

NOTES

Virulence of the Baculovirus of *Oryctes rhinoceros* from Ten Locations in the Philippines and in Western Samoa

Oryctes rhinoceros larvae in the Philippines infected with the baculovirus of *Oryctes* occasionally develop a brown color before death (B. Zelazny, *FAO Plant Prot. Bull.* **25**, 73-77, 1977). This symptom has not been observed in Western Samoa where the baculovirus was introduced from Malaysia in 1967 (K. J. Marschall, *Nature (London)* **225**, 288-289, 1970). In the present paper the virulence of baculovirus isolates from these two countries is compared, and the frequency of the appearance of the brown color is studied.

The baculovirus was isolated from *Oryctes rhinoceros* larvae from six locations in the Philippines: isolate PA from Albay Province, Southern Luzon; isolate PB and PF from two locations near Tacloban, Island of Leyte; isolate PC and PD from two locations near Davao, Island of Mindanao; and isolate PE from San Pablo, Central Luzon. In Upolu, Western Samoa, the baculovirus was isolated from an *Oryctes rhinoceros* adult (isolate SA) and from larvae in three locations up to 50 km apart (isolates SB, SC, and SD). The isolates were propagated while carefully avoiding contaminations. Their virulence against 2-week-old, third instar *O. rhinoceros* larvae was tested by adding to the larval food (decaying kapok wood mixed with dry cowdung) 0.2, 0.6, 2, or 20 ppm of virus-killed ground-up larvae which had been stored frozen for up to 3 weeks. The virulence of the isolates PB, PC, and SA against *O. rhinoceros* adults was tested by injecting into each beetle 0.1 ml of sterile water containing 0.05, 1, 10, or 200 pl of hemolymph collected from larvae with advanced virus infections and stored frozen for up to 3 weeks. After 2 weeks the adults were examined for baculovirus infections with bioassay (B. Zelazny, *J. Invertebr. Pathol.* **20**, 235-241, 1972). In each repli-

cate, 10 insects were inoculated per dosage. LD₅₀ values were estimated with the maximum likelihood method using the combined data from all replicates. For each isolate the average periods of lethal infection of the replicates were combined to a mean period with standard error. One-half-, 2-, and 9-week-old larvae were inoculated with the isolates PA, PB, or SA as before (30 larvae/dosage) and were observed for the appearance of the brown color.

In all experiments no mortalities occurred among a total of 390 control larvae, and no baculovirus infections were detected in 90 control beetles. During the first 5 weeks after inoculation, isolate PB caused on average 8.7 ± 0.7 (SE, $N = 3$), 5.8 ± 1.2 ($N = 10$), 12.7 ± 1.5 ($N = 3$), 11.0 ± 0.0 ($N = 3$), and 11.3 ± 1.2 ($N = 3$) more dead larvae per replicate than isolate PF, SA, PE, PC, and PD, respectively. These differences were significant ($P < 0.02$). For larvae the estimated LD₅₀ values for all replicates ranged from 3 (isolate PB) to 22 (isolate PD) ppm of virus-killed larvae in the food (Table 1). The number of infections caused by the isolates PB, PC, and SA in beetles were not significantly different, but as for larvae the LD₅₀ value was estimated to be lower for isolate PB than for isolate PC or SA (Table 1). The isolates often caused significantly ($P < 0.05$) different periods of lethal infection in larvae (Table 1). Before death from the baculovirus, a brown color only appeared in the 9-week-old larvae inoculated with isolate PA (4 out of 32) or PB (5 out of 25) but not in younger larvae or in larvae inoculated with the isolate SA. Virus-infected larvae developing the brown color on average lived significantly ($P = 0.001$) longer (30.0 ± 1.0 days, SE, $N = 9$) than virus-infected larvae remaining white (20.9 ± 1.1 days, SE, $N = 48$).

TABLE 1

VIRULENCE OF THE BACULOVIRUS OF *Oryctes rhinoceros* FROM 10 LOCATIONS IN THE PHILIPPINES AND WESTERN SAMOA

Isolate	Origin	No. of replicates	LD ₅₀ value	Mean period of lethal infection (days ± SE) ^a
Tests with <i>Oryctes rhinoceros</i> larvae ^b				
PB	Leyte Island (Phil.)	10	3	20.2 ± 0.9 (N = 10) a
PF	Leyte Island (Phil.)	3	6	21.1 ± 1.1 (N = 3) ab
PA	Southern Luzon (Phil.)	8	11	21.5 ± 1.8 (N = 8) ac
SA	Upolu (W. Samoa)	11	11	15.2 ± 1.2 (N = 11)
SB	Upolu (W. Samoa)	3	13	26.2 ± 1.8 (N = 3) cd
SC	Upolu (W. Samoa)	3	14	27.7 ± 2.1 (N = 3) ce
SD	Upolu (W. Samoa)	3	15	29.0 ± 0.9 (N = 3) def
PE	Central Luzon (Phil.)	3	16	29.4 ± 1.0 (N = 3) deg
PC	Mindanao Island (Phil.)	3	21	26.1 ± 2.4 (N = 3) bcfg
PD	Mindanao Island (Phil.)	3	22	25.2 ± 3.9 (N = 3) acfg
Tests with <i>Oryctes rhinoceros</i> adults ^c				
PB	Leyte Island (Phil.)	3	0.16	
PC	Mindanao Island (Phil.)	3	0.41	
SA	Upolu (W. Samoa)	3	0.86	

^a Means followed by the same letter are not significantly different ($P > 0.05$).

^b The larval food contained either 0.2, 0.6, 2, or 20 ppm of virus-killed larvae; 10 larvae were inoculated per concentration per replicate; the LD₅₀ values are given in parts per million and were based on the mortalities during the first five weeks after inoculation.

^c The beetles were injected with 0.1 ml of sterile water containing either 0.05, 1, 10, or 200 pl of hemolymph from virus-infected larvae; LD₅₀ values are given in pikoliters and were based on baculovirus infections 2 weeks after inoculation.

It is concluded that isolates of the baculovirus of *Oryctes rhinoceros* from the Philippines and from Western Samoa can cause significantly different mortalities and periods of lethal infection in *O. rhinoceros* larvae, thus they might have different impacts on the *O. rhinoceros* population.

KEY WORDS: *Oryctes rhinoceros*; baculovirus isolates; virulence of insect virus.

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