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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PP 1/264 (2)

Efficacy evaluation of plant protection products

PP 1/264 (2) Principles of efficacy evaluation for mating disruption pheromones

Specific scope

This Standard describes the general principles of trial design for the efficacy evaluation of mating disruption techniques based on pheromones. Generally, these techniques are based on female sex pheromones, but others could be used, for example aggregation pheromones which attract both sexes for mating or, in rare cases, where males produce sex pheromones.

Pheromones and other types of semiochemicals may also be used in other pest control methods. These include use as repellents and in mass trapping techniques (with or without chemical insecticides). Whilst not directly covered under this guideline, a number of the practical aspects relating to trial design and assessment methods may also be relevant considerations for other semiochemical techniques.

This Standard should be read in conjunction with EPPO specific Standard PP 1/314 *Evaluation of mating disruption techniques against Lepidopteran pests in grapevine, pome and stone fruits under field conditions* and on the *Evaluation of mating disruption techniques against Lepidopteran pests in grapevine, pome and stone fruits under semi-field conditions* (in preparation)[1].

Specific approval and amendment

First approved in 2008-09.

Second revision approved in 2019-09 following adoption of EPPO Standards PP 1/314 *Evaluation of mating disruption techniques against Lepidoptera pests in grapevine, pome and stone fruits under field conditions* and the preparation of the Standard on the *Evaluation of mating disruption techniques against Lepidoptera pests in grapevine, pome and stone fruits under semi-field conditions* (in preparation).

1. Introduction

Mating disruption techniques may be based on several possible mechanisms. One common approach is false trail following, based on using high levels of female insect sex pheromones. These disrupt the male insect's sensory ability to locate and therefore mate with females. Prevention or delay of mating can cause significant impairment of breeding success with commensurate benefits in reducing subsequent crop damage. Because sex pheromones are typically species-specific, the mating disruption products usually target one pest species. It is also possible to have a product mixture containing more than one pheromone, each specific to an individual species. In such cases, data (from preliminary trials, published sources and field trials) should demonstrate the activity of each component against each individual species. Furthermore, careful consideration should be given if claims of extrapolation to other species are proposed, particularly for pheromones involved in mating disruption because these are typically species-specific.

Most techniques are based on the principle of releasing high rates of pheromone, creating a localized high concentration area within which males cannot locate individual females. Example techniques include simple manually placed dispensers, programmed 'puffers', sprayable formulations, micro-encapsulated formulations or luring males into a non-lethal trap where they are coated with a pheromone-loaded powder. The emerging males are unable to detect females, act as false lures and point sources of further pheromone release.

These techniques are very specialized and present particular challenges when considering trial design. A conventional design based around randomized small plots, replicated treatments and direct comparisons with a reference product and untreated controls is not usually practical. This guideline has therefore been developed to provide general guidance on those factors which should be considered when generating data and designing field trials.

[1]For EU Member States, SANTE/12815/2014 rev. 5.2 May 2016 *Guidance document on semiochemical active substances and plant protection products* provides additional guidance on semiochemicals and addresses the risk assessment areas. For efficacy, it refers to this EPPO Standard.

One of the challenges to the success of the technique is overcoming the border effects of immigration of mated females. There is also a need to prevent potential treatment interference between plots. For these reasons, sites cannot be split into conventional plots and replicated treatments are often impractical. Untreated and treatment areas need to be separated, usually by some distance. The extent of the separation of plots will depend on the biology of the individual pest species, in particular flight behaviour and typical distances travelled. Plot size for the pheromone treatments may also be significant (e.g. several hectares for some pests) in order to achieve reliable performance. Usually, the larger the area treated, the more effective the technique.

All these factors make comparisons of damage and population levels between any untreated, pheromone or reference insecticide-treated areas less accurate. It is particularly important therefore to gain information on the pest history and damage levels from previous seasons at the chosen site.

The effectiveness of the technique is also dependent on population density, and monitoring provides important information in this respect, both at the trial sites and making use of any local/regional monitoring programmes. Consideration needs to be given to appropriate trap (and product dispenser) placement. This is dependent on many factors, including those related to the trial site and the behaviour of the target.

Biology is of key importance when considering trial design and particularly factors relating to mating behavior, e.g. movement distances of adults (which will affect plot size), mating and egg-laying sites, spatial distribution of population within the crop, what the sex ratio is, whether unmated females lay viable eggs, whether males are polygamous and how many matings the male can achieve. The trial design must address potential border effects through the immigration of mated females into treated areas, and also prevent interference between treatment plots.

