

FEEDING PREFERENCE, FECUNDITY AND EGG HATCH OF RICE STINK BUG¹ ON
ARTIFICIAL DIET, RICE AND ALTERNATE HOST GRASSES

T. Rashid, D. T. Johnson and J. L. Bernhardt

319 AGRI, University of Arkansas, Department of Entomology, Fayetteville AR 72701

ABSTRACT

Feeding preference, mating frequency, fecundity and egg hatch of rice stink bug, *Oebalus pugnax* (F.) were examined in the laboratory. Adult rice stink bugs were allowed to feed on one of five different food sources including rice, *Oryza sativa* L.; barnyardgrass, *Echinochloa crusgalli* (L.); dallisgrass, *Paspalum dilatatum* Poir.; ryegrass, *Lolium* spp; and a new artificial rice flour diet. Initially, a higher percentage of females fed on all food sources than did males. Ryegrass and dallisgrass were fed on less frequently than rice or barnyardgrass. The insects mated twice as frequently and laid more eggs on rice panicles than on rice flour diet. However, rice flour diet was comparable to ryegrass and dallisgrass both in mating frequency and number of eggs laid per female. Egg hatch was higher from females reared on natural hosts than on rice flour diet. However, percentage egg hatch was similar for females fed rice flour diet or ryegrass. Rice flour diet may provide an acceptable alternate food source for rice stink bug adults in the laboratory but needs to be improved to make it as acceptable as barnyardgrass or rice.

INTRODUCTION

Rice stink bug, *Oebalus pugnax* (F.), is an important pest of rice, *Oryza sativa* L., in the southern United States. In addition to rice, it has been reported to have a wide host range of gramineous plants (Douglas 1939, Odglen and Warren 1962). Rice stink bug exhibits variable feeding responses to different grass species with rice being the most favorable (Naresh and Smith 1984). The average number of eggs laid by a rice stink bug female during its lifetime ranged from 73 to 491 on barnyardgrass, *Echinochloa crusgalli* (L.) (Odglen and Warren 1962, Nilakhe 1976) and as high as 915 on rice (Nilakhe 1976).

Artificial diets are important in laboratory cultured insect colonies. Brewer and Jones (1985) have reported failure of a meridic diet for rearing rice stink bug. Mittler and Dadd (1963) reared aphids on artificial diets fed through parafilm M[®] membranes. Landes and Strong (1965) and Raulston and Auclair (1968) successfully reared *Lygus hesperus* (Knight) on bean juices or chemically defined diet fed through stretched parafilm membranes in sachet form.

The objective of this experiment was to compare rice stink bug mating frequency, fecundity potential and egg hatch among three wild host grasses and a new rice flour diet.

Hemiptera: Pentatomidae¹

MATERIALS AND METHODS

Adult rice stink bugs and late instar nymphs were collected during May 2000 from rice fields and grass hosts at Stuttgart, AR, and taken to the University of Arkansas Agricultural Experiment Station (AES), Fayetteville in 19-liter plastic pails covered with 64-mesh nylon cloth. Combinations of excised panicles of different grasses including ryegrass, *Lolium* spp, barnyardgrass and dallisgrass, *Paspalum dilatatum* Poir., collected from fields at AES were provided as a food source to adults and late instar nymphs. The colony was held at the AES at room temperature of $25.6 \pm 2^\circ\text{C}$ and 14:10 L:D photoperiod.

Ten grams stone ground white rice flour (Bob's Red Mill Natural Foods, Milwaukie, OR) and 5g sucrose (granulated sugar) were added to 100ml of boiling water with continuous stirring. The solution was removed from the hot plate when it became viscous. When the temperature dropped to 50°C , 1.2g Vanderzant-Adkisson[®] vitamin mix (Bio-Serv, Frenchtown, NJ) was incorporated into the diet solution. The diet was held at room temperature until cooled.

PM-992 parafilm M[®] (American National Can, Menasha, WI) was cut into sections 12.9cm^2 in size and then stretched. Approximately 5ml of diet was poured onto each stretched parafilm piece. Both sides of the parafilm were joined, pressed firmly together and then the ends were twisted. Stretched parafilm packets containing the rice flour diet were punctured with #7 insect pins mounted on a cork to induce and stimulate rice stink bug feeding. A 20-cm long straw was vertically attached to a plastic bottle lid which served as a platform. A hole was drilled through the lid to insert one end of the straw. Two parafilm packets containing diet were tied together facing each other on the straw with 5cm distance between each pair of the packets. Three pairs were tied to each straw.

