Pesquisa Agropecuária Tropical
ISSN: 1517-6398
ISSN: 1983-4063
evnovaes@gmail.com
Universidade Federal de Goiás
Brasil

Barcellos de Lima, Crislaine; Dionei Grützmacher, Anderson; de Bastos Pazini, Juliano; Felisberto da Silva, Fernando; Schneid Afonso da Rosa, Ana Paula; da Silva Martins, José Francisco
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Pesquisa Agropecuária Tropical, vol. 49, 2019, pp. 1-7
Universidade Federal de Goiás
Goiânia, Brasil

DOI: https://doi.org/10.1590/1983-40632019v4953397

Research Article

Development of *Oryzophagus oryzae* (Costa Lima) in rice cultivars[1]

Crislaine Barcellos de Lima[2], Anderson Dionei Grützmacher[2], Juliano de Bastos Pazini[2], Fernando Felisberto da Silva[3], Ana Paula Schneid Afonso da Rosa[4], José Francisco da Silva Martins[4]

ABSTRACT

Insect-resistant cultivars are essential for improving the management of pests such as *Oryzophagus oryzae* (Coleoptera: Curculionidae), which significantly decreases flooded rice yields. However, the degree of benefit brought to the crop varies with the type of resistance. This study aimed to evaluate the resistance of rice cultivars to *O. oryzae*, inferring about antixenosis and antibiosis effects on the development of larvae and adults. Under natural infestation conditions, a biennial experiment was carried out using a latin square design, with six treatments (cultivars) and plots with 30 plants (five rows of six plants equidistant 20 cm) irrigated with water depth of 15 cm. The number and weight of larvae by size, as well as the number, weight, emergence rate, and sex ratio of adults, were evaluated using standard soil, plant and root samples. Except for adult sexual ratio, the cultivars differed ($p \leq 0.05$) for the other variables, whose relationship evidenced antibiosis effects at the larval stage, making possible to conclude that: the BRS Firmeza cultivar has resistance to *O. oryzae* due to antixenosis and/or antibiosis effects; BRS Ligeirinho is susceptible to this insect species, resulting in a relatively higher number of well-developed larvae and adults; and IRGA 417 shows an antibiosis action, restricting the adult emergence rates. The weight of adults gives a greater accuracy to the evaluation of the harmful effects of rice cultivars on the *O. oryzae* development.

KEYWORDS: *Oryza sativa*, rice water weevil, integrated pest management.

RESUMO

Desenvolvimento de *Oryzophagus oryzae* (Costa Lima) em cultivares de arroz

Cultivares resistentes a insetos são essenciais à melhoria do manejo de pragas como *Oryzophagus oryzae* (Coleoptera: Curculionidae), altamente prejudicial à cultura do arroz irrigado por inundação. O grau de benefício, porém, depende do tipo de resistência. Objetivou-se avaliar cultivares de arroz quanto à resistência a *O. oryzae*, inferindo sobre efeitos de antixenose e antibiose no desenvolvimento de larvas e adultos. Em condições naturais de infestação, instalou-se um experimento bienal em delineamento de quadrado latino, com seis tratamentos (cultivares) e parcelas de 30 plantas (cinco linhas de seis plantas equidistantes 20 cm) irrigadas com lâmina de água de 15 cm. Por meio de amostras-padrão de solo, plantas e raízes, foram avaliados o número e peso de larvas por classe de tamanho, bem como o número, peso, taxa de emergência e razão sexual de adultos. Exceto para a razão sexual, as cultivares diferiram ($p \leq 0,05$) quanto às demais variáveis, cuja relação evidenciou efeitos de antibiose à fase larval e possibilitou concluir que: a cultivar BRS Firmeza possui resistência a *O. oryzae* por efeitos de antixenose e/ou antibiose; BRS Ligeirinho é suscetível, abrigando alta população larval e propiciando melhor desenvolvimento de larvas e adultos; e IRGA 417 possui resistência do tipo antibiose, que restringe a taxa de emergência de adultos. O peso de adultos atribui maior precisão à avaliação de efeitos prejudiciais de cultivares de arroz ao desenvolvimento de *O. oryzae*.

PALAVRAS-CHAVE: *Oryza sativa*, gorgulho-aquático, manejo integrado de pragas.

INTRODUCTION

Rice (*Oryza sativa* L.) has social and economic importance as a staple cereal, in Brazil. Around 80 % of the national rice production comes from irrigated crops grown in 1.2 million hectares in the southern part of the country (Conab 2017). In this region, the insect species *Oryzophagus oryzae* (Costa Lima) (Coleoptera: Curculionidae) is harmful to the crop plants and produces at least two generations per year (Martins & Cunha 2015).

