COMPATIBILITY OF CpGV WITH BIOFUNGICIDES AND *BACILLUS THURINGIENSIS* SUBSP. *KURSTAKI*

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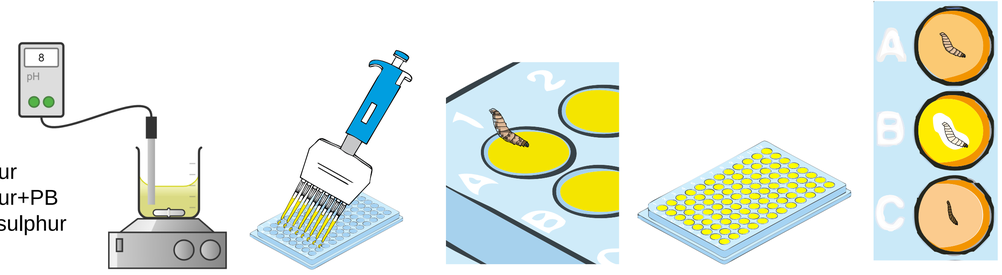
INTRODUCTION

- The codling moth *Cydia pomonella* granulovirus, CpGV, has recently been used in our region to control codling moth, particularly since an increase number of apple growers have adopted low risk pest control methods.
- For effective control, CpGV must be applied repeatedly covering a period when other pests and diseases are present, which will lead to mixture pesticide applications.
- During this period, biofungicides are sprayed mostly to control apple scab and the bioinsecticide *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) to control obliquebanded leafroller, *Choristoneura rosaceana* (Harris).
- This study focused on the virus activity when the CpGV is mixed with currently used biopesticides.
- We evaluated in laboratory bioassays, the compatibility of the CpGV with biofungicides and *Btk* at field concentrations.

MATERIALS AND METHOD

Mixtures:
CpGV only
CpGV+PB
CpGV+Sulphur
CpGV+Sulphur+PB
CpGV+Lime sulphur
CpGV+ Btk



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1. Mixing	2. pH Measuring	3. Mixture Deposition	4. CM Introduction	5. CM Incubation	6. Observation
CpGV added ▪ 1 min after fungicides/ Btk dilution	Time after mixing ▪ T1= 1 min ▪ T15 = 15 min ▪ Replicates : 3	6 µl Mixture deposition on 200µl of CM diet/ 300 µl well Drying period before CM introduction: 1 h	1 CM neonate larvae/ well Total : 64 neonates/ mixture Replicates : 4	25 ± 1°C; 65 ± 5% RH; 16:8 (L:D) Larval mortality: 7 d post CM introduction Alive CM : ▪ Reacting to stimulation of a brush (A) Dead CM ▪ Virus-induced mortality non-reacting, whittish liquid around or coming out the larvae (B) ▪ Unknown cause of mortality, non-reacting, without a whittish liquid (C)

Table 1. Commercial bioinsecticides mixed with CpGV for larval bioassays at field concentrations

Commercial products	Active ingredients	Doses (/ha)	Concentrations (/L)
Virosoft[MC] CP4	C. pomonella granulovirus strain CMGv4	0.25 L	3.56 x 10^9 Obs
Bioprotec PLUS®	Bacillus thuringiensis kurstaki strain EVB113-19	1.8 L	0.65 ml
Potassium bicarbonate technical	Potassium bicarbonate	4 kg	8 g
Kumulus® DF	Sulphur	5 kg	10 g
Lime sulphur	Calcium polysulphide	916 ml	9.16 ml

Statistical Analysis

GLM with treatments, time and treatments*time as factors with Tukey as a comparison test, for the pH variable and GLM with binomial distribution and treatments as factors and the Bayesian approach as comparison test for the morality variable. Values are expressed as mean ± 95 % confidence intervals.

RESULTS

Mixture CpGV + Biofungicides

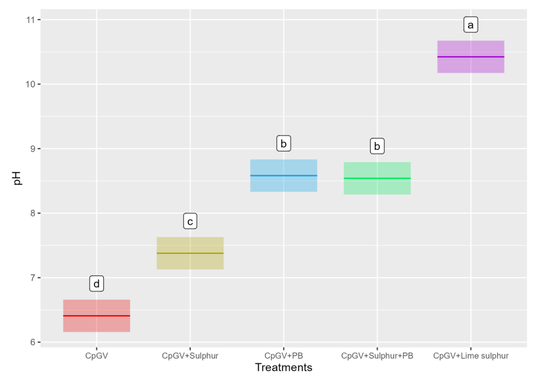

Figure 1. Potential of hydrogen (mean ± 95 % CI) for each treatment taken by a pH-meter after the preparation of the mixture.

