

## Resistance of codling moth against *Cydia pomonella* granulovirus (CpGV): State of knowledge

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### Abstract

*Cydia pomonella granulovirus (CpGV) based biocontrol agents are highly effective and environmentally benign control measures for codling moth (CM). In recent years, CpGV has become a corner stone for CM control both in the organic and integrated production of apple and pears. In 2005, first reports on CM populations with a dramatically decreased susceptibility to CpGV products were published. Since then, the resistance gene could be located on the Z-chromosome of CM, methods for resistance monitoring were developed and new isolates overcoming CpGV resistance have been identified, tested and registered. In this contribution the present knowledge of intensive research on CpGV resistance will be presented.*

**Keywords:** Baculovirus, codling moth, biological control, geographic distribution, resistance mechanism, biological diversity

### Introduction

The codling moth (CM, *Cydia pomonella* L.), is one of the most harmful insect pests in organic apple and pear production. The application of *Cydia pomonella* granulovirus products (CpGV) and pheromone based mating disruption are the most commonly used control measures of CM in organic production. CpGV is an extremely specific and highly virulent pathogen of CM and has a comparable efficacy to many chemical insecticides. In recent years, several local CM populations with a dramatically decreased susceptibility to CpGV products have been reported from Germany and from France (Fritsch et al., 2005; Sauphanor et al., 2006; Asser-Kaiser et al., 2007). These populations were found in organic apple plantations, where intensive CpGV application failed in CM control.

Single pair crosses between a susceptible laboratory strain and a genetically homogenized strain originating from a resistant field population revealed that resistance is based on a single, dominant gene that is located on the Z-chromosome (Asser-Kaiser et al., 2007; Asser-Kaiser et al., 2010). Intensive search for alternatives to the conventionally used CpGV isolate culminated in the finding of new isolates overcoming CpGV resistance (Jehle et al., 2006; Zingg, 2008; Eberle et al., 2008; Berling et al., 2009). Several of these isolates have been tested in the field (Kienzle et al., 2007; Zingg, 2008; Berling et al., 2009) and are being registered for CM control in different European countries.

Several research projects funded by the Bundesprogramm Ökologischer Landbau (BÖL) (2006-2009) ([www.apfelwickler.de](http://www.apfelwickler.de)) and the European Commission (Craft Project SustainCpGV, [www.sustaincpgv.eu](http://www.sustaincpgv.eu)) (2006-2008) were initiated to provide scientific solutions for this emerging problem.

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Jehle et al. (2006a) identified several important questions to be solved in order to address the problem of CpGV resistance:

- 1) What is the distribution of CpGV resistance in different growing areas?
- 2) What is the mode of inheritance of CpGV resistance?
- 3) What is the mechanism of CpGV resistance?
- 4) What are the alternatives to the conventionally used CpGV products?

In the following, we will provide an overview about the current state of knowledge regarding these questions.

### **Distribution of CpGV resistant CM populations**

Knowledge on the distribution of CpGV resistant CM populations is a prerequisite to understand the potential dynamic of its spread. For the identification of CM populations with CpGV resistance it is important to have reliable methods of analysis. Two methods were developed and applied to identify CM population with resistance to CpGV. (1) Classical LC50 determination was performed from the F1 offspring of diapausing larvae. These larvae were collected from cardboards placed at the trunks of trees in autumn (Fritsch et al., 2005). (2) Testing at a discriminative concentration was developed for larvae directly isolated from infested apples. This method allowed first conclusions within a few days or weeks (Schulze-Bopp and Jehle, unpublished). In total about 40 orchards with CpGV resistant animals could be identified in different European countries, e.g. Germany, France, Italy, Switzerland and the Netherlands. It appears that there is a wide geographic distribution but that resistance occurs only in single orchards, sometimes only in parts of orchards. Thus, the genetic factor responsible for CpGV resistance may be geographically widely distributed but is only present in very low frequencies in non resistant populations. This finding further supports the hypothesis, that CpGV resistance can be very efficiently selected.

