Infection of the plum fruit moth, *Cydia funebrana* (*Lepidoptera: Tortricidae*) by Cydia pomonella granulovirus (CpGV)

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Abstract

The Cydia pomonella granulovirus (CpGV), a member of the family Baculoviridae, is effectively used worldwide for controlling C. pomonella (codling moth) larvae in apple orchards. Although CpGV is known to be highly specific thus not affecting non-target organisms, a few studies have shown that CpGV can infect other Cydia spp. and species in the family Tortricidae, in particular if viral dosages are substantially increased. The plum fruit moth, Cydia funebrana is regarded as one of the key pests of plum in Europe, with biological control being severely hold back mainly due to a lack of available and efficient control agents. To test if infection of plum moth larvae by CpGV is in principal possible, viral suspensions of different CpGV isolates were sprayed at a concentration of 10^7 occlusion bodies/ml on mature plums in the laboratory. Sterile water was used as a control. Freshly hatched C. funebrana larvae were allowed to feed and to bore into these fruits and were assessed for mortality within a period of 3-14 days. A substantial number of dead and liquefied larvae were present in the virus treatments and absent in the control. Presence of CpGV in the cadavers was confirmed with CpGV specific primers in polymerase chain reactions (PCR) and subsequent sequence analysis of obtained PCR products. This result opens up new possibilities for C. funebrana control in organic plum production.

Keywords: Plum fruit moth, granulovirus, CpGV

Introduction

The plum fruit moth, *Cydia funebrana* (Lepidoptera: Tortricidae) is an oligophagous insect, attacking different host plants within the family Rosaceae, such as the fruits of plum, cherry and peach. This species is regarded as one of the key pests of plum in central Europe (Dickler 1991). Larvae feed inside the fruits causing fruit discoloration, gummosis, premature ripening and fruit drop (Alford 1987), ultimately resulting in a reduced marketability and significant yield reductions.

As a prerequisite for control, pheromone traps are used effectively to monitor adult flight activity and make treatment recommendations. In organic plum production, *C. funebrana* can be controlled by the use of sex pheromones for mating disruption. In addition, the use of *Bacillus thuringiensis* products or egg parasitoids such as *Trichogramma* spp. offer further control options. Trials are currently being conducted using entomopathogenic fungi or nematodes. However, in general, most of the biological control agents being tested up to now, showed a varying efficiency and practicability in the field, making organic plum production particularly difficult.

The Cydia pomonella granulovirus (CpGV), a member of the family Baculoviridae, is effectively used worldwide for controlling *Cydia pomonella* (codling moth) larvae both in integrated and organic apple orchards. CpGV is estimated to be used on approximately 100.000 ha in Europe annually (Eberle & Jehle 2006). For infections to occur, virus

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occlusion bodies must be ingested by the larvae, with neonate larvae being more susceptible towards infection than older larval stages.

Although CpGV is known to be highly specific towards *C. pomonella*, a few studies have shown that CpGV can indeed infect other *Cydia* spp. (such as the pea moth *C. nigricana* and the Oriental fruit moth *C. molesta*) and species in the family Tortricidae (such as the pine shoot moth *Rhyacionia buoliana* or the false codling moth *Cryptophlebia leucotreta*) (for a review see Lacey *et al.* (2008)). In most of the bioassays, however, extremely high rates of virus dose were required to infect and subsequently kill the larvae.

As the availability of a virus product against the plum fruit moth would be a significant step towards efficient *C. funebrana* control in organic plum production, we tested if infection of plum moth larvae by CpGV is in principal possible, and if different CpGV isolates were showing altered levels of efficiency.

Material and Methods

Virus isolates, insects and treatments

Preparations of 10 different CpGV isolates were obtained from Andermatt Biocontrol.

During late July and August 2009 last instar *C. funebrana* larvae or pupae were obtained from various sources in southern Germany (see Acknowledgements) and were kept in the laboratory until adult emergence. Male and female moths were kept together in plastic cylinders and were offered ripe plums for egg deposition. Plums containing eggs were kept at 23°C and 16:8 L:D. Single laid eggs were marked on the plum surface and were daily monitored for embryonic development. When egg development had reached the black-head stage, suspensions of 10 different virus isolates were sprayed on the plum each at a concentration of 10^7 occlusion bodies (OBs)/ml using a fine airbrush sprayer. Sterile water was used as a control. Freshly hatched *C. funebrana* larvae were allowed to feed and to bore into these fruits. Since plums were often containing several eggs (up to 40), four additional plums were treated with virus suspensions or water, respectively, in the same way and were put together with the egg-containing plum in a plastic container of 13 x 8 x 6 cm in size. Four replicates were set up for each virus isolate.

Since plums were frequently infested with *Monilia* or other fungi causing fruit rot, larvae were assessed for mortality within a varying period of 4-14 days by dissecting larvae out of the respective plums. Living larvae or cadavers were put in single Eppendorf tubes and were stored frozen at -80°C. Larvae of the control plums were handled accordingly. Corrected efficacy was calculated using the formula of Schneider-Orelli (Püntener 1981).

