MADEX

Technical Information Sheet for Distributors

The codling moth, *Cydia pomonella*, is the most destructive pest in apple and pear production world wide. Other crops attacked by the codling moths are quinces, walnuts, apricots, peaches, almonds, kakis, medlars and oranges. The damage is caused by the larvae entering the fruit and penetrating to the core.

MADEX is a biological insecticide containing the *Cydia pomonella* granulovirus (CpGV) as the active ingredient, which is a natural occurring pathogen of the codling moth. Andermatt BIOCONTROL AG is successfully producing MADEX since many years. MADEX is registered and distributed in many countries all around the world.

The Codling Moth – General Information

The life cycle of the codling moth, *Cydia pomonella*, is starting in spring, when temperatures are rising. The last –instar larvae, having over-wintered in the bark of the trunk, start to pupate. After pupation, the adult moths are flying, usually in the evening times. The females of the first generation deposit up to 300 eggs mainly on the leaves. If a second or third generation is formed, the females of the later generations can deposit their eggs directly on the fruits. The speed of the maturation of the females and also the development of the eggs depend of the temperature. Thus, the first appearance and egg deposing are much earlier in warmer climate, and also the hatching of the larvae is earlier. Within 5 to 15 days after egg deposition the larvae hatch and crawl around in search for fruits. The larvae chew their way into the fruits, usually entering by the blossom end, by the stalk end or at contact points between the fruits. Their spiral tunnel finally leads towards the core. After the larvae reach the 5th instar, they leave the fruit, and go for pupation in their cocoons at the base of the trunk.

Depending on the climate condition, a second or third generation can be formed per season, until the larvae go for hibernating at the base of the trunk. Not all individuals of a population will go for a second or third generation. It is known that a part of the population will go for hibernation after just one generation, at least in regions of moderate regions.

Figure 1: *Cydia pomonella* larva entering an apple. The larva puts the first bites of the apple skin aside.

The Codling Moth Granulovirus (CpGV)

The *Cydia pomonella* granulovirus or codling moth granulovirus (CpGV) belongs to a family of insectpathogenic viruses called baculoviruses. Baculoviruses can only be found in arthropod species and are known to have a very narrow host range. CpGV is a natural occurring pathogen of *Cydia pomonella*. Apart from *Cydia pomonella*, CpGV infects only very few species as *Cydia nigricana* or *Rhyacionia buoliana*. The virus is characterised by a virion containing the DNA, which is encapsulated in an occlusion body (OB). The OB protects the virion from destructive environmental impacts. The size of a single virus particle is not bigger than 400 nm (Figure 2).

Figure 2: *Cydia pomonella* granulosis virus (CpGV). The virion is embedded in a protective occlusion body.
The larvae have to ingest the viruses to get infected. No infection through the insect integument is possible. In the alkaline environment of the larval midgut, the OBs get dissolved and release the virions, which are able to penetrate the cells of the epithelial layer in the midgut. Inside the host cell nucleus, the viral DNA is getting incorporated into the host genome, which leads to the replication of new viral DNA. The new produced viruses get propagated to other parts of the larva. Within a few days the viruses infest most organs of the host. The larva stops feeding and often leaves its tunnel. At its death, the infected larva is melting and releasing billions of new viruses into the environment, which can infect new larvae (Figure 3).

Figure 3: *Cydia pomonella* larva (L1 left, L5 right) at its latest stage of CpGV-infection. The larval bodies are melting and releasing millions to billions of new CpGV occlusion bodies into the environment.

The place and time point of virus ingestion are important factors for an optimal CpGV application. During hatching, the larvae do not eat the egg shell. Afterwards, the young larvae crawl around searching for fruits and suitable entering sites. During this period of time (30 minutes to 1-2 days), the larvae can get infected by drinking dew droplets or eating leaf surface. Anyway, after crawling around on virus contaminated leaves and fruit surfaces, the larvae will carry enough virus material on their bodies into the tunnel. By this way the tunnels get contaminated by the viruses. The larvae that are feeding inside the tunnel finally ingest the viruses.

The infectivity of CpGV is high. Lab experiments revealed 50% mortality in codling moth larvae that ingested only one or two virus particles. At a high virus dosage, the larvae already get killed at first instar. None the less, the young larva may cause small stings on the fruits before they die, as they usually only get infected after starting feeding on the fruit. Larvae that get killed by the CpGV release billions of new virions into the environment (Figure 3). This dispersion of viruses is also called horizontal transmission. At lower virus concentration, the larvae survive the first larval stages, but get killed at later larval instar. Although these larvae already caused deep damage on the fruit, the low CpGV concentration reduces the pest population further. Some infected codling moth larvae can survive and complete their cycle, but pass on the virus to its offspring that can be later killed by the virus. This mode of transmission is known as vertical transmission. A minor part of the larvae infected by CpGV get not immediately killed by the virus. These viruses can stay inactive in the host until enfeebled, or stress (e.g. overwintering, starve, other diseases) will lead to an activation of the viruses that kills its host (latent infection).