Trials conducted against low population densities may have to be conducted over several seasons to reveal differences between treated and untreated areas. A proportion of the trials may usefully explore the efficacy of the mating disruption treatment against moderate to high population densities when used as part of a season-long control programme with a range of other insecticide treatments. This information may then be used to support advice to growers. This includes comparison with a reference plot using only the same number of 'reduced' insecticide treatments alone in order to demonstrate the benefit of the mating disruption component of the combined programme.

2. Site location

Site choice is a very important element in the trial design. Various factors will affect the behaviour, distribution and immigration of the pest into the site. These include geography, topology, plot shape, wind direction, neighbouring crops, crop planting distances, crop height, management methods and the presence of any crop storage areas. Ideally, a single large site of sufficient size to contain untreated, treated and reference treatment with appropriate spatial separation to limit plot interference is preferred. In practice, spatially separated sites in which individual plots are situated are preferable which limit interference by immigration of mated females. Plots within a trial should ideally have crops of the same variety, similar age and crop structure, and have a comparable history of pest damage.

Details on variety, age (perennial crops) and growth stage should be recorded. A range of varieties that mature at different times can be important for targets with several generations per season. Including late varieties assists in demonstrating effectiveness across all generations.

2.1. Site maps

A site map may be useful to compare trial sites and put levels of control achieved into context. Together with knowledge of pest biology, it can also identify likely immigration hot spots, which aids both placement of monitoring traps and dispensers, and application of any appropriate insecticide barrier treatments. Site maps are particularly useful when the adult flight activity can take place over wide areas. An example of a site map is provided in Appendix 1 for a large-scale test mating disruption site and illustrates the information that may be recorded. It does not include examples of test product treatments as this will very much depend on the nature of the product, pest and site.

2.2. History of pest damage on site

Because of the need for separation between treatments and the difficulties in monitoring populations and measuring effectiveness (see below), it is particularly important to record the pest history at each site. This should include past monitoring records and past crop damage/yield losses that have occurred at the site to assist in determining both effectiveness of the treatments and allowing comparisons between plots.

3. Design and layout of the trials

The problem of interference, both between mating disruption plots and also with untreated plots, means that plots need to be separated by a minimum distance. The distance required will depend largely on the flight activity, movement of the individual pest species and prevailing wind direction but should generally be a minimum of 100 m and for some species may need to be greater. Plots do not necessarily need to be located in separate sites but could be spatially separated within a larger block.

Mating disruption techniques require large plots, with the actual size dependent on the target species and typical migration distance. The untreated controls and the reference insecticide treatments should, where possible and practical, have comparable population densities.

Whilst replication is preferred where practical, large-scale trials may be unreplicated but a suitable number of trials should be conducted to allow appropriate support of product claims.

4. Application of treatments

4.1. Information on pest pressure

An untreated control may be included to give a direct measure of pest population density and level of crop damage. It may be possible to make the assessment in an adjacent plot. As described, it may be necessary to have a spatial separation between the mating disruption test plots and the untreated plot. Assessments made in the untreated plot should be considered alongside other sources of information on pest history and regional monitoring records (see sections 2.2 and 5.2).

4.2. Reference products

Where possible and if available a reference mating disruption treatment should be included at a location near to the test sites. Conventional insecticide reference treatments can also provide useful information on pest populations during that season and can be applied in smaller blocks.

4.3. Barrier treatments

Insecticide treatments may be used as barriers to try and isolate the plot or reduce immigration in hot spot areas, or reduce initially very high populations within the site. It may also be necessary to control other pests with insecticides to prevent misleading interpretation of plant or fruit damage. In these cases, the product chosen should where possible have as little impact as possible on the particular target pest. The site maps and reference to monitoring information (see section 5.2) can be used to identify the likely areas of immigration where barrier treatments may be required and the timing of such treatments.

4.4. Test treatments

The distribution of pests within a crop may not be uniform across the site and dispenser placement should reflect this. Three of the common reasons for the technique not working are too high a population within the site, immigration into the site by already mated females and a high influx of adults of either sex from nearby high-population areas. The site map will indicate likely immigration points and determine where a higher number of dispensers (and monitoring) may be required. The edges of the plots may be particularly vulnerable because, for example, female Lepidoptera tend to lay their eggs in the first suitable location. A significant proportion of crop damage may occur close to these areas and buffer zones or discard areas may be useful to ensure results from the net plot are not compromised by such events. Alternatively, spatially separating plots to isolated crop areas may be more practical. Persistence of effect, particularly whether a second placement of dispensers will be required later in the season, should also be examined.

