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# Effects of High Temperature on the Development of *Laodelphax striatellus* (Homoptera : Delphacidae) and on Its Intracellular Yeastlike Symbiotes

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*Laodelphax striatellus* harbours intracellular yeastlike symbiotes in the fat body, transmitting them to the next generation through the female ovary. High temperature, 35°C, destroyed the yeastlike symbiotes in the mycetocytes. Under the continuous high temperature no adults were obtained. The population of symbiotes in the 5th-instar nymphs, which were previously exposed to the high temperature for 3 days after hatching (heat treatment), was reduced; approximately one twentieth of that in the normal 5th-instar nymphs. These heat-treated insects showed poor growth in spite of sufficient sucking of rice plant juice. More than 70% of the heat-treated 5th-instar nymphs did not become adults and a number of them died. In some of them, the adult cuticle was not deposited or only deposited in part. The high temperature also exerted an influence on fecundity of this species. The heat-treated insects seem to be useful for the further study to explore the role of the yeastlike symbiotes.

## INTRODUCTION

A number of plant sucking insects among Hemiptera-Homoptera possess internal microorganisms (BUCHNER, 1965). Many authors have mentioned that endosymbiotes may play an important role in host's nutrition or metabolism. In the course of studies on the artificial diet of aphids (DADD and MITTLER, 1966; DADD and KRIEGER, 1968; EHRHARDT, 1968; SRIVASTAVA and AUCLAIR, 1971), it has become generally accepted that the small number of amino acids required and the non-requirement of sterol in aphids is due to the supply of nutrients from their intracellular symbiotes.

Since the intracellular symbiotes of some insects are transmitted through the ovary to the next generation and they are found in all of the host's life, the precise study of the nutrition or metabolism of these insects is difficult. In order to distinguish the metabolism of the insect itself from that of the symbiotes, the cultivation of the symbiote or the creating of aposymbiotic insects from which symbiotes are eliminated is required. For the removal of the intracellular symbiotes from the insects, two different ways seem to be available: chemical treatment and physical treatment. Administrations of chemicals such as antibiotics, disinfectants and bacteriolytic enzymes, are useful methods

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for the elimination of the prokaryotic symbiotes (BROOKS and RICHARDS, 1955; MALKE, 1964; EHRHARDT, 1966; EHRHARDT und SCHMUTTERER, 1966). For physical treatment, there are X-ray irradiation (SCHWEMMLER, 1973) and exposure to high temperature (GLASER, 1946; BROOKS and RICHARDS, 1955; HUGER, 1956).

The smaller brown planthopper, *Laodelphax striatellus*, harbours yeastlike symbiotes in the mycetocytes in the fat body, which are transmitted to the next generation via the female ovary (NASU, 1963; NODA, 1977). The yeastlike symbiotes transmitted into the embryo propagate in the mycetocyte with the host development and attain the maximum number at an early stage of the ovipositional period of the female adult (NODA, 1974).

In the present paper, the effectiveness of high temperature for the elimination of the yeastlike symbiotes was investigated. In the first place, the effects of high temperature on the development of *L. striatellus* and on the symbiote population were studied. Other host activities affected by high temperature were also explored.

## MATERIALS AND METHODS

*Rearing conditions.* *Laodelphax striatellus* (FALLÉN) was reared on rice seedlings in a glass bottle (ca. 17 cm in height, 9 cm in diameter) under a constant condition of 25°C and a 16L:8D photoperiod for stock culture. Newly hatched nymphs were taken from the stock culture and were individually moved into glass tubes (ca. 104 mm in length ,10 mm in diameter) to allow sucking a rice seedling of a germinating stage. The rice seedlings were changed every five days. Insects were also reared in a group in the glass bottle. They were placed in a constant temperature box subjected to a 16L photoperiod. Insects were reared in the following four different thermal conditions; (A) at 25°C ,(B) at 35°C, (C) at 35°C for the first 3 days and at 25°C for the rest of the host's life, (D) at 35°C for 3 days from 6 day to 9 day after hatching (approximately corresponding to 3rd instar stage) and at 25°C for the rest of the host's life.

