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Endosymbiosis of Phloem Sap Sucking Planthoppers with special Reference to *Sogatodes orizicola* (MUIR) feeding on *Oryza sativa* L.

With 9 text figures

As in aphids and other groups of insects with sucking mouth parts, microbial symbiots have been shown in the bodies of planthoppers (Auchenorrhyncha). It has however been emphasized by MÜLLER (1949) that only phloem sap sucking species need endosymbiots or intracellular microorganisms, and that such symbiosis is not present in Typhlocybinae, specialized in feeding cell sap. The reason might be that, unlike the parenchyma suckers, the phloem sap sucking species lack a proteinase in their salivary glands (SAXENA 1955). In contrast to the Aphididae, there is much greater variety in the symbiotic relations of planthoppers, and this has prompted MÜLLER (1962) to study the phylogenetic ramifications in a paper entitled „Neuere Vorstellungen über Verbreitung und Phylogenie der Endosymbiose der Zikaden" (Recent concepts concerning the spread and phylogeny of endosymbiosis in cicadas).

The endosymbiots of phloem sap sucking planthoppers include various groups of microorganisms, and several of these may be found in a particular species. Most prominent are bacteria-like microorganisms which SCHWEMMLER (1973) categorizes as primitive protokaryotic „protoplastoids" (the name he gives to the intracellular main-(a)-symbiot of *Euscelis plebejus* FALL. is *Protoplastoides buchneri* SCHWEM. while he calls the minor-(t)-symbiot *P. vagonis* SCHWEM.). According to STANARIUS (1978) these organisms belong to mycoplasms. In addition, there are the yeast-like symbiots (YLS), probably conidia of Ascomycetes (MÜLLER 1972), which MAHDIHASSAN (1976) calls „Cicadomyces".

The following groups of symbiots can be found in cicada species according to MÜLLER, H.-J. (1949, 1962), MÜLLER, J. (1972):

— Essential symbiots
- main symbiots
- a-symbiots: spherical bacteroids mostly surrounded by mycetoms (including x-symbiots in Fulgoromorpha);
- H-symbiots: yeast-like symbiots (YLS) mostly of an oblong shape and embedded in fat tissue (syncytial fat body formation: Hs) or populating separate organs (in general called mycetom: Ho). According to MAHDIHASSAN (1976) the YLS are simply protoplasmatic residues, a concept which has found no response so far.
- minor symbiots: these occur only together with main symbiots and mostly populate a double mycetom:

- ● t-symbiots (only in Cicadomorpha)—bacteria-like, enclosed in a mycetocyte which may become a migratory cell (F-symbiots of Fulgoromorpha).

— Complementary symbiots

- ● accompanying symbiots—can occur only together with main symbiots and are mostly limited to a systematic host group: k-, m-, n-, o-, g-symbiots;
- ● accessory symbiots—are mostly limited to specific host species and not yet sufficiently integrated into the body of the host (some with a parasitic character): β-, γ-, υ-, π-, τ-, and ω-symbiots.

So far symbiots have been found in about 50 species of planthoppers (MAHDIHASSAN 1976, MOREAU and BOULAY 1967, KÖRNER 1969a, 1978, GIL-FERNANDEZ and BLACK 1965, CHIEN-CHUNG CHEN et al. 1981a, NODA 1974, 1977, ERMISCH 1960, MÜLLER 1957, 1962, 1969, 1972, 1973, HAMON 1971, SANDER 1968, SCHWEMMLER 1973, ORENSKI et al. 1965, QUAYUM 1968, MITSUHASHI 1975, SCHWEMMLER and HERRMANN 1979, 1980, and others).

With regard to frequency distribution MÜLLER (1962) gives the following percentages for the cicada species he studied: there were a-symbiots in over 64 %, YLS in 36.7 %, x-symbiots in 30 %, f-symbiots in 29 % and t-symbiots found in 26 % of all cases. The picture for the number of symbiot species per cicada species was as follows: about 3 % were apo-symbiotic (Typhlocybinae), 5 % mono-symbiotic, 55 % disymbiotic, 30 % trisymbiotic and 6 % tetra to hexasymbiotic.

The question of whether the „Blochmann bodies" (LANHAM 1968) are really symbiots in the widest sense of the word (STEINHAUS 1967), or whether they are better described as plasmids (LEDERBERG 1952), pseudovitelli etc. is still under discussion. SCHWEMMLER (1973) compares the endosymbiotic protoplastoids and rickettsia with mitochondria and emphasizes the following features:

While mitochondria are permanently attached to a cell, the endosymbiots are attached with a short interruption during the infection of the egg: mitochondria can not be cultivated in vitro, whereas protoplastoids and rickettsia can normally be cultivated to some extent by way of tissue culture when certain cell metabolites are added (as apparently practiced by SCHWEMMLER et al. 1973 with the endosymbiots of *E. plebejus*): mitochondria may be described as xenoreproductive and endosymbiots as semi-selfreproductive. On the other hand, mitochondria and protoplastoids are similar in their physiologic behaviour and possibly use the same mechanism to control their pH and pO (osmotic pressure) status.

STANARIUS (1978) in his comparison covers viruses and mycoplasms apart from bacteria and notes that, unlike viruses, mycoplasms can be cultivated on a cell-free medium and, unlike bacteria which possess cell walls have only a lipoprotein membrane with a thickness between 7 and 12 nm. Finally, mycoplasms are highly sensitive to tetracyclines whereas penicillin has no effect.

