RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (1) 168-179, 2024
http://doi.org/10.54910/sabrao2024.56.1.15
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PRE-HARVEST DARK SPOTS HARM THE RICE GRAINS QUALITATIVELY AND QUANTITATIVELY

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SUMMARY

Rice grain dark spots are harmful, damaging the grains and, eventually, affecting the grain quality. Dark spots appear due to insect pests of the family Pentatomide, which feed on caryopsis tissues with a bite-puncture from the upper lip of their oral apparatus transformed into a needle. Mechanical damage to the grain leads to the penetration of microbes into the grain tissues, resulting in its darkening. The practical study aimed to determine the effects of the ripening stage on the ratio of dark spots on rice grains and their damage. Experiments assessed the influence of panicle age on rice grain damage caused by the southern green stink bug (*Nezara viridula* L.). Data recording on various parameters progressed by placing one and three bugs on the rice panicle. After crop maturity, evaluating the cut panicles continued based on empty grain content, grain size, and damaged grains. Other treatments had bugs placed during the flowering and five, 10, 15, 25, and 30 days after. Higher rice grain damage occurs when infested with three stick bugs in the first 10–15 days after flowering, revealing this period was the most vulnerable. Infection at later stages of ripening caused no significant damage to rice grains. The scientific literature does not provide information on dividing rice varieties into groups according to the degree of grain damage in the field in the form of dark spots. Based on the results, groups of pecky rice appeared for the first time according to the degree of damage, depending on the infection period, i.e., surface type, medium degree, and extensive damage type to the rice grains.

Keywords: Rice, pecky rice, stink bug (*Nezara viridula* L.), grain dark spots, grain quality traits, grain sterility, grain size

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: September 8, 2023; Accepted: December 11, 2023.

Citation: Tumanyan NG, Tkachenko MA, Kumeiko TB, Chizhikova SS (2024). Pre-harvest dark spots harm the rice grains qualitatively and quantitatively. *SABRAO J. Breed. Genet.* 56(1): 168-179 http://doi.org/10.54910/sabrao2024.56.1.15.

Key findings: The results showed an incisive increase in rice grain damage and sterility and a decrease in grain size after infecting rice plants in the first 15 days after flowering with stink bugs (*Nezara viridula L.*), revealing this period was the most vulnerable. By damaging the grains at the milky phase, the damage classification was with deep penetration into the caryopsis.

INTRODUCTION

In recent decades, the expanding and intensifying harmfulness of stink bugs (Heteroptera: Pentatomidae) in various regions has led to significant losses in agriculture production. The enhancement in ferocity of shield insects might refer to their inherent migration potential, climate change, and unintended introduction of phytophages (Krinski and Foerster, 2017a, b, and c).

Grain quality is one of the main issues that needs a focus for rice producers and consumers due to technological properties and consumer advantages. Technical properties of rice grains comprised grain parameters, such as grain size, fracturing, flour content, and damaged grains. Consumer advantages are grain appearance, organoleptic properties during preparation, and nutritional rewards. High-quality rice production can be under a wide range of germplasm with high manufacture and processing technologies.

The discovery of the rice stink bug, *Oebalus pugnax*, dates back to 1775. During rice ripening in the field, the appearance of dark spots on grains linked with stink bugs damages the flowers, seeds, fruit coats, and grain endosperms in rice-growing countries. During that damage, fungal and bacterial microflora also develop, which darkens the caryopsis tissues at the puncture site. Various representatives of stink bugs became pests, i.e., *Oebalus pugnax F.*, *Oebalus poecilus* (Dallas), *Oebalus insularis* (Stal), *Hypatropis inermis*, and *Eysarcoris ventralis* (Westwood) (Gomez-Sousa and Meneses-Carbonell, 1980; Albuquerque, 1993; Blackman and Stout, 2017).

In Brazil, the widespread bugs are the *Oebalus poecilus* and *Tibraca limbativentris* Stål stink bugs (Albuquerque, 1993; Krinski and Foerster, 2017a, b, c). The *Tibraca limbativentris* Stål is more widespread in the Brazilian rainforest regions of Southwestern Para State, Amazon (Krinski *et al*., 2015). In Japan, reports of the *Leptocorisa chinensis* and *Nezara viridula* L. were also existing in the rice fields (Kazuhiko *et al*., 2022). In South America, the bug *Oebalus poecilus* is the chief pest of rice crops (Halteren, 1972; Sutherland *et al*., 2002); in the south of the USA, in Mississippi, Arkansas, Louisiana, and Texas states, the shield bug *Oebalus pugnax* F. (Hemiptera: Pentatomidae) are prevalent. The *Oebalus poecilus'* first occurrence was in 2007 in Florida rice fields (Cherry and Nuesly, 2010). In South and Central America, *Oebalus poecilus* has four generations per season, spaced four weeks apart. The intensity of their reproduction in the fields resulted from weather conditions influences (Sutherland and Baharally, 2003).

