

## EFFECTS OF RICE PANICLE AGE ON QUANTITATIVE AND QUALITATIVE INJURY BY THE RICE STINK BUG (HEMIPTERA: PENTATOMIDAE)

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### ABSTRACT

Greenhouse experiments were conducted to evaluate the effects of panicle age on quantitative and qualitative injury caused by rice stink bug, *Oebalus pugnax pugnax* (Fab.), infestations on rice, *Oryza sativa* L. The effects were measured at two infestation levels (one and two bugs per panicle) and compared with an undamaged control. Percentage of empty grains and average weight of filled grains (quantitative injury) and percentage of pecky rice (qualitative injury) were evaluated at grain maturity. Regardless of infestation level, insect feeding during anthesis and the early milk stage of grain development (first 8 d after anthesis) caused substantially higher numbers of empty grains than feeding during later grain development and the control. Average grain weights were lower in infestations during anthesis and milk stage and higher in infestations during later grain development and the control. Pecky rice was significantly higher during late milk and soft dough stages, 9-16 d after anthesis, compared with remaining stages of grain development and the control. Injury was greater in the experiment in which panicles were infested with two bugs. Pecky rice was associated with highly significant reductions in germination of the grains. The data suggest that rice is most vulnerable to rice stink bug injury during the first two weeks after anthesis, and that the major effects of stink bug feeding change as panicles age.

**Key Words:** *Oebalus pugnax pugnax*, *Oryza sativa*, rice stink bug, injury, anthesis, rice panicle age

### RESUMEN

Experimentos en invernadero fueron realizados para evaluar los efectos de la edad de la panícula en daños cualitativos y cuantitativos causados por la chinche hedionda del arroz., *Oebalus pugnax* (Fab.), en arroz, *Oryza sativa* L. Estos efectos fueron medidos a dos diferentes niveles de infestación (uno y dos insectos por panícula) y comparados con un testigo sin daño. El porcentaje de granos vacíos y peso medio de los granos llenos (daño cuantitativo) y el porcentaje de arroz picado (daño cualitativo) fueron evaluados durante la madurez del grano. Independientemente del nivel de infestación, daños causados por la alimentación de los insectos durante la antesis y el período inicial de desarrollo de grano lechoso (primeros 8 días después de la ocurrencia de la antesis) resultaron en un mayor número de granos vacíos que durante estadios de desarrollo del grano más avanzados y el testigo. El peso medio del grano fue más bajo cuando las infestaciones se produjeron durante la ocurrencia de la antesis y el grano lechoso y más alto en infestaciones durante estadios de desarrollo más avanzados y en el testigo. La ocurrencia de grano picado fue significativamente más alta durante los estadios de desarrollo de grano lechoso avanzado y masa blanda (9-16 días después de la ocurrencia de la antesis), en comparación con los otros estadios de desarrollo y el testigo. Daños fueron mayores en el experimento en el cual las panículas fueron infestadas por dos insectos. Arroz picado resultó en reducciones de la germinación del grano muy significativas. Estos datos sugieren que el arroz es más vulnerable a daños causados por la chinche hedionda del arroz durante las primeras dos semanas después de la ocurrencia de la antesis, pero los efectos de la alimentación de las chinches cambian con la edad de las panículas.

Translation by the authors.

The rice stink bug (Hemiptera: Pentatomidae), *Oebalus pugnax pugnax* (Fab.), is one of the most injurious pests of rice in the southern United States (McPherson & McPherson 2000). It is common in the United States east of the Rocky Mountains and as far north as Minnesota and New York (Sailer 1944). It is attracted to rice during repro-

ductive phases of growth, in particular during grain development (Douglas 1939; Rashid et al. 2006). Both adults and nymphs feed on developing grains (Naresh & Smith 1983). Feeding results in losses in yield and reduction in grain quality (Odglen & Warren 1962; Swanson & Newsum 1962; Bowling 1963). The entire contents of

the rice grain may be removed during the milk stage, resulting in false grains, or a portion of the content may be sucked out, resulting in atrophied grains (Swanson & Newsom 1962). Feeding during soft and hard dough stages leaves a chalky discolored area around the feeding site and rice so affected is called *pecky rice* (Harper et al. 1993). Fungi often enter the punctures made by rice stink bug (Hollay et al. 1987; Lee et al. 1993). Pecky rice easily breaks during milling, lowering the percentage of whole grains and, thus, the market value of the product (Odglen & Warren 1962). If pecky rice does not break during milling, it will appear in head rice, resulting in inferior quality of rice (Harper et al. 1993). For a brown rice sample to qualify as US #1 or US #2, it should contain no more than 1 or 2% pecky rice, respectively (Fryar et al. 1986). Feeding also results in losses due to reduced viability of the grain (Swanson & Newsom 1962).