The symbiots inhabit different regions in the body of planthoppers, and the picture includes diffuse intracellular patterns, syncytial populations in the fat body, and inclusions in the mycetom or mid-gut epithelium and in mycetocytes which may unite into mycetoms, particularly where combinations of these patterns occur. Mycetocytes and syncytia in these cases are giant cells surrounded by an epithelium which accommodates the endosymbiots. The epithelium provides a link with the tracheas that are present in large numbers (KÖRNER 1978). In the bacteriodal forms there is a predominance of mycetocytes and mycetoms or similar organs (as in Aphididae). Double mycetoms often occur in the presence of symbiot combinations. The essential feature seems to be, however, that these organs are primarily found near the gonads (KÖRNER 1969c, 1978, GIL-FERNANDEZ and BLACK 1965, ERMISCH 1960).

1. Symbiot activity

Endosymbiots in planthoppers seem to perform a variety of functions quite similar to those of their counterparts in Aphididae (FRÖHLICH 1988). In the cicada too they apparently affect processes related to embryogenesis and especially the biosynthesis of chitin. It has been reported by SCHWEMMLER et al. (1973) that lysozyme and Tetracycline-treated females of *E. plebejus* lay aposymbiotic eggs from which develop „capital embryos" without abdomens. It is the assumption of the authors that treatment with Tetracycline inhibits translation in the synthesis of protein while Penicillin interferes with the cross-linking of cell walls and lysozymes cause lysis of the 1,4-glucosidic bond of amino acetal polysaccharide in the Murein of the bacterial cell wall. From this they conclude that symbiotic protoplastoids are a necessary condition for normal embryogenesis. Semi-aposymbiotic and aposymbiotic females were inhibited in their reproduction, vitality and development, and a total loss of symbiots was fatal. When aposymbiotic cicadas were raised on different types of diets it was found that the endosymbiots also produce amino acids (Cysteine) and cholesterol and transmit these substances to their host, a situation very similar to that prevailing in Aphididae. In addition, they are essential in converting final products from the protein and nucleic acid metabolism of their hosts and thus responsible for detoxification or N recycling. They therefore are important in the normal pattern of ontogenesis so that endosymbiosis seems to be compulsory.

Finally, the symbiots are vital in the anabolism of their hosts. The accumulation of uric acid crystals in aposymbiotic specimens shows their importance also for catabolism (SCHWEMMLER and HERRMANN 1979). In the absence of symbiots death is caused by a rising pH and pO regulation, and physiologic and chemical poisoning is eventually brought about by the accumulation of catabolites and a lack of anabolites.

Since hatchable embryos develop in the eggs of cicadas even after the elimination of the symbiot mass, which does not happen in Aphididae, SANDER (1968) and KÖRNER (1978) have concluded that the symbiots of planthoppers may be dispensable for their hosts during embryogenesis but are essential for metabolism especially at larval development.

2. Protokaryotic symbiot transmission

The transmission of protokaryotic symbiots is an intraovarial process in planthoppers which occurs after the development of specific forms of infection in the body of the females, in this case an infection of the ovarioles (MÜLLER 1972). On the whole, the infectious process is more complex in this group of insects than in Aphididae.

For example, the bacteria-like symbiots of cicadas exist in two forms, one vegetative and the other infectious. The former, like the symbiots of aphids, has two membranes—a plasma membrane (M_1) and a modified bacterial cell wall (M_2). There is also a third „envelope membrane" (M_3) formed by the host organism (KÖRNER 1969c, HOUK and GRIFFITHS 1980). Only M_1 and M_2 can be found in the infectious forms. The close relation between ontogenesis and the symbiot cycle has been demonstrated by SCHWEMMLER et al. (1973) who studied symbiot infection in *E. plebejus*. They found the symbiotic infection mass (symbiot ball—SB) at the round end of the egg, surrounded by periplasm. It remains connected to the embryo throughout germinal development, has a diameter from 50—60 µm and consists of some 40—50 protoplastoid spherical or oval main-(a)-symbiots and 150 eosinophile minor-(t)-symbiots. The diameter of the main symbiots is from 3—5 µm, that of the minor ones between 1 and 2 µm. Twenty hours after the egg laying, the SB is embedded

in blastodermic epithelium. The first mononuclear primary or a_1-cells develop from blastomeres (vitellophages) between the 3rd and 4th day and transform into primary or a_1-mycetocytes after infection. In each of the 25 or so a_1-mycetocytes of the primary mycetom one finds up to 30 a-symbiots partly incorporated and partly resulting from intramycetocary multiplication. No extracellular symbiot reproduction has been observed (SCHWEMMLER et al. 1973, KÖRNER 1978).

On the 5th day, as the germ band shortens, prospective symbiot cells of mesodermic origin are observed (50—70 t-cells), which are characterized by a plasma space of about 1 µm. They migrate to the primary mycetom where infection by t-symbiots occurs, resulting in t-mycetocytes with about 30 t-symbiots each by incorporation and intramycetocary multiplication. These a- and t-mycetocytes form a complete primary mycetom (PM). From the 7th day onwards, secondary or a_2-cells enter the PM from the mesodermal genital ridge to make up the next symbiot generation. They have two nuclei and incorporate the a-symbiots while the a_1-mycetocytes degenerate. The PM is transformed into an unpaired transitory mycetom (TM or pseudo-mycetom) where both the a- and t-symbiots develop into transparent and partly lobulate vegetative forms with a size of 8 µm by intramycetocary growth. Around the 10th day, as the embryo unfolds and for some time thereafter, the TM separates into a collective or double mycetom (DM) consisting of an a- and t-organ each. In the ready-to-hatch embryo, finally, one finds lateral mycetoms (LM) between the 2nd and 5th abdominal segment with a lenght of 0.02 mm (SCHWEMMLER et al. 1973, KÖRNER 1978).

