

Successful control of codling moth resistance to *Cydia pomonella* granulovirus (CpGV)

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Abstract

Cydia pomonella granulovirus (CpGV) is indispensable for an efficient and environmentally sound control of codling moth (*Cydia pomonella* L.) in organic pome fruit production. By molecular means, CpGV isolates can be distinguished in five phylogenetic groups A-E. During the last decade more than 40, mainly organic apple plantations with codling moth populations resistant to CpGV products have been identified in different European countries. By genetic analyses and resistance testing three different types of resistance (type I-III) were revealed; they differ in the mode of inheritance as well as in the resistance mechanism. Type I resistance is Z chromosomal inherited and is targeted only against CpGV isolates of the genome group A. Type II resistance is autosomal inherited and also targeted against A, D and E. Finally, type III resistance appears in certain aspects like a mixture of both type I and type II resistance. Currently, different commercial CpGV isolates are used for codling moth control and their efficacy toward different resistance types was tested. This presentation will provide an update on the current knowledge of CpGV resistance.

Keywords: Codling moth, granulovirus, resistance, inheritance, resistance testing

Cydia pomonella granulovirus

The *Cydia pomonella* granulovirus (CpGV) is a dsDNA virus, highly virulent and specific to codling moth (CM), *Cydia pomonella*. CpGV is harmless to non-target organisms and commercial CpGV products have been used since more than 25 years in CM control in organic apple and pear production (Lacey et al., 2008). Today, CpGV products are registered as biocontrol agents in virtually all pome fruit growing countries. CpGV isolates have been isolated from different areas in the world, including Mexico, Canada, Europe, Caucasus Area and China. These isolates have been distinguished by DNA endonuclease restriction analysis or more recently by full genome sequencing (Wennmann et al., 2017). All known CpGV isolates are classified into five phylogenetic genome groups A – E (Gebhardt et al., 2014, Wennmann et al., 2017). The most widely used commercial isolate was the Mexican isolate CpGV-M, belonging to the genome group A.

Three types of CpGV resistance

Since 2005 field resistance of CM populations against CpGV products has been observed in Europe, mainly in Germany but also in France, Italy, Switzerland Austria, Czech Republic and The Netherlands (Fritsch et al., 2005, Sauphanor et al., 2006; Berling et al., 2009). When resistance testing was performed with neonate CM larvae, it was found that some of these field populations of CM were more than 1,000 times less susceptible to CpGV-M than typical susceptible CM populations. By further resistance testing and genetic analyses two other resistance types were identified and finally classified in three different resistance types I-III (Table 1).

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Type I resistance, which is geographically most widely distributed, follows a Z-chromosomal, dominant inheritance and is targeted against CpGV-M and other isolates from genome group A. Type II resistance, so far only detected in a couple of orchards in Germany, follows an autosomal, dominant mode of inheritance and is targeted against all CpGV genome groups, except genome group B. Type III resistance was observed in a single CM field population from Germany and is also directed against different CpGV isolates, mainly from the genome groups A and E. Crossing analyses of type III resistant CM suggested a complex polygenic inheritance pattern with some mixed characteristics of type I and type II resistance.

Table 1: Summary of three types of field resistance of CM to CpGV.

Field Population	Selected Strain	Inheritance Type	Resistance Type	References
CpR	CpRR1	Z-linked , dominant, monogenic	Type I	Asser-Kaiser et al., 2007
NRW-WE	CpR5M/ CpR5S	Autosomal , dominant, monogenic	Type II	Jehle et al., 2017; Sauer et al., 2017a
SA-GO	CpRGO	Autosomal/Z-linked , Dominant, polygenic	Type III	Sauer et al., 2017b

Efficacy of commercial resistant-breaking CpGV products

When it was realized that certain CpGV genome groups appear to be resistance-breaking, novel commercial products were developed and eventually registered. Currently, a number of improved CpGV products, containing resistance-breaking isolates are available, i.e. MadexMAX[®] with CpGV isolate 0006, MadexTOP[®] with CpGV isolate V15 and Carpovirusine EVO2[®] with CpGV isolate R5.

We performed laboratory experiments using neonate CM larvae from different susceptible and resistant CM strains to test the efficacy of these commercial isolate in 7-day and 14-day bioassays (Figure 1).