### **Inheritance and mechanism of CpGV resistance**

Next to the distribution of CpGV resistance, information on the mode of inheritance is important to understand the population dynamics of resistance. Understanding the CpGV resistance on molecular level will be an important key in sustaining the utility of CpGV products that are presently on the market and those which will be marketed in the future. Two classic approaches were followed to determine the mode of resistance. (1) Mass crossing experiments between a resistant wild population (CpR) and a susceptible laboratory strain (CpS) of CM suggested that CpGV resistance is semi-dominant, polygenic and autosomally inherited (Eberle & Jehle, 2006). (2) This finding, however, was contradicted when a genetically homogenous CM strain (CpRR1) that derived by inbreeding from CpR was crossed with CpS individuals in single pair crossings and tested with a discriminative concentration. The results of these experiments clearly showed that CpGV resistance is linked to the sex chromosome Z and follows a monogenic inheritance pattern (Asser-Kaiser et al., 2007). Meanwhile, this finding could also be corroborated for the original strain CpR in single pair crossings (Asser-Kaiser et al., 2010). It was concluded that CM populations with a fixed resistance gene cannot be controlled anymore by using conventional CpGV products. The very high resistance ratio and the efficient mode of inheritance via the Z sex-chromosome favour a fast selection of resistant individuals under continued selection pressure. Therefore the need to change to differing control measures and other CpGV isolates is inevitable in resistant orchards.

Resistance to CpGV-M was also observed when budded virus of CpGV was directly injected into the hemocoel of resistant CM individuals, thereby circum-passing the midgut (Asser-Kaiser and Jehle, unpublished). This finding clearly indicated that not a change in the midgut but a cellular factor must be responsible that CpGV is not able to replicate in resistant CM individuals. Presently, this genetic factor is searched for by different molecular approaches. As soon as the resistance gene is identified, a genotypic resistance monitoring can be developed.

### **New CpGV isolates overcoming resistance**

All CpGV products, which had been registered in Europe, were based on the so-called Mexican (M) isolate, CpGV-M (Tanada, 1964). It was proposed that alternative isolates with differing virulence might be critical in the resistance management based on different CpGV strains (Jehle et al., 2006a). In 2006, the novel isolate CpGV-I12 was identified showing a very good efficacy against resistant CM larvae in bioassays (Jehle et al., 2006b; Eberle et al., 2008). This isolate originated from Iran and was tested in the field (Zingg, 2008; Berling et al., 2009). Also in 2006, Andermatt Biocontrol selected a CpGV virus (termed Madex Plus) by subsequent passage of CpGV-M through resistant CM larvae. Madex Plus received registration in Switzerland in December 2007 and might be registered along with other new isolates in other European countries (Kienzle et al., 2007; Zingg, 2008). In addition, several new CpGV isolates, which fully or partly overcome resistance, were isolated. By genome characterization and genome mapping different genome types of CpGV could be identified (Berling et al., 2009; Eberle et al., 2009). This diversity in CpGV genotypes is the capital for the further development and improvement of CpGV products and is the genetic basis of new CpGV products, which are currently in the registration process.

### **Virulence management of CpGV isolates**

The success and sustainability of CpGV products in the future will depend on the implementation of strategies to avoid selection of resistance and to exploit the natural genetic diversity of different CpGV isolates. This diversity is of outstanding importance as it offers not only new tools in CM control but also provides a unique model to study micro-evolutionary processes in the virus insect interaction between CpGV and CM, as well as of viruses and host organisms in general. A wise application of new CpGV isolates includes that not only a single genotype is applied but that a mix – different isolates at the same time or consecutively - of different CpGV viruses will be considered.

In addition, it is important to note that any control strategy – whether chemical or biological – that depends on a single product or a single mode of action is prone to selection of individuals that are not susceptible to this agent. Therefore, it will be of greatest importance for the control of codling moth in organic farming to diversify the control measures and to integrate all available control methods, e.g. mating disruption, but also harvesting and destroying infested apples during the growing season and controlling the over-wintering larvae using entomopathogenic nematodes. By applying these techniques it should be possible to sustain the efficacy of one of the most effective biological control agents in organic fruit production.

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