During the nymphal stage differentiation continues in the kidney-shaped LM. The a-organ transforms into a-syncytium and the spherical nuclei form lobules, while the t-organ does not change its cell boundaries and nuclear shapes; the a- and t-organs form separate envelope epithelia for demarcation. The first oocyte infection begins about two days after the adult molt of the female with the penetration of symbiots into the follicular cells at the rear end of the egg. From there they eventually reach the space between the follicular epithelium and the periplasm (SCHWEMMLER et al. 1973, KÖRNER 1969b, 1972, 1978, SCHWEMMLER and HERRMANN 1980). It would seem important in this connection to cite SANDER (1968) and KÖRNER (1969b) who observed that the embryonic mycetoms (primary and secondary mycetocytes) develop as if under normal conditions even when the symbiots have been experimentally removed, i. e. when there has been no infection.

3. Planthopper species populated exclusively by YLS

The picture appears somewhat simpler in the case of planthoppers inhabited exclusively by yeast-like symbiots. Some of these species play a particular role in the transmission of viruses in rice and include *Nilaparvata lugens* STÅL, *Laodelphax striatellus* (FALL.), *Sogatella furcifera* HORV. and *Sogatodes orizicola* (MUIR). The species also known as rice planthoppers like *Nephotettix cincticeps* UHLER and their relations do not fall in this category as they are populated by bacteria-like a- and t-symbiots (MITSUHASHI and KONO 1975).

N. lugens is known as virus vector of grassy stunt disease (Philippines), *L. striatellus* of stripe disease and blackstreaked dwarf (Japan), *S. furcifera* transmits a number of viruses and *S. orizicola* as vector of white leaf spot disease hoja blanka (North, Central and South America).

NODA (1977) has found YLS of an oblong oval shape (ca. 13 µm long) in the fat body of *L. striatellus*, which probably reproduce by budding. The reaction of their nuclei is Feulgen positive, and their RNS and protein reaction are also positive. In the

course of postembryonic development their number increases and does so, initially, in the body of females. Fewer are found in male specimens. Infection occurs at the rear end of the primary oocytes through the epithelial plug of the ovary. In the last phase of embryonic development the symbiots invade the abdominal fat body. In females, they can be detected only there and in the ovarioles but not in the thorax or head region. The pattern of distribution is as follows (NODA 1977):

L_2 stage—symbiots scattered in the fat body
L_3 stage—symbiots merged into small groups
L_4 and L_5 stage—localized in large groups to a growing extent

The following description can be given of the transovarial passage and embryo infection:

The symbiots infect the epithelial plug between the vitellarium and pedicle and penetrate into the ovariole. Then they proceed to the primary oocyte at the rear end and form a symbiot ball (SB) before yolk production finishes. The SB is surrounded by a membrane and becomes embedded into the yolk near the rear end of the egg (Fig. 1 and 2), where it can be detected immediately after the eggs have been laid. Two days later it has moved to the front end, migrates back to the rear end in the course of embryonic development and eventually enters the fat tissue.

Fig. 1: Infection of yeast-like symbiots at the rear end of primary oocytes in *Laodelphax striatellus* (E = epithelial plug, S = symbiot ball, P = primary oocyte — from NODA 1977)

NODA (1974) has developed the following formula for determining the total number of symbiots in the individual stages including the eggs, after homogenization in an 0.8 % salt solution and a count taken in the THOMA champer:

$$\text{Total no. of symbiots} = \frac{a\,(x+m)}{n \cdot v} \text{ per specimen or egg}$$

where a = number of symbiots in v
m = total weight of homogenized insects
n = no. of homogenized specimens or eggs
v = volume of insect homogenate in the hemocytometer
x = volume of salt solution needed for homogenization.

Fig. 2: Symbiot ball (S) in the posterior part of the egg in the female of *Laodelphax striatellus* (from NODA 1977)

In cases where x is much greater than m (e.g. eggs or L_1 stage), m can be neglected.

The number of symbiots increases as the host develops, while their number per µg of fresh wt. remains constant in the post-embryonic stages (about 140—170). At the L_5 stage the female nymph has a larger number of symbiots than its male counterpart. After molting the number found in brachypterous and macropterous males drops sharply, while in females it rises consistently for a start and reaches a maximum at the beginning of the egg laying period (240,000 in brachypterous forms and less in macropterous ones). Then the population density goes down (Fig. 3).

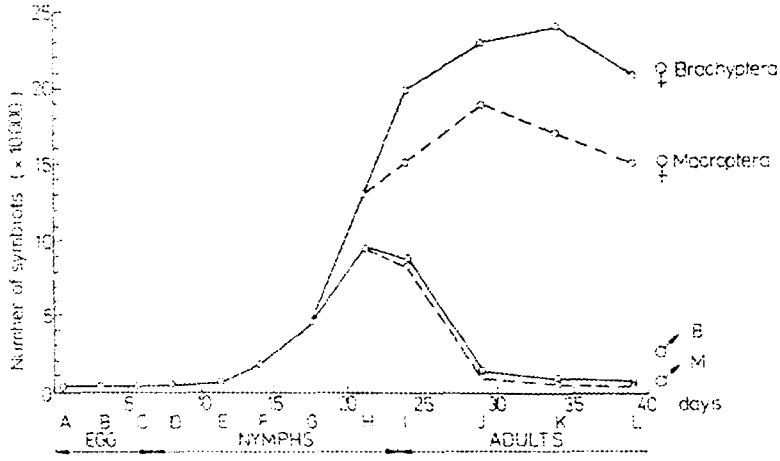

Fig. 3: Changes in the population density of symbiots throughout the development of *Laodelphax striatellus* (A = 12—24 h, B = 72—84 h, C = 130—142 h old eggs; D = 1st, E = 2 nd, F = 3 rd, G = 4 th, H = 5 th nymphal stage; I = 0—1 d, J = 5—6 d, K = 10—11 d, L = 15—16 d old adults; acc. to NODA 1974)

Histologic studies have shown that most symbiots accumulate around the ovaries of maturing females. This seems to be related to the transovarial passage, just as the greater population density in the bodies of females must be ascribed to egg production. The rapid drop in the symbiots populating the bodies of males suggests that their function has ended and the balanced relation between host and symbiot is disturbed or has become superfluous.

