

evaluation of delayed effect of food shortage, however, has remained to be solved. Field and/or laboratory experiments which are designed to assess the delayed effect of food shortage should be conducted in the future.

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### Preliminary Histological Observation and Population Dynamics of Intracellular Yeast-Like Symbiotes in the Smaller Brown Planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae)<sup>1</sup>

Many insect species possess internal flora of microorganisms including bacteria, yeasts, etc. These specialized microorganisms are commonly referred to as symbiotes, and have long been considered to be closely related with the host physiology (RICHARDS and BROOKS, 1958; KOCH, 1960; BUCHNER, 1965).

The present paper reports the histological observations and the population changes of yeast-like symbiotes in a life cycle of the smaller brown planthopper, *Laodelphax striatellus* (FALLÉN).

Insects were reared on rice seedlings under a constant condition of 25°C and 16 hr daily illumination. For histological preparations, whole insects were fixed in BOUIN's solution, washed in 90% ethanol, and dehydrated in butanol. After embedded in paraffin wax, they were sectioned at 6 μm. The sections were stained with MAYER's hematoxylin-eosin, gentian violet, or GIEMSA buffered at pH 5 with citrate buffer. For counting the yeast-like symbiotes at each stage, insects or eggs were homogenized gently with 0.8% saline solution. Using a THOMA's hemacytometer, the number of symbiotes was calculated by

the following formula:

$$\text{Total No. symbiotes} = a(x+m)/n \cdot v$$

(per insect or egg)

a : number of symbiotes in v.

m : total weight of insects homogenized.

n : number of insects or eggs homogenized.

v : volume of the insect homogenate sampled in the hemacytometer.

x : volume of the saline solution used to homogenize insects or eggs.

As x is much larger than m, m may be neglected in eggs and 1st instar nymphs.

The yeast-like symbiotes were found in the fat body of the abdomen, but not in the other portions of insect body. They were elongated oval-shaped, and 13 μm length on an average. In the nymphs, the symbiotes were scattered in groups over the fat body. In the female adult, most of the fat body was occasionally occupied by the symbiotes (Fig. 1A). The symbiotes were also observed in the eggs.

The change of numbers of the symbiotes at successive stage of host is shown in Fig. 2. The total number of symbiotes increased steadily with development of the host. However, the number of symbiotes per μg of fresh weight of nymph was mostly constant (about 140-170/μg) during nymphal development. In the 5th instar stage, the female nymphs possessed more symbiotes than the male nymphs. After emergence, the symbiotes declined rapidly in numbers in the male adults

<sup>1</sup> *Appl. Ent. Zool.* **9** (4): 275-277 (1974).

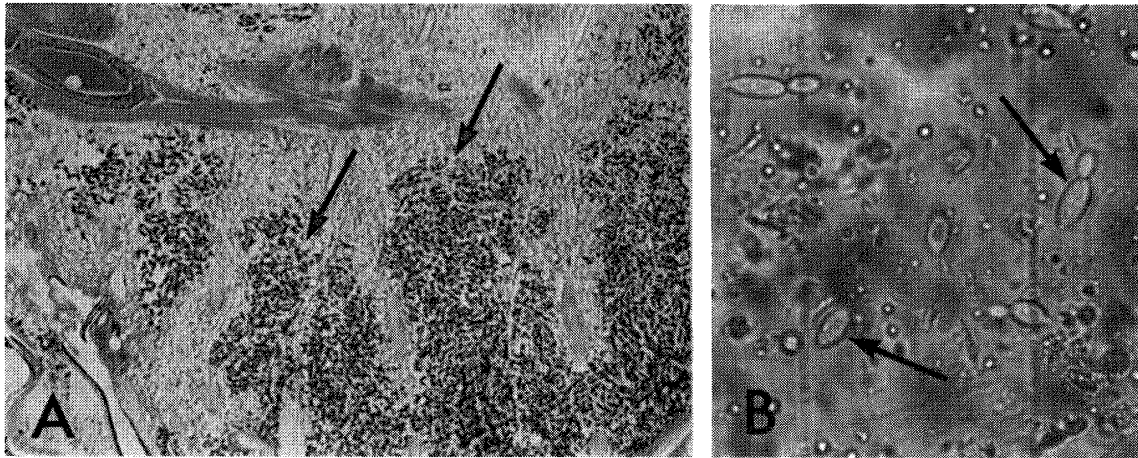


Fig. 1. Micrographs of the yeast-like symbiotes (arrows) in the smaller brown planthopper. A, abdominal section of the brachypterous female; B, symbiotes in the homogenate of the saline solution in the hemacytometer.

