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Regulation of Yeast-Like Endosymbiotes in the Rice Brown Planthopper *Nilaparvata lugens* Stål (O: Homoptera, F: Delphacidae)

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Abstract

The Delphacid *N. lugens*, widely known as rice brown plant-hopper (BPH) is the most damaging pest on rice. A bacterium and yeast (*Candida* sp.) are associated with this insect as endosymbiotes. We studied the yeast population in the host insect from egg to adult stage using histochemistry and light microscopy. The position and numbers of symbiotes were studied. The initial inoculum of the yeast in the egg multiplied 10-fold by the end of the embryonic development. During the post-embryonic development, the build-up was gradual in the nymphal instar. The symbiotic load was reduced during the summer months. Further, it was observed that in the presence of entomophagous parasitoids such as *Elenchus* sp. and Dryniids, in adult BPH the number of yeast came down drastically and much of the host cells previously occupied by the symbiotes became symbiote-free. In eggs, the symbiotes disappeared completely in the event of parasitisation by *Anargus optabilis*; an egg parasitoid of BPH eggs.

Keywords: yeast endosymbiotes, BPH, build-up, temperature, parasitism-influence

Abbreviations: BPH: Brown plant-hopper, LS: Longitudinal Section, YLO: yeast-like organisms

1. Introduction

Both bacteria and fungi as endosymbiotic partners in insect hosts have been the objects of interest to many biologists for quite some time (Korner, 1969; Schwemmler, 1980). The rice brown planthopper (BPH), *Nilaparvata lugens* Stål, one of the major pests on rice, is known to carry an endosymbiotic yeast which has been implicated with host insect nutrition (Nasu et al., 1981), and its embryonic development (Lee and Hou, 1987; Chen et al., 1981, and Sogawa, 1982). Implications such as the above mean the host insect to have the command over regulating the activities of the symbiotes, particularly their population-load in the host system. It is important to note that these symbiotes are prone to numerical change as per the physiological needs of the host insect that harbours them.

The present paper deals with such population changes when the host physiology is influenced by known biotic and abiotic factors, such as parasitoids, host-food, prevailing temperature and humidity.

2. Materials and Methods

To assess the initial symbiotic inoculum in the eggs of BPH, the primary oocytes located above the pedicel of each ovariole with distinctly formed symbiotic ball (SB) at the posterior pole were carefully removed from the gravid females. Placing a coverglass gently over the separated SB, a small quantity of saline was added along the edges of the coverglass. When sufficient quantity of saline was flooded by capillary action, the SB was ruptured by applying gentle pressure over the coverglass. Counts of yeast-like organisms (YLO) were taken in the entire area of the coverglass using a binocular microscope at $\times 100$ magnification.

During the embryonic development of the egg, the multiplication of symbiotes in the eggs was studied at various stages that could be distinguished by means of visible developmental characters, as shown by Nasu and Suenaga (1958).

The multiplication of symbiotes during post embryonic development of BPH was investigated by the method of Noda (1974). The influence of the following factors on the host regulation of symbiotic load was studied.

1. Fifth instar males/females from field populations during the period April 1986 to May 1987 were used to estimate seasonal fluctuations in the symbiotic populations.

2. BPH eggs parasitised by *Anargus optabilis* Perkins were examined for symbiotic load as against that present in normal eggs. The number of YLOs was counted in individual eggs by thoroughly squashing them with a drop of saline.
3. Similarly, adult BPH parasitised by a drynid, *Haplogonatopus orientalis*, a Trombidiid mite and an undetermined species of *Elenchus* (F: Strepsiptera) were examined for symbiotic load as compared with healthy insects.
4. The symbiotes were also estimated BPH females with multiple parasites such as drynid and strepsipterons.

3. Statistical Methods Used

For analysing change in number of symbiotes during embryonic and post-embryonic development of BPH, the data were analysed using the available computer programme for polynomial regression.

4. Results

Multiplication of YLOs during embryonic development of the host insect

The initial YLO-inoculum housed in the symbiotic ball (SB) measuring a mean of 69.6 μm in diameter in the restricted primary oocyte was 201.2/egg, increasing to 327.73 in an hour-old egg. The size of SB itself increased to 82.8 μm. This increase was maintained and the number at blastokinensis was 1102.27/egg. Further observation during the three phases of egg-pigmentation stage also showed a gradual increase in symbiotic population (Tables 1&2).

