PHEROMONES AND MATING DISRUPTION

With a view to fulfilling its aim of spread the use of innovative, low environmental impact defence methods throughout the agricultural world, CBC (EUROPE) Ltd has joined forces with Shin-Etsu Chemical Co Ltd in order to develop and market a number of pheromone-based mating disruption products designed to counter the damage caused by Lepidoptera.

Over the last few years, heightened public awareness vis-à-vis environmental issues has led to a considerable reduction in the use of agrochemicals products in the field of crop protection. Indeed legislative measures have restricted both the level of residues allowed in crops productions and the number and type of molecules which may be used.

Thus, protection of the environment and the safeguarding of human health have been the main reasons for the development of both integrated and organic crop protection systems and, consequently, the gradual increase in the use of substances known as **sex pheromones**.

CHEMICAL COMMUNICATION BETWEEN LEPIDOPTERA

Insects are highly reliant on their olfactory system as a means of communication.

Indeed, insects used olfactory communications to regulate such vital processes as choice of mate, selection of host plant, choice of site for eggs laying, identification of location of prey, etc..

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This behaviour exploits a vast range of semiochemical (from the Greek term *semeion or* signal) substances: chemical substances which enable the exchange of information between organisms.

When interaction takes place between organisms from different species (and thus the message is interspecific), a semiochemical substance known as an allelochemical is generated. However, when interaction takes place between two organisms from the same species (and thus the message is intraspecific), the semiochemical substance released is known as a **pheromone**.

PHEROMONES

Chemical communication between insects of the same species was first hypothesized at the end of the 17th century, although real proof of this idea was only provided in the 19th century by the French naturalist Henri Fabre, who verified that virgin female Lepidoptera were able to attract males over long distances.

Nevertheless, it was only at the end of the 1950s that the German chemist Butenandt isolated and characterized the first insect pheromone, that of the silk worm *Bombyx mori*, using abdominal tissue from a large number of virgin females.

During the same period, Karlson and Luscher also proposed a definition of these substances which is still valid today: pheromones are substances secreted into the external environment by an individual and received by a second individual of the same species in which they provoke a specific reaction.

These substances many be divided into a number of categories, according to the functions performed:

- **Aggregation pheromones**: substances which increase the density of the number of insects of a certain species in the area around the source of smell. Many different types of behaviour have been linked to these substances, especially in social Hymenoptera (bees and wasps) and Coleoptera Scolytidae (beetles).
- **Dispersal pheromones**: stimulate flight and other defence mechanisms. Examples are found in bees and aphids.
- **Sex pheromones**: regulate the behaviour which encourages the two sexes to come together and mate. These substances may be used both to locate partners at long distances (sexual attraction) and to coordinate the "short distance" reproductive activities of males and females (courtship pheromones).

Of all the semiochemical substances identified, sex pheromones are those which have been most intensively studied and, consequently, most exploited in practical applications.

CHEMICAL SPECIFICATIONS

From the chemical point of view, the sex pheromones of Lepidoptera are classified as medium-long chained, oxygenated hydrocarbons with the following characteristics:
- straight chain composed of 10-20 carbon atoms;
- saturated, with up to 3 double bonds;
- chemical category: alcohols, acetates or aldehydes.

With a few exceptions, these molecules are all part of a well-defined group of straight-chained substances ending in one of the three functional groups.

The chemical specifications of the substances belonging to each of these series varies as the number of carbon atoms are increased or double bonds are introduced. This highly predictable variance is shown for alcohols in the following table:

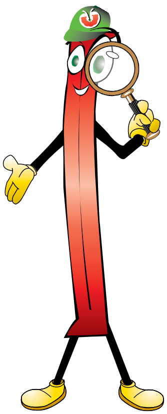
ALCOHOL	MELTING POINT (°C)	BOILING POINT (°C)	PROPERTIES	USES
1-Octanol	from -16° to -17°	194°-195° (760 mm Hg)	Colourless liquid	Cosmetics
1-Decanol	6,4°	233° (760 mm Hg)	Viscous liquid	Production of solvents, herbicides, surfactants
1-Dodecanol	24°	259° (760 mm Hg)	Viscous liquid	Production of wetting agents
1-Tetradecanol	38°	167° (760 mm Hg)	White crystals	Production of wetting agents, emollients for creams
1-Hexadecanol	49°	344° (760 mm Hg)	White crystals	Cosmetics
1-Octadecanol	59,4°	210° (15 mm Hg)	White grains	Cosmetics
(Z)-9-Octadecen-1-ol	13°-19°	195° (8 mm Hg)	Oily liquid	Production of detergents, antifoams