Stink bug (*O. Insularis*) is the rice pest in Central and South America in the Caribbean (Gomez-Sousa and Meneses-Carbonell, 1980; Guharay, 1999; Cherry and Nuesly, 2010). The white-spotted stink bug, *Eysarcoris ventralis* (Westwood) (Pentatomidae), became reported as one of the major pests attacking the rice crop worldwide. In Northern Iran, identifying the stink bug *Eysarcoris ventralis* first in rice crops happened in 2017 and 2018. Biting-puncturing of rice grains by adults and nymphs caused various symptoms of empty grains and black spots. The insects also infect the alfalfa, wheat, and grapes (Jalaeian *et al*., 2019).

The brown marble bug (*Halyomorpha halys* Stal) is a pest for numerous annual and perennial crops. In Russia, its first recorded emergence transpired in 2014. In Northern Italy, the first evidence that damage the rice grains in panicles appeared in 2017 (Lupi *et al*., 2017). The first sighting of *Hypatropis inermis* in highland rice (cultivar Cambará) in Novo Progresso, State of Pará, Brazil, came out, and the search for insect pests commenced in 2010 and 2011 using entomological grids and visual searches in rice plants (Krinski *et al*., 2015). Past experimental data had shown the stages of rice panicle development influenced by the bug (*Oebalus pugnax*), and the highest rice losses emerged at the flowering phase, specifically in the milky and early waxy phases (Awuni *et al*., 2015; Blackman and Stout, 2017).

In Brazil, with irrigated, floodplain, and upland farming systems, stink bugs are highly harmful, feeding on rice grains at the nymph stage in the milky, waxy, and full ripeness phases, while in the milky phase, the panicle sterility reaches 83%. Based on the rice cultivars, the variations in the degree of damage by stink bugs *O. Poecilus* and *O. Ypsilongriseus* also appeared (Ferreira *et al*., 2002). Several intensities of damage to rice crops arose; however, the yield damage intensity depended upon the insect population. The rice cultivar 'Cheniere' showed more damage compared with another cultivar 'Kaybonnet' by the stink bug (*Oebalus pugnax*); however, the larvae surviving the adult stage did not differ among the rice cultivars (Blackman and Stout, 2017).

In recent decades, the world has seen an expansion of the ranges and increases in the harmfulness of stink bugs. In the southern regions of Russia at the beginning of the year 2000, a sharp increase was notable in the harmfulness of *Nezara viridula* in vegetables, fruits, berry crops, and soybeans. The bugs began to spread from Northern Africa in the second half of the 20th century as one of the first pentatomides, moving to the North, enhancing its harmfulness to crops (Karpun *et al*., 2022).

In Russia, the issue of stink bugs that cause dark spots on rice grains has yet to reach an unambiguous resolution. Significant damage in the rice yield was apparent in 2012 in the Krasnodar region, Russia, up to 20%–30%, decreasing the profitability of rice growing (Tumanyan *et al*., 2021; Mukhina *et al*., 2022; Mulyaningsih *et al*., 2023). Federal Scientific Rice Centre, Krasnodar, Russia, monitors the intensity of grain damage in different rice farms of the region and also evaluates the rice breeding material in connection with the 'rice black spot' (Chizhikova *et al*., 2014). From pecky rice grain representatives, the fungal microflora's isolation included *Alternaria tenuis*, *Trichothecium* sp., and *Fusarium moniliforme*; however, in small quantities in *Penicillium* sp., *Rhizopus* sp., *Aspergillus flavus oryzae*, *Cladosporium* sp., *Phoma* sp., and *Drechslera hawaiiensis* (IRRI – International Rice Research Institute, Philippines) (Fanyan *et al*., 2002; Kumeiko and Tumanyan, 2020). It showed that grain damage in the form of dark spots leads to varying degrees of decrease in grain quality (Tumanyan *et al*., 2021, 2022).