As an adult, *O. oryzae* is known as rice water weevil and has autochthonous populations. At the beginning of flooding, new rice fields are infested

1. Received: Jun. 07, 2018. Accepted: Dec. 21, 2018. Published: Jun. 25, 2019. DOI: 10.1590/1983-40632019v4953397.
2. Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Departamento de Fitossanidade, Capão do Leão, RS, Brasil. *E-mail/ORCID*: crislainebarcellos@hotmail.com/0000-0002-3189-1243, adgrutzm@ufpel.edu.br/0000-0002-5464-9337, julianopazzini@hotmail.com/0000-0002-4350-1708.
3. Universidade Federal do Pampa, Itaqui, RS, Brasil. *E-mail/ORCID*: fernando.silva@unipampa.edu.br/0000-0003-1229-896X.
4. Empresa Brasileira de Pesquisa Agropecuária (Embrapa Clima Temperado), Pelotas, RS, Brasil. *E-mail/ORCID*: ana.afonso@embrapa.br/0000-0003-4146-0612, jose.martins@embrapa.br/0000-0003-1901-4050.

by weevils leaving hibernation sites. These insects feed on rice seedling leaves, but usually cause no economic losses. Egg laying occurs in the leaf sheath aerenchyma, about 2 cm below the irrigation water surface (Prando 1999, Moreira 2002). Larvae hatch around 7 days later and, in the second instar, they move from leaf sheaths to roots for feeding, what decreases the rice production by 10-20 % (Martins & Prando 2004). After nearly 30 days, larvae pupate in cocoons fixed to the roots, and adults emerge about ten days later (Martins & Cunha 2015).

At each rice cropping season, a large quantity of chemical insecticides is applied to seeds for *O. oryzae* management, mainly those of the pyrazole group (e.g., fipronil), increasing production costs and environmental contamination risks (Martins et al. 2017). The use of cultivars resistant to rice water weevil is indicated to reduce potential negative impacts from the chemical control (Stout et al. 2009, Martins & Cunha 2015).

Rice resistance to *O. oryzae* involves antixenosis, antibiosis or tolerance (Martins & Terres 1995), which is also used against infestations by the North American rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae) (N'Guessan et al. 1994a and 1994b, Lupi et al. 2013). Tolerance is the ability of plants to withstand damage by insect pests, maintaining the crop yield without affecting the behavior and development of phytophagous insects (Lara 1991). In rice, tolerance to damage by *L. oryzophilus* and *O. oryzae* is attributed to the emergence of new roots (N'Guessan & Quisenberry 1994, Martins et al. 2004).

Conversely, antixenosis and antibiosis are associated with plant physical and chemical factors interfering with insect biological processes (Panda & Khush 1995, Smith 2005). Antixenosis involves host selection, reducing plant colonization. In rice, antixenosis may reduce egg laying by *L. oryzophilus* and hence larval density in leaf sheaths (Stout & Riggio 2002). The effects of antibiosis start from insect feeding on plants, reducing its metabolism and limiting its size and vigor. In rice, antibiosis may cause death to *L. oryzophilus* larvae in leaf sheaths, reducing its population in the roots (Smith & Robinson 1982, Stout et al. 2013). In some cases, it is difficult to distinguish between antixenosis and antibiosis, mainly in cultivars with both resistance types (Lara 1991, Baldin et al. 2019).

Antibiosis overcomes antixenosis, in terms of reducing the population of rice water weevil, by killing larvae or restricting their growth, before or after root infestation (N'Guessan et al. 1994a), resulting in less damage to growing crops. In addition, a lower larval growth, as for *L. oryzophilus* in the Nira cultivar (Smith & Robinson 1982), may restrict adult emergence, as for *O. oryzae* in the Dawn cultivar (Martins et al. 2001), and result in a lower autochthonous population and lower reproductive potential, reducing infestation levels and future crop damages.

Studies on the effects of antixenosis and antibiosis on rice water weevil are challenging under laboratory conditions. The analysis of these two effects are focused on egg and larval stages (Bowling 1972, Prando 1999, Moreira 2002), even considering that relatively small populations of adults that could be reared in the laboratory (Zhang et al. 2004). These limitations are even greater from larvae migration to the roots until the end of the pupal stage, when laboratory manipulations could be lethal. Another complication is to simulate natural conditions of equilibrium for irrigation water quality, volume and temperature, as well as for the amount of roots, soil and shoots of rice plants.