In the present experiments carried out on *L. striatellus*, the words "high temperature" and "normal temperature" refer to 35°C and 25°C, and the signs (A), (B), (C) and (D), indicate the thermal conditions mentioned above, respectively. The term "heat treatment" means the exposure to 35°C for the first 3 days after hatching.

*Counting method of the yeastlike symbiotes.* The number of the yeastlike symbiotes were calculated by means of a haemacytometer (NODA, 1974).

*Microscopy.* Fresh insects were fixed in Bodian II mixture or Bouin's mixture, and dehydrated in butanol. They were embedded in paraffin and were cut at 5 to 7 μm. The sections were stained with Mayer's Haematoxylin-eosin or PAS reaction (NODA, 1977).

For electron microscopy, fresh insects were fixed in 2.67% $OsO_4$ for 2 hr and post-fixed in 6% glutaraldehyde for 1 hr in 0.067 M s-collidine buffer (BENNETT and LUFT, 1959). They were dehydrated in ethanol series and embedded in Epon 812 (COULTER, 1967). Ultrathin sections were made by Porter MT-1 ultramicrotome with a glass knife. The sections were stained with uranyl acetate and lead citrate and examined by Hitach HU-12 electron microscope.

*Oviposition.* The number of eggs produced by two groups of females reared under different thermal conditions, (A) and (D), were counted. The brachypterous female adults of both groups were respectively reared for 5 days with normal male adults.

They were then transferred to the glass tube containing a rice sheath. The number of eggs laid in the sheath for 24 hr were counted under a binocular.

*Honeydew excretion.* The amounts of honeydew deposited by the normal (A) and the heat-treated (C) 5th-instar nymphs were compared according to SŌGAWA's method (1970) for *Nilaparvata lugens*. The 15 nymphs were introduced into a plastic cup (ca. 37 mm in height, 47–65 mm in diameter) to allow them to suck a rice plant which was grown in a cup containing soil. A filter paper was put below their sucking site so that it absorbed the honeydew droplets discharged by insects for 24 hr. The filter paper was then dipped into acetone solution of ninhydrin and dried in a heat drier. The honeydew droplets become visible by ninhydrin owing to the amino acids in them.

## RESULTS

*Development under high temperature*

Preliminarily, 2nd-instar nymphs were reared at four different temperatures, 25, 30, 35 and 40 °C. The nymphs normally grew up to adults at 25°C and 30°C, showing no marked difference in growth. At 35°C the growth of the insects was delayed and the number of the yeastlike symbiotes was reduced strongly. Many insects died at 40°C. From the above preliminary observations, two temperatures were selected for further experiments; 25°C for normal temperature and 35°C for high temperature.

At the normal temperature the insects metamorphosed into adults in about 13 days after hatching (Fig. 1A). Under the continuous high temperature, some 3rd-instar nymphs did not proceed to the next instar. No adults, but nymphs staying at the 3rd, 4th or 5th instar, could be observed in 24 days after hatching (Fig. 1B). The growth curves of 3rd to 4th and 4th to 5th instar showed gentle slopes indicating that the intervals between the moults were long and the ecdyses occurred sparsely. The mortality was high and some insects died during ecdysis. The nymphs raised under continuous high temperature were small in size and especially had a smaller abdomen. The skin colour was yellowish and whitish, and no nymph had dark pigments in its skin.

Since rearing under the continuous high temperature seemed to be a considerably harsh condition for the insects, the exposure to the high temperature was limited to the first 3 days after hatching. The development of the heat-treated insects showed poor synchronization and the intervals between two moults in later stages were longer than those of the normal insects (Fig. 1C). In 25 days after hatching, 20% of the insects became adults and some insects still remained in the 5th-instar stage. In this rearing no nymphs was alive by the 29th day and 21.5% of adults emerged. The heat-treated nymphs had the smaller abdomen and the pale yellow skin. The treated 5th-instar nymphs appeared to show a slow growth of the wing disks. The 5th instar nymphs which lived for longer periods had rather a big abdomen. But if they became adults, the female abdomen was small and the ventral part did not swell even at the oviposition period. Some insects died during metamorphosis, e.g. shedding the half of their old cuticle or the cuticle of their legs and abdominal tip (Fig. 2).