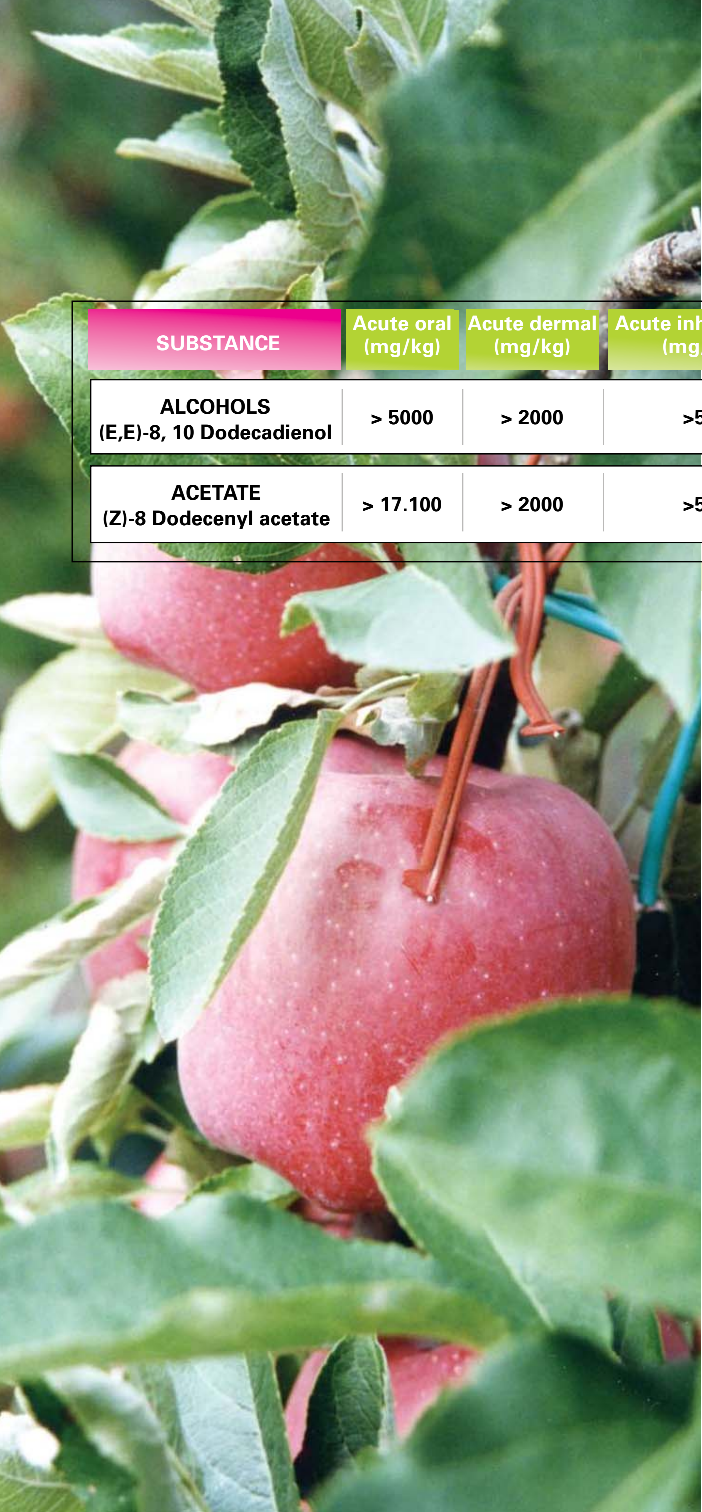
As can be seen, an increase in the number of carbon atoms leads to an increase in the melting and boiling points. The last substance shows the effect of the introduction of a double bond on the physical specifications of the molecule.

The following table shows the physical-chemical specifications of the *Cydia pomonella* (Codling Moth) pheromone.

