Spatial and temporal distribution of South American fruit fly in vineyards[1]

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ABSTRACT

The South American fruit fly *Anastrepha fraterculus* (Wied., 1830) is one of the major insect pests of economic importance in vineyards of Southern Brazil. Understanding species behavior and knowing the moments when their population peaks occur can help producers and technicians to define management strategies. This work was carried out the spatial and temporal distribution of the *A. fraterculus* in two commercial vineyards of variety 'Moscato Branco' for two crop seasons. To evaluate the *A. fraterculus* distribution, we used the mass trapping system with handmade traps (transparent plastic bottles of polyethylene terephthalate – PET), baited with hydrolyzed protein CeraTrap™. The evaluations were performed every two weeks, counting the total number of adults found per trap in each vineyard. From the number of insects caught per trap, data analysis was performed using geostatistics, through semivariograms. The spatio-temporal fruit fly distribution was evaluated by thematic maps, using the inverse square distance interpolation. The semivariograms showed that most of the reviews were 'pure nugget' effect, indicating the absence of spatial data dependence. The spatio-temporal distribution maps allow us to assert that *A. fraterculus* shows invasive behavior in the vineyard, with its entry from the edges to the center, associated with the fruit ripening.

Keywords: *Anastrepha fraterculus*, *Vitis vinifera* L., inverse distance weighting, spatial variability.

INTRODUCTION

The South American fruit fly *Anastrepha fraterculus* (Wiedmann, 1830) (Diptera: Tephritidae) is one of the main species of pest insects associated with grapevine in Southern Brazil (Formolo *et al*., 2011; Zart *et al*., 2011). However, lack of knowledge and characterization of *A. fraterculus* lesions in grapes, besides the confounding of these with injuries caused by other pests, such as thrips (Thysanoptera) (Formolo *et al*., 2011), relegated for many years the importance of this insect. In recent years, with market demand for increased fruit quality, *A. fraterculus* has become a significant pest, mainly in white skin grape varieties (Zart *et al*., 2011), in which their injuries damage the visual appearance of the fruits and jeopardize the sale.

The damage of the insect occurs by the adult female oviposition injuries (punctures), and larval feeding and development galleries (Soria, 1985; Zart *et al*., 2011). Furthermore, Soria (1985) related that bacterial colonies and fungus that were released at the time puncture, are capable to change, through the enzymatic action, berries compounds into substrates assimilated by the larvae, making them unfit for human consumption or reducing the quality of the final product after processing. Machota Junior *et al*. (2013) identified plant pathogens associated with the *A. fraterculus* in vineyards and reporting the presence of several wild species to the plant, such as *Botrytis cinerea*, *Cladosporium* spp., *Colletotrichum* spp. and *Penicillium* spp., a factor that increases the fruit fly

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relevance in the crop for the potential dispersion of disease-causing pathogens.

Currently, it is known that the nominal species *A. fraterculus*, in fact, represents a cryptic species complex. In this way, the morphotype "Brazilian-1" or *Anastrepha* sp.1 aff. *fraterculus* is widely distributed in a biogeographic area that includes Southern Brazil, with evidence of studies of low genetic variability and full sexual compatibility. (Hernández-Ortiz *et al*., 2012). In Southern Brazil, there are many research information about the temporal distribution and population fluctuation of the South American fruit fly in vineyards over time (Nondillo *et al*., 2007; Chavarria *et al*., 2009; Formolo *et al*., 2011). However, there is little information about the spatial distribution of tephritids inside these areas, being this necessary to understand insect-plant-environment interactions (Gyenge *et al*., 1999) and to establish correct management strategies.

One way to study the spatial distribution of insects in a crop is through mapping using Geographic Information System (GIS), along with geostatistics and interpolators. In the case of fruit flies, studies were performed in Spain (Alemany *et al*., 2006; Muñoz & Marí, 2009), Mexico (Utgés *et al*., 2011), Caribbean (Epsky *et al*., 2010), Italy (Sciarretta & Trematerra, 2011), Greece (Castrignanò *et al*., 2012), Hawaii (USA) (Leblanc *et al*., 2012), Portugal (Pimentel *et al*., 2014) and Brazil in guava *Psidium guajava* L. orchard (Jahnke *et al*., 2014) and in an urban area with forest fragments (Garcia *et al*., 2017). However, in vineyards, there is no information about the spatial distribution of fruit flies.