The 3-day exposure which was applied to the 6-day-old nymphs delayed their adult emergence slightly (Fig. 1D). All live insects developed into adults, though some resulting adults had a little smaller abdomen and a slightly yellowish skin.

Table 1 shows the number of individuals which proceeded to the next stage: from 3rd to 4th, from 4th to 5th and from 5th instar nymph to the adult. In the normal in-

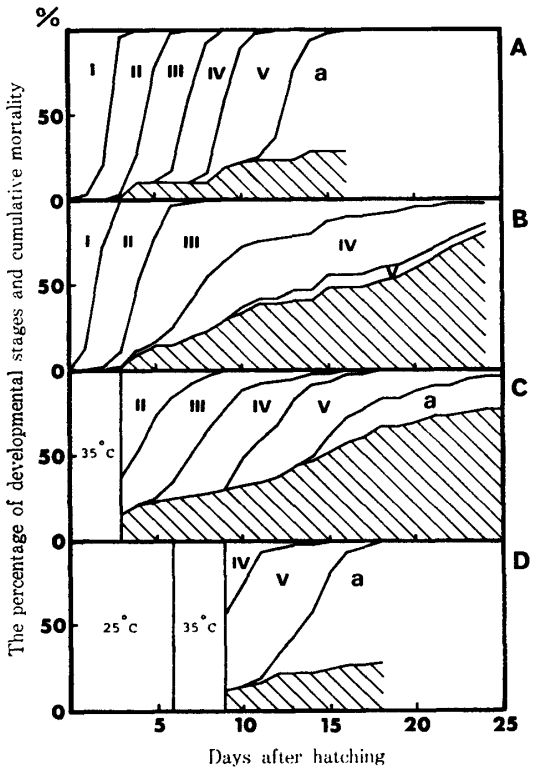



Fig. 1. Growth curve and mortality under different thermal conditions. I, II, III, IV, V and *a* show the respective nymphal instars and adult. Mortality is shown in oblique lines. In A and B, the dead insects on the day of outset of rearing are excluded. A, 25°C; B, 35°C; C, 35°C for the first three days after hatching and 25°A for the rest of the life; D, 35°C from 6 to 9 day and 25°C for the rest of the life.

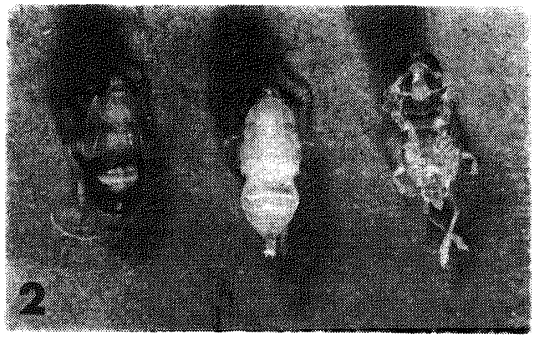



Fig. 2. Abnormal adult moult of the heat-treated 5th instar nymphs. Insects were exposed to high temperature for first 3 days after hatching. Left, there is black colouration under the nymphal cuticle; Centre, the legs and the abdominal tip are shed but the cuticle is not split; Right, the insect is dead halfway through ecdysis.

sects (A), more than 85% of insects in each instar moulted to the next stage. Under the continuous high temperature (B), only 13.9% of 4th-instar nymphs could proceed to the 5th-instar stage. In the heat treatment (C), the insects were not much affected by the high temperature in the development from 3rd to 4th and from 4th to 5th instar.

Table 1. THE RATE OF DEVELOPMENT UNDER DIFFERENT THERMAL CONDITIONS[a]

| Thermal conditions[b] | No. of insects examined | 3rd-4th | 4th-5th | 5th-adult |
|---|---|---|---|---|
| A | 60 | 53/54 (98.1) | 46/53 (86.8) | 43/46 (93.5) |
| B | 63 | 36/53 (67.9)[c] | 5/36 (13.9)[c] | 0/ 5 ( 0.0)[c] |
| C | 65 | 44/47 (93.6) | 36/44 (81.8) | 13/36 (36.1)[c] |
| D | 76 | | | 54/62 (87.1) |

[a] Calculated from the rearings of Fig. 1. The number of insects that proceeded to the next stage against the total number of the insects in each stage. The number in parenthesis shows the percentage.