Little effort has been made to develop nonchemical controls for rice stink bug for several reasons, including the short period of rice plant susceptibility (heading to harvest, approximately 30 d for most varieties), the high mobility of the bug, the low economic thresholds, and the relatively low cost of chemical controls (Way 1990). However, several of the chemical pesticides used for controlling stink bugs may be removed in the future due to label revision, to cancellation because of environmental and human safety concerns, or to costs of the registration process (Todd et al. 1994; McPherson & McPherson 2000). Thus, investigations of alternate methods of control are needed.

The susceptibility of a crop plant to injury from pest insects usually varies with the stage of the crop. In rice, although numerous attempts have been made to quantify the relationship between densities of rice stink bugs and injury (Odglen & Warren 1962; Swanson & Newsom 1962; Bowling 1963; Robinson et al. 1980; Harper et al. 1993; McPherson & McPherson 2000; Rashid 2003), surprisingly few studies have attempted to quantify the changes in rice susceptibility that occur as rice panicles age and grains mature. Previous studies that have investigated the influence of panicle age or grain stage on susceptibility to stink bug injury were conducted in the field, where the presence of parasites, pathogens, and weeds might have influenced results (Odglen & Warren 1962; Rashid 2003; Tindall et al. 2004). The objective of this study was to evaluate the effects of panicle age and grain maturity on the quantitative and qualitative injury caused by rice stink bug feeding in a controlled environment on panicles of the rice variety 'Cocodrie', a recently-released and widely-planted variety. Effects were measured for two infestation levels of rice stink bug. Effects on germination of infested grains also were evaluated.

## MATERIALS AND METHODS

### Qualitative and Quantitative Injury

Experiments were conducted during the summer of 2003 in a greenhouse on the campus of Louisiana State University, Baton Rouge, LA. Rice (*Oryza sativa* cv 'Cocodrie') was planted in pots and grown in the greenhouse from Mar to Jul. Rice for the first experiment was planted on Mar 19 and for the second experiment on Mar 25. Pots were 7" inches in height and 7" in diameter. Growth medium was a mixture composed of 4 parts soil: 2 parts peat moss: 1 part sand: 1 part vermiculite. Each pot was supplied with approximately 3.5 g of 23:12:12 NPK fertilizer at planting. Plants were watered as needed during the experiments. Natural lighting was the only source of light. Temperature ranged from 25 to 35°C in the greenhouse throughout these experiments.

Rice stink bugs were collected from heading or headed rice as well as barnyard grass at the LSU AgCenter Rice Research Station, Crowley, LA. Bugs were maintained on panicles of barnyard grass in the laboratory for approximately 2 d and only bugs showing no signs of disease or damage were used in experiments.

Experiments were initiated by tagging a large number of panicles at the anthesis stage (approximately 1 d after initial emergence of panicle) on Jun 9 (experiment one) and Jun 13 (experiment two). Panicles were randomly assigned to the following treatments: infestation at 1, 5, 9, 13, 17, and 21 d after anthesis. In the first experiment, each panicle was infested with one female rice stink bug at the appropriate day for 4 d. In the second experiment, panicles were infested with two female rice stink bugs per panicle at the appropriate day for 4 d. In both experiments, bugs were placed inside muslin cloth sleeves enclosing a rice panicle and tied at the bottom. Panicles serving as controls were enclosed by muslin cloth without stink bugs. Bugs were removed from the muslin cloths after 4 d and the muslin cloth was again put back on the panicle until harvest. Treatments were arranged in a completely randomized design with 18 replications in the first experiment (one bug per panicle) and 10 replications in the second experiment (two bugs per panicle).

Rice panicles were in the anthesis stage (Counce et al. 2000) during approximately the first 4 d after tagging (Patel, personal observation). Panicles then advanced into the milk stage (Counce et al. 2000) approximately 5 to 12 d after tagging. The soft dough (Counce et al. 2000) stage ran approximately from 13 to 17 d after tagging and then gradually progressed into the hard dough stage.