The influence of the degree of grain damage on the technological quality traits needs more studies, and the criteria for predicting the development of harmfulness of stink bugs for the rice crop also requires development. Therefore, it is vital to determine the most harmful effects of stink bugs on caryopsis in connection with the period of caryopsis maturation. In this regard, the appropriate study aimed to assess the nature of the damage by stink bugs during the flowering - full maturation stages on grain sterility, grain size, intensity, and degree of damage to rice grains under controlled conditions.

MATERIALS AND METHODS

Meteorological conditions

The sum of average daily temperatures and an average of ten-day air temperatures are critical factors in the formation of rice grain quality. For rice, the sum of average daily temperatures ranges from 2000 °C to 3000 °C, 520 °C during a seedling phase, and 700 °C at the onset of the filling phase. Rice yield formation periods in 2021 and 2022 had different weather conditions (Table 1). The sum of effective temperatures in 2021 and 2022 by the end of the grain filling period (last week of August) reached 1623 °C and 1637 °C, respectively, which was significantly higher than the long-term average temperature. The average ten-day temperatures during 2021 and 2022 were higher (26.1 °C and 27.6 °C, respectively) than the long-term average (21.6 °C). In the initial period of maturation (the first ten days of August), the maximum average

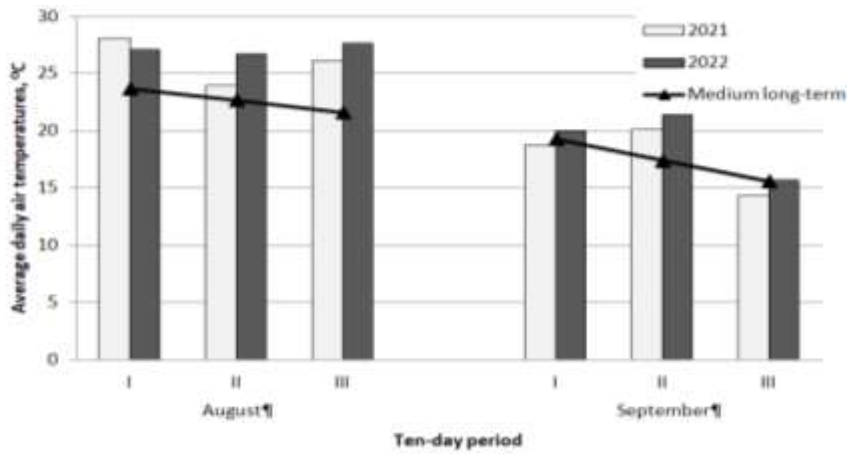


Figure 1. Average daily air temperatures in August - September 2021 and 2022.

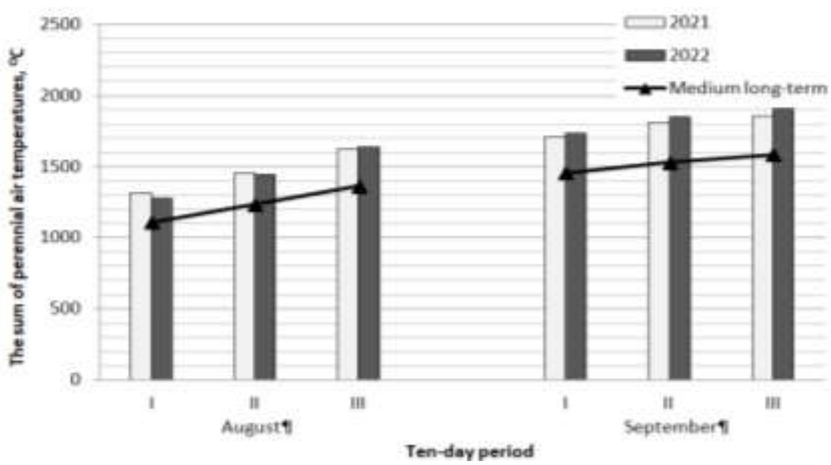


Figure 2. The sum of perennial air temperatures in August - September 2021 and 2022.

ten-day temperature (28.1 °C) was in 2021. In August, the temperatures were significantly higher during both years than the long-term average temperature (Figures 1 and 2).

Plant material and study site

The genetic material used during the study was the short-grain rice cultivar "Rapan." The variety of Rapan occupies up to 30% of the sown area of rice; hence, it served as a material for research. The experiments commenced in 2021 and 2022 on the territory of the Federal Scientific Rice Centre, Krasnodar region, Krasnodar, Russia, on a vegetation site. The collected stink bugs came from the Federal Scientific Rice Centre rice fields.

