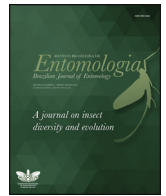




SOCIEDADE BRASILEIRA
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FUNDADA EM 1937

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Mapping of spatiotemporal distribution of *Tibraca limbativentris* Stal (Hem.: Pentatomidae) in flooded rice crop in Southern Brazil



Helen da Silva Costa^a, Enio Júnior Seidel^a, Juliano de Bastos Pazini^{b,*}, Augusto Maciel da Silva^a, Fernando Felisberto da Silva^b, José Francisco da Silva Martins^c, José Alexandre Freitas Barrigossi^d

^a Universidade Federal de Santa Maria (UFSM), Centro de Ciências Naturais e Exatas (CCNE), Departamento de Estatística, Cidade Universitária, Santa Maria, Rio Grande do Sul, RS, Brazil

^b Universidade Federal do Pampa (UNIPAMPA), Campus Itaqui, Itaqui, Rio Grande do Sul, RS, Brazil

^c Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, RS, Brazil

^d Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Embrapa Arroz & Feijão, Santo Antônio de Goiás, Goiás, GO, Brazil

ARTICLE INFO

Article history:

Received 11 October 2018

Accepted 18 April 2019

Available online 9 May 2019

Associate Editor: Regiane Bueno

Keywords:

Insect ecology

Multiquadric equations

Oryza sativa L.

Pest monitoring

Rice stem bug

ABSTRACT

The rice stem bug, *Tibraca limbativentris* Stal (Hem.: Pentatomidae), is one of the main insect pests in Brazilian rice crops. Knowledge of its spatiotemporal distribution can support the development of an effective sampling system and improve IPM strategies. This study aimed to map the spatiotemporal distribution of rice stem bug in flooded rice crop in Southern Brazil. Flooded rice crop was scouted for two seasons to estimate insect densities. Four occurrence categories were observed: no insect, only adults, only nymphs, and both phases. The relationship between phenological stages and insect categories was established. Interpolation (mapping) of occurrence categories of *T. limbativentris* was performed by multiquadric equations. In two seasons during the rice cycle, the results indicate that rice stem bug “adults” were the most abundant category until the mid-vegetative phase of the rice; “nymphs” were the most abundant from the end of vegetative phase; “adults + nymphs” occurred from the beginning of reproductive phase; there were no rice stem bugs in more than 66% of the area, as they were most concentrated near the edge of the crop. The information presented here provides further knowledge about *T. limbativentris* spatiotemporal dynamics that can be applied to improve IPM strategies, such as developing sampling plans and localized control measures at the edge of rice fields.

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Introduction

Rice (*Oryza sativa* L.) is the most consumed food and the second most cultivated cereal in the world, with an area of 168 million hectares. Among the ten largest producers in the world, Brazil is the only rice producer not located on the Asian continent (FAOSTAT, 2016). Rice is socioeconomically important in Brazil as a staple food in the population's diet. About 80% of its domestic production comes from flood-irrigated fields in 1.2 million hectares in the South region, where the state of Rio Grande do Sul accounts for more than 70% of Brazilian rice production (CONAB, 2018).

Tibraca limbativentris Stal (Hem.: Pentatomidae) is a species harmful to rice fields in different countries distributed throughout the Neotropical region (Martins et al., 2004). In addition, it is among the five economically important species of phytophagous stink bugs with a high potential for propagation in countries that have not yet recorded their occurrence, such as the United States (Panizzi, 2015). In Brazilian rice fields, rice stem bug is an insect pest of primary occurrence that can cause grain yield losses of up to 90% (Ferreira et al., 1997) due to the feeding of adults and nymphs on plant stems (Krinski and Foerster, 2017). “Dead-heart” symptoms arise when they pierce the stems in the vegetative stages, while “white panicle” symptoms occur when they pierce the already developed stems in the reproductive stages (Ferreira et al., 1997; Reunião, 2016).