CHIEN-CHUNG CHEN et al. (1981a) have also found rod-shaped yeast-like symbiots (13—15 µm long, 5—6 µm wide) in the fat body of *N. lugens*, which reproduce by budding. While evenly distributed throughout the body in the L_1 and L_2 stages, they accumulate to an increasing extent in the fat body from the L_3 to the adult stage. Similar to *L. striatellus* and *S. furcifera*, *N. lugens* has no special mycetom, and the cells inhabited by symbiots merge into syncytia.

The infection is intraovarial/transovarial. From the rear of a newly laid egg the symbiot ball migrates to the opposite end two days after the egg has been laid, in the course of embryonic development. Once blastokinesis is over, it returns to its original position. The SB bursts and the YLS accumulate in the abdominal part of the embryo. Figure 4 shows the changes in the population density of symbiots as the host develops, as determined by the method of NODA (1974).

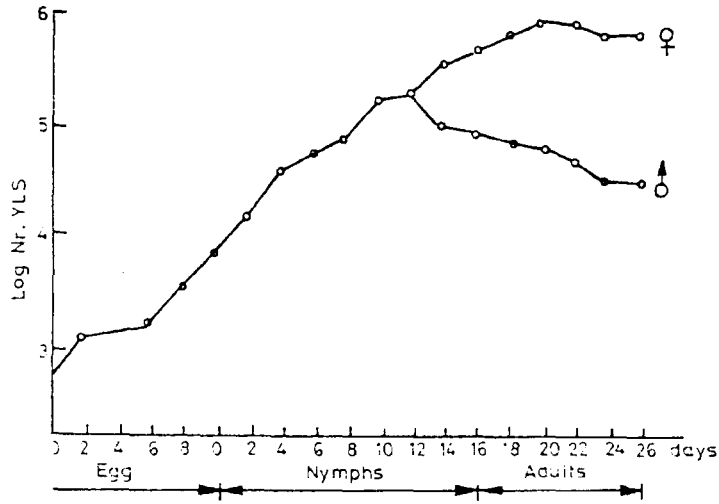

Fig. 4: Changes in the population density of symbiots in *Nilaparvata lugens* (acc. to CHIN-CHUNG CHEN et al. 1981 a)

NODA and SAITO (1979) exposed the larvae of *L. striatellus* to temperatures of 35 °C for three days immediately after hatching. The result was a reduction of the symbiots. The insects treated in this manner did reach the L_5 stage, but a certain percentage died then, while others did not molt and therefore could not complete metamorphosis to the adult stage as they had no normal adult cuticle. Sterol analysis suggested that the symbiots are a major source of sterol for the host insects by supplying 24-methylene cholesterol.

A test was then made to see if the above-mentioned defect could be repaired by feeding sterols and ecdysteron to heat-treated planthoppers (NODA and SAITO 1979). Newly hatched heat-treated larvae were kept at 25 °C and given cholesterol, β-sitosterol and campesterol with rice leaves. Even though their development was somewhat

retarded, 61.6 % of the nymphs fed cholesterol reached the adult stage as against only 15.2 % with β-sitosterol. Although the insects receive β-sitosterol from the rice plant, its conversion into cholesterol requires several stages which were not completed in the course of development. The success achieved with cholesterol, on the other hand, indicates that the symbiots supply *L. striatellus* with 24-methyl cholesterol.

As a supplement to this test, dorsal topical application of ecdysteron was used on heat-treated L_5 stages 19 days after hatching (optimal dosis 10^{-2} µg), which in some cases led to the development of a new cuticle and thus stimulated the adult molt. Since cholesterol is a precursor in the production of ecdyson, the synthesis of the latter suffers when the former is in short supply, and metamorphosis is inhibited (NODA et al. 1979).

Apart from heat treatment, antibiotics were also tested for their suitability in obtaining aposymbiotic planthoppers. *N. lugens* were given Polyoxine S (0.3 %), Chloramphenicol (0.1 %), Cycloheximide (0.1 %), Blasticidin-S (0.2 %), Nystatin (0.1 %) and Ampicillin trihydrate. In the case of Chloramphenicol the symbiots were reduced by 47.5 % (including abnormal forms) and mortality was 34.7 %. The corresponding figures for Polyoxine S were 44.4 % and 30.7 % and for Cycloheximide 55.9 % and 61.1 %, respectively. It is assumed that Polyoxine tretment inhibits chitin biosynthesis, whereas Cycloheximide leads to lysis of the symbiots (CHIEN-CHUNG CHEN et al. 1981b) – (see also Table 1).

Table 1:

Effect of antibiotics on symbiots of planthoppers (review of literature)
BLS = bacteria-like symbiots; YLS = yeast-like symbiots

1) = ORENSKI et al. 1953; 2) = MITSUHASHI 1975;
3) = CHIEN-CHUNG CHEN et al. 1981b; 4) = KUSUMI et al. 1980;
5) = SCHWEMMLER et al. 1973; 6) = SCHWEMMLER 1974

