Effect of mixtures with other products on the efficacyof codling moth granulovirus (CpGV)

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Abstract

The Cydia pomonella *granulovirus (CpGV) is one of the most important agents for the control of codling moth in organic farming. In practice, CpGV products are often applied in tank mixtures together with other fungicidal or plant strengthening products to reduce the costs of separate applications. Some of these agents have a high pH value in solutions and therefore may have a negative effect on the stability of the CpGV occlusion bodies. Therefore, we examined whether such mixtures influence the activity of CpGV. In laboratory tests, CpGV was mixed with different fungicidal products (Cuprozin, Netzschwefel Stulln), plant strengtheners (Steinhauers Mehltauschreck, Wasserglas, VitiSan, Cocana, Myco-Sin, HF-Pilzvorsorge, CutiSan, Omniprotect and Armicarb), and other compounds, such as Molke, Düngal, lime sulphur, Ventex and Prev-B-2. After an exposure of four hours, virus activity was calculated from larval mortality determined in bioassays with neonate codling moth larvae. A significant loss of virulence was only found in mixtures under strong alkaline conditions higher than pH 11. Four agents, sodium silicate (Wasserglas) and lime sulphur as well as Omniprotect and Ventex should not be used with CpGV products in tank mixtures.*

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

Introduction

The *Cydia pomonella* granulovirus (CpGV) is a highly important and widely used agent to control of codling moth, *C. pomonella*, in organic and integrated farming (Huber, 1998). In the past, it was common practice in organic fruit growing to use tank mixtures of CpGV products and wettable sulphur (e.g. Netzschwefel Stulln) for the combined control of codling moth and apple scab. The combination of both agents in mixtures had no detrimental effect on the viral activity. Hence, CpGV products could be applied in short intervals without the need of separate applications. Currently, not only fungicides like Cuprozin, Netzschwefel Stulln and lime sulphur are applied against powdery mildew and apple scab, respectively, but also several plant strengtheners are widely used in organic apple production. Treatments with potassium carbonate and bicarbonate (VitiSan and Omniprotect), sodium bicarbonate (Steinhauers Mehltauschreck) and water glass (sodium silicate) became important applications, too. Furthermore, products of clay (Myco-Sin and CutiSan) and a special kind of whey (Sprühmolkepulver) are currently tested for preventive application. Calcium chloride (Düngal) is often used for prevention of bitter pit during the hatching period of the second generation of codling moth. With the increasing number of products applied during the hatching period of codling moth larvae, the question arose, whether these products can also be applied in tank mix with CpGV without loss of virus activity. In general, CpGV is highly stable due to a proteinaceous occlusion body surrounding the virus particle.

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However, the occlusion body becomes destabilized at extreme (especially high) pH values and virus activity gets lost under such detrimental conditions (Jaques, 1977). To avoid any virus inactivation, mixing with some of the above mentioned anti-fungal agents is often not recommended because of the pH value of the tank mixture.

Recently, the effect of ten agents on the activity of CpGV was tested under standardized laboratory conditions at the Julius Kühn Institute (JKI) in Darmstadt (Fritsch *et al*., 2008). Here, we report the results of testing the miscibility of further ten commercial products with CpGV.

Material and Methods

Commercial products

The commercial agents tested in mixtures with CpGV are listed in Table 1. For the performance of the bioassays the products were prepared in the same manner as recommended for field application but for a water volume of 200 ml. The calculated dosage is based on a water volume of 300 l per hectare and 2 m tree crown height.

Virus

The *Cydia pomonella* granulovirus (CpGV) used in the bioassays is a descendent from the isolate CpGV-M ("Mexican isolate") (Tanada, 1964). It was propagated in host insects and purified by the method described by Huber (1981). The isolate CpGV-M was provided from the DLR Rheinpfalz (Agricultural Service Center Palatinate, Neustadt/Weinstr.).

