

Field EAG measurements of sprayable pheromone for mating disruption of *Sesamia nonagrioides*

Uwe T. Koch, Mathias Ascherl, Martina Weber

FB Biologie der Universität Kaiserslautern, Postfach 3049, 67653 Kaiserslautern, Germany

Abstract: Results of measurements of airborne pheromone concentrations in maize fields in southern France treated for mating disruption of *Sesamia nonagrioides* Lef. with a sprayable formulation are reported for the period from 1997 to 1999. In order to obtain short sampling times, results without delay and limited equipment cost, a portable field EAG measurement system was used. The concentration measurements were made from the day of spray treatment over a period of up to 10 days in intervals of 1 to 3 days in different fields. The results show a consistent picture of the gradual decay of the pheromone concentration over time.

Key words: pheromone, mating disruption, sprayable formulation, field EAG, portable electroantennogram, pheromone concentration, time dependence, concentration decay

Introduction

In the development of a sprayable pheromone formulation, one of the key questions is to assess the time span over which the sprayed product is actively suppressing mating activity in the target pest. Apart from observations of trap shutdown, one would like to have a reliable measurement of pheromone concentration in the field. The standard analytical method (Flint, 1993) consists of sampling large amounts of air, trapping the pheromone on an adsorbing agent and analyzing the eluate by GC-MS. This method yields absolute pheromone concentration values, but sample processing is time consuming and results are often available only after considerable delay.

The field EAG measurement system developed in our lab is capable of providing reliable pheromone concentration measurements, yielding results within an hour after the measurement. It has been used to measure pheromone concentrations in vine yards (Milli, 1990; Sauer, 1991; Karg, 1992; Färbert, 1992, 1995; Termer, 1992; Karg *et al.*, 1990, 1995; Karg and Sauer, 1995; Koch *et al.*, 1992, 1995), in cotton fields (Cardé *et al.*, 1993; Färbert and Koch, 1993; Färbert, 1995; Färbert *et al.*, 1996, 1997), in pea fields (Bengtsson *et al.*, 1994), in apple orchards (Milli, 1993, 1994; Suckling *et al.*, 1994; Milli *et al.*, 1997; Koch *et al.*, 1997; Witzgall *et al.*, 1999), in

forests attacked by gypsy moths (Thorpe *et al.*, 1999 unpublished), in cranberry bogs (Polavarapu *et al.*, 1999 unpublished), and in alpha alpha fields (Cardé *et al.*, 2000, unpublished). Since 1993, our system makes use of a sophisticated calibration system, and a special signal superposition technique to suppress the influence of plant odors and other non-pheromone airborne stimuli on the pheromone concentration measurements. The fast measurement cycle and short evaluation time permit to follow up on the development of the pheromone concentration on a day by day basis, to construct a decay-curve and even signal the moment when retreatment would be advisable, in accordance with trap shutdown data.

Methods

The field EAG system used in these experiments has been described in detail in Färbert *et al.* (1996, 1997). It consists of an excised antenna of *Sesamia nonagrioides* placed in a special antenna holder. The holder is mounted in an antenna chamber 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 placed at the tube upper entrance removes all stimulating odor components from the incoming air. Three calibration sources, consisting of glass syringes, containing a vial with a pheromone-oil mixture (Sauer, 1989), 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. Therefore, it cannot be used as a reliable measure for pheromone concentration. While the filter remains open, additional calibration pulses are released. The additional responses of the EAG signal to the superimposed calibration puffs are used to calculate the airborne pheromone concentration using a mathematical model of the antenna and the data from the dose-response curve (calibration). The repetition of the calibration every 50 seconds avoids errors which could arise from the continuous change of the antenna's sensitivity.

An important parameter is the threshold of a given antenna. This is the pheromone concentration at which the antenna ceases to yield clear responses. Pheromone concentrations below this threshold cannot be detected by the field EAG measurement system. Antenna thresholds vary from one male to another and vary also over the time of the antenna's use. Some correlations between the quality of antennae and the general fitness of the male have been observed, but clear effects of changes in the rearing method on antenna quality have not been established.