Placement of both monitoring traps (see section 5) and dispensers within the crop should also take account of pest biology, for example where flight activity is concentrated within the crop. Again, an explanation in the trial reports on the placing of both test dispensers and monitoring traps within the crop should be provided.

5. Mode of assessment and monitoring

5.1. Meteorological and edaphic data

During the test period, meteorological data should be recorded, including wind speed and direction. The latter is especially important because it influences pest movement and pheromone distribution.

5.2. Monitoring and estimating population densities

Monitoring pest populations has two purposes. The first is to determine the appropriate timing for any necessary treatments by providing information on adult flight/migration activity and an indication of local population levels (see section 4). Second, in the mating disruption plots the monitoring data may additionally be used as an indication of the product effectiveness.

Monitoring adult flight/migration activity should be based on traps located in the plots or in the margins of the experimental field (away from any treated buffer areas). Different semiochemical traps are described below and regardless of the type used these should be of known effectiveness (e.g. commercially available traps or semiochemical traps described in published literature).

The use of pheromone traps in the mating disruption plots, as the sole determinate of effectiveness or pest population density, should be avoided. The numbers caught in these monitoring traps can be misleading because if the test pheromone disruption product is working effectively, the males may not be able to locate the monitoring traps ('trap shut down'). As such, numbers in monitoring traps cannot be used for decision making on, for instance, the necessity or timing of treatments.

Alternatively, the monitoring traps could include other standard semiochemicals that are not based on sex pheromones, including kairomone lure traps (e.g. fruit extracts such as pentyl acetate or acetic acid) or light traps. Data from these traps can be useful because they are not affected by high concentrations of the sex pheromones, delivered by the test product, interfering with the males' ability to locate the monitoring trap. Such traps may attract both males and females and may not be species-specific, therefore it is important to record the presence of other species. Light traps may also be used as an alternative monitoring tool and again are not sex/species-specific.

Regional monitoring data should be provided where available because it also provides an indication of general pest pressure during that season.

In any case, it should be noted that there is not always a strong positive correlation between trap catches and the subsequent crop damage therefore catches in the monitoring traps cannot be a reliable indicator of population density and assessment parameter for effectiveness (see also section 5.3).

5.3. Assessing effectiveness

5.3.1. Crop damage

Because of the difficulties of using population levels as an indication of product effectiveness, the principle assessment is based on crop damage, whether qualitative or quantitative. Because mating disruption techniques are generally species-specific, care is needed to establish that crop damage relates to the particular target and not confuse it with other pest damage. Reference has also been made (see section 4.5) to the fact that crop damage may not be uniform throughout the plot and the importance of being able, where possible, to compare with typical damage from previous years.

5.3.2. Other indicators of effectiveness

The information on crop damage can be very usefully supported by other assessments. For example, use of mating tables with tethered virgin females to estimate reduction in mating can be a particularly useful indication of the success of the technique. Assessments of population reservoir at the end of the trials can also be useful if data from the previous year is available for comparison, e.g. if appropriate, using tree bands to determine numbers of larvae in diapause overwinter. Also, the content of pheromones in the dispensers (weight loss due to evaporation of the active substance) at the end of the trials may be of interest (for dispenser-based products) as an indication of dispenser efficiency over the season.

Statistical analysis should normally be done using appropriate methods which should be indicated. If statistical analysis is not done the reason for this should be justified. See EPPO Standard PP 1/152 *Design and analysis of efficacy evaluation trials*.

6. Crop safety assessments

It is accepted that these techniques (other than spray applications) are unlikely to cause significant crop damage. However, they do result in localized high concentrations of pheromone, possibly in combination with the release of other formulation components. Some types of dispenser are based on impregnated powder, with the possibility of spillage, or ‘puffer’ release packs. There is evidence with the latter that these can sometimes cause localized limited symptoms of damage. It is therefore considered appropriate to make visual assessments of phytotoxicity symptoms on leaves, and effects on russeting where appropriate, in the local areas where dispensers are placed.