One objective of the spatio-temporal analysis is to monitor changes in the spatial distribution of fruit fly populations over time (Midgarden *et al*., 2014). In general, different types of spatio-temporal analysis are possible, including trends, pre and post (Mitchel, 2009), where trends indicate whether the population is increasing or decreasing or the direction and pattern of insect movement; while pre and post patterns show conditions before and after an event or action – like an insecticide application, for example – and attempt to evaluate this impact.

Knowledge of the fruit fly infestation pattern on vineyards could help farmers to define management strategies prioritizing outbreaks of infestation. Thus, this study aimed to evaluate the spatio-temporal distribution of *Anastrepha fraterculus* morphotype "Brazilian-1" in the grapevine.

MATERIAL AND METHODS

The study was performed during 2013/2014 and 2014/2015 crop seasons, in two commercial vineyards variety 'Moscato Branco', located at the municipality of Farroupilha, RS, Brazil. The two vineyards were conducted in the trellis system. The first one, named Area 1 (geographic coordinates 29º08'24" S; 51º22'41" W; elevation 617 m) presented spacing of 1.5 m between plants and 2.5 m between rows, with 0.47 ha area. The other, named Area 2 (29º08'40" S; 51º22'23" W; 563 m) presented spacing of 1.5 m between plants and 2.4 m between rows, with 1.09 ha area.

The vineyards were distant about 600 m apart, being in the same watershed and showing similar mesoclimatic conditions. On the edges and near the vineyards there were other fruit trees, such as peach *Prunus persica* L. Batsch and sweet orange *Citrus sinensis* (L.) Osbeck, in Area 1, and grapevine variety 'Isabel' *Vitis labrusca* L. in Area 2. In both areas, there was the presence of native Atlantic forest in the surroundings (Figure 1), a natural landscape of the region.

To evaluate the spatial distribution of fruit fly adults in vineyards we used handmade traps (2,000 mL polyethylene terephthalate – PET plastic bottles with four holes 7-mm diameter during the 2013/14 crop season; and 600 mL PET plastic bottles with two holes 7-mm diameter during the 2014/15 crop season), receiving 300 and 200 mL of undiluted hydrolysed protein Ceratrap™ (Bioiberica S.A., Barcelona, Spain), respectively. Every 30 days during the study, only the evaporated volume was completed, and the solution was not exchanged.

Based on the mass trapping technique, the traps were distributed equidistantly, every two crop lines and spaced at 12 m between plants, in a density of 120 traps per ha. Traps were set in the vineyard supporting wires (trellis system), hanging at 1.5m above the ground and positioned under the canopy of vines.

The experiment initiated in the first half of December 2013 in the 2013/14 crop season, finished at the harvest and installed again in the first half of November 2014 during the 2014/15 crop season. The traps location was obtained by a Glonass and GPS navigation signal receiver (Garmin®, model Etrex 30).

For the pest management, it was made only one application of the lambda-cyhalothrin insecticide (Karate 50 CS, 50 mL of commercial product/100 L) in Area 1 at the beginning of January 2015. In other areas and crop seasons, no insecticide applications were made.

The traps were serviced every 15 days, recording the number of fruit flies captured. The insects caught by the traps were placed in labeled vials containing 70% ethanol for subsequent sorting, counting and identification. The fruit fly specimens of the genus *Anastrepha* Schiner were sexed and identified using the identification key of Steyskal (1977) and Zucchi (2000). From these data, exploratory data analysis was performed using geostatistics, through semivariograms. The adjustments were made by theoretical mathematical models, using the software GS+® (Gamma Design Software). The semivariograms models were adjusted based on the lowest residual sum of squares (RSS) and the better coefficient of determination (R-square or R^2). From the data fit to a mathematical model, the semivariograms parameter were defined: Nugget effect (C_0), Sill value (C_0+C_1) and Range of influence (A_0). The Degree of spatial dependence (DSD) was calculated according to the methodology proposed by Cambardella *et al.* (1994).

The spatial distribution was evaluated by maps made using the ArcGis 10.1 (ESRI) software. The interpolator used for map generation was the inverse distance square (IDS) algorithm.

RESULTS AND DISCUSSION

Of the 2,382 Tephritidae specimens collected in the current study, all insects belonged to the genus *Anastrepha* Schiner and were identified as *Anastrepha fraterculus* (Wiedmann, 1830), corroborating other studies carried out in Southern Brazil (Nava & Botton, 2010; Garcia & Norrbom, 2011; Nunes *et al*., 2012; Dias *et al*., 2013; Pereira-Rêgo *et al*., 2013; Bortoli *et al*., 2016).