The relative pheromone concentration units used in our experiments 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. This concentration value has been measured to be in the order of 1 ng/m^3 in the case of *Cydia pomonella*. 1 ng/m^3 is a concentration value usually found in successful mating disruption experiments involving standard dispensers.

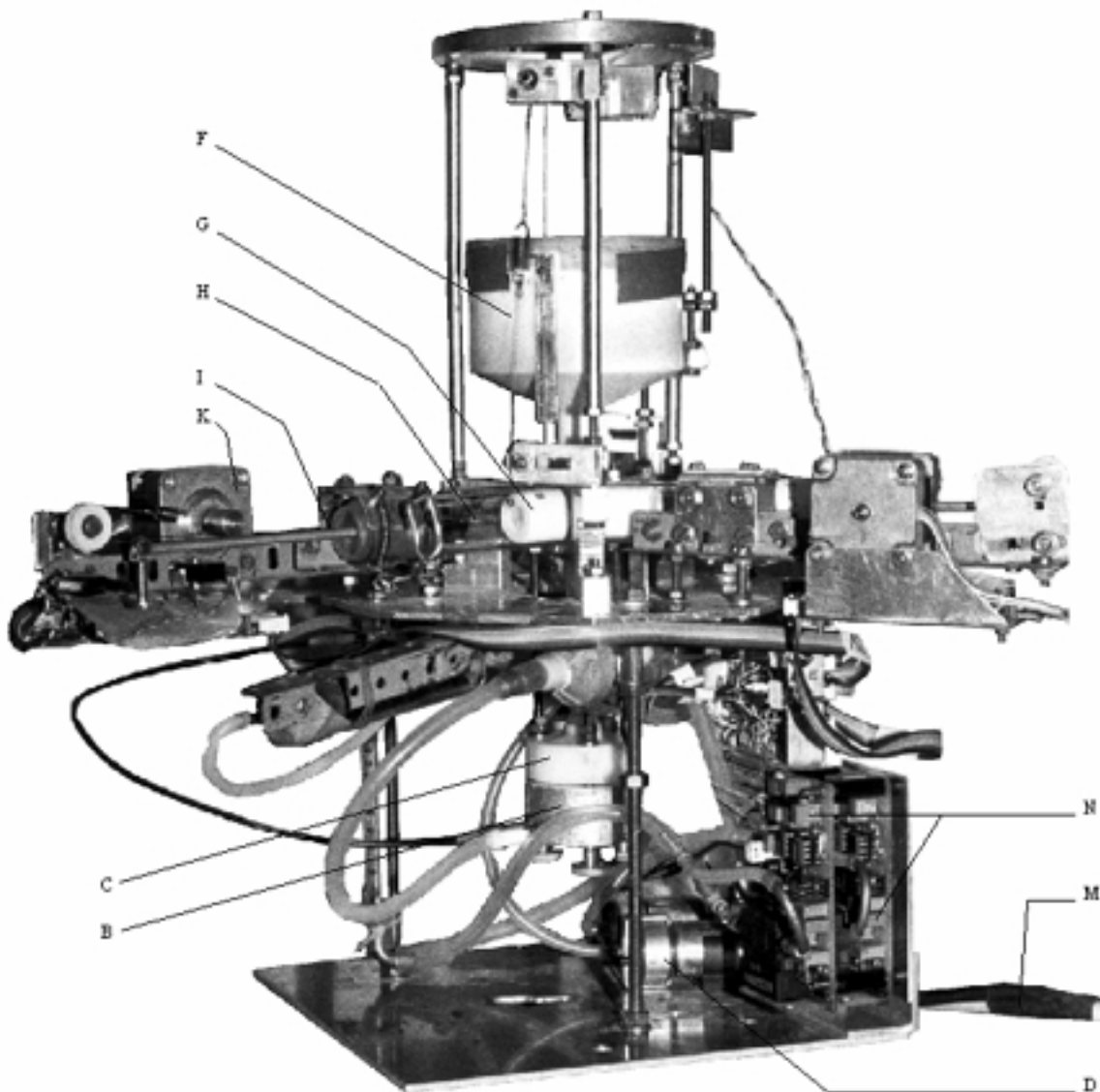


Figure 1. View of the EAG measurement probe: B: antenna chamber, lower part; C: antenna chamber, upper part; D: suction pump; F: charcoal filter; G: teflon vial with mixture of pheromone and oil; H: calibration syringe; I: piston rod; K: step motor; M: main cable; N: flow regulator

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 positioned on a pole between 0.3 and 2.8 m high. Wind velocity and direction are recorded in 40 ms intervals by two sensitive vector anemometers, one mounted on the EAG probe, the other at 2.8 m height.

Measurement sites and conditions for EAG measurements

In 1997, one field (St.Avit) in the area of Revel was measured on the day of the treatment and during the following 3 consecutive days.

In 1998, three fields (UE 2, NPP 2, and NPP 5) in the Revel area were routinely measured to follow the development of the pheromone concentration over time: Each field was measured on 5 to 7 individual days in a time span of 0 to 9 days after treatment

In 1999, we measured pheromone concentrations in 4 different fields (Peyre, Lassentiat, Caucou and Miracle) in the area of Saverdun (Ariège). In order to track the development of the pheromone concentration in each field, we took measurements on three or four days, most often with one or two days of pause. Apart from days with batches of rainfall, the most stringent reason for the pauses was the extensive irrigation.