Panicles were gently harvested by hand at maturity and individually placed in plastic Ziploc

bags. All panicles were taken out of the Ziploc bags and air-dried on a lab bench at room temperature for one week. Panicles were then individually threshed by hand. Filled and empty grains were separated manually; partially filled grains were counted as filled. The numbers of empty and filled grains per panicle were counted and the data were used to calculate the percentages of empty and filled grains in each treatment. Total weight of the filled grains also was determined. The weight and number of the filled grains per sample were used to determine the average weight of a filled grain per treatment. Hulls were removed mechanically from the rough rice samples by a McGill Sheller (H. T. McGill Inc., Houston, TX). The resultant samples were separated visually into pecky vs. nonpecky rice and then weighed separately. All chalky discolored grains were classified as "pecky." Weights of pecky and pecky plus nonpecky rice were used to calculate the percentage of pecky rice for each treatment (time of infestation).

#### Effects on Germination

Pecky and nonpecky grains from the experiment in which one bug per panicle was used to injure rice were used for the germination experiment. Grains were included from panicles infested 1, 9, and 17 d after anthesis as well as those from the control. The effects of rice quality (pecky vs. nonpecky), time of infestation (1, 9, or 17 d after anthesis), and their interaction were tested in this experiment. For each of the eight treatment  $\times$  time combinations, five replicates of 20 grains were placed in a  $5 \times 4$  matrix in 100 mm  $\times$  15 mm sterile Petri dish (BD Falcon™, BD Biosciences, Franklin Lakes, NJ), lined with three layers of germination paper (Anchor Paper Co., St. Paul, MN) saturated with 8 ml distilled water. Grains were treated with Quadris 2.08 SC (Syngenta Crop Protection, Greensboro, NC), a fungicide, and covered with two layers of Kimwipe tissue paper to ensure uniform hydration. Closed dishes were incubated at 100% relative humidity for 14 d at 30° C in darkness. Radical emergence was the criterion for germination. The number of grains germinated during the 14 d was recorded for each Petri dish.

#### Data Analysis

Data from the two experiments with different infestation levels were analyzed separately. Data on quantitative (percentage of empty grains and average weight of filled grains) as well as qualitative injury (percentage of pecky rice) were analyzed by multivariate analysis of variance with the MANOVA statement and Wilks' Lambda statistic in PROC GLM of SAS (SAS Institute 1996). This statistic tested the null hypothesis of no

overall significant treatment (time of infestation) effect on all three response variables. Correlations among response variables were assessed with Pearson correlation coefficients produced by PROC CORR of SAS (SAS Institute 1996). Then, each of these response variables was individually subjected to analysis of variance by PROC GLM and the Tukey HSD test for means separation (SAS Institute 1996). Germination data were subjected to two-way analysis of variance and were analyzed with PROC GLM of SAS (SAS Institute 1996).

## RESULTS

#### MANOVA Procedure and Pearson Correlation Coefficients

The multivariate analysis suggested that treatment (time of infestation) had an overall significant effect on the response variables (percentage of empty grains, average weight of filled grains, and percentage of pecky rice) in both experiments (Wilks' Lambda, one rice stink bug per panicle:  $F_{183, 332} = 36.47$ ,  $P < 0.001$ ; Wilks' Lambda, two rice stink bugs per panicle:  $F_{187, 174} = 61.71$ ,  $P < 0.001$ ). Pearson correlation coefficients revealed that only the percentage of empty grains and average weight of filled grains were significantly correlated with each other. This correlation was stronger at the higher infestation level ( $r = -0.5245$ ,  $P < 0.001$  [one bug/panicle],  $r = -0.7548$ ,  $P < 0.001$  [two bugs/panicle]).

#### Percentage of Empty Grains

The percentage of empty grains in panicles decreased as time of infestation after anthesis increased in both experiments (one rice stink bug per panicle:  $F_{67, 119} = 31.25$ ,  $P < 0.001$ , Fig. 1; two rice stink bugs per panicle:  $F_{67, 63} = 81.11$ ,  $P < 0.001$ , Fig. 1). In both experiments, the percentage of empty grains was statistically greater in panicles infested 1 d after anthesis compared with that in panicles infested during later grain development and panicles in the control. Regardless of infestation level, the percentage of empty grains in panicles infested 1 d after anthesis was approximately 2 times greater than the percentage in panicles infested 9 d after anthesis. In both experiments, infestation of panicles for 4 d beginning 1 and 5 d after anthesis produced greater percentages of empty grains compared with panicles infested 13, 17, and 21 d after anthesis and panicles in the undamaged control. In the two bugs per panicle experiment, panicles infested 9 d after anthesis also produced a greater percentage of empty grains than panicles infested during later grain development and panicles in the control. Infestation of panicles 13, 17, and 21 d after anthesis did not produce any significant reductions in

