Biological Control

# Carpovirusine granulosis virus formulation: control of resistant strain of codling moth and study of the vertical transmission of the virus

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*Abstract:* Carpovirusine is a biological larvicide based on *Cydia pomonella* granulosis virus. This product is formulated in a specially dedicated plant in the southwest of France where the virus production is obtained by natural mass rearing of codling moth infected by the virus. Carpovirusine was developed in western Europe in 1993. After 10 years of experimental trials and studies conducted with scientific researchers and apple growers, key data have been generated. With these results we can promote the product as a tool for managing resistance to existing insecticides and for controlling existing codling moth population. A comparative efficacy study has been conducted on codling moth strain resistance in comparison with regular chemical insecticide. No change in the efficacy level was recorded during this study. Another study has been conducted in the laboratory on codling moth larvae treated with a sublethal dose rate of Carpovirusine. The surviving larvae were bred under favorable conditions. Repetition of Carpovirusine sublethal treatments on 9 generations of codling moth did not induce any resistant strain selection into the population. Calliope and the French National Institute for Agronomy conducted a study based on the monitoring of 2 groups of apple codling moth, one treated at L3 larval stage by sublethal CpGV and the other one untreated. A thermal stress was applied on the next generation. On both populations dead larvae were counted to evaluate the transmission of the virus from one generation to the next. Mortality in untreated group was 9.5% vs. 50.8% in the population from infected parent group. In top fruit orchards, several orchard trials confirmed this decline in the overwintering larvae population resulting from the vertical transmission of the virus throughout the next generation.

## Introduction

Carpovirusine, a biological insecticide based on the granulovirus of *Cydia pomonella*, has been registered since 1993 in France for the control of apple, pear and walnut codling moth. Registration has been granted in main apple growing countries in various continents (Europe, Africa, America). EPA registration and state registrations in Washington, Oregon and Michigan for apple codling moth control were granted in 2002. Registration in California is pending.

The virus is produced by environmentally safe codling moth mass rearing in a special facility located in Pau, in southwest France. Carpovirusine contains $10^{13}$ virus granules per liter. The concentration of product to be applied is 0.1 l per 100 l spray (0.1%).

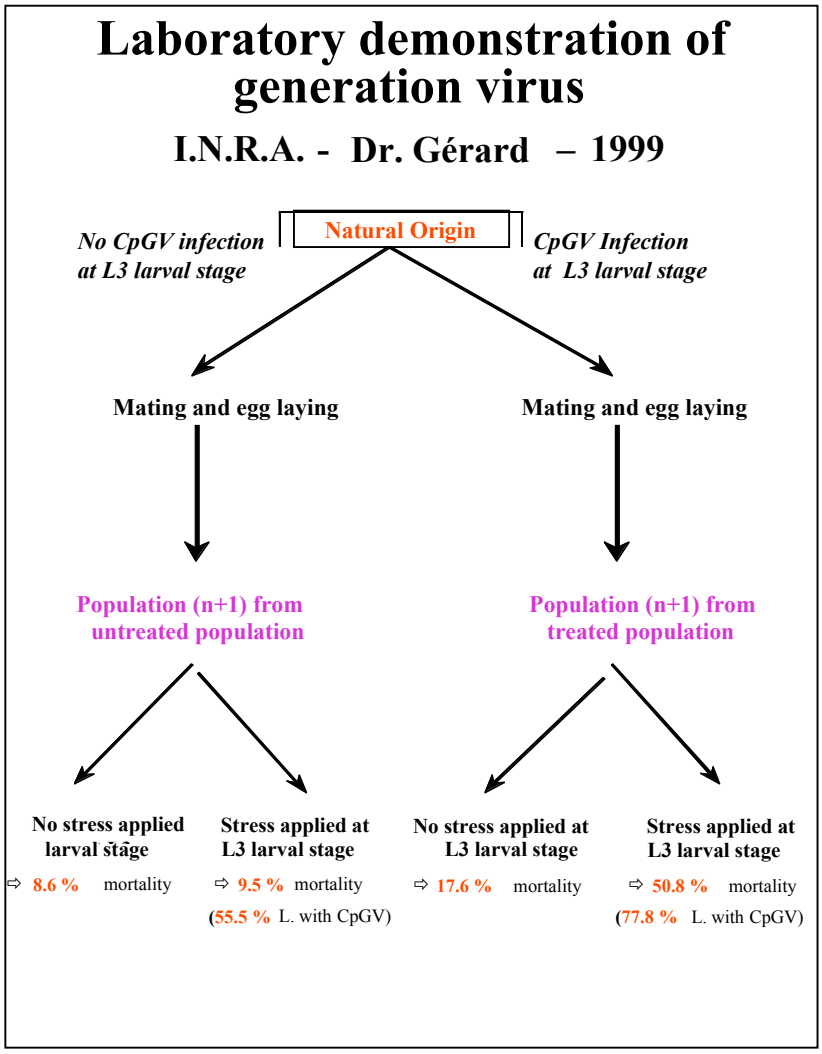
Today insecticide resistance is a major issue for agriculture with the negative consequences of both poor crop protection and increase in pesticides used. Control of apple

codling moth is a perfect example of the resistance issue. Studies conducted over the past 10 years have shown that the codling moth has developed a cross-resistance to several insecticides in many orchards. Insecticide resistance management is an important part of integrated pest management (IPM) strategies to retard or avoid this insecticide resistance.