The measurements were always made in the early morning hours (6:00 to 9:00) in order to record the pheromone levels in wheather conditions close to the ones existing at the flight time of *Sesamia*. Care was taken to measure always at a position in the field where the wind was coming from a major part of the treated field in order to load up the air with a representative amount of pheromone. In order to establish a vertical concentration profile, three probe heights were routinely used: 30 cm (ground level), 150 resp. 100 cm (within the maximum foliage) and 270 cm (above the canopy). The plant height was around 220 cm in 1998 and 200 cm in 1999.

One important disturbance in the measurements was the irrigation. Measurements could not be made during or within 6 hours after irrigation. In the Revel area (1997 & 1998), this posed not a major difficulty, since irrigation was scheduled once a week or once every 4 to 5 days. In the department of Ariège, irrigation turned out to be a problem. Since the type of maize in the Ariège fields was for seeding purposes, farmers were maintaining a high rate of irrigation to keep the maize turgid, since this is an advantage in the different castration operations. As a consequence, irrigation was present almost every other day. In one case, we measured directly after a rainfall and were not able to detect any pheromone. The pheromone signal reappeared the next day, however. Since then we tried to schedule our 1999 measurements in such a way that they were not influenced by the irrigation.

Results

Figure 2 shows a representative measurement result from the measurement site NPP2 (1998). The graph shows a clear pheromone signal in the range of $0.9 \cdot 10^{-6}$ to $2.0 \cdot 10^{-6}$ relative units, well about the individual antenna threshold levels. The concentration is very similar near the ground (30 cm) and in a medium height (150 cm) near the maximal density of the foliage. In all cases, there is a clear drop of the concentration at 270 cm probe height, which is somewhat above the canopy height of ca. 200 cm. This indicates that within the foliage, the pheromone is evenly distributed from the ground to the canopy. Above the canopy, the wind mixes the pheromone with fresh air and thus the pheromone content is diluted.

