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Effects of nitrogen nutrient on the behavior of feeding and oviposition of the brown planthopper, *Nilarpavata lugens*, on IR64

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Abstract: We determined the preferences of feeding and oviposition of the brown planthopper (BPH), *Nilaparvata lugens*, to rice plants of IR64 with different nitrogen content, the changes in preferences of BPH populations fed successively on plants with high (200N) or low (0N) nitrogen fertilizer, and the influences of nitrogen content on the spatial distribution patterns of feeding and oviposition. In choice test, all females from four populations reared on both 200N and 0N rice plants preferred significantly to feed and oviposite on plants with high nitrogen content, while no marked difference in nymph feeding preference was found among the rice plants. The positions of feeding and oviposition were shifted gradually from the bottom to upper of leaf sheath, and then to leaf blade with the reduction of nitrogen content in host plants. Both nymphs and adults located at higher position of plants and fed much more frequently on 0N rice plants than that on 200N rice plants, and differed markedly among BPH populations. The highest feeding frequency was found in the nymphs, which reared successively on 0N rice plants, on 0N rice plants, implying that feeding frequencies of BPH populations were related closely to their previous host plants.

Key words: brown planthopper; nitrogen; feeding; oviposition; behavior
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氮营养对褐飞虱在 IR64 稻株上取食和产卵行为的影响(英文) . 浙江大学学报(农业与生命科学版), 2005, 31(1): 62-70

摘 要: 研究了褐飞虱对不同含氮量的 IR64 稻株的取食和产卵选择性、寄主含氮量对褐飞虱种群选择性的影响以及在不同含氮量稻株上取食和产卵的空间分布 . 结果表明, 无论褐飞虱饲养在高氮和低氮稻株上其雌成虫均显著偏爱在含氮量高的稻株上取食和产卵, 而若虫对不同含氮量稻株的取食选择性无显著差异 . 随着稻株含氮量的下降褐飞虱取食和产卵的位置从叶鞘底部逐渐向上部转移, 直至叶片中脉 . 在低氮

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稻株上若虫和成虫均偏向于较高的位置取食和产卵, 而且不同的种群有明显差异. 饲养在低氮稻株上的褐飞虱种群在低氮稻株上的取食频次最高, 褐飞虱种群的取食频率与其前期饲养的寄主有关.

关键词: 褐飞虱; 氮营养; 取食; 产卵; 行为

Feeding behavior is the first step to acquire nutrients and is the foundation to carry out a series of physiological activities for the maintenance of insect populations, and the oviposition is the last course and the final goal to complete their life cycle of insects[1, 2]. Feeding and ovipositing behaviors composed the most important characteristics in insect ecological and biological systems. They are managed and regulated mainly by the genetic properties, however, they may be affected and controlled by environmental factors such as temperature, moisture, habitats, morphological and chemical components of host plants, especially by the nutrients, such as nitrogen, sugar, amino acids, and semiochemicals in host plants[3-8]. Nitrogen including amino acids and proteins, and sugar are two major kinds of the herbivore feeding regulators, which can adjust the feeding rate on different host plants[1, 6].

Feeding physiology of the brown planthopper (BPH), *Nilaparvata lugens*, and its relation to the host plant had been reviewed[7]. BPH was attracted much more effectively to feed and oviposit on those rice plants applied with nitrogen fertilizer, and could produce more honeydew[8-10], less probing behaviors and more eggs[9]. Kanno *et al*[11] monitored the feeding activity of BPH using ^{32}P and found that the feeding amounts and honeydew excretion on the high nitrogen plants were increased by 3-7 and 7 times, respectively. On the susceptible rice, TN1 with different rate of nitrogen fertilizer, the honeydew excretion of BPH fluctuated with the increase of the nitrogen. The increase in nitrogen fertilization of plants up to 100 kg/hm^2 resulted in the increase in the honeydew excretion, while further increasing the nitrogen dose reduced honeydew excreted by BPH[12]. However, BPH populations in paddy fields may be affected in temporal and spatial by the nitrogen nutrient. As the results, the BPH feeding and ovipositing behaviors may be changed or modified in the successive populations fed continuously on host plants, and in spatial distributive pattern of insects located and eggs laid on the host plants with different nitrogen regimes.

