwind edge of an apple orchard. This reduced pheromone concentration may explain in part why the success of mating disruption is always reduced at the borders of a treated area. In order to have the same pheromone density in all parts of an orchard, one would need a border treatment over a zone with a width considerably wider than hitherto recommended.

Apart from further investigations on pheromone distributions in different types of plant arrangements and climate situations, the field EAG with proper calibration and evaluation systems have been successfully used to determine the lifetime of sprayable pheromone formulations (Koch, in prep.).

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