Group	Action	Preparation	Test animal	Symbiot type	Concentration	Type of application	Results	
Aminoglycosides	bactericidal	Kanamycin	*Macrosteles fascifrons*	BLS		in vitro culture	symbiots found sensitive	1)
			Laodelphax striatellus	YLS		in vitro culture	no effect	2)
		Streptomycin	*L. striatellus*	YLS		in vitro culture	no effect	2)
ß-lactam antibiotics	bactericidal	Ampicillin trihydrate	*Nilaparvata lugens*	YLS		in vitro culture	no effect	3)
		Penicillin	*L. striatellus*	YLS		in vitro culture	no effect	2)
			Euscelis plebejus	BLS	1000 ppm	artificial diet	symbiots damaged hosts	5)
Chloramphenicol	bactericidal	Chloramphenicol	*M. fascifrons*	BLS	30 µg/ Petri dish	in vitro culture	symbiots found sensitive	1)
			N. lugens	YLS	1000 ppm	via plant	47.5 % reduction of symbiots 34.7 % mortality of hosts	

Table 1 (Continued)

Group	Action	Preparation	Test animal	Symbiot type	Concentration	Type of application	Results	
Methene-amine		Mandelic-amine	*M. fascifrons*	BLS		in vitro culture	symbiots found sensitive	1)
Novobiocin	bacteri-cidal	Novobiocin	*M. fascifrons*	BLS	5 µg/ Petri dish	in vitro culture	symbiots found sensitive	1)
			L. striatellus	YLS		in vitro culture	no effect	2)
Polyene and polyene macrolide	fungi-cidal	Amphotericin B	*L. striatellus*	YLS: L_3-1	30 µg	in vitro culture	growth inhibited at 25 °C	4)
				L_3-2	30 µg	in vitro culture	growth inhibited at 37 °C	4)
		Nystatin	*N. lugens*	YLS	1000 ppm	via plant	no effect	3)
Tetra-cyclines	bacteri-cidal	Tetra-cycline	*E. plebejus*	BLS	1000 ppm	artificial diet	25 % reduction of symbiots 50 % mortality	5)
			E. plebejus	BLS	100 ppm	root application	85 % normal embryos	
					1000 ppm	via plant	25 % normal embryos	
					250 ppm	via watering	95 % normal embryos	
		Cyclo-heximide (Actidon)	*L. striatellus*	YLS: L_3-1	2.5 µg 25 °C	in vitro culture	symbiots killed	4)
				L_3-2	30 µg 25 °C	in vitro culture	symbiots killed	4)
			L. striatellus	YLS	0.001 — 0.01 mg/ml	in vitro culture	symbiots growth inhibited	2)
		Chlor-heximide	*N. lugens*	YLS	1000 ppm	via plant	55.9 % reduction of symbiots 30.7 % mortality	3)
	Chitin synthesis inhibitors	Polyoxine S	*N. lugens*	YLS	3000 ppm	via plant	44.4 % reduction of symbiots 30.7 % mortality	3)
		Blasticidin-S	*N. lugens*	YLS	2000 ppm	via plant	no effect	3)
			L. striatellus	YLS	0.001 — 0.01 mg/ml	in vitro culture	symbiot growth inhibited	2)
		Mycostatin	*L. striatellus*	YLS	0.001 — 0.01 mg/ml	in vitro culture	symbiot growth inhibited	2)
		Sarcomycin	*L. striatellus*	YLS			no effect	2)

Reference should also be made to the attempted isolation of symbiots from the abdomen of planthoppers for cultivation in vitro. MITSUHASHI (1975) subjected eggs of *L. striatellus* to surface sterilization by dipping them in 70 % ethyl alcohol (1 min), followed by rinsing in sterile aqua dest. The eggs were then placed on culture medium (MGM-401 for tissue culture), the chorion removed with the aid of fine needles, the embryo and yolk were spread and incubated at 25 °C. At that time some of the symbiots were in budding. Hypha growth then occurred until mycelium was produced. The mycelium immersed in the medium was gray, while that which remained in air was cotton-white. This mycelium was transferred to MGM-401 agar, upon which it increased in size and developed a dark pigment near the edge. Such results were, however, obtained only with planthoppers caught in the open and not with specimens bred in the laboratory.

Similar studies were conducted by KUSUMI et al. (1979, 1980) who used eggs of *L. striatellus*. These were sterilized for 3 min in 0.2 % Hyamine T, rinsed with sterile aqua dest. and dipped for 3 min in 75 % ethanol. After this treatment the eggs were homogenized in sterile water, the homogenate was transferred to nutrient agar plates with modified Grace TC medium, and incubation at 25 °C followed. This led to the isolation of two different YLS which were then identified as L_s-1 and L_s-2 with the aid of immunologic techniques:

L_s-1: multiplies by budding and forms white colonies, the cells are ellipsoidal 4.7 × 2.2 µm,

L_s-2: multiplies also by budding, forms yellowish-white colonies, the cells are oblong rods measuring 1.5 × 7.6 µm (KUSUMI et al. 1979).

The following differences were noted with regard to the effect of temperature on growth:

L_s-1: The temperature range for growth in vitro was between 22 and 33 °C, with no growth above or below these limits. The optimal range was from 25–31 °C.

L_s-2: The temperature range for growth in vitro was between 22 and 40 °C, with no growth above or below these limits. The mycelium developed well between 25 and 35 °C, the optimum being 30 °C.

A temperature of 37 °C had a lethal effect on L_s-1 but only a static one on L_s-2.

The effect of the following antibiotics was also tested—Cycloheximide, Amphotericine B, Tetracycline and Chloramphenicol. The latter two had no effect at all, while Amphotericine B inhibited the growth of L_s-1. Cycloheximide killed both symbiots (2.5 µg at 25 and 37 °C, respectively), but L_s-2 showed increased sensitivity only at temperatures above 25 °C.

MITSUHASHI (1975) also made a study of how antibiotics and antiseptics affected the growth of these YLS in vitro. While there was no effect from Kanamycin, Streptomycin, Sarcomycin, Novobiocin and Penicillin, Mycostatin, Actidon (Cycloheximide) and Blasticidin inhibited the growth of YLS at concentrations from 10^{-3}—10^{-2} mg/ml. The same was true of the antiseptics methyl-p-hydroxbenzoate, methyl toquinon and Mezonine at concentrations from 10^{-3}—10^{-2} mg/ml (Table 1).