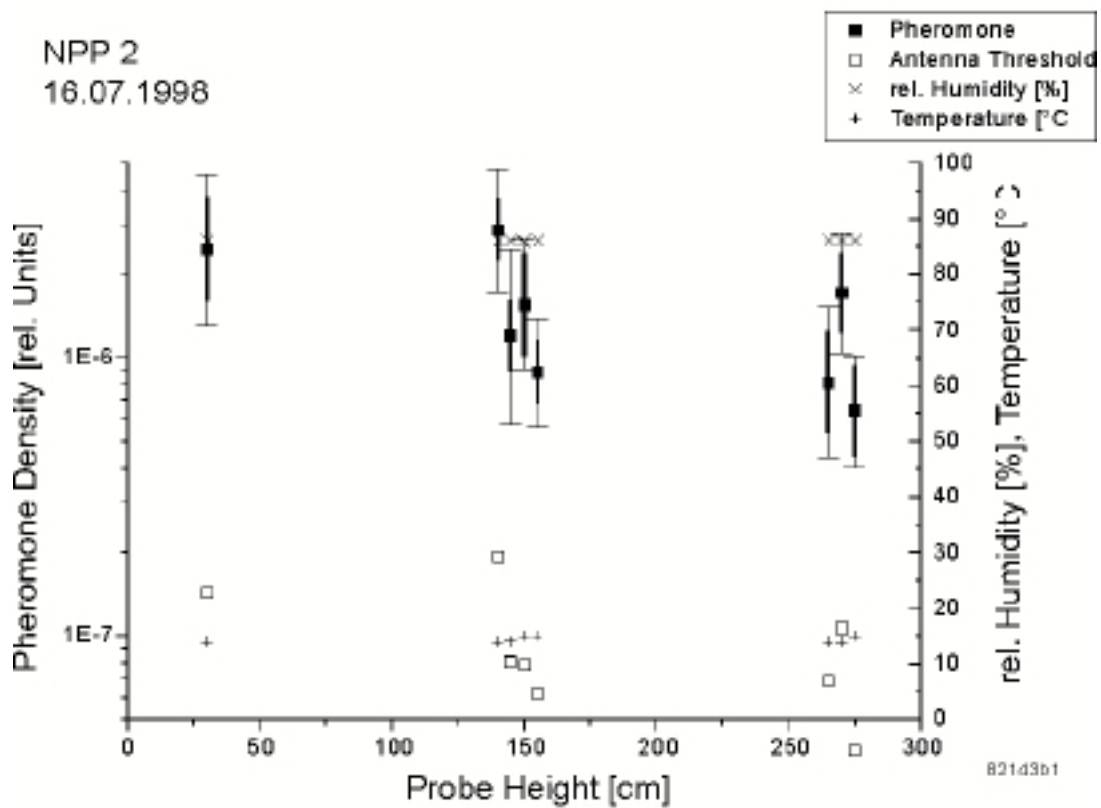


Figure 2. Typical measurement result from a field treated with spray formulation. The pheromone concentration is plotted versus probe height. The thin error bars with caps indicate the confidence interval of each individual measurement. The thick black error bars (without caps) represent the confidence interval of the mean of several measurements as an indicator of the variability of the individual pheromone density readings.

In order to gain an overview over the performance of the spray formulations at each measurement location, the measurement results from probe heights 30 and 100 cm of

each measurement day were averaged. These averages were plotted versus time after the treatment. Figs. 3, 4 and 5 show the results from NPP2, NPP5 and UE2 as examples. The figures show a continuous decay of pheromone concentration over time.

