

Modeling population dynamics of yeast-like symbionts (Ascomycota: Pyrenomycetes: Clavicipitaceae) of the planthopper *Delphacodes kuscheli* (Hemiptera: Delphacidae)

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Abstract *Delphacodes kuscheli* establish mutualistic relationship with yeast-like symbionts (YLS) that live in the fat body and are necessary for host survival and reproduction. We estimated for a host of age t , its body weight, $W_{(t)}$, and the number of YLS per host, $YLS_{(t)}$. The host body weight was calculated as: $W_{(t)} = Lm/[1 + e^{-(d-kt)}]$, (Lm = the maximum observed weight, and d and k are constants), and the fat body was considered a fixed proportion of $W_{(t)}$. We calculated the number of YLS per unit host body mass: $\alpha_{(t)} = YLS_{(t)}/W_{(t)}$. We also calculated the number of YLS per host, $cYLS_{(t)}$, and analyzed the pattern of variation in both sexes adapting the expression of the logistic model: $cYLS_{(t)} = KN_0e^{rt}/K + (e^{rt} - 1)N_0$, (N_0 = initial number of YLS, r = intrinsic per capita rate of natural increase, and K = variable carrying capacity). In females the carrying capacity varied according to a constant proportion of the host's weight: $K_{(t)} = \alpha W_{(t)}$. In males $\alpha_{(t)}$ was considered a decreasing function of the host age: $K_{(t)} = \alpha_{(t)} W_{(t)}$. The coefficients N_0 , α , and r were subjected to parameterization. We found that the patterns of $W_{(t)}$ and $YLS_{(t)}$ of *D. kuscheli* were similar to other planthoppers. In females YLS increased up to the adult stage and then

remained almost constant, varying similarly to individual weight. In males YLS increased up to the 5th instar nymph as the individual weight did, but the number of YLS decreased in the adult stage and the correlation was not so good. The calculated number of YLS per host matches reasonably well with the number estimated experimentally both in females and males. This is the first study that quantified and modeled the dynamics of YLS endosymbionts in a Neotropical planthopper pest. The models will be used in future studies for better understand the experimental reduction of YLS in young nymphal stages.

Keywords Argentina · Maize · Yeast-like symbionts · Planthoppers · *Delphacodes kuscheli*

1 Introduction

The interaction between microorganisms that live in the cytoplasm of special fat body cells (mycetocytes) and their sap-feeding host insects is unquestionably mutualistic: microorganisms supply essential amino acids, lipids, and vitamins required by the insects, and the insect is required by the microorganisms for their very existence (Douglas 1998). The interaction is highly complex, often involving developmental mechanisms of the host that ensure transfer of symbionts between generations, mechanisms for controlling symbiont proliferation and location, as well as specialized cell types and host organs (Buchner 1965; Moran 2007; Kono et al. 2008; Nishikori et al. 2009; Vigneron et al. 2014).

Plant sap-sucking hemipterans, harbor obligate, intracellular symbiotic microorganisms to compensate a restricted diet (xylem or phloem sap) deficient in some essential nutrients (Moran et al. 2005; Bauman 2005). Planthoppers (Hemiptera: Fulgoromorpha) of the family Delphacidae are

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phloem sap feeders (Denno and Roderick 1990); they harbor obligate intracellular symbiotic yeast-like symbionts, YLS (subphylum Ascomycota, Class Pyrenomycetes, family Clavicipitaceae) (Noda et al. 1995; Suh et al. 2001) especially in the mycetocytes formed by fat body cells of abdomen (Noda et al. 1995; Xet-Mull et al. 2004). They grow by budding and are vertically transmitted to the next generation by transovarial infection (Chen et al. 1981a; Cheng and Hou 2001; Michalik et al. 2009). The YLS appears to play a role in the nitrogen metabolism of the host through recycling of uric acid (Sasaki et al. 1996; Hongoh and Ishikawa 1997) and supply of the main source of sterol (ergosta-5,7,24(28)-trienol) (Wetzel et al. 1992; Noda and Koizumi 2003). Recently, it was suggested that YLS symbionts can determine or mediate hopper virulence on rice plants and that symbiont functions could change over successive generations of selection on both resistant and susceptible plants (Lu et al. 2004; Chen 2009; Ferrater et al. 2013, 2015).

Different experimental methods to suppress the YLS population have shown the importance of these symbionts in development, reproduction, and embryonic development (Noda and Saito 1979; Lee and Hou 1987), so that their occurrence is absolutely necessary for the survival and reproduction of the host (Chen et al. 1981b). The quantification of the abundance of YLS per host throughout its life cycle was studied in two important rice planthoppers pests in Asia: *Laodelphax striatellus* (Fallén) (Noda 1974, 1977; Noda and Saito 1979) and *Nilaparvata lugens* Stål (Chen et al. 1981a, b). Symbiont population dynamics also has been documented in other insects such as aphids (Hemiptera: Aphididae) (Koga et al. 2003), mealybugs (Hemiptera: Pseudococcidae) (Kono et al. 2008) and beewolves (Hymenoptera: Crabronidae) (Kaltenpoth et al. 2010).

The planthopper *Delphacodes kuscheli* Fennah, 1955- whose generic status is being revised- (Hemiptera: Delphacidae), is a native species widely distributed in Argentina, particularly between latitude 32°–35° S (Remes Lenicov and Virla 1999). It feeds on phloem sap of different cultivated and wild gramineous plants (Brentassi and Remes Lenicov 2007) and it is the most important vector of Mal de Río Cuarto virus (MRCV) (Remes Lenicov et al. 1985; Remes Lenicov and Paradell 2012), which affects maize (*Zea mays* L.) production in the major productive areas of maize in Central Argentina and particularly in the south-west of Córdoba Province (Lenardón et al. 1998). Biological studies on this species allowed the detection of the YLS particularly in the abdominal fat body and in the posterior pole of primary oocytes of *D. kuscheli* (Brentassi et al. 2010, 2014). In the present work we quantified the abundance of YLS per *D. kuscheli* according sex and throughout the host life cycle, and we also analyzed YLS population growth patterns using the well-known logistic model.

