The Future of *Cydia pomonella* Granulovirus in Biological Control of Codling Moth

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Abstract

Resistance of codling moth against Cydia pomonella *granulovirus (CpGV) products has alarmed growers, extension services, the CpGV producers and the scientific community. During the last two years many activities were initiated in Germany and in Europe to understand this phenomenon and to overcome this problem. Meanwhile, first important results about the distribution, mode of inheritance and the efficacy of novel CpGV isolates overcoming CpGV resistance became available. This contribution will provide an overview about the different developments and the progress made towards an improvement of CpGV application in the future.*

Keywords: codling moth, CpGV, resistance, new isolates

Introduction

In nearly all apple growing areas in the world the codling moth (CM, *Cydia pomonella* L.) is one the most important pests. Without control, CM infestation can result in a complete loss of marketable fruits and a severe economic damage to the growers. Hence, environmentally sound control measures are of utmost importance.

CM control products based on the *Cydia pomonella* granulovirus (CpGV) has provided organic growers a highly effective and environmentally benign control agent. Together with mating disruption, CpGV application is the corner stone of CM control in organic apple production. Meanwhile CpGV products are also becoming a key component of integrated production systems. CpGV (virus family *Baculoviridae*) is extremely pathogenic to CM and allows efficient damage control on apples. CpGV products have been registered in most European countries, in North and South America, as well as in South Africa and New Zealand. Since its discovery by Tanada (1964), who originally isolated CpGV from infected larvae originating in Mexico (CpGV-M) further natural isolates originating from England (CpGV-E) and Russia (CpGV-R) have been identified (Crook et al., 1985, 1997). All CpGV products commercialized in Europe contain the isolate CpGV-M as an active ingredient.

Recently, single orchards with CM populations showing an about 1000-fold decrease in susceptibility to CpGV were observed in Germany and France (Fritsch et al., 2005, Sauphanor et al., 2006). This finding was very alarming as it threatened the CM control in these orchards and because until then field observations of baculovirus resistance were extremely rare (Cory & Myers, 2003). In a concerted action including growers, extension services, CpGV producers and scientists from different disciplines initiated research activities to understand the resistance phenomenon and to overcome this problem. Presently, research consortia, sponsored by the Bundesprogramm Ökologischer Landbau (BÖL) (www.apfelwickler.de) and the European Commission (Craft Project SustainCpGV, www.sustaincpgv.eu), deal with this problem.

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Jehle et al. (2006a) outlined several key issues, which need to be solved in order to sustain the efficacy of CpGV products:

1) Analysing the distribution of CpGV resistance in different growing areas
2) Determining the inheritance pattern of CpGV resistance genes
3) Development of molecular markers of CpGV resistance alleles
4) Determining the mechanisms of CpGV resistance
5) Isolation and development of improved CpGV strains
6) Development of strategies to manage the resistance against CpGV

Meanwhile some of these tasks have been successfully completed, some are still in progress. In the following I will provide an overview about the status of this work.

Analysing the distribution of CpGV resistance in different growing areas

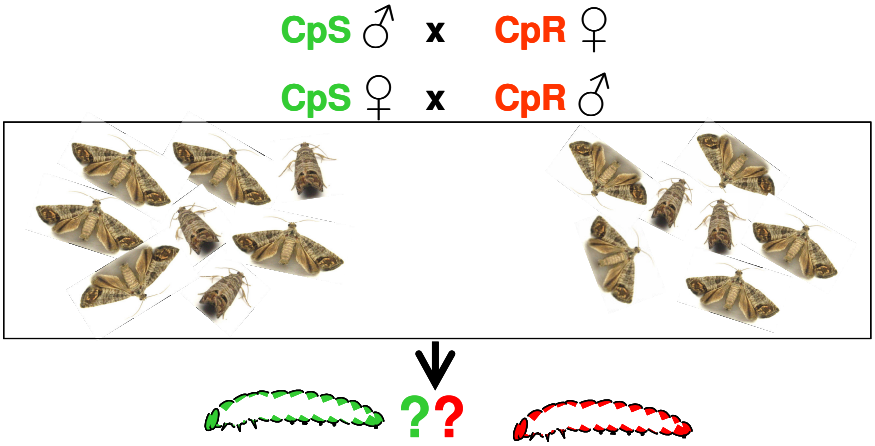
Determining the susceptibilities of different CM populations helps to identify local CM populations with CpGV resistance and may provide evidence about the potential dynamic of spread of this phenomenon. In the framework of the above mentioned BÖL and SustainCpGV projects and in collaboration with local extension services, more than 40 populations originating from Germany and many other European countries have been reared and subsequently tested for their CpGV susceptibilities by the Institute of Biological Control, Julius Kühn-Institute, Darmstadt (Germany) (compare unpublished contribution to the discussion: Susceptibility of populations of *Cydia pomonella* against *Cydia pomonella* Granulovirus in organic orchards in Europe by A. *Schmitt, Julius Kühn Institute Darmstadt,* this meeting*).* In summary, more than 35 orchards in Germany, France, Italy, Switzerland, and the Netherlands were identified, where CM show very low CpGV susceptibility. Hence, CpGV resistance is geographically widely distributed but very local since always single orchards but never larger growing areas are affected. From this it can be concluded that (1) the genetic factor responsible for CpGV resistance may be geographically widely distributed but appears at very low frequencies in non resistant populations, (2) CpGV resistance may be effectively selected for under CpGV selection pressure, if the resistance gene is present in the CM population.

