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Influence of male age on producing rival calls in brown planthopper *Nilaparvata lugens* Stál (Homoptera: Delphacidae)

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Abstract
Brown planthopper (BPH) *Nilaparvata lugens* is an imperative insect pest of rice crop throughout the Asia. Both BPH male and female rely on substrate borne vibrational signals prior to mate. Often such mating communication is affected by rivalry signals of other existed males of variable age and population. Therefore, the current study observed the effect of age and population density on production of male rival calls. The results illustrated that the male rival calls were only produced in the presence of other male. No single male rival call was produced when a pair of a matured male and female of five days old was placed on rice plant. However, production of rival calls increased with increasing the age of BPH male and maximum number of (90%) male produced rival calls at the age of fifth days. Similarly, the population density affected the behaviour of male for producing the rival calls. A maximum number of male produced rival calls, when population of seven virgin males were reared together prior to experiment. Such results showed that the presence of rivalry signals during mating communication is a crucial part of mating behaviour of brown planthopper and only observed among males. Understanding the influence of these factors on mating behaviour of BPH is essential as it serves as baseline for exploiting the mating signals of BPH to reduce the population density below the economic injury level.

Keywords: Brown planthopper (BPH); rival signals; rice plant, insect age, insect population density

1. Introduction
Brown planthopper (BPH), *Nilaparvata lugens* (Stàl) is a serious pest of rice that has caused significant yield losses over the years especially in Asian countries [1, 2]. Both nymphs and adults like other homopterian [3a, b] suck the cell sap and their feeding damage in rice field is referred as "hopper burn". They are also responsible for transmitting the plant viral diseases such as rice ragged stunt virus (RRSV) and rice grassy stunt virus (RSGV) [4]. Both adult male and female exhibit substrate borne vibrational signals prior to mate, that plays a crucial role in the mating behaviour of brown planthopper [5]. During such mating communication; competition between males has also been commonly observed in numerous planthopper species [6a]. Often, age and population density play a significant role in the intensity of competitive behaviours displayed by competing individuals.
In BPH, competitions are displayed in the form of rival calls and physical harassment by dominant males. Using these rival calls, males try to disrupt the on-going mating communication between a pair of dueting male and female and very often mating disruption is successfully achieved [6b]. The specificity and functional importance of the above highlighted competitive communication behaviour in BPH males, further presents an option for it to be manipulated for developing effective pest control measures.
Therefore, this study was attempted to understand the effect of male age and density on production of rival calls in BPH thus, enabling to devise a technique that will be helpful in future to disrupt the vibrational communication between BPH male and female.

2. Materials and Methods
2.1 Collection and rearing of BPH
Insects were collected randomly using a sweep net from the rice fields of Tanjung Karang, Selangor, Malaysia and with an aspirator from Field-10, Universiti Putra Malaysia during 2014-15. The collected specimens were brought to the glass house at Department of Plant

Protection, Faculty of Agriculture, Universiti Putra Malaysia and reared in two wooden cages (90 x 42 x 42 cm) covered with mesh for proper aeration at 28 ± 2 °C, 70% Relative Humidity and photoperiod of 12D:12L. Both temperature and relative humidity were recorded on simple graph paper using a hygrothermograph (Mechel -15 + 45 °C, Italy) placed inside the glass house. Throughout the study, insects were reared on the rice variety of MR-219. This produced the main culture for the two insect populations.

Insects used for the following experiments were obtained from the main culture as mentioned above and rearing of virgin insects was further performed. As age and population density are known to affect the production of rival calls, the influence of both these factors were first observed by subjecting insects to two different rearing procedures as described below.

To observe the influence of age on production of rival calls, newly emerged virgin adult males were reared on fresh rice seedlings at a population density of five adults per rearing jar (Figure 1a). Insects reared in this experiment were within one day to seven days old and each rearing jar represented specific age groups. For experimental purpose, four age-groups were selected such as one day old, three days old, five days old and seven days old to observe the production of rival calls. Meanwhile, to study the influence of population density on production of rival calls, newly emerged virgin adult males were reared at different adult densities until five days (Figure 1b). The rearing densities were one, three, five and seven males per rearing jar.

