



Quantitative and qualitative damages of *Oebalus poecilus* on irrigated rice in southern Brazil¹

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ABSTRACT

Oebalus poecilus is one of the most important pests of irrigated rice in Brazil. However, the impact of this species on the cultivar IRGA 424 RI, which is the most used, is unknown. Hence, the objective of this work is to evaluate the damage caused by *O. poecilus* on cv IRGA 424 RI. Panicles of this cultivar were infested for seven days at the R5 stage, using a randomized block design, considering the following factors sex, insect reproductive stage and insect density (number/panicle). In order to analyze the effect of these factors, the qualitative and quantitative damage caused by stink bugs to panicles were evaluated. An interaction was found between the sex and developmental stage for quantitative variables, in which reproductive females were responsible for increasing the damage in comparison to pre-reproductive females and the males, while the greatest qualitative damage was caused by reproductive insects regardless of sex and by females, despite the reproductive stage. Density of only one infesting insect has already increased the qualitative damage, while significant losses on the weight of the grain were verified with the infestation of two insects, demonstrating that IRGA 424 RI is susceptible to the attack of these stink bugs.

Keywords: IRGA 424 RI; *Oryza sativa* Linnaeus; rice stink bug; IPM; insect plant interaction.

INTRODUCTION

Rice stink bug, *Oebalus poecilus* (Dallas) (Hemiptera, Pentatomidae), is one of the main pests of rice crop in Brazil, occurring both in upland and flood irrigated crops (Barrigossi, 2008; SOSBAI, 2016). The importance of this bug relies on the fact that nymphs and adults feed by sucking the endosperm contents of the spikelets, causing direct and indirect damages to the grains (Blackman & Stout, 2017). The impact of this insect feeding varies according to the phenological stage of the plant (Espino *et al.*, 2007; Krinski & Foerster, 2017). During flowering (R4) and milky-grain (R5) stages, the number of empty spikelets may be increased, their weight may be reduced by partial or complete removal of the endosperm, favoring micro-organisms contamination, causing spots on the spikelets and on the grain as well as to reduce seed

germination (Ferreira *et al.*, 2002; Krinski & Foerster, 2017). In the soft dough phase (R6), in addition to favoring fungal contamination and causing stains on the grains, they can also weaken them structurally, which facilitates the breakage during the grain processing, reducing the percentage of whole grains (Barrigossi, 2008).

Abiotic factors such as temperature and relative humidity of the air and biotic factors, such as the presence of natural enemies and the susceptibility of cultivars may also affect the damage intensity (Barrigossi, 2008; SOSBAI, 2016). Several studies have demonstrated differences among genotypes of rice in relation to total spikelet loss (qualitative and quantitative) due to attack of bugs, however, they were carried out using cultivars adapted to the Central-West region or even to an upland rice system (Silva *et al.*, 2018; Ferreira & Barrigossi, 2006; Krinski & Foerster, 2017).

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Almost 25% of the area destined to the production of rice in irrigated environment in Brazil is cultivated with cultivar IRGA 424 RI (IRGA, 2017; CONAB, 2017). The expressive use of a single cultivar is mainly due to the fact that it presents high yield potential besides the resistance to herbicides of imidazolinones group and to rice blast (*Magnaphorthe oryzae*). In addition it presents a great tillering capacity (IRGA, 2014). Although it is the most used cultivar in Brazil, little is known about the feeding damages caused by *O. poecilus* on the rice panicles. Therefore, the objective of this work is to evaluate the quantitative and qualitative damages of *O. poecilus* on this rice cultivar IRGA 424 RI.

MATERIAL AND METHODS

The experiment was conducted in a rice commercial crop during 2016/17 harvest, in the county of Itaqui (29° 07' 31" S; 56° 33' 11" W) in the region of Fronteira Oeste, in the state of Rio Grande do Sul. The climate in the region is classified as "Cfa", subtropical, warm temperate, with well distributed rainfall and well-defined seasons. Over the experiment, the average temperature was 24.9 °C.