Resistance of apple codling moth to several chemical insecticide families was first noted in 1992 in the USA, and in 1996-1997 in France (Sauphanor et al.) resistance was recorded to diflubenzuron, pyrethroids and OP insecticides. In Switzerland, Dr. Charmillot demonstrated in 1996 that an orchard population of apple codling moths had developed resistance to diflubenzuron, with a positive cross-resistance to azinphosmethyl and deltamethrin, even if pyrethroids were not registered against codling moth in this country. Effectiveness of diflubenzuron, fenoxycarb, tebufenozide, phosalone, indoxacarb, chlorpyrifos-methyl, deltamethrin and methoxyfenozide was strongly reduced in several Swiss orchards.

**Laboratory Study Proving the "Vertical" Transmission of the Granulovirus**

An additional study on virus transmission was conducted by INRA using 600 larvae split into two populations, one untreated and the other treated at L3 with a 5.76 g/mm$^2$ (equivalent to the $LC_{50}$). In order to activate the viral replication, a thermal stress (quick temperature variation from 32 to 5°C and return) was applied at the L3 larvae stage on half of the descendent moths issued from the two parent populations, nontreated and treated. A subsequent viral detection by PCR was conducted 2, 5, 7, 9 and 11 days after the thermal stress.




The results showed that the granulovirus was detected from only the 7th day onward after thermal stress. The virus was present and dormant in larvae and the stress induced its replication and activity. Cumulative mortality of 9.5% over 10 days was recorded in the untreated batches, while the level reached 50.8% in the treated population and the percentage of these dead larvae carrying the granulosis virus was 77.8%. An analysis was carried out after enzymatic digestion to determine the genetic identity of the virus activated by the stress factor. A comparison of the enzymatic restriction profiles obtained by enzyme restrictions Eco R1 and Bam H1, respectively, from the virus activated in larvae and from the standard virus used for the infection of the parents shows the same molecular identity of these two strains. This confirms that the virus activated by thermal stress is the same virus used for the initial infection and that the virus transmitted from one generation of codling moth to the next one is latent.

## Consequence in Orchards of the "Vertical" Transmission of *Cydia pomonella* Granulovirus

Introduction of the granulovirus has shown that, due to the unique biological replication properties of granulovirus, it is possible after several years to reduce codling moth populations in the treated areas with a profitable impact for beneficial insects and thus a reduction in cost of the crop protection.

Experiments conducted during the "Verger 2000" ("Orchard 2000") IPM program throughout France between 1983 and 1993 provide a good illustration of the successful use of the CpGv. In 30 out of 45 trials carried out with CpGv, a decrease in the population of codling moths was recorded. These observations were completed with a two-year laboratory study on overwintering larval populations. Assessment of mortality, fecundity and weight of pupae shows a significant mortality due to the virus in overwintering larvae and a reduction in the emergence of populations treated with the granulovirus.

Certain comments should be made to clarify these results:

- Carpovirusine was used on orchards where codling moth population was monitored year after year. The codling moth population is highly variable in France, varying from one flight in the north of France to three flights in the south, with cross resistance to many chemical insecticides. In some areas, all orchards were managed according to current IPM practice.
- At the same dose rate of $10^{13}$ granules/ha and intervals of 10 to 12 days between treatments, on 45 orchards with low or moderate pest infestation, 1.6% of attacked fruits were observed at harvest. During these trials it was also noted that, in 63% of orchards treated over several successive years with Carpovirusine, the *Cydia pomonella* population was stable or declining.