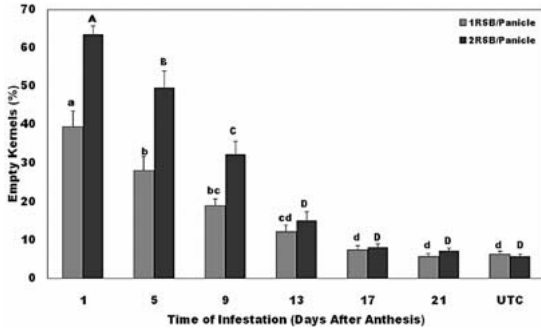


Fig. 1. Mean percentage ( $\pm$ SE) of empty kernels in rice panicles infested for a period of 4 d beginning 1, 5, 9, 13, 17, or 21 d after anthesis and in panicles from the untreated control (UTC). Two bars at each infestation time represent data from two experiments with infestation levels of one or two rice stink bugs (RSB) per panicle. Means within each infestation level followed by same lower or upper case letter did not differ significantly at  $\alpha = 0.05$  (Tukey, HSD).

the percentage of filled grains compared with the control in either experiment. Panicles in the control averaged 6-7% empty grains in the two experiments. Feeding by two rice stink bugs produced at least 1.5 times as many empty grains as feeding by one bug in panicles infested 1, 5, and 9 d after anthesis.

Average Weight of Filled Grains

Treatment significantly affected the average weights of filled grains in rice panicles infested with one rice stink bug per panicle ( $F_{67,119} = 6.45, P < 0.001$ , Fig. 2) as well as two rice stink bugs per

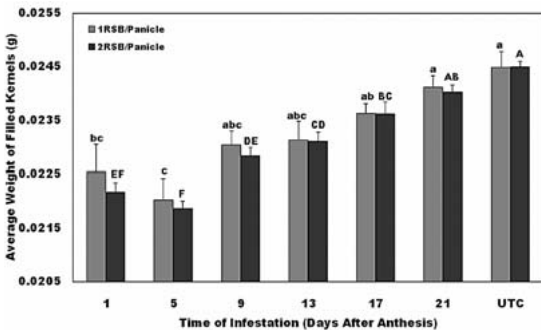


Fig. 2. Average weight (g) of filled kernels ( $\pm$ SE) in rice panicles infested for a period of 4 d beginning 1, 5, 9, 13, 17, or 21 d after anthesis and in panicles from the untreated control (UTC). Two bars at each infestation time represent data from two experiments with infestation levels of one or two rice stink bugs (RSB) per panicle. Means within each infestation level followed by same lower or upper case letter did not differ significantly at  $\alpha = 0.05$  (Tukey, HSD).

panicle ( $F_{67,63} = 33.86, P < 0.001$ , Fig. 2). Average weights generally increased with the time of infestation after anthesis in both experiments. In the one rice stink bug per panicle experiment, panicles infested 1 and 5 d after anthesis had lower average weights compared with panicles infested 21 d after anthesis and panicles in the control. In the two rice stink bugs per panicle experiment, panicles infested 1 d after anthesis had lower average weights compared with panicles infested 13, 17, and 21 d after anthesis and panicles in the control. In the same experiment, panicles infested 5 d after anthesis had lower average weights compared with panicles infested during later grain development and panicles in the control. When infested with one rice stink bug per panicle, there were reductions of 8% and 10% in average weights in panicles infested 1 and 5 d after anthesis, respectively, compared with the control; these reductions were 10% and 11% with two rice stink bugs. This result suggests that feeding during the anthesis, milk, and soft dough stages of grain development reduced the average weights of filled grains, with more injury during early milk stage. Reductions in average weights were high in panicles infested for 4 d beginning 1, 5, and 9 d after anthesis and low thereafter, demonstrating that the first 12 d after anthesis were the most critical for injury in terms of reduced grain weight.