The study used cultivar IRGA 424 RI, sown on September 29, 2016, at a density of 80 kg.ha⁻¹ in a minimum and sloped cultivation system. Fertilization used 400 kg.ha⁻¹ of 5-20-25 (N-P-K) formulation as the base fertilizer and at the V3/V4 stage, 72 kg.ha⁻¹ of N (urea) was applied in side dressing. Weed control was performed using imazethapyr and imazapique herbicides at doses recommended by Agrofite (MAPA, 2018). For insect control, a pyrethroid insecticide (lambda-cyhalothrin, 40 mL.ha⁻¹) applied on 01/10/2017 and 02/16/2017 was used, following the technical recommendations for irrigated rice cultivation (SOSBAI, 2016).

In the milky stage (R5), when the panicle presented at least 50% of the grains at this stage (Counce *et al.*, 2000), cages made of PET bottles (2 L) were fixed in wooden stakes, randomly placed near the plants, 50 meters away from the edge of the crop. A panicle with no visible damage signs was isolated in each cage. The experiment used *O. poecilus* adult males and females, at pre-breeding and reproductive phase, which were obtained from a colony maintained in Entomology Laboratory of the Universidade Federal do Pampa (UNIPAMPA). Soon after the emergence in laboratory, the insects were sexed and individualized. Adults up to four days of age were considered pre-reproductive. To obtain the reproductive ones, couples were formed and isolated in Petri dishes until observed the first oviposition.

The isolated panicles were infested with insects of the same sex and stage, at densities of one, two and four individuals, besides the control treatment without

infestation. The insects remained in them for seven days. The cages were inspected on a daily basis for removal of eggs and dead insects, which were replaced by individuals of the same sex and stage.

After a one-week infestation, the bugs were removed and the cages remained near the plants until harvest (R9). On this occasion, the panicles were harvested with a cut right below the flag leaf insertion and individually wrapped in paper bags. In the laboratory, they were manually threshed, and moisture corrected to 13%. Next, the evaluation was performed, recording the number and weight of spikelets per panicle (NSP and SW) and number of stings per spikelet (NSS). The percentage of stung spikelets (SP) and empty spikelets (ES) was calculated. In addition, the average weight of full spikelets (AWFS) and weight reduction percentage (WR) were calculated from the spikelets that were full (containing intact or atrophied grains), compared to control (uninfected panicle). Afterwards, the grains were manually husked and the percentage of grains with qualitative damages was calculated using the formula: grain weight (stung + chalky + atrophied + broken) x 100/ total grain weight.

A randomized block design was used in factorial design (2 x 2 x 4): stage (pre-reproductive and reproductive), sex and densities (zero, one, two and four insects) in three blocks. Data were submitted to the Shapiro-Wilk normality test and, when necessary, the *arc sen* $\sqrt{x/100}$ transformation was used. The factors were compared by the Tukey's test at a 5% probability of error by the software Assistat® 7.7 (Silva & Azevedo, 2016). Data on the density were also analyzed by regression analysis using SigmaPlot® 10.0 software.

RESULTS AND DISCUSSION

No influence of the stage of reproductive development, sex and density of infesting insect was found on the number of spikelets formed per panicle when compared to the control ($F = 0.0040$; $gl = 1$; $p = 0.9498$), with an average of 108 spikelets/panicle. The number of spikelets to be produced is mainly related to the genetic characteristics of the cultivars and is not influenced by the attack of the bugs since this occurs after the panicle emission by the plant, when the number of spikelets has already been determined. Similarly, in other rice cultivars, Silva *et al.* (2018) and Krinski & Foerster (2017) also found no influence of feeding on the number of spikelets formed.

An interaction was found between the stage of reproductive development of the insects and sex on the variables panicle weight (PW) ($F = 12.0201$; $gl = 1$; $p = 0.0019$), weight of full spikelets (WFS) ($F = 4.3919$; $gl = 1$; $p = 0.0468$), reduction in the spikelet weight (RSW) ($F = 5.9324$; $gl = 1$; $p = 0.0226$) and empty spikelets (ES) ($F = 5.313$, $gl = 1$, $p = 0.0301$) (Table 1).

In panicles infested with insects in the pre-reproductive stage, males and females caused similar damages to PW, WFS, RSW and percentage of ES, however, such damages were different when the bugs were in the reproductive phase, when the females were responsible for the significantly reduction in PW and the AWFS, reaching over the 42% reduction on the weight of spikelets, as well as, to increase by over 20% the percentage of empty spikelets in the panicles when compared to the males.

