SPATIAL PATTERNS AND ASSOCIATIONS OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) AND ITS PARASITOID *DORYCTOBRACON AREOLATUS* (HYMENOPTERA: BRACONIDAE) IN ORGANIC ORCHARDS OF *PSIDIUM GUAJAVA* AND *ACCA SELLOWIANA*

Simone Mundstock Jahnke*, Emerson Medeiros Del Ponte, Luiza Rodrigues Redaelli
and Diogo Ricardo Goulart Pereira Rego

Programa de Pós-graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, 91540-000, Porto Alegre, Brazil

*Corresponding author; E-mail: mundstock.jahnke@ufrgs.br

ABSTRACT

The objective of this study was to determine the spatial patterns and associations of the South American fruit fly (*Anastrepha fraterculus* (Wiedemann); Diptera: Tephritidae) and its parasitoids in organic orchards of common guava and pineapple guava. The field study was conducted from Feb to Mar 2010 in 2 organically-managed orchards, one of common guava (*Psidium guajava* L.) (Myrtaceae) and the other of pineapple guava [*Acca sellowiana* (O. Berg) Burret] (Myrtaceae), in Rio Grande do Sul (RS) state, Brazil. Fruits were sampled from all trees at 2 sampling occasions, spaced 15 days apart, just before fruits were in the final maturation period. On each tree, 10 fruits were randomly collected from the entire canopy and held in the laboratory until the flies had pupated. Heterogeneity of insect count data was analyzed by fitting theoretical distributions to the data and calculating dispersion indices. The spatial arrangement was evaluated with SADIE. Local spatial associations were measured using a SADIE association index (I_t^a). In a common guava orchard the emerged individuals were *A. fraterculus*, and its parasitoid, *Doryctobracon areolatus* (Szépligeti) (Par) (Hymenoptera: Braconidae) with a parasitism rate of 8.3%. The same 2 species occurred in a pineapple guava orchard, where the parasitism rate was 25.5%. The variances of the data for both the *A. fraterculus* and *D. areolatus* were greater than the corresponding means for most cases, hence the significance of both the dispersion index (I) and the values of k of the negative binomial suggested an aggregated distribution pattern. On the other hand, the clustering indices (v_i and v_j) and I_t^a suggested a random spatial pattern of *A. fraterculus* and the parasitoid for most situations (orchards and sampling times). Spatial association indices revealed significant associations for 5 of the 12 pair-wise comparisons, 3 in the common guava orchard and 2 in the pineapple guava orchard.

Key Words: spatial distribution, *Anastrepha* sp., parasitism, Myrtaceae

RESUMO

O objetivo do estudo foi determinar os padrões espaciais e associações das moscas-das-frutas e seus parasitoides em pomares de goiaba comum e goiaba-serrana. O estudo foi realizado entre fevereiro e março de 2010, em um pomar de goiaba (*Psidium guajava* L.) (Myrtaceae) e outro de goiaba-serrana [*Acca sellowiana* (O. Berg) Burret] (Myrtaceae), no Rio Grande do Sul, Brasil. Foram amostradas todas as árvores em duas ocasiões de amostragem, espaçadas em torno de 15 dias, no período final de maturação. Em cada árvore, dez frutos foram coletados aleatoriamente da copa e mantidos em laboratório até a formação dos pupários das moscas. A heterogeneidade dos dados de contagem de insetos foi ajustada às distribuições teóricas e índices de dispersão. O arranjo espacial foi avaliado com SADIE. A associação espacial foi medida utilizando um índice de associação SADIE (I_t^a). No pomar de goiabas as moscas emergidas foram da espécie *Anastrepha fraterculus* (Wied.) (SAFF) e os parasitoides, *Doryctobracon areolatus* (Szépligeti) (Par) (Hymenoptera: Braconidae) com uma taxa de parasitismo de 8,3%. Em goiaba-serrana, ocorreram as mesmas espécies com uma taxa de parasitismo de 25,5%. A variância dos dados, para tando a mosca-das-frutas SAFF e *D. areolatus* foi mais elevada do que a média para a maioria dos casos, apontada pelo índice de dispersão (I) e os valores de k da binomial negativa, sugerindo um padrão agregado de distribuição. Por outro lado, os índices de agrupamento (v_i e v_j) e I_t^a sugeriram um padrão espacial aleatório da mosca-das-frutas (SAFF) e do parasitoide (Par) para a maioria das situações (pomar e época de amostragem). Os índices de associação espacial revelaram associações significativas em cinco das doze comparações de pares, sendo três no pomar de goiaba e dois no pomar de goiaba-serrana.