Research methodology

Rice seeds' planting in vessels proceeded their growing from May to September. The nutrient medium was the soil from rice paddies. The arable horizon of the experimental plot of the Federal Scientific Rice Centre had the following characteristics: pH (7.5) and various nutrients, i.e., the total humus content (4.2), easily hydrolyzable nitrogen (7.3 mg/100 g), total nitrogen (0.22%), mobile phosphorus (2.9 mg/100 g), total phosphorus (0.25%), exchangeable potassium (37.4 mg/100 g), and total potassium (1.2%). During rice planting, adding about 3.5 g of NPK fertilizer to each pot had a ratio of 23:12:12, regularly watering the rice plants. The light source was natural, and the daytime air temperature at the site ranged from 25 °C to 36 °C. Night temperatures were 18 °C–27 °C.

One variant had one bug placed on two panicles, and the second variant had three bugs placed on plants after one, five, 10, 15, 23, and 30 days of flowering. In both experiments, bugs and panicles put in transparent paper sleeves had the bottom tied, with panicles without bugs used as controls. On the fifth day, each case had the bugs removed from the isolators, leaving the panicles in the isolators. The distribution in both variants continued with 10 repetitions according to a randomized scheme.

Cutting all the panicles by hand when fully mature continued with drying on a laboratory bench at room temperature for three days, with the panicles threshed by hand after. Counting the number of empty, filled, and partially filled grains in one panicle ensued. Grain sterility (%) calculation in each variant also occurred. Determining the total mass of filled and partially filled grains helped calculate the grain size. The mass and number of filled grains in the sample provided the average weight of the filled grain in each variant.

The research based on the rice quality laboratory of the Federal Rice Research Center used the following methods: the mass of 1000 completely dried grains determined according to GOST 10843-73 using an ELVIZ-2 moisture analyzer, an ASESh-8-2 air-thermal measuring device, an automatic seed counter SLY-C, and electronic laboratory scales Cas CUW-420H. The intensity of damage assessment followed the relative content of rice grains with dark spots on the pericarp and expressed as a percentage according to STO 46429990-025-2016. Grains with dark spots from all samples continued isolation and weighing to calculate the percentage of damaged grains. In visualizing the damage to the caryopses in the endosperm, the seeds incurred peeling and got photographed. Then, cutting the seeds with a blade in a dorsoventral direction followed before taking their pictures. The image scale is 1:7, 1:5 (A4).

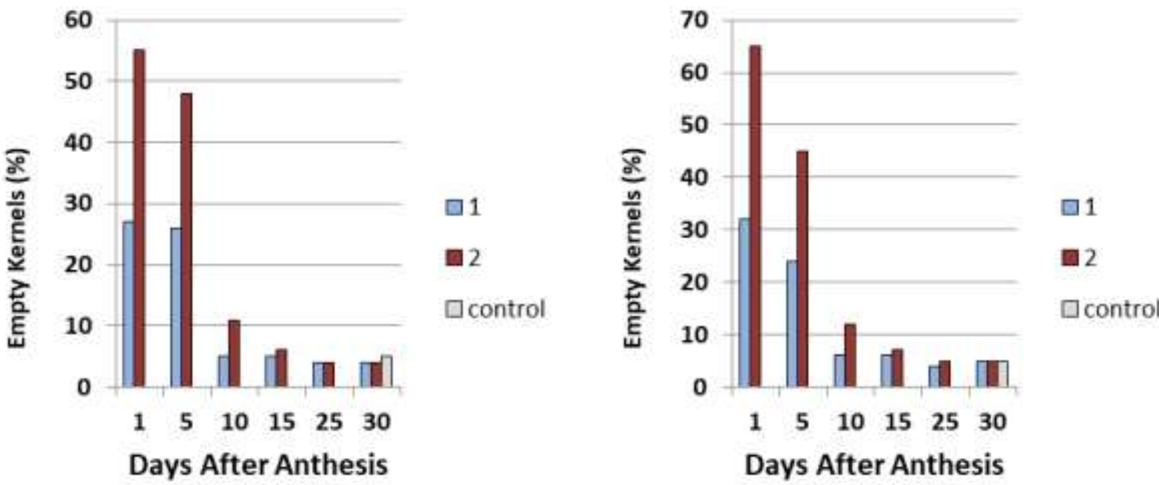
Statistical analysis

Statistical and mathematical processing and compilation of all the recorded data on various parameters ran through the Microsoft Excel program.