The control of the rice stem bug is usually carried out with pyrethroid and neonicotinoid insecticides (Martins et al., 2016). Adequate monitoring of insect population growth in rice fields is essential for a correct decision to control and reduce crop damage. For this, determining the spatiotemporal distribution of this

* Corresponding author. Present address: Universidade Federal de Pelotas (UFPEL), Faculdade de Agronomia “Eliseu Maciel” (FAEM), Departamento de Fitossanidade (DFs), Campus Universitário, Avenida Eliseu Maciel, Caixa Postal 354, CEP 96010-900, Pelotas, Rio Grande do Sul, RS, Brazil.

E-mail: julianopazzini@hotmail.com (J.B. Pazini).

insect is important information for Integrated Pest Management (IPM), as well as the establishment of effective sampling processes and potential for localized control actions, to minimize production costs and risks of environmental contamination by insecticides.

The spatial distribution of an organism is an ecological characteristic largely influenced by birth, mortality, and migration rates (Toledo et al., 2006). Spatial distribution is the form these organisms are dispersed in their natural habitat (Pazini et al., 2017). In a pioneering study, Costa and Link (1992) assessed the spatiotemporal distribution of rice stem bug adults and emphasized that the vegetative and reproductive phenological stages of rice plants may have influenced their model of dispersion in the crop. Recently, Alves et al. (2016) pointed out that the immature and adult stages of this insect may have different dispersions, not always found on the same plant or sampling point in rice fields of northern Brazil. Pazini et al. (2017) found an aggregate spatial distribution for adults, nymphs, and adult and nymph phases together, regardless of plant phenology in rice paddies of southern Brazil. These studies evidenced quantitative differences in the occurrence of *T. limbativentris* at different times of the rice crop cycle (phenological stages), e.g. non-occurrence of the insect, presence of adults only, presence of nymphs only, or coexistence of both phases.

Traditional techniques of Geostatistics by linear kriging have been applied in Agricultural Entomology to map the distribution of organisms in a space when the target variables of the study are insect density (insect counts) (Pazini et al., 2015a; Alves et al., 2016; Pias et al., 2017; Pasini et al., 2018). However, there are no studies for categorical data, such as the occurrence of pest insects in the immature or adult phase or the coexistence of these phases predominant in crop segments throughout the crop cycle. Understanding the way that the rice stem bug is distributed in rice fields in the space and time provided by mapping of occurrence categories or life-cycle stages of the insect (adults, nymphs, and adults + nymphs) observed on plants can enable farmers to improve their monitoring of commercial crops, leading to safer management decisions.

Bönisch et al. (2004) used indicator kriging of interpolation soil properties expressed by categorical data. However, this approach shows weaknesses if any of the categories has a low occurrence ratio, leading to problems in the geostatistical interpolations. Recently, Yamamoto et al. (2012), in a theoretical study, recommended the use of multiquadric equations to map categorical variables, emphasizing this approach is more efficient than Geostatistics.

Thus, due to the need to improve sampling procedures for the rice stem bug in commercial irrigated rice crops and the higher exploration and applicability of interpolation methods for categorical variables, this study aimed to map the spatiotemporal distribution of rice stem bug in flooded rice crop. This study is in line with recent efforts to understand the spatiotemporal distribution of rice stem bug to support the development of an effective sampling scheme and consequently the IPM practice in commercial rice fields.

Material and methods

Study area

The study was conducted in the 2010/11 and 2011/12 seasons in a commercial rice production crop of a Luvisol with an average slope of 4.8% and located in Itaquí, Rio Grande do Sul, Brazil (29°09'56.52" S and 56°29'20.06" W). This municipality is part of the "Western Frontier" rice region, which is the largest cereal-producing region in Brazil, with about 321 thousand hectares and an average yield of 8327 kg ha⁻¹ in the 2016/17 season (IRGA,

2018). The predominant regional climate is "Cfa", a subtropical, warm temperate climate with well-distributed rains and well-defined seasons, according to the Köppen–Geiger classification.

