# Impact of different Agents on the Efficacy of Codling Moth Granulovirus in Tank Mixtures

E. Fritsch[1]; K. Undorf-Spahn[1], J. Huber[1], J. Kienzle[2]

**Abstract**

*In the control of codling moth it is common to combine the granulovirus with other agents, especially fungicides, in spray application. Therefore the knowledge about the influence of these agents on the efficacy of the virus in tank mix is very important. Studies on this subject were part of a project supported by BMELV (German Federal Ministry for Food, Agriculture and Consumer protection) at the Institute for Biological Control of JKI in Darmstadt.*

*The granulovirus of* Cydia pomonella *(L.) (CpGV) was mixed with 10 different agents at concentrations as applied in the field. After the exposure the virus activity was calculated from larval mortality determined in bioassays with neonates of a susceptible codling moth strain.*

*Only two agents with a pH of 11 (sodium silicate (water glass) and calcium polysulfide (lime sulphur)) reduced the virulence of CpGV significantly.*

**Key words:** Plant protection, codling moth granulovirus, *Cydia pomonella*, tank mixture, virus inactivation

## Introduction

With the increasing resistance of codling moth strains against *Cp*GV it is even more important than before that the *Cp*GV applied in the field is not completely or even partly inactivated by tank mixture with other products (Asser-Kaiser *et al.*, 2007). In the first years of *Cp*GV application in organic fruit growing, the main fungicide was wettable sulphur. Since it was well known, that a tank mixture of wettable sulphur and *Cp*GV was possible, it became common practice to combine fungicidal treatments with *Cp*GV. Due to this, *Cp*GV could be applied in short intervals without the need of separate applications. Today, there are more fungicides to be considered, as, e.g. lime sulphur and copper which is used in low concentrations also after blossom. With increasing infestation of sooty blotch even other treatments as potassium, sodium bicarbonate and water glass (sodium silicate) became important. It was not so clear, if there would be no inactivation of *Cp*GV in tank mix with these substances. Furthermore, mainly in the region of Lake Constance, a special kind of whey with rather acid pH was used against spider mites. Calcium chloride is often used against bitter pit during the second generation of codling moth. Also in this case the question of miscibility with *Cp*GV arose. The aim of this study was to test if different products lead to an inactivation of *Cp*GV if applied in tank mix.

## Commercial products

The following commercial products used in organic apple production were tested with regard to effects on virus stability.

[1] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany, jürg.huber@jki.bund.de
[2] Apfelblütenweg 28, D-71394 Kernen, Germany, jutta@jutta-kienzle.de

In practice the calculation of the dosage is based on 2 m high trees and a water volume of 300 l per hectare. For bioassays the products were prepared with the same dosages before *Cp*GV was added.

Table 1: Commercial formulations used together with *Cp*GV in tank mix for control of codling moth.

| Commercial products | Dosage in 300 litre per ha | Concentration per 100 ml water | H-ion concentration pH |
|---|---|---|---|
| Whey | 14 kg | 4,67 gr | 4 |
| Sodium bicarbonate | 5 kg | 1,67 gr | 8.5 |
| Sodium silicate (water glass) | 5 litre | 1,67 ml | 11 |
| Calcium chloride (Düngal) | 20 litre | 6,67 ml | 7 |
| Potassium bicarbonate (Vitisan) | 5 kg | 1,67 gr | 9 |
| Potassium bicarbonate (Armicarb) | 5 kg | 1,67 gr | 8.5 |
| Copper liquid (Cuprozin) | 0,66 litre | 0,22 ml | 7 |
| Copper WP (Cuprozin WP 24158) | 0,44 kg | 0,148 gr | 7 |
| Sulphur (Stulln) | 4 kg | 1,33 gr | 7 |
| Lime sulphur | 15 litre | 5,0 ml | 11.5 |
| Water | - | 100 ml | 7 |

**Virus**

The granulovirus of the codling moth used in the bioassays is a descendent from the *Cp*GV collected in Northern Mexico ("Mexican strain") (Tanada, 1964). It was propagated in host insects and purified by the method described by Huber (1981).

**Test insects**

Larvae of a laboratory strain of the codling moth, *Cydia pomonella*, served as test insects in the bioassays. This laboratory strain has been established more than thirty years ago at the Institute for Biological Control of the BBA in Darmstadt. The rearing method has been described by Bathon (1981).