Test insects

Larvae of a laboratory strain (CpS) of the codling moth, *C. pomonella*, served as test insects in the bioassays. This laboratory strain is derived from Andermatt Biocontrol, Switzerland. The rearing method has been described by Bathon (1981).

Bioassay method

For the bioassays, each commercial agent was dissolved in 200 ml of water. After adding CpGV (2.4×10^5 occlusion bodies/ml), the suspensions were incubated at room temperature for 4 hours. The pH of the each suspension was determined by colour-fixed indicator sticks (Roth, Art. Nr. C731 pH 4.5 – 10 and MERCK, Art. Nr. 9541 pH 2.5 – 4.5).

Larval mortalities of about 70% after 7 days and 100% after 14 days, respectively, were expected at the chosen virus concentration. Additionally, a water suspension of CpGV was prepared as a positive control. The virus activity in the mixtures was determined in bioassays with first instars (L1) of codling moth using the method described by Huber (1981). After incubation of the agents with CpGV occlusion bodies, aliquots of the prepared suspensions were mixed with an artificial diet (Ivaldi-Sender, 1974) and poured into bioassay trays (LICEFA, Bad-Salzuflen, Germany) with 50 separate cells (1.5 x 1.5 x 2 cm). The next day one neonate larva was placed into each cell. The covered trays were incubated at 26°C, 60-70% relative humidity and a 16 hr photoperiod. Larval mortalities were recorded after 7 and 14 days. The mortality data of larvae exposed to the mixtures and to the positive control were compared by using ANOVA (Proc GLM; SAS 9.2; Scheffe's test).

Results and discussion

The effect of different commercial agents used in organic fruit production (Table 1b) on the activity of CpGV was investigated in bioassays by means of larval mortalities recorded after 7 and 14 days. After CpGV had been mixed with these agents for 4 hours, the mortality of most mixtures caused mortalities of about 70% after 7 days and about 98% after 14 days, respectively. These

data corresponded to those of the positive control (CpGV suspended in water) (Figure 1, Figure 2). A substantial reduction of mortality was only observed for the plant strengthener Omniprotect and the agent Ventex. In the bioassay evaluated after 7 days, no virus killed larvae were found for both products (Figure 1). After 14 days, the larval mortality reached 22% (Ventex) and 35% (Omniprotect), respectively (Figure 2). This suggested that the activity of CpGV was negatively affected by these two agents.

Table 1a: List of commercial products tested in 2007 in mixtures with codling moth granulovirus (CpGV) and the miscibility with CpGV in tank mixtures. Given are the field application rates of the products and the pH values of the mixtures determined by colour-fixed pH indicator sticks.

a) Products tested in 2007 (Fritsch et al., 2008)			
Commercial products (ingredients)	**Application rate per ha for 300 liter water and 2 m crown height**	**Hydrogen ion concentration (pH)**	**Miscibility**
Whey (Sprühmolkepulver)	14 kg	4.0	yes
Steinhauers Mehltauschreck (Sodium bicarbonate)	5 kg	8.5	yes
Water glass (Sodium silicate)	5 l	11.0	no
Düngal (Calcium chloride)	20 l	7.0	yes
VitiSan (Potassium bicarbonate)	5 kg	9.0	yes
Armicarb* (Potassium bicarbonate) * Registration pending	5 kg	8.5	yes
Cuprozin flüssig (Copper hydroxide liquid formulation)	0.66 l	7.0	yes
Cuprozin WP (Copper WP 24158)	0.44 kg	7.0	yes
Netzschwefel Stulln (Sulphur)	4 kg	7.0	yes
Lime sulphur (Polisenio)	15 l	11.5	no

The pH measurement showed low hydrogen ion concentrations (pH 11.5) for the virus suspensions containing Omniprotect and Ventex. No effect on viral activity was observed for the suspensions with higher hydrogen ion concentrations ranging from pH 3.8 to pH 10. Previous investigations showed that the chemical compounds water glass and lime sulphur resulted also in a high pH value of the mixtures and also significantly reduced the virus activity (Fritsch *et al.*, 2008) (Table 1a). Studies on the stability of a nucleopolyhedrovirus of *Heliothis zea* (corn earworm) reported also pH dependent effects. In this case, virus infectivity was reduced when the occlusion bodies were 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 occlusion body surrounding the virus particles. At strong alkaline conditions the occlusion body protein will be dissolved.