In both seasons, the crop was planted via minimal tillage by sowing the rice cultivar "IRGA 417" in the first week of October, at a density of 60 seeds per linear meter and spacing of 0.17 m between rows. Fertilization (N–P–K) consisted of the application of 286 kg ha⁻¹ of 4–17–27 at sowing time, 150 kg ha⁻¹ of 45–0–0, 15 days after seedling emergence (before flooding the rice paddy), and 75 kg ha⁻¹ of 30–0–20 at the differentiation of the floral primordia stage. The phytosanitary management was performed according to the technical recommendations for irrigated rice cultivation (Reunião, 2010), but with no insecticide application. In the first season, during rice post-harvest in mid-February, the crop was subjected to two plowings and one leveling harrow to destroy crop residues. In the 2011/12 season, crop residues were destroyed with a knife roller.

The physiognomy of the background vegetation and other elements that composed the landscape include an eucalyptus forest on the southern border; a road for circulation and a corridor for cattle flow on the north; a high presence of spontaneous plants, predominantly Apiaceae, Asteraceae, Cyperaceae, and Poaceae on the west; a local access road with a small extension of an eucalyptus forest and a high incidence of plants similar to those already mentioned at the east.

Rice stem bug monitoring

To monitor *T. limbativentris* in the crop in both seasons, a regular grid with equidistant georeferenced sampling points was previously established as in Kuno (1991). The monitoring included biweekly samplings during the vegetative and reproductive phenological stages of the rice plants (V₄ to R₉) (Counce et al., 2000), as well as samplings during the post-harvest because the insect can be found in the crop residues (Botta et al., 2014a). In both seasons, the spacing between sampling points was 10 m, totaling 693 sampling points in 2010/11 and 352 sampling points in 2011/12. A metal square of 0.5 × 0.5 m was placed on each sampling point, and the plants inserted in this square were visually examined for four occurrence categories: (I) non-occurrence of the insect, (II) presence of adults only, (III) presence of nymphs only, and (IV) coexistence of both phases.

Analysis and interpolation

An indicator variable for each occurrence category was created to indicate its chance of occurrence or non-occurrence in a certain region (sampling point in the crop). This variable assumed values of 0 or 1, representing its absence or presence at the sampling date, respectively (Bönisch et al., 2004; Yamamoto et al., 2012).

After obtaining the indicator variable, the frequency of each category was observed at different sampling dates (phenological stages of rice plants). Subsequently, the symmetry test was performed to verify whether the frequencies of occurrence categories were similar at each sampling date, two to two, in both seasons. The symmetry test ($p < 0.05$) were performed using the package "rcompanion" of R software (R Development Core Team, 2018).

The interpolation of occurrence categories of the rice stem bug was carried out by multiquadric equations (Yamamoto et al., 2012). The multiquadric equations were used to interpolate a non-sampled point by means of a linear combination, whose weights were obtained by solving a system of linear equations. This system of equations used a radial basis function of distances between sampled points and points to be estimated, and the considered radial basis function was the generalized multiquadric function (Yamamoto et al., 2012).

Table 1

Frequencies of categories of the rice stem bug *Tibraca limbativentris* (count and percentage) and symmetry tests applied to the comparison between sampling dates (phenological stages) in flooded rice crop in Southern Brazil, 2010/11 crop season.

Sampling dates (phenological stage ^b)	Categories			
	I ^a	II	III	IV
<i>12/13/10 (V₈) a</i>				
Count (n)	532	52	101	8
Percentage (%)	76.77	7.50	14.57	1.15
<i>12/27/10 (V₁₀/V₁₁) b</i>				
Count (n)	482	17	188	6
Percentage (%)	69.55	2.45	27.13	0.87
<i>01/18/11 (R₁) c</i>				
Count (n)	322	115	148	108
Percentage (%)	46.46	16.59	21.36	15.58
<i>02/12/11 (R₉) d</i>				
Count (n)	246	143	174	130
Percentage (%)	35.50	20.63	25.11	18.76
<i>02/15/11 (P.h.^c) a</i>				
Count (n)	525	32	120	16
Percentage (%)	75.76	04.62	17.32	02.31
Average percentage (%)	60.81	10.36	21.10	7.73

Sampling dates followed by different lowercase letters differ significantly by symmetry test ($p < 0.05$).