Tables 2 to 4 show the build-up of YLOs during the post-embryonic development of BPH. There was an increase in symbiotic population as it grew through 1st, 2nd, 3rd and 4th instar nymphal stages. The polynomial regression worked out for the nymphs, males and females separately, showed the existence of a significant relationship between insect age and YLO density, indicating a progressive increase in YLO number with the development of the host. A gradual increase in the symbiotic load was observed in adult females during the preovipositional period while the density in the males was noted to be rapidly decreasing from the day of their emergence. The females built up the yeast population to its maximum within 20 to 22 days after their emergence. The post-ovipositional period among the older females showed considerable decrease in YLO.

Table 1. Change in symbiote number during embryonic development at BPH

Development stage of egg	Hours after egg laying	Mean* YLO/egg
Initial inoculum in undeposited ovum	0.0	201.00 (14.178)
Blastoderm stage	2.0	328.00 (18.082)
	14.0	429.00 (20.692
Germband stage	26.0	589.00 (25.073)
	65.0	753.00 (27.488)
Blastokinensis stage	85.0	1101.00 (33.295)
Eye pigmentation stage	112.5	1207.00 (34.637)
	137.5	1688.00 (40.990)
	157.5	2288.00 (47.690)

* Mean values of 15 observations
Figures in parentheses are means of square root transformed values ($\bar{y}$)
Coefficient of determination (R^2) = 0.995
Standard error of estimate (SEE) = 0.6959

Explanation – Statistics

Polynomial regression worked out at polynomial of degree 1 was found to be sufficient, which results in the following equation.

$Y = 17.1326 + 0.1800 \times \pm$ standard error of estimate (SEE), 0.6959 and the coefficient of determination (R^2) being 0.905, where Y = symbiote density and X = time in hours.

Before using polynomial regression analysis, the stability of the variances for each developmental stage of the insect was examined. Since the variations were found to be unstable, the y values were transformed by the square root transformations. After the transformation, the variances were found to be stable. Hence, polynomial regression analysis was carried out for the $\bar{y}$ value against the x values instead of y against x. Similar procedure was adopted for data given in Tables 2&3.

Table 2. Changes in symbiote number during the post-embryonic development of BPH (upto fourth instar nymphal stage)

Age of the insect (in days)	Mean* symbiote number $\times 10^2$ per insect
0.0	0.363 (59.953)
2.0	0.558 (74.656)
4.0	1.335 (115.640)
6.0	3.045 (174.760)
8.0	5.529 (234.992)
10.0	7.703 (277.499)
12.0	10.378 (322.140)

* Mean of 15 replications
Figures in parentheses are means of square root transformed values.
Coefficient of determination (RE) = 0.996
Standard error of estimate (SEE) = 5.947

Symbiotic load as influenced by parasitism in host insects

The influence of parasitism of the host insect by four internal parasitoids such as a Mymariid egg-parasitoid, a strepsipteron, a drynid and a Trombidiid mite on the symbiotic load was studied. In the host eggs parasitised with the mymariid, *Anargus optabilis*, the number of YLO decreased with the growth of the parasitoid, starting from 1066.7/egg during the first instar of the parasitoid to a mere 192.6/egg when the parasitoid reached its second instar. During the later stages of parasitism, YLOs disappeared completely.

With regard to the influence of the remaining three parasitoids, either individually or together as multiple parasites, similar reduction in density could be seen (Table 4). As compared with non-parasitised hosts multiparasitised with Dryniids and *Elenchus* together, and those superparasitised by *Elenchus*, showed drastic reduction in symbiotic load.

Histologically, the effect of parasitism on host insects could be observed as much pronounced. The neotenic female *Elenchus* was found to occupy the whole abdomen of the BPH, pushing the ovary and mycetocytes to the periphery of the host abdomen (Figs. 1&2).

Table 3. Changes in symbiote number during post-embryonic development of BPH (In males and females from 5th instar onwards).