In addition, it was observed that panicles exposed to pre-reproductive males had their weight reduced in comparison with those that remained with the reproductive ones, however, insects at this stage did not significantly decrease the other variables (Table 1). Conversely, for females, a significant reduction in PW and AWFS and an increase in RSW and ES were observed when they were in the reproductive stage, compared to those in the pre-reproductive stage.

Such results show similarity in the quantitative damages caused by pre-reproductive insects, regardless of sex. However, from the maturation of the reproductive organs, the damage is reduced in males, possibly because they have already ingested the necessary amount of nutrients to reach the sexual maturity and fulfill their biological reproduction function. In females, in the reproductive stage, the energy demand is continuous to ensure the copulas and the egg production, which results in a greater intake of nutrients and, consequently, potentiated quantitative damages.

Few are the works relating sex and stage of reproductive development of the bugs to feeding behavior or damage in rice crops. In this context, the works of Bowling stands out (1979). This author, working with the Pentatomid *O. pugnax* (F.), recorded that females fed about twice as much as males, however, the reproductive stage of insects was not considered. The author attributed the results to the larger size of the females compared to the males.

O. poecilus females are approximately 10% larger than the males, however, in the present study, the quantitative

damages on the spikelets were more pronounced only when the females were in the reproductive stage, suggesting that copulation, oocyte maturation and oviposition are activities that require a greater amount of resources, which is supported by the fact that no significant effect was observed on PW, WFS, RSW and ES when they were pre-reproductive stage compared to males.

According to Parra *et al.* (2009), the production of eggs or progeny in insects involves the accumulation of energy and nutrients by the female, causing them to increase consumption when compared to males. When evaluating the reproductive effect on feed intake on *Oncopeltus fasciatus* (Dallas) (Hemiptera, Lygaeidae), Slansky-Junior (1980), indicated that reproductive females fed more and converted up to 47.2% of the assimilated feed for egg production, in contrast to the only 10% converted by the pre-reproductive ones.

No significant interaction between the factors of reproductive development and sex ($p > 0.05$) was found for the variables related to qualitative damages, such as number of stings per spikelets, percentage of stung spikelets and damaged grains; only the isolated effect of each factor was significant. Reproductive insects, regardless of sex, were responsible for increasing the number of stings per spikelet and the percentage of stung spikelets, as well as for damaging more than 25% of total grains compared to pre-reproductive ones (Table 2). Regarding the sex factor, the values of all variables were higher when the panicles were exposed to the females in comparison to the males, regardless of the reproductive development stage (Table 2).

It is important to note that in the variables presented in Table 1, which represent the quantitative damages caused by the insects, there was a significant interaction between the stage and sex factors, while in Table 2, which is associated to the qualitative damages caused by injuries on the grains, that do not directly influence their weight, only the isolated factors was significant. This fact indicates that, for females, regardless their reproductive stage, the percentage of stings spikelets was the same (qualitative

Table 1: Average (\pm SE) weight of the panicle (PW), weight of full spikelet (WFS), reduction spikelet weight (RSW) and percentage of empty spikelet (ES) of rice (*Oryza sativa*) cv. IRGA 424 RI infested with *Oebalus poecilus* males and females, in the pre-reproductive and reproductive stages

		PW (g)	WFS (mg)	RSW (%)	ES (%)
Pre-reproductive	Males	1.71 \pm 0.19 Ab*	22.9 \pm 1.19 Aa	23.9 \pm 4.84 Aa	25.1 \pm 5.07 Aa
	Females	2.03 \pm 0.15 Aa	22.8 \pm 1.03 Aa	24.0 \pm 4.19 Ab	24.6 \pm 4.00 Ab
Reproductive	Males	2.21 \pm 0.19 Aa	23.1 \pm 1.02 Aa	22.3 \pm 4.17 Ba	23.5 \pm 3.68 Ba
	Females	1.44 \pm 0.17 Bb	19.6 \pm 1.53 Bb	42.6 \pm 6.20 Aa	45.4 \pm 7.47 Aa

*Means followed by the upper-case letter compare sexes within each stage and the lower-case letter compare stages within the same sex by the Tukey's test at 5% of probability error. SE = Standard Error.

damage), however, the consequence of this feeding on weight (quantitative damage) was more pronounced in the reproductive females than in the pre-reproductive ones.