^a Occurrence categories of rice stem bug = I – no insect, II – adult, III – nymphs, and IV – adult + nymphs.

^b Phenological stage according to Counce et al. (2000).

^c Rice stem bug sampling in post-harvest. Crop residues not destroyed.

The interpolation by multiquadric equations was performed with Geovisual 5.0 software using an algorithm that estimated the type of occurrence of the rice stem bug at a non-sampled point and selected that with the highest probability of occurrence (probability higher than or equal to 72%) from the estimated probabilities for each type of categorical variable (Yamamoto and Landim, 2013). In addition to the spatial interpolation of occurrence categories of the rice stem bug, the uncertainty associated with the interpolation was also estimated (Yamamoto and Landim, 2013).

Results and discussion

In the 2010/11 season, on average, *T. limbativentris* did not occur in 60.81% of the sampled points. The presence of adults, nymphs, and both phases occurred in 10.36, 21.10, and 7.73% of the sampled points, respectively (Table 1). In the 2011/12 season, on average, the rice stem bug did not occur in 73.01% of the sampled points, while the presence of adults, nymphs, and both phases occurred in 12.22, 11.08, and 3.69% of the sampled points, respectively (Table 2). These results were similar to those obtained by Alves et al. (2016), who reported the non-occurrence of rice stem bug in 67.78% of the sampling points distributed in the crops, as well as the occurrence of adults, nymphs, and both (adults + nymphs) in 14.40, 14.59, and 3.23% of the sampling points, respectively.

The symmetry test for both seasons showed different occurrence rates of *T. limbativentris* at different phenological stages in which samples were taken. When comparing the sampling dates, distinct counting behaviors of each occurrence category of the insect were observed in both seasons, except for the dates of 12/13 and 02/15 in the 2010/11 and 01/07 and 01/21 in the 2011/12 season, not differing significantly (Tables 1 and 2).

The variability at each occurrence category of rice stem bug at the different sampling dates indicates an association between occurrence categories and the phenological stages, with the phenological stages of the rice an important factor for insect prevalence and rice damage (Hickel et al., 2016; Krinski and Foerster, 2017). The spatiotemporal variability of economically important arthropods in agriculture has been reported as strongly related to the phenological stages of crops (Nava et al., 2018). The occurrence of

T. limbativentris has been reported in rice fields when plants reach a growth level suitable for feeding, which occurs from the V₄ stage and increases rapidly as generations develop, with a higher occurrence from the beginning of tillering (V₄) to flowering (R₄), and a reduction in the post-harvest (Pazini et al., 2015a).

The thematic mapping of the spatiotemporal behavior of occurrence categories of the rice stem bug in commercial flooded rice in two agricultural seasons obtained by means of multiquadric equations is in Figs. 1 and 2. The reduced zones of uncertainty associated with these mapping accurately determined the spatiotemporal distribution of the occurrence categories of insect in the rice fields.

At the sampling dates of 12/13 in the 2010/11 season (Fig. 1A) and 11/19, 12/03, and 12/17 in the 2011/12 season (Fig. 2A–C), which correspond to the stages from V₄ to V₈/V₉, a predominance of the category I (non-occurrence of insects), in more than 75% of the sampled points was observed in the rice paddy. Initially, the rice stem bug population is low, resulting only from the migration of part of the post-hibernating population since the total abandonment of the winter refuges surrounding the crop can be extended to January, depending on biotic and abiotic factors (Botta et al., 2014a).

The incidence of category II, composed only of *T. limbativentris* adults, occurred at the beginning of tillering of rice plants in marginal crop areas (edges) (Fig. 2A–C) by the migration of post-hibernating adults from adjacent vegetation, where they remain in the off-season (Costa and Link, 1992). A photoperiod higher than 12 h and temperatures higher than 22 °C determine the abandonment of hibernation sites, migration to rice plants, and consequent stem bug proliferation in rice crops (Botta et al., 2014a). Landscape characteristics, with the presence of plants of the Apiaceae, Asteraceae, Cyperaceae, and Poaceae families, which are preferred hibernation refuges for the rice stem bug (Botta et al., 2014b; Hickel et al., 2016) in the areas surrounding the studied rice fields, the low innate movement capacity of post-hibernating adults, the great need to feed on the already formed stems, and mating partners may explain their higher concentrations at the crop edges. Monitoring of different stink bug species (Hemiptera: Pentatomidae) in different agricultural crops found higher densities in samples near the crop edges, with a significant effect on spatiotemporal behavior of insects (“edge effects”) (Espino and