This paper deals with the preferences of feeding and oviposition to host plants with different nitrogen content, the changes in preferences of successive BPH populations on rice plants with high or low nitrogen, and the influences of nitrogen on the spatial distribution patterns of feeding and oviposition.

1 Materials and methods

1.1 Host plants

In all cultures and experiments, rice varieties, Taichong Native 1 (TN1), susceptible to BPH and IR64, moderate resistance to BPH were used. Seeds were sown at 15 days intervals to ensure adequate supply of host plants of specific stages for experiments.

For cultures and experiments, 3 or 4 ten-day-old rice seedlings were transplanted into clay pots (size No. 3, Diameter 14 cm or size No. 0, Diameter 7 cm) filled with garden soil. 30%, 30% and 40% of total nitrogen fertilizer, ammonium nitrate, were applied quantitatively on 7 days after transplanting, tillering and reproductive stage, respectively. IR64 plants with four rate of nitrogenous fertilizer, 200, 100, 50 and 0 kg/hm^2, named as 200N, 100N, 50N and 0N, respectively, were cultured. The exact amount of nitrogenous fertilizer applied in different size pots was calculated based on the amount of soil in each pot and quantified by a series of containers with various sizes. Any pesticide was free in all growth period of rice. The water irrigation was managed regularly.

An electronic chlorophyll meter (SPAD-502, Minolta camera Co., Osaka, Japan) was calibrated for the assessment of tissue nitrogen. At different rice growth stages, eight uppermost fully expanded leaves were selected at random from each nitrogen regime and the SPAD readings recorded. The tillers bearing these leaves were removed and placed separately in brown paper bags. The samples were placed immediately in an oven at 110 ℃ for 30 min and dried at 80 ℃ for 48 hours until constant weight. Nitrogen content was determined by micro-Kjeldahl digestion and distillation. A regression of tissue nitrogen content (%) as the dependent variable and SPAD meter reading as the independent variable was established and used to convert SPAD readings. Pooling data from all plant growth stages, the relationship between leaf nitrogen content and leaf SPAD readings was found to be

$$N\% = 0.1151\ \mathrm{SPAD} - 1.2772$$

$(F = 162,\ P < 0.001)$

Where N% is the nitrogen content in percentage and SPAD is the chlorophyll meter reading. This linear model was used to predict the nitrogen content of plants in all the experiments.

1.2 Insect

BPH adults were collected from rice fields in Laguna, Philippines and reared in an oviposition cage with susceptible variety, TN1, as the host plant. On every Monday and Thursday, 45-60 day-old plants in pots were placed into this cage and removed 24 hours late. The potted plants with BPH eggs were placed in a cage with TN1. Those populations were designed as G0.

To establish different BPH populations on rice plants with different nitrogen regimes, newly molted BPH adults from G0 were introduced into cages (L 50 cm ×W 38 cm ×H 80 cm) with opening top and side windows attached with fine-mesh nylon cloth, containing 45-60 day-old IR64 rice plants. Two host plants, 0N and 200N, were used. Populations that were maintained on each respective nitrogen regime for more than one generation were designated as G1, G2 and so on. Populations under different nitrogen regimes were labeled. The first generation of BPH population reared on 200N and 0N rice plants were named as 200NG1 and 0NG1, respectively.

1.3 Preferences of the feeding and oviposition

1.3.1 Preference to rice plants 45 day-old plants in pots were trimmed to main stem and one primary tiller, and the SPAD readings were recorded. Four pots of rice plants, 200N, 100N, 50N and 0N, were arranged randomly in a plastic tray (L 35 cm, W 30 cm and H 14 cm) and covered with the cylindrical plastic cage (H 80 cm) with fine nylon mesh on the top and one 3 cm-long-cut on side wall for BPH infestation. Water level in trays was kept to the rims of clay pots in order to let insects move among plants randomly. Fifteen gravid brachypterous female adults and twenty-five 3rd instar nymphs of BPH from the population maintained on TN1 rice plants were introduced in the middle of the cage. The numbers of BPH including female adults and nymphs located on each plant were recorded at 24, 48 and 72 h after infestation. After 72 h, the SPAD values of rice leave were measured again and the eggs laid on rice plants were counted by dissecting the plant tissues under the binocular microscope. The preferences of feeding and oviposition to host plants were expressed by the total number of BPH located and eggs laid on one plant in 72 h. All treatments were replicated eight times in the greenhouse in International Rice Research Institute, Philippines. The natural photoperiod of approximately L12∶D12 was maintained.