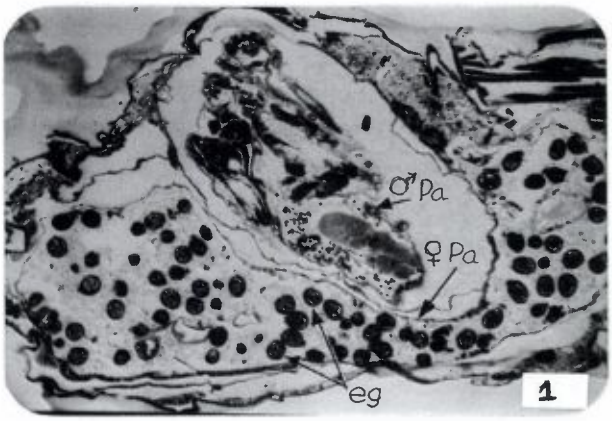
Age of the insect (in days)	Mean* of symbiotes $\times 10^4$/insect	
	males	females
14.0	11.988 (345.990)	19.103 (437.050)
16.0	8.583 (292.930)	23.464 (484.290)
18.0	6.983 (264.230)	35.953 (599.480)
20.0	5.696 (238.320)	54.639 (739.020)
22.0	3.900 (197.450)	52.547 (724.720)
24.0	3.278 (180.950)	47.310 (687.720)
26.0	2.717 (154.620)	40.729 (638.150)
28.0	–	28.971 (541.430)
Coefficient of determination (R^2)	0.998	0.997
Standard error of estimates (SEE)	2.921	5.687

* Mean of 15 replications
Figures in parentheses are means of square root transformed values.

Influence of crop-season on YLO population in the host insect

The pattern of YLO presence in the host insects during April 1986 to May 1987 (Fig. 5) shows drastic reduction in YLOs during the summer months (April to June) with mean temperature 29.2, 32.3 and 32.4°C, respectively. During subsequent months, with fall in temperature, a gradual build-up of symbiotic load could be seen, reaching maximum during November. The multiple regression (Table 5), clearly showed a highly significant negative and positive co-efficient for temperature and relative humidity, respectively.

Similar influence of temperature and RH on the YLO-inoculum was also evident in the eggs of BPH with enhanced symbiotic load during the cooler months of November and December.


♂Pa
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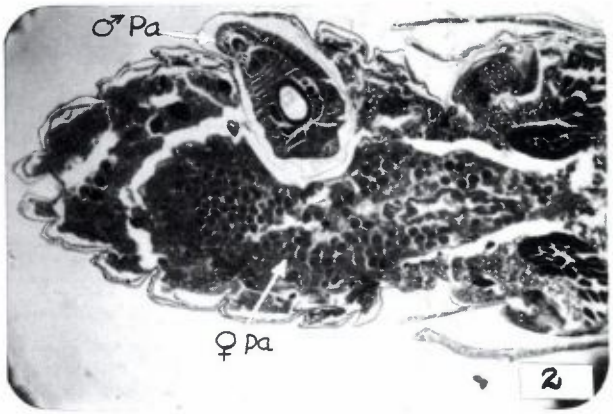
1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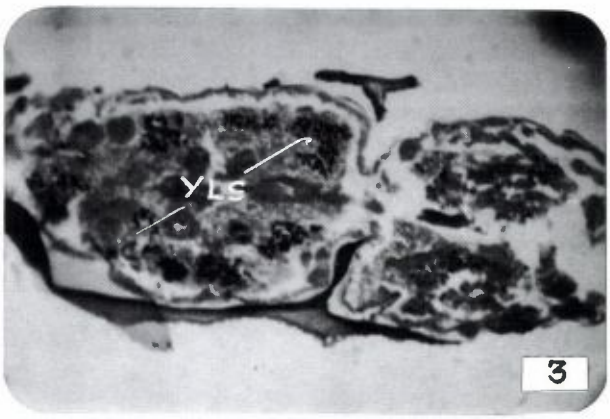
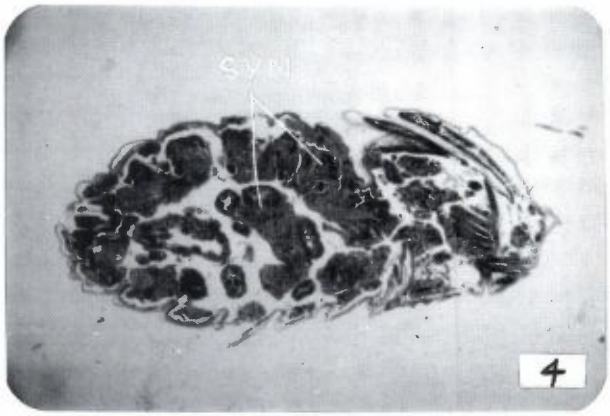

YLs
3



Figures 1–4. (1& 2) L.S. of female BPH infected with *Elenchus* sp.×140
pa = parasite (*Elenchus*)
eg = eggs of *Elenchus*.
(3) L.S. of third instar lymphal BPH×140
YLO = Yeast-like organisms
(4) L.S. of adult BPH showing formation of syncytium fat bodies×70
Syn = syncytium.