These results do not meet those obtained by Machado *et al.* (2014) who, worked with the pentatomid *Tibraca limbativentris* Stål in irrigated rice. They found that reproductive females were responsible for increasing the amount of volatiles released by the plants after feeding, compared to the males. The authors suggested that this plant response could be associated with the longer time spent by the females during feeding. Yet, according to Moraes *et al.* (2005), when females of the Pentatomid *Euschistus heros* F. fed on soybeans plants, they produced a greater amount of saliva than males and nymphs. According to Houseman *et al.* (1985), the saliva of sap-sucking insects, mainly, as in Pentatomidae, is composed of lysosomal enzymes, which have the capacity to digest food, facilitating the feeding process. Thus, the release of a greater quantity of saliva may be associated with the larger injuries caused by the females observed in the present study.

On the other hand, Espino & Way (2007) found similar damages among *O. pugnax* males and females and attributed this result to the short period of exposure to panicles (48 hours); however, the authors did not specify the stage of reproductive development of insects. In the present study, the bugs remained in contact with the panicles for a longer period (seven days), which was sufficient to detect the distinct effect between the sexes and also between the stage of reproductive development.

In relation to the density of infesting insects, the presence of two individuals on the panicle resulted in reductions of 32.7 and 14.7% in panicle weight and in the average weight of full spikelets, respectively, however, with the increase from two to four insects there are no significant reduction to these variables (Figure 1).

These damages vary according to the stage when the bugs attack, and soon after the flowering stage, the number of empty spikelets increases, but as grains develop, they become less consumed by the insects, which are not able to make them completely empty,

however, they reduce the content of the endosperm and its weight, as a consequence (Espino & Way, 2007; Barrigossi, 2008; Krinski & Foerster, 2017). In the present study, as insect density raised, the amount of completely empty and lighter spikelets increased, mainly as a result of competition among individuals, since only one development stage was used.

There was observed a reduction of 32.7% in the weight of the two-insect infested panicle, a value greater than that recorded by Ferreira & Barrigossi (2006), 27.7%, when they evaluated 20 genotypes of irrigated rice also infested with two *O. poecilus* adults. The authors exposed the panicles at R5-R9 stage to the insects, whereas in this work this only occurred in the most susceptible stage of the spikelets (R5) (Krinski & Foerster, 2017), thus the damage caused by the bug feeding on IRGA 424 RI was more pronounced, indicating that this cultivar is more susceptible to the attack of these insects than the genotypes evaluated by the authors. In Mississippi, USA, Awuni *et al.* (2015) recorded an average reduction in panicle weight of 29.8%, when panicle were infested for seven days by two *O. pugnax* adults at milky stage, indicating similarity in the damage caused by these two species, although the cultivars and climate regions are different.

The infestation of rice panicle by one or two stink bug caused a significant increase in the percentage of stung and empty spikelets and damaged grains in comparison to the control. However, adding more insects up to four, there was not observed any increases on the damage (Figure 2). In the non-infested panicles, about 15% of SW were recorded, which may be explained by the fact that they were isolated and protected only at the R5 stage, which may have allowed some prior contact of naturally occurring bugs in the area, although in the isolation, the target was the panicles without visible signs.

When the infestation occurred with one insect/panicle, the percentage of stung spikelets was upper than 43%. However, when the panicles were infested with two and four insects/panicle, although the percentage was also increased to 56.6 and 62.9%, respectively), those increments were not significant.

Table 2: Average number (\pm SE) of stings per spikelet (NSS) and average percentage (\pm SE) of stung spikelet (SS) and damaged grain (DG) of rice (*Oryza sativa*) cv. IRGA 424 RI as a function of the infestation of *Oebalus poecilus* males and females in the pre-reproductive and reproductive stages.

		NSS	SS (%)	DG (%)
Stage	Pre-reproductive	1.57 \pm 0.08 B*	46.4 \pm 3.90 B	18.7 \pm 2.05 B
	Reproductive	1.85 \pm 0.08 A	62.9 \pm 2.81 A	25.16 \pm 2.40 A
Sex	Males	1.60 \pm 0.09 b	48.5 \pm 4.14 b	18.3 \pm 2.30 b
	Females	1.83 \pm 0.08 a	60.7 \pm 3.11 a	25.5 \pm 2.01 a

*Means followed by the same upper-case letter do not differ within each stage, means followed by the same lowercase letter do not differ between the sexes by the Tukey's test at 5% of probability error. SE = Standard Error.