1.3.2 Preference to positions of rice plants

Potted rice plants at vegetative (35 day-old) and reproductive (65 day-old) growth stages were trimmed to one main stem, recorded SPAD readings and covered with mylar cages. Leaf sheaths were marked in middle with Pilot® super color marker. Ten 4-5th instar nymphs and gravid female adults were introduced into cages. The numbers of BPH located on the bottom and upper of leaf sheaths, and leaf blades were recorded after 24 h of infestation, and the numbers of eggs laid on rice plants were counted by dissecting the plants under the binocular microscope. Tests were conducted at (26±2)℃ in laboratory. The proportion of BPH located and eggs laid at different positions on host plants was calculated in percentage. Eight replications were set up in each rate of nitrogen fertilizer.

1.4 Feeding behavior

45 day-old rice plants were placed individually in test tubes (∅2 cm, H 15 cm). One 4th instar nymph or newly molted female adult was introduced into a test tube. 1% Eosin water solution was prepared to mark feeding marks, and the feeding marks were counted under the binocular microscope based on the color dyed by Eosin.

1.5 Data analysis

Values in percentage were transformed by arcsine before analysis. Linear regressions were analyzed in IRRISTAT Ver. 4.0. Analysis of variance (ANOVA) and Duncan's multiple range test were performed with SAS package using PROC ANOVA or PROC GLM.

2 Results

2.1 Oviposition preference

2.1.1 Preference to host plants　In choice test experiments, all females from BPH populations rearing on rice plants both with high (200N) and low (0N) nitrogen fertilizer preferred to lay their eggs on the host plants with higher nitrogen content (Fig. 1). Significant ($P < 0.0001$) linear regressions between the number of eggs laid and nitrogen content of rice plants were obtained in all four BPH populations. No observed differences in oviposition preferences were found among tested four populations, with the exception of the relative lower values of R^2 and F were regressed in the populations on 200N than those on 0N. The regression model for all data was found to be $E = 85.036N - 82.602$ ($F = 280.21$, $R^2 = 0.7488$, $P < 0.0001$), where E is the number of eggs laid and N is the nitrogen content of

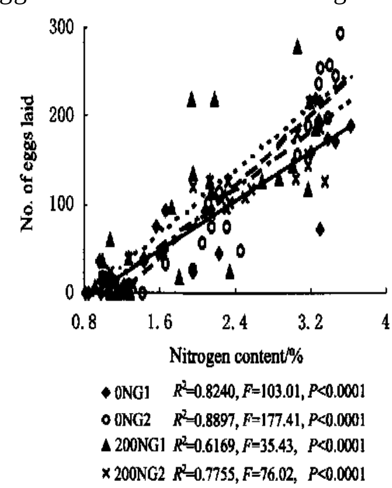

Fig. 1 Oviposition preference of BPH populations on rice plants with different nitrogen content

rice plants.

2.1.2 Preference to positions of host plants

The similar trend of oviposition preference to the bottom and upper leaf sheath, and leaf blade was found at the vegetative and reproductive rice growth stages (Fig. 2 and Fig. 3). The proportion of eggs laid increased at the bottom of leaf sheath and decreased in the upper of leaf sheath and leaf blade with the increase of nitrogen content in host plants ($P < 0.01$). These implied that BPH females prefer to lay their eggs in the lower part of leaf sheath. However, with the reduction of the nitrogen content, BPH eggs were laid at the higher part of leaf sheath and in the leaf blade.