5. Discussion

Distribution and multiplication of YLOs through the developmental stages of BPH

The preliminary histological studies indicated that the yeast-like symbiotes were not distributed in the abdominal fat cells of the host insect at random. A disciplined arrangement was evident right from the third instar nymph, indicating a high level of perfection in the distribution in BPH (Figs. 3&4). A larger distribution of symbiotes closer to the region of ovaries evidently facilitates transmission of symbiotes.

Nasu and Suenaga (1958) were the first to provide details on embryonic development of the host in relation to the position of the mycetocyte. Chen et al. (1981) went a step further to determine symbiote population during

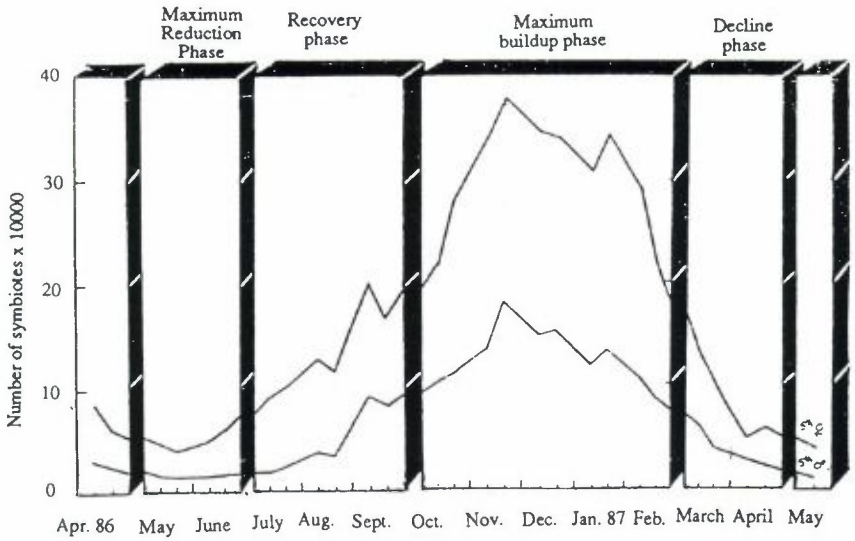


Figure 5. Hypothetical population phases of BPH in Ammamalainagar based on the changes in density of their endosymbiotes.

Table 5. Relationship between symbiote number in female BPH nymphs and seasonal temperature/relative humidity

	Table of coefficients, Standard Errors & 't' values		
Factors	Beta	Standard Error	't' values
Intercept	28.2851	39.6387	0.7136
Temperature (x_4)	−2.5854	0.5990	4.3164**
Relative humidity (x_2)	0.8422	0.3272	2.5737*

Coefficient of determination = 0.8401
Corrected coefficient of determination = 0.8268
$F = 63.0396$; $Y = (28.2851 - 2.5854x_1 + 0.8422x_2) \times 10^4$

embryonic development of BPH at two-day intervals. In the present study, the population was recorded at still shorter intervals of time (2–15 hr) to recognise the behaviour of symbiotes at various stages of egg development. The increase seems to be gradual right from the time of infection. The increase in the size of symbiote ball from 69.6 μm to 82.8 μm further shows the active multiplication of the symbiotes from the early stage of egg development.

The changes in the position of mycetocyte during embryonic development of BPH are so fine and definite that they could be taken as indirect indicators of various developmental stages of the embryo. Compared to data available

on embryonic development of BPH (Nasu and Suenaga, 1958; Mochida and Okada, 1979) the development appears to be rather rapid in the present study. This is perhaps due to the high temperature and humidity to which the host insects were exposed.

The pattern of multiplication of symbiotes during post-embryonic development of BPH was similar to those reported earlier for a related Delphacid, the smaller brown planthopper, *Laodelphax striatellus* (Noda, 1974). The population of symbiotes recorded is almost in corroboration with the results reported from Taiwan by Chen et al. (1981) for the adults of *N. lugens*. The observed difference in the symbiote number in the nymphal stages from that of Chen et al. (1981) could be due to ecological variations existing in the sites of experimentation to which the host insects were exposed. Chen et al. (1981) carried out the experiments at 25±2°C while the present experiments were at 28±2°C. The present observations are in contrast to that observed by Sogawa (1982) who reported a huge number of symbiotes in BPH, 10 times more than the present observation. The cause for such a huge difference is not understood.

As reported by Chen et al. (1981), the symbiotic load was more in newly emerged females than in males and the population increased only in the case of females, understandably for the purpose of transmission to offspring (Noda, 1974).