In the exploratory analysis of the data through semivariograms, it was observed that in both areas and crops, most of the samples presented the pure nugget effect (PNE), indicating the absence of spatial dependence and characterizing random distributions (Table 1).

PNE is commonly reported in Entomology studies, since spatial dependence may occur on a smaller scale than those used in some experiments (Liebhold *et al*., 1993). The PNE was also observed in other insect species, *Atta* spp. (Hymenoptera: Formicidae) in *Eucalyptus* forest (Lasmar *et al*., 2012), *Gymnandrosoma aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae) in the citrus orchard (Carvalho *et al*., 2015) and *Metamasius hemipterus* (Coleoptera: Curculionidae) in oil palm field (Dionisio *et al*., 2015). In the case of leaf-cutting ants *Atta* spp., the dominant influence of some soil characteristics (aeration and humidity, for example) favor the development and survival of the ant's colony, which results in the nest's aggregation. Carvalho *et al*. (2015) report that fruits attacked by *G. aurantiana* are distributed in aggregate form in the citrus orchard, as observed for *M. hemipterus* in oil palm *Elaeis guineensis* plantation (Dionisio *et al*., 2015).

In this way, PNE indicates that there is no spatial dependence, a random distribution, or the sampling spacing used is higher than necessary to reveal spatial dependence. This unexplained variability may result from undetected measurement errors or microvariations (Cambardella *et al*., 1994), considering the need to reduce the distance between the sampling points (handmade traps) to detect this dependence. Another point to consider is the low efficacy of monitoring *A. fraterculus* using food attractants is due, in part, to the limited range radius of traps up to 10 m (Nascimento *et al*., 2000).

For the evaluations where it was not possible to fit a theoretical semivariogram model and that presented no spatial dependence, it was not possible to use a geostatistical interpolator. In these cases, to maintain a temporal sequence of distribution maps, for all evaluations was chosen the interpolator inverse distance square (IDS) algorithm, which performs the estimation of the variable throughout space, determining weights at each of the *n* closest points (Jimenez & Domecq, 2008). This interpolator proved to be efficient when used to evaluate the effect of the landscape elements and host plants on medfly *Ceratitis capitata* (Wied., 1824) (Diptera: Tephritidae) distribution in 500 ha area composed of several fruits (Sciarretta & Trematerra, 2011).

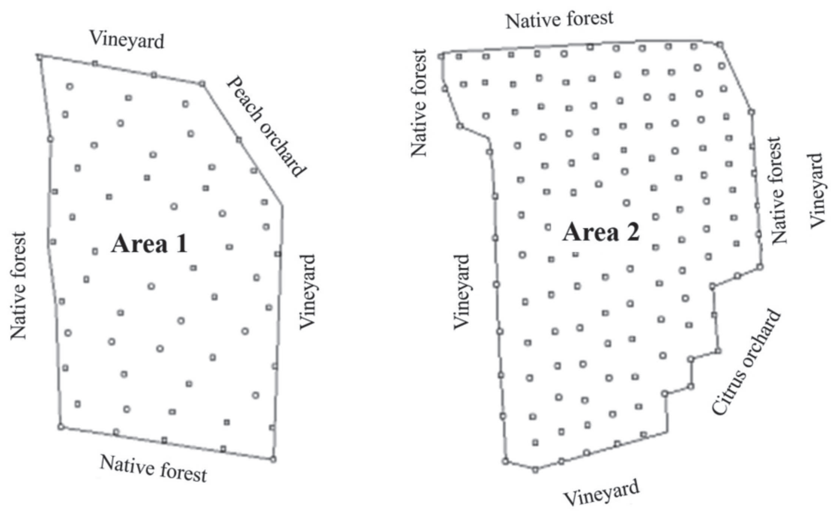

Figure 1: Schematic maps of the two vineyards (Area 1 and 2) of *Vitis vinifera* L. variety 'Moscato Branco', with the representation of the fruit crops present in the surroundings (without scale). Points on the map represent the distribution of the handmade PET traps.

In some evaluations it was possible to adjust to a theoretical model, however, due to the data oscillated between the assessments and the crop seasons showing no characteristic behavior, no inferences were made about the range or adjusted models. Through the distribution maps, it is observed that there were variations about the *A. fraterculus* spatial distribution (between the areas) and temporal distribution (between the harvests) (Figure 2, 3, 4 and 5).