Percentage of Pecky Rice

Pecky rice as a percentage of the total weight of the de-hulled grains in each rice panicle is shown in Fig. 3. In both experiments, controls had approximately 3% pecky rice. This result indicated

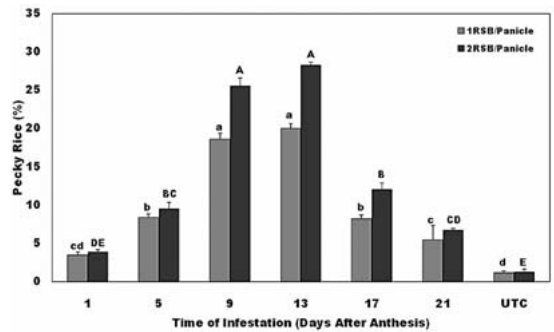


Fig. 3. Mean percentage ( $\pm$ SE) of pecky rice in rice panicles infested for a period of 4 d beginning 1, 5, 9, 13, 17, or 21 d after anthesis and in panicles from the untreated control (UTC). Two bars at each infestation time represent data from two experiments with infestation levels of one or two rice stink bugs (RSB) per panicle. Means within each infestation level followed by same lower or upper case letter did not differ significantly at  $\alpha = 0.05$  (Tukey, HSD).

that pecky rice was caused by factors in addition to rice stink bug. The percentage of pecky rice in panicles differed with the time of infestation after anthesis in both experiments: (one rice stink bug per panicle:  $F_{67, 119} = 138.92$ ,  $P < 0.001$ , Fig. 3; two rice stink bugs per panicle:  $F_{67, 63} = 200.23$ ,  $P < 0.001$ , Fig. 3). In both experiments, the percentage pecky rice was greater in panicles infested 9 and 13 d after anthesis compared with that in panicles in all other treatments and the control. Similarly, the percentage pecky rice was greater in panicles infested 5 and 17 d after anthesis compared with that in panicles infested 21 d and 1 d after anthesis as well as that in the control. The percentage of pecky rice in panicles infested 1 and 21 d after anthesis did not differ, and infestation at d 1 did not differ from the control. In both experiments, the percentage pecky rice in panicles infested 9 or 13 d after anthesis was at least 2 times greater than that in panicles infested 5 or 17 d after anthesis and approximately 4 times greater than in panicles infested 1 or 21 d after anthesis or that in the control. Thus, rice stink bug caused pecky rice injury when rice panicles were infested for 4 d at 5 to 21 d after anthesis, with the most severe injury inflicted in panicles infested on d nine and d 13. Incidence of pecky rice was higher in the two bugs per panicle experiment.

#### Percent Germination of Infested Grains

Peckiness was associated with reductions in the germination of rice grains ( $F_{17, 32} = 935.03$ ,  $P < 0.001$ ), but the level of reduction did not differ with time of infestation ( $F_{37, 32} = 0.61$ ,  $P < 0.6118$ ). There was no quality of rice  $\times$  time of infestation interaction ( $F_{37, 32} = 1.05$ ,  $P < 0.3860$ ). This result indicates that qualitative injury by rice stink bug feeding reduced germination by nearly the same amount at all times of infestation after anthesis as well as in the control. Germination of nonpecky grains averaged 89% while that in pecky grains was 43%.

#### DISCUSSION

The data in these experiments showed that rice grains became less susceptible to quantitative injury (yield loss) by the rice stink bug as the grains developed. Feeding during anthesis and the milk stage produced significantly higher percentages of empty grains than did feeding during later grain development. This finding supports past field work by Pantoja et al. (2000) with a related species, *Oebalus ornatus* (Sailer), and by Rashid (2003) and Swanson & Newsom (1962) with the rice stink bug that showed severe losses in rice yields resulting from rice stink bug feeding during the flowering and the milk stage compared with feeding during the soft dough stage. This re-

sult is partly explained by the feeding method of this bug, which sucks out the contents of grains in the milk stage (Odglen & Warren 1962). The exact feeding mechanism of the rice stink bug on rice grains at anthesis is not reported in the literature. Previous work by Every et al. (1990) indicated that the wheat bug, *Nysius huttoni* White, could suck sap rich in amino acids and sugars from the ovary of wheat seeds at late anthesis. Lee et al. (1993) found that rice stink bug feeding during anthesis restricted further grain development. Rice stink bug feeding also reduced the average weights of filled grains during anthesis and the milk stage (first 12 d after anthesis). Feeding during the milk stage has been shown to produce atrophied grains (Swanson & Newsom 1962; Robinson et al. 1980), which probably was a major contributing factor to the reduced average weights during the milk stage. Fryar et al. (1986) stated that many pecky rice grains weigh substantially less because they are not fully developed. Therefore, it is likely that the higher percentages of pecky rice infested during the milk and soft dough stages in our experiments significantly contributed to the reduced average weights during those stages. Previous work by Fuchs et al. (1988) indicated that rice stink bug infestation during grain development in sorghum reduced the weight and size of the seeds.