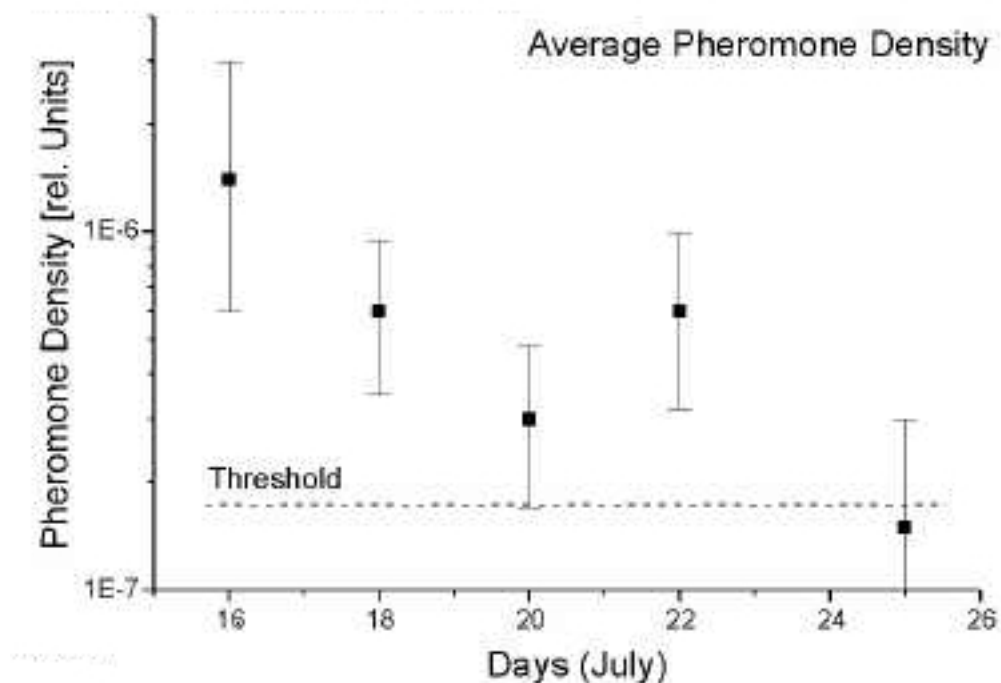


Figure 3. Development of average pheromone concentration over time after spray treatment on July 16 1998 at NPP2. After about 8 days, the pheromone concentration had decayed to the threshold, which was about 10 times below initial concentration.

Considering the different averaged concentration curves from the individual fields, several common characteristics can be discerned. The high values found shortly after treatment go down to the threshold level and seem to stay there. This does not mean, however, that the pheromone level stays constant at this value. Rather, we must assume that the pheromone concentration decays further. But whenever the antenna threshold is reached, the decay cannot be tracked any more, since the measurement system cannot distinguish between „pheromone“ and „no pheromone“ below the threshold. Any hypothesis about the dynamics of the decay therefore can only be tested in the range between initial concentration and threshold.

For all EAG graphs, we chose a logarithmic concentration scale. In such a diagram, a decay following an exponential law must appear as a straight line with a negative slope. An exponential decay can be a valuable first approximation to the time course of the pheromone concentration decay process since it is the adequate description in a system where the rate of evaporation is proportional to the amount of substance remaining.