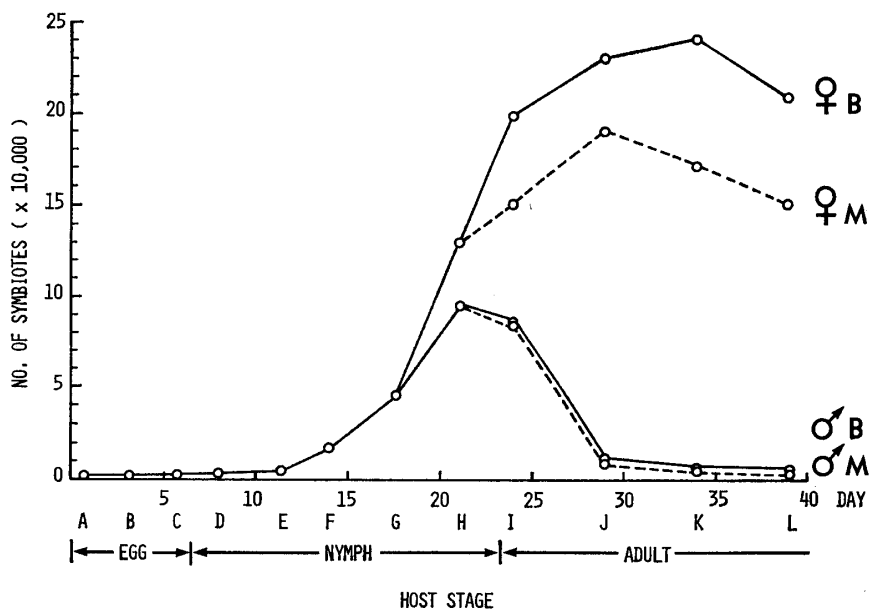


Fig. 2. Population changes of the yeast-like symbiotes during a life cycle of the smaller brown planthopper. A, B, C, eggs of 12-24, 72-84, 130-142 hr after oviposition, respectively; D, E, F, G, H, nymphs of 1st, 2nd, 3rd, 4th, 5th instar, respectively; I, J, K, L, adults of 0-1, 5-6, 10-11, 15-16 days after emergence, respectively; ♀ B, brachypterous female; ♀ M, macropterous female; ♂ B, brachypterous male; ♂ M, macropterous male.

of both brachypterous and macropterous forms. On the other hand, in the female adult their number continuously increased after emergence, and attained a maximum in the early stage of ovipositional period: in this stage 240,000 symbiotes were recorded in one brachypterous female. Afterward, they gradually decreased. The population of symbiotes in the brachypterous female was

maintained at a higher level than in the macropterous female. The histological sections disclosed that a lot of symbiotes aggregated around the ovary of the mature female. These facts seem to be associated with the transovarial passage of the symbiotes (NASU, 1963).

In view of the evidence that population density of symbiotes is maintained at a definite level

during nymphal stages, the mechanisms to regulate the symbiotes propagation seem to exist, as mentioned by BUCHNER (1965) and HINDE (1971). As has been indicated by EHRHARDT (1966), it seems reasonable to assume that the higher population level of symbiotes in the female adults than in the male ones is connected with the egg production. Moreover, the rapid decline of symbiotes population in the male adults indicates an involvement of a certain factor to destroy the balanced relationship between host and symbiotes.

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### The Multiplication of Western X Mycoplasma-like Organism in the Brain of a Leafhopper Vector, *Colladonus montanus* (Homoptera : Cicadellidae)

Lesions are observable in the brain of infectious leafhoppers, *Colladonus montanus*, that transmit Western X-disease (WHITCOMB et al., 1967). The lesions are masses of the Western X mycoplasma-like (WXM) organisms (NASU et al., 1970), which appear to multiply in the brain of *C. montanus* at an unusually high rate in comparison to other tissues. The multiplication of WXM in the brain of *C. montanus* has, therefore, been studied more closely.<sup>2</sup>

An extract from infected leafhoppers was injected into healthy nymphs of *C. montanus*. The nymphs were then transferred to healthy celery to serve as an experimental stock of insects for a 40-day testing period. To follow the development of the disease after injection, five leafhoppers were

sacrificed at intervals of two or three days. The ganglia (i.e., the brain, suboesophageal ganglion, thoracic ganglion, and abdominal ganglia) were dissected and fixed for 30 min at 4°C in 1% osmium tetroxide in veronal acetate buffer at pH 6.8. The fixed specimens were then dehydrated through a graded ethanol-propylene oxide series and embedded in SPURR low-viscosity medium (SPURR, 1969). Thin sections were cut on a Porter-Blum MT-2 ultramicrotome using glass knives. The sections were stained with saturated uranyl acetate solution for 60 min and observed with a Hitachi HU-12 electron microscope.

The following phenomena were observed in thin sections of injected leafhopper brains. Seventeen days after injection, large spherical electron transparent bodies of WXM were first observed in the cytoplasm of the glial cells of the brain. By the 23rd day, the bodies had increased in number, and small numbers of similar bodies were detected in the suboesophageal gang-

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<sup>2</sup> The leafhopper injection, sampling, and fixation was done at the University of California, Berkeley.