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Short Communication

Effect of Age on Reproductive Behaviour of the Brown Planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidea)

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Authors' Contribution
AMA, AZAA and FNK designed the study plan. IK helped in insect identification (mature, adults, virgin and gravid). JH and QA composed, revised and proof read the manuscript. IAR statistical analysed and interpreted the data. MAL collected and reared the insects and helped in performing the lab experiments.

Key words
Brown planthopper, Mating behaviour, Age, Male dance, Female response

ABSTRACT

The insect age plays a significant role in the intensity of competitive behaviours among the individuals. The courtship behaviour of brown planthopper (BPH) is followed by numbers of strides by both genders and influenced with age. In the present study, the age of male for 1-7 days and female 1-10 days was selected to observe the different parameters {male response time (MRT), male arrival time (MAT), male arresting time (MATt), number of times male extended genitalia (MEG), total pre-mating time (TPMT), mating duration (MD), female calling latency and female rejecting rate}. In the results, a maximum MRT 13.14±1.77 min was observed in D1. The BPH males approached to the female with jerky walking steps up and down with a minimum time of 0.24 ±0.03 min in D3. After successful male arrival, male went for a maximum arresting time of 3.19±0.51 min by female in D1. Though, all the males extended their genitalia for successful copulation but D1 male did not only take maximum time under female arresting but it also extended genitalia with maximum number of 5.80±0.37 times. Overall, a maximum pre-mating time of 16.70 ± 0.55 min and minimum 3.39 ± 0.17 were observed in D1 and D5 males. The mating duration was lower in early ages (D1 and D2) and became advanced / peaked in mid ages (D5 and D6) which later decreased again in D7. The physical behaviour of BPH females did not show any mating response during the first and second day of emerging. However, the lowest calling latency was observed in D9 (2.2 ± 0.3) and D10 (2.2 ± 0.3) and maximum in D3 (12.1 ± 1.5). The correlation coefficient also indicated strong with negative relationship (r= -0.92) between male age and total premating time and strong with positive relationship (r=0.95) between male age and mating percentage.

A billion households in Asia and Africa relies on rice systems as their main source of employment and livelihoods. In fact, rice crops are also on the front line in the fight against world hunger and poverty (Van Nguyen and Ferrero, 2006). A major constraint in achieving self-sufficiency in the rice-producing country is a pest problem. The crop is subjected to attack by more than 100 species of insects and 20 of them can cause economic damage. Brown planthopper (BPH) *N. lugens* (Hemiptera: Delphacidae) is one of the most important insect pests of rice throughout Asia (Heong and Hardy, 2009) and are commonly called delphacid planthoppers (Mochida and Okada, 1979). They usually attack the basal portion (stem) of the plant and damage plants directly by feeding, thus causing a characteristic yellowing of tissues (leaves) known as "hopperburn". They also indirectly act as vectors for a variety of plant pathogens and transmit the most common diseases such as Rice Ragged Stunt Virus (RRSV) and Rice Grassy Stunt Virus (RGSV) (Cabauatan *et al*., 2009). BPH resistance to insecticides has increased the probability of outbreaks. The pest has also shown resistance to a number of improved genes in host plants (Peñalver Cruz *et al*., 2011). Although biological agents have shown an impressive role to suppress the pest population, all these control measures still could not provide a complete solution to combat the pest outbreak. The mating behaviour of BPH comprises

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multiple steps and initiates by female abdominal vibration (FAV) and male response by dancing on a rice plant in search of vibrated female (Ichikawa and Ishii, 1974). These responses exhibited by both sexes are affected by age and quality of food as well as environmental changings but still depth studies are required to confirm the bonding of each mating parameters in relation to their age particularly in male because females in many animal species often mate with males of a particular age (Brooks and Kemp, 2001) and older males are more successful at competing for mates or acquiring resources important for female reproduction (Rasmussen *et al*., 2008). In addition, sexual maturity of insects progresses with increasing age and it is one of the imperative features that elicit the change in the mating behaviour of insects. Often, age plays a significant role in the intensity of competitive behaviours displayed by competing individuals. Beside this, the methods relying on behavioural disruption, such as those aiming to disrupt mating, may require extensive knowledge of the particular pest and be vitiated by slight changes in the behaviour of the species (Polajnar *et al*., 2015). There are a number of biological and environmental factors responsible for disturbing or delaying the mating response of numerous insect pests. There have been several studies on BPH; however, only a few studies in the past on their mating behaviour in concern with its age have been carried out. Therefore, this study presents the role of BPH male age and their related mating. If the control of mating behavior is possible, the damage to plants might be successfully reduced.