There are at least two explanations for the decrease in quantitative injury to rice grains as the grains matured. First, rice stink bugs may feed less as grains develop and harden. Second, stink bug feeding may be equal on grains of different ages, but grains may become less susceptible to injury from rice stink bug feeding as they mature.

Incidence of empty grains and reductions in weights of filled grains were greater under the higher infestation level, particularly during anthesis and the milk stage. Studies by Swanson & Newsom (1962), Robinson et al. (1980), and Rashid (2003) with the rice stink bug also found significant reductions in the total weight per grain at higher infestation levels compared with lower infestation levels. This finding supports previous greenhouse research with the rice bug *Leptocorisa oratorius* (F.) that showed a negative correlation of rice yield to bug density (Jahn et al. 2004). Yield losses in this latter study resulted from increased numbers of empty and partially filled grains under higher infestation levels.

The data for percentage of pecky rice (qualitative injury) revealed two valuable pieces of information. First, in contrast to the results for quantitative injury, the highest levels of pecky rice occurred in grains infested during the soft dough stage. Severe qualitative injury, at both infestation levels, occurred in panicles infested during the soft dough stage (13 d after anthesis). Panicles infested during the late milk stage (9 d after anthesis), which had a significant number of

grains in the soft dough stage, and panicles infested during the hard dough stage (17 and 21 d after anthesis) also had considerable pecky rice. The vulnerability of the soft and hard dough stages is probably explained by the fact that this bug removes a portion of the contents of grain, leaving a discolored area around the site. Previous studies have shown that grains attacked during the soft and hard dough stages resulted in pecky rice (McPherson & McPherson 2000; Harper et al. 1993); although not as common, pecky rice was also reported in grains attacked during the milk stage in the field (Odglen & Warren 1962).

Second, the presence of pecky rice in the controls in both experiments suggests that peckiness is caused by factors in addition to rice stink bug feeding, perhaps fungi (McPherson & McPherson 2000). It is clear, however, that rice stink bug feeding was a major factor contributing to pecky rice in infested panicles in the current experiments, either directly or indirectly by facilitating the entry of microbes. The rice stink bug is known to vector several pathogens through its stylets in a transient manner (Hollay et al. 1987). Lee et al. (1993) demonstrated that discoloration in pecky rice resulted from fungi that were introduced when rice stink bug was feeding. Maretti & Peterson (1984) demonstrated that rice stink bug feeding was a major factor in grain discoloration, although *Bipolaris oryzae* (Breda de Haan), a fungus that causes brown spot, was a primary cause of some grain discoloration and was one of several microbes that colonized grains through feeding punctures. *Nematospora coryli* Peglion, a fungus capable of causing discolored areas, has also been noted (Way 1990).

Pecky rice germinated at a significantly lower rate than nonpecky rice, indicating that injury due to rice stink bug feeding and/or microbes associated with pecky grains may have injured the embryo of the attacked grains. It is also possible that microbes present within the pecky grains interrupted the germination process, although no visible sign of differences in the microbial growth between pecky and nonpecky grains were observed during the germination test. A previous study has documented reductions in viability of grains because of rice stink bug feeding (Swanson & Newsom 1962). In this study, grains that were atrophied or injured at the proximal (germ) end had reduced viability. Apparently, the embryo is extremely sensitive to injury by the rice stink bug. Rice stink bug attack during grain development in sorghum reduced seed germination (Fuchs et al. 1988). Although the seed cleaning process would eliminate much of the seed severely atrophied by rice stink bug injury, observed reductions in germination were substantial enough to prevent certification of seed for commercial sale, which has an acceptable limit of 85% germination (Douglas & Tullis 1950).

Rice producers have long relied on synthetic insecticides to control rice stink bugs (McPherson & McPherson 2000). Concerns about the toxicity of insecticides to non-target organisms, continued availability of currently registered insecticides, and adverse effects of insecticides on the environment have prompted investigations of alternative strategies for management of the rice stink bug. The short window of vulnerability of the rice plant to rice stink bug (approximately 30 d for most varieties) has been an important factor in restricting research in the development of nonchemical control measures (Way 1990). The current available action thresholds for rice stink bug in rice (30 bugs per 100 sweeps for the first two weeks of heading and 100 bugs per 100 sweeps from the dough stage until two weeks before harvest (Johnson et al. 1987) accounts to some degree for age-related changes in grain susceptibility to injury. However, more precise information such as that reported here on the susceptibility of rice panicles may be important for the refinement of the current thresholds and for the development of alternative management strategies for the rice stink bug.

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