To some extent, the difficulties in performing laboratory assays to assess the resistance of rice cultivars to *O. oryzae* can be overcome by direct field studies, exposing the cultivars to natural infestations. In the field, the number of larvae per standard cylindrical samples of soil, plants and root has been a key variable for assessing resistance to rice-infesting weevils (Smith & Robinson 1982, Martins & Terres 1995, N'Guessan et al. 1994a, Stout et al. 2013, Maisarah et al. 2018). Given that this variable indicates isolated or combined effects of antixenosis and antibiosis during oviposition and larval feeding on leaf sheaths and roots, it does not always allow the correct distinction between the resistance types, especially in cases in which antixenosis causes death by starvation (Panda & Khush 1995) and reduces the number of larvae. However, this limitation can be overcome by adopting the methodology proposed by the International Rice Research Institute for studies on antibiosis for rice water weevil in the field. This methodology is based on calculating the number, weight and percentage of larvae classified by size (Heinrichs et al. 1985). In addition, the physiological inadequacies that remain at the end of the larval phase, including antibiosis effects (Smith 2005), and that are transferred to the pupal phase, reducing the insect viability, as is the case in *O. oryzae* (Martins et al. 2001), can be used to evaluate this type of resistance. Therefore, the application of variables that are inherent to the adult phase, other than the emergence rate (Heinrichs et al. 1985), and that can be recorded under field conditions, may help to elucidate the effects of antibiosis on *O. oryzae* and resistance of rice to this weevil species.

Thus, this study aimed to evaluate the resistance of rice cultivars to *O. oryzae*, inferring about the possibility of isolated or combined effects of antixenosis and antibiosis on the development of the insect.

MATERIAL AND METHODS

The experiment was carried out in October 2009 (first cropping season) and November 2010 (second cropping season), in the margin of a 104-hectare area containing a typic Albaqualf soil, common in flooded rice fields, in Capão do Leão, Rio Grande do Sul state, Brazil, by transplanting young plants produced in a screened greenhouse.

Six rice cultivars with differences in the development cycle, i.e., the period from the seedling emergence to flowering, composed the treatments, which included two cultivars with a very short cycle (BRS Atalanta and BRS Ligeirinho; development cycle of 95-100 days), three cultivars with a short cycle (BRS Firmeza, BRS Querência and IRGA 417; development cycle of 110-120 days) and one cultivar with an intermediate cycle (BRS Pelotas; development cycle of 130 days). To ensure an equal probability of infestation of the cultivars by adults of *O. oryzae* in a multiple-choice test (Silva et al. 2003) in both crops, the experiment was developed in a latin square design, in an area (31º48’45’’S; 52º27’59’’W) with flat soil surface. BRS Atalanta was included as a standard of resistance to *O. oryzae* (Martins et al. 2001).

In the first cropping season (October 21, 2009), at 20 days after the emergence, rice plants were transplanted to the field, in soil covered by a 2-cm irrigation water depth. The experimental plots contained 30 plants (five rows of six plants equidistant 20 cm) and were spaced at 100 cm on the larger and smaller sides. For ten days after transplanting, the irrigation water depth was gradually increased until stabilizing at 15 cm, to create conditions for the natural infestation of the cultivars by *O. oryzae*, when the plants reached the development stage V_5 (Counce et al. 2000), with a height of approximately 25 cm.

At 33 days after the stabilization of the irrigation water depth, near the peak of the larval population (Carbonari et al. 2000), eight standard cylindrical soil samples, one plant and root samples were collected in each plot, in the same positions, according to a standard method (Heinrichs et al. 1985) modified by Neves et al. (2011).

Immediately after the collection, four samples were individually ground and immersed in water inside a nylon mesh sieve for separating and counting the *O. oryzae* larvae. The larvae of each soil sample were kept refrigerated in glass tubes containing water up to 5 days after collection for categorization (C) of the size (t) [C_1, small ($0 < t \leq 3$ mm); C_2, intermediate ($3 < t \leq 6$ mm); C_3, large ($t > 6$ mm)], as proposed by Heinrichs et al. (1985) and adopted by Botton et al. (1996) and Wu & Wilson (1997). Shortly thereafter, the larvae were dried on filter paper for 3-5 min, to record the weight (mg).