Table 2
Frequencies of categories of the rice stem bug *Tibraca limbativentris* (count and percentage) and symmetry test applied to the comparison between sampling dates (phenological stages) in flooded rice crop in Southern Brazil, 2011/12 crop season.

Sampling dates (phenological stage ^b)	Categories			
	I ^a	II	III	IV
11/19/11 (V₄) a				
Count (n)	337	15	0	0
Percentage (%)	95.74	4.26	0.00	0.00
12/03/11 (V₆) b				
Count (n)	313	39	0	0
Percentage (%)	88.92	11.08	0.00	0.00
12/17/11 (V₈/V₉) c				
Count (n)	280	69	3	0
Percentage (%)	79.54	19.60	0.85	0.00
01/07/12 (V₁₁) d				
Count (n)	214	13	114	11
Percentage (%)	60.79	3.69	32.39	3.12
01/21/12 (R₁) d				
Count (n)	205	9	116	22
Percentage (%)	58.24	2.56	32.95	6.25
02/02/12 (R₅) e				
Count (n)	170	103	28	51
Percentage (%)	48.29	29.26	7.95	14.49
02/15/12 (R₉) f				
Count (n)	188	95	49	20
Percentage (%)	53.41	26.99	13.92	5.68
02/29/12 (P.h.^c) g				
Count (n)	349	1	2	0
Percentage (%)	99.15	0.28	0.57	0.00
Average percentage (%)	73.01	12.22	11.08	3.69

Sampling dates followed by different lowercase letters differ significantly by symmetry test ($p < 0.05$).

^a Occurrence categories of rice stem bug = I – no insect, II – adult, III – nymphs, and IV – adult + nymphs.

^b Phenological stage according to Counce et al. (2000).

^c Rice stem bug sampling in post-harvest. Crop residues destroyed.

Way, 2008; Tillman et al., 2009; Reay-Jones et al., 2010; Macfadyen and Muller, 2013; Pilkay et al., 2015; Venugopal et al., 2014, 2015; Nguyen and Nansen, 2018; Pasini et al., 2018).

The knowledge that post-hibernating adults of *T. limbativentris*, which initiate rice colonization at the beginning of plant tillering, are concentrated spatiotemporally in the marginal extension of the crop influenced by the edge has several applications in IPM. The results indicate that sampling efforts of *T. limbativentris* should be concentrated at the entrance areas of the insect, starting from the rice field edges at the beginning of the tillering period (Pazini et al., 2015a). The development of pest monitoring plans stratified and directed along the rice field edges from plant tillering should be considered to increase the efficiency and reduce sampling efforts (Nguyen and Nansen, 2018).

In addition, it optimizes the effective use of localized control measures (Barrigossi et al., 2001), such as insecticide sprayings on the edge extension of the crop, where the pest is concentrated at the beginning and first half of tillering (Reay-Jones et al., 2009; Toews and Shurley, 2009; Venugopal et al., 2014). The directed spraying of insecticides is an effective strategy to control agricultural pests, helping to reduce the production costs and risks of contamination in the agroecosystem by significantly reducing the insecticide amount (Nguyen and Nansen, 2018) compared to conventional sprayings scheduled throughout the crop, as usually employed for *T. limbativentris* in southern Brazil (Martins et al., 2016). Because the plants are 25–40 cm high and have sparse leaves until mid-tillering, the probability of the sprayed insecticide reaching the stem bugs between rice stems is high (Meus et al., 2012). The chemical control carried out during this period would significantly eliminate the rice

stem bugs before the beginning of their reproductive activity and oviposition, avoiding a rapid population growth.