Panicles and stems of barnyardgrass, ryegrass and dallisgrass collected from AES were cut to the same length as that of the straws used for rice flour diet packets. The panicles were placed in glass vials containing water and plugged with cotton.

Vertically held straws with rice flour diet packets and panicles of all three grasses in glass vials were randomly placed equidistant in a (50cm × 25cm × 30cm) glass arena and covered with metal screen. Rice stink bug adults were held without food on water-moistened cotton for 8h prior to the experiment (Naresh and Smith 1984). Twenty adult rice stink bugs were used in each of eight replications. Males and females were tested separately. The insects were released into the arena and observed for feeding 1, 3, 6, 9, 12 and 24h post-introduction, respectively at $25.6 \pm 2^\circ\text{C}$ and 14:10 L:D photoperiod. An individual was considered 'feeding' when observed for at least one minute with mouthparts inserted into the food source.

The rice stink bugs were collected and held according to the protocol described previously. The experiment was conducted at a room temperature of $25.6 \pm 2^\circ\text{C}$, 14:10 L:D photoperiod. The panicles of rice from plants grown in the greenhouse and grasses (barnyardgrass, ryegrass and dallisgrass) that were collected from AES were cut 20-30cm below the panicle bases. The bases of the panicles were merged into water in glass bottles and secured with paper towels to prevent entry of the rice stink bug individuals into the bottles. Rice diet was prepared according to procedures described previously and diet packets were tied on straws. The straws and bottles holding the food source were placed on the bottom of the cage. Late fifth-instar male and female rice stink bug nymphs were identified by the presence or absence, respectively, of red testes visible through the abdominal sterna (Nilakhe 1976) and separated into cages on rice diet or one of the host grass panicles. After the molt to adult, 15 pairs of rice stink bugs (24-h old) were randomly selected and transferred into the cages consisting of 19-liter plastic pails containing one of the food sources and covered with 64-mesh nylon cloth. The panicles and the rice diet were replaced every 2 days. The cages were observed at 0800 and 2000h CDT for eggs and any

mating pairs. Eggs were collected, counted and incubated. The number of days required for at least 90% hatch from each egg mass (one egg mass for each of three replicates given > 25 eggs per mass) was recorded. Data were analyzed with ANOVA and means separated with student's *t*-test (SAS Institute 2002).

RESULTS AND DISCUSSION

Numbers of male and female rice stink bugs observed feeding were significantly greater ($F = 130.29$; $df = 3, 383$; $P < 0.0001$) on rice flour diet and barnyardgrass than on either ryegrass or dallisgrass throughout the observation period (Table 1). One hour after release, overall male feeding (32.5%) was significantly less than that observed for females (55.5%) ($F = 14.24$; $df = 1, 63$; $P = 0.0004$) (Table 1). However, at 3 h or more post-introduction, male feeding (> 66%) was comparable to female feeding (> 74%) on all hosts. Overall, significantly more females than males fed ($F = 6.65$; $df = 1, 383$; $P = 0.01$) during a 24-h period. This study agrees with the previous host preference studies of rice stink bugs where the initial female response to feeding stimuli was more rapid than that of males (Naresh and Smith 1984).

TABLE 1. Feeding Preference (\pm SE) of 20 Male or Female Rice Stink Bugs, *Oebalus pugnax* (F.), on Different Food Sources in Laboratory.