The remaining four samples were kept intact. However, the plant shoot was cut to a height of 20 cm. At the collection date, the samples from each plot were grouped and submerged (10 cm) in water in plastic buckets (30 cm in diameter x 50 cm in height) covered with voile, for collecting and counting emerging adults of *O. oryzae* from the roots (Martins et al. 2001). The first count occurred at 10 days after the collection and continued at 5-day intervals for 35 days, between 1:00 p.m. and 3:00 p.m., when the temperature tended to be higher, inducing the adults to move to the top of the buckets. The water from the buckets was periodically removed with a siphon (with an 80-cm hose and a screen at one end) and replaced to avoid the development of algae, which reduce the oxygen supply to larvae and pupae in the roots, and hinders visualizing the emerging adults. The adults removed from each bucket on their respective dates were kept refrigerated for up to 5 days in plastic pots for weighing (mg). Before weighing, the sex ratio of adults [number of females/ (number of females + number of males)] present in 50 % of the plastic pots from each bucket was calculated. Adult sexing was performed using a stereomicroscope (40x magnification) equipped with micrometric eyepieces and involved measuring body length, rostrum width and ventral morphological differences of insects (convex and concave area in the fifth sterna of females and males, respectively), as proposed by Prando (1999), with modifications. In the second cropping season (October 21, 2010), the experiment was performed at the same site as the first season, with the following changes: seedling transplantation at 15 days post-emergence; stabilization of the irrigation water depth at 15 cm; use of plants at the development stage V_4, with a height of approximately 20 cm; and sampling (soil, plants and roots) at 40 days after stabilization of the irrigation water depth.

In both cropping seasons, in addition to the number, weight and size of larvae and/or adults of *O. oryzae*, the adult emergence rate (%), i.e., the ratio between the total number of larvae and the total number of emerging adults in each sample, was measured.

All statistical analyses were performed using the R software (R Development Core Team 2015). The number and weight of larvae and weight of adults of *O. oryzae* (x) normalized by the transformation in $\sqrt{x}$ and the adult emergence rate (%) normalized by transformation into arc sine $\sqrt{x/100}$ were subjected to analysis of variance, combining data from the first and second cropping seasons. Since there was no significant effect of rows and columns of the Latin square experimental design, regarding the number and weight of larvae and adults of *O. oryzae*, the data were analyzed as a randomized block design. In this case, the plot rows arranged parallel to the margin of the experimental area where *O. oryzae* adults predominate were considered blocks. The means were compared using the Scott-Knott test ($p \leq 0.05$), because of the significance of the F-test.

RESULTS AND DISCUSSION

The combined analysis for the number of *O. oryzae* larvae of different sizes (C_1, C_2 and C_3) revealed significant differences ($p \leq 0.05$) among the rice cultivars. All cultivars, proportionally, contained more intermediate size larvae (C_2) than small (C_1) and large size (C_3) larvae. The cultivars with significant differences in the number of larvae of intermediate size formed three groups: BRS Atalanta and BRS Firmeza (less infested); BRS Pelota and BRS Querência (moderately infested); and BRS Ligeirinho and IRGA 417 (most infested) (Table 1).

The difference in larval populations among the rice cultivars may be due to antixenosis and antibiosis effects, revealing a possible preference for oviposition or inhibition of larval development (Table 1). It has been shown that there may be less colonization of foliar sheaths by neonatal *L. oryzophilus* larvae, if antixenosis restricts oviposition (Stout & Riggio 2002), and that fewer larvae reach the roots if they are affected by lethal factors of antibiosis during feeding in the sheaths (Smith & Robinson 1982, Stout et al. 2013). The antixenosis and antibiosis effects, including lethal effects (Panda & Khush 1995, Smith 2005), may occur in the remainder of the larval phase, compromising the insect metabolism.

The significant lower number of intermediate size larvae in BRS Atalanta and BRS Firmeza (Table 1), approximately 60 % lower than in the other cultivars, was due to the effects of antibiosis, in addition to probable antixenosis effects (Panda & Khush 1995, Smith 2005). These results agree with the lower weight of intermediate size larvae in the BRS Atalanta, BRS Firmeza and BRS Querência cultivars and the lower weight of large size larvae in BRS Firmeza and BRS Querência. For BRS Atalanta and BRS Firmeza, possible effects of antibiosis would arise from the fact that both cultivars contain genes from the Dawn cultivar (Gomes & Magalhães

Table 1. Number and weight of *Oryzophagus oryzae* larvae collected in plant roots of six flooded rice cultivars distributed in three size (t) classes (C)[1].