The most recent research worth mentioning is that done by VON DER HEYDE (1985) on the effect of neem products on rice cicadas. Specimens included *N. lugens*, *S. furcifera* and *Nephotettix virescens* (DIST.), i.e. two carriers of YLS, the latter species with bacteria-like symbiots. The fact that neem extracts had a marked influence on nymphs and females of *N. lugens* and *S. furcifera* depending on concentration, but no effect on *N. virescens*, may be related to the different types of symbiots. Unfortunately, the desired confirmation of an effect on the existing planthopper population was not obtained in field experiments.

Finally, mention should be made of tests to establish the effect of different formulations of Buprofezin on *N. lugens* in irrigated rice (ASAI, KAJIHARA, MAEKAWA 1984). Very good and persistent control was reported even with low concentrations.

4. Endosymbiosis in *Sogatodes orizicola* (Muir)—Delphacidae

S. orizicola plays an important role in rice growing all over America as it transmits the virus that causes white leaf disease of rice. This species is one of a group all inhabited by yeast-like symbiots which have not been detected either in the thorax or head region but are limited to the abdominal fat body and ovarioles of the females. The symbiots are oval in shape, with an average length of 11.8 µm (8.3—15.0 µm) and a width of 4.6 µm (4.0—6.7 µm). This is very similar to the data that have been reported for the symbiots of *L. striatellus* and *N. lugens* (see above). Microscopic studies have shown that they multiply by budding (Fig. 5). Their distribution in

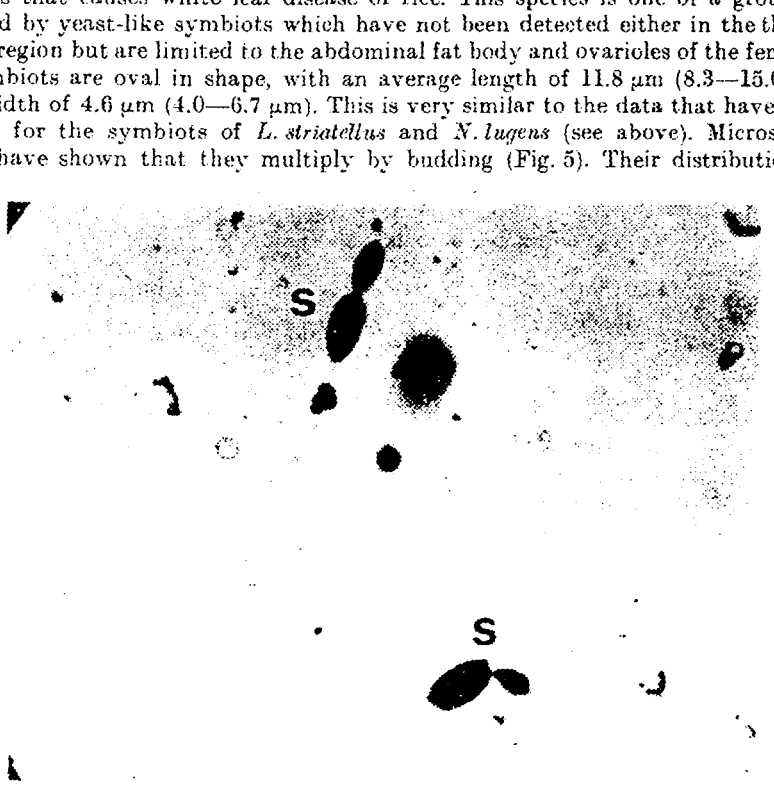

Fig. 5: Budding symbiots (S) of *S. orizicola* (methylene blue stain, 1225 x)

the body at individual stages of development follows the pattern reported by Noda (1977) for *L. striatellus*—scattered in comparatively uniform diffusity across the fat body at the L_1 and L_2 stage, concentration growing at the L_3 stage and centering on clearly defined syncytial regions beginning with L_4 and particularly at stage L_5. In female specimens, these concentrations are found particularly around the ovaries (Fig. 6).

The growth of the symbiot populations at the various stages was determined using Noda's (1974) method (Fig. 7, Table 2). It was found that the number of symbiots approximately doubled during embryonic development, followed by a drastic sevenfold jump at the L_1 stage. Thereafter the populations approximately doubled again from one stage to the next, but after L_4 this trend continues up to L_5 only for the male line with almost a tripling of numbers for the females at L_5. This growth is parallel with the increase in body weight during the post-embryonic stages of development. After the adult molt the population density of the male symbiots drops sharply while that of the females continues to rise and reaches a maximum at the beginning of egg laying. Histologic sections of the female abdomen show most of the symbiots in the syncytia clustered around the ovaries. As opposed to population

Fig. 6: Syncytium (syn) in the fat body of female of *S. orizicola* filled with symbiots (Microtome section 7 µm)