CYDIA POMONELLA PHEROMONE	
Boiling point	110° - 120° C/2 mm Hg
Specific gravity (25°C)	0,857
Refractive index	1,467
Viscosity (25°C)	22,9 c.s.
Flash point	91°C
Vapour pressare (25°C)	1,428 x 10 - 2 mm Hg
pH	5,6

Cydia pomonella
(Codling Moth)





TOXICOLOGICAL SPECIFICATIONS

From the toxicological point of view, Lepidoptera pheromones are characterized by a low level of toxicity, as is shown by the examples in the following table:

SUBSTANCE	Acute oral (mg/kg)	Acute dermal (mg/kg)	Acute inhalation (mg/L)	Skin irritation (1)	Eye irritation (2)	Mutagenicity (Ames test)
ALCOHOLS (E,E)-8, 10 Dodecadienol	**> 5000**	**> 2000**	**>5**	**2.13**	**9.0**	**Negative**
ACETATE (Z)-8 Dodecenyl acetate	**> 17.100**	**> 2000**	**>5**	**0.96**	**5.3**	**Negative**

(1) Draize scoring system. Maximun score is 8.0

(2) Draize scoring system. Maximun score is 110

The following table provides toxicological data regarding molecules of the most commonly used agricultural insecticides:

SUBSTANCE	Acute oral (mg/kg)	Acute dermal (mg/kg)
Azinphos-methyl	**16,4**	**250**
Fenitrothion	**800**	**890**
Flufenoxuron	**> 3.000**	**> 2.000**
Tebufenozide	**> 5.000**	**> 5.000**

ENVIRONMENTAL FATE

All semiochemicals, and especially pheromones, dissipate rapidly into the environment, mainly through volatilization and degradation. Indeed, the ongoing presence of such substances in nature would counter the ability of insects' olfactory systems to receive subsequent communications. Straight-Chained Lepidoptera Pheromones (SCLP) are rapidly transformed via oxidation of the double bonds of the chain of carbon atoms and by other types of oxidizing degradation. The enzymes which bring about the degradation of the pheromone residues are present throughout nature.

MATING DISRUPTION

The **method** which exploits these substance in order to control insects and especially Lepidoptera, in agricultural contexts is universally known as **“mating disruption”**.

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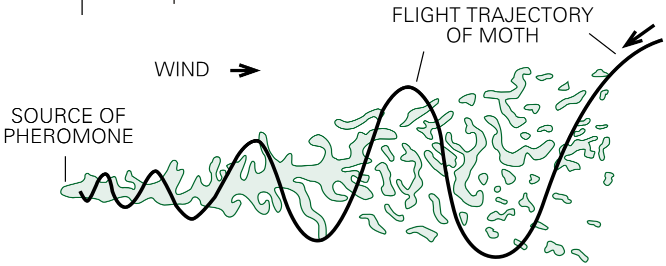
The behaviour of Lepidoptera during mating has been the subject of considerable study over the years.
Mating commences with the release of a specific pheromone by the female. When received by the male, this pheromone causes the male to move in a zig-zag flight against the wind towards the source of the pheromone.



The emission of a uniform concentration of synthetic pheromones in the environment disguises the natural pheromones, making them indistinguishable by the male insect and thus causes a change in behaviour.

The mating disruption method is based on the release into the environment of a synthetic version of the natural pheromone. Emitted in sufficient quantities as to create a concentration of the substance in the air (a few nanogrammes per m^3), the synthetic pheromone compromises the ability of the targeted male insects' olfactory systems to receive the stimulus of the natural pheromone. This effect may be the result of the following mechanisms:

a) sensorial inhibition leading to loss of ability to locate the female by the male insect;
b) masking of natural olfactory traces;
c) competition between artificial and natural sources of the pheromone (false trails following).

Implementation of the mating disruption technique generates both a **reduction in the total number of couplings (as a result of sensorial inhibition**, the masking of natural olfactory traces and competition between synthetic and natural pheromones) and a delay in mating (with a consequent reduction in overall fertility of the female). The combination of these two effects leads to a reduction in the overall reproductive capacity of the target insect and consequently a reduction in the population.

FUNDAMENTAL ASPECTS OF THE MATING DISRUPTION TECHNIQUE

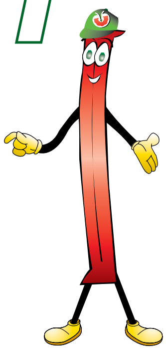
In addition to the biological features of the target insect, 3 groups of factors influence the effectiveness of the mating disruption technique:

1. **pheromone dispenser characteristics;**
2. **concentration of the pheromone in the field;**
3. **features of the treated area and other application aspects.**

1. Pheromone dispenser characteristics

Pheromone dispensers are specially designed to release molecules of pheromone into the surrounding area by exploiting a number of physical-chemical principles. Their technical construction is therefore extremely important.