2 Materials and methods

2.1 Plants and insects

Experiments were conducted on *Avena sativa* L., a preferential feeding and oviposition host plant of *D. kuscheli*, and the most important winter host plant where outbreaks of insect populations were registered (Remes Lenicov et al. 1991; Ormaghi et al. 1993; Boito and Ormaghi 2008). The plants (5–6 per pot) used for the experiments were grown from seeds in plastic pots (11 cm diameter and 12 cm high) containing sterilized fertile soil and kept free of insects until used in the trials. Plants at vegetative stage of 3–4 leaves were used in the experiments, and at weekly intervals new ones in the mentioned vegetative stage were replaced with them.

D. kuscheli individuals were originally collected in oat crops in Río Cuarto (Córdoba Province, Argentina) to establish a laboratory colony. Several generations were reared on oat, under controlled conditions (24 °C ± 1 °C, 40–50 % RH and a 16 h L:8 h D photoperiod) in a rearing room of the División Entomología, Facultad de Ciencias Naturales y Museo, UNLP. For the experiments we used insects in the following developmental stages: young and embryonated eggs, nymphs (1st to 5th stages) and adults in pre-reproductive, reproductive and post-reproductive stages. Apart from their developmental stages, the insects were also characterized by their ages from oviposition expressed in days, *t*, and each developmental stage were associated with a mean age: oviposition (*t* = 0), young eggs (*t* = 4), embryonated eggs (*t* = 7), 1st instar nymph (*t* = 10), 2nd instar nymph (*t* = 13), 3rd instar nymph (*t* = 16), 4th instar nymph (*t* = 20), 5th instar nymph (*t* = 25), pre-reproductive adult (*t* = 28), reproductive adult (*t* = 38), and post-reproductive adult (*t* = 50) (Brentassi 2004).

2.1.1 Eggs

To obtain the eggs 3 pots with a 3–4 oat plants covered with a cylindrical PET cage (4 cm diameter and 18 cm high with a fine mesh top) were prepared. Five gravid females from the colony were transferred to each pot for oviposition. After 48 h, females were removed. Eggs were obtained from dissection of leaf tissues with fine needles, under a LEICA EZ5 stereoscopic microscope.

2.1.2 Nymphs

Nymphs in the five instars were used, within 24 h period after moulting. We began with 100 first instar nymphs (<24 h from hatching) that were transferred to individual glass tubes (10 × 1 cm diameter) containing fresh leaf pieces of oat. Individual insects were checked daily for ecdysis and survivorship and the following 5 stages were selected: 1st instar nymph, 2nd instar nymph, 3rd instar nymph, 4th instar

nymph, and 5th instar nymph. Fifth-instar nymphs were separated according to sex using the stereoscopic microscope.

2.1.3 Adults

Upon emergence adults of the same age were discriminated by sex and the following maturation stages, according to females, considered: pre-oviposited (28 days old), peak oviposited (38 days old), and post-oviposited (50 days old). Peak oviposition represents the maximum reproductive effort when a maximum of 20 eggs per day per female were oviposited (Brentassi 2004).

2.2 The weight of an individual host as an indicator of the amount of fat body

As mentioned, the fat body of an insect is a dynamic tissue where YLS are found living inside vacuoles of certain cell types, in permanent symbiosis (Dean et al. 1985; Arrese and Soulagues 2010).

We considered that the environment where YLS could be found was the fat body mass of the host, as well as in the oocytes of reproducing adult females. As we did not directly measure the fat body mass of each host, we considered that the body weight of *D. kuscheli* represents a proportion of the amount of its fat body content. This assumption was based on the work by Lease and Wolf (2011) who found that in adult insects, arachnids and arthropods in general, the lipid content shows an isometric scaling relationship with respect to body mass. For that purpose, hosts in all nymphal instars (1st to 5th) and adults in the different mentioned stages were individually weighted (number of replicates varied between 10 and 30). For the egg stage, instead of individual eggs, we weighted groups ($n = 5$) of 50 eggs each and the weight of an individual egg was calculated dividing the total group weight by 50. We used a four digit-balance (Acculab Sartorius Group, ALC210.4) and the weight was expressed in mg.

The weight of an individual host of age t , $W_{(t)}$, increased during its life cycle, and we used the Day (1966) growth model to describe it: $W_{(t)} = Lm/[1 + e^{-(d-kt)}]$, where, t is the age (in days from oviposition) of the different developmental and reproducing adult stages, Lm represents the maximum observed weight, and d and k are constants. It was shown that this model is a particular case of a generalized von Bertalanffy model (Day 1966). Only for peak oviposited females, apart from its weight we added the weight represented by the number of mature oocytes calculated as: $WE_{(t=38)} = 20 \cdot W_{(t=4)}$, where 20 represents the mean maximum number of eggs deposited during peak reproduction (Costamagna et al. 2005) and $W_{(t=4)}$ the weight of an individual young egg.

The differences between the weight of males and females in the 5th instar nymphs and in the maturation adult stages were tested by a two-way ANOVA. Homoscedasticity was

analyzed by means of the Levene's test and normality of data by means of the Lilliefirs test.