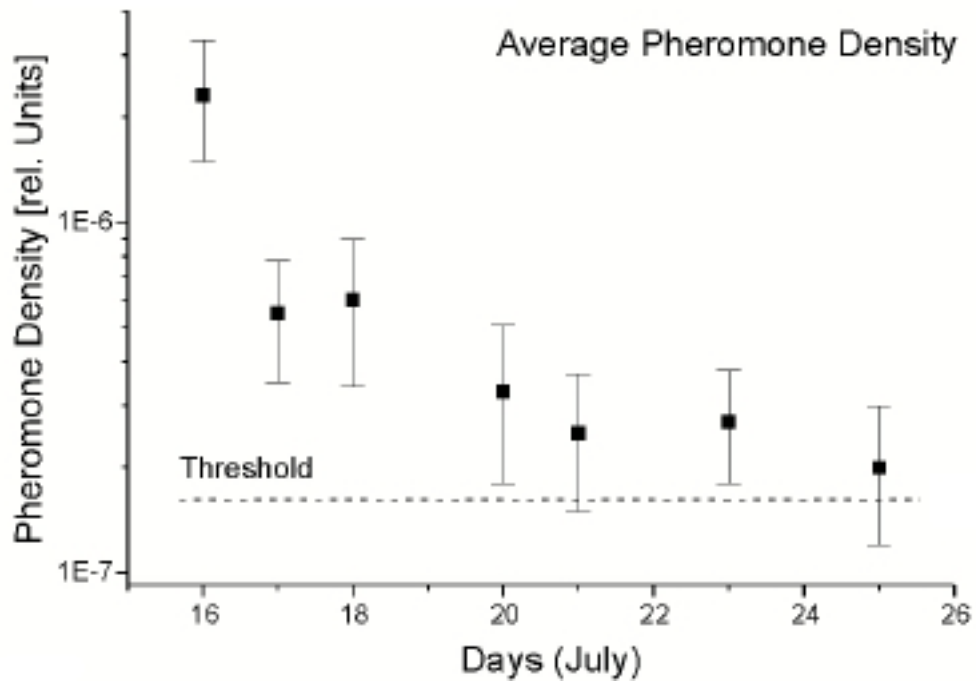


Figure 4. Development of average pheromone concentration over time after spray treatment on July 16 1998 at UE2. Note the very high initial concentration. After about 8 days, the pheromone concentration had decayed to a level about 10 times below initial concentrations

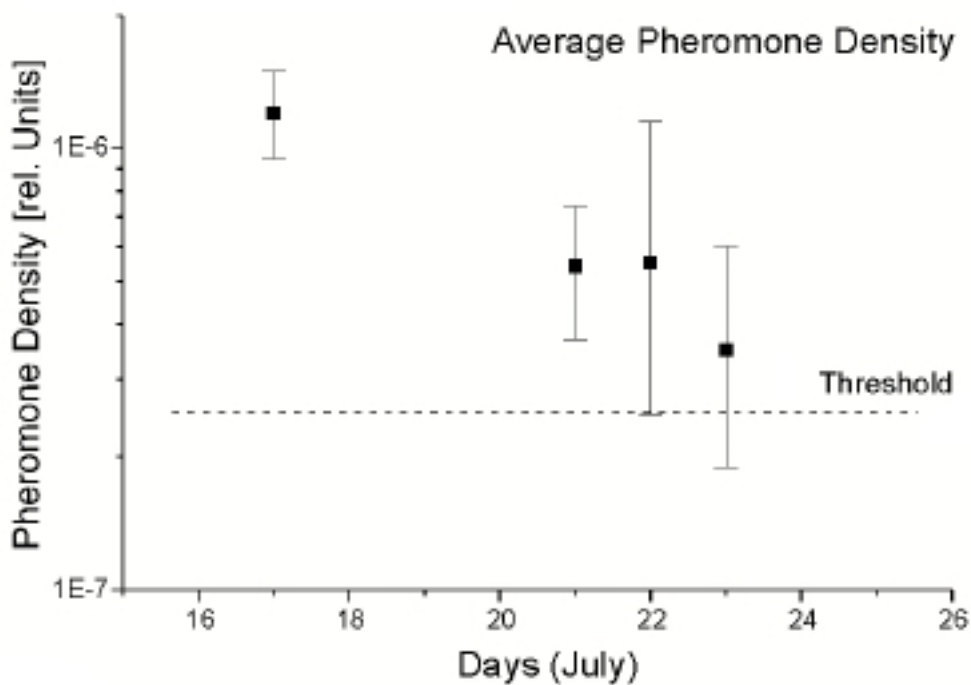


Figure 5. Development of average pheromone concentration over time after treatment at NPP5. After ca. 7 d, the pheromone concentration has reached threshold and cannot be tracked further

Assuming that this decay dynamics is valid, we can fit straight lines into the time course graphs recorded from all the different fields and years. The slope of these graphs yield a time constant, i.e. the time it takes for the concentration to decay to $1/e$ of its initial value. The results of these fits plotted in Fig 6 show a slight increase in the time constant between 1998 and 1999, indicating that the sprayed product showed a slightly longer lifetime in 1999. However, the very large error bars have to be considered, so that a significant time constant difference between 1998 and 1999 can hardly be established. Apart from differences in antenna quality, the larger errors in 1999 stem mainly from the restrictions in possible measurements imposed by irrigation and rain.

