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Simulated Sunlight-UV Sensitivity of Engineered Juvenile Hormone Esterase and Scorpion Toxin Recombinants of the Nuclear Polyhedrosis Virus of *Autographa californica*[1]

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ABSTRACT: There has been an effort over the last decade to enhance the effectiveness of wild type baculoviruses using genetic engineering. Wild-type viruses are extremely sensitive to sunlight-ultraviolet, but, what about engineered, recombinant baculoviruses? We found that insertion of a foreign gene did not result in recombinant baculoviruses being more or less sensitive to simulated sunlight-UV than a parental wild-type baculovirus. The half-life of activity for all recombinants and the wild-type parental isolate we tested was within that previously reported for other baculoviruses.

There has been an increased effort over the last decade to expand the host range, virulence, or rate of mortality of baculoviral insecticides via genetic engineering (Miller et al., 1983; Kirschbaum, 1985; Kondo and Maeda, 1991; Wood and Granados, 1991; Bonning and Hammock, 1992, 1994). Engineering of the baculoviral genome has included the insertion of genes expressing insect-selective toxins, enzymes or hormones (Maeda, 1989; Hammock et al., 1990; Merryweather et al., 1990; Stewart et al., 1991; McCutchen et al., 1991; Maeda, et al., 1991; Tomalski and Miller, 1991; Bonning et al., 1992). Persistence of the inherent insecticidal activity after field application of a viral insecticide, however, may be as, or more significant than enhancement of its activity. Sunlight ultraviolet radiation has been widely reported as the most destructive environmental factor affecting field persistence of viruses and thereby their potential usefulness as microbial insecticides (Ignoffo, 1992). The possible effects of sunlight ultraviolet on the insecticidal activity of recently engineered recombinant baculoviruses, however, has never been determined. This short communication reports on the simulated sunlight-ultraviolet (SUV) sensitivity of a parental, wild-type clone (Ayres et al., 1994) and three, genetically engineered, recombinants of the nuclear polyhedrosis virus of *Autographa californica* (AcMNPV).

Materials and Methods

One recombinant (AcAaIT) was engineered to express the insect toxin from the Algerian scorpion, *Androctonus australis* (Stewart et al., 1991; McCutchen et al., 1991). The other two recombinants designated AcJHE-KK and AcJHE-SG (Bonning and Hammock, 1994) were engineered to express modified, stabilized coding sequences of juvenile hormone esterases originally obtained from *Heliothis virescens* (Hanzlik et al., 1989). In the recombinant virus AcJHE-KK, two lysines (at position 29 and 522), were replaced by arginines. In the recombinant AcJHE-SG the catalytic serine was replaced by glycine (Ward et al., 1992). All of the recombinant baculoviruses used in these experiments were polyhedrin-positive, with expression of the inserted foreign gene driven by a duplicated viral p10 promoter.

Polyhedral inclusion bodies (PIB) of the wild-type clone (AcC6) and the recombinants (AcAaIT, AcJHE-KK, and AcJHE-SG) were propagated in mid-instar *Trichoplusia ni* larvae and then semi-puri-

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

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Table 1. Effect of simulated-sunlight-ultraviolet radiation (SUV) on insecticidal activity of a parental (AcC6) and recombinant strains (AcAaIT, AcJHE-KK, AcJHE-SG) of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV).

AcMNPV strain	SUV-exposure[a]	Percent mortality[b] (mean ± SEM)[d]	Percent OAR[c] (mean ± SEM)[d]
AcC6	Shielded	92.0 ± 1.4 a	
	Nonshielded	47.7 ± 7.3 b	51.8 ± 7.5 a
AcAaIT	Shielded	90.0 ± 4.3 a	
	Nonshielded	42.0 ± 7.1 b	50.3 ± 8.7 a
AcJHE-KK	Shielded	93.0 ± 2.6 a	
	Nonshielded	51.9 ± 8.9 b	55.8 ± 8.1 a
AcJHE-SG	Shielded	95.1 ± 3.1 a	
	Nonshielded	37.8 ± 8.3 b	43.0 ± 10.5 a
None	none	0.5 ± 0.5 c	

[a] Exposed for 4 hr to SUV.

