

# CIRCE – an Addition to the Toolbox for Assessment / Improvement of Mating Disruption

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The tools for investigation of new active ingredients and / or formulations for use in mating disruption have been classically known to be insect flight tunnels, field experiments and air sampling methods. While flight tunnels are key instruments to identify and optimize pheromone mixtures for lures, they may not always reveal optimal mixtures of ingredients for mating disruption, because the axiom „the best attractant is the best disruptant“ has been shown to be wrong in several cases.

Field experiments (comparing damage between treated and untreated plots over a planting season) suffer from a well known catalog of experimental drawbacks, and yet are required by many registration authorities.

With air sampling methods, pheromone density levels in successful mating disruption plots have been measured. Air sampling however lacks speed and flexibility to tackle measurement tasks like pheromone transport by wind, three dimensional distribution profiles of pheromone density or short term transients.

We propose to make more use of four additional tools, which interact to yield valid results in short time: 1) Standardized release rate measurements, 2) Field EAG, 3) Production of dispensers with reduced release rates, and, most prominent, 4) CIRCE<sup>3</sup>, a Cage method for Investigation of pheromone effects by Release and Capture, described by Eric Doye.

1) Standardized release rate measurements are recommended for pheromone dispensers of all kinds. They are performed in special wind tunnels featuring constant temperature, wind speed and relative humidity. A chain transport mechanism ensures that all dispensers measured perceive the same average air turbulence conditions and also transports each dispenser to the weighing scale (0.01 mg resolution) within the wind tunnel environment. The weight loss data gained this way give direct release rate values when the dispenser contains only one ingredient. In other cases, a system collecting all released materials on an adsorbing cartridge is used, and the collected materials are analyzed by GC. These devices give reliable release rate data independent of weather conditions within a 5 day time scale. They also permit to measure temperature and wind dependence of release rates.

2) The field EAG has been successfully used to measure horizontal pheromone density profiles, vertical profiles, Pheromone displacement by wind, decay of concentration in spray formulations, and storage of pheromone on plant surfaces. The field EAG has new applications when determining the pheromone profiles in applications where the plants are very different from the fields studied so far. In situations where the pheromone dispensers are not surrounded by plant leaves, the pheromone plumes expand in the turbulent air without interaction with the plant surfaces. The Pheromone density in this case is very variable, and the EAG signals tend to be sharp pulses caused by individual pheromone puffs. In this case, it is not possible to give an average pheromone density level because the characteristics of the EAG are extremely nonlinear. In addition, the overall precision and repeatability of EAG results cannot be better than 10 to 15% in good cases, which is about the same precision level as achieved with air sampling methods.

3), 4) CIRCE is a relatively new tool which however has already been adopted by a large number of -mainly applied- research centers. It relies on the idea, that a calling female in a

trap should not be able to catch any male if the pheromone treatment is sufficient to have efficient mating disruption. In practice, a large flight cage ( e.g. 6 to 8 m<sup>3</sup> volume) is placed in the middle of a treated field with a representative part of plants inside. At least one female baited trap is placed into the cage, and a large number (e.g. 50) of males are released into it. At the same time, a similar cage is used in an untreated field (far enough to avoid air transport of pheromone) as control. This is essential to document female calling as well as searching capabilities of males. After 2 to 3 days, no more catches are noted in the control cage, and the catches in treated and control cages are evaluated. An effective way to cancel out asymmetries in microclimate and other cage properties is to remove the dispensers in the treated area, place them around the former control cage and repeat the release-recapture experiment. This is especially easy, if only a minimum surface was treated for the experiment in an otherwise pheromone free environment.

The question, „what is a minimum treated surface?“ can be answered using the field EAG to measure how far one has to go inside a treated field to have stable pheromone levels. In the case of vineyards and apple orchards, these measurements yield approx. 10 m for wine and approx. 25 m for apple. We therefore estimate a treated surface of approx. 50\*50 m as a minimum treated surface. To treat such an area, only about 100 dispensers are needed. This means that dispensers with special properties can be made to test defined problems, such as: „what is the minimum release rate needed for dispensers to ensure trap shutdown?“ To tackle this problem, dispensers with specially reduced release rates were manufactured. Their release rate was measured under standardized conditions as described above. The recapture quotient (recaptures in treated cage divided by recaptures in control cage) was measured using CIRCE. Plotting release rate versus recapture quotient yields a „dose response curve“ of the system, in which the „threshold release rate“ (i.e. the release rate which is just sufficient to suppress male catches in the cage) can be determined. This release rate then can be regarded as the minimum release rate necessary for efficient mating disruption.

Testing new ingredients (e.g. pheromone precursors, additional bouquet components, „enhancers“) which are hoped to increase the efficiency of mating disruption can be done in the following way: apply dispensers of the standard pheromone with a release rate producing a recapture quotient of about 50% in two test fields equipped with cages. In one test field, add dispensers filled with the extra ingredient. If these are enhancing mating disruption efficiency, a reduction in the recapture quotient should be seen. To symmetrize, switch the extra dispensers to the other field.

More applications of this tool box are evident: Comparing attractivity of artificial lures to females, measuring lifetime of a spray formulations, measuring communication disruption in fields treated with „Puffer“ systems, measuring effects of airborne pheromone drifting from a treated field to an adjacent nontreated field, assessing recapture quotients in exposed positions (field edges, different heights, varying dispenser density and distribution....)

It seems that the CIRCE method backed by standardized release rate and field EAG measurements avoids the drawbacks of the classical methods flight tunnel and field test, yet combines their advantages, resulting in: a good representation of the natural environment, an integrative measurement of undisturbed behavior, a high sensitivity (10% difference in pheromone density level can be resolved), good reproducibility, highly significant statistics (50 males !), simple evaluation, short test duration, and -last not least- low cost. Registration authorities in Germany have begun to consider CIRCE data and standardized release rate measurements as relevant for registration. CIRCE has only one disadvantage, which will be addressed in the talk.

<sup>3</sup> In the ancient Greek epos „Odyssee“, CIRCE was an attractive woman endowed with witchcraft, who tried to keep Odysseus on her island forever.