2.3 Quantification of the abundance of YLS per host

The abundance, considered as the number of YLS per host, in the stages mentioned was estimated using a haemocytometer (Neubauer Chamber, BOECO Germany) according to Noda (1974). Insects were individually macerated with a steel micropestle in an Eppendorf tube (2.5 ml) in which 0.5 ml of sterile water was added. The homogenization was carried out with manual agitation and the content was filtered with a fine mesh before used. The number of YLS present in the 25 large squares of each central grid of the chamber were counted and then averaged. This number multiplied by 10^4 and by 0.5 allowed us to estimate the total number of YLS in the count (i.e. the total number of YLS per host) (Undeen and Vávra 1997). The number of replicates was: 1st instar ($n = 14$), 2nd instar ($n = 13$), 3rd instar ($n = 11$), 4th instar ($n = 22$), 5th instar males ($n = 18$), 5th instar females ($n = 12$), and for both sexes of each pre-oviposited, peak oviposited and post-oviposited adults ($n = 10-20$). For young and mature eggs, instead of individuals we used groups of 30 eggs ($n = 5$ in each case) and the number of YLS per egg was obtained dividing by 30 the total number of YLS per group.

For male and female hosts of age t we calculated the number of YLS per unit host body mass, $\alpha_{(t)} = YLS_{(t)}/W_{(t)}$, where $YLS_{(t)}$ represents the number of YLS per host of age t , and compared the mean values by means of the t test. Excluding the egg stage (young egg $YLS_{(t=4)}$ and embryonated egg $YLS_{(t=7)}$), we plotted $\alpha_{(t)}$ on host age t if $\alpha_{(t)}$ was independent of host's age (i.e., represents a constant proportion of the host's weight) the correlation between $\alpha_{(t)}$ and the host age t would not differ from zero. In this case the mean value, α , would represent a situation where the number of YLS per unit host body mass was independent of host's age (and host weight), as occurred through nymphal period in the symbiotic interaction YLS-*N. lugens* studied by Chen et al. (1981a).

For a host of age t , we also calculated the rate of increase, $a_{(t)}$, as: $a_{(t)} = [YLS_{(t+\Delta t)} - YLS_{(t)}]/\Delta t \cdot [YLS_{(t+\Delta t)} + YLS_{(t)}]/2$, where Δt represents the duration of the t^{th} instar or developmental host stage expressed in days, and $YLS_{(t)}$ and $YLS_{(t+\Delta t)}$ represent the number of YLS per host in two successive stages or instars characterized by their ages t and $t + \Delta t$, respectively. The estimation was made during the intervals: embryonated eggs-1st instar nymph, up to 3rd-4th instar nymph, where the increase of YLS was higher. We analyzed $a_{(t)}$ for density-dependence by plotting $a_{(t)}$ on the corresponding number of YLS per host, indirectly estimated by means of $W_{(t)}$ which was not used in the calculation of $a_{(t)}$.

2.4 The models for describing the number of YLS per host according to sex

In females of other planthopper species, the number of YLS per host through their life cycles (Noda 1974; Chen et al. 1981a) exhibited a growth pattern that, after a moderate increase, accelerates and then slowed down up to a plateau reached in the adult stage. Following those results, we considered that for *D. kuscheli* females a logistic type model would be adequate to describe the number of YLS per host throughout its life cycle. The parameter estimates below and their equations for abundance of YLS per *D. kuscheli* are summarized in Table 1. We calculated the number of YLS per host of age t , $cYLS_{(t)}$, using the Verhulst-Pearl logistic model: $cYLS_{(t)} = KN_0e^{rt}/K+(e^{rt} - 1)N_0$ (Royama 1992), where N_0 represents the initial number of YLS, r represents the intrinsic per capita rate of natural increase of YLS, and K represents the carrying capacity. As mentioned by Seidl and Tisdell (1998) exogenous environmental forces and/or variation in population size may, in general terms, cause variation in the carrying capacity of a population. So, our model was adapted considering that the carrying capacity K varies along the life cycle according to a constant proportion of the host's weight, α , i.e., $K_{(t)} = \alpha W_{(t)}$. Apart from that, we hypothesized that no time delay would be necessary to include in the model because the increase in the carrying capacity would be synchronic with the increase of YLS. In a second version of the model, developed

for describing $cYLS_{(t)}$ in *D. kuscheli* males, we relaxed the assumption of α being a constant irrespective of host's age, and allowed it to be a decreasing function of the latter, $\alpha_{(t)}$, so: $K_{(t)} = \alpha_{(t)}W_{(t)}$. We based this assumption following the patterns described for other planthoppers (Noda 1974; Chen et al. 1981a, b), as well as possible mechanisms developed by insects host for controlling symbiont proliferation and location (Buchner 1965; Kono et al. 2008; Nishikori et al. 2009; Vigneron et al. 2014).

The coefficients (N_0 , α , and r) were subjected to parameterization, i.e., the fit of the model to sampled data to estimate the model's parameter values and initial conditions using an iterative technique that minimizes the differences between calculated and observed data by means of the Solver tool from Microsoft Excel® software. The already adjusted coefficients Lm , d and k of the Day (1966) growth model, and the coefficients that describe $\alpha_{(t)}$ as a decreasing function of the host's age, which in turn were calculated values to give the best fit, were not subjected to parameterization.