In the 2013/2014 crop season, the entry site of adult fruit flies in Area 1 was next to the peach orchard *Prunus persica* L. and one of the edges with native forest (Figure 1 and 2). The peach fruits harvest ended in early January, making adults migrate to the vine area in search of food and a favorable environment for their reproduction. During this period, the culture was in the green berries phenological stage (Eichhorn & Lorenz, 1984). Although this phenological stage is not suitable for the larval development, adults of fruit fly can perform oviposition injuries (punctures), causing deformation and falling berries (Zart *et al*., 2011). Even after harvesting the fruit trees located around the vineyards, it was observed that some fruits remained in the area, not being harvested and maintained in the plant or on the ground. These fruits, in hypothesis, allowed the development of *A. fraterculus* larvae that later caused an increase in the adult fruit fly population in the vineyards.

Regarding to the edge of the native forest, native host plants (such as Surinam cherry *Eugenia uniflora* L. (Myrtaceae) and loquat *Eriobotrya japonica* (Thumb.)

Table 1: Parameters for semivariogram adjustment of *Anastrepha fraterculus* assessments in two *Vitis vinifera* L. variety 'Moscato Branco' commercial vineyards. Crop seasons 2013/2014 and 2014/2015

Sampling date	Model	C_0	C_0+C	A_0	RSS	R²	DSD
				2013/2014 crop season			
				Area 1			
01/03				PNE			
01/17	Exponential	0.49	1.29	65.70	0.05	0.85	Medium
01/31				PNE			
02/14				PNE			
02/27				PNE			
03/14	Spherical	1.29	4.05	66.10	0.48	0.93	Medium
				Area 2			
01/03				PNE			
01/17				PNE			
01/31	Exponential	0.0001	0.10	9.00	0.01	0.82	High
02/14	Gaussian	2.44	12.88	174.59	0.22	0.99	High
02/27	Gaussian	1.38	4.77	201.61	0.09	0.94	Medium
03/14	Gaussian	20.60	92.20	133.36	25.70	0.97	High
				2014/2015 crop season			
				Area 1			
11/28	Spherical	0.15	0.33	60.40	2.891E-05	0.99	Medium
12/12				PNE			
12/22				PNE			
01/09				PNE			
01/23				PNE			
02/06				PNE			
02/23				PNE			
				Area 2			
11/28	Linear	0.36	0.89	131.4	0.61	0.80	Medium
12/12				PNE			
12/22				PNE			
01/09	Exponential	0.001	0.37	7.0	0.04	0.77	High
23/01	Gaussian	0.23	2.48	357.32	0.06	0.91	High
02/06	Gaussian	1.48	5.96	287.34	0.22	0.91	High
02/23	Linear	5.17	7.33	130.0	9.76	0.91	Medium

Note: PNE = Pure nugget effect; C_0 = Nugget effect; C_0+C = Sill; A_0 = Range; RSS = Residual sum of squares; R² = Coefficient of determination; DSD = Degree of spatial dependence.

Lindley (Rosaceae) were identified at the vineyards' borders. In the neighboring properties, there were peach *Prunus persica* L. Batsch (Rosaceae) and grape *Vitis* spp. L. (Vitaceae) commercial orchards allowing the fruit fly occurrence and development (Garcia & Norrbom, 2011; Bortoli *et al*., 2016). In this case, the species would carry out migratory movements between the forest plants towards the vineyard.

In the next crop season (2014/2015), in Area 1 was observed that the spatial and temporal behavior of the species was influenced by the lambda-cyhalothrin insecticide application, being held in early January. After this period, it was verified the insects returned to colonize the Area, with a unique pattern of movement, being its presence identified in all quadrants of the vineyard (Figure 4).

It is important to note that the grapevine has not been registered as a preferred plant host for *Anastrepha fraterculus* (Zart *et al*., 2011). It has been observed that, in general, the population of a particular species of fruit flies remains near their preferred hosts (Carvalho, 2005), on the edges, and their movement and orientation respond to the favorable host's maturation (Christenson & Foote, 1960).

The results found during the two crop seasons in Area 1, agree with studies demonstrating that host plants provide an ecological corridor that supports the spread of fruit fly within crops (Midgarden & Lira, 2006; Sciarretta & Trematerra, 2011; Leblanc *et al*., 2012; Pimentel *et al*., 2014).

