High Temperature as a Stressor of *Spodoptera litura* Latent Virus

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Abstract

Different types of insects were exposed to a range of temperatures to evaluate natural cyclic outbreaks of nucleopolyhedrovirus occurring in larvae of *Spodoptera litura* at the experiment fields of the Faculty of Agriculture, Kagoshima University, southern Japan, during 1997 through 2008. Results suggested likely latency in vertical transmission of sublethal viral infection. It was demonstrated that high temperature acted as a stressor of latent virus harbored by *S. litura* larvae, and mortality rates varied depending on insect generation.

Key words: nucleopolyhedrovirus latency, temperature, *Spodoptera litura*, vertical transmission.

Introduction

Latent virus infections defined as the ability of a virus to survive in a host without causing recognizable symptoms have intrigued researchers ever since the phenomenon was first documented (reviewed by PODGWAITE and MAZZONE 1986). However, it is only with the more recent molecular techniques that it has become possible to detect low levels of the virus infection (IL' INYKH and UL' YANOVA 2005). BURDEN *et al*. (2002) hypothesizes that latent baculovirus may be low persistent, sublethal infections resulting from survival from initial baculovirus exposure.

Virulence of latent virus infections in insects may be triggered by several factors including physico-chemcal factors, rearing conditions, insecticides and foreign viruses (FUXA *et al.* 1999, PODGWAITE and MAZZONE 1986). KOUASSI *et al.* (2009a) demonstrated that *S. litura* harbored a latent virus that can be activated by the foreign nucleopolyhedrovirus (NPV) from *Mythimna separata* NPV G.

In fields of Kagoshima University, a cyclic outbreak of diseased larvae of *S. litura* from NPV was observed during August when the temperatures are relatively high, and initial assumptions of the probable relationship between temperature and occurrence of insect disease coined. To verify the major causes triggering the outbreak of the disease, different types of insects were exposed to varying temperatures, and the relationship between temperature and the virulence of the latent virus harbored by *S. litura* larvae was then assessed.

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Material and Methods

Insects

Laboratory stock of *S. litura* collected from fields in Kagoshima Prefecture in 1997 were reared continuously in the laboratory at ca. 25℃ on an artificial diet, Insecta-LFS (Nihon Nosan Co. Ltd).

Randomly chosen egg masses were divided into two batches; the first batch was reared until third instar under 25℃ with artificial diet while the second batch was ground in DNA extraction buffer (0.15 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, 1% SDS, pH 8.0), incubated at 65℃ for 20 min and centrifuged at 16,000 *g* for 10 min. DNA in the supernatant was then extracted by phenol-chloroform, collected by ethanol precipitation suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and then used in a nested PCR reaction using the primer sets SlNPVlef-8F3/ SlNPVlef-8R3 and SlNPVlef-8Fnes /SlNPVlef-8Rnes as described by KOUASSI *et al.* (2009a). To avoid contamination by exogenous viruses or DNA, sterilized disposable plastic ware including microtubes, homogenizers, tips and petri dishes were used for all the experiments.

The populations derived from egg mass controlled positive and negative for the virus were considered as "diseased" insects, and "healthy" insects, respectively.

Third-instar larvae from "healthy" populations were fed on an artificial diet treated with SpltNPV S at 1.7×10^4 PIB/ml, a concentration considered lower than the median lethal concentration (3.78×10^4 PIB/ml) estimated by KOUASSI *et al.* (2009b). Survivors were sexed and kept separately in different containers for adult emergence and apparently healthy and active moths selected and paired for mating and egg laying, placing each pair in a container. The egg-masses were then divided into two batches and subjected to similar treatments as described earlier. The population derived from this egg-mass was considered as "infected" and randomly sampled larvae from this latter population monitored by nested PCR according to the procedure described earlier.

Bioassays

Third-instar *S. litura* of diseased, healthy, and infected insects were reared at 8 ℃, 25℃, and 32℃ in 14-h light and 10-h dark photo periods. Thirty newly molted larvae were allowed to feed individually on the artificial diet for 48 h and mortality monitored daily until death or pupation. Tissue smears were prepared from dead larvae and examined for the presence of occlusion bodies (OBs) under a Nikon Alplaphot-2 phase contrast microscope.

Data analysis

The effect of temperature was checked by Chi-squares test and variations between each temperature level and between-population compared by Fischer's exact probability test with Bonferroni correction using JMP 7 software (SAS-INSTITUTE 2007). Dead larvae that did not contain OBs were not analyzed.

Results

PCR amplification SpltNPV *lef-8* gene fragment from progenies of infected *S. litura*

PCR amplification of the SpltNPV *lef-8* gene fragment was performed on individual progenies (larvae) of *S. litura* infected with SpltNPV to detect *S. litura* nucleopolyhedrovirus in "infected" insect populations. Although no product was visible in the first PCR reaction, nested PCR of every sample yielded a 217-bp fragment (Fig. 1).

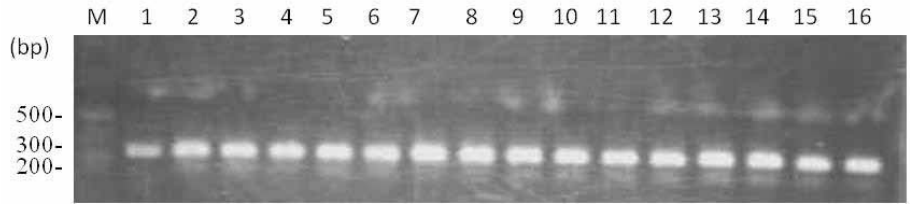

Fig 1. Detection of SpltNPV *lef-8* 217-bp fragment by nested PCR.
Lane 1, PCR positive control (SpltNPV DNA); lanes 2 to 16, DNA from offsprings of healthy laboratory stock of *S. litura* that have been slightly infected with SpltNPV; lane M, 100 bp ladder size marker.