UV radiation is the most destructive environmental factor on CpGV. Exposed to the sunlight, the half-life period of CpGV is only 2 days. In shaded areas or protected by dust, the lifespan of CpGV is much longer. In the soil, CpGV is known to survive for several years.

MADEX Product Information

MADEX is a biological insecticide containing CpGV at a concentration of \(3 \times 10^{13}\) viruses per liter. The viruses are produced in vivo (see Figure 4). The formulation only contains organic additives; therefore the application of MADEX produces no chemical residues in the food chain and can be used in organic farming. Apart from the viruses, the formulation contains also larval bodies and diet residues, which improves the protection from UV radiation and consequently the lifespan of the viruses in the environment. As CpGV is highly specific to *Cydia pomonella* and no beneficial organisms are affected, the use of MADEX is environmentally safe.

Figure 4: In-vivo production of CpGV on infected codling moth larvae for the mass-production of MADEX.

MADEX is an excellent tool for both damage and population control. MADEX is a larvicide. Codling moth larvae that are treated with a high dosage of MADEX at an early instar (L1) will die in a short time. Nevertheless, small superficial damage can not be prevented, as the larvae have to start feeding on the fruit and get not instantly killed due to the mode of action of the virus. Depending on the fruit varieties, such superficial damages, or “stings”, heal up well leaving only small scars on the fruit (Figure 5). In comparison to chemical insecticides, MADEX has a much higher
long-term impact on the codling moth population. The virus will multiply in the host and disperse in the environment after killing the larvae. Due to the relatively slow mode of action and the high mortality up to the end of a generation cycle, the efficacy of the virus is underestimated by only assessing the damage on the fruits. Due to the possibility of vertical transmission or latent infection of the virus, the treatments of MADEX can reduce the population even in the next generations. In contrast, larvae that survived a chemical treatment will have no barrier, complete their development and reproduce without further population control. The control of the codling moth population with MADEX is sustainable.

Figure 5: Damages caused by *Cydia pomonella* on apple. Healthy codling moth larvae cause full damage by entering the apple to the core (left). Larvae infected at early instar may cause superficial damage (“sting”) before getting killed by CpGV due to the mode of action of the virus (right).

MADEX is environmentally safe and causes no hazardous residues on fruits, plants and in the environment. Firstly, the CpGV do not infect any beneficial insects, vertebrates or plants. Furthermore, the viruses get inactivated in the sunlight by the UV radiation. The content of microbial contaminants of MADEX is deriving from larval bodies and diet in the formulation. No human pathogenic germs are detected in MADEX. The level of aerobic mesophile germs is not higher than $10^7$ CFU per ml, which is still in a threshold range accepted for many culinary products. The CFU contamination on the fruits after a MADEX application is very low due to the low MADEX volume and very high dilution effect. The very small amount is negligible compared to natural occurring flora of microorganisms on the fruits.

**MADEX Application Strategies**

The precise timing of the first application of MADEX against the young larvae of the first generation is a crucial factor for a successful damage control. When applied too early, the viruses get inactivated by UV radiation. When applied too late, the larvae have already entered the fruits without contact to the viruses. Therefore the highest virus concentration is necessary immediately before the first larvae are hatching. The swarming of the adult moth can be monitored with pheromone traps. As soon as the evening temperatures (at 9 pm) are >18°C, the females start with oviposition. From this time point, the hatching can be calculated by the sum of day degrees (DD). The maturation of the eggs usually takes 90 DD. We recommend the first MADEX application already after 86 DD (see Box 1).

**Box 1: The first MADEX application easily calculated!**

Use the sum of day degrees (DD), which lay above the zero point of development (for codling moth: 10°C):

$$DD = \frac{T_{\text{max}} + T_{\text{min}}}{2} - 10^\circ C$$

**Example:**

The daily temperature maximum is 25°C and the minimal temperature is 15°C. Therefore:

$$DD = \frac{25^\circ C + 15^\circ C}{2} - 10^\circ C = 10^\circ C.$$  

The first MADEX application is indicated when the sum of DD after oviposition reaches 86 DD. In this example with an average DD of 10°C, MADEX should be applied after 8 to 9 days. The higher the average day temperature, the earlier MADEX has to be applied.

The standard dosage of MADEX is 100 ml per hectare. It is recommended to repeat the application after 8 days of sunshine. Two cloudy or partially sunny days count as one day of full sunshine. Usually 3 applications per generation are necessary. Of course, application rate and strategies can be adapted to the specific condition of pest pressure and to the use of other control measures. At lower pest pressure the second and following applications can be sprayed with a lower concentration of MADEX or bigger intervals. Nevertheless, the first application at the standard application of 100 ml per hectare will give the best effect for damage control (see Figure 6 and Box 2).