Palavras Chave: distribuição espacial, *Anastrepha* sp., parasitismo, Myrtaceae

In economic entomology and insect ecology, studies on spatial patterns have long been based on the fit of insect count data from quadrat-based sampling to known theoretical distributions, such as Poisson or Negative Binomial (Elliott 1983; Krebs 2000) as well as on the calculation of several indices based on the mean and variance of quadrat counts (Elliott 1983). Although these analyses are useful to depict spatial distribution patterns of insect populations, they are actually a measure of dispersion (not really pattern measurements) because their arrangement in relation to one another is unknown, and so variations within the region cannot be recognized.

Several methods that take into account the relative location of sampling sites have been developed for depicting spatial patterns and a few examples include spatial analysis by distance indices (SADIE) (Perry 1995), methods based on quadrat variance, spatial autocorrelation, distance based joint-counts/network method and geostatistics (Perry et al. 2002).

Studies on spatial patterns of Tephritidae are scarce and are mostly based on trap counts. Examples include spatial patterns of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) using large grid of traps with trimedlure (Israely et al. 1997), spatial distribution of adults of *Bactrocera oleae* (Gmelin) in olive orchards using food attractant-based traps (Dimou et al. 2003), spatial and temporal patterns of *B. dorsalis* (Hendel) adults captured in traps and described by indices of dispersion and regression models (Soemargono et al. 2011), and spatial patterns of released sterile males in IPM programs (Economopoulos & Mavrikakis 2002; Meats 2007). Most of those studies showed different levels of aggregation of the pest influenced by the attraction of the traps, and spatial patterns influenced by oviposition driven by substrate choice are not well understood.

Nevertheless, knowledge on the spatial distribution of insect larvae in fruit is useful when developing an IPM strategy that includes definition of where to locate traps or toxic baits, to release natural enemies or to define sequential sampling plans (Norris et al. 2003). Nestel et al. (2004), for example, studied the spatial patterns of fruit flies using a geographical information system to visualize hot-spots of damage and make decisions on augmentation of mass-trapping in specific area of the agricultural landscape.

Anastrepha fraterculus (Wied.) (Diptera: Tephritidae), the South American fruit fly (SAFF), is an important native pest of commercial fruit crops and native fruit trees (Kovaleski et al. 2000) in South America, and it is especially associated with Myrtaceae host species (Malavasi & Zuchi 2002) migrating from one host plant to another throughout the year (Salles 1996). In many other species of Tephritidae, the females oviposit in maturing and ripening fruit, and the larvae hatch inside the

fruit where they complete their development. As the fruit ripens and rots, it falls to the ground, the fully mature larvae leave the fruit and burrow into the soil to pupate (Salles 2000). *Anastrepha fraterculus* may cause serious crop injury and economic losses when conditions are favorable for pest development. Chemical pesticide options are available and frequently used in conventional production (Carvalho et al. 2000), but pesticide use is under governmental regulation to minimize pesticide levels on fruit. Obviously synthetic insecticides are not an option for producers targeting organic fruit markets.

The presence of native host trees, like common guava and others Myrtaceae, nearby commercial fruit orchards may increase the population size of *A. fraterculus* (Kovaleski et al. 1999). Among the most frequent native parasitoids, *Doryctobracon areolatus* (Szépligeti) (Par) (Hymenoptera: Braconidae), parasitizes larvae of first and second instars of several Tephritidae species (Nunes et al. 2011).

The availability of parasitoids that recognize and attack a wide range of pests of several crops is the most desirable tool in a biological control program (Carvalho et al. 2000). The parasitism rate is influenced by many factors, and, in the case of fruit flies, the spatial arrangement of hosts is considered a key factor (Salles 1996). Knowledge of the spatial distribution and patterns of both insect pests and their natural enemies may be used as an indicator of parasitoid effectiveness (Denno et al. 2011) and provide critical information for implementing an IPM-based strategy (Barbosa & Perecin 1982). Moreover, it allows the acquisition of an understanding of the underlying processes, such as responses to environmental heterogeneity or intraspecific and interspecific interactions, i.e., competition, predation and reproduction (Thomas et al. 2001).