The dispensers produced by Shin-Etsu Chemical Co Ltd are plastic micro-capillary type containers. The release of the synthetic pheromone into the



specification of the pheromones

environment is governed by two factors: the speed of permeation of the pheromone through the walls of the dispenser and the speed of evaporation of the pheromone into the air. While the latter of these factors (speed of evaporation) varies according to the average air temperature and the average wind speed in the area treated, the former (speed of permeation) mainly depends on the way in which the dispenser is constructed (and, especially, the type of plastic used and the thickness of the dispenser walls).
Shin-Etsu Chemical Co Ltd's commitment to research and development has enabled the design of a capillary-type dispenser whose walls may be varied in thickness, unlike other dispersal mechanisms, during production of the pheromone. Its design also ensures that the dispenser remains impregnated with the active ingredient even when its contents have been depleted.
The overall surface area from which the pheromone is released thus remains constant throughout the period of treatment and permeation remains continuous as long as external wind speeds and air temperatures remain the same. Over the years, Shin-Etsu Chemical Co Ltd has identified the types of plastic most suited to dispersal of the various types of molecule.

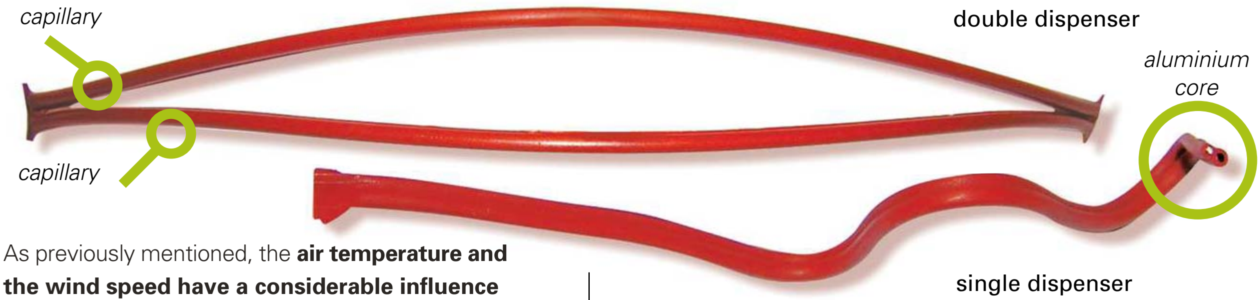
Effect of air temperature on the entity of release and on the concentration of the pheromone in the field.

SEASON	Release (g/hectare/day)	Concentration (ng/m^3)
SUMMER	4,3	20
SPRING	1,9	19

Effect of wind speed on quantities of synthetic pheromone released and on the concentration of pheromone in the field.

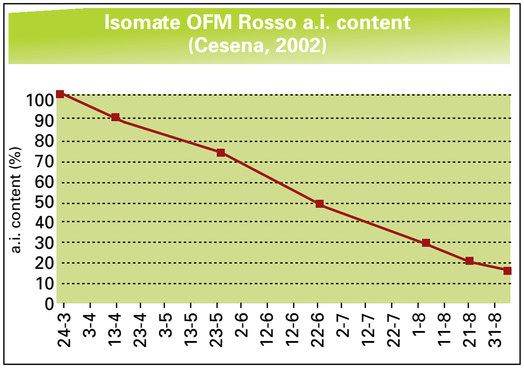
WINDINESS	Wind speed (m/sec)	Release (mg/hr/ha)	Concentration (ng/m^3)
A. WINDY AREA	2,5	235	1,2
B. NON-WINDY AREA	1	204	2,5
A/B	2,5	1,15	0,48

The following two graphs – release life and release rate – show the quantities of pheromone released by dispensers and are based on the results of samples collected from dispensers throughout the season and laboratory analysis of their residual content of active ingredients.