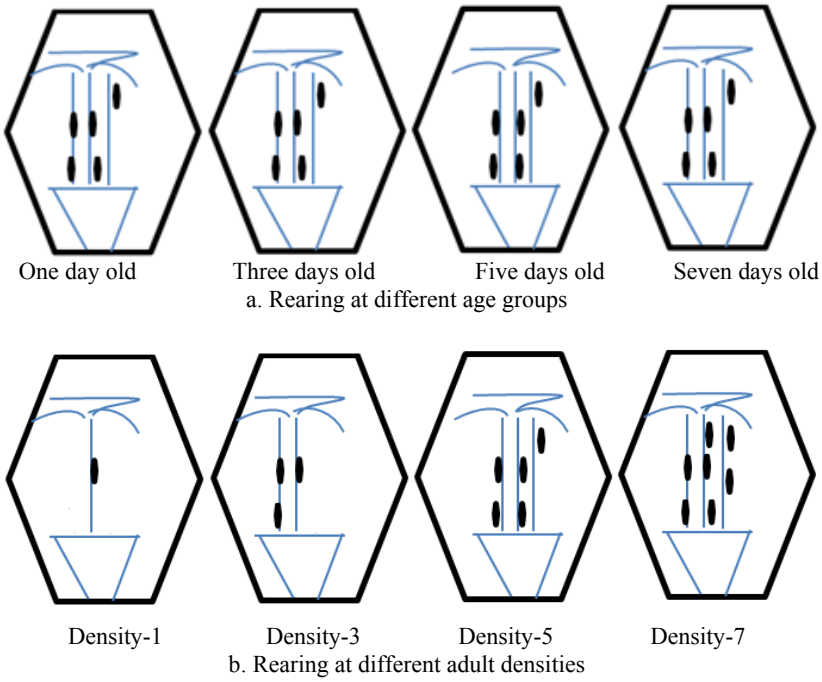

Fig 1: Two different rearing methods of virgin adult male brown planthopper

2.2 Recording of BPH male rival calls

The introduction of BPH males on rice plant and initial setup for mating behaviour was similarly followed as mentioned by [7]. Males from both rearing groups (age and population density) were introduced onto 30-40 days old rice plants individually or in pairs to observe the number of males that produced rival calls. Throughout the experiment, released male BPH were stimulated by five days old female. Leaf blades of both plants were pre-connected, therefore permitting the transfer of mating (vibratory) responses in both sexes. The observation time for each replication was 10 minutes and ten replications for single and pairing males were observed. The produced rival calls were recorded using a laser vibrometer under 100 W fluorescent lights. The signals released by both sexes were recorded using a laser vibrometer (OMITRON VH 300+, Model-329, Bruel and Kjær) and the results were stored directly onto a hard drive through FFT Analyser (Model-3560-C, B and K Denmark) Signal spectral analysis performed by means of Pulse 110 Lab. Shop 16.1.0 (B & K Denmark) and monitored on laptop connected to FFT analyser.

2.3 Statistical analysis

The effect of age and population density on the production of male rival calls was observed using ten paired and ten single males from each group. The experiment was conducted in Complete Randomized Design (CRD). All collected data were transformed through square root transformation and analysed using one-way analysis of variance (ANOVA). Least significant difference (LSD) at 0.05 probabilities was used to separate the means with significant differences. All data were presented in percentage and analysis was done using Statistical Analysis Software version 9.3 (SAS Institute Inc. 2009).

3. Results and Discussion

3.1 Sample characteristics

Skewness and Kurtosis were not normally distributed with the values of 1.30 and -26.22 for male rival calls within different age groups and 0.00, -26.33 within different groups of population density thus data were square root transformed prior to statistical analyse.