[b] Larval mortality after 10 days exposure at 30 ± 1°C to 1.0 PIB/mm^2 of diet surface.

[c] OAR = percent original activity remaining is the ratio of nonshielded to shielded times 100.

[d] Average of 4 replicates/isolate/SUV-exposure using 35–50 larvae/replicate. Values with same letter are not significantly different (ANOVA and Scheffe's test $P < 0.05$).

fied using differential centrifugation (Ignoffo, 1964). Counts of PIB (mean ± SEM × 10^8/ml) of the purified stocks, stored in distilled water at 5°C, were: clone AcC6, 11.4 ± 1.4; AcAaIT, 7.6 ± 0.5; AcJHE-KK, 6.9 ± 0.5; and AcJHE-SG, 14.3 ± 0.4. For the SUV studies both aluminum foil-shielded and nonshielded PIBs of clone AcC6 and the recombinants were exposed on glass cover-slips to SUV for 4 hr (Ignoffo and Garcia, 1992) (Preliminary bioassays after 1 and 2 hr of SUV-exposure indicated no differences between any of the isolates). After exposure to SUV the PIB were washed from the cover-slips, and the suspension diluted to provide 1.0 PIB/mm^2 of diet surface. We previously determined that bioassays, using 24-hr-old *T. ni* larvae, 1.0 PIB/mm^2 and 4 hr of SUV-exposure resulted in a ca. 50% loss in insecticidal activity of PIB. Average percent mortality (after 10 days of exposure at 30 ± 1°C) was based on use of 35–50 larvae/treatment/replicate and 4 replicates/treatment. Means and standard errors were calculated (ABSTAT™) and treatments were compared using ANOVA and Scheffe's test of significance (Anderson-Bell, Parker, CO). Percent original activity remaining (% OAR) was calculated as the ratio of nonshielded to shielded larval mortality ×100.

Results and Discussion

None of the recombinants (AcAaIT, AcJHE-KK, AcJHE-SG) were significantly more or less sensitive to SUV ($P < 0.05$) than the parental, wild-type clone AcC6 (Table 1). The mean-percent (±SEM) OAR of AcC6 was 51.8 ± 7.5. The % OAR of the recombinants AcAaIT, AcJHE-KK, and AcJHE-SG averaged 50.3 ± 8.7, 55.8 ± 8.1, and 43.0 ± 10.5%, respectively. The percent larval mortality of the shielded samples, of all the viral isolates exposed to SUV (wild-type and recombinants), averaged ca. 92% while nonshielded, SUV-exposed PIB averaged ca. 45%. Mortality of control larvae averaged less than 1%. The half-life of 3 to 4 hr for all recombinants and the parental strain is within that previously reported for other baculoviruses with similar inactivation rates (Ignoffo, 1992).

In summary, insertion of a foreign gene did not result in the recombinants AcAaIT, AcJHE-KK, AcJHE-SG being more or less, sensitive to SUV than the parental wild-type. Similar results were obtained using a polyhedral envelope-deleted isolate of AcMNPV (Ignoffo et al., 1995). Although we did not anticipate that recombinants exposed to SUV would behave any differently than the wild-type, it was necessary to document this by experimentation. At this time however, it is too early to generalize from these specific results for all engineered recombinants such as gene deletion (O'Reilly and Miller, 1991), host range expansion (Maeda et al., 1993), replacement of polyhedrin (Wood et al., 1993) or replacement of p10 (Williams et al., 1989). Indeed, recombinants AcAaIT and AcJHE-KK have produced less PIB than the wild-type AcC6 (Ignoffo and Garcia, 1996; Kunimi et al., 1996). Because of the relative lack of data on the environmental stability of novel, genetically modified baculoviruses, studies on persistence should be determined prior to their use as viral insecticides.

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