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NOTE

Lethal Ratios: An Optimized Strategy for Presentation of Bioassay Data Generated from Genetically Engineered Baculoviruses

Baculoviruses are the subject of increasing interest with respect to their potential as insect pest control agents. A number of baculoviruses are already in use against pests on crops for which some cosmetic damage has minimal impact on the product (P. F. Entwistle and H. F. Evans, "*Comp. Insect Physiol. Biochem. Pharmacol.*" (G. A. Kerkut and L. I. Gilbert, Eds)., Vol. 7, pp. 347–412, Pergamon Press, New York). However, for application against most crop pests, the time taken to kill needs to be reduced. A baculovirus may take 4 to 9 days at 20 to 25°C to kill its host.

Genetic engineering enables insertion of foreign coding sequences into the baculovirus genome to speed the time of kill and provide an alternative means of insect pest control. To date, the nuclear polyhedrosis viruses of *Autographa californica* (AcNPV) and *Bombyx mori* (BmNPV) have been the prime candidates for engineering. AcNPV is of particular importance as its host range includes some major pest species in the genera *Heliothis*, *Trichoplusia*, and *Spodoptera*.

Introduction of the coding sequences for insect hormones or enzymes can disrupt development of the larva, causing rapid mortality and thereby facilitating use of the engineered baculoviruses of rapid-kill insecticides. The coding sequence for diuretic hormone was introduced into BmNPV (S. Maeda, *Biochem. Biophys. Res. Commun.* 165, 1177–1183, 1989), and juvenile hormone esterase has been incorporated into the AcNPV genome (B. D. Hammock, B. C. Bonning, R. D. Possee, T. N. Hanzlik, and S. Maeda, *Nature* 344, 458–461, 1990). Baculoviruses expressing insect-specific toxins from the scorpion *Androctonus australis* (L. M. D. Stewart, M. Hirst, M. Lopez Ferber, A. T. Merryweather, P. J. Cayley, and R. D. Possee, *Nature* 352, 85–88, 1991; B. F. McCutchen, P. V. Choudary, R. Crenshaw, D. Maddox, S. G. Kamita, N. Palekar, S. Volrath, E. Fowler, B. D. Hammock, and S. Maeda, *Biotechnology* 9, 848–852, 1991), from *Bacillus thuringiensis* (A. T. Merryweather, U. Weyer, M. P. G. Harris, M. Hirst, T. Booth, and R. D. Possee, *J. Gen. Virol.* 71, 1535–1544, 1990), and the mite *Pyemotes tritici* (M. D. Tomalski and L. K. Miller, *Nature* 352, 82–85, 1991) have also been produced with varying degrees of success as insecticidal agents.

Lethal times and lethal doses (or survival times and survival doses, "The Biology of Baculoviruses," (R. R. Granados and B. A. Federici, Eds.), Vol. 2, CRC Press, Boca Raton, FL, 1986) are generally determined to evaluate the effectiveness of any new recombinant. However, the absolute numbers for LT_{50} and LD_{50} may be misleading. Situations have arisen where the efficacy of viruses from different laboratories have been compared based on time to death. Since LT_{50} and LD_{50} vary dramatically among bioassays run in different laboratories, one could use ratios of the test virus to the wild-type virus as shown below to determine lethal ratio for time (LRT) or lethal ratio for dose (LRD) at the 50 and 90% levels. When such ratios for bioassay data are presented in addition to primary data, comparison of different viral constructs will be simplified since they take into account the relative potency of the wild-type virus (Table 1). Ratios have also been used for comparison of the relative toxicities of different chemicals, the relative susceptibilities of populations, and in pesticide resistance (J. L. Robertson and H. K. Preisler, "Pesticide Bioassays with Arthropods," CRC Press, Boca Ra-

TABLE 1

Lethal Ratios for Time (LRT) for Recombinant Baculoviruses Expressing an Insect-Specific Scorpion Toxin

Insect species	Temp (°C)	Reported LT_{50} (hr) (95% confidence units): Wild-type virus[a]	Scorpion toxin virus	LRT_{50}
Trichoplusia ni	23	113 (112–115)	86[b] (85–87)	0.761
Heliothis virescens	27	125 (117–134)	88[c] (82–95)	0.704
Heliothis virescens	30	81 (79–82)	62[d] (59–64)	0.765

[a] *Autographa californica* nuclear polyhedrosis virus strain C6 (AcNPV C6).

[b] AcST-3 (L. M. D. Stewart, M. Hirst, M. Lopez Ferber, A. T. Merryweather, P. J. Cayley, and R. D. Possee, *Nature* 352, 85–88, 1991).

[c] AcAaIT (B. F. McCutchen, P. V. Choudary, R. Crenshaw, D. Maddox, S. G. Kamita, N. Palekar, S. Volrath, E. Fowler, B. D. Hammock, and S. Maeda, *Biotechnology* 9, 848–852, 1991) [a] and [b] are engineered to express the insect-specific neurotoxin from the venom of the North African scorpion *Androctonus australis* Hector.

[d] M. D. Betana and B. F. McCutchen, unpublished data for AcAaIT.

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ton, FL, 1992). Of course, even when using the ratio method, standardized assay conditions will increase the reliability of the comparison.

$$\text{Lethal ratio for time 50\% (LRT}_{50}) = \frac{\text{LT}_{50}\text{ test virus}}{\text{LT}_{50}\text{ wild-type virus}},$$

$$\text{Lethal ratio for dose 50\% (LRD}_{50}) = \frac{\text{LD}_{50}\text{ test virus}}{\text{LD}_{50}\text{ wild-type virus}}.$$

KEY WORDS: Bioassay; genetically engineered baculovirus; lethal ratio.

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