Investigations on the mechanism of CpGV resistance in *Cydia pomonella*

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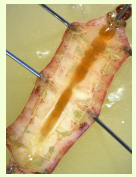
Introduction

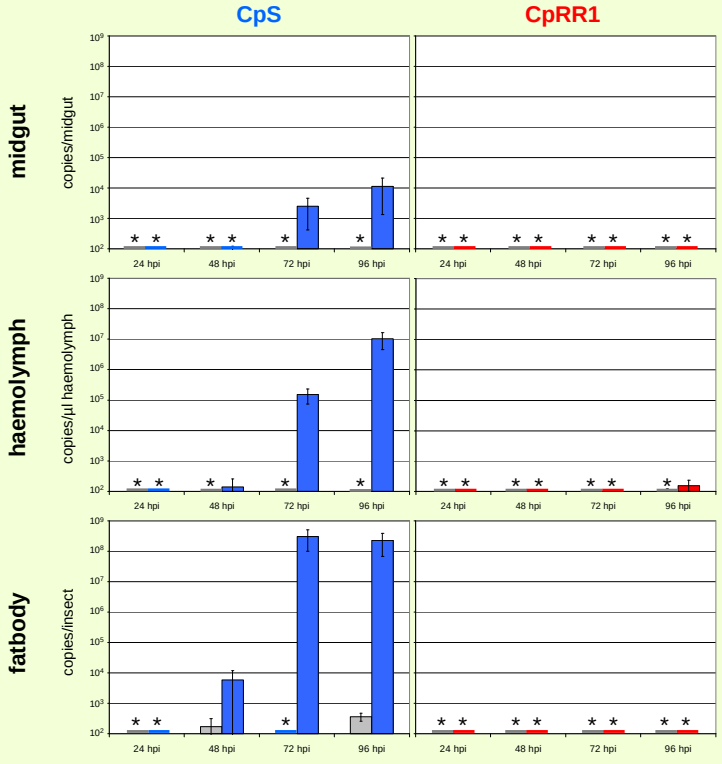
- For more than 20 years *Cydia pomonella* granulovirus (CpGV) has been successfully used for the control of codling moth (CM) in organic and integrated production.
- Recently, codling moth populations with an up to 1,000-fold decreased susceptibility to CpGV have been observed in Germany, France, the Netherlands, Italy and Switzerland. The resistance is inherited by a single dominant gene which is located on the Z-Chromosome (Asser-Kaiser et al. 2007, Science 317: 1916-1918).
- Three different experimental approaches were followed to investigate the mechanism involved in CpGV resistance.

1) Tissue specific virus replication



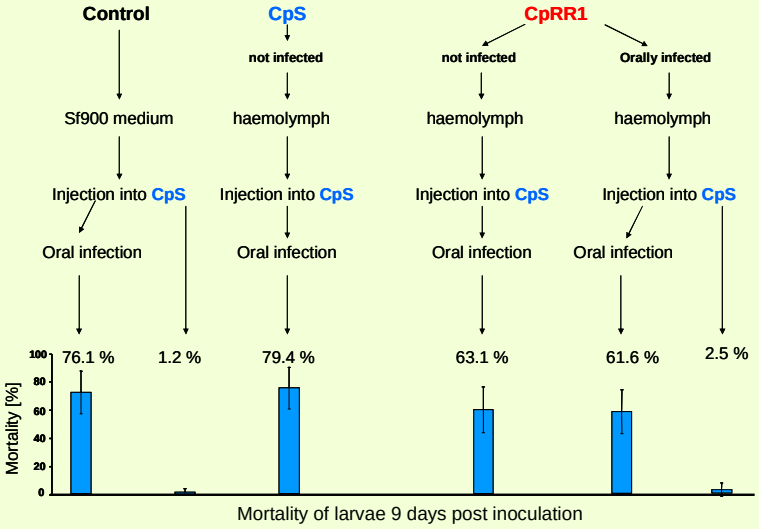
Preparation of tissue samples

To compare the spread of CpGV infection, both resistant (CpRR1) and susceptible (CpS), CM larvae (L5) were orally infected with 1000 CpGV OBs. Control larvae obtained virus-free food. 24, 48, 72 and 96 hours post inoculation (hpi) samples of the midgut, haemolymph and fatbody were taken from 5 larvae per treatment. DNA of these samples was isolated and the number of CpGV genomes was estimated with QPCR using granulin specific primers.



2) Transfusion of haemolymph

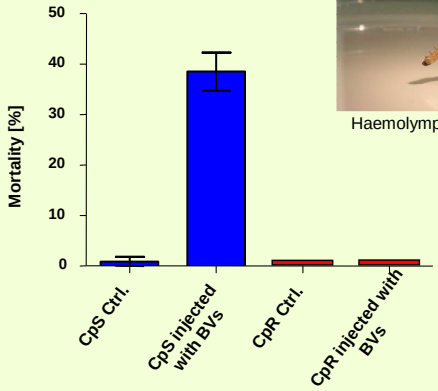
Humoral and cellular immune responses as a reason for resistance were investigated: Haemolymph of CpS larvae (L4), as well as of infected and not infected resistant CpRR1 larvae (L4) were injected into CpS. Sf900 medium was injected to control larvae. Afterwards these larvae were fed with a piece of diet either contaminated with 1000 OBs or free of virus. Mortality was scored 9 days later.



3) Intra-haemocoelar infection

In order to determine whether resistance is located in the midgut only, peroral infection was by-passed by injecting budded virus (BV) of CpGV into the haemocoel of CpS and CpR larvae. First, CpS larvae (L4) were infected orally with 1000 OBs. 5 days later haemolymph of these larvae was extracted and the concentration of BV was measured using QPCR. 10^5 BVs in 2 µl Sf900 were injected into the hemocoel of 20-25 CpS and CpR instars (L4). 2 µl Sf900 medium was injected into control animals. Mortality was scored after 12 days. 5 replicates were performed.

Haemolymph injection



Results and Conclusions

1) In none of the isolated cell types of CpRR1 virus replication could be detected. Hence infection is already blocked at the midgut and not further spread, or resistance is present in each of the cell types.

2) Haemolymh injection of CpRR1 into CpS cannot convey resistance in CpS. Thus, no factor in the haemolymph wich induces resistance could be identified.

3) Intrahaemocoelar injection of BVs does not overcome resistance. Accordingly, resistance is not restricted to the midgut but also to secondary infection.

As replication is impaired in midgut cells (1) and in secondary infection (3) but no immune response was observed (2), it is proposed that virus replication is affected in all cell types, suggesting a virus-cell incompatibility in resistant CMs.