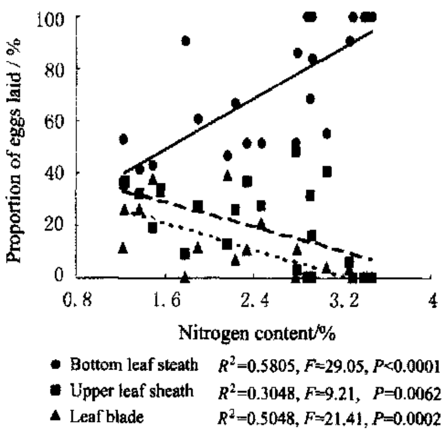

Fig. 2 Proportion of BPH eggs laid on leaf sheath and blade of rice plant with different nitrogen content in vegetative growth stage

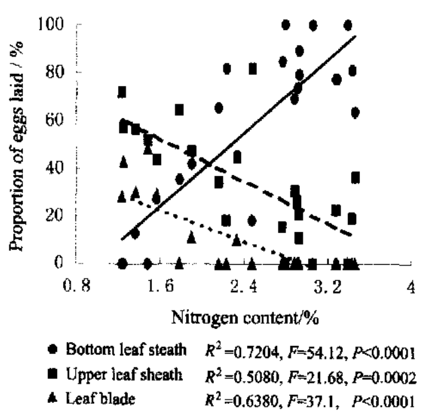

Fig. 3 Proportion of BPH eggs laid on leaf sheath and blade of rice plant with different nitrogen content in reproductive growth stage

2.2 Feeding preference

2.2.1 Preference to host plants Feeding preferences of BPH nymphs and female adults maintained on susceptible variety TN1 were determined in the free-choice test experiments. Results indicated that more adults preferred to feed on the host plants with higher nitrogen content. The adult densities were significantly ($P = 0.0001$) related to the increase of nitrogen content in host plants. However, no marked difference was found in nymphs ($P = 0.0825$) (Fig. 4). Similar results in nymphs ($P > 0.05$) and female adults ($P < 0.0001$) were found in the populations reared continuously on 200N (high nitrogen) plants and 0N (low nitrogen) plants for 1 and 2 generations (Fig. 5 and Fig. 6).

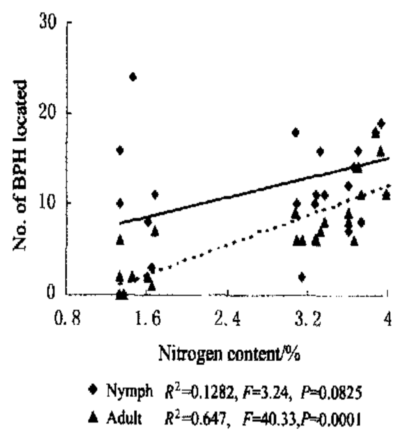

Fig. 4 Feeding preference of BPH from TN1 on rice plants with different nitrogen content

2.2.2 Preference to positions of host plants

The relationships between nitrogen content in host plants and the proportion of BPH located were positive at the bottom of leaf sheath and negative on the leaf blade. However, the slope values of the regression were higher in female adults than that in nymphs

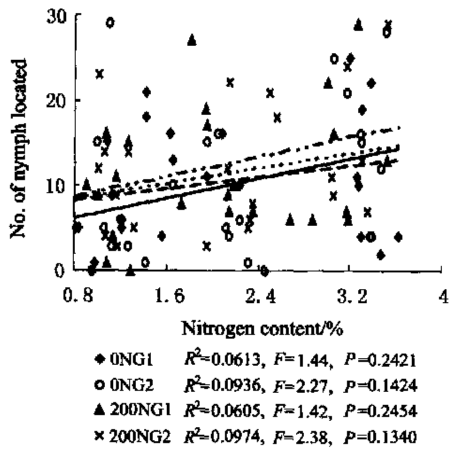

Fig. 5　Nymph feeding perference of BPH populations to rice plants with different nitrogen content