We did not validate the model due to the absence of independent data (Haefner 1996). Instead we compared the parameterized values of the coefficients with the corresponding estimated values in laboratory experiments by means of the t_s test that compares a single value (calculated) with an estimated sample: $t_s = (Y_1 - Y_2)/s [(n_2 + 1)/n_2]^{1/2}$, where Y_1 and Y_2 represent the calculated and estimated values, respectively, s represent

Table 1 Parameter estimates (or their equations) for abundance of YLS per *D. kuscheli*

| Parameter | Symbol | Value or equation | Units | Source |
|---|-----------------|---|------------------------------------|----------------------|
| Nr of YLS per host (YLS changes with host age) | YLS Number | $cYLS = KN_0e^{rt}/K+(e^{rt} - 1)N_0$ (Royama 1992) | | |
| Time | t | $t = 1, 2, \dots, 50$ | Day (host age from oviposition) | |
| Rate of per capita natural increase | $\bar{a}_{(t)}$ | 0.2129 (both sexes) | day ⁻¹ | This work (1) |
| Carrying capacity | $K_{(t)}$ | Female hosts : $\alpha W_{(t)}$, and $\alpha = 167,989.2$ Male hosts : $\alpha_{(t)}W_{(t)}$, and $\alpha_{(t)} = [-115,937 \ln(t) + 486509] \cdot W_{(t)}$ | Number | This work (2) |
| Initial number of YLS per host egg | N_0 | 1,210.5 (both sexes) | Number | This work (3) |
| Mean number of YLS per host weight | α | 179,600.3 (female hosts) 133,698.6 (male hosts) | Number/mg | (Day 1966) This work |
| Mean maximum host weight | W_m | 1.2488 (female host) 0.7804 (male host) | mg | This work (4) |
| Constant | d | $d = \ln [W/0.023 - 1]$ $d = 3.8705$ (female host) $d = 3.4894$ (male host) | abstract number | This work (5) |
| Rate of host weight increase | k | 0.2052 (female host) 0.2561 (male host) | day ⁻¹ | This work (6) |

¹ Rate of per capita natural increase in female hosts, calculated minimizing sum of square differences (SSQ)

² Carrying capacity, estimated in the laboratory

³ Initial number of YLS per host egg, estimated in the laboratory

⁴ Mean maximum host weight, estimated in the laboratory

⁵ Constant d , calculated minimizing SSQ

⁶ Rate of host weight increase, calculated minimizing SSQ

the standard deviation of the estimated values and n_2 the number of replicates (Sokal and Rohlf 1995). In the case of female hosts, the values estimated in laboratory experiments to be compared to the parameterized values (N_0 , α , and r) were, respectively: the number of YLS recorded in young eggs (i.e., $YLS_{(t=4)}$), the mean number of YLS per unit host body mass (α), and the mean rate of increase during the intervals embryonated eggs–1st instar nymph, up to 3rd instar nymph–4th instar nymph (\bar{a}). In the case of male hosts, the values estimated in laboratory experiments to be compared to the parameterized values were N_0 and \bar{a} , and in both cases were the same as for females. The number of YLS per unit host body mass decreased with age (see below) instead of being independent of host age, and no mean value was estimated.

3 Results

3.1 The weight of an individual host as an indicator of the amount of fat body

The mean body weight of successive developmental stages of the host, which as mentioned was considered to represent a fixed proportion of its fat body, is shown in Fig. 1. The weight increased during pre-imaginal stages, while once in the adult stage, remained approximately constant except in peak-reproducing females whose weight increased due to the mature oocytes. The mean weight throughout the life cycle of *D. kuscheli* was: young egg: $W_{(t=4)} = 0.0233$ (SD = 0.0028),

embryonated egg: $W_{(t=7)} = 0.0233$ (SD = 0.0028), 1st instar nymph: $W_{(t=10)} = 0.0686$ (SD = 0.0135), 2nd instar nymph: $W_{(t=13)} = 0.189$ (SD = 0.0102), 3rd instar nymph: $W_{(t=16)} = 0.230$ (SD = 0.0534), 4th instar nymph: $W_{(t=20)} = 0.702$ (SD = 0.0928), 5th instar nymph male: $W_{(t=25)} = 0.7389$ (SD = 0.0864), 5th instar nymph female: $W_{(t=25)} = 1.030$ (SD = 0.1137), pre-oviposited adult male: $W_{(t=28)} = 0.6650$ (SD = 0.4210), pre-oviposited adult female: $W_{(t=28)} = 1.065$ (SD = 0.3317), peak oviposited adult male: $W_{(t=38)} = 0.9425$ (SD = 0.1706), peak oviposited adult female: $W_{(t=38)} = 1.6802$ (SD = 0.1718), post-oviposited adult male: $W_{(t=50)} = 0.7750$ (SD = 0.0354), and post-oviposited adult female: $W_{(t=50)} = 1.220$ (SD = 0.1442).

In those instars or stages where hosts were discriminated by sex, (5th nymphal instar and adults of 28, 38, and 50 days old from oviposition), females (1.2488 mg; SD = 0.31) were heavier than males (0.7804 mg; SD = 0.28) ($F = 28.537$, $df = 40$, $P = 0.000004$).

The weight of a host of t days from oviposition, $W_{(t)}$, estimated by the Day's (1966) growth model was also presented in Fig. 1. In males the model predicts the same weight throughout all adult stages considered. In females, the model also predicts the same weight throughout adult stages, except in the case of peak-reproducing females whose value must be added to the total weight of the mature oocytes. In this case, the total weight of peak-reproductive female was: $W_{(t=38)} = 1.6802$ mg. Subtracting 0.466 mg which correspond to the weight of 20 mature oocytes (0.0233 mg per young oocyte), the body weight of the adult female was $1.6802 - 0.466 = 1.2142$ mg. When we grouped all adults independently of their ages (except peak oviposited females), differences between their mean



Fig. 1 Weight of different stages of *D. kuscheli* (in mg) expressed in days from oviposition. The lines are calculated values: the black line represents the weight of female hosts and the dashed line the weight of the females plus the weight of 20 mature oocytes, while the grey line represents the weight of male hosts. The symbols represent: YE (young eggs), ME (mature eggs = embryonated eggs), 1st (first nymphal instar), 2nd

(second nymphal instar), 3rd (third nymphal instar), 4th (fourth nymphal instar), 5th (fifth nymphal instar), Ad-28 (Pre-Reproductive adults, 28 days after oviposition), Ad-38 Peak- Reproductive adults, 38 days after oviposition), and Ad-50 (Post- Reproductive adults, 50 days after oviposition). (Confidence intervals at 95 %)

weight and that of the fifth instar nymph was not significantly different ($F = 0.65$, $df = 46$, $P = 0.424$).

