

Biological Control

Enhancing the Insecticidal Potential of Viral Pesticides for Codling Moth

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The codling moth, *Cydia pomonella* (L.), is an economically important pest of apples, pears, and cherries. Increased attention has been given to control strategies other than chemical in order to minimize the toxic effects on non-target organisms and the environment and to help break the pattern of insecticide resistance. Codling moth granulosis virus (CpGV) has been studied over the past 30 years as a viable alternative to chemical control. However, death by viral infection may take up to two weeks. Our goal is to genetically alter the CpGV to enhance its killing power (virulence) and/or persistence.

We want to insert a bacterial ice nucleating gene into CpGV. This gene encodes for a protein which causes spontaneous freezing at temperatures of -2 to -5°C (28 to 23°F). If a codling moth larva was infected with CpGV with the ice nucleation gene, lethality would be ensured by one of two ways: (1) death by granulosis viral infection or (2) death by freezing. Death by freezing would occur because the ice nucleator protein causes freezing at temperatures higher than codling moth supercooling temperatures. Treatments with engineered CpGV targeted at the second generation could greatly reduce the diapausing populations of codling moth.

This past year, we have broadened this project to explore the potential of spider venom toxins (nerve poisons) to genetically enhance the killing power of both *Autographa californica* multi-nucleopolyhedrovirus (AcMNPV) and CpGV as insecticides for codling moth and other orchard pests. AcMNPV genetically engineered with the spider venom toxins *Diguetia* and *Tegenaria* (aggressive house spider) and injected into larvae of lepidopteran pests have been demonstrated to significantly reduce both feeding times and survival times over larvae injected with wild-type AcMNPV (Hughes et al. 1997, Krapcho et al. 1995). Whereas the ice nucleation gene requires a drop in ambient temperature in order for it to be an effective killing agent for codling moth, the toxin genes would work during any part of the growing season. The system for expression of foreign genes has been more optimized in AcMNPV than in CpGV. Thus the potential for enhancement of the killing power would be greater for AcMNPV, even though CpGV is a more specific and more virulent viral vector to codling moth than AcMNPV (Lacey et al. 1999).

We have established a test system with AcMNPV which was genetically engineered with the ice nucleating gene and assayed for expression. Last year, larvae of codling moth, obliquebanded leafroller, and cabbage looper were injected with the engineered virus, with some tests repeated in 1999. Larvae injected with tissue culture medium and/or wild type AcMNPV served as controls. The injected larvae were subjected to a whole body supercooling point analysis to determine the effect of expression of the ice nucleating gene on supercooling point. The engineered virus was passed through cell culture and amplified before the 1999 tests on codling moth and cabbage looper. All species showed a marked increase in their supercooling points when injected with the engineered virus, with the cabbage looper showing the greatest increase in temperature (Tables 1-3). Moreover, the amplified engineered virus preparation used in 1999 resulted in codling moth freezing at higher temperatures than in 1998 (from a high of -10.9°C [12.4°F] in 1998 to a high of -7.6°C [18°F] in 1999, Table 1).

We are currently conducting preliminary feeding bioassays with the genetically

engineered virus. In addition, progress has been made in cloning the spider venom toxin genes to be inserted into our test virus AcMNPV. Once we test the genes in AcMNPV, then we will proceed to genetically transform codling moth granulosis virus with both the ice nucleation gene and the spider venom toxic genes.

Table 1. Whole body supercooling point analysis (°C) for codling moth. Sample size is in parentheses.

Injected with	1998		1999	
	24hr	48 hr	24 hr	48 hr
Tissue culture medium	-16.4 (30)	-17.1 (26)	-16.3 (9)	-19.3 (10)
Wild type AcMNPV	-18.1 (10)	-18.8 (10)	-	-
AcMNPV + ice nucleating gene	-11.3 (20)	-10.9 (17)	-9.1 (9)	-7.6 (10)

Table 2. Whole body supercooling point analysis (°C) for cabbage looper. Sample size is in parentheses.

Injected with	1998		1999	
	24 hr	48 hr	24 hr	48 hr
Tissue culture medium	-15.2 (10)	-15.8 (10)	-13.4 (10)	-12.6 (5)
AcMNPV + ice nucleating gene	-3.8 (10)	-2.9 (8)	-3.6 (9)	-3.5 (5)

Table 3. Whole body supercooling point analysis (°C) for obliquebanded leafroller (1998 data). Sample size is in parentheses.

Injected with	Photoperiod	24 hr means	48 hr means
		(sample size)	(sample size)
Tissue culture medium	Short day	-16.1 (8)	-14.0 (9)
AcMNPV + ice nucleating gene	Short day	-7.4 (10)	-6.7 (8)
Tissue culture medium	Long day	-11.6 (10)	-11.5 (10)
AcMNPV + ice nucleating gene	Long day	-10.7 (10)	-6.8 (10)

Short day = 8L:16D

Long day = 16L:8D

REFERENCES

- Hughes, P.R., H.A. Wood, J.P. Breen, S.F. Simpson, A.J. Duggan, and J.A. Dybas. 1997.** Enhanced bioactivity of recombinant baculoviruses expressing insect-specific spider toxins in lepidopteran crop pests. *J. Invert. Pathol.* 69:112-118.
- Krapcho, K.J., R.M. Kral, B.C. Vanwagenene, K.G. Eppler, and T.K. Morgan. 1995.** Characterization and cloning of insecticidal peptides from the primitive weaving spider *Diguetia canities*. *Insect Biochem. Molec. Biol.* 25:991-1000.
- Lacey, L.A., P.V. Vail, and D.F. Hoffmann. 1999.** Comparative activity of baculoviruses against the codling moth, *Cydia pomonella*, and three other tortricid pests of tree fruit. *J. Invert. Pathol.* 80(1):64-68.