[b] Shown in the legend in Fig. 1.

[c] Some of the remaining insects that did not proceed to the next stage were dead.

However, only 36.1% of the 5th-instar nymphs could metamorphose while the rest of the insects were dead or remained in the 5th-instar stage. This indicates that the heat treatment exercises an influence on the adult emergence.

*Adult moult in the heat-treated insects*

As the heat-treated 5th-instar insects showed low rate of adult emergence and some of them died during ecdysis, figures were collected for time of emergence or time of death after final nymphal ecdysis. The normal 5th-instar nymphs ecdysed from 3 to 5 days after final nymphal ecdysis, on an average 3.65±0.08 day (Fig. 3). On the other hand, heat-treated 5th-instar nymphs became adults sparsely and longer period

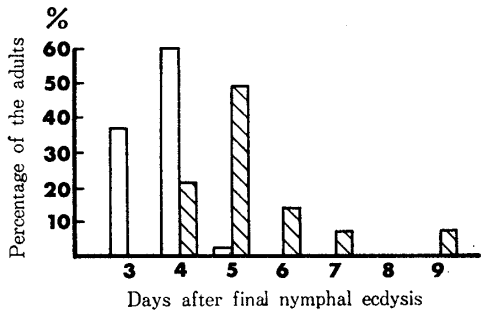



Fig. 3. The duration of the 5th instar in the heat-treated adults. This is calculated from the rearing of Fig. 1. White column, normal insects; obilique line, heat-treated insects. The adult in 9th day after final nymphal ecdysis emerged 27 day after hatching.

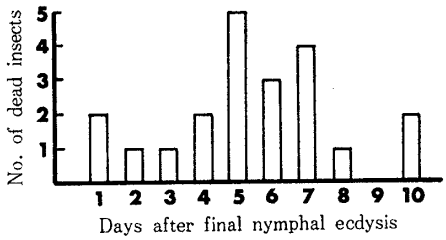



Fig. 4. The duration of the heat-treated 5th instar nymphs from the final nymphal ecdysis to the death. This is calculated from the rearing of Fig. 1C.

Table 2. Adult Cuticle Deposition of the Heat-Treated 5th Instar Nymphs

| Stages | No. of insects examined | Non-deposition | Deposition[a] |
|---|---|---|---|
| 1 Day after final nymphal ecdysis | 6 | 6 | |
| 3 Day | 10 | 10 | |
| 5 Day | 10 | 5 | 5 |
| 7 Day | 10 | 4 | 6 |
| 9 Day | 9 | 6 | 3 |
| More than 35 day after hatching | 10 | 10 | |

[a] The insects in which the new cuticle was deposited in a small part of the body are also treated as deposited ones.

was needed to moult, on an average 5.36±0.34 day (Fig. 3).

The heat-treated 5th-instar nymphs were largely dead in 5 to 7 days (Fig. 4). The death of the nymphs was observed in large quantity during the adult emergence, indicating that there is a relation between the adult moult and death in the heat-treated 5th-instar nymphs.

Therefore, the cuticle of the heat-treated 5th-instar nymphs reared in the glass bottle in a group was examined in paraffin sections. The deposition of adult cuticle was checked. The relation between the day after final nymphal ecdysis and the adult cuticle deposition is shown in Table 2. In 1-day-old heat-treated 5th-instar