Food Source	Time (CDT)					
	0900	1100	1400	1700	2000	0800
	Males					
Barnyardgrass	2.8 \pm 0.4a	4.8 \pm 0.3a	4.5 \pm 0.5a	5.5 \pm 0.3a	4.9 \pm 0.6a	5.1 \pm 0.4a
Rice Flour Diet	1.8 \pm 0.5ab	3.8 \pm 0.7ab	4.5 \pm 0.6a	4.5 \pm 0.7a	4.6 \pm 0.6a	4.0 \pm 0.5b
Dallisgrass	0.9 \pm 0.2b	2.6 \pm 0.2b	2.5 \pm 0.3b	2.5 \pm 0.3b	1.9 \pm 0.4b	2.3 \pm 0.3c
Ryegrass	1.0 \pm 0.3b	2.1 \pm 0.4c	1.6 \pm 0.3b	1.9 \pm 0.2b	1.8 \pm 0.3b	2.1 \pm 0.2c
Mean Response	1.6	3.3	3.3	3.6	3.3	3.4
% Feeding	32.5	66.5	65.5	72.0	66.0	67.5
	Females					
Barnyardgrass	4.4 \pm 0.5a	5.9 \pm 0.5a	5.8 \pm 0.6a	5.8 \pm 0.5a	5.9 \pm 0.4a	6.1 \pm 0.5a
Rice Flour Diet	2.9 \pm 0.4b	4.6 \pm 0.5a	4.9 \pm 0.6a	5.1 \pm 0.5a	5.0 \pm 0.5a	5.1 \pm 0.7a
Dallisgrass	2.0 \pm 0.4b	2.4 \pm 0.4b	2.3 \pm 0.3b	2.5 \pm 0.3b	2.0 \pm 0.4b	2.0 \pm 0.4b
Ryegrass	1.8 \pm 0.4b	2.3 \pm 0.4b	2.3 \pm 0.3b	2.0 \pm 0.4b	1.9 \pm 0.2 \pm b	2.3 \pm 0.4b
Mean Response	2.8	3.8	3.8	3.9	3.7	3.9
% Feeding	55.5	76.0	76.5	77.0	74.0	77.5

*Means in same column with different letters are significantly different ($P < 0.05$, Student's *t*-test)

Overall mean number of eggs increased with days after eclosion (Fig. 1). Significantly more ($F = 76.36$; $df = 1, 629$; $P < 0.0001$) eggs were collected at 0800h compared to those collected at 2000h CDT. Mean number of eggs laid per day significantly differed ($F = 4.8$; $df = 4, 314$; $P = 0.0008$) among food sources (Table 2).

Significantly more mating ($F = 115.85$; $df = 1, 629$; $P < 0.0001$) occurred at 2000 than at 0800h CDT. Overall, mating frequency was significantly higher ($F = 11.4$; $df = 4, 314$; $P < 0.0001$) on rice than on barnyardgrass. Mating on barnyardgrass was greater than on ryegrass, dallisgrass or rice diet, which were all similar (Table 2).

FIG. 1. Mean Number of Egg Masses Laid per Day by Female Rice Stink Bug, *Oebalus pugnax* (F.) ($n = 15$, Each with 15 Females) During a 21-Day Period While Maintained in the Laboratory on *Oryza sativa* L., *Echinochloa crusgalli* (L.), *Paspalum dilatatum* Poir., *Lolium* spp and a new artificial rice flour diet at 25.6°C, 14:10 L:D Photophase.