In summary, the use of CpGV in combination with the products lime sulphur, water glass, Omniprotect and Ventex in tank mixtures is not recommended for application in organic production. A negative impact of the other tested products on CpGV was not observed under the described conditions.

Table 1b: List of commercial products tested in 2011 in mixtures with codling moth granulovirus (CpGV) and the miscibility with CpGV in tank mixtures. Given are the field application rates of the products and the pH values of the mixtures determined by colour-fixed pH indicator sticks.

b) Products tested in 2011			
Commercial products (ingredients)	**Application rate per ha for 300 liter water and 2 m crown height**	**Hydrogen ion concentration (pH)**	**Miscibility**
Cocana (28% Potassium, soap based on coconut oil)	8 kg	10.0	yes
PREV-B 2 (Oil of orange)	4 l	7.0	yes
Myco-Sin (clay)	8 kg	3.5	yes
	2.4 kg	3.5	
HF-Pilzvorsorge (Oil of fennel and other plant extracts)	4 l	6.5	yes
CutiSan (Kaolin)	2 kg	7.0	yes
Omniprotect (Potassium carbonate)	6 kg	11.5	no
Ventex (Potassium carbonate)	9 kg	11.5	no
Düngal (Calcium chloride)	20 l	7.0	yes
	10 l	7.0	
Cuprozin Progress (Copper hydroxide)	2 l	7.0	yes
Cuprozin 2720 WP (Copper hydroxide)	1.42 kg	7.0	yes