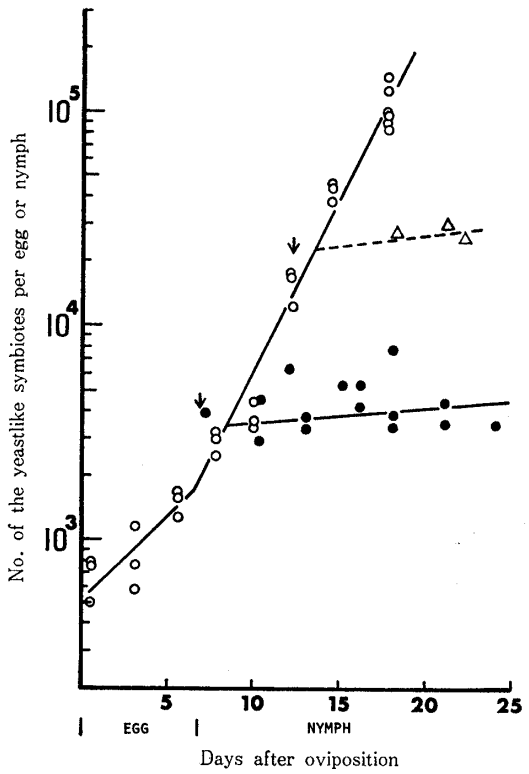
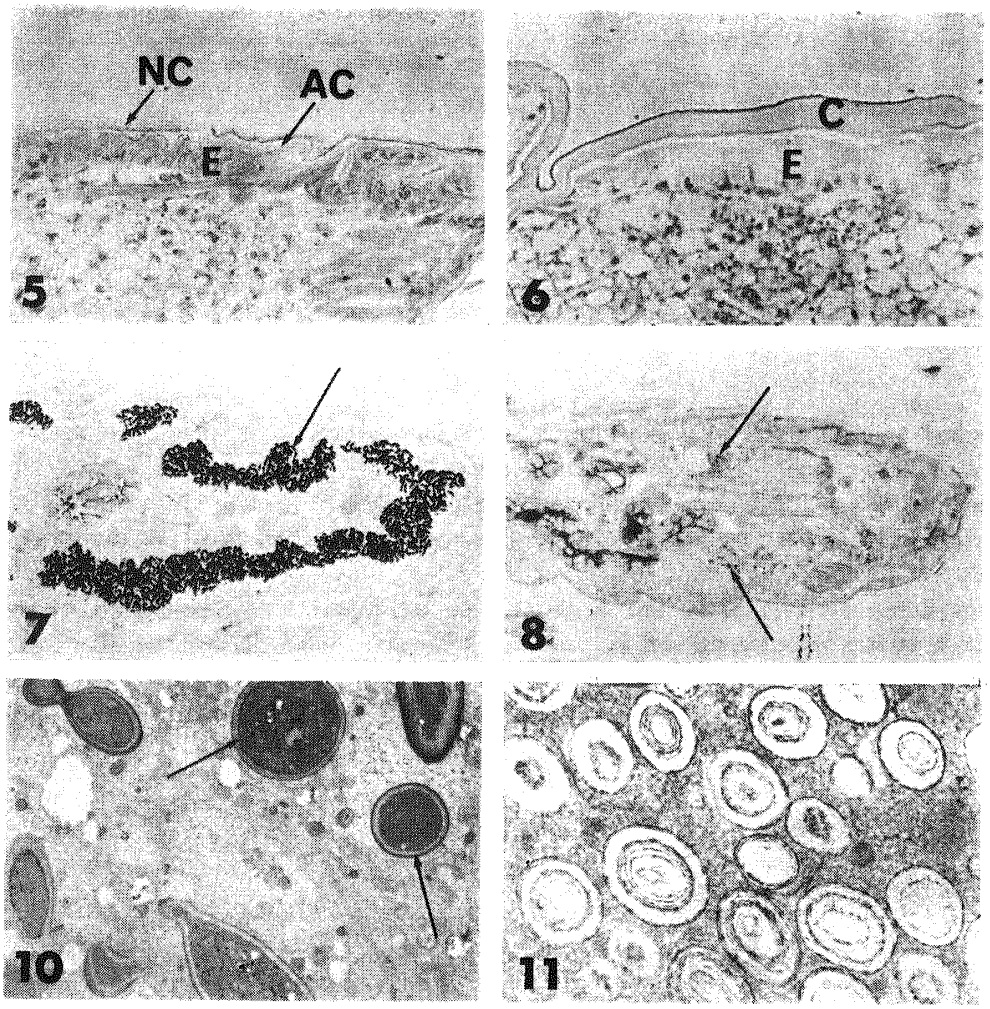



Fig. 9. The number of yeastlike symbiotes after heat treatment. The high temperature was given insects for 3 days at the point of an arrow. ○, normal insects, plotted from the data of Noda (1974); ●, heat-treated insects; △, high-temperature-exposed insects.