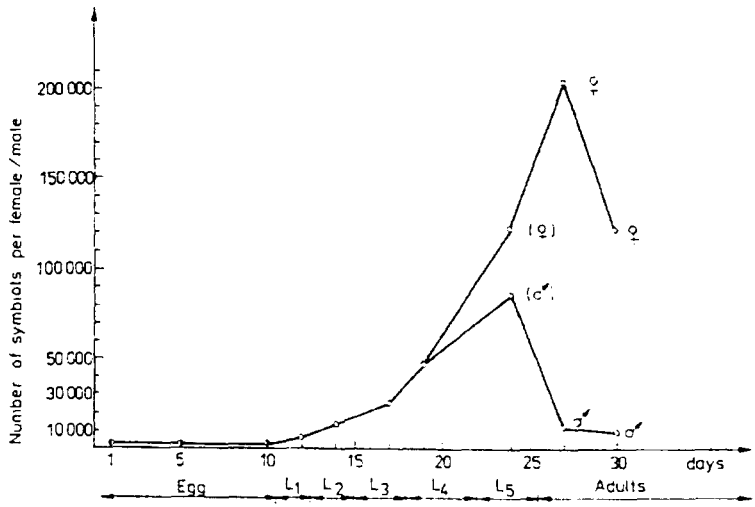

Fig. 7: Population growth of symbiots in the development of *S. orizicola* (macropterous females and males) (L_1—L_5 = larval stages)

growth with increasing body weight, their number per unit fresh weight remains relatively constant. It is given as 140—170 per µg of fresh wt. in the case of *L. striatellus* (see above) and varies between 150 and 190 ($\bar{x} = 165$) for *S. orizicola* in our studies. This is, however, true only for females and not for male insects (Table 2).

The transmission of symbiots in *S. orizicola* obviously follows the pattern of transovarial passage and embryonic infection described by NODA (1977) for *L. striatellus*.

Table 2:

Comparison between development stage, fresh weight and no. of symbiots (as determined by the method of NODA 1974)

Days after egg laying	Stage	Fresh wt. µg	No. of symbiots	No. of symbiots per µg of fresh weight
1	egg	·	300—400	·
5	egg	·	700	·
10	egg	·	800	·
12	L_1	38	5600	147,4
14	L_2	55	13300	241.8
17	L_3	160	24300	151.9
19	L_4	295	45800	157.9
24	L_5 ♂	670	85000	(126,9)
24	L_5 ♀	900	121000	134.4
27	adult ♂	645	11700	(18.1)
27	adult ♀	1300	204200	157.1
30	adult ♂	640	9300	(14.5)
30	adult ♀	1075	120700	(112.3)
				$\bar{x} = 165.1$

With the aid of sagittal sections through the abdomen, the existence of symbiot balls (SB) and symbiots has been demonstrated in the eggs of females before they start of egg laying (Fig. 8). The SB (diameter ca. 25 µm) is clearly visible near the round rear end of the sickle-shaped eggs on the day of laying (Fig. 9). It comprises up to 400 symbiots with an average length of 8.1 µm and a width of 3.1 µm. The eggs were kept in sterile physiologic solution of common salt for observation of changes in the position of the SB. In the course of embryonic development, the ball migrated to the front end and then changed position as the embryo turned. With the completion of embryogenesis it is found in the abdominal fat tissue where it dissolves. The symbiots first populate individual fat cells which then merge into syncytia as the population grows.

Table 1 reflects the influence of certain antibiotics to symbiots of planthoppers as already mentioned, where Tetracycline affects especially bacteria-like symbiots of *E. plebejus*, while Chloramphenicol and Chlorheximide have an effect on yeast-like ones of *L. striatellus* and *N. lugens* (SCHWEMMLER et al. 1973, SCHWEMMLER 1974, CHIEN-CHUNG CHEN et al. 1981b). Chloramphenicol but also Nystatin, Sulfachin and Thicoper also had a certain effect to the symbiots of *S. orizicola* after root application with permanent exposition in preliminary tests involving antibiotics, fungicides and sulphonamides, which could not be confirmed in all cases in further tests using other types of application.

Based on the observation that egg infection by symbiots starts at the beginning of the ripening phase of the eggs after hatching of the adult females, rice plants in special rearing cages were sprayed (2 ml/cage) by products and concentrations summarized in Table 3. After drying of the spraying film (after about 30 min) 5 freshly hatched females and males each were applied to the treated plants. Together with their off springs they remained there up to the end of the test (about 35 days), so far phytotoxic influences by the chemical products or high population density of the planthoppers do not force to add new plants to the treated ones. The effect of these products observed bases mainly on the uptake together with the plant sap, for a minor part on the contact with the treated plant, because the planthoppers did not become sprayed directly.

Fig. 8a

Fig. 8b

Fig. 8a: Egg with symbiot ball (sb) in the abdomen of a female of *S. orizicola* (microtome cross section 7 µm). — Fig. 8b: Egg with symbiot ball (sb) in greater enlargement (620 x, microtome cross section 7 µm)

Fig. 9a

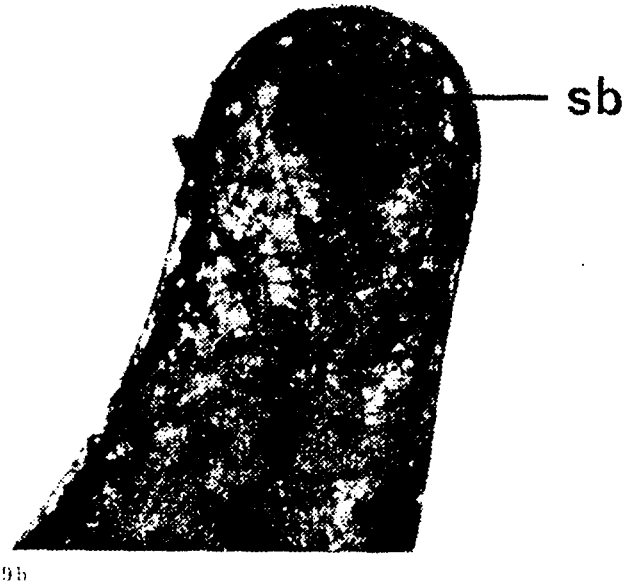

Fig. 9b

Fig. 9a: Newly laid egg of *S. oryzicola* with symbiot ball (sb). — Fig. 9b: Round end of egg with symbiot ball (sb) — (greater enlargement)

Table 3:

