

## Monitoring codling moth resistance to *Cydia pomonella* granulovirus (CpGV) in organic fruit growing in Germany

E. Fritsch<sup>1</sup>, K. Undorf-Spahn<sup>1</sup>, J. Kienzle<sup>2</sup>, J. Zimmer<sup>3</sup>, Goertz, S.<sup>3</sup>, B. Benduhn<sup>4</sup>,  
C. Adolphi<sup>4</sup>, C.P.W. Zebitz<sup>5</sup>, J.A. Jehle<sup>1</sup>,

### Abstract

*Cydia pomonella* granulovirus (CpGV) products are indispensable for an efficient and environmentally friendly control of codling moth (*Cydia pomonella*) in organic apple and pear production. Since the first occurrence of field resistance against commercial CpGV products, intensive research was initiated to understand the mechanism of resistance and to identify new CpGV isolates with resistance-breaking traits. Such resistance-breaking CpGV isolates can be used to provide solutions for orchards affected by CpGV resistance as well as to develop resistance management strategies. Here, we report the current status of 15 years of CpGV resistance monitoring in Germany.

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

### Introduction

*Cydia pomonella* granulovirus (CpGV) is one of the main tools used for codling moth (CM) control in organic pome fruit production. In 2004, first codling moth populations with resistance to commercial CpGV products were recorded in Southern Germany (Fritsch et al., 2005). Since then, resistance monitoring was started in different regions in Germany. In all organic orchards raising suspicion of a CpGV resistance to growers and/or extension services, codling moth infestation was assessed by the cooperating partners (University of Hohenheim, DLR Rheinpfalz and OEON e.V.). In this assessment, infested apples were collected and sliced to monitor the number of living larva inside and the number of “stopped injuries”. Whereas “stopped injuries” are characteristic for effective CpGVs, they are rare in case of CpGV resistance. If this initial assessment gave reason to suspect that the CM population might be resistant, several hundreds of infested apples with living larvae were collected and the larvae were subjected to resistance testing at the Julius Kühn Institute (JKI) in Darmstadt. In recent years, extensive resistance testing from samples of more than 100 orchards and genetic analyses revealed three different types of resistance (type I-III); they differ in their mode of inheritance as well as in the resistance mechanism. Type I resistance is Z chromosomal inherited and is only directed to CpGV isolates of the genome group A. Type II resistance is autosomal inherited and is directed against the genome group A, D and E CpGVs. Finally, type III resistance appears in certain aspects like a mixture of both type I and type II resistance (Asser-Kaiser et al., 2007; Jehle et al., 2017; Sauer et al., 2017ab). Five phylogenetic lines of CpGV isolates (genome groups A-E) are known (Eberle et al., 2009; Gebhardt et al.; 2014; Wennmann et al.; 2017). More recently, additional isolates with novel genetic diversity (genome group F-G) were isolated in China (Fan et al. 2020ab).

---

<sup>1</sup> JKI, Institute for Biological Control, Heinrichstraße 243, 64287 Darmstadt

<sup>2</sup> Apfelblütenweg 28, 71394 Kernen, jutta@jutta.kienzle.de

<sup>3</sup> DLR Rheinpfalz, Campus Klein-Altendorf2, 53359 Rheinbach

<sup>4</sup> OEON, Moorende 53, 21635 Jork

<sup>5</sup> University of Hohenheim, Institute for Phytomedicine, Department of Applied Entomology, D-70593 Stuttgart

## Material and Methods

Initially, CM larvae were trapped in the field as diapausing larvae using corrugated cardboards. After overwintering, the progeny of the adults was tested for resistance in laboratory bioassays by means of  $LC_{50}$  estimations and/or using a single diagnostic concentration of CpGV adapted to L1 larvae (Asser-Kaiser et al., 2007; Schmitt et al., 2013). In 2013, a laboratory test system was developed that allowed a direct testing of wild CM larvae extracted from infested apples collected in the field. The aim of this direct test is to provide fast information about suspected resistance during the growing season (Schulze-Bopp & Jehle, 2013).