The details of the mode of infection of the oocytes and the entry of symbiotes in matured embryo of BPH have been reported by Chen et al. (1981). Therefore, the present study did not probe such aspects in detail, except for a brief verification of these results, namely, the mode of infection. Present experimental data generally confirmed their results. Regarding the infection of primary oocytes, Chen et al. (1981) were not sure whether every primary oocyte was infected in BPH. In the present study it was observed that every primary oocyte was infected with YLOs. It appears that in a normal healthy delphacid the transmission of symbiote occurs as a regular uninterrupted event, regulated in such a way that every oocyte gets infected before passing through the pedicel. This proves that the host 'system' has an amazing control over the timing of the event so that no oocyte passes into the oviduct without getting its share of symbiotes. But the factor(s) controlling these events is/are to be identified.

Influence of crop-season on YLOs population

A close examination of the pattern of symbiote numbers in the fifth instar male and female and the percent egg hatch during different months suggest a possible link between the symbiotic number and the abundance of BPH in this

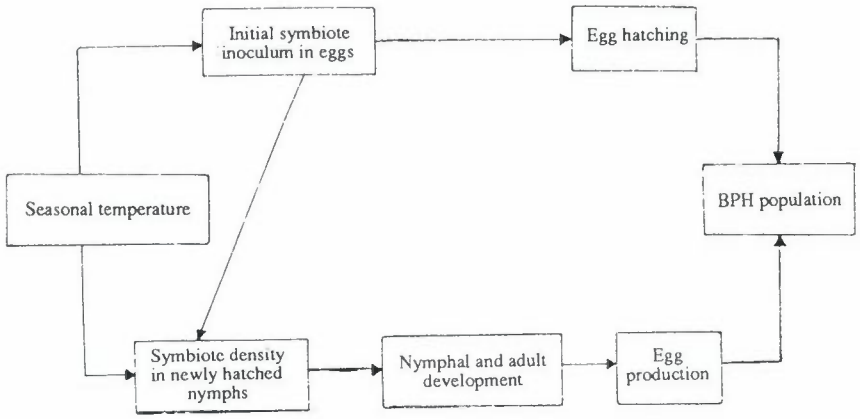


Figure 6. Possible role of symbiotes in the population build-up of BPH

region of South India during certain parts of the year. Conversely, it could be that the symbiote number in BPH seems to be much influenced by the host exposure to changes in seasonal temperature, and in turn, the symbiote number seems to influence the host population itself. Taking these possibilities into account, the occurrence of BPH in this tract may be divided into four different phases viz., build-up phase, decline phase, maximum reduction phase, and recovery phase (Fig. 5). Thus it appears that the symbiotes must be influenced by seasonal temperature fluctuation which in turn acts as a force in deciding the levels of BPH incidence through the year.

The possible role of seasonal temperature in the build-up of BPH population is indicated in the flow chart (Fig. 6). The seasonal temperature, perhaps, in combination with RH appears to influence the initial inoculum of symbiotes in eggs which in turn might influence egg hatching, and determine symbiote density in newly hatched nymphs. Nymphal and adult development further depend on the symbiote density in them. The fecundity of females also seems to be influenced by the prevailing temperature and RH to which these insects are exposed, ultimately deciding the BPH population in a particular season.

Influence of internal parasitoids on YLO's populations

Anargus optabilis, being a true egg parasitoid, is considered an active feeder of yolk spheres in the host eggs (Sahad, 1984). Probably with their increased feeding activity, the symbiote number was considerably disturbed. However, the symbiotes were not seen in the alimentary canal of the parasitoid, so in all probability, the parasitoid may not be consuming the symbiotes directly.

The presence of rod-like structures observed in the parasitised eggs is another aspect that needs further probing.

Regarding the effect of occurrence of *Elenchus* sp., the heavy reduction of symbiotes observed (Table 4) could not be treated as the result of direct feeding by the parasitoids, since they normally obtain their nourishment by diffusion through the integument (Clausan, 1972). It is assumed that the reduction could be a sex-specific one or due to possible production of certain enzymatic substances by the parasites. The reduced number of symbiotes due to super parasitism by *Elenchus* and multiple parasitism by *Elenchus* and drynid is understandable considering the cumulative effect of their presence.

The reduction of over 30% in symbiote number to drynid was not surprising considering the fact that it is supposed to feed directly on the internal tissues of BPH.

In the present study the trombidiid mite had the least effect on symbiote. Though Narayanasamy and Baskaran (1983) reported the occurrence of Trombidiid mite on nymphs and adults of BPH, its effect as parasitoid on symbiotic number is not evident.

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