Effect of antibiotics and fungicides to the number of offsprings and the duration of their development during F_1- and F_2-generation

Xenobioticum	mode of action	concentration, ppm	mode of application	effect to F_1-generation	effect to F_2-generation
OTC	bactericide	1000	sprayed	reduced number of insects compared to the control	equal number of insects like the control
Penicillin	bactericide	1000	sprayed	reduced number of insects compared to the control	equal number of insects like the control
Nystatin	fungicide	1000	sprayed	equal number of insects like the control	retardation of development at least 6 d
Griseofulvin	fungicide	1000	sprayed	equal number of insects like the control	less offsprings compared to the control
Chloramphenicol	bactericide/bacteristatic	1000	sprayed	reduced number of insects compared to the control, mortality after 9 d = 70 %	less offsprings compared to the control, retardation of development around 3 d
Cycloheximide	antifungal	100	sprayed	less phytotoxic, no larva hatched	no F_2-generation
		50	sprayed	less phytotoxic, small amount of larva hatched, died in L_1 stage	no F_2-generation
		10	sprayed	less insects compared to the control, development retarded up to 5 d, mortality after 7 d = 85 %	considerable less insects compared with control, retardation of development around 4 d
Thiroper (Carbendazim)	fungicide	1000	sprayed	retardation of embryo genesis for about 2—3 d	equal number of insects like the control
Antracol (Propineb/ Lindane)	fungicide/ insecticide	2000	sprayed	equal number of insects like the control	number of insects smaller than the control
Morestan (Chinomethionat)	fungicide/ acaricide	300	sprayed	no larva developed	no F_2-generation
		200	sprayed	equal number of insects like the control	number of insects considerable less compared with the control

In result OTC, Penicillin and Thicoper showed actually a certain effect to the F_1-generation, but became leveled again in F_2 compared to the control. In contrast Nystatin, Griseofulvin and Antracol showed effects to the F_2-generation but with low importance at all. The effect of Cycloheximide (100 and 50 ppm) and Morestan (300 ppm) is distinctly demonstrated by the absence of the development of larva in the following generation or the hatched insects died, respectively, thus a F_2-generation could not come up. Worth mentioning are also Chloramphenicol (100 ppm) and Morestan (200 ppm) under this aspect.

To illustrate the effect of these products to symbiots, the latter ones became counted according to NODA (1974)—see above. It was proved that the number of symbiots of the survived planthoppers showed no significant differences at the test end as well as tests between after about 20 days compared with the control. The same result came up after staining with methylene blue and sodium citrate (shake 0.01 g methylene blue with 10 ml aqua dest., add 2 g sodium citrate, stir and filtrate, add aqua dest. to filtrate to give 100 ml). Dead protoplasma will turn into an intensive blue, while living cells remain hyaline. From this fact comes the conclusion: either the symbiots become quickly killed by the active ingredient of these products and the affected planthoppers die immediately, while by insufficient action of the a. i. the population density of the symbiots in the hosts become soon balanced, or there is no symbioticidic principle. In all cases the described test arrangement is not usable for proving of special symbioticidic effects.

Summarized it becomes visible, that the infection process and the change of the population density of the symbiots during the development of the host as well as the mode of action of the symbiots and their importance for development processes seems to be relatively similar for the planthopper species with yeast-like symbiots discussed in this paper.

Summary

After a brief survey of the various forms of symbiosis between cicadas and microorganisms, the symbiotic activities and the transovarial transmission of bacteroid symbiots at the example of *Euscelis plebejus*, the carriers of yeast-like symbiots are dealt with. These are species of cicadas mainly from the family Delphacidae that are known as vectors of rice viroses. The distribution of the symbiots in the body of the different instars and of the adults, their increase during the development of the cicadas, the significance of their metabolic activities for the host organism, the form of their transmission to the offspring and their reactions to antibiotics and other xenobiotics of *Laodelphax striatellus*, *Nilaparvata lugens* and *Sogatodes orizicola* are compared and found to correspond in many respects.

Zusammenfassung

Nach einer kurzen Übersicht über die vielfältigen Formen der Symbiose zwischen Zikaden und Mikroorganismen, die symbiontischen Aktivitäten und die transovariole Übertragung bakterienartiger Symbionten am Beispiel von *Euscelis plebejus* gilt im Folgenden die Aufmerksamkeit den Trägern hefeartiger Symbionten. Es sind als Vektoren von Reisvirosen bekannte Zikadenarten vornehmlich aus der Familie der Delphacidae. Verglichen werden die Verteilung der Symbionten im Körper der Entwicklungsstadien und der Adulten, ihr Populationszuwachs im Verlaufe der Zikadenentwicklung, die Bedeutung ihrer Stoffwechselaktivitäten für den Wirtsorganismus, die Form der Übertragung auf die Nachkommen sowie ihre Reaktion auf Antibiotika und andere Xenobiotika von *Laodelphax striatellus*, *Nilaparvata lugens* und *Sogatodes orizicola*, wobei sich weitgehende Übereinstimmungen zeigen.

Резюме

После короткого обзора о разнообразных формах симбиоза между цикадами и микроорганизмами, симбиотической активности и трансовариольном переносе бактериовидных симбионтов на примере *Euscelis plebejus* внимание уделяется носителям дрожжевидных симбионтов. Это в первую очередь виды цикад семейства Delphacidae, известных как переносчики вирусных болезней риса. Сравнивали распределение симбионтов в разных стадиях развития, увеличение популяций в течение развития цикад, значение активности их обмена веществ для организма хозяина, форма переноса симбионтов на потомство хозяев, а также их реакцию на антибиотики и другие ксенобиотики *Laodelphax striatellus*, *Nilaparvata lugens* и *Sogatodes orizicola*, причем установили практически полное совпадение.

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