The fruit fly's spatial distribution in the initial evaluations in Area 2 in the 2013/2014 crop season did not present an entry site or a directed movement in the area, probably due to the surrounding environment being predominantly occupied by vineyards (Figure 1 and 3). In the 01/17 evaluation, were observed fruit fly's infestation foci distributed inside and on the edges (Figure 3), where it was located a variety 'Isabel' vineyard. Starting in February, it was observed that the entry of the fruit fly by this border was intensified. After this period, fruit fly cap-

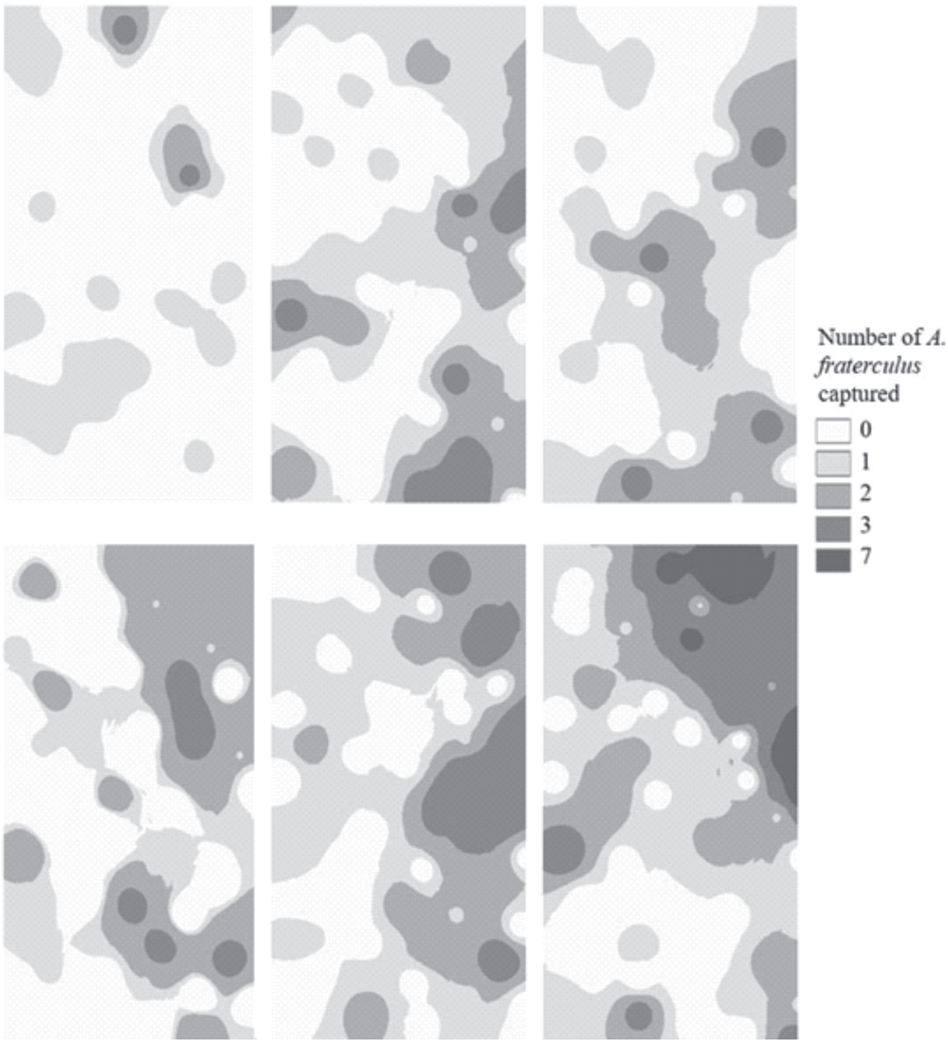

Figure 2: Thematic maps are indicating the spatial and temporal distribution of *Anastrepha fraterculus* in grape *Vitis vinifera* L. variety 'Moscato Branco', in Area 1. 2013/ 2014 Crop season. Note: letters from A to F represent dates of evaluations. A = January 3; B = January 7; C = January 31; D = February 14; E = February 27 and, F = March 14, 2014.

tures increased gradually, coinciding with the fruit ripening, when the fruit fly population was distributed spatially through almost the entire area (Figure 3).

In the region of this study, the grapevine variety 'Isabel' fruits maturation process starts in January, and the harvest starts at the end of February (Camargo, 2004). One of the hypotheses is that this crop, when in the process of maturation, released compounds attracting fruit fly adults that were found in the native forest near the study area. Since these insects did not find adequate oviposition and feeding substrates in this place, they migrated to the area under evaluation. Zart *et al*. (2011) found that in variety 'Isabel', South American fruit fly larvae did not complete the development, being this an inadequate substrate, whereas larvae of this insect were able to form galleries and to develop in the variety 'Moscato Embrapa'.