Regarding the ES values, when no bug infestation occurred, 10% of the spikelets were empty, which may have been attributed to the genetic characteristics of the cultivar itself, which normally presents about 17% of sterility (IRGA, 2017). Similarly to what had occurred with the stung spikelets (SS), the density of an insect had already significantly increased the percentage of ES, but the increase from two to four insects did not cause significant changes.

These results may be related to the competition effect among individuals, where infestation of only one insect (without competition) increased the values of these two variables in comparison to the control, possibly because they had a greater amount of resource available to the insect. When a competition situation was maintained between two insects/panicle, there was not observed a significantly larger amount of sting spikelets, but they were able to significantly raise the values of ES. Panicles submitted to intense competition (four insects.panicule⁻¹), provided less amount of resource for each individual,

which possibly reduced the damages caused by them in this condition, considering that the increase in density did not significantly increase the values of EP and ES, as well. According to competition theory, two organisms tend to compete for available food resources, and this interaction depends on density, spatio-temporal occurrence and ecological similarity (Kaplan & Denno, 2007). Thus, in this context, the density of infesting insects was a key factor of competition, considering that the other factors were isolated.

When the infestation was of two bugs/panicle, the percentage of stung spikelets (SS) was 56.6%, value smaller than 70.8% recorded by Ferreira *et al.* (2002), in five irrigated rice genotypes also infested with two *O. poecilus* individuals. Also, with the same insect density, the percentage of empty spikelets (ES) found by the authors was 31.7%, value close to 33.4% here observed. However, Ferreira *et al.* (2002) infested the panicles over the entire grain development period, which corresponds to about 40 days, whereas in the present study, the

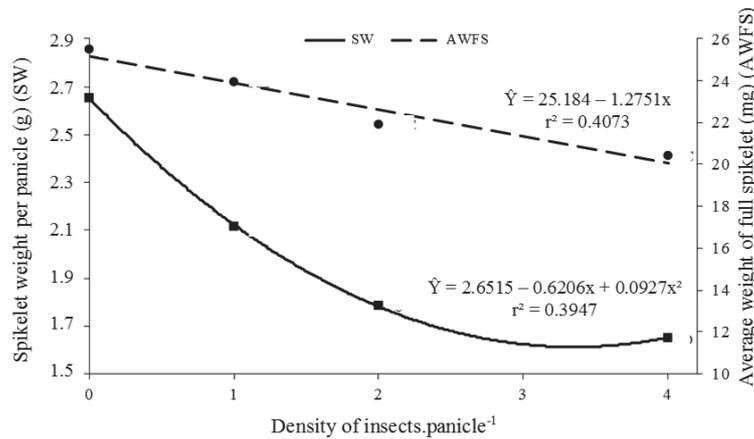


Figure 1: Spikelet weight per panicle (SW) and average weight of full spikelet (AWFS) of rice (*Oryza sativa*) cv. IRGA 424 RI as a function of different densities of *Oeobalus poecilus* infestation.

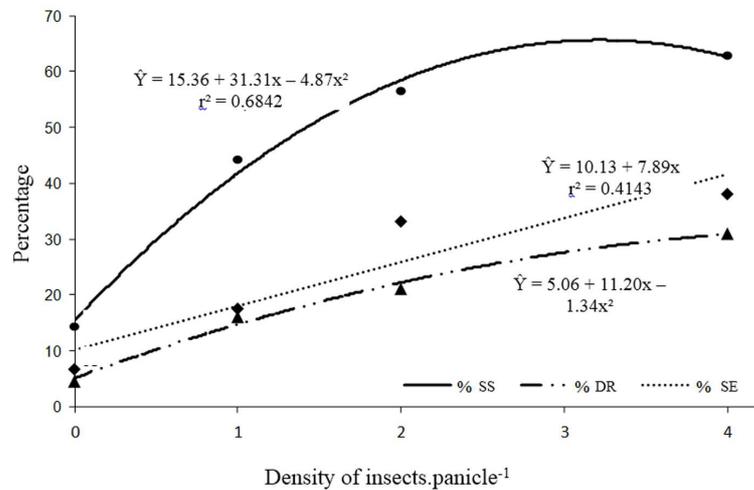


Figure 2: Percentage of stung (SS), empty (SE) and damaged rice grains (DR) (*Oryza sativa*) cv. IRGA 424 IR, as a function of the different densities of *Oeobalus poecilus* infestation.

infestation was only at the R5 stage and for seven days. In Ferreira *et al.* (2002), the bugs had a longer feeding period, which was reflected in the greater number of spikelets (qualitative damages), but even so, the values of empty spikelets were similar (quantitative damages), demonstrating that in the cultivar IRGA 424 RI, *O. poecilus* significantly reduced the weight of the spikelets in a shorter time.