RESULTS AND DISCUSSION

The latest study aimed to evaluate the damage by the stink bug to rice seeds of rice cultivar Rapan during the flowering and maturation periods based on empty grains, grain size, and the content of damaged grains under controlled conditions of the growing area for different levels of infestation with the stink bug (*Nezara viridula* L.). Previously, various damage degrees to rice grains by stink bugs were noticeable from the flowering and seed-ripening stages (Patel *et al*., 2006). The study revealed a decline in grain sterility at the later stages of infection. In isolators, rice sterility also decreased at each latter infestation period after flowering in infestations of one and three bugs per panicle (Figure 3).

The bugs stayed on the rice panicle for five days. Control panicles averaged 2%–5% empty grains in two experiments. The panicle infected on the first day after flowering, compared with panicles infected on the fifth and 10th day, gave a higher number of empty grains in 2021. For the first variant, it was more than 5.5 times (on the 10th day), while for the second option, 1.2 and 5.0 times. For the first option in 2022, it was more than 1.3 and 5.3 times, while for the second option, 1.4 and 5.4 times. When infecting the panicles on the 15th, 25th, and 30th day, the sterility differed slightly from the sterility value obtained in the control. By comparing options 1 and 2, the influence of the number of bugs on the number of empty grains in the panicle was apparent. By infecting on days one, five, and 10 in 2021 and 2022, the damaged grains in the second option were 1.9–2.2 times higher. The infection of panicles with bugs 15 and 20



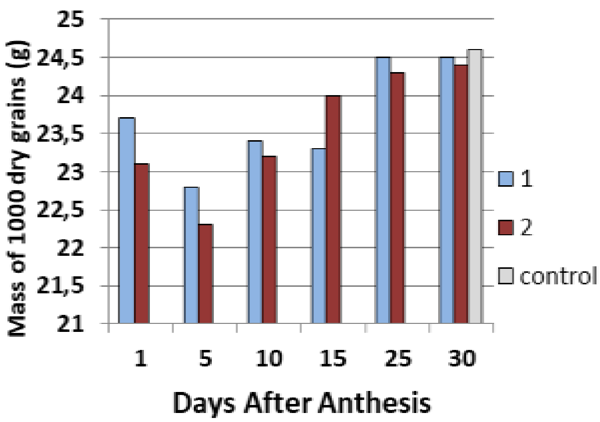
2021 2022

Figure 3. Percentage of empty grains in the panicles with bugs placed on 1–30 days after flowering for five days (option 1 – 1 bug, option 2 – 3 bugs) and without bugs (control).

days after flowering revealed a nonsignificant increase in the index. Previously, Awuni *et al*. (2015) and Blackman *et al*. (2017) findings showed an increase in empty grains when infecting crops with *Oebalus pugnax* F.

Feeding during the flowering and the milky stages, the percentage of empty grains was significantly higher than feeding during the latter stages of grain development. The frequency of empty grains and weight reduction of filled grains were greater, with a higher level of infection, especially during flowering and milky stages. $F_{2,37}$ = 77,72, P < 0.05; $F_{2,37}$ = 236,02, P < 0.05, respectively in 2022; $F_{2,37}$ = 80,17, P < 0.05; $F_{2,37}$ = 264,08, P < 0.05, respectively in 2021. Given that f_{obs} > f_{cr}, the null hypothesis was acceptable on the significant influence of the factor on the obtained results in the experiments (the null hypothesis attained rejection about the equality of group means, with the group means differing significantly). The presented results were consistent with the conclusions made in past studies with the rice bug *Leptocorisa oratorius* (F.) in greenhouses that showed a negative correlation of rice yield with insect density due to an increase in empty grains (Jahn *et al*., 2004). Infection of panicles with one and two *Oebalus pugnax* bugs (Fab.) significantly affected the average weight of filled grains in rice panicles (Patel *et al*., 2006).