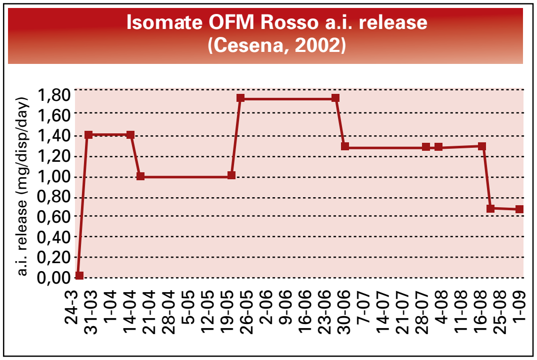


As previously mentioned, the **air temperature and the wind speed have a considerable influence** on the design of the dispensers.

Release life graph: indicates the quantity of active ingredient present in the dispenser at the moment of sampling in percentage of initial content (100%).



Release rate graph: indicates the rate of release of the content of the dispenser into the atmosphere in mg/dispenser/day. The curve expresses the difference in content of the dispensers at two sampling dates. This quantity (in mg) is distributed over the number of days between the two sampling dates.



2. Concentration of the pheromone the field

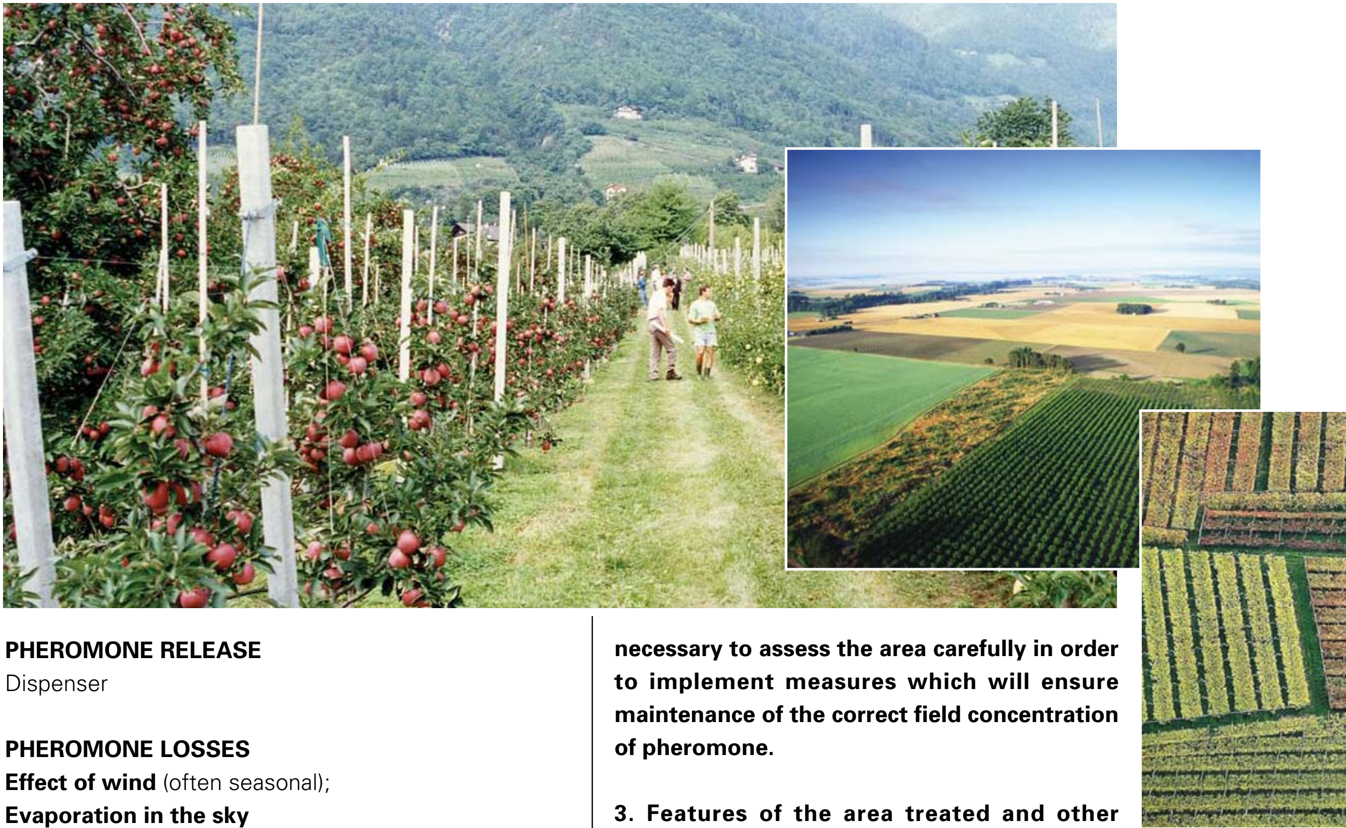
Interruption of olfactory communications between males and females requires the constant presence of a minimum concentration of pheromone throughout the period of activity of the insect. Once released by the dispensers, the pheromone spreads into the atmosphere forming a cloud which envelops the orchard.