In this study, we hypothesized that 1) fruit fly larvae show an aggregate spatial pattern driven by various factors such as nutritional status of fruit, insolation and presence of native hosts and that 2) fruit fly parasitoids, whenever present, show a spatial pattern similar to their hosts. The objective of this study was to determine the spatial patterns and associations of *A. fraterculus* and its parasitoid *D. areolatus* in organic orchards of common guava (*Psidium guajava* L.) (Myrtaceae) and pineapple guava [*Acca sellowiana* (O. Berg) Burret] (Myrtaceae). Also we tried to deduce the underlying mechanisms leading to the observed patterns and the implications for insect management.

MATERIALS AND METHODS

Study Area

The field study was conducted from Feb to Mar 2010 in 2 relatively small organically-man-

aged orchards in Rio Grande do Sul state, Brazil. One orchard consisted of common guava and the other consisted of pineapple guava, also known as feijoa. The common guava orchard had 4 rows with 5 trees and 2 rows with 4 trees, totaling 28 trees. They were spaced 3 m between rows and 2 m within row. It was located in the experimental area of the Centro Agrícola Demonstrativo, Porto Alegre (S 30° 07' -W 51° 05'). The pineapple guava orchard had 3 rows with 11 trees, totaling 33 trees. They were spaced 2 m between rows and 3 m within row. This orchard was located in the fruit production region of Montenegro (S 29° 40' W 51° 32').

Sampling and Insect Identification

In each orchard, fruit we sampled from all trees at 2 sampling occasions spaced 15 days apart, just before fruits were in the final maturation period. At the common guava orchard, fruit were sampled on Mar 6 and Mar 18 and at the pineapple guava orchard, on Feb 26 and Mar 11. On each tree, 10 fruit were randomly collected from the entire canopy and taken to the laboratory where fruit were individually rinsed with a 0.03% hypochlorite solution, and placed in a plastic jar (1 L) filled with 10 g of sand. The jars were kept in a growth chamber at room temperature until pupation.

After 12 to 24 days of incubation, the jars were opened and the sand was visually inspected. All tephritid pupae detected were transferred into glass containers (100 mL) containing wet sterilized sand on the bottom where the pupae were kept until emergence. The emerged flies and parasitoids were identified based on morphology (Malavasi & Zucchi 1999) and the non-emergent pupae were dissected. The number of pupae, flies and parasitoids that emerged or that were identified during dissection were counted. The unidentified pupae were discarded. Total parasitism was considered in this study. All tephritid pupae detected were transferred into glass containers until emergence. The emerged flies and parasitoids were identified based on morphology and the unclosed pupae were dissected.

Spatial Distribution and Pattern Analyses

Heterogeneity Analyses

Heterogeneity of insect count data were analyzed by fitting the data to theoretical distributions, and by calculation of dispersion indices for each orchard and assessment time. The Poisson and negative binomial distributions were fitted to the count data. Chi-square tests were used to test the goodness-of-fit of the observed distributions. For the negative binomial distribution, the dispersion parameter (k) was estimated. For characterizing dispersion of the data, the dispersion index (I), also known as variance-to-mean ratio, was used to measure the dispersion of a probability distribution (Krebs 2000). Also, the Morisita's index (I_δ) was calculated (Elliott 1983) because all sampling units had an equal number of samples.

Correlation-Type Analysis

The spatial arrangement of fruit fly and parasitoids was evaluated with SADIE (Perry 1995). SADIE takes into account the location of the trees and the counts of individuals per tree to evaluate the spatial arrangement of the individuals. Similar to a correlation-type analysis, SADIE results reflect the spatial arrangement of the insects at the sampling unit level (tree) and above, but depend on the pre-existing level of heterogeneity in the data set. In SADIE, the distance to regularity (D_r) is the minimum total distance that an individual would need to move to achieve the same number in each tree. The degree of non-randomness within a set of data is quantified by comparing the observed spatial pattern with rearrangements obtained after random permutations of the individuals among the quadrats. The proportion (P_a) of randomized samples with distance to regularity as large as or larger than the observed value D_r, can be used for a one-sided test of spatial aggregation (at the significance level of 5%). An overall index of aggregation is given by $I_a = D_r/E_a$ where D_r is the distance to regularity for the observed data, and E_a is the mean distance to regularity of the randomized samples $I_a > 1$ indicates an aggregated pattern.