In the first evaluations of the 2014/2015 crop season in Area 2, it was observed that the distribution of the species occurred initially (11/28) on the vineyard's edge, near the native forest (Figure 1 and 5).

In sequence, the number of insects found in the area reduced about the first evaluation and was distributed by some points of the area, individually, not assuming a characteristic pattern. In February (02/06), adults of the fruit fly begin to invade the area through the edges, two with native forest and one with a vineyard. On the next evaluation (02/23), when the variety 'Moscato Branco' harvest was carried out, the insect population was already distributed throughout the area.

Regarding the two evaluated areas, although close and with similar characteristics by the presence of native forest and fruit fly hosts (Table 2). It should be noted, however, the presence of orange trees in Area 2 can act as a natural repository of fruit flies, especially in a period of low availability of host fruits and that coincides with the beginning of the orange/citrus harvest (Garcia & Norrbom, 2011; Nunes *et al*., 2012; Bortoli *et al*., 2016). Another factor that may have affected insect density and distribution of the South American fruit fly was the size of areas so that Area 2 (1.09 ha) was about 2.3x bigger than Area 1 (0.47

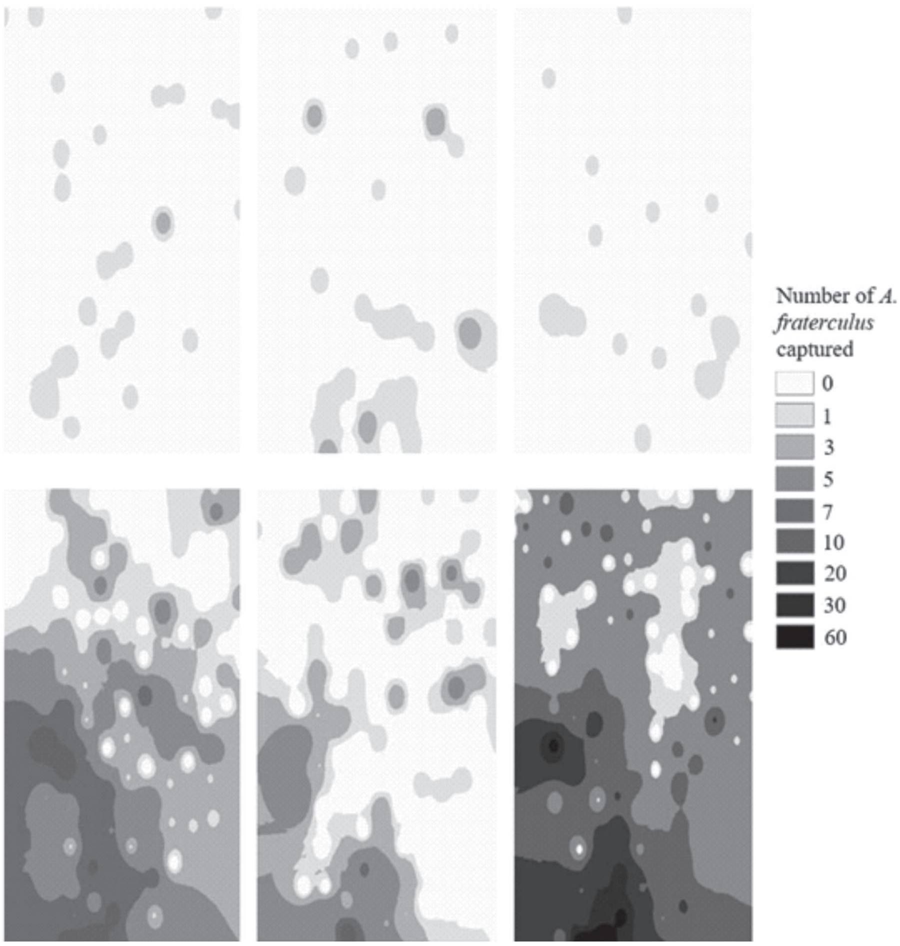

Figure 3: Thematic maps are indicating the spatial and temporal distribution of *Anastrepha fraterculus* in grape *Vitis vinifera* L. variety 'Moscato Branco', in Area 2. 2013/ 2014 Crop season. Note: letters from A to F represent dates of evaluations. A = January 3; B = January 7; C = January 31; D = February 14; E = February 27 and, F = March 14, 2014.

ha), and the first had a higher number of adult fruit flies captured than in Area 1.