Figt 5. Cuticles and epidermal cells of the 7-day-old heat-treated 5th instar nymph. AC, adult cuticle; E, epidermal cells; NC, nymphal cuticle.

Fig. 6. Cuticle (C) and epidermal cells (E) of a heat-treated nymph more than 35 days of age.

Fig. 7. Abdominal vertical section of the nomal 5th-instar nymph. (stained by PAS reaction; arrow, yeastlike symbiotes)

Fig. 8. Abdominal vertical section of the heat-treated 5th-instar nymph. (stained by PAS reaction; arrow, yeastlike symbiotes)

Fig. 10. Electron micrograph of mycetocytes of the normal insects. (arrow, yeastlike symbiotes)

Fig. 11. The residual body of the yeastlike symbiotes in the mycetocytes of heat-treated 2nd-instar nymph.

nymphs, apolysis was never found and the nymphal cuticle itself was thin. Three-day-old nymphs did not show the deposition of the new cuticle. In 5-day-old 5th-instar nymphs, some formed new cuticle mainly in their legs. In the 7th day after final nymphal ecdysis, 6 insects out of 10 deposited new cuticle in some parts of the body or the whole body (Fig. 5). The deposition of adult cuticle was often observed in the legs even though not in the other part. The heat-treated nymphs that were