Cultivar	Number per sample[2;4]			Weight (mg)[3;4]		
	C_1	C_2	C_3	C_1	C_2	C_3
BRS Atalanta	6.92 aB	11.22 cA	2.88 aB	3.92 aB	6.21 bB	14.25 cA
BRS Firmeza	4.69 aB	8.98 cA	0.79 aB	3.28 aB	7.69 bA	9.06 dA
BRS Ligeirinho	5.01 aB	24.00 aA	3.44 aB	5.20 aC	10.79 aB	17.28 bA
BRS Pelota	3.03 aB	20.77 bA	2.80 aB	4.22 aC	9.96 aB	21.05 aA
BRS Querência	5.54 aB	22.11 bA	4.84 aB	3.02 BC	7.43 bB	10.47 dA
IRGA 417	3.18 aB	26.89 aA	2.74 aB	3.22 aC	9.09 aB	13.99 cA
CV (%)		42.42			23.92	

[1] C_1: small ($0 < t \leq 3$ mm); C_2: intermediate ($3 < t \leq 6$ mm); C_3: large ($t > 6$ mm). [2] Mean number of larvae per sample in the first and second cropping seasons. [3] Mean weight or larvae per size class. [4] Means with equal lowercase letters in the columns and uppercase letters in the rows indicate absence of significant differences ($p \leq 0.05$) by the Scott-Knott test.

Junior 2004), which is a source of resistance to *L. oryzophilus* (Heinrichs et al. 1985). Furthermore, the Dawn cultivar is less infested by *O. oryzae* when insects cannot choose the host plant, evidencing typical effects of antibiosis that maintain normal its levels of rice yield (Silva et al. 2003).

In IRGA 417 and BRS Querência, despite the higher total number of larvae per sample, the mean larval weight was lower, with values similar to those found in BRS Atalanta and BRS Firmeza (Table 2). Therefore, the discrepancy between the total number of larvae and the mean weight of larvae in IRGA 417 and BRS Querência may explain the action of some effects of the antibiosis of both cultivars on the larval phase. In contrast, in BRS Ligeirinho and BRS Pelota, a highest larvae mean weight was observed, suggesting the absence of antibiosis effects in these cultivars (Table 2).

The number of emerging adults of *O. oryzae* per sample (Table 2), lower in BRS Atalanta, BRS Firmeza and IRGA 417 than in the other rice cultivars, might be due to different seasonal deleterious effects on the larvae, which affected the ratio between the total number of larvae and emerging adults. Mainly the situation of IRGA 417, with more larvae but a lower number and emergence rate of adults (Table 2), can characterize the presence of strong antibiosis mechanisms.

There were significant differences ($p \leq 0.05$) among the rice cultivars, in relation to the weight of emerging adults of *O. oryzae* per sample, and this variable was used for the first time for evaluating the resistance of rice cultivars to rice water weevil. The mean weight of emerging adults of both sexes was used because the sex ratio of *O. oryzae* was similar among the six cultivars. As the sex ratio of *O. oryzae* was the same in the six cultivars (Table 2), the analysis of the mean weight of adults of both sexes is justified (Table 2).

Under natural infestation conditions, the mean weight of adults of *O. oryzae* may indicate the total harmful effects of rice cultivars (antixenosis and/or antibiosis) on insect development, from the beginning of the larval phase to the end of the pupal phase. The interrelation analysis of adults weight with the total number of larvae and adults per sample may help to assess mainly antibiotic effects on the larvae fed on leaf sheaths and rice roots. In this respect, BRS Firmeza, with fewer larvae and lower adult weight, had a degree of resistance to *O. oryzae* similar to that of the standard resistant BRS Atalanta cultivar, evidencing isolated or synergistic effects of antixenosis and antibiosis. In turn, BRS Ligeirinho, with more larvae and higher weight of larvae and adults, was susceptible to insect infestation. BRS Pelota and BRS Querência, with more larvae and intermediate weight of adults, evidenced a moderate degree of resistance, which, although did not decrease the emergence of adults, reduced the size to some extent. IRGA 417, with more larvae and a lower number and weight of adults, showed a strong antibiosis effect, which might have reduced the larval and pupal viability, resulting in a lower emergence rate and adult body mass (Table 2).

It is evidenced that, depending on the host rice cultivar of *O. oryzae*, the impairment of larval metabolism, due primarily to antibiotic factors, persists in the other phases of the biological cycle of the insect, and this characteristic is relevant for integrated pest management. Thus, BRS Firmeza, that demonstrates qualitative antibiotic effects,

Table 2. Number and weight of larvae and adults, emergence rate and sex ratio of *Oryzophagus oryzae* in plants of six cultivars of flooded rice.