In the early stages of infection, a decline in grain size was prominent. In options 1 and 2, the significant reduction in grain size occurred during infection on the first day after flowering, by 1.6 and 2.2 g, respectively (Figure 4). For 2022, in an experiment with one and three bugs on panicles and infected on the fifth and 10 days after flowering, the grain size lowered by 2.0 and 1.4 g and 2.4 and 1.5 g, respectively. However, after 15 and 20 days, the dynamics of the decrease in 1000-grain weight of completely dried grains was not so obvious compared with the control: by 1.0 and 0.2 and 1.2 and 0.4 g. In the experiment with one bug, a decrease in seed weight during infection emerged on the first, fifth, ninth, 15th, and 23rd day by 6.4%, 8.0%, 5.6%, 4.0%, and 0.8%, respectively, while in the experiment with three bugs, by 8.8%, 9.6%, 6.0%, 4.8%, and 1.6%, respectively. Comparing the grain size values in two options during infection in the corresponding periods, a higher dynamics of the decrease in the trait was evident in the option with three bugs by 2.4%, 1.6%, 0.6%, 0.8%, and 0.8%, respectively. In 2021, the dynamics of the trait decline ranged from 0.4% to 2.5%. The results



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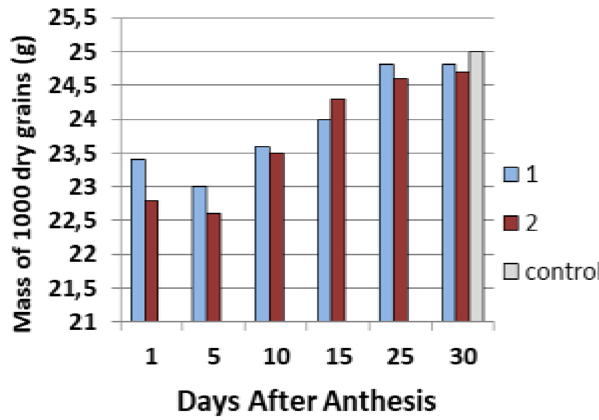

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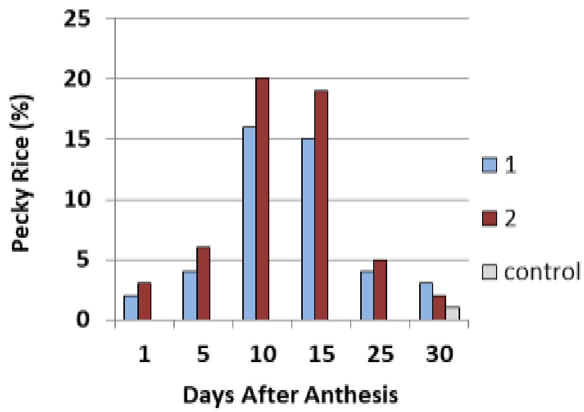
Figure 4. Average grain weights in the panicles with bugs placed on 1–30 days after flowering for five days (option 1 – 1 bug, option 2 – 3 bugs) and without bugs (control).

obtained correspond to the previously gathered data, and the feeding of the bug during and immediately after flowering, in milk ripeness, inhibits the development of the seed, leading to a decrease in grain size and an increase in unfulfilled grains (Robinson *et al*. 1980; Lee *et al*., 1993; Rashid, 2003).

The results further indicated that placing stink bugs on the panicle at the stages of flowering, milky, and soft dough reduced the average weight of the filled grains, with the maximum reduction in grain weight observed at the early milky stage. However, the decrease in average weight was high in panicles infected for five days on the first, fifth, and 10th days after flowering and lowest after that. It revealed that the first 15 days after flowering are the most critical for damage in reducing grain weight and, consequently, reducing the rice grain yield. Infecting with a different number of stink bugs, it was apparent that the weight loss was more intense in the infection by three stink bugs than with one. $F_{2,37}$= 50,45, $P < 0.05$, $F_{2,37}$ = 52,13, $P < 0.05$, respectively, in 2022; $F_{2,37}$= 66,77, $P < 0.05$, $F_{2,37}$ = 96,43, $P < 0.05$, respectively, in 2021. On average, the group means differed significantly. With fobs > fcr, the null hypothesis gained acceptance on the significant influence of the factor on the results (the null hypothesis reached rejection on the equality of group means, and the group means differed significantly). A similar negative correlation between the mass of filled rice grains and the stink bug density occurred earlier (Jahn *et al*., 2004; Patel *et al*., 2006).