- pH values statistically different between biopesticides ($p < 0.001$), but not statistically different between T1 and T15 ($p = 0.6$) with no interaction between time and biopesticides ($p = 0.78$).
- pH values differed between all treatments ($p < 0.0001$), but not between CpGV + PB and CpGV + Sulphur + PB ($p = 0.9992$).

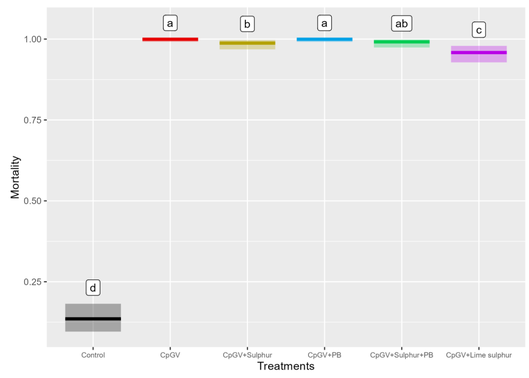

Figure 2. Mortality (mean ± 95 % CI) of codling moth neonates observed 7 days post CM introduction.

- Neonate mortality was significantly reduced in the CpGV + sulphur and the CpGV + lime sulphur mixture ($p < 0.001$).
- Highest neonate mortalities were observed but non significantly between CpGV, CpGV + PB and CpGV + sulphur + PB mixture.
- Lowest neonate mortality in mixture was observed in the CpGV + Lime sulphur.

Mixture CpGV + *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*)

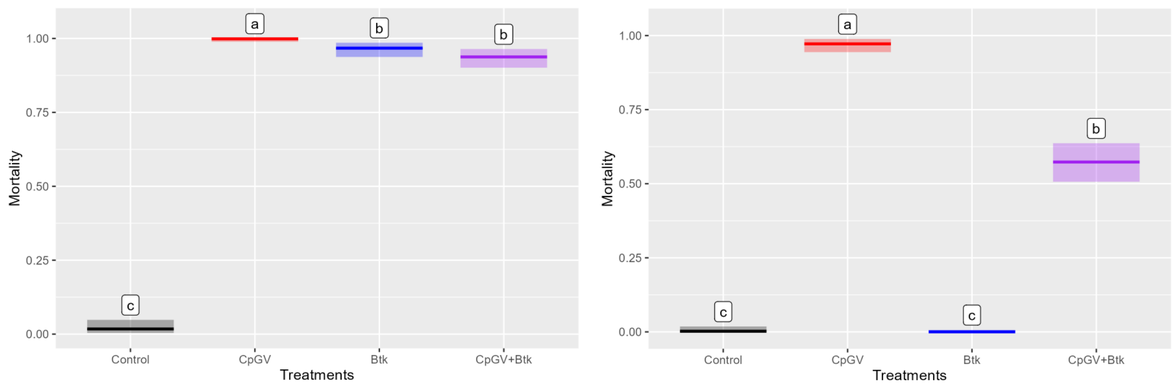

Figure 3. Total (left) and virus-induced (right) mortality (mean ± 95 % CI) of codling moth neonates observed 7 days post CM introduction.

- Total mortality was significantly higher in the CpGV treatment than in *Btk* ($p = 0.001$) and in CpGV + *Btk* ($p < 0.001$) treatments.
- Virus-induced mortality was significantly lower in the CpGV-Btk mixture than CpGV alone ($p < 0.001$).

DISCUSSION

- The significant mortality reduction of neonates in the mixture with lime sulphur formation could be associated with a pH value greater than 10, known to dissolve the viral occlusion bodies (Harvey and Volkman 1983). Reduction of codling moth mortality was also observed when CpGV was mixed with fungicides under alkaline conditions (Fritsch et al. 2012).
- However, when CpGV was mixed with sulphur formulation, alone or with potassium bicarbonate, a reduction, was observed in the mortality of neonate but the pH could not be associated with the reduction.
- The mortality reduction observed in the CpGV + sulphur (pH < 8) could not be associated with the dissolution of the viral occlusion bodies in alkaline conditions suggesting that sulphur played a role in the mortality reduction of neonate. However, the addition of PB to the CpGV + sulfur mixture had reduced the neonate mortality. The role of sulphur is unclear, but it is known to be involved in the process of some virus penetration through the cell membrane (Cheng et al. 2020).
- Mixing *Btk* with CpGV reduced the total mortality of neonates but more extensively the virus-induced mortality. Such reduction was also observed by Chancey et al. (1973), suggesting an inhibition of feeding or an inhibition of viral passage into the midgut cells. Inhibitory feeding effect of *Btk* on larvae may have resulted in reduction of CpGV intake by the larvae and reduction in number of virus that induced mortality (Pingel and Lewis, 1999). In the other hand, both entomopathogen cause pathology in the midgut and *Bt* acts more rapidly than baculovirus (Knowles, 1994; Granados and William, 1986), which may have caused more rapidly the mortality of neonates than CpGV.

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