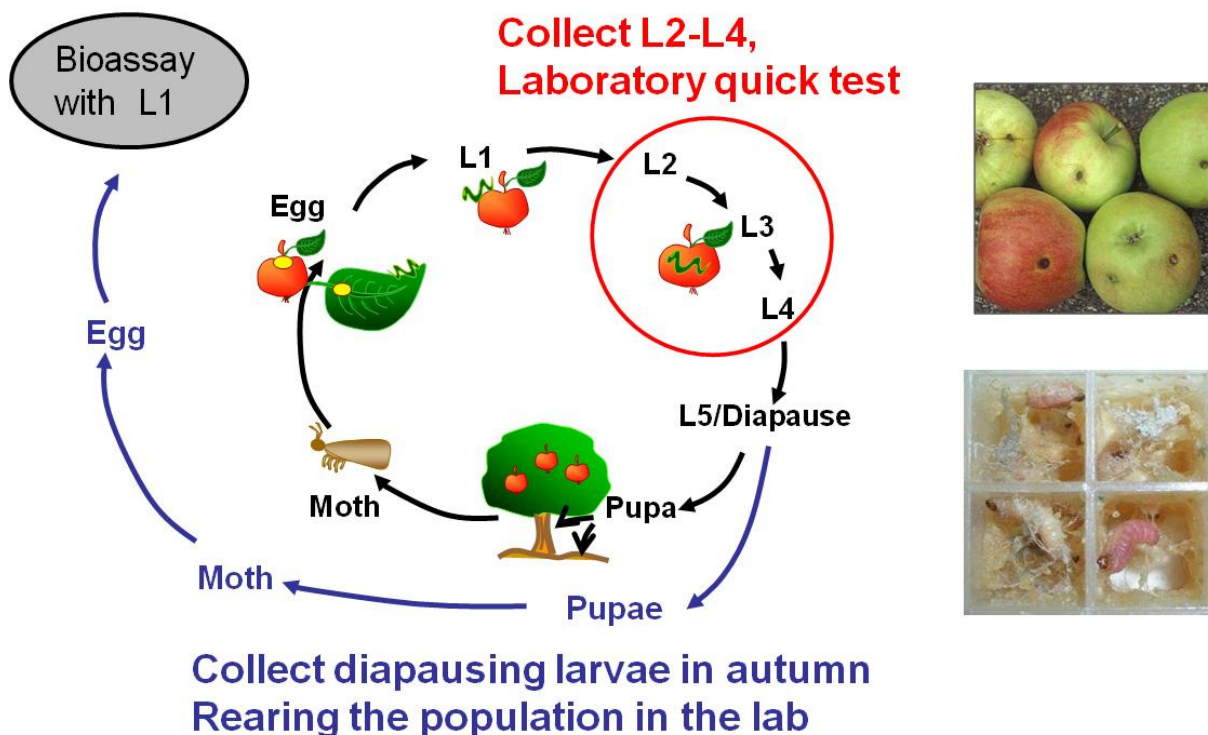


Fig. 1. Resistance testing by collecting either diapausing larvae and testing the progeny L1 larvae in a bioassay (Schmitt et al., 2013) or by performing a quick test by extracting living L2-L4 larvae from infested apples and exposing them to a discriminating CpGV concentration adapted to older larvae (Schulze-Bopp & Jehle, 2013)

## Results and Discussion

Until 2013, 22 resistant codling moth populations with type I resistance were confirmed. In most of these orchards, CpGV products containing a resistance-breaking CpGV isolate of genome group E were sufficient to control resistant CM. However, it was noticed that the fruit stings seemed more frequent and deeper than in apples from orchards without resistance. Codling moth larvae resistant to genome group A CpGV died off slightly later when resistance-breaking genome group E CpGV was applied. In consequence, from 2007 all organic orchards were treated with the resistance-breaking products containing group E CpGV to prevent a further spread of type I resistance.

In 2008, in West Germany and in Saxony, two orchards were confirmed to express resistance type II and type III, respectively. Since 2010, the number of orchards with type II

resistance increased from year to year. Most of them were located in Northern Germany but also some orchards in Central Germany, the Lake Constance area and in South Baden have been identified.

In 2019, two CM populations with type II resistance were identified in Saxony. Recently, most CM populations with type I resistance seemed to develop type II resistance. Growers with verified type II resistance have been allowed using experimental products containing type B CpGV according to Article 53 of Regulation 1107/2009 (Emergency situations in plant protection).

Currently, there are 55 organic orchards with resistant codling moth populations, representing less than 10 % of the area under organic fruit growing in Germany. For these growers solutions are needed. So far, the combined action of research, extension services, growers and CpGV producers allowed overcoming CpGV resistance and to provide suitable strategies for CM control.

Table 1: Occurrence of CpGV resistance in organic apple growing in Germany 2004 – 2019, verified in laboratory bioassays of L1 larvae by LC<sub>50</sub> estimation or resistance testing and/or direct testing of L2-L4 larvae extracted from apples.

Year	Verified Resistance		Total
	Type I	Type II	
2004 - 2009	22	2	24
2010 - 2016	-	14	14
2017 -2019	-	17	17
2004 - 2019	22	33	55

## Acknowledgements

We acknowledge financial support of BMEL (2809OE097) and DFG (Je245/14-1).

## References

- 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.
- Eberle, K. E., Sayed, S., Rezapanah, M., Shojai-Estabragh, S., Jehle, J. A. (2009). Diversity and evolution of *Cydia pomonella* granulovirus (CpGV). *Journal of General Virology* 90, 662-671.
- Fan, J.; Wennmann, J.T.; Wang, D.; Jehle, J.A. (2020a). Novel diversity and virulence patterns found in new isolates of *Cydia pomonella* granulovirus from China. *Applied and Environmental Microbiology* 86(2). DOI: 10.1128/AEM.02000-19.
- Fan, J.; Wennmann, J.T.; Wang, D.; Jehle, J.A. (2020b). Single nucleotide polymorphism (SNP) frequencies and distribution reveal complex genetic composition of seven novel natural isolates of *Cydia pomonella* granulovirus. *Virology*, 541. DOI: 10.1016/j.virol.2019.11.016.
- 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.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. *Proc. Natl. Acad. Sci. USA*, 111, 5711–15716.

- 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.
- 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.
- Schmitt, A.; Bisutti, I. L.; Ladurner, E.; Benuzzi, M.; Sauphanor, B.; Kienzle, J.; Zingg, D.; Undorf-Spahn, K.; Fritsch, E.; Huber, J.; Jehle, J. A. (2013). The occurrence and distribution of resistance of codling moth to *Cydia pomonella* granulovirus in Europe. *J. Appl. Entomol.*, 137, 641-649.
- Schulze-Bopp, S.; Jehle, J. A. (2013). Development of a direct test of baculovirus resistance in wild codling moth populations. *J. Appl. Entomol.* 137, 153-160.
- Wennmann, J.T.; Radtke, P.; Eberle, K.E.; Gueli Alletti, G.; Jehle, J.A. (2017). Deciphering single nucleotide polymorphisms and evolutionary trends in isolates of *Cydia pomonella* granulovirus. *Viruses* 9 (8), 227. DOI: 10.3390/v9080227.

**Citation of the full publication**

The results presented have been published in Sauer et al. 2017 ab.