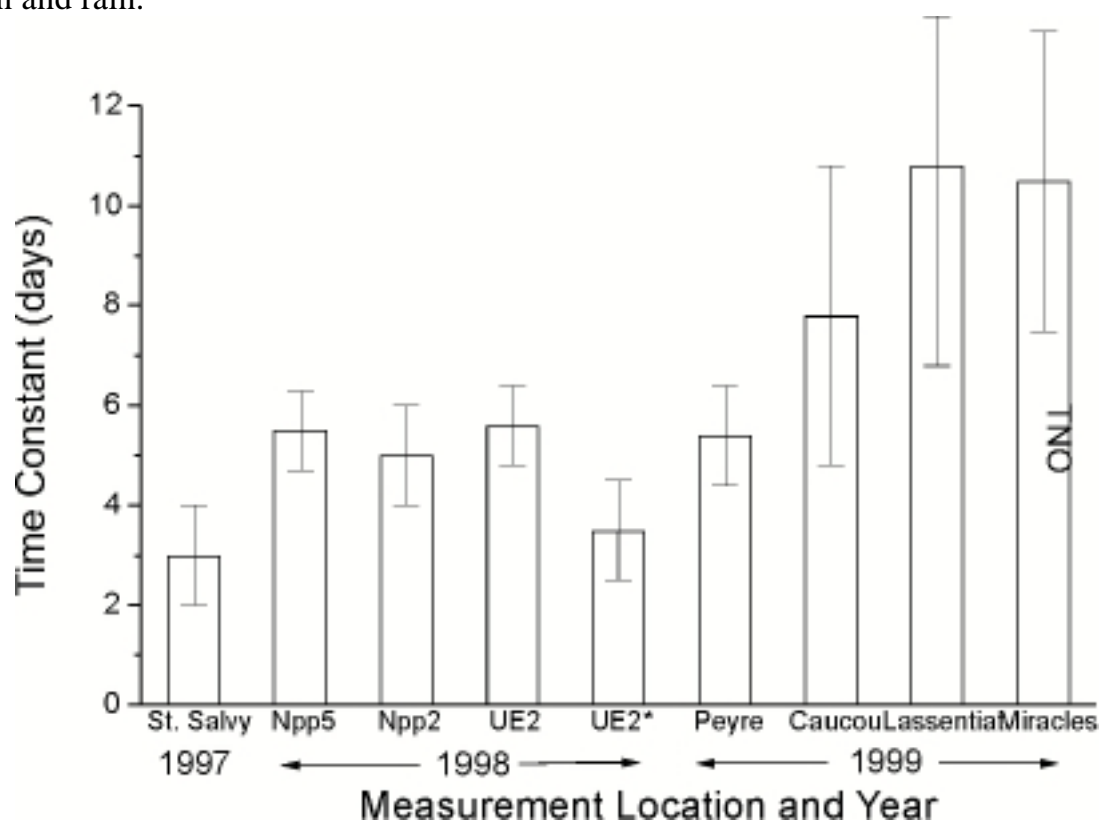


Figure 6. Time constants of pheromone concentration decay in the fields measured in 1997-1999. A slight trend to longer time constants can be observed from 1998 to 1999. The larger error bars in 1999 are mainly due to the restrictions in measurement schedule imposed by irrigation and rainy weather. The value of UE2* stems from an alternate evaluation including the very first data point.

The results of the EAG measurements 1997-1999 show the the pheromone concentration decays exponentially with time constants of 5 to 8 days. A slight tendency for longer time constants seems to be visible for the 1999 NPP formulation. The TNO formulation performs as well or better than the NPP, however the precision of these results should be improved. Strong reductions of the pheromone concentration were

observed in 1999 immediately after irrigation. A return to previous concentration values was found when the maize leaves had dried. This effect might reduce the efficacy of mating disruption in regions with very frequent irrigation or rainfall.

A sprayable formulation capable to evaporate the pheromone more evenly over time would be advantageous for further improvement of mating disruption in maize fields.

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