Determining the inheritance pattern of resistance genes

Information of the genetic background of the CpGV resistance is important for understanding the dynamics of resistance development as well as for developing appropriate measures to manage resistance. The mode of inheritance of CpGV resistance was determined by mass crossing experiments (Eberle & Jehle, 2006) and single pair crossing experiments (Asser-Kaiser et al., 2007) between a susceptible and a resistant CM strain, followed by back-crossings and susceptibility tests of the offspring. These crossing experiments allowed telling whether the inheritance of resistance is recessive or dominant, linked to the sex chromosomes or to the autosomes, polygenic or monogenic. From both approaches it was concluded that resistance is incomplete dominant. Mass-crossing experiments performed with a non-homogenous resistant CM population (which still contained susceptible individuals) initially suggested that the resistance gene(s) may be located on an autosome (Eberle & Jehle, 2006). However, single-pair crossing experiments performed with a genetically homogenized resistant CM strain led to a different conclusion and unequivocally demonstrated that the allele responsible for CpGV resistance is sex-linked and located on the Z-chromosome of the moth (Asser-Kaiser et al., 2007). In most Lepidoptera as well as in *C. pomonella*, the sex chromosomes are ZW in females and ZZ in males. The dominance is concentration-dependent.

From this genetically homogenized strain it was concluded that the resistance ratio based on the mean lethal virus concentration (LC_{50}) for neonate larvae exceeded a factor of 100,000 in both resistant females (genotype Z^RW) and homozygous males (Z^RZ^R) when compared to susceptible CM larvae; heterozygous males (Z^RZ^S) still exhibited a more than 1000-fold resistance. From these findings it can be 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 favours a rapid selection of resistant individuals under continued selection pressure, in case of low ecological costs of resistance.

A) Mass crossing experiment



B) Single pair crossing experiment

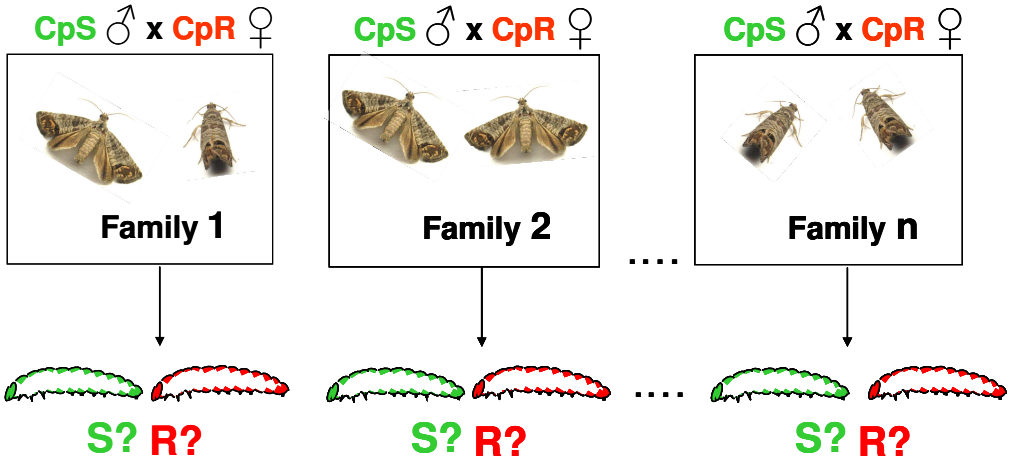


Fig. 1. Crossing scheme for mass crossing and single pair crossing experiments. (**A**) In mass crossing experiments, about 30 males and 30 females were allowed mating in reciprocal crosses. The offspring of this cross and of their back-crossings to susceptible larvae were subjected to full range bioassays varying from 100 to 100 000 OB/ml. (**B**) In single pair crossings individual susceptible males and resistant females from a genetically homogenized CM strain CpRR1 [= (CpRxCpR)x(CpRxCpR)] were used. Resistance testing was done at a discriminative concentration of $5.8x10^4$ OB/ml (Eberle & Jehle, 2006; Asser-Kaiser et al., 2007).