Typically, studies of the spatial and temporal distribution of the fruit fly are carried out in large areas (Sciarretta & Trematerra, 2011). The present study is the first one carried out with this objective in the region of Serra Gaucha, characterized by the production of fruits in small rural properties. In the two experimental areas, both small, the largest catches were obtained at the edges of vineyards. This result in a sharp border effect, very difficult to be minimized under these conditions and that may have favored the occurrence of PNE.

In the hypothesis, the highest catch rates in Area 2 are explained by the greater total number of traps in the area

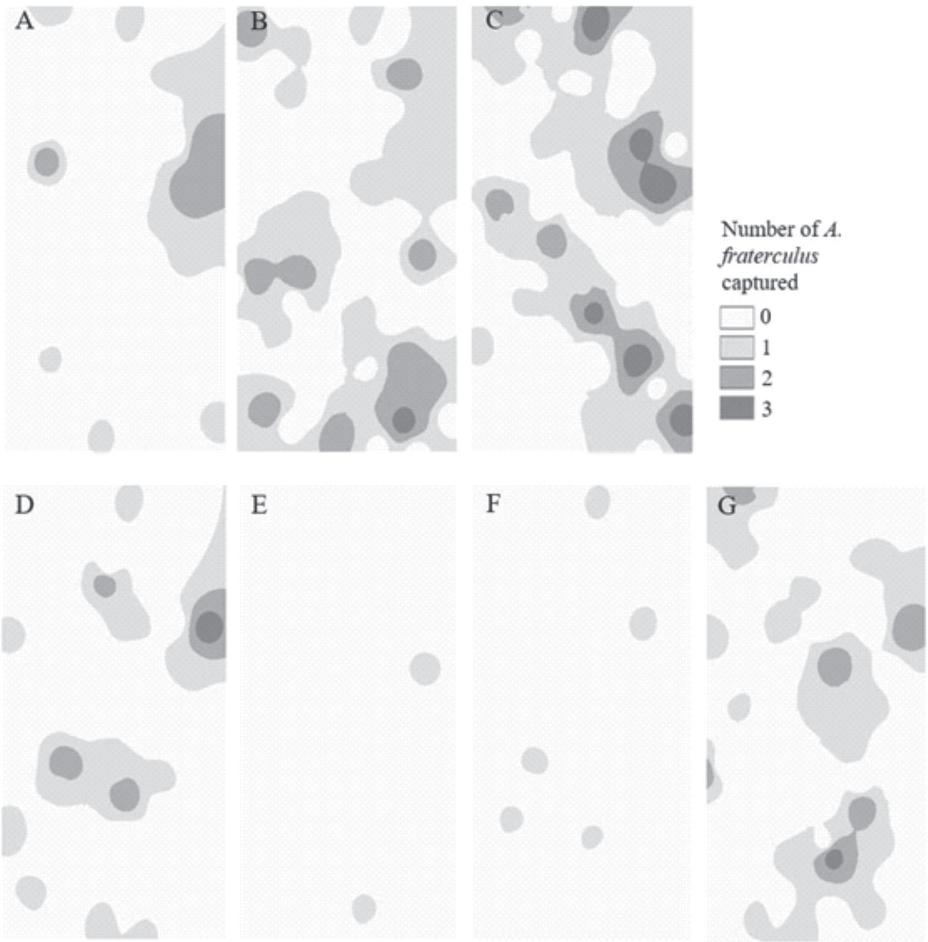

Figure 4: Thematic maps are indicating the spatial and temporal distribution of *Anastrepha fraterculus* in grape *Vitis vinifera* L. variety 'Moscato Branco', in Area 1. 2014/ 2015 Crop season. Note: letters from A to G represent dates of evaluations. A = November 28; B = December 12; C = December 22, 2014; D = January 9; E = January 23; F = February 6; and G = February 23, 2015.