**Bioassay method**

For the assays each agent was dissolved in 100 ml of water at the amount given in table 1. *Cp*GV was added to these suspensions ($1.5 \times 10^6$ G/ml) and held at room temperature for 4 hours corresponding to the conditions during field application. Additionally a water suspension of *Cp*GV was prepared as a control. The pH was determined by colour-fixed indicator sticks.

Samples were removed 4 hr after mixing and assayed for infectivity using the bioassay method described by Huber (1981). Therefore, aliquots of the prepared solutions were incorporated by thorough mixing into an artificial diet (Ivaldi-Sender, 1974) kept at a temperature of 45 °C in a water bath. The bioassay diet was dispensed into special boxes (LICEFA, Bad-Sulzuflen, Germany) with 50 separate cells (1.5 x 1.5 x 2 cm) and the next day one neonate larva was placed in each cell.

The boxes were covered with a layer of tissue paper, a polyethylen sheet, and a hard-plastic cover, and fixed with two rubber bands. The boxes were incubated at 26°C, 60-70% RH with a 16 hr photoperiod and larval mortalities were recorded after 14 days. Only copper wettable powder (Cuprozin WP 24158) was tested in a bioassay incubated for 7 days. For each bioassay the solutions were diluted so that the *Cp*GV concentration in the diet was equivalent to a $LC_{90}$.

From the mortality data of the bioassays virus activity was calculated by using the dose response regression line of a standard bioassay.

**Results and discussion**

In the bioassays the larval mortality was recorded and used for the calculation of the viral activity. These data are presented in figure 1. Obviously only two products, water glass and lime sulphur reduced the viral activity substantially. After exposure in the water glass solution the virus retained 27% of its original activity. After exposure in lime sulphur solution, the activity was only 14 %. In the suspensions of these chemical compounds the viruses were exposed to high H-ion concentrations of pH 11. The other products ranging from pH 4 to pH 9 had no inactivating effect on the viruses and caused larval mortalities similar to that of *Cp*GV suspended in water. The new product Cuprozin wettable powder tested in a 7 day bioassay showed as well as Cuprozin liquid no reduction of virulence (figure 2).

Studies on the stability of a nucleopolyhedrosis virus of *Heliothis zea* (corn earworm) showed that its infectivity was reduced when the virus was buffered at pH 2 or 12 but was unaffected at pH 5, 7, or 9 (Gudauskas & Canerday, 1968). The stability of baculoviruses is due to the intact inclusion body embedding the virions. At strong alkaline conditions the inclusion body will be dissolved. Jaques (1977) reported that residues of various chemicals (bicarbonates, sodium, potassium and calcium) found in dew on leaves can have an effect on baculoviruses.

Our results show that *Cp*GV should not be combined in tank mix with lime sulphur or water glass in order to avoid an inactivation. Any effect of tank mix with the other commercial products in the tested dosage is not to be expected.