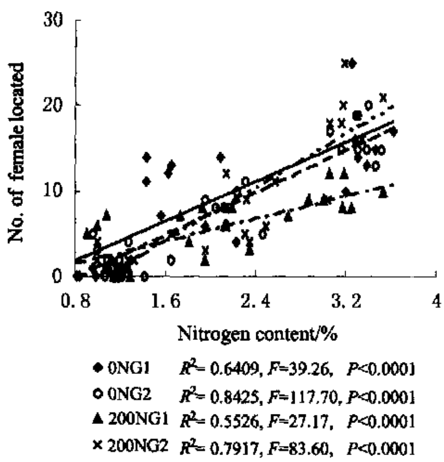

Fig. 6　Female adult feeding preference of BPH populations to rice plants with different nitrogen content

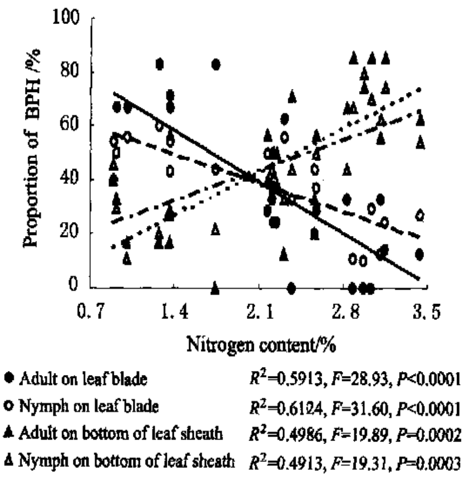

Fig. 7　Proportion of BPH located on leaf blade and bottom of leaf sheath of rice plants with different nitrogen content

(Fig. 7). It implied that both nymph and adult fed on the lower parts of host plants with higher nitrogen content, and the higher parts of plants were the feeding sites on the rice plants with lower nitrogen content.

2.3　Feeding behavior

2.3.1　Feeding frequency　　Feeding frequency of BPH on rice plants was expressed as the number of feeding marks. Results showed that both nymphs and adults fed much more frequently on 0N rice plants than that on 200N rice plants (Table 1). The significant differences in number of feeding marks in 24 h between on 200N and 0N rice plants were found in nymphs (F = 143.38, P < 0.0001) and adults (F = 9.45, P = 0.0036). The highest feeding frequency of nymphs was recorded on 0N rice plants in those nymphs reared previously on 0N rice plants, while the lowest frequency was monitored on 200N rice plants in those insects reared successively on 200N rice plants. However, the highest adult feeding frequency was recorded on 0N rice plants in the BPH from TN1 population, and these adults maintained successively on 0N rice plants had the lowest feeding frequency on 200N rice plants. On the same diet plants, there were significant differences among BPH populations in nymphs (F = 12.56, P < 0.0001) and adults (F= 14.45, P < 0.0001). Statistical differences in interaction of population and diet were analyzed in adults (F = 5.74, P = 0.0008), and not in nymphs (F = 1.08, P = 0.3774). Results indicated the feeding mechanism differed between nymphs and adults and was related closely to the diet plants and their previous host plants, as well as to their adaptation to host plants.

2.3.2 Feeding position Proportions in percentage of feeding marks on the bottom of leaf sheath (%) of rice plants with different nitrogen fertilizer were listed in Table 2. On 200N rice plants, proportions on the bottom of leaf sheath of feeding marks produced by both nymphs and adults were more than 50%, and no significant differences in nymphs (P= 0.1897) and in adults (P = 0.2567) were found among BPH populations. On 0N rice plants, however, the proportions on the bottom of leaf sheath both in nymphs and adults were less than 50%, and significant differences in nymphs (P< 0.0001) and in adults (P= 0.0024) were found among BPH populations. Comparing to the BPH populations from 200N and 0N rice plants, feeding positions of nymphs were no significant difference (F= 2.17, P= 0.0871), however those were significant in adults (F= 4.69, P= 0.003).