Figure 6: MADEX standard application. Three applications with the full dosage (100 ml/ha) on one *Cydia pomonella* generation. Spraying interval of 8 days of full sunshine.
Depending on the local situations, different strategies can lead to a successful codling moth control with MADEX:

**MADEX can be intensively sprayed** with a reduced dosage of 50 ml per hectare but in shorter intervals of 6 sunny days resulting 4 to 6 treatments per generation. Such a strategy is often followed in organic farming systems. Of course this spraying schedule is more time consuming, but can reduce the total amount of MADEX needed during the season. Furthermore it guarantees a more equal distribution of virus level over the time. But also with such a strategy, the first application is recommended to be sprayed at full dosage of 100 ml per hectare, in order to have the best result in the damage control. (see Figure 7 and Box 2)

The combination of MADEX and mating disruption is a widespread strategy of the codling moth control. Particularly in small orchards with a high probability of infestation by surrounding undisturbed orchards or in areas with a generally high infestation of codling moth, mating disruption as a single tool for population control is not suitable. But in combination with MADEX, an excellent control of the codling moth population is possible. Depending on the infestation, one to six treatments of MADEX at a dosage of 50 ml per hectare are necessary (see figure 8).

**MADEX can be used in combination with chemical insecticides.** This strategy is particularly followed to reduce the risk of resistance of the codling moth towards chemical insecticides. Usually MADEX is applied on the first generation in order to reduce the pest population. The first MADEX application with 100 ml per hectare is followed by several MADEX applications depending on the infestation level. At the peak of the first and second flight, the use of a fast-acting chemical insecticide can be sprayed to prevent fruit damage. It is also possible to continue with MADEX applications to hold down the pest population, and await the extend of the second flight to decide if a further chemical treatment is necessary. In order to reduce the initial population, a treatment with an ovicide or the installation of mating disruption is a further option for a combination with MADEX and other insecticides (see figure 9).

**Box 2: Fruit damage reduction**

Data from field trials in Italy, by Pasqualini and Civolani, 2000

<table>
<thead>
<tr>
<th>Fruits with damage in %</th>
<th>Efficacy in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>15.9 c -</td>
</tr>
<tr>
<td>Azinphos-methyl SC</td>
<td>2.5 ab 84.3</td>
</tr>
<tr>
<td>MADEX standard</td>
<td>1.8 ab 88.5</td>
</tr>
<tr>
<td>MADEX intensive</td>
<td>1.5 ab 90.9</td>
</tr>
<tr>
<td>other CpGV product</td>
<td>4.0 b 74.8</td>
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MADEX applied in standard (3 x 100 ml/ha) and in intensive (1 x 100 ml/ha and 3 x 50 ml/ha) applications reduced the fruit damage with the same or better efficacy as the chemical control Azinophos-Methyl (2 x full dosage) and other CpGV product (3 x full dosage). Means in one column with a different letter differ significantly (P<0.05)

**Figure 7: MADEX intensive spraying.** After the first application with the full dosage (100 ml/ha) (big arrow), MADEX is applied in shorter intervals (6 days of full sunshine) at half dosage (50 ml/ha) (small arrows)

**Figure 8: MADEX combined with mating disruption.** After early installation of mating disruption (green arrow), MADEX is applied at half dosage (50 ml/ha) (red arrows).

**Figure 9: MADEX combined with chemical pesticides.** After the first application with the full dosage (100 ml/ha), MADEX is applied at half dosage (50 ml/ha) (red arrows). Chemical pesticides are applied on eggs and/or during the peak of flights of Cydia pomonella moths (blue arrows).
MADEX Resistance Management

Resistance management is necessary where CpGV is continuously used during several years. First occurrence of resistant codling moth population towards CpGV has been observed in Germany in orchards that have been treated exclusively with CpGV over many years. Until now, resistance problems are locally very restricted, but affect all CpGV products equally. Resistant codling moths are more than 1000 times less susceptible towards the applied CpGV strain. In order to prevent resistance problems, use MADEX in combination with other control measurements as mating disruption or other pesticides.

Resistance towards CpGV can be broken! Andermatt BIOCONTROL AG is continuously developing its virus products and offers new solutions. Andermatt BIOCONTROL is able to provide you MADEX Plus, an advanced CpGV product, which has an excellent efficacy against the resistant codling moth populations. In case of resistance problems and further question on this topic, please contact our experts.