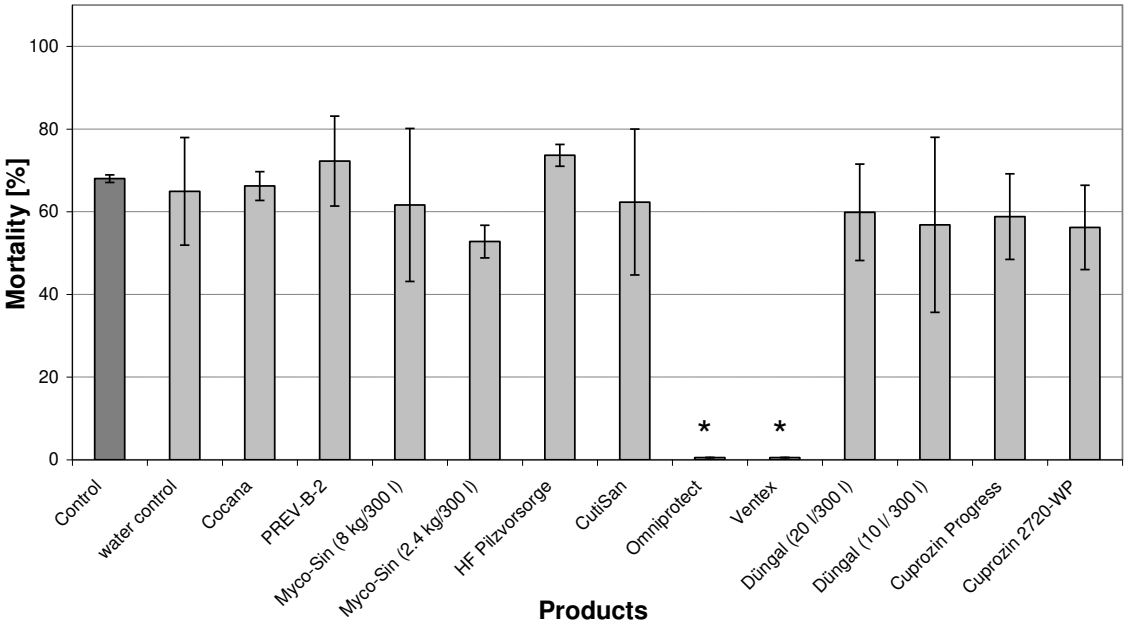

Figure 1: Activity of CpGV after 4 hours exposure in spray mixtures of different products at room temperature, calculated from larval mortality after 7 days in bioassays. Given is the mean mortality of neonate codling moth larvae from 3 replicates. The vertical lines indicate the standard error. For the control assay the CpGV was mixed directly into the bioassay medium. For the water control the virus was resuspended in water and exposed for 4 hours at room temperature before the bioassay was conducted. *The virus activity in these product suspensions differs significantly from the control and the other products at the level of $P< 0.05$ (Scheffe´s test, SAS 9.2; ANOVA, Proc GLM).

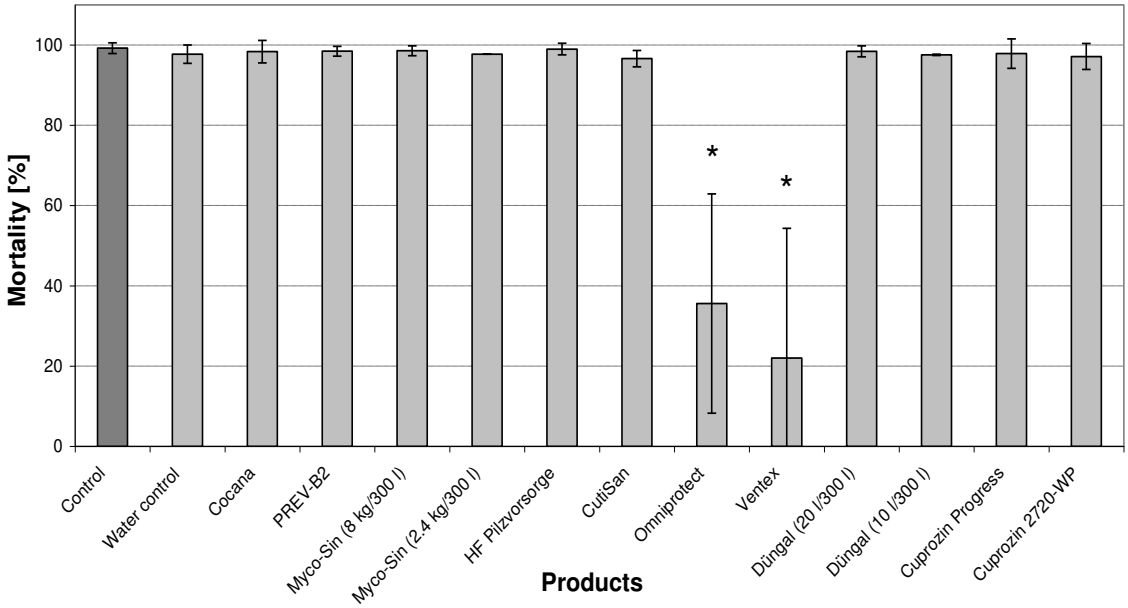

Figure 2: Activity of CpGV after 4 hours exposure in spray mixtures of different products at room temperature, calculated from larval mortality after 14 days in bioassays. Given is the mean mortality of neonate codling moth larvae from 3 replicates. The vertical lines indicate the standard error. For the control assay the CpGV was mixed directly into the bioassay medium. For the water control the virus was resuspended in water and exposed for 4 hours at room temperature before the bioassay was conducted. *The virus activity in these product suspensions differs significantly from the control and the other products at the level of $P< 0.05$ (Scheffe´s test, SAS 9.2; ANOVA, Proc GLM).

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