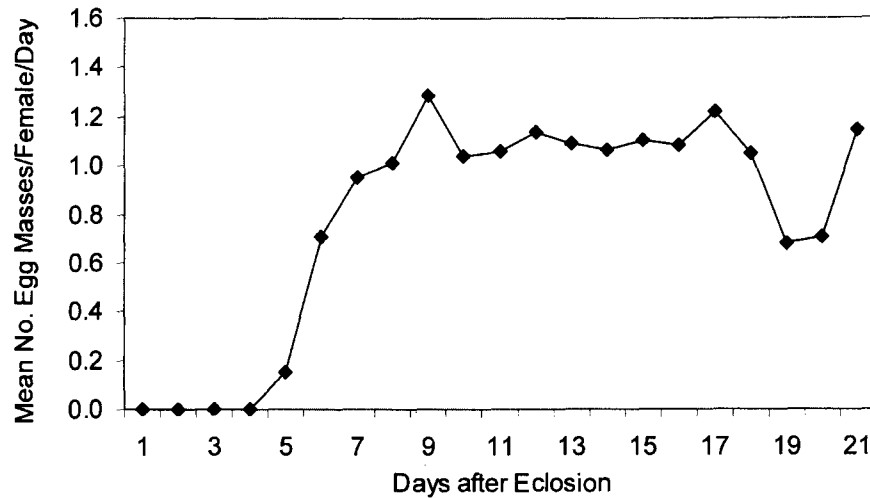


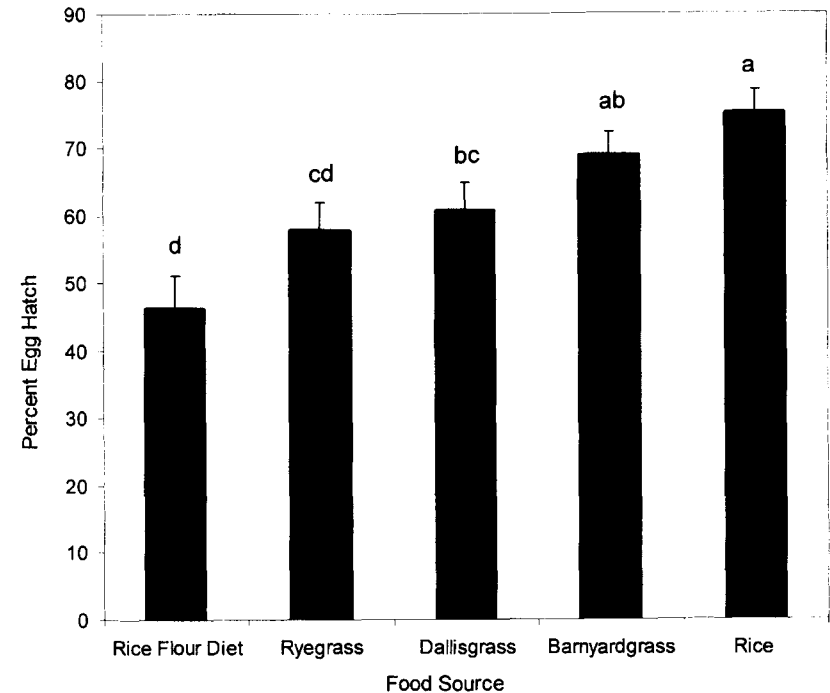
TABLE 2. Mean Number (\pm SE) of Matings and Eggs Laid per Day by 15 Pairs of Rice Stink Bugs, *Oebalus pugnax* (F.), on Different Food Sources in Laboratory During a Period of 21 d.

Food Source	Time (CDT)		Total
	0800	2000	
	<u>No. of Matings</u>		
Rice	1.6 \pm 0.1a	2.6 \pm 0.16a	4.2 \pm 0.2a
Barnyardgrass	1.4 \pm 0.09ab	2.2 \pm 0.14b	3.6 \pm 0.2b
Dallisgrass	1.2 \pm 0.1bc	1.9 \pm 0.14bc	3.1 \pm 0.2bc
Ryegrass	0.9 \pm 0.07c	1.8 \pm 0.13c	2.7 \pm 0.18c
Rice Flour Diet	0.9 \pm 0.07c	1.6 \pm 0.13c	2.5 \pm 0.18c
	<u>No. of Eggs</u>		
Rice	9.1 \pm 1.2a	3.6 \pm 0.6a	12.7 \pm 1.5a
Barnyardgrass	7.8 \pm 0.9ab	2.8 \pm 0.5ab	10.6 \pm 1.2ab
Dallisgrass	6.5 \pm 0.8b	2.8 \pm 0.6ab	9.3 \pm 1.1b
Ryegrass	5.8 \pm 0.8bc	2.3 \pm 0.4ab	8.1 \pm 1.0bc
Rice Flour Diet	4.4 \pm 0.8c	1.6 \pm 0.4b	6.0 \pm 0.9c

^aMeans in same column with different letters are significantly different ($P < 0.05$, Student's t -test)

Egg hatch also significantly differed ($F = 7.1$; $df = 4, 201$; $P < 0.0001$) among females reared on different food sources with rice being the most efficient food source (Fig. 2). The 42.2% egg hatch recorded for females reared on rice flour diet was significantly less than that of females reared on rice (75.2%), barnyardgrass (68.9%) or dallisgrass (60.6%) but was not significantly different from ryegrass (57.7%) (Fig. 2).

FIG. 2. Percent Hatch of Rice Stink Bug, *Oebalus pugnax* (F.) eggs ($n = 3$ Egg Masses; Given > 25 eggs per Mass) as Affected by Adult Feeding on Different Food Sources While Maintained in the Laboratory at 25.6°C, 14:10 L:D Photophase.



Rice flour diet may be an acceptable alternate food source for adult rice stink bugs in the laboratory comparable to natural hosts such as ryegrass. Efforts to rear early instar rice stink bug nymphs on rice flour diet were unsuccessful. The early instar nymphs fed on diet packets for an extended period of time (2-3 weeks) but no growth was observed and additional molts did not occur. Further studies are necessary to determine the dietary requirements of the early instar rice stink bug nymphs to overcome the nutritional or feeding stimulant deficiencies in the diet presented here.

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