Nymphs only (III) were predominantly verified in up to 33% of the sampling points between the end of the vegetative stage (V₁₀ and V₁₁) and the beginning of the reproductive period (R₁) (Figs. 1B and 2D, E). These nymphs originated from post-hibernating individuals and represent the first summer generation of the rice stem bug in the crop. The thematic maps showed the beginning of the incidence of this category remains at small aggregations near the occurrence areas of post-hibernating adults (Figs. 1A and 2C). Ferreira et al. (1997) pointed out that first instar nymphs are usually grouped around egg-masses and apparently do not feed. However, from the second instar, nymphs are less clustered and presented more dispersion for feeding, spreading spatially, as observed in this study (Figs. 1B and 2D, E).

The occurrence of the category IV (adults + nymphs), started markedly at the R₁ stage, but extending to the end of the crop cycle at stage R₉, corresponding to the complete maturity of panicle grains (Figs. 1C, D and 2E, F, G). In this period, the lowest indices of category I (non-occurrence of the insect) were also observed, indicating the almost absolute colonization of the area (Tables 1 and 2). The higher frequency of sampling points on the maps with the coexistence of adults and nymphs from the beginning of the reproductive stage is caused by the emergence of a new population of nymphs from adults of the first summer generation, which characterizes the second summer generation of the rice stem bug. In addition, this coexistence indicated the occurrence of overlapping insect generations in the rice crop. The adult population of the second summer generation of *T. limbativentris*,