Molecular diagnosis of CpGV infection

In order to confirm presence of CpGV in dead *C. funebrana* cadavers, DNA was extracted i) from the virus suspensions used for treatments of the plums and ii) from living larvae or cadavers from CpGV treated or untreated plums using the Master Pure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies). Obtained DNA was amplified in a standard polymerase chain reaction (PCR) using primers specific for the CpGV granulin gene (NesPRCP1U and NesPRCP1L; J. Jehle, pers. communication). Amplified products were loaded on a 1% agarose gel and were visualized via SybrSafe staining of the gels under UV light. A couple of PCR products were purified from the agarose gels using HiYield PCR Cleanup Kit (SLG) and were sequenced at AGOWA, Berlin.

Results

A substantial number of dead and liquefied larvae were present in the different virus treatments and absent in the control. In the control, a mortality of 6% of the larvae was recorded, while in most of the cases the mortality of larvae in the different virus treatments was considerably higher, varying between 4 and 66%. Corrected efficacy was calculated using the formula of Schneider-Orelli (Fig.1).
While CpGV isolates V20 and V03 were showing no or only a very little efficacy in laboratory trials, isolates V15 and V18 inflicted a substantial mortality among the *C. funebrana* larvae, with efficacies of 58 and 63%, respectively.

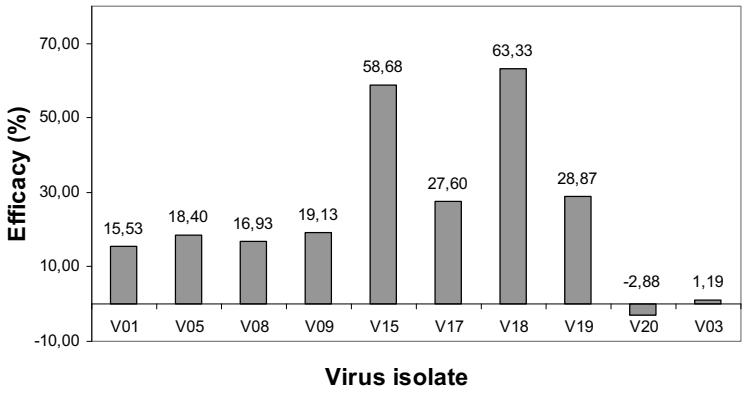

Fig. 1: Efficacy (%, according to Schneider-Orelli) of 10 different CpGV virus isolates on neonate *C. funebrana* larvae in laboratory trials using plums treated with 10^7 OBs/ml. Four replicates were set up for each virus isolate.

Molecular confirmation of CpGV infection

DNA was extracted both from living or dead *C. funebrana* larvae obtained from control plums and from plums treated with the different virus isolates. In addition, virus DNA was purified from the original virus suspensions used for treating plums. DNA was PCR amplified with primers specific for the CpGV granulin gene and products were visualized on an agarose gel.

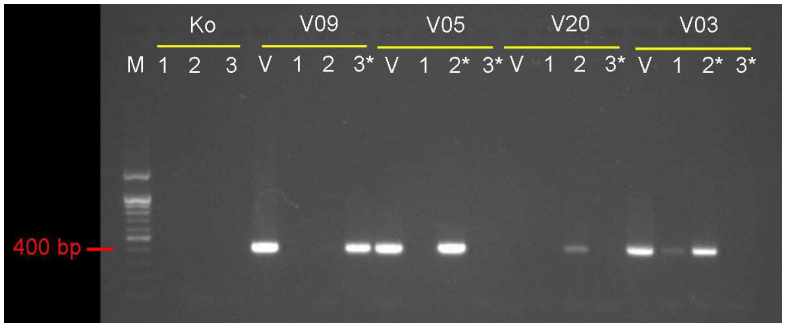

Fig. 2: Confirmation of CpGV infection in *C. funebrana* larvae based on amplification of a 422 bp fragment of the CpGV granulin gene. Three *C. funebrana* individuals were assessed from the control (Ko) and from treatments with four different virus isolates. Lane "V" shows amplification products obtained from the original viral suspension. Individuals marked with an * were still alive at the time of mortality assessment. The 400 bp fragment of the molecular weight marker (M) is marked on the left.

A single specific band of the correct size of 422 bp was visible in DNAs from most of the virus solutions as well as in *C. funebrana* cadavers or living larvae dissected out of the CpGV treated plums. No amplification products were obtained from dead or from living larvae of the untreated controls. Sequence analysis of the obtained PCR products showed 96% identity to the CpGV granulin gene entry in GenBank.

Discussion

The results presented here demonstrate that an infection of *C. funebrana* with CpGV is in principle possible. Efficacies calculated on the basis of the number of dead larvae reached a maximum of 63%, however, diagnosis and confirmation of CpGV infection based on PCR amplification and sequence analysis of a specific fragment revealed, that a substantial number of plum fruit moth larvae which were still alive at the time of mortality assessment were as well infected by CpGV. Since in some cases experiments had to be terminated earlier than planned due to an increasing rotting of overripe plum fruits, one could expect a maybe higher mortality if a longer duration of the experiments would have been possible.

Plum fruits were treated with virus concentrations of 10^7 OBs/ml. This is about 10^3 times higher than the CpGV inoculum that is used for discriminating CpGV resistant and CpGV susceptible *C. pomonella* individuals (Asser-Kaiser *et al.* 2007). It also confirms a number of studies that a significantly higher CpGV virus dosage is required to kill other insects than *C. pomonella* (Lacey *et al.* 2008). Future trials will thus assess concentration - mortality relationships against first and second generation *C. funebrana* larvae both in the laboratory as well as in the field.

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