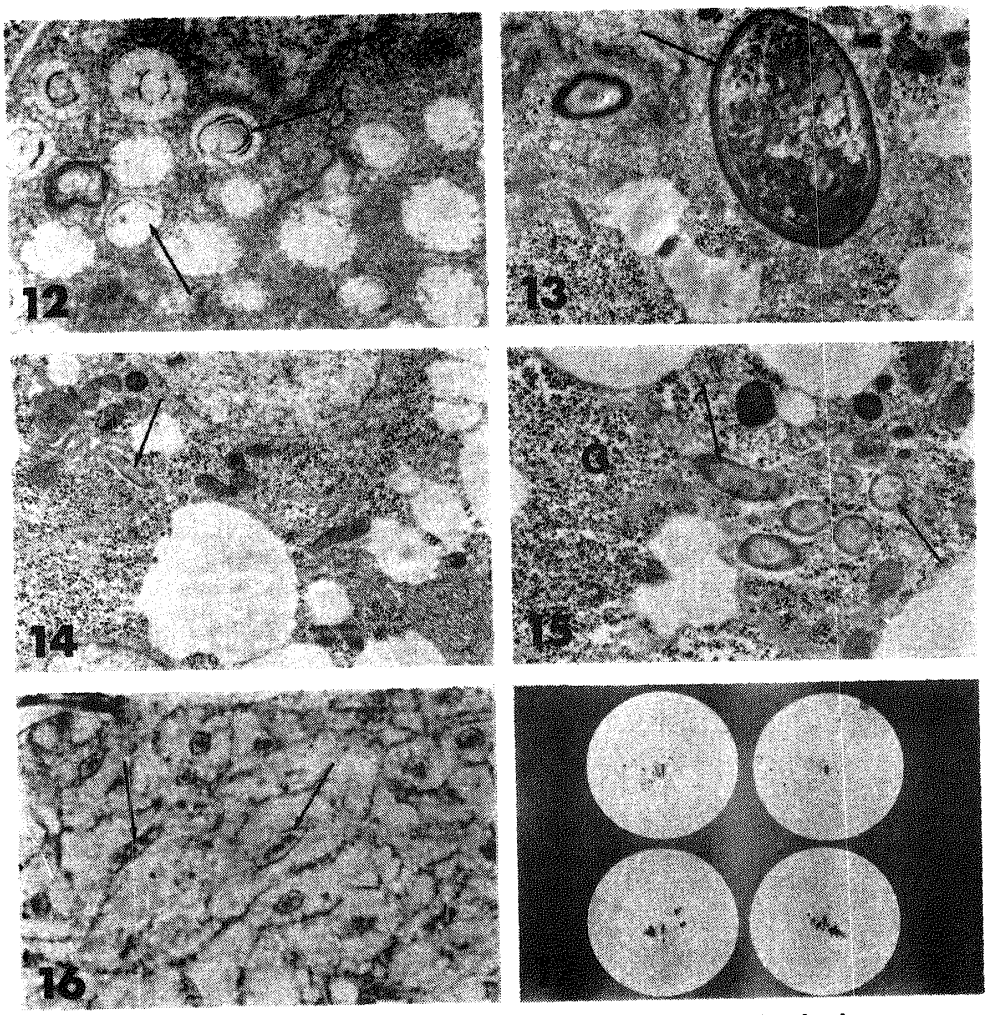



Fig. 12. Almost completely destroyed yeastlike symbiotes (arrow) by the heat treatment.
Fig. 13. Electron micrograph of the mycetocytes of the heat-treated 2nd instar nymph. (arrow, yeastlike symbiote)
Fig. 14. Electron micrograph of the mycetocytes of the heat-treated insect. (arrow, bacterium)
Fig. 15. Bacteria (arrow) in the mycetocyte of the heat-treated insect. (G, glycogen)
Fig. 16. Abnormal nuclei of the mycetocytes in the heat-treated 5th instar nymph.
Fig. 17. Honeydew excreted by the normal (above) and the heat-treated (below) 5th-instar nymphs. 15 insects excreted for 24 hr.

more than 35 days old did not form the adult cuticle, although their epidermal cells were thick (Fig. 6).

*Effect of heat treatment on symbiotes and mycetocytes*

The normal *L. striatellus* harbours the yeastlike symbiotes in the mycetocytes in the fat body (Fig. 7), and the number of the symbiotes increases during nymphal stages of the host (NODA, 1974). The heat-treated 5th-instar nymphs possessed only a few symbiotes (Fig. 8). The number of symbiotes per insect after heat treatment was calculated (Fig. 9). It was found that the symbiotes had increased only slightly

after exposure. The treated 5th-instar nymphs usually possessed less than 5,000 symbiotes on an average; approximately 1/20 of those in the normal 5th-instar nymphs. Thus, high temperature treatment applied for 3 days to the nymph in the earliest stage reduced greatly the population of the yeastlike symbiotes.

Electron microscopical observations of the normal insects revealed that the yeastlike symbiotes were usually electron dense and had a thick cell wall, surrounded by a membranous envelope (Fig. 10). In the heat-treated nymphs, many dead symbiotes were observed (Fig. 11). These residual bodies contained whorled structures. Some were completely destroyed (Fig. 12). Some symbiotes had a cell wall, but their cytoplasm was partly transparent and contained small vesicles (Fig. 13). Even the yeastlike symbiotes showing a normal outer shape seemed to be injured physiologically.

The normal *L. striatellus* also harbours bacteria. They were found both in the intercellular space and in the mycetocytes. The heat-treated insects also possessed these prokaryotic microorganisms (Fig. 14, 15). No marked influence of the heat treatment was observed to these bacteria, except for a few of them which were surrounded by membranous structures in the mycetocyte.

The mycetocytes of the heat-treated insects showed a few differences from the normal ones. In the heat-treated mycetocytes, glycogen granules were rich and seen in clumps (Fig. 14, 15). A light microscopical observation of the heat-treated insects also showed that some mycetocytes contained elongated nuclei (Fig. 16).

*Feeding and oviposition*

In order to explore whether the previous exposure to the high temperature affects feeding of the insects, the honeydew excretion of the heat-treated 5th-instar nymphs was compared with that of the normal nymphs. The honeydews discharged by 15 nymphs in 24 hr are shown in Fig. 17. There was no large difference in quantity between honeydews deposited by the two groups. This indicates that the heat-treated insects suck the rice plant juice sufficiently and their feeding was not reduced by the previous heat treatment.