The following table shows the concentrations of pheromone necessary to disrupt various species of Lepidoptera.

As can be seen, these concentrations are so small that they are recorded in nanogrammes per m^3, while the quantities released by the various dispensers are recorded in milligram's per day, as shown in the dispersal curve graph above.

TARGET INSECT	Concentration of pheromone (ng/m^3)	Active substance
P. gossypiella	2-5	Z,Z/E-7,11-16-Ac
A.orana	5-10	Z,11-14-Ac
C. pomonella	3-8	E,E-8-10-12-OH
S. hector	< 1	Z,Z-3,13-18-Ac
P. xylostella	< 1	Z-11-16-Ac+Z-11-16-Al

In order to understand these differences, it is necessary to analyze the "balance of pheromones in the field", comparing the input (pheromone release in the environment) with the outputs (the causes of pheromone loss or dissipation) of pheromones in the field.



PHEROMONE RELEASE

- Dispenser

PHEROMONE LOSSES

- **Effect of wind** (often seasonal);
- **Evaporation in the sky** (as a result of high temperatures);
- **Orchard edges**;
- **Field orography** (relief of land);
- **Decomposition** (UV, bacteria)
- **Absorption by soil and plants** (depending on the structure of the pheromone);
- **Particular and specific conditions** (presence of roads, flows of water, etc.)

As can be seen, there is only one input (the dispenser), yet there are many causes of loss of the pheromone. **In order to reduce these losses, prior to applying the method in orchards, it is necessary to assess the area carefully in order to implement measures which will ensure maintenance of the correct field concentration of pheromone.**

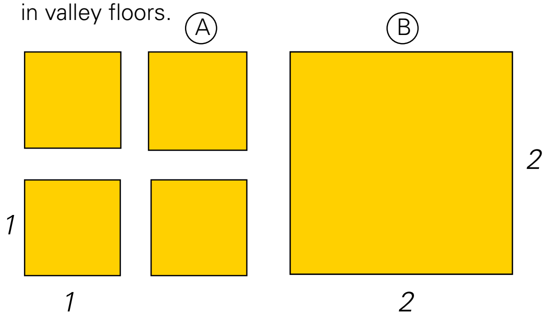
3. Features of the area treated and other application aspects

Size, shape and slope

The mating disruption technique gives its best results in large, flat, regular-shaped orchards whose trees are all of more or less of the same height. However, good results may also be achieved in smaller orchards following the implementation of a number of precautions such as expanding the area treated to include surrounding crops or increasing the suggested dosage.

Regular-shaped orchards (ideally square and flat)

enable the generation of a homogeneous cloud of pheromones and thus limit lateral loss of the active ingredient. Indeed, as the overall total area of regular-shaped orchards increases, their edge have less and less incidence.
This concept is clarified in the following diagram which compares the incidence of the perimeter of two orchards of the same surface area, one of which is uniform and the other of which is fragmented.
The slope is also an important element to be considered. Indeed, the relief of an orchard greatly affects the rate of displacement of the pheromones which, being heavier than air, tend to accumulate in valley floors.



PARAMETERS	SITUATION A	SITUATION B
PERIMETER	16	8
AREA	4	4
P/A	4	2

In order to counter this phenomenon, it is necessary to apply the dispensers in an irregular pattern with more dispensers in the upper part of the valley floor and fewer in the lower part of the valley, thus leaving the overall dosage unchanged.

Tree size

Better results are achieved in orchards of small trees as they are easier to envelop in a cloud of pheromone. Indeed large trees, often accompanied by large target pest populations, have high volume of foliage that make homogeneous coverage by pheromones rather difficult. Thus, while trees of less than 4 metres in height may be treated by positioning the dispensers in their upper third, taller trees require the positioning of two layers of dispensers: 1/3 of the dispensers in the upper part of the tree and 2/3 of the dispensers in the lower branches.