Cultivar	Larvae		Adults			
	Number per sample[1;6]	Weight (mg)[2;6]	Number per sample[3;6]	Weight (mg)[3;6]	Emergence rate (%)[4;6]	Sex ratio[5;6]
BRS Atalanta	21.02 b	8.13 b	8.73 b	1.94 c	41.17 a	0.55 a
BRS Firmeza	14.46 b	6.68 c	5.44 b	2.08 c	37.26 a	0.53 a
BRS Ligeirinho	32.45 a	11.09 a	11.21 a	2.51 a	34.37 a	0.45 a
BRS Pelota	26.60 a	11.74 a	9.58 a	2.21 b	35.92 a	0.53 a
BRS Querência	32.49 a	6.97 c	11.52 a	2.30 b	34.86 a	0.48 a
IRGA 417	32.81 a	8.77 b	7.92 b	1.92 c	23.73 b	0.51 a
CV (%)	21.53	23.92	39.57	9.72	16.71	9.34

[1] Total number (sum) of small, intermediate and large size larvae. [2] Mean weight of larvae from three size classes. [3] Number and mean weight of emerging adults per sample submerged in water for 35 days after collection. [4] Emergence rate [(number of adults/number of larvae) * 100)]. [5] [Number of females/(number of females + number of males)] in 50 % of collected adults. [6] Means with equal lowercase letters in the columns and uppercase letters in the rows indicate the absence of significant differences ($p \leq 0.05$) by the Scott-Knott test.

conditioning less growth (weight) of larvae and adults, may reduce the potential of larval damage to the roots (Silva et al. 2003) and the population of native (autochthonous) adults that infest the future rice fields (Martins et al. 2001, Awmack & Leather 2002). It is possible that the lower weight of adults results from the competition for food among larvae and not from the qualitative antibiotic effects. Therefore, the lower weight of adults could be caused by a larger larval population in the roots, which would result in a lower larvae growth rate, as observed in the connection between the smaller size of *L. oryzophilus* larvae and the lower relative feeding rate in the roots (Wu & Wilson 1997). However, in the present study, opposite situations in two cultivars did not confirm the effect of larval competition for food on adult weight: BRS Firmeza, with fewer larvae, did not produce adults with a higher weight; and BRS Ligeirinho, with more larvae, did not produce adults with a lower weight.

Despite physiological differences among rice plants at a similar period after the emergence, depending on the duration of the development cycle of the cultivars, which may change the degree of resistance to insects (Panda & Khush 1995), these differences apparently did not affect the resistance of the cultivars to *O. oryzae*. It should be pointed out that BRS Atalanta and BRS Ligeirinho, which have a very short development cycle, were resistant and susceptible, respectively, whereas BRS Pelota, with an intermediate development cycle, and BRS Querência, with a short cycle, had the same degree of resistance (Table 2). The fluctuation of the *O. oryzae* population may be similar among some rice cultivars, regardless of the length of the development cycle and degree of resistance (Carbonari et al. 2000). However, this information does not eliminate the need to advance the knowledge about the effect of phenological aspects of rice plants on the metabolism of *O. oryzae* in the larval and adult phases.

It is worth noting that the adults weight has not been considered in pioneering studies on rice resistance to rice water weevil (Smith & Robinson 1982, Martins & Terres 1995) or in more recent studies (Lupi et al. 2013, Stout et al. 2013, Vyavhare et al. 2016, Maisarah et al. 2018). Thus, it is important to determine the applicability of this variable in the identification of antibiosis effects to *O. oryzae*, evaluating variables such as adult emergence rate, egg fecundity and viability, and chemical compounds present in rice plants.

CONCLUSIONS

1. The BRS Firmeza cultivar is resistant to *Oryzophagus oryzae* by antixenosis and/or antibiosis effects;
2. BRS Ligeirinho is susceptible to *O. oryzae*, harboring a high larval population and propitiating a better development of larvae and adults;
3. IRGA 417 has an antibiosis-type resistance to *O. oryzae*, which restricts the adult emergence rates.

ACKNOWLEDGMENTS

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for granting a PhD. scholarship to the first author; to the trainee of the Universidade Federal de Pelotas (UFPel), Germano Tessmer Büttow, for assistance to collect research data; and to Elton Rogério Nolasco Fonseca, employee of the Embrapa Clima Temperado (CPACT), for taking care of the experiment in the field.

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