When susceptible CpS larvae were tested, virus-induced mortality ranged between 91% (isolate V15) and 100% (0006) after seven days and between 99% (R5) and 100% (0006 and V15) after 14 days. No significant differences (ANOVA, post-hoc Tukey HSD test, $P < 0.05$) were observed. Type I resistant CpRR1 larvae showed mortality between 62% (V15) and 98% (0006) after seven days and between 86% (V15) to 98% (0006, R5) after 14 days. For the type II resistant strains CpR5M and CpR5S, larval mortality was only between 8% to 17% for the isolates R5 and 0006 after seven days. Even after 14 days mortality did not increase to more than 37%. Only V15 was able to cause significant mortality of type II resistant larvae of about 50% after seven days and more than 85% after 14 days (ANOVA, post-hoc Tukey HSD test, $P < 0.05$). Neonate larvae with type III resistance (CpRGO) showed mortality of about 64% for 0006, about 53% for R5, and 91% for V15 after seven days. After 14 days exposure, mortality increased to 80% for 0006 and R5 and to 100% for V15.

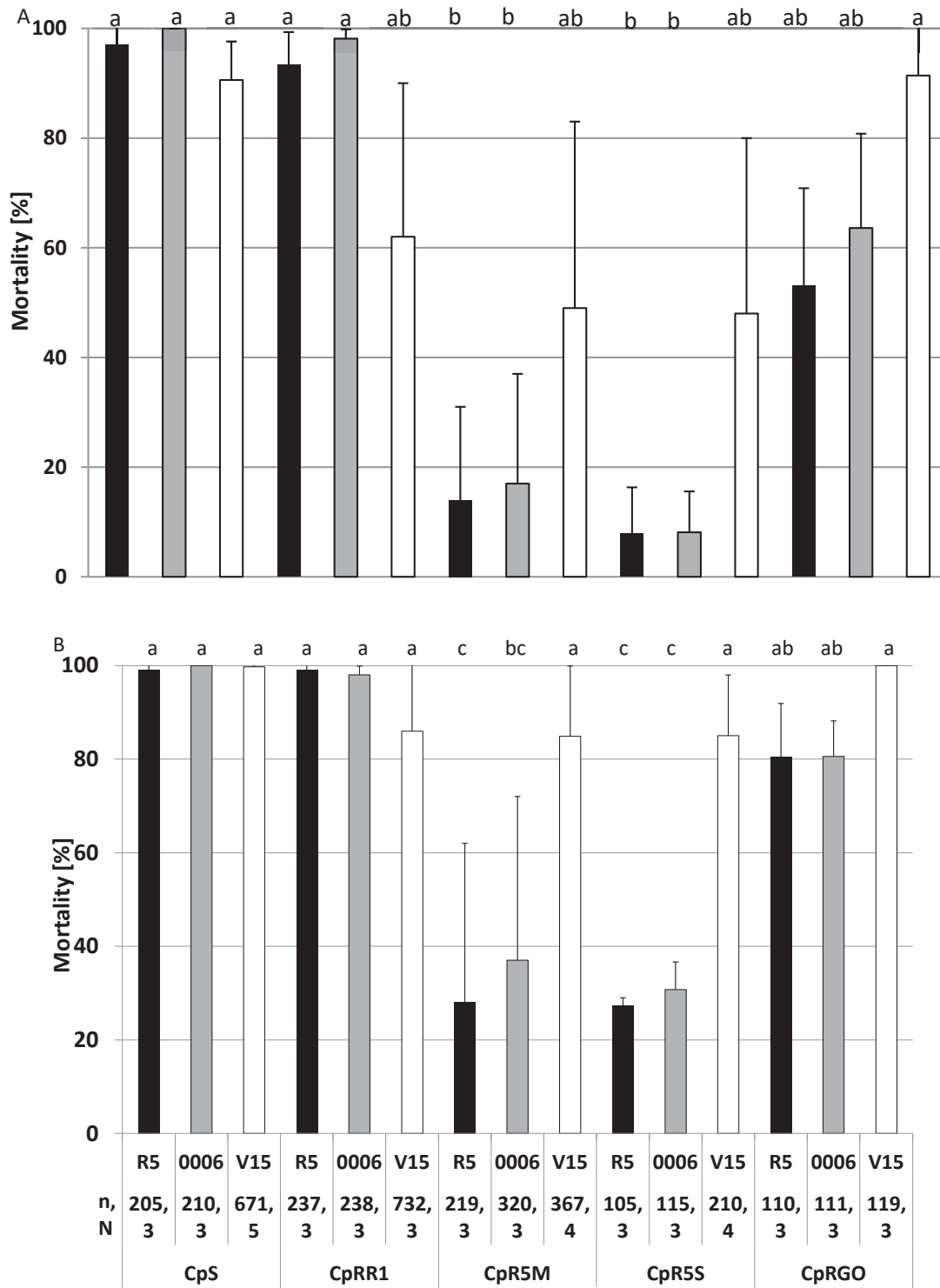


Figure 1: Susceptibility of different CM strains to different commercial resistance-breaking CpGV isolates, R5 (black bars), 0006 (grey bars), or V15 (white bars). Mortality was determined for neonates of CpS, CpRR1 (type I), CpR5M and CpR5S (type II) and CpRGO (type III resistance) exposed to commercial CpGV isolates at the discriminating concentration of 5.8×10^4 OB/ml artificial diet. Abbott (1925) corrected mean mortality and standard deviation (error bars) were determined at seven days (A) and 14 days (B) post infection. The total number of tested individuals (n) and independent replicates (N) are given below the chart. Columns marked by different letters differed significantly (ANOVA, post-hoc Tukey HSD test, $P < 0.05$).

Conclusions

Three types of resistance (type I - III) have been identified; they show a considerable difference in their susceptibility to CpGV isolates belonging to different genome groups. Testing of commercial products showed that isolates 0006 and R5 did not break type II resistance, whereas V15 caused high mortality in all resistant CM strains.

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References

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.
- Asser-Kaiser, S., Fritsch, E., Undorf-Spahn, K., Kienzle, J., Eberle, K. E., Gund, N. A., Reineke, A., Zebitz, C. P. W., Heckel, D. G., Huber, J., Jehle, J. A. (2007). Rapid emergence of baculovirus resistance in codling moth due to dominant, sex-linked inheritance. *Science* **318**, 1916-1918.