3.2 Effect of male age on production of rival signals in BPH

Male rivalry calls were successfully recorded from rice plants with laser (Figure 2) and the influence of age on the number of males producing rival calls in BPH is as given in Figure 3. None of the single adult male from all the selected age groups produced rival calls and similar results were also observed

even in pairings between one day old males (F= 0.00; dF= 3, 36; Anova, $P \leq 0.05$). However, results were significantly different in production of male rival calls within paired insect of different age groups (F= 9.00; dF= 3, 36; Anova $P \leq 0.05$). The highest percentage of male 90.0 ± 0.1 (n=10) produced rival calls at the age of five days and the lowest percentage of 50.0 ± 0.2 (n=10) at seven days old. Non-significant difference in number of rival calls in three (n=10; 60%) and seven (n=10; 50%) days old virgin males was observed. Interestingly, the same mature five days old males did not produce rival calls when they were introduced individually on rice plant. This showed that rival calls are only displayed by males when they occur in pairs.

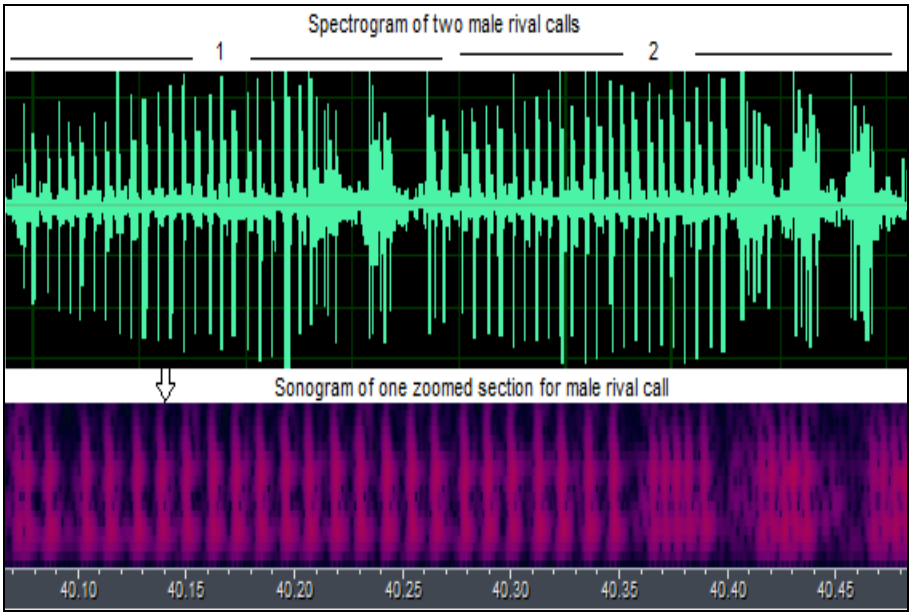

Fig 2: Male rival calls of *Nilaparvata lugens* (Stál) recorded from rice plant through laser vibrometer