Level of infestation

The mating disruption technique is most effective in the presence of low initial levels of infestation. In orchards with medium-high or high initial levels of infestation it may be necessary to integrate the mating disruption with the use of insecticides. Indeed, high levels of infestation increase the possibility of casual meetings between insects, mating and eggs laying.
In such cases, the insecticide's choice should include substances of a low environmental impact which safeguard the presence of beneficial insects, yet ensure indirect control of other phytophagous insects in the orchard ecosystem.

Dispensers application

The dispensers must be installed in the field in spring prior to the start of the flight of the males of the target species.
This in order to act on the very first male adults and thus ensure precocious containment of the relative population.

The dispensers should be distributed as uniformly as possible with the exception of areas featuring particular orographic conditions or characterized by the presence of tall trees.
A greater number of dispensers must be installed in the rows of trees bordering the edges of orchards, and on the first tree in each row, in order to compensate for the greater loss of pheromones occurring in these areas.

Efficacy assessment
The application of the mating disruption method requires frequent monitoring in the field. Indeed, only by means of constant monitoring is it possible to assess the development of the phytophagous population and thus to act in time (to prevent a problematic situation from getting out of hand). The first level of control requires the use of monitoring traps which should be placed in the centre of the area treated as well as in areas of particular risk such as orchard edges and at the top of slopes where it is more difficult to maintain a correct concentration of the pheromone. Traps set in treated areas normally catch next to no insects. However this is only to be considered a preliminary indication of the correct functioning of the method, rather than foolproof evidence of its success as a total trap shutdown requires a far lower concentration of pheromones than the concentration necessary to significantly reduce mating, especially in the presence of large insect populations.

As damage to crops may occur even when no insects are captured in the monitoring traps, it is of absolute importance to assess the presence of damaged buds or fruits in the areas treated on a frequent basis. Indeed, in such a way it will be possible to satisfy any need for the implementation of further crop protection methods as soon as possible.

Knock-down spray
Elimination of the damage-causing insects should only be pursued when their presence exceed the thresholds specifically established for the control of each insect.

For more details of the monitoring methods and the characteristics of each CBC (EUROPE) Ltd product, please visit **www.cbceurope.it.**

USE OF THE MATING DISRUPTION METHOD IN EUROPE

Over the last few years, research has focused on discovering the most effective mix of pheromones and plastic polymers per each species of Lepidoptera and the best method of ensuring their constant release into the atmosphere

The physical-chemical characteristics of the Shin-Etsu dispensers are specific to each species.

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Testimony of **Shin-Etsu Chemical Co. Ltd and CBC (EUROPE) Ltd** 's commitment to research in the area of mating disruption is the increase in the number of products made available to the market by these companies since the appearance of the ISOMATE C dispenser (mating disruption of *Cydia pomonella)* in 1989. Information sheets relative to these products are attached to this guide. In addition, Shin-Etsu and CBC also have a series of products attending licensing and others still at the testing stage.

Use of the mating disruption method to control fruit and vine-damaging Lepidoptera has increased constantly over the last ten years for the same reasons as those which have determined its success in Italy.

In the European Union, pheromone dispensers are most widely used on vines. Indeed, mating disruption is used on a surface area of over 35,000 hectares to control *Lobesia botrana* and of over 60,000 to simultaneously control *Lobesia botrana* and *Eupoecilia ambiguella*.

The main drupaceous crop-damager is the *Cydia molesta* which, over the last few years, has also begun to attack pomes. At present, a surface area of circa 10,000 hectares is controlled using mating disruption techniques. Another problem regarding drupaceous trees is the containment of another increasingly insistent Lepidoptera, the *Anarsia lineatella.* Indeed, a surface area of over 2,000 has already been treated (using a specific pheromone) to combat damage by this insect.

The main pome-damaging insect is the *Cydia pomonella* whose containment using the mating disruption technique has expanded to the point that an estimated surface area of circa 30,000 hectares is now treated using this method.
Another Lepidoptera upon which the use of pheromones has generated interesting results is the fruit-damaging *Zeuzera pyrina.* A surface area of circa 2,000 hectares has already been treated to prevent damage by this insect.

Over the last few years there has been a considerable increase in **the request for multifunctional dispensers** (devices which are able to control several insects simultaneously using just one dispenser) which would led to a considerable saving in terms of cost of both product and man hours.

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