- Berling, M., Blachère-Lopez, C., Soubabère, O., Lery, X., Bonhomme, A., Sauphanor, B. & López-Ferber, M. (2009). *Cydia pomonella* granulovirus genotypes overcome virus resistance in the codling moth and improve virus efficiency by selection against resistant hosts. *Applied and Environmental Microbiology* **75**: 925-930.
- Fritsch, E., Undorf-Spahn, K., Kienzle, J., Zebitz, C.P.W. & Huber, J. (2005). Apfelwickler-Granulovirus: Erste Hinweise auf Unterschiede in der Empfindlichkeit lokaler Apfelwickler-Populationen. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **57**: 29-34.
- Gebhardt, M., Eberle, K.E., Radtke, P. & Jehle, J. A. (2014). Baculovirus resistance in codling moth is virus-isolate dependent and the consequence of a mutation in viral gene pe38. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)* **111**: 15711-15716.
- Jehle, J.A., Schulze-Bopp, S., Undorf-Spahn, K., Fritsch, E. (2017): Evidence for a second type of resistance against *Cydia pomonella* granulovirus in field populations of codling moths. *Applied and Environmental Microbiology* **83**(2): e02330-16.
- Lacey, L.A., Thomson, D., Vincent, C. & Arthurs, S.P. (2008). Codling moth granulovirus: a comprehensive review. *Biocontrol Science and Technology* **18**: 639–663.
- Sauphanor, B., Berling, M., Toubon, J.-F., Reyes, M., Delnatte, J. & Allemoz, P. (2006). Carpocapse des pommes. Cas de résistance au virus de la granuloose en vergers biologique. *Phytoma-La Défense des Végétaux* **590**, 24-27.
- Sauer, A., Fritsch, E., Undorf-Spahn, K., Nguyen, P., Marec, F., Heckel, D., Jehle, J.A. (2017a). Novel resistance to *Cydia pomonella* granulovirus (CpGV) in codling moth shows autosomal and dominant inheritance and confers cross-resistance to different CpGV genome groups. *PLoS ONE* **12**(6): e0179157.
- Sauer, A.J., Schulze-Bopp, S., Fritsch, E., Undorf-Spahn, K., Jehle, J.A. (2017b). A third type of resistance to *Cydia pomonella* granulovirus in codling moths shows a mixed Z-linked and autosomal inheritance pattern. *Applied Environmental Microbiol* **83**:e01036-17.
- Wennmann, J., Radtke, P., Eberle, K.E., Gueli Alletti, G., Jehle, J.A. (2017). Deciphering single nucleotide polymorphisms and evolutionary trends in isolates of the *Cydia pomonella* granulovirus. *Viruses* **9**(8): 227

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