For spray applications directly on the crop, crop safety assessments should be made. For further details see EPPO Standard PP 1/135 *Phytotoxicity assessment*, which contains sections on individual crops.

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Appendix 1 – Example of the use of site maps

Site maps may provide useful guidance both in determining appropriate monitoring and placement of pheromone dispensers, and as reference when interpreting trials data. An example map showing the type of information that may be useful is provided. Maps may also be based on satellite photographs. The example illustrated in Figure 1 is for a Lepidopteran orchard pest, but the type of information recorded is relevant for other semiochemicals and insect pests.

In this example, the position of the treatment plot is indicated, along with the placement of the monitoring traps. Insecticide treatments have also been made to provide barriers against immigration from adult moths. Both these treatments and the placement of the monitoring traps reflect the likely highest risk areas, which are from a neighbouring organic orchard where no insecticide treatments are used. The untreated area is in a conventional orchard.

For simplicity this map represents the situation prior to placement of any treatment dispensers within the 3 ha plot, but these could be added. It is also possible that standard insecticide treatments, which could be of much smaller plot size, could also be included within other areas of this orchard. The site map also illustrates some of the other information required on the trial orchard and surrounding orchards, including typical insecticide programmes.

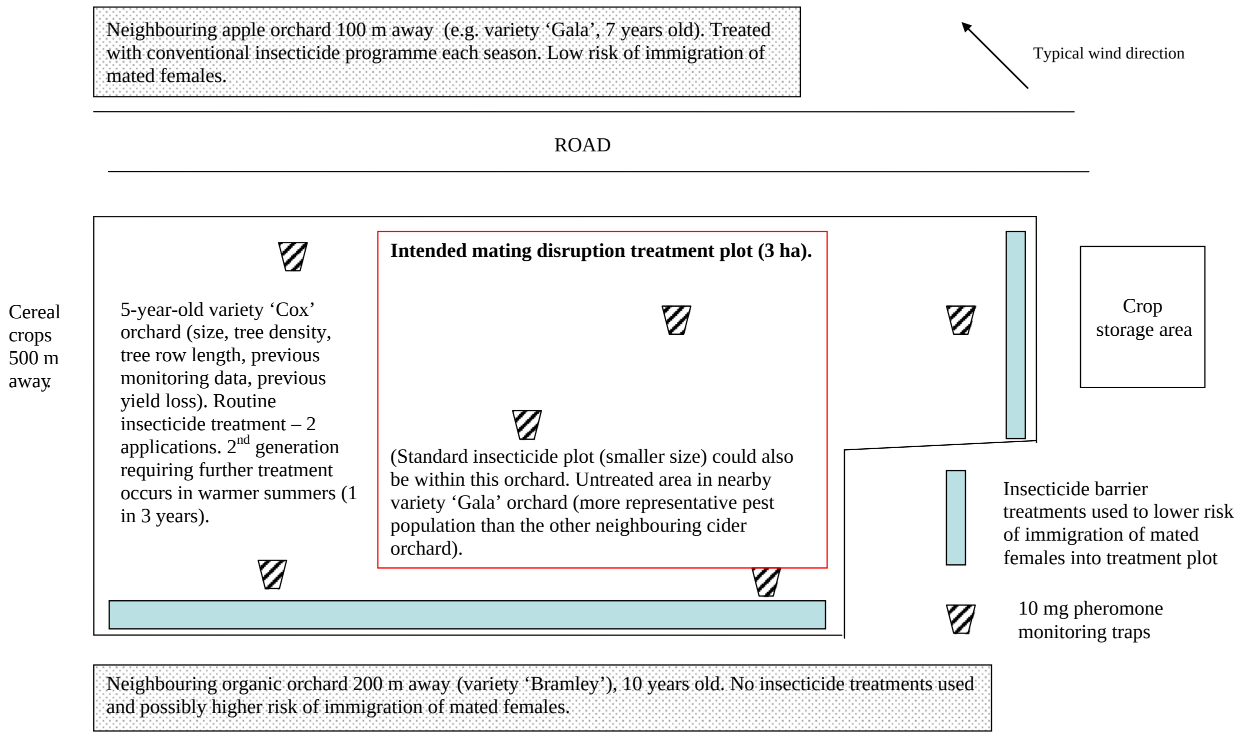

Fig. 1 Example of mating disruption treatment using an orchard site to place pheromone dispensers to control Lepidopteran damage. [Colour figure can be viewed at wileyonlinelibrary.com]