Materials and methods

The reared culture of *N. lugens* was taken from the Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tando Jam and further kept in wooden cages (90 x 42 x 42 cm) at 28 ± 2°C and 60-70 % relative humidity in the insectary during 2018-2019. The newly emerged adults were separated from the main culture to maintain their virginity. The sexes of adult insects were identified based on their genitalia and then kept in separate plastic jars having fresh rice seedlings inside it. Brachypterus males and females were taken to observe the effect of age in the mating behaviour of BPH. The male age (1 to 7 days) paired with 5 days female and female age (1 to 10 days) paired with 5 days male were selected. The insects were allowed to settle on two 30-40 days old rice plants with connected leaf blades (to permit interaction) under 100 W fluorescent lights. The mating behaviour of insects was observed between 1700 to 2100 hours. However, the efficiency of different aged male and female was observed in time with digital stop watch (SDW-219; Malaysia) based on male response time (MRT), male arrival time (MAT), male arresting time (MATt), number of times male extended genitalia (MEG), total pre-mating time (TPMT) and mating duration (MD) with naked eye as this study was based on physical behaviour of BPH regarding their mating efforts. The experiments were conducted in Complete Randomized Design (CRD) using five replications per treatment 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. In addition, two mating parameters such as total pre-mating time and successful mating percentage was correlated with male age using Pearson correlation coefficient (r). All analysis was done using Statistical analyser software (Statix 8.1).

Results and discussion

The influence of the variable age of male in mating efforts of BPH was observed significantly different (MRT, F=26.59; MAT, F=16.39; MATt, F=8.50; MEG, F=27.86; MD F=4.02 and TPMT F=77.37 with dF=6, 28 at p<0.05). Each mating attempt or parameter was examined with uneven duration and observed influenced by male age (Table I). A maximum time to response (MRT) was observed in D1 and observed earlier as male age advanced. The BPH males responded with jerky walking steps up and down and sometimes they were observed stationary. However, the time noted for males of D1 to D5 was not significantly different except for D6 and D7 males with minimum time by D3 male. Such responses showed that different age pairing combinations played an imperative role in displaying variability in mating responses of BPH. A female's behaviour is usually more complex than males as it invests more in offspring than males (Ayasse *et al*., 2001; Fortes and Consoli, 2011) thus its behaviour changes in accordance with the activity of its corpora allata with aging. After successful male arrival, male went for arresting time by female in which female scrutinised male thoroughly. During this period, male tried to connect its genitalia with female genitalia for successful copulation. Virgin males of variable age attempted a number of times to get over the female for mating but five days old virgin females took time and did not allow males swiftly. Nevertheless, male arresting time in virgin males of D3 to D7 was almost the same, though minimum time of 1.04±0.08 min was observed in D5. D1 male did not take maximum time under female arresting only, but it also extended genitalia maximum number of times that displayed maximum number of efforts (unsuccessful) attempted by young one day (D1) old male. However, more successful males were D5 to D7; those who extended one or two times their genitalia and were successfully accepted by females. This permitting act could be important for aging female

Table I. Age wise mating behavioural response of male *N. lugens* (Stàl).

Age (days)	Male calling latency (min)	Male searching time (min)	Male arresting time (min)	No. of times male extended genitalia	Mating duration (min)	Pre-mating time (min)
D1	13.14±1.92^{a}	0.36±0.06^{c}	3.19±0.51^{a}	5.80±0.37^{a}	1.02±0.12^{c}	16.70±0.55^{f}
D2	10.65±0.67^{ab}	0.37±0.02^{c}	2.12±0.06^{b}	4.20±0.37^{b}	1.14±0.12^{bc}	13.41±1.22^{e}
D3	8.69±0.64^{b}	0.23±0.03^{c}	1.64±0.22^{c}	3.00±0.32^{c}	1.24±0.10bc	10.59±0.61^{d}
D4	5.04±0.49^{c}	0.36±0.06^{c}	1.33±0.22^{c}	2.20±0.37d^{e}	1.43±0.16^{ab}	5.12±0.46^{bc}
D5	3.1 ±0.37^{de}	0.68±0.13^{b}	1.04±0.83^{cd}	1.60±0.24^{e}	1.84±0.23^{a}	3.39±0.18^{a}
D6	2.12± 0.27^{e}	0.68±0.14^{b}	0.96±0.24^{d}	1.20±0.20^{e}	1.92±0.23^{a}	4.03±0.33^{ab}
D7	2.24±0.33^{e}	1.19±0.14^{a}	1.41±0.24^{c}	1.40±0.24^{e}	1.49±0.18^{ab}	4.01±0.31^{ab}

Means followed by different letters within the same row are significantly different (p≤0.05).