3.2 Quantification of the abundance of YLS per host

In female hosts the number of YLS increased up to the onset of the adult stage and then remained almost constant, varying similarly to individual weight. In consequence, the weight of individual hosts of age t , $W_{(t)}$, was highly correlated with the number of YLS/host of the same age, $YLS_{(t)}$: $r = 0.9405$, ($F = 61.3608$, $df = 8$, $P = 0.00006$). In male hosts the number of YLS increased up to the 5th instar nymph as the individual weight did, but unlike females, the number of YLS decreased in the adult stage and the correlation was not so good as that of the females ($r = 0.6963$); ($F = 5.4410$, $df = 8$, $P = 0.048$) (Fig. 2).

The mean number of YLSs per host along its life cycle was: young egg: $YLS_{(t=4)} = 1,210.5$; (SD = 386.8), embryonated egg: $YLS_{(t=7)} = 4,652.5$ (SD = 491.4), 1st instar nymph: $YLS_{(t=10)} = 25,714.3$ (SD = 15,455.5), 2nd instar nymph: $YLS_{(t=13)} = 29,230.8$ (SD = 24,481.8), 3rd instar nymph: $YLS_{(t=16)} = 30,000$ (SD = 25,224), 4th instar nymph: $YLS_{(t=20)} = 58,068.2$ (SD = 26002.9), 5th instar nymph male: $YLS_{(t=25)} = 99,166.7$ (SD = 46241.1), 5th instar nymph female: $YLS_{(t=25)} = 120,833.3$ (SD = 21,698.7), pre-oviposited adult male: $YLS_{(t=28)} = 30,000$ (SD = 63,290.8), pre-oviposited adult female: $YLS_{(t=28)} = 234,444.4$ (SD = 108,231.7), peak oviposited adult male: $YLS_{(t=38)} = 58,734.4$ (SD = 55,357.1), peak oviposited adult female: $YLS_{(t=38)} = 263,636.4$ (SD = 105,097.4), post-oviposited adult male: $YLS_{(t=50)} = 6,346.2$ (SD = 6,342.3),

and post-oviposited adult female: $YLS_{(t=38)} = 247,023.8$ (SD = 62,177.3). In the 5th instar nymph the number of YLS did not differ between females and males ($t = 1.1018$, $df = 13$, $P > 0.20$), but in adults the mean number of YLS in females: 248,360.2 (SD = 14,642.3), was greater than the mean number of YLS in males: 33,901.1 (SD = 19,795.9) ($t = 12.32$, $df = 4$, $P < 0.001$, two tails test).

The mean number of YLS per host body weight (all stages considered) did not differ between host sex: in females, $\alpha = 179,600.3$ YLS/mg (SD = 88,920.4); in males, $\alpha = 133,698.6$ YLS/mg (SD = 105,728.4) ($t = 0.000006$, $df = 18$, $P > 0.20$, two tails test). In females the correlation coefficient between the number of YLS per host body weight and the host age expressed in \log_e from the 1st instar nymph, did not differ from zero: ($r = -0.1576$); ($t = -0.3909$, $df = 7$, $P = 0.7094$). So, in females α was independent of host's age and was represented by its mean value: $\alpha = 194,160.2$ (SD = 94,628.6) YLS/mg. In contrast, in male hosts the correlation was significantly lower than zero: ($r = -0.9363$), ($t = -6.5342$, $df = 7$, $P = 0.0006$). The corresponding regression equation was: $\alpha_{(t)} = -193,724 \ln(t) + 744,476$.

Concerning the per capita net rate of increase of YLS, $a_{(t)}$, when we plotted $a_{(t)}$ on the host weight, $W_{(t)}$, we found a negative relationship in both sexes. This result is consistent with the stabilization or decrease in the number of YLS in the host adult stages. However, only in males the relationship was significant: $a_{(t, \text{male})} = -0.1245 \log_e W_{(t)} + 0.0736$, ($r = -0.6813$), ($t = -2.4626$, $df = 7$, $P = 0.0433$), while in females it did not: $a_{(t, \text{female})} = -0.0717 \log_e W_{(t)} + 0.0813$, ($r = -0.6358$), ($t = -2.1792$, $df = 7$, $P = 0.0657$). The average