The pecky rice content in dehusked grains appears in Figure 5. In both options, the control samples were characteristic of the damaged grains (1.2%–1.8%), indicating not only bugs influenced the emergence of dark spots on the caryopsis. The content of pecky rice in panicles differed based on the time of infection after flowering in both experiments. In both options in 2021 and 2022, the rice damage was fiercer in panicles infected on the 10th and 15th day after flowering compared with panicles at all other infection periods and the control variant. The intensity of rice grain damage one day after flowering was slightly higher than in the control option (2% and 4% compared with 1%). In 2022, in the first and second options, by infecting on the 10th and 15th day, the intensity of grain damage was maximum (16%, 15%, 27%, and 26% higher than the control), and in 2021 (by 15%, 14%, 19%, and 18%, respectively). Thus, the grain damage in the second option was higher than in the first option, and the stink bug caused intense damage to rice grains for five days by infecting five to 15 days after flowering; however, it was less severe when infected on



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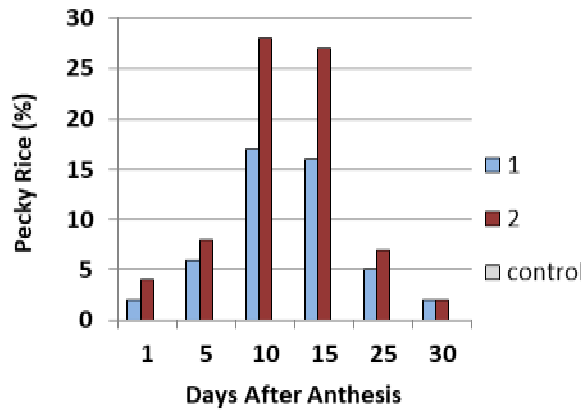

2022

Figure 5. Percentage of pecky rice in the panicles with bugs placed on 1–30 days after flowering for five days (option 1 – 1 bug, option 2 – 3 bugs) and without bugs (control).

the 25th day. The presented results of the relationship between the periods of infection of rice with the shield bug *Nezara viridula* showed that a significant increase in the intensity of grain damage occurs during the initial period of infection after flowering, which corresponds to past findings (Robinson *et al*., 1980; Lee *et al*., 1993; Rashid, 2003; Patel *et al*., 2006).

The data revealed that the damaged rice percentage was higher during infection in the first half of maturation, and increasing the number of bugs from one to three per panicle, the content of pecky rice in the panicles increased. The maximum lesion level was visible 10–15 days after anthesis in the milky and soft dough stages. One rice stink bug per panicle – $F_{2,37}$= 188.52, $P < 0.05$, three bugs per panicle - $F_{2,37}$ = 439,44, $P < 0.05$ in 2022; $F_{2,37}$= 165.86, $P < 0.05$, three bugs per panicle - $F_{2,37}$ = 76,11, $P < 0.05$.

The stink bugs placing period (1–15 days after flowering) significantly affected the percentage of empty grains, average weight of filled grains, and percentage of pecky rice by placing one and three bugs per panicle in the experiment. In the study, photographic images of hulled grains surface and their sections at the sites of damage at different stages of infection of rice panicles ensued (Figures 1-3). The figures show photographs of grains at a scale of 1:7 and 1:5 (A4). According to the nature of the damage, the rice grains classification comprised three groups based on the degree of damage: surface type, medium type, and extensive type of grain damage. The surface type was regular damage 20 days after flowering, from the beginning of the waxy ripeness. In this case, the grain acquires hardness, and the damage was not as significant as in the other two types. In such grains, removing surface-damaged tissues transpired during grinding (Figure 6). The rice grains division into groups based on the degree of damage from stink bugs is interesting in connection with the assessment of the period of damage that occurred and the assessment of the possibility of eliminating stains during the rice products preparation. However, the authors have not found data in the scientific literature on the characteristics of the degree of damage: the size of the spot and the penetration deep into the tissues of the grain of damage depending on the damage period after flowering. However, this indicator seemed valuable since it can give an idea of the intensity of damage to the crop grain at the initial stage of rice ripening (Mukhina *et al*., 2022).

At medium and extensive damages, unusual mealy areas of loose starchy parenchyma appear from the area directly under the puncture up to the entire grain

Figure 6. Surface type of rice grain damage.

endosperm (Figures 7 and 8). In the pertinent study, during infection, rice grain formation was immediately after flowering and up to the 15th day of maturation, which was in milky ripeness. Such grains bore rejection during processing since it is already unmarketable during grinding, and the damaged areas will remain in the polished rice. For the first time, risk groups with pecky rice were distinguishable according to the degree of damage depending on the infection period, i.e., surface type, medium degree, and extensive damage to rice grains (Mulyaningsih *et al*., 2023).