Table II. Age wise mating behavioural response of female *N. lugens* (Stàl).

Days	FAV latency (min)	Rejection rate	Pre-mating time (min)
D1	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
D2	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
D3	12.1±1.5^{b}	1.6±0.2^{b}	13.7±1.4^{b}
D4	10.5±0.7^{b}	1.4±0.5^{b}	11.6±0.8^{ab}
D5	7.8±1.4^{c}	1.4±0.6^{b}	9.4±1.4^{b}
D6	5.6±0.7^{cd}	1.0±0.3^{bc}	6.8±0.7^{c}
D7	2.6±0.4^{d}	0.8±0.2^{c}	4.2±0.6^{d}
D8	2.7±0.4^{d}	0.6±0.2^{c}	4.2±0.4^{d}
D9	2.2±0.3^{d}	0.2±0.2^{cd}	1.0±0.1^{e}
D10	2.2±0.2^{d}	0.2±0.2^{cd}	1.1±0.0^{e}

Means followed by different letters within the same row are significantly different (p≤0.05).

who already has a shortened preference window for the selection of best males thus allowing them to increase its reproductive potential. Whereas males do not often refuse copulation with vibrating females after locating them (Claridge *et al*., 1984), however this response could be affected by physiological factors like age or changes in environmental condition (Silva *et al*., 2012). In last, a minimum pre-mating time (3.39±0.17 min) was observed in D5 males with maximum mating duration of 1.92 ± 0.22 min in D6 old males. The mating duration was lower in early ages (D1 and D2) and reached at a peak in mid ages (D5 and D6) which later started to decrease a bit again in D7. In male cotton leafhopper *A. devastans* (Dist.), male Caribbean fruit flies *Anastrepha suspensa* (Loew) and in the previous study on male BPH, the effects of age on the sexual response of male have also been reported (Ichikawa, 1979; Kumar and Saxena, 1986; Aluja *et al*., 2009). This again highlights that too young and old males are not at the peak of mating efficiency and are less preferred by matured five days old females (Butlin, 1993; De Luca and Cocroft, 2011). Besides, the physical behaviour of BPH females did not show mating response during the first and second day of emerging (Table II). The first mating response by displaying female abdominal vibration (FAV) was noticed on the third day (D3) with a maximum calling latency of 12.1±1.5 mint. The results further showed that the imitativeness of females for courtship (calling latency) increased in terms of short period as older females responded early as compared to younger or middle age females. Similarly, the rejection rate also decreased (0.8 to 0.2) with increasing age (D7 to D10) of females which further assured less premating time in older females (4.2 to 1.1 min) as compared to young females but it also could be possible due to mating response of male partners. Meanwhile, overall rejection rate, FAV latency and pre-mating time were observed maximum in D3 females as compared to the rest of selected females. The correlation co-efficient also indicated strong with negative relationship (r= -0.92) between total premating time and male age and strong with a positive relationship (r=0.95) between male age and mating percentage (Table III). Female abdominal vibration is also seen as a guide for the male to locate the female depending upon the vibrating frequency (Ichikawa, 1979; De Luca and Cocroft, 2011). The maximum percentage of receptiveness was mostly observed in five to seven days old females and her willingness often correlated with male fitness (age). In BPH, females' mate for a limited number of times, whereby two to three times of mating is quite sufficient for their entire life to fertilize their eggs (Mochida and Okada, 1979; Heong and Hardy, 2009). Therefore, they were found to be selective in choosing their partner; often scrutinizing either male size or male call to ensure the fittest male sires the progeny. Unlike females, males show post-copulatory mating response because they often can mate with a maximum of nine females within 24 h (Mochida and Okada, 1979;

Heong and Hardy, 2009). Often, the aged female of nine days old showed less interest in long mating duration and was observed kicking and repelling the male to dislodge immediately after a short mating duration when paired with a different aged group of males.

Table III. Correlation co-efficient matric between male age and mating success in *N. lugens* (Stål).

Male age (days)	Correlation co-efficient (r^2)	p value	Remarks	Relation
Total pre-mating time (Mints.)	-0.92	0.003	Highly significant	Negative linear
Successful mating (%)	0.95	0.001	Highly significant	Positive linear

Conclusion

It is concluded that all the mating parameters of both sexes were influenced by age. The best age for exhibiting maximum mating response was D5 for both adults.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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