Table 2: List of hosts of *Anastrepha fraterculus* near two commercial vineyards of *Vitis vinifera* L. variety 'Moscato Branco'– Area 1 and 2 – and its fruiting period. Farroupilha, RS, Brazil. Crop seasons 2013/2014 and 2014/2015

Area	Fruit fly hosts	Fruiting period
1	*Eugenia uniflora* L. (Myrtaceae)	February – March
	Eriobotrya japonica (Thumb.) Lindley (Rosaceae)	July – September
	Prunus persica L. Batsch (Rosaceae)	October – February
	Vitis spp. L. (Vitaceae)	January – February
2	*Eugenia uniflora* L. (Myrtaceae)	February – March
	Eriobotrya japonica (Thumb.) Lindley (Rosaceae)	July – September
	Vitis spp. L. (Vitaceae)	January – February
	Citrus sinensis (L.) Osbeck (Rutaceae)	July – November

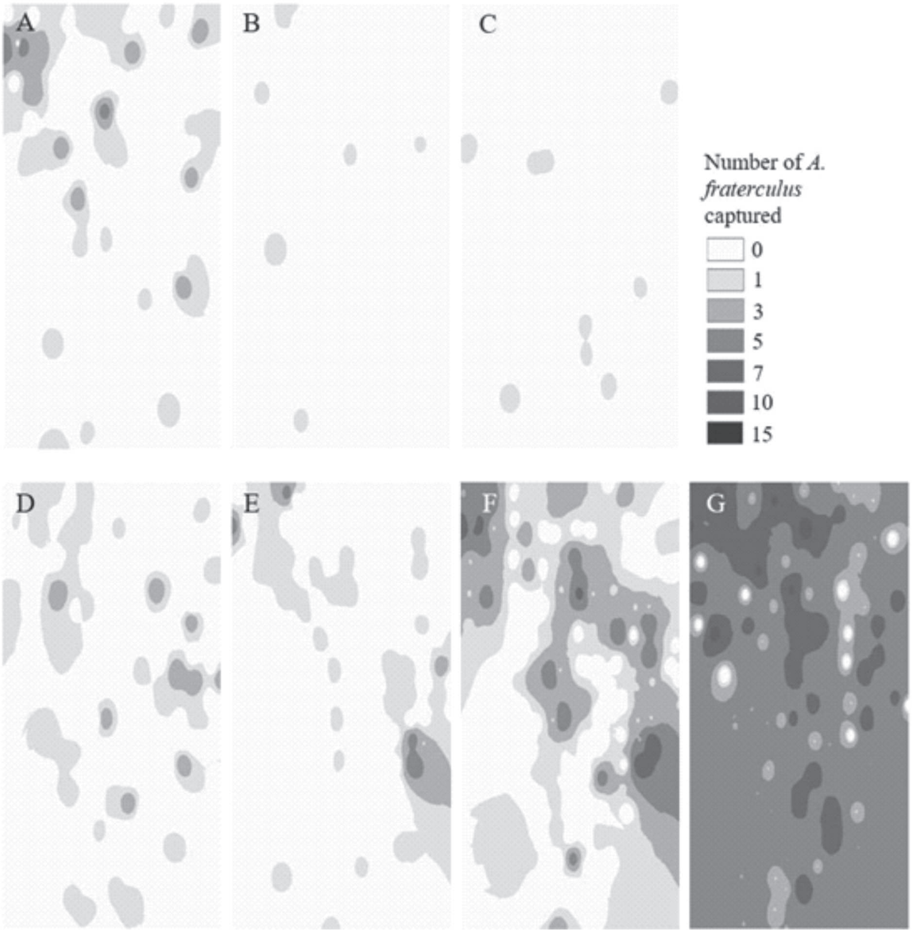

Figure 5: Thematic maps are indicating the spatial and temporal distribution of *Anastrepha fraterculus* in grape *Vitis vinifera* L. variety 'Moscato Branco', in Area 2. 2014/ 2015 Crop season. Note: letters from A to G represent dates of evaluations. A = November 28; B = December 12; C = December 22, 2014; D = January 9; E = January 23; F = February 6; and G = February 23, 2015.

and the greater number of host plants attractive for the fruit flies located at the edges (Kovaleski *et al*., 1999; Manoukis *et al*., 2014). These conditions generated a removal effect, causing the catches to occur in the traps closest to the edge, reducing the number of insects as they moved away from the edge. This fact proves that the incursion of *A. fraterculus* in the vineyards occurs mainly in the period of ripening fruit stage with insects coming from outside to inside the area, as previously reported in apple orchards (Kovaleski *et al*., 1999). Understanding this behavior is fundamental to determine the right moment to install mass trapping traps and use control strategies.

CONCLUSIONS

1. Thematic maps that use the inverse square of the distance allow to showing the spatial distribution of *A. fraterculus* in grapevine in small areas.

2. Adults of *A. fraterculus* shows spatial distribution located in foci with invasive behavior in the vineyard, with its entrance from the edges to the center.

3. *A. fraterculus* temporal distribution is related to the maturation of the fruits in the vineyard when there is an increase in its population in the areas.

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