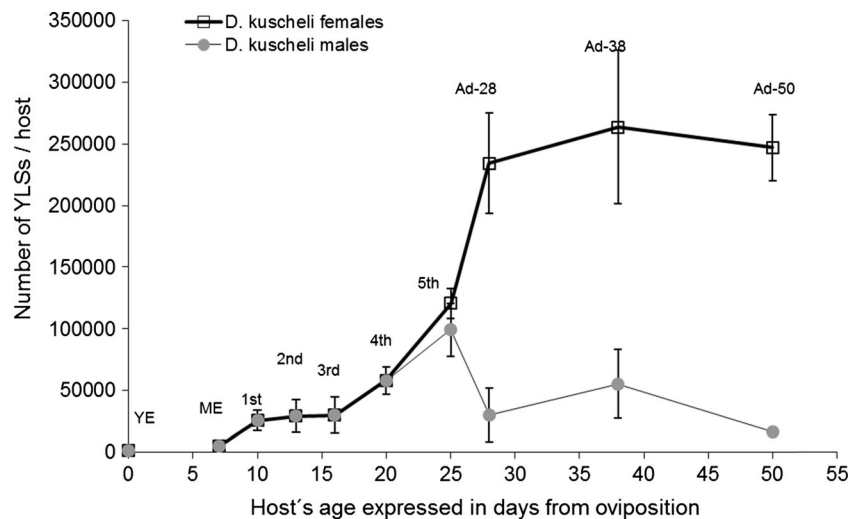


Fig. 2 Number of YLS per host along its life cycle and according to sex plotted on the host's age expressed in days from oviposition. The symbols represent: YE (young eggs), ME (mature eggs = embryonated eggs), 1st (first nymphal instar), 2nd (second nymphal instar), 3rd (third nymphal instar), 4th (fourth nymphal instar), 5th (fifth nymphal instar), Ad-28

(Pre-Reproductive adults, 28 days after oviposition), Ad-38 Peak-Reproductive adults, 38 days after oviposition), and Ad-50 (Post-Reproductive adults, 50 days after oviposition). (Confidence intervals at 95 %)

value of the first five rates (from embryonated eggs to 4th instar nymph) that were equal for both sexes was $\bar{a} = 0.2129$ (SD = 0.2047).

3.3 The models for describing YLS dynamics in female insects

In females the number of YLS per host of age t , $cYLS_{(t)}$, is shown in Fig. 3. Except in the egg stage, in only three cases (1st, 2nd and 5th instar nymphs) the calculated values were out the 95 % confidence interval of the estimated values. In the pre-imaginal stages the rate between the number of YLS in two successive ages, $cYLS_{(t+\Delta t)}/cYLS_{(t)}$, fluctuated between $2.53 = cYLS_{(t=7)}/cYLS_{(t=4)}$ and $3.11 = cYLS_{(t=26)}/cYLS_{(t=20)}$. Once in the 5th instar and in the adult stage, the rate fluctuated between $1.35 = cYLS_{(t=28)}/cYLS_{(t=25)}$ and $1.01 = cYLS_{(t=50)}/cYLS_{(t=38)}$ indicating the stabilization of $cYLS_{(t)}$. The rapid increase of YLS matched the increase in female host body weight: $1.73 = W_{(t=7)}/W_{(t=4)}$ and $1.56 = W_{(t=20)}/W_{(t=16)}$. Once in the 5th instar and in the adult stage, the rate fluctuated between $1.11 = W_{(t=28)}/W_{(t=25)}$ and $1.02 = W_{(t=50)}/W_{(t=38)}$ also indicating the stabilization of the female host weight.

The estimated value for the initial number of YLS corresponded to young eggs ($No = 1210.5$) did not differ significantly from the parameterized value ($No = 175.13$), ($t = 2.444$, $df = 4$, $0.1 > P > 0.05$). The estimated mean number of YLS per host weight ($\alpha = 167,989.2$ did not differ from the parameterized value ($\alpha = 228,803.2$) ($t = 0.618$, $df = 7$, $P > 0.20$), while the estimated mean per capita rate of natural increase, $\bar{a} = 0.2129$, did not differ significantly from the parameterized value ($r = 0.3105$), ($t = 1.9683$, $df = 4$, $P > 0.10$, two tails test), which determines a duplication time (with respect to $No = 1210.5$) of 2.23 days if the increase of YLS was exponential.

3.4 The models for describing YLS dynamics in male insects

In males, the carrying capacity was written as a decreasing function of a host age: $K_{(t)} = [-115,937 \text{ Ln}(t) + 486509] \cdot W_{(t)}$ and the calculated number of YLS per host of age t , $cYLS_{(t)}$, decreased in the adult stages. Except in the egg stage, the calculated values, corresponding to the 1st and 5th instar nymph, and the pre-oviposited and post-oviposited adults were out the 95 % confidence interval of $YLS_{(t)}$ (Fig. 4).

The estimated value for the initial number of YLS corresponded to young eggs was the same as for females ($No = 1210.5$), and significantly smaller than the parameterized value ($No = 3141.43$), ($t = 4.557$, $df = 4$, $0.02 > P > 0.01$). The parameterized value of the intrinsic per capita rate of natural increase of YLS was: $r = 0.1955$ which determines a duplication time (with respect to $No = 1210.5$) of 3.55 days if the increase of YLS was exponential, and did not differ from the mean estimated value ($\bar{a} = 0.2129$), ($t = 2.2809$, $df = 4$, $P > 0.05$, two tails test).

4 Discussion

In host-parasite interactions Bush and Holmes (1986) proposed a hierarchical classification scheme, and according to these authors the lowest level was denominated an infrapopulation, which refers to all individuals of a parasitic species in a host individual at a particular time. In this study we dealt with a mutualistic interaction between a planthopper (host) and intracellular yeast-like mycetocyte microorganisms, YLS (endosymbionts). We quantified and modeled the number of YLS per host throughout its life cycle, and in a sense it could

Fig. 3 Estimated (black squares) and calculated (continuous curve) number of YLS per female host of age t , $cYLS_{(t)}$ (confidence intervals at 95 %). The parameterized values of the coefficients were: $r = 0.3105$, $No = 175.13$ YLS/egg, and $\alpha = 228,803.2$, while the estimated values were: $No = 1210.5$ YLS/egg, and $\alpha = 194,160.2$ (SD = 94,912.3). The dashed line represents the carrying capacity