The organization of clusters into patches (neighborhoods of units with counts larger than the average density m) or gaps (neighborhoods of units with counts $< m$) was analyzed by mapping clustering indices attributed to each sampling unit, i.e., tree (Perry et al. 1999). The index v_i measures the degree to which the unit contributes to a patch, whereas v_j is defined similarly but for a gap and by convention takes a negative value. As a general rule, large v_i values were those > 1.5 and small v_j values those < 1.5, where these values pertain to members of a patch or gap, respectively. A more formal test of degree of clustering was provided by comparing the average values of v_i and v_j with their corresponding values for randomization. Contour maps were drawn from these values provided by SADIE. For each situation (orchard × sampling date) SADIE analysis was performed with a total of 2,028 randomizations.

Spatial Association Analysis

Two populations may be positively associated spatially, disassociated or occur randomly with

respect to each other. In this study, local spatial association was measured using the SADIE association index (I_t^a), which is based on similarities between the clustering indices of 2 populations measured at the kth sample unit (Perry & Dixon 2002). A coincidence of 2 patches or 2 gaps indicates a positive value of I_t^a (association), while a negative association (dissociation) results from a patch coinciding with a gap in both populations. The overall spatial association P_a was calculated as the mean of local values of the 2 populations. The significance of I_t^a was tested by randomization, with values reassigned among sample units, after a small-scale autocorrelation in cluster indices from either population. At the 5% level, the statistic $P < 0.05$ indicated significant association while $P \geq 0.975$ indicated significant disassociation. A total of 6 pair-wise spatial associations were analyzed, for each orchard, considering: fruit flies and parasitoids in the same or different sampling occasions; fruit flies at 2 sampling occasions and parasitoids at the 2 sampling occasions.

RESULTS

The data on the numbers of *A. fraterculus* fruit flies and *D. areolatus* parasitoids that emerged, densities of fruit infestation and parasitism rates are presented in Table 1.

Distribution Fitting Analysis

For both plant hosts, the mean counts of the *A. fraterculus* and *D. areolatus* were always greater at the first sampling time than at the second sampling time. The highest mean count of *A. fraterculus* per tree was found in the common guava orchard, which was about 3 times greater than the number found in the first sampling in the pineapple guava (Table 2). *Doryctobracon areolatus* was found to have similar mean counts per tree in both orchards and sampling times.

The variances of the data for both the *A. fraterculus* and *D. areolatus* were greater than the means for most cases, hence the significance of both the dispersion index (I) and the values

for the dispersion parameter (k) of the negative binomial suggested an aggregated distribution pattern of the organisms among trees. Likewise, the Morisita index (I_δ) was significantly greater than 1.0, suggesting a high degree of aggregation of counts for both *A. fraterculus* and its parasitoid at the tree level (Table 2). Among the situations, the highest heterogeneity of the dispersion index ($I > 25$) was found for *D. areolatus* in the common guava orchard, second sampling time, and for *A. fraterculus* in the pineapple guava orchard, first sampling time (Table 2). The lowest heterogeneity ($I < 2$ and $k > 0.2$) was detected for *D. areolatus* counts at the common guava orchard, second sampling time (Table 2).

Spatial Patterns

Spatial analysis based on distance indices showed patterns that did not fully agree with patterns defined by the distribution fitting analysis. The aggregation statistics (I_a) and clustering indices (v_i and v_j) suggested a random spatial pattern of *A. fraterculus* and its parasitoid for most situations (orchard and sampling times) (Table 3). The exception was the distribution of *A. fraterculus* data from the second sampling date at the common guava orchard, which showed a marginal evidence of aggregation at the 5% level for the aggregation statistics ($I_a = 1.35$, $P = 0.032$); this situation was the one with the highest evidence of aggregation detected by the heterogeneity analysis (Table 3). The clustering maps for each of the 4 situations clearly depicted low numbers of patches and gaps, ranging from 1 to 3 relatively small patches or gaps (Figs. 1 and 2). Higher numbers of large areas of patches and gaps were found for the fruit fly data at the second sampling date at the common guava orchard (Fig. 1B), where the fly's aggregation was detected.

Spatial Associations between *A. fraterculus* and *D. areolatus*

Spatial association indices revealed significant associations for 5 of the 12 pair-wise com-

TABLE 1. NUMBER OF COLLECTED FRUITS OF COMMON GUAVA (CG, *PSIDIUM GUAJAVA*) AND PINEAPPLE GUAVA (PG, *