Development of molecular markers of resistance alleles

The development of molecular markers of resistance will allow monitoring the frequency of resistance alleles in CM populations before resistance becomes an apparent character of the population. By using the individuals obtained in the different single-pair crossing experiments a linkage mapping is presently performed by a collaborative effort of the University of Hohenheim, the Max-Planck-Institute of Chemical Ecology in Jena and the Forschungsanstalt Geisenheim.

Determining the mechanisms of resistance

Understanding the CpGV resistance at molecular level will be pivotal for sustaining the utility of CpGV as a biopesticide. Different approaches ranging from the identification of the resistance gene, comparing the virus pathogenesis in both susceptible and resistant individuals by light and electron microscopy, comparing the immune status of susceptible and resistant individuals are presently in progress in the frame work of the BÖL and SustainCpGV project.

Isolation and development of improved CpGV strains

All CpGV products registered in Europe are based on the so-called Mexican (M) isolate, CpGV-M. As CpGV does not represent a completely homogenous assembly of identical genomes but is more likely a meta-population of slightly differing genotypes it was proposed that other isolates may overcome the observed resistance to CpGV-M. It was envisaged that alternative isolates with differing virulence may be critical in the resistance management based on different CpGV strains (Jehle et al., 2006a). Indeed, a novel CpGV isolate termed CpGV-I12 was identified and showed superior efficacy against resistant CM larvae in laboratory bioassays (Jehle et al., 2006b). This isolate originated from Iran and was 2007 tested in the field (Zingg, 2008). In 2006, Andermatt Biocontrol succeeded to select a CpGV virus (termed Madex Plus) by subsequently passaging CpGV-M through resistant CM larvae. Madex Plus has been tested in the field in 2006 and 2007 and received registration in Switzerland in December 2007 (Kienzle et al., 2007; Zingg, 2008). Meanwhile three other CpGV isolates overcoming CpGV resistance have been successfully tested by our laboratory. This diversity in CpGV genotypes are highly promising and will lead to new CpGV products based on alternative isolates. It will be important that these isolates receive registration as soon as possible. The broader the genetic basis of CpGV products is in the future, the lower the risk of resistance development will be.

Development of strategies to manage the resistance against CpGV

The development of resistance management strategies will be an important task for the future application of CpGV products. Resistance management has to consider the extremely reduced susceptibility of CM in case of resistance as well as the differing virulences of different CpGV isolates. With the identification of the mode of inheritance and the characterization and testing of novel CpGV isolates first successful steps have been made. Although novel CpGV isolates are in the pipeline, it will be important for the successful usage of CpGV products in the organic production that CM control is not put solely on CpGV products but on different pillars, at least mating disruption, harvesting and destroying infested apples during the growing season. Control of the over-wintering larvae using entomopathogenic nematodes may be another promising tool (see contributions in this volume).

A further important step will be the implementation of resistance monitoring measures, e.g. by applying molecular markers to be developed as mentioned above. However, these genotypic markers will be only suitable for known resistance alleles. In addition to this a fast and reliable method for screening the susceptibilities of CM populations to conventional and novel CpGV isolates are essential. We have developed and validated a bioassay, which allows testing the susceptibility of second to fourth instar larvae directly taken from infested apples (Schulze & Jehle, unpublished). This method has the advantage that larvae of the first and second generation can be tested directly and results are already available after two weeks. By using this approach in the year 2007, we have tested about 2500 larvae extracted from 9122 apples from 21 orchards in Germany, Switzerland, Italy and the Netherlands. We are presently planning to commercialize this methodology and provide it to farmers interested in testing.

Conclusions

Although resistance of CM to CpGV has been documented in several orchards in different European countries it can be concluded that these cases are still rather singular and isolated and that the general situation is far away from an area-wide problem. In nearly all orchards, CpGV-M based products still do an excellent job. However, those growers who suffer from CpGV resistance in their orchards are in need of fast help. The experimental application of new CpGV isolates was a first step. The next step has to be the registration and application of the new isolates before CpGV resistance further spreads. We are just at the very beginning of understanding the host response to CpGV infection and the genetic factors defining the virulence of CpGV. Though the new findings and developments reported here are very promising and give legitimate reason for optimism, more research and new developments are essential that we do not loose this long-term battle.

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