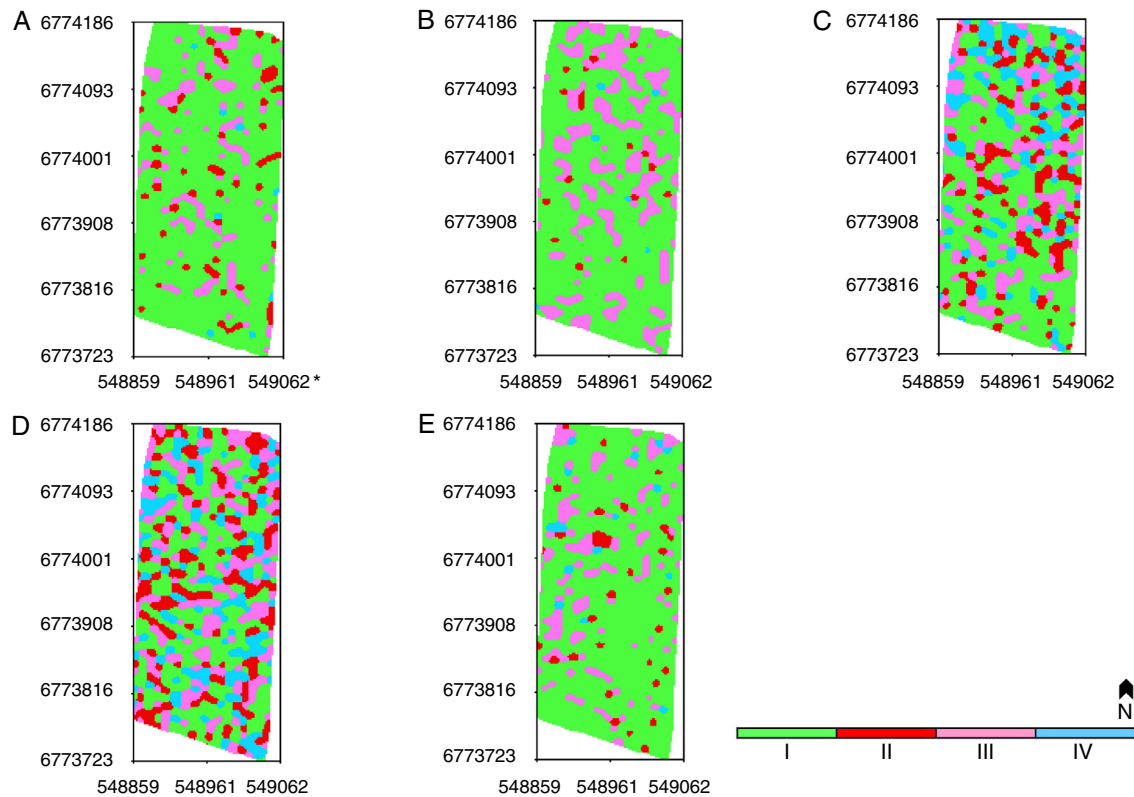


Fig. 1. Interpolation maps by multiquadric equations of spatiotemporal distribution of occurrence categories of *Tibraca limbativentris* [I = no insect (green), II = adult (red), III = nymphs (pink), IV = adult + nymphs (blue)] in flooded rice crop in Southern Brazil, 2010/2011 crop season. *Thematic maps: (A) 12/13/10 [V₈]; (B) 12/27/10 [V₁₀/V₁₁]; (C) 01/18/11 [R₁]; (D) 02/12/11 [R₉]; (E) 02/15/11 [post-harvest = crop residues not destroyed]. Phenological stage according to Counce et al. (2000).

which are composed of the pre-hibernating population, has a low potential to damage the crops due to the advanced phenological cycle of the plants. The rice stem bug can produce up to three generations in rice crops and the third is usually complete in crop residues and plants adjacent to the crop at the beginning of the off-season (Botton et al., 1996; Ferreira et al., 1997; Botta et al., 2014a).

The reduction of stem bug population was only detected in post-harvest samples, with category I (non-occurrence of the insect) in more than 75% of the sampled points (Tables 1 and 2). The reduction of the post-harvest population, detected in the 2011/12 season (Table 2, Fig. 2H), was significant compared to the previous season (Table 1, Fig. 1E) mainly due the mortality or scape generated by the destruction of crop residues by the knife roller hours after the rice harvest. Thus, the anticipation of crop management operations in post-harvest of commercial rice fields may help to reduced population of pre-hibernating adults. The incorporation of crop residues is

an efficient technique of mechanical control of insect pests in several crops and avoids favorable conditions of winter refuge for the *T. limbativentris* population during the off-season of the rice crop (Pazini et al., 2012).

In the context of the IPM in Brazilian rice crops, the results obtained in this study are an essential and pioneering approach demonstrating the spatiotemporal distribution of *T. limbativentris*. With the recent advances in the knowledge about this pest population dynamics in rice agroecosystem (Botta et al., 2014a; Pazini et al., 2015a, 2015b, 2015c, 2015d, 2017; Alves et al., 2016), these studies can provide scientific bases for the development of efficient monitoring techniques and more environmentally correct pest management actions (site-specific management), reducing the production costs with emphasis on diminishing insecticide use that can cause environmental contamination.