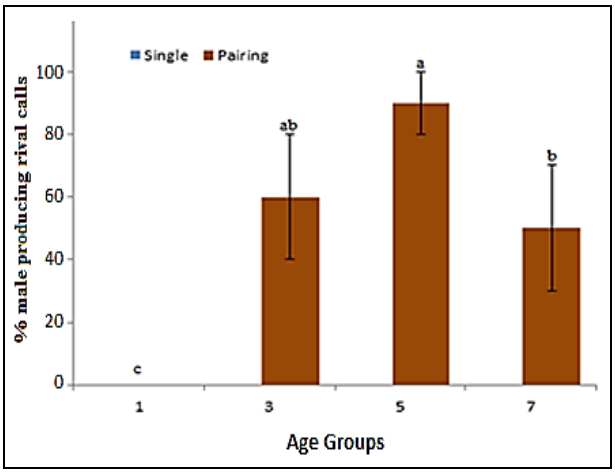

Fig 3: Percentage of BPH males producing rival calls at variable age groups

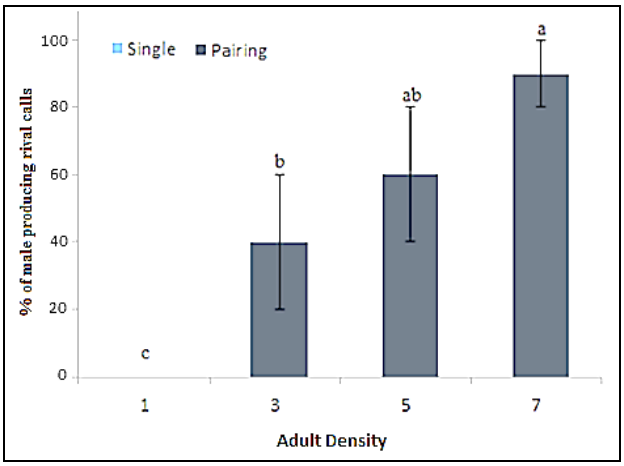

Fig 4: Percentage of BPH males producing rival calls at variable adult densities

Means followed by different letters within the same coloured bar gram are significantly different ($p \leq 0.05$).

3.3 Effect of male population density on production of rival calls in BPH

The adult density during rearing also affected the behavioural response of BPH virgin males in producing the male rival calls (Figure 4). The response was significantly different (F= 8.90; dF= 3, 36; Anova $P \leq 0.05$) at variable adult densities. The highest percentage of 90.0 ± 0.1 (n=10) male produced rival calls when reared at a maximum density of seven adults per rearing jar and minimum percentage of 40.0 ± 0.2 (n=10) at three adults per rearing jars. The percentage of male producing rival calls increased as adult density increased and decreased as adult density decreased during rearing of males.

Means followed by different letters within the same coloured bar gram are significantly different ($p \leq 0.05$).

However, results were same as observed at different age groups with no single male rival call recorded when males from different population densities groups were observed alone or reared alone before experimentation. This exhibited that like age, male adult density also played an imperative role in male-male competition for the production of rival songs.

Male-male communication in Homopterous seems to have been largely ignored except for some instances of *Achorotile albosignata* [8]. It is now recognised that apart from communication between male and female for mating, male rival calls are also prominent reproductive behaviour found in leaf and planthoppers which exhibit male-male competition. These signals are also referred to as agnostic vibrational signals used by males during their rivalry vibrations [9, 10]. The

first pioneering effort to explore this rivalry signal was presented by [11] in *N. lugens* and most recently in *S. titanus* [6b] and 'Pentatomid' *Dichelops melacanthus* [12].

Similarly, the rivalry signals of *N. lugens* recorded in this study using the laser vibrometer, has produced detailed information about the rivalry characteristics of BPH with influential aspects of age and population density. The results of this study revealed that rival signals were only produced by paired males when they were stimulated by the on-going duet of male and female. As the density of males increased during rearing, behavioural changes were displayed by males in the form of increased production of rival calls and maximum competition between males. Similarly, the effect of age was also imperative, the maximum aggressive behaviour for producing rival calls was observed in five days old male and such behaviour declined with the increasing age of male.

A competition between male abundance has also been recorded in wandering spider *Cupiennus getazi* in which it was observed that the presence of more males generated higher percentage of the agonistic signals [13]. Previously, [11] also reported that agonistic signals in *N. lugens* were not produced when a single male was present but only when there was a group of individuals. As competition started between pair of males, rival calls were produced and males were also observed to come closer and perform physical provocation at each other. Such hassling behaviour of males continued until one of them stopped calling songs and distanced itself from the vibration source. These violent body attacks with aggressive rival calls were also previously reported in *N. lugens* and *H. obsoletus* even in the absence of females on the plant [11,6b]. This showed that within groups, a male may be inhibited from calling by other males as a result of dominant hierarchy.

4. Conclusion

Rivalry calls of BPH male were observed influenced with age and population density of males. However, only males produced this competitive behaviour whereas no single male produced rival call in the absence of other male even at maturity age of five days old. The maximum percentage of 90% males produced rival calls when attained at the age of five days old and similarly less population density of male before experiment did not much develop such competitive behaviour among virgin BPH males.

5. Acknowledgement

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