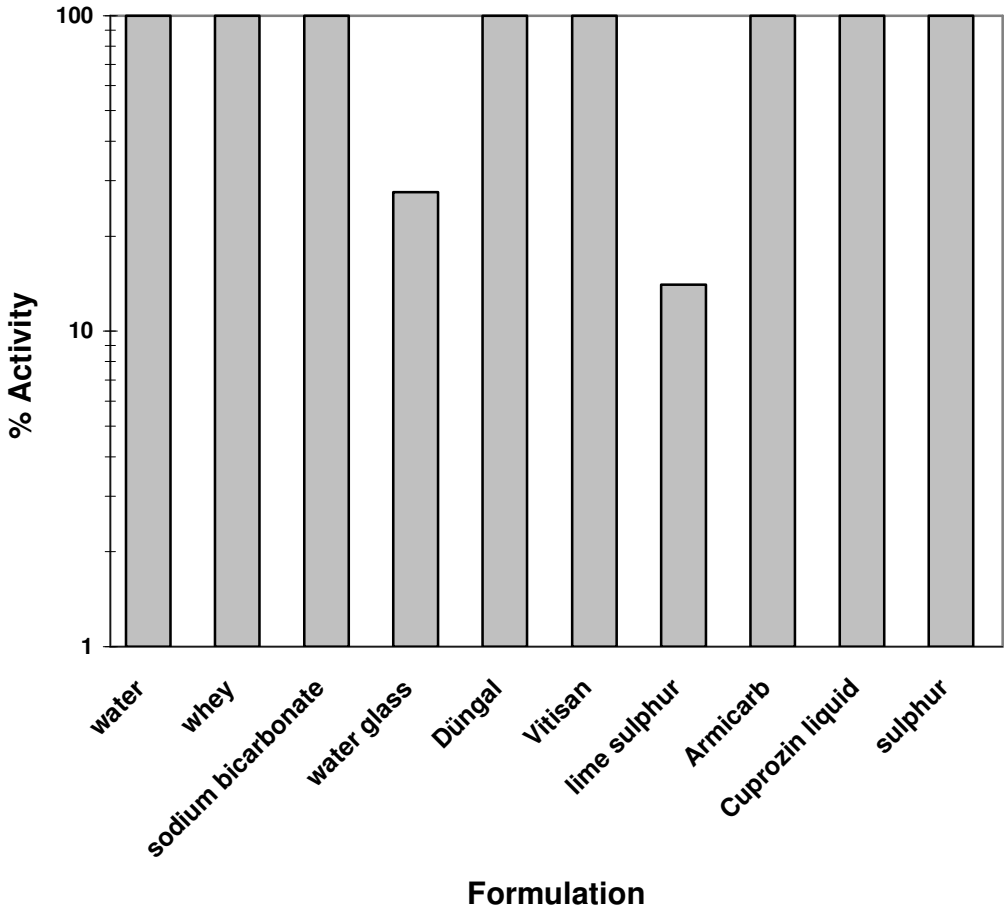


Figure 1: Activity of *Cp*GV after 4 hr exposure to different formulations, calculated from larval mortality after 14 days in bioassays.

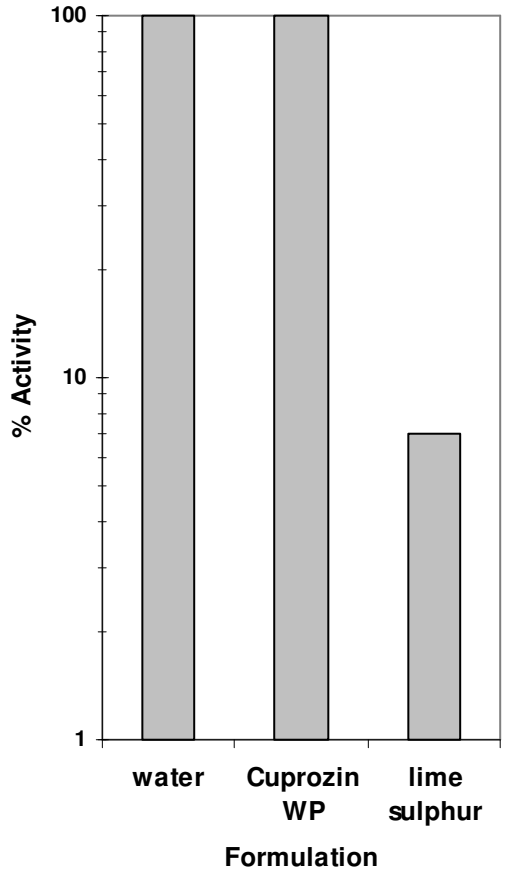


Figure 2: Activity of *Cp*GV after 4 hr exposure to different formulations, calculated from larval mortality after 7 days in bioassays.

## 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. *Sience* **318**: 1916-1918.

Bathon, H. (1981). Zur Zucht des Apfelwicklers, *Laspeyresia pomonella* (L.) (*Lep., Tortricidae*), auf einem künstlichen Nährmedium. *Mitt. dtsch. Ges. allg. angew. Entomol.* **2**: 136-140.

Gudauskas, R. T. & Canerday, D. (1968). The effect of heat, buffer, salt and H-ion concentration, and ultraviolet light on the infectivity of *Heliothis* and *Trichoplusia* nuclear polyhedrosis viruses. *J. Invertebr. Pathol.* **12**: 405-411.

Huber, J. (1981). Apfelwickler-Granulosevirus: Produktion und Biotests. *Mitt. dtsch. Ges. allg. angew. Entomol.* **2**: 141-145.

Ivaldi-Sender, C. (1974). Techniques simples pour élévage permanent de la tordeuse orientale, *Grapholita molesta* (*Lep., Tortricidae*), sur milieu artificiel. *Ann. Zool. Ecol. Anim.*, **6**: 337-343.

Jaques, R. P. (1977). Stability of entomopathogenic viruses. *Misc. Publ. Entomol. Soc. Am.* **10**: 99-116.