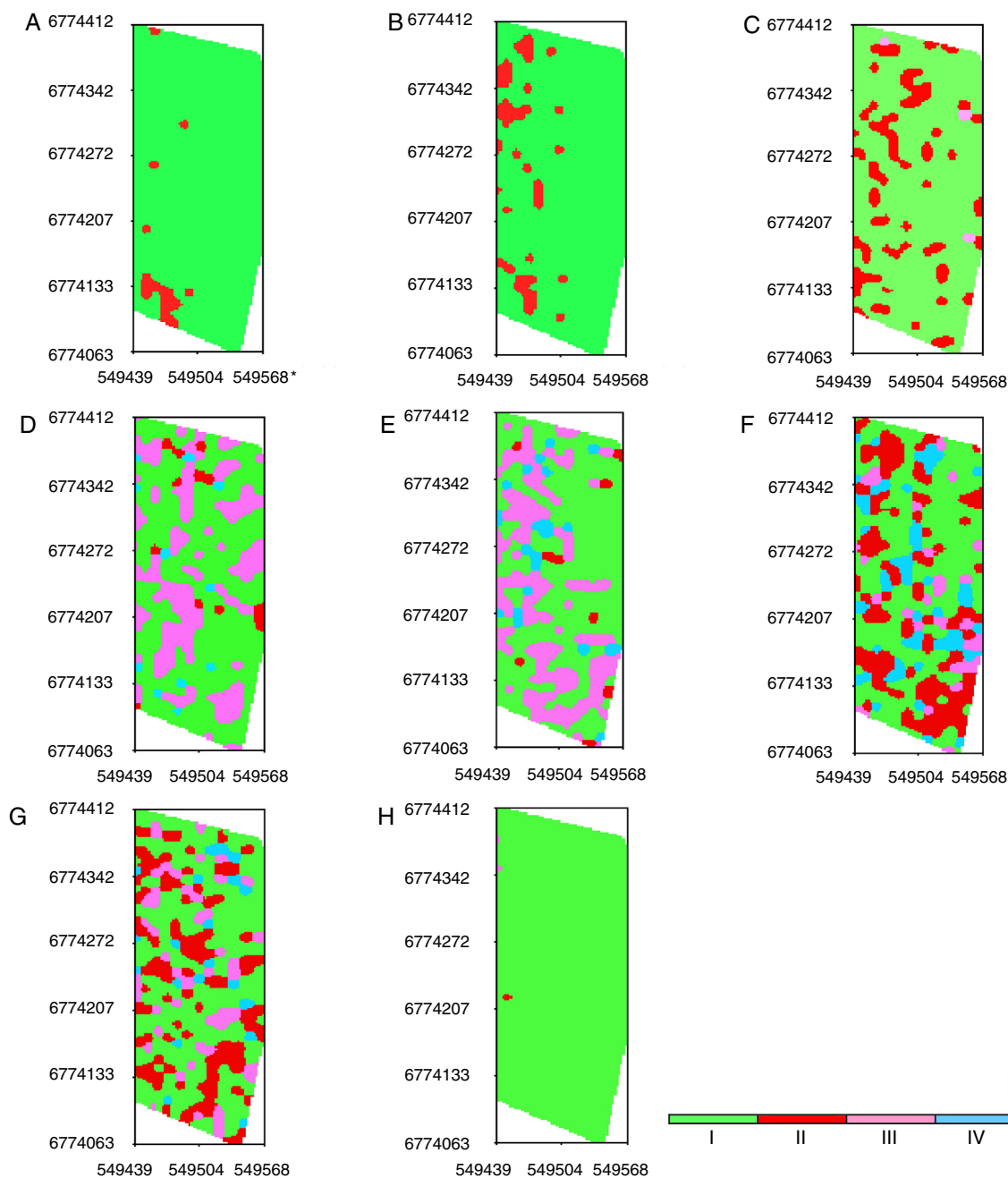


Fig. 2. Interpolation maps by multiquadric equations of spatiotemporal distribution of occurrence categories of *Tibraca limbativentris* [I = no insect (green), II = adult (red), III = nymphs (pink), IV = adult + nymphs (blue)] in flooded rice crop in Southern Brazil, 2011/2012 crop season. *Thematic maps: (A) 11/19/11 [V₄]; (B) 12/03/11 [V₆]; (C) 12/17/11 [V₈/V₉]; (D) 01/07/12 [V₁₁]; (E) 01/21/12 [R₁]; (F) 02/02/12 [R₅]; (G) 02/15/12 [R₉]; (H) 02/29/12 [post-harvest = crop residues destroyed]. Phenological stage according to Counce et al. (2000).

Conclusions

In a rice crop, it can be expected that more than 66% of the area will have no rice stem bug. “Adults” of rice stem bug are the most abundant insect category until mid-vegetative phase of rice crop while “nymphs” are the most abundant from the end of the vegetative phase. The adult category completely concentrates at edge of the field until mid-vegetative phase of the rice crop, which indicates that localized control measures could be used to effectively eliminate the pest before population growth.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution statement

FFS, JFSM, and JAFB conceived and designed the research. JBP and HSC conducted the experiments. EJS, AMS, and HSC analyzed data. HSC and JBP wrote the manuscript. All authors contributed in

revising and preparing the paper for submission. All authors approved the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to thank the entomology laboratory members at UNIPAMPA (LABENTO) for their support in carrying out this research. Special thanks to Robson Antônio Botta and Rodrigo Rübenich for their full assistances in the field. We thank the Pitangueira Rice Company for supplying the rice fields for this study and their full courtesy and cooperation in this research.

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