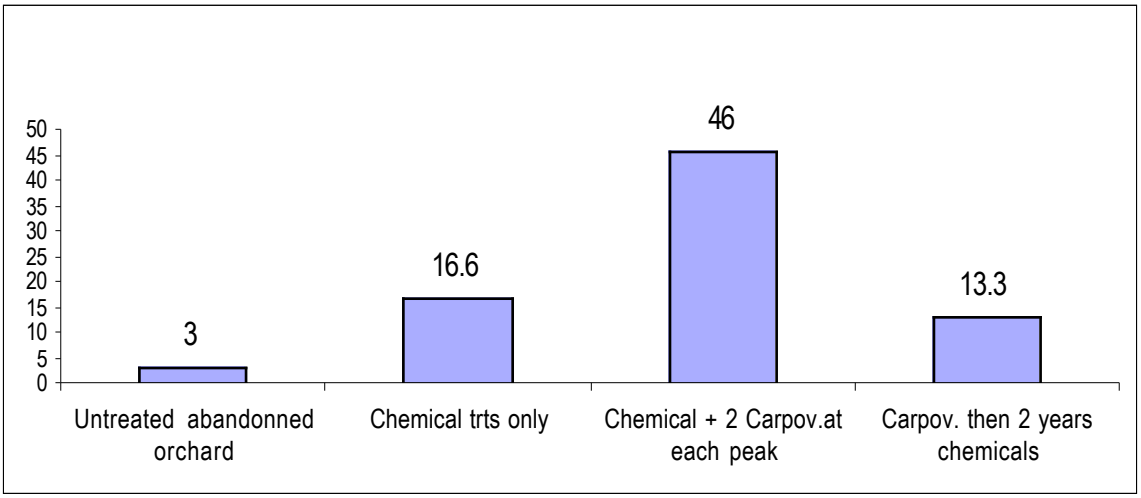



**Fig. 1.** Presence (%) of granulovirus in overwintering larval population according the insecticide program applied.

With chemical treatments, the granulovirus is present in 16.6% larvae. With a combination of chemical and granulovirus at each flight peak, 46% larvae are contaminated with the virus.

A proportion of larvae infected die during the winter and contribute to the reduction of the codling moth population in the orchards. The infected and surviving larvae are able to transmit the granulovirus to their descendents. In the plots without re-infection with virus for 2 years, the virus level remained at 13% in the population, exhibiting this vertical transmission of the virus from one generation to the next. Studies under laboratory conditions using serology diagnostic method (Elisa) and a Polymerase Chain Reaction (PCR) method allow us to predict the codling moth granulosis virus activity. Its mode of action can be described with three sequences:

- First the larvicide activity.
- Then a secondary effect inducing mortality on overwintering larvae and a consequent reduction of adult moth population the following spring.
- Finally, transmission of the granulosis virus to the next generation.

When compared to a chemical larvicide, Carpovirusine offers additional benefits which obviously fit nicely in a integrated pest management program.

## Efficacy of Granulovirus on *Cydia Pomonella* Sensitive and Resistant Strains to Chemical Insecticides

The sensitivity study of granulovirus to various *Cydia pomonella* strains, either resistant to several chemical insecticides or a sensitive strain, is shown below.

**Table 1.** Sensitivity of one sensitive and one resistant strain of *Cydia pomonella* granulovirus

| Strain | Number of trials | $LC_{50}$ granules/mm$^2$ | Confidence interval 5% granules mm$^2$ |
|---|---|---|---|
| Sensitive | 9 | 2.1 | 1.7 – 2.6 |
| Resistant | 3 | 2.2 | 1.6 – 3.1 |

The results of the titration tests did not show any significant difference in sensitivity of two larval strains to granulovirus.

## Test to Select a Resistant Strain of *Cydia pomonella*

An attempt to select a resistant strain to granulovirus under laboratory conditions was conducted. After the 9th generation, the trials were stopped due to loss of fecundity and fertility of remaining surviving females. The following table shows the LC 50 of the granulovirus for the 3rd, 6th and 9th generations ($G_3$, $G_6$, $G_9$).

**Table 2.** $LC_{50}$ obtained by selection of *Cydia pomonella* to granulovirus by successive treatments

| Generation treated | $LC_{50}$ | Confidence interval 5% |
|---|---|---|
| G3 | 5.3 | 2.8 – 10.6 |
| G6 | 3.7 | 1.7 – 9.2 |
| G9 | 0.5 | 0.2 – 2.1 |

Resistance to granulovirus has not been established, even with repeated use of Carpovirusine over numerous generations.

## Conclusion

Besides the larvicide effect on the larvae directly treated, in Europe Carpovirusine provided a clear reduction of overwintering larvae populations, and a subsequent reduction in codling moth emergence has been recorded. The attempt to select a resistant strain of *Cydia pomonella* to the granulovirus failed in spite of repeated applications over numerous generations of codling moths. No difference in sensitivity to the granulovirus was recorded for either sensitive or chemical-resistant strains of *Cydia pomonella* granulovirus. These three properties allow Carpovirusine to be used in integrated pest management programs to provide efficient protection specifically oriented against *Cydia pomonella,* while remaining harmless for the environment. For a replacement of OP compounds in the future conventional programs as well as for a combination with mating disruption techniques, Carpovirusine is the product of choice for these future strategies.

## References Cited

**Biache, G., M. Guillon, and H. Quénin.** 1998. Biological control of *Cydia pomonella* with Carpovirusine trial results.

**Biache, G., M. Guillon, and A.M. Waffelaert.** 1998. Mise en évidence des effets différés du virus de la granulose du carpocapse dans un programme de lutte intégrée.

**Biache, G., S. Perchat, H. Quénin, and P. Aupinel.** 1999. La Granulose du Carpocapse. Une méthode de détection de la maladie virale par amplification génomique.

**Charmillot, P.J., and D. Pasquier.** 1999. Carpocapse des pommes: premier cas de résistance au diflubenzuron en Suisse.

**Guillon, M., and G. Biache.** 1997. IPM Strategies for control of codling moth. Interest of CpGv for long term biological control of Pest complex in orchards.

**Sauphanor B. et al**. 1998. Coping with insecticide resistance in fruit production: the example of codling moth resistance in Europe.