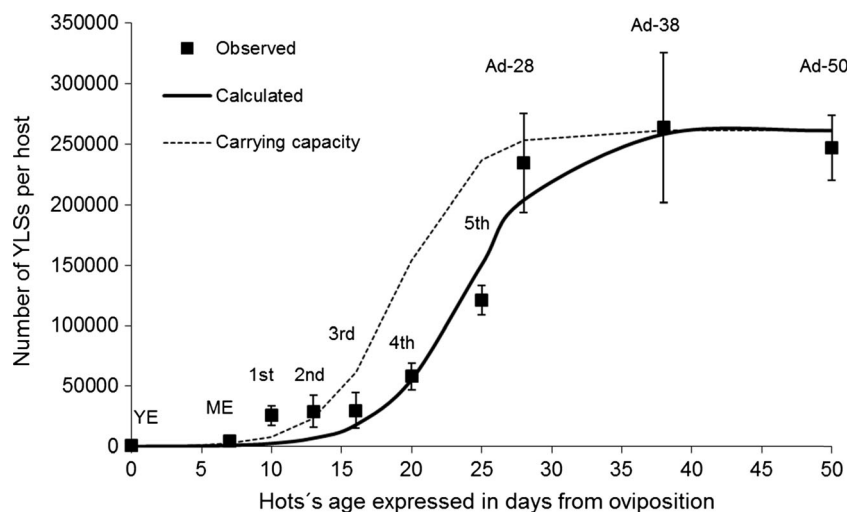
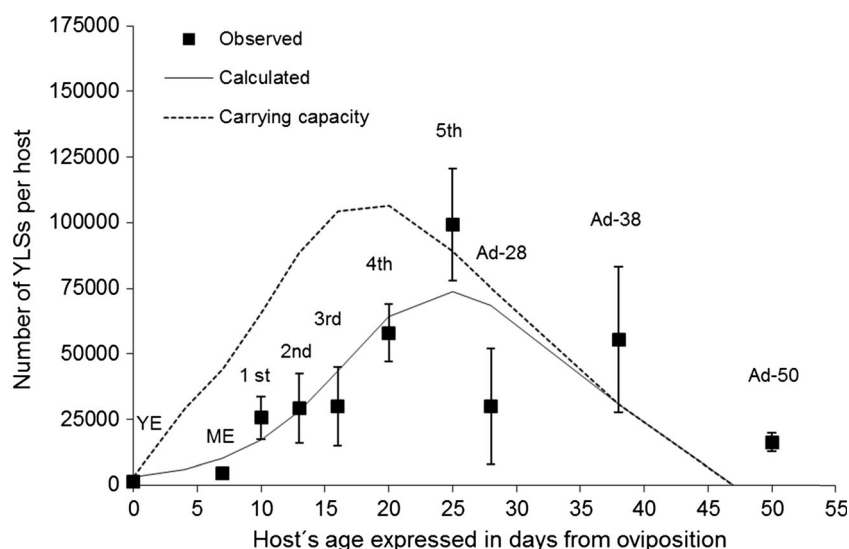


Fig. 4 Estimated (black squares) and calculated (continuous curve) number of YLS per male host of age t , $cYLS_{(t)}$ (confidence intervals at 95 %). The scale in the ordinate is half that from females in Fig. 3. The parameterized values of the coefficients were: $r = 0.1955$, $N_0 = 3141.3$ YLS/egg, while the estimated values for the initial number of YLSs was: $N_0 = 1210.5$ YLS/egg). The dashed line represents the carrying capacity



be considered as the dynamics of an infrapopulation of YLS along the development of the planthopper host.

The YLS play an essential role in supporting planthopper nutrition and development (Noda et al. 1995; Chen 2009) and it was suggested that the number of YLS was maintained at a definitive level during nymphal stages of the host through mechanisms that regulate their propagation (Chen et al. 1981a). Once in the adult stage of the planthopper *L. striatellus*, as was observed in our study, it was registered a rapid decline of YLS in males, which was attributed to certain factor/s, which may destroy the balanced relationship between host and symbionts (Noda 1974). Degradation mechanisms regulating the population of symbionts have been mentioned in other insects such as mealybugs (Hemiptera: Coccoidea: Pseudococcidae) (Kono et al. 2008), the pea aphid *Acyrtosiphon pisum* (Harris) (Nishikori et al. 2009), cereal weevils (Coleoptera) (Vigneron et al. 2014) and other insect-microbe symbiotic associations (Buchner 1965). To date the mechanism/s which regulates symbionts populations remains unknown.

Concerning YLS-*D. kuscheli* system, the YLS live in permanent symbiosis inside mycetocytes, a particular cell type of the insect fat body. The fat body is a dynamic tissue distributed throughout the body involved in the synthesis and utilization of energy reserves in response to insect's demands and considered an organ of great biosynthetic and metabolic activity (Arrese and Soulages 2010). In our study, we quantified and modeled the number of YLS throughout the life cycle of *D. kuscheli* according to sex adapting one expression of the logistic model depicted by Royama (1992). We considered that the fat body of the host represents the

main resource where the YLS live and reproduce and the fat body was indirectly estimated by the host body weight, based on the study by Lease and Wolf (2011) who demonstrated that the lipid content of adult insects, arachnids, and arthropods in general shows an isometric scaling relationship with respect to body mass.

We found that the weight of *D. kuscheli* increased during pre-imaginal stages similarly to the increased recorded in the planthopper *N. lugens* by Chen et al. (1981a). Once in the adult stage and in both sexes the weight of *D. kuscheli* remained approximately constant, except in the peak-oviposition stage of females due to development of oocytes. We also found that *D. kuscheli* adult females were heavier than the adult males, in agreement with Zera and Denno (1997) who found that in Hemiptera the fat tissue is more abundant in females than in males and with the recently studies of Wan et al. (2015), who reported that the body weight of newly emerged female adults of *L. striatellus* (Fallén) was significantly greater than that of newly emerged male adults. Furthermore, Lease and Wolf (2011) evidenced that female insects and arachnids generally have higher lipid contents than males.