MADEX Recommendations of Use

No additives are necessary. MADEX is ready to use. It is not necessary to add sugar, skimmed milk or any other additives. The application timing is much more important than the use of any additives. In order to protect the viruses from UV exposure, it is recommended to spray MADEX in the evening. Formulation compounds already serve as an adequate UV protection. The use of sugar or molasses as feeding stimulant improves the performance of MADEX. But field trials revealed that, MADEX applied without additives is also highly effective compared to other CpGV products (see Box 3)

MADEX can be used in tank mixes with other pesticides, like wettable sulphur, chemical fungicides and insecticides, but not with Myco-San and Myco-Sin, Ulmasud and copper products. Be careful that the pH of the tank mix lies between 5 and 8. Particularly, the alkaline environment will destroy the protective occlusion bodies of the viruses which leads to a quick inactivation of the viruses. Therefore avoid tank mixes with substances as lime sulphur or soaps.

The tank mix with copper has destructive effect on the viruses, and therefore should be avoided. The spraying of copper a few days before or after a MADEX application is acceptable. It should be noted that the negative effect of copper on MADEX is much less important than to miss the right time point of a MADEX application, particularly of the first application. Field trials revealed a reduction of 10 to 15% of the efficacy of MADEX (100 ml per ha) when sprayed in a tank mix with copper (1kg/per ha).

MADEX has a good rainfastness. The surface of the virus particles is lipophilic, which favours a strong adherence to the plant surfaces. Therefore no washing-off effect by rain is to be expected and no additives are necessary to improve rainfastness.

High temperatures after a MADEX application are not a limiting factor for the CpGV efficacy. The influence of the UV radiation in the field is much more important and will reduce the amount of viable viruses much faster than the high temperature does. In the long-term, high temperatures reduce the viability of the viruses, which is important for storing MADEX before application.

MADEX has to be stored in the refrigerator. At temperatures below 5°C, MADEX can be stored for at least two years. If stored in a deep freezer (below -18°C), MADEX can be stored for many years without loss of activity. The half life time of MADEX at room temperatures (26°C) is about 6 months. At 40°C the inactivation is much faster. The storage conditions during transport or at the end-user place can be temporarily sub-optimal, but can be accepted for a few days. Nevertheless, high temperatures should be avoided, when ever possible. We recommend storing MADEX always in the refrigerator.
below 5°C. At -18°C, MADEX is still liquid and can be immediately used for spraying without unfreezing. Once opened, the bottle can be stored further at low temperatures without loss of quality.

MADEX Quality control

Bioassays as quality control of a CpGV product. Several CpGV products are on the market, with different content of active ingredient, volume and formulation. The counting of the number of virus particles under the microscope is difficult and the results strongly vary between different counting persons. Furthermore, counting can not distinguish between active and inactive viruses. The activity of a CpGV product can be assessed by using bioassays in artificial diet. Andermatt BIOCONTROL AG is carrying out such bioassays for the quality control of each produced MADEX batch and guarantees its high activity for each batch in comparison to a standard. Furthermore each batch is proved to be free of human pathogens and not to exceed the microbial contamination threshold of 10^7 CFU per ml.

MADEX Efficacy Trials

Field trials are necessary to demonstrate the real efficacy of MADEX in comparison to other control measures and competitive products. The influence of UV radiation and other environmental factors on the performance can only be assessed in the field.

The monitoring of the fruit damage can be assessed by counting the attacked fruits. Due to the slow mode of action of the virus, infected larvae can still cause superficial damage on the fruit before getting killed by the virus. Therefore, it is important to differ between superficial damages and full damages to the core, to assess the efficacy of the CpGV. Furthermore, also the fallen fruits have to be assessed, as a major part of the fully damaged fruits fall down before harvest.

The monitoring of the codling moth density is important, because MADEX has a strong effect on the pest population. The codling moth population can be assessed by using traps of corrugated card boards. Double strips of corrugated card boards are fixed on the basis of the tree stems at a height of 10 to 20 cm (see figure 10). The hibernating larvae will spin inside of the strips. As a part of the larvae of the first summer generation may already go directly for hibernation, the traps should be installed before this time point. The traps are collected in the autumn and the diapausing larvae found in the traps are counted.

Figure 10: Installation of cardboard traps for the assessment of the codling moth population (Figure: M. Baggiolini)

MADEX Swiss Quality and International Certification

MADEX is included on the input list of Swiss Research Institute of Organic Agriculture (FiBL) and has been confirmed as a product analogous the European regulation for producers of organic farm inputs (Regulation EEC No. 2092/91).

MADEX is produced in the facilities of Andermatt BIOCONTROL AG in Switzerland. The company Andermatt BIOCONTROL AG is certified according to ISO 9001.

MADEX is registered and distributed in many countries all around the world.

Contact our experts

In case that you have any question on the use or on other technical issues of MADEX, please do not hesitate to contact our experts at Andermatt BIOCONTROL AG.

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