To investigate whether the fecundity is affected by the previous high temperature, the number of eggs laid in 24 hr by the female adults were examined. The high

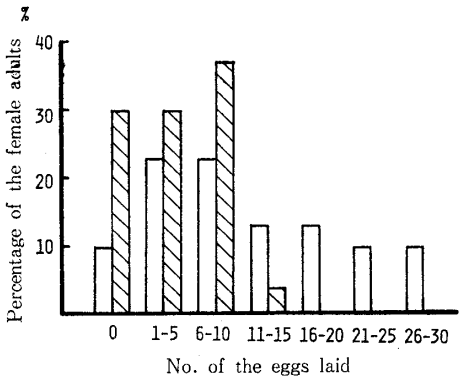



Fig. 18. The number of egg laid by the normal (white column) and the high-temperature-exposed (oblique line) brachypterous female adults. The high temperature was given from 6 to 9 day after hatching.

temperature was exposed to the 6-day-old nymphs for 3 days, and the resultant female adults were used. A normal brachypterous female adult laid 11.7 eggs in a day on an average, and the number of eggs ranged from 0 to 29 (Fig. 18). On the other hand, a high-temperature-treated brachypterous female adult laid 4.5 eggs in a day on an average, and the number of eggs ranged from 0 to 11. When the insects that laid no eggs were excepted, the average numbers of eggs laid by the high-temperature-treated and the non-treated insects were 6.7 and 13.7, respectively. These results show that the high temperature reduced the fecundity of the resultant female adults.

## DISCUSSION

High temperature has been used by others to produce aposymbiotic insects (GLASER, 1946; BROOKS and RICHARDS, 1955; HUGER, 1956). In the bed bug, *Cimex lectularius*, the mycetomes were almost free of symbiotes after 2 weeks at 35°C (CHANG, 1974). In these experiments, rather higher temperature or longer exposure time were employed in comparison with those of the present study. The symbiotes studied by the above authors are prokaryotes, and while *L. striatellus* also has bacteria both in mycetocytes and in intercellular spaces between mycetocytes. These bacteria were not much affected by the heat treatment. It was the yeastlike symbiotes in the mycetocytes that were largely destroyed by the heat. More severe temperature treatment showed unfavorable direct effects on the host insects. If the host-symbiote relationship is finely integrated, complete elimination of symbiotes may result in host death.

After 3 days at 35°C, many insects failed to become adults, remaining in the 5th-instar or dying. This seemed to result from failure of the adult cuticle to form normally. Heat-treated 5th-instar nymphs moulted in 4 to 7 days after the previous nymphal ecdysis, and most deaths occurred on the 4th to 8th days. In these experiments death appeared to be related to adult emergence. The heat-treated 5th-instar nymphs formed adult cuticle slowly. Nymphs surviving more than 35 days could not form adult cuticle, which suggests that the longevity may be a consequence of failure to elaborate new cuticle. The heat-treated 5th-instar nymphs could be grouped into three categories: those moulted successfully to adults, those died during ecdysis, and those lived a rather long time without deposition of adult cuticle.

Besides this deleterious effect, the heat treatment reduced the number of yeastlike symbiotes to 1/20 of that in the normal 5th instar. The fecundity was also poor in the females exposed to the high temperature. The heat-treated insects seemed to take up sufficient rice plant sap because they excreted a normal quantity of honeydew. Thus heat did not cause inadequate intake of nutrition but rather a certain physiological change in the insects. WIGGLESWORTH (1952) demonstrated that the moult of *Rhodnius prolixus* was completely prevented at 36°C and half of the 4th-instar nymphs could not moult at 35°C. Subsequently this phenomenon was fully investigated by OKASHA (1968a, b, c). Although the cessation of moulting in *R. prolixus* could be reversed by replacing the insects at normal temperature, this was not the case in *L. striatellus*. Thus the effect of heat appears not to be the same in these two species. The harmful effect on the adult moult in the planthopper came out long after heat treatment. This seems to indicate that the high temperature did not act directly on the insect physiology but rather indirectly through the reduction of the yeastlike

symbiotes. In other words, the heat appears to cause the above harmful effects, destroying the close relationship between host and symbiotes. Generally elimination of symbiotes reduces offspring production (BROOKS and RICHARDS, 1955; EHRHARDT, 1966). The yeastlike symbiotes in *L. striatellus* may make an important contribution to the host emergence or oviposition through providing with uncertain substances, which are indispensable to this planthopper. Heat-treated *L. striatellus* with a reduced population of yeastlike symbiotes might be useful for further study on the role of the symbiotes.

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