We described adequately the body weight of *D. kuscheli* along its life cycle using the model of Day (1966). The body weight, $W_{(t)}$, and the number of YLS per host weight at age t , $\alpha_{(t)}$, were the two components of the carrying capacity, $K_{(t)}$, of the logistic model. The equations used to represent $\alpha_{(t)}$ were determinate by an empirical fit to data, and differed between the sexes. In female hosts the equation that best fitted the data suggested that $\alpha_{(t)}$ was independent of host's age and was represented by a constant. In male hosts the equation indicated a decreased of $\alpha_{(t)}$ as the hosts aged. The carrying

capacity curve of female hosts exhibited a saturation trend similar to the function that described the female body weight, $W_{(t)}$, as: $K_{(t)} = W_{(t)} \cdot \alpha$, with α constant. In contrast, the carrying capacity of male hosts exhibited an optimal curve type whose maximum value occurred in the 5th nymphal instar. This could happen due to the consumption of the fat body in the adult stage of the insect, however in females this could be balanced by the development of oocytes as another resource for YLSs.

The calculated number of YLS per host matches reasonably well the number of YLS estimated experimentally. In females it continuously increased after emergence stabilizing once in the adult stage, except at the peak-reproductive stage where it exhibited the maximum value. The maximum value was possibly in relation to the development of ovaries and the transovarial transmission of YLS as was suggested for others delphacids species by Noda (1974) and by Chen et al. (1981a). In males the calculated number of YLS per host also matches reasonably well the data estimated experimentally, and was lower than in females: the maximum number of YLS was attained at the 5th nymphal instar and then declined rapidly. However, the body weight in the 5th instar nymph and in the adult stages remained approximately constant, which implies, following our assumption, that the fat body mass would have remained unchanged. Notwithstanding, the number of YLS calculated and experimentally estimated, decreased which could be due to a decreased not in the amount of basic fat body cells but of mycetocytes.

In two planthopper species: *L. striatellus* and *N. lugens* similar patterns were observed in the number of YLS per host, as well as in the number of YLS per host weight (Noda 1974; Chen et al. 1981a). In reference to *L. striatellus*, as was mentioned before, it was suggested that the rapid decline of YLS in adult males would involve a certain factor, which may destroy the balanced relationship between host and symbionts (Noda 1974). In our models the mentioned unknown factor/s were implicitly considered in terms of $\alpha_{(t)}$, one of the factors used in the calculation of the carrying capacity K .

Following these trends, we showed that in females, the host body weight was highly correlated with the number of YLS per host, while in males due to the reduction in the number of YLS/host in the adult stage; the correlation was not so good. Recently Wan et al. (2015) also reported differences between females and males of the planthopper *L. striatellus*: only in females a significant increase in the abundance of YLS and in their body weight was found. These observations suggest that in planthoppers differences between sexes exists in relation to their weight and also in the abundance of YLS.

The evolutionary ecological aspect of the male-specific symbiont reduction has been documented in insects. Kono et al. (2008) reported that in the development pathway of males of two species of mealybugs: *Planococcus kraunhiae* and *Pseudococcus comstocki*, the endosymbiotic system degenerated progressively and the endosymbionts were almost lost in adult males. Males of these species neither feed nor grow but just molt and metamorphose. Considering the putative nutritional roles of the endosymbionts for the sap feeding host insects the endosymbiotic system is plausibly not necessary for males of the mealybugs. Similar male-specific absence of the endosymbiotic system has been reported in soldiers and males of an eusocial aphid, *Colophina arma* (Aoki) by Fukatsu and Ishikawa (1992). One explanation for the lack of the endosymbiont in these morphs, which do not feed, is the "host's selection hypothesis" which assumes that the host reject the symbiont's infection or disrupt the infecting symbiont. It considers the nutritional interaction between the host and symbiont. The plant phloem sap is nutritionally quite unbalanced, and the most important role of the symbiont seems to synthesize the essential nutrients and supply the host with them (Sasaki et al. 1991). If the host does not feed, the symbiont should be unnecessary. In such a case, the symbiont may be harmful rather than unnecessary since to harbor endosymbionts might cost energy and resources. Furthermore adult males need far less resources than adult females not only because they are smaller but also because to produce sperm costs far less than to produce eggs (Fukatsu and Ishikawa 1992). The rapid decline of YLS in adult males of *D. kuscheli* could be explained in this context. If to harbor endosymbionts might cost energy and resources, it is conceivable that adult males of *D. kuscheli* reduce the amount of YLS contrary to adult females. Further, in cases where the adults do not feed at all, like some aphids and scale insects, the symbionts should be unnecessary and only the male lacks the symbionts (Toth 1933; Toth 1937; Lampel 1959 in Fukatsu and Ishikawa 1992).

As mentioned, similar patterns in the number of YLS, experimentally estimated in female and male hosts, were reported in two rice pests in Asia: *L. striatellus* (Noda 1974, 1977; Noda and Saito 1979) and by Chen et al. (1981a, b) in *N. lugens*. However, to our knowledge this is the first study that quantified and modeled the dynamics of YLS endosymbionts in a Neotropical planthopper pest. The models developed here for male and female hosts, will be used in future studies as reference for better understanding the experimental reduction of YLS in young nymphal stages as well as in studies that quantify the *D. kuscheli* YLS abundance in field conditions.

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Compliance with ethical standards

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