Stage Independent Insecticidal Effects of a Recombinant Baculovirus on *Bombyx mori* Larvae[1]

Taro OHKAWA, Bill F. MCCUTCHEN, Terry N. HANZLIK, Shizuo George KAMITA, Hiromi SASAGAWA,[2] Prabhakara V. CHOUDARY, Bruce D. HAMMOCK and Susumu MAEDA

Department of Entomology, University of California, Davis, California 95616, U.S.A.

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Baculoviruses are potentially safe alternatives to synthetic chemical insecticides. The agricultural use of baculoviruses, however, has been limited due to their relatively slow speed of insect killing. Recombinant baculoviruses expressing genes encoding diuretic hormone (MAEDA, 1989), juvenile hormone esterase (HAMMOCK et al., 1990), insect specific scorpion toxin (MAEDA et al., 1991; MCCUTCHEN et al., 1991; STEWART et al., 1991), and insect paralytic mite toxin (TOMALSKI and MILLER, 1991) show increased insecticidal properties, i.e. a shortened time of insect killing, compared to wild type viruses. At present the expression of insect specific neurotoxins appears to be the most promising for improving the insecticidal activity of beculoviruses. In the present paper, we examine the effects of the expression of an insect specific neurotoxin by the baculovirus *Bombyx mori* nuclear polyhedrosis virus (BmNPV) in silkworm larvae at various stages of development. These studies are important since it has been shown that the susceptibility of insects to chemical insecticides often changes throughout insect growth.

Silkworms (*B. mori*) were reared on artificial diet at room temperature. BmAaIT, a recombinant BmNPV carrying an insect-specific scorpion toxin (AaIT) gene from *Androctonus australis* (MAEDA et al., 1991); Bm20, a polyhedrin-deficient recombinant BmNPV carrying a 2 kbp long insect derived DNA fragment without coding capacity (MAEDA, unpublished); and BmM14, a polyhedrin-deleted BmNPV mutant (MAEDA et al., 1991), were propagated in BmN cells as described previously (MAEDA et al., 1985). Larvae were starved for 6 h prior to injection and anesthetized by immersion in an ice-cold water bath for approximately 5 min. In order to infect larvae, a viral suspension (10^7 PFU/ml) containing 6 μg/ml of kanamycin was injected subcutaneously into the larvae using an elon-

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[2] Present address: *Department of Insect Physiology, National Institute of Sericultural and Entomological Science, Oowashi, Tsukuba 305 .Japan*

gated glass capillary. Either 0.3, 1, 3, or 20 μl of the viral suspension (or culture media as a control) was injected into the body cavity of 2nd, 3rd, 4th, or 5th (last) instar larvae (5 larvae per instar), respectively, so that a titer of approximately 100 to 200 PFU/μl of hemolymph was obtained. Larvae were injected with budded viral particles rather than fed occluded viral particles in order to avoid any secondary effects, e.g. attachment to the midgut cells, inherent in the viral transportation process through midgut tissues. Furthermore, it is well known that late instar silkworm larvae are highly resistant to oral infection.

Following injection, larvae were observed every 6–12 h for AaIT-specific symptoms, e.g. continuous rotations of the head, dorsal arching, body tremors, etc. (see MAEDA et al. (1991)). Bm20 and BmM14 showed lethal times similar to that reported for wild type BmNPV (MAEDA et al., 1991). AaIT-specific symptoms were recognized at about 40 h post infection (p.i.) in 2nd instar larvae injected with BmAaIT. In 3rd, 4th and 5th instar larvae, AaIT-specific symptoms were initially observed between 40 and 50 h p.i. All BmAaIT-infected larvae of all instars exhibited AaIT-specific symptoms 24 to 36 h prior to death.

The time to death of all BmAaIT-infected instars (2nd–5th) that were tested was about 30% faster compared to wild type BmNPV-infected larvae (Fig. 1). The LT_{50} of BmAaIT compared to wild type BmNPV was decreased by 42%, 34%, 36% and 30% in 2nd, 3rd, 4th and 5th instar larvae, respectively. Although insects often become more resistant to chemical insecticides as they develop, the toxicity of BmAaIT remained constant. A 5 to 25% decrease in body weight at approximately 24 h prior to death was also found in all larval instars infected with BmAaIT compared to wild type BmNPV infected larvae. Although all of the test larvae were infected by subcutaneous injection in these experiments, similar effects are expected following per os infection of agricultural pest insects (e.g. *Heliothis virescens*; see MCCUTCHEN et al. (1991)) assuming that the recombinant virus can efficiently penetrate into the body cavity of all instars. These results indicate that recombinant baculoviruses may be better able to efficiently control pest insects throughout their developmental stages compared to chemical insecticides.

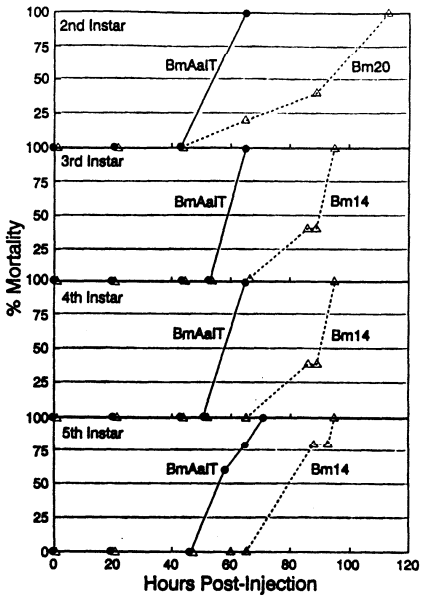

Fig. 1. Mortality of 2nd, 3rd, 4th and 5th instar silkworm larvae infected with AaIT-expressing or control viruses. Dark circles represent the percentage of dead larvae after injection with BmAaIT; open triangles represent the percentage of dead larvae after injection with control (polyhedrin minus) virus (either BmM14 or Bm20).

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REFERENCES

HAMMOCK, B.D., B.C. BONNING, R.D. POSSEE, T.N. HANZLIK and S. MAEDA (1990) *Nature* **344**: 458–461.

MAEDA, S. (1989) *Biochem. Biophys. Res. Commun.* **165**: 1177–1183.

MAEDA, S., T. KAWAI, M. OBINATA, H. FUJIWARA, T. HORIUCHI, Y. SAEKI, Y. SATO and M. FURUSAWA (1985) *Nature* **315**: 592–594.

MAEDA, S., S.L. VOLRATH, T.N. HANZLIK, S.A.

HARPER, K. MAJIMA, D.W. MADDOX, B.D. HAMMOCK and E. FOWLER (1991) *Virology* **184**: 777–780.

McCUTCHEN, B.F., P.V. CHOUDARY, R. CRENSHAW, D. MADDOX, S.G. KAMITA, N. PALEKAR, S.L. VOLRATH, E. FOWLER, B.D. HAMMOCK and S. [illegible] (1991) *Bio/Technology* **9**: 848–852.

STEWART, L.M., M. HIRST, M.L. FERBER, A.T. MERRYWEATHER, P.J. CAYLEY and R.D. POSSEE (199[illegible] *Nature* **352**: 85–88.

TOMALSKI, M.D. and L.K. MILLER (1991) *Nature* **352** 82–85.