In addition, a 33.4% of empty spikelets were recorded, a value greater than 22% found by Krinski & Foerster (2017), in the upland system when evaluating cv. Cambará, at R5 stage, with infestation with two *O. poecilus* adults/panicle during seven days. Such differences may be associated to the different environments where the works were conducted. The authors worked in the Northern region of Pará State and the present study was conducted in Fronteira Oeste in Rio Grande do Sul State. In addition, the genetic characteristics of the cultivars, such as productive potential and insect damage may have affected these results. Cultivars adapted to the cultivation under irrigated system were improved to reach productivity over 10 t.ha⁻¹, while those of highlands reach around 4.5 ton.ha⁻¹ (CONAB, 2017). The goal of the genetic improvement to increase yield may have led to reducing the plant natural resistance to other factors such as insect damage, that seems to have occurred with IRGA 424 RI.

The percentage of damaged grains (DG) significantly ($p < 0.0001$) increased as the density of the bugs increased, reaching the maximum at 31% with four insects/panicle. It was observed 21.7% of damaged grains with infestation of two insects/panicle, a value close to 18.6% found by Awuni *et al.* (2015), with the same density of *O. pugnax* during a week at the R5 grain stage, suggesting similar damages of these two species or the susceptibility of the cultivars evaluated in these two studies are similar.

The R5 is the most susceptible stage to insect attack because it is the grain filling period, facilitating the feeding process which increase the quantitative losses in the production (Patel *et al.*, 2006). Moreover, the most expressive damages caused at the R5 stage were reported to *Oebalus insularis* (Stål) infestation in Panama (Rodriguez *et al.*, 2006). However, Patel *et al.* (2006) suggested that according to the genetic potential of the cultivars, they would be able to tolerate insect attack in the panicles because of a compensatory response of the plant as a function of bug feeding. Bugs do not feed on parts of plants that may limit the production of photoassimilates, as the chewer insects do, and translocation of a larger amount of photoassimilates to the attacked grains may occur in some cultivars. This process could reduce the quantitative damages caused by feeding, especially in

the milky stage (R5). Nevertheless, it seems that this fact does occur in the cultivar IRGA 424 RI, which presented a large amount of spikelets with completely removed (empty) endosperm.

Another aspect to be considered is the objectives of the genetic improvement programs. They search to increase yield by selecting plants with higher tiller development. So, a single plant produce a larger number of tiller, resulting in several panicles with different maturation stages (Awuni *et al.*, 2015). This characteristic is present in IRGA 424 IR, which maintains the plants for a longer period of time, with panicles in the most susceptible stage to the stink bugs attack, increasing the insect damage on rice.

Considering the large area cultivated with this rice cultivar in Brazil recently and its susceptible to the *O. poecilus* attack, there should be warned that the expansion of the cultivated area based on a single cultivar make this system fragile and unsustainable. In addition, for irrigated system, there is no registered insecticides to control this specie, which makes the situation more vulnerable.

This work has also demonstrated the influence of different stages of reproductive development and sex of insects on their ability to cause quantitative and qualitative damages to the rice crop. Such information may support a recommendation for adopting more adequate strategies and new guidelines for the management of these insects in an irrigated rice system. Also, the search for alternative tools to improve the control and management of this stink bugs in the rice field should be a priority.

CONCLUSIONS

Females of *O. poecilus* at reproductive stage are the most quantitative damaging individuals on IRGA 424 RI cultivar under irrigated system.

Qualitative damage is significant just with one infesting insect/panicle. Quantitative losses reach the maximum with just two Stink bug /panicle infestation.

Rice grain of cultivar IRGA 424 RI at milk stage is susceptible to *O. poecilus* attack.

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