Table 1 Feeding frequency of BPH populations on rice plants with different nitrogen fertilizer

Feeding marks/d

BPH population generation		TN1 > 15	0N rice plants		200N rice plants		F	P
			1	3	1	3		
Nymph	200N	15.9 b	17.9 ab	19.1 a	14.9 b	14.8 b	3.3	0.021
	0N	20.4 b	26.7 a	25.8 a	21.7 b	22.2 b	7.21	0.0002
Adult	200N	19 a	12.6 c	12.4 c	14.2 bc	16.2 b	13.45	< 0.0001
	0N	24.1 a	18.5 b	15.9 b	16.6 b	18.6 b	14.16	< 0.0001

Table 2 Proportions of feeding marks on the bottom of leaf sheath %

BPH population generation		TN1 > 15	On 0N rice plants		On 200N rice plant		F	P
			1	3	1	3		
Nymph	200N	55.58	63.0	54.71	61.19	53.45	1.63	0.1897
	0N	30.37 b	25.31 c	20.06 c	36.34 a	28.91 bc	25.91	< 0.0001
Adult	200N	69.69	71.33	70.86	70.73	70.60	1.98	0.2567
	0N	42.3 a	21.28 c	26.62 bc	31.32 ab	31.28 ab	5.08	0.0024

3 Discussion

Insect behavior is generally determined by the closed genetic programs. The number of publications dealing with learning in phytophagous species is also increasing rapidly[13]. Hypothesis, that a preference is induced as the result of an experience with high-nitrogen plants, indicated that the selection of feeding and oviposition of herbivores to host plants with higher nitrogen content can be induced by rearing previously on plants of high nitrogen. This hypothesis may be proved by a herbivorous fly, *Liriomyza trifolii* that had been exposed previously to tomato plants of high nitrogen preferred to feed and oviposit on high nitrogen plants, whereas flies previously exposed to plants with the least nitrogen showed no preference[14]. In this experiment, however, BPH reared successively on rice plants both with high and with low nitrogen content for two generations showed the preference to high nitrogen content plants, all four populations tested have the same trend in feeding and ovipositing preference (Fig. 1 and

Fig. 6). Results implied that the preference of BPH to host plants with high nitrogen content was not induced by previous hosts, and is attributed to the genetic characters. The nymph feeding frequency showed slightly the learning response to their previous host plants. Lower feeding frequency was recorded on 200N rice plants in those nymphs fed previously on 200N rice plants, while higher feeding frequency was found on 0N rice plants in those nymphs fed previously on 0N rice plants (Table 1). However, the difference in feeding frequency of BPH population maintained on TN1 should be related to the resistance of IR64 to BPH, since the resistance of rice plants could affect greatly on the feeding response of BPH[15, 16].

The process of host plant selection by phytophagous insects is a lock-key system, in which the lock refers to the host plant and the key stands for the complex sensory pattern of the insect[4]. Only when this pattern corresponds to some innate standard is an appropriate behavioral response triggered[17]. The host plant recognition process is not simply a matter of responding to a positive signal or avoiding a negative signal. The process is the result of an integration of numerous plant chemical and insect internal factors[4]. The great difference in reception of nerve pulse produced by environmental stimulation through olfactory organs and the diversity in nutritional demands of various growth stages resulted in the differences in the feeding preference to host plants[2]. Nymphs of BPH responded non-sensitively to nitrogen content in host plants and the great selection of adults to rice plants with high nitrogen content (Fig. 4-6) may be attributed to their differences in the olfactory organs, and not in nutritional demands, since they have the same feeding physiology and feeding process[7].

The contents of nutrients such as amino acids in rice sap are so low compared to their needs that BPH have to suck much more sap for their better life performance[8, 9]. The increase of amino acid content in rice sap and more succulent plants by the application of nitrogenous fertilization improved greatly the nutritional condition and ovipositing space for sap-sucking hoppers[18]. So adult females selected host plants with high nitrogen content to oviposit for the best life performance of the progenies. The excessive application of the nitrogen fertilizer resulted in thick canopy and more succulent plants can contribute to the successful oviposition[19]. Obvious few eggs were laid on the nitrogen-deficient rice plants compared to on those with nitrogen fertilizer[9], however, in non-choice test, females can lay their eggs on the relative soft and thickness place such as at the upper part of leaf sheath and the midribs of leaf blade (Fig. 2 and Fig. 3), since the eggs were laid into leaf sheath and leaf blade. The changes in position of eggs laid shifted from the bottom of leaf sheath to leaf blade may be considered to be the adaptation of BPH to nitrogen-deficient factor.

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