Effect of temperature

Numbers of dead third-instar larvae of the three population of *S. litura* exposed to different temperatures are shown in Table 1. Mortality was influenced by temperature and/or the status of insects populations ($\chi^2 = 186.21$, df = 11, $p<0.01$). Death in "Healthy" and "Diseased" insects were significantly different from death in "Infected" insects ($p<0.05$). Moreover, a significant difference was recorded between "Infected" insects samples ($p<0.05$).

Table 1. Number of dead third-instar larvae of the three population of *S. litura* exposed to different temperatures

Insect population	Temperature (℃)		
	8	25	32
Healthy	0	0*a**	0*A*
Diseased	0	0*a*	0*A*
Infected 1	0	1*a*	7*A*
Infected 2	0	22*b*	23*B*

* Numbers followed with the same italic letter are not significantly different by fisher's exact probability test with Bonferroni correction ($\alpha = 0.05$).

Discussion

Disease is defined as a condition in which a state of physiological equilibrium of an organism with its environment becomes unbalanced due to non infectious or infectious causes (FUXA and TANADA 1987). There have numerous reports of sporadic outbreaks of NPV infection in natural populations of insects that appear to be caused by the activation of latent NPV infections (LONGWORTH and CUNNINGHAM 1968, EVANS and HARRAP 1982). Latency describes the state of a virus that does not produce obvious signs of infection (HALE AND MARGAM 1988). Latency involving insect viruses has been fairly reviewed; the first report on latency deals with a slow spread of polyhedral virus disease of nun moth *Lymantria monacha* in insect population without affecting all the individuals of the population (PODGWAITE and MAZZONE 1986). Depending on external conditions, this chronic course would often change suddenly into an active or acute form. Latency provides another mechanism for vertical transmission of virus from one generation to another. FUXA *et al.* (1992) confirmed that vertical transmission, from parents to progenies, may play an important role in natural epizootics, with observation of polyhedra in larvae, pupae, and adults of *Spodoptera frugiperda* whose parents had survived exposure to *S. frugiperda* nucleopolyhedrovirus. It has been suggested that such infections can be activated by a number of stress factors such as low temperature, over crowding and poor diet (SMITH 1963, LONGWORTH and CUNNINGHAM 1968), although it has proved experimentally difficult to test this hypothesis in every case (MCKINLEY *et al.* 1981). Recent progress in molecular biology techniques has however made it possible to obtain fundamental data on the detection of latent viruses in different insect species as well as on the mechanism of induction for the latent infections (COOPER *et al.* 2003, HUGES *et al.* 1994, HUGES *et al.* 1993, HUGES *et al.* 1997).

Based on detection of viral DNA by nested PCR amplification and sequencing of a *lef-8* fragment, KOUASSI *et al.* (2009a) demonstrated that 20 % and 22.6 % of eggs and larvae of laboratory stocks and wild of *S. litura* insects was latently infected by SpltNPV and that the virus could be activated by a foreign NPV.

The detection of SpltNPV gene in "infected" insects exclusively by nested PCR suggests that the latency is in fact low level, sublethal infections resulting from survival from initial baculovirus exposure as hypothesized by BURDEN *et al.* (2002).

Results of the present study established that high temperature activated the virus provoking death in larvae, confirming that high temperature was a major factor triggering activation of latent virus.

It was observed that although "Diseased" laboratory insects were controlled positive for SpltNPV, they did not die under high temperatures while on the contrary, mortality occurred with "Infected" populations. This may be attributed to the low level of virus borne by the "Diseased" laboratory stock, indicating a likely negative correlation between generation stage and virus transmission. Thus, the transmission

rate of the virus from generation to generation was likely to decrease.

Differences in mortality rate between "Infected" insect samples showed that the transmission rate to offspring differed from a parent to parent even when exposed to the same viral concentration. However, intrinsic immunity system between samples can not be ruled out as a mostly likely explanation for the observed variations.

It has been demonstrated that latent infection can be activated by stress factors such as low temperature (LONGWORTH and CUNNINGHAM 1968). However, in the present study, no mortality was recorded under low temperature (temperature below 20℃). The outbreak of *S. litura* occurred when temperature are high because the species is thermophilic. Therefore, at low temperature, the density of the insects is low and hence disadvantageous for the virus to be activated and released in the environment, since its release would lead to horizontal transmission to surviving larvae with likely extinction of the host. Therefore, it can be hypothesized that the activation of virus by temperature varies between insect species.

From the present study, it can be concluded that the population dynamic of *S. litura* is influenced by the couple temperature-latent virus. At high temperature when insect population is very large, the latent virus is activated and transmitted horizontally via occlusion bodies that are released after death of infected individuals. Thus the outbreak of virus appears to be a likely phenomenal natural control of the insect population. At low temperature, the virus remained inactive; ensuring the survival of the host for vertical transmission of the virus from parents to offspring. Thus the dispersal of the virus is mainly ensured by the adults which are capable of flying farther from the home population. The results of the present study call for additional studies on other stress factors such as the humidity, which reaches maxima during the hot summer months in Kagoshima, southern Japan.

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