The results of the relationship between the periods of rice infection with the stink bug (*Nezara viridula* L.) showed that significant differences in the indicators of grain sterility and intensity of grain damage occurred in the initial period of infection after flowering, corresponding to previously obtained data. The feeding of the bugs during and immediately after flowering stops the further development of seeds, and the nutrition in milky ripeness decreases grain size and increases unfulfilled grains (Robinson *et al*., 1980; Patel *et al*., 2006). The decrease in the degree of grain damage in the latter stages of seed maturation was probably due to the less attractiveness of the hard caryopsis and the difficulty for insects in piercing the tissues of the caryopsis (Lee *et al*., 1993; Rashid, 2003).

High grain sterility and a decrease in grain size were noteworthy, with an increase in the number of bugs on the panicles, especially in the early stages of grain development, which confirms the data through a negative correlation between the grain yield and density of stink bugs and the correlation between sterility, grain filling, and stink bug density (Jahn *et al*., 2004). The presence of pecky rice was notable, with the absence of bugs on the panicles, which may be due to damage to the grain by fungi. It is also well-known that the emergence of a dark spot on the panicle is due to the penetration of pathogenic microflora through the caryopsis tissue damaged by a bug bite (McPherson and McPherson, 2000). Thus, with the predominant role of stink bugs in the manifestation of rice grain damage in the form of dark spots, the influence of pathogenic microflora, for which the appropriate conditions for damage were favorable, cannot be excluded (Patel *et al*., 2006).

Thus, according to the results of the study in the field, the growing season of rice is of great importance, during which grain damage occurs (flowering, milk ripeness, and wax ripeness of grain) and the density of stink bugs increase in rice crops, which is consistent with previously obtained results. In further studies on the problem of pecky rice, a proposal will proceed to evaluate the reaction of various varieties, including new ones, to grain damage in the field to develop an assessment methodology for the breeding process.

Figure 7. Medium type of grain damage

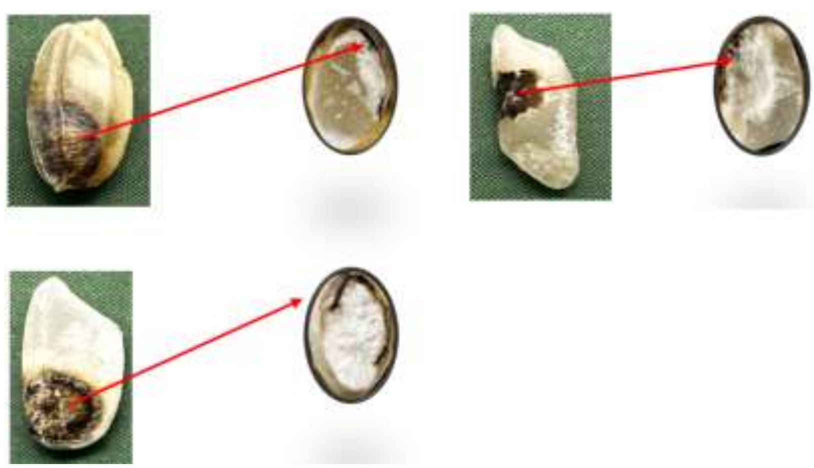

Figure 8. Extensive and severe type of grain damage.

CONCLUSIONS

The damage by stink bugs (*Nezara viridula* L.) to the grains of two rice cultivars during the flowering - full ripening periods gained scrutiny on grain sterility, grain size, content of pecky rice under controlled conditions of the growing area, and different levels of bug infestation. An increase in grain damage in the form of dark spots was noteworthy in the initial infection period with the stink bugs and with an increase in their density on the panicles. Pecky rice groups were distinguishable according to the intensity of damage based on the infection period, i.e., surface type, medium type, and extensive grain damage. With a mass settlement of stink bugs during the formation of a panicle and the initial stage of maturation, a significant decrease in rice grain quality and yield and a decrease in the profitability of production is possible. The information obtained on the colonization of crops by insects will allow both to predict the grain quality and to apply the protective measures in time.

ACKNOWLEDGMENTS

The work received financial support from the RSF grant, KNF No. 22-26-20098, "Influence of 'black

spotting' of rice on the yield quality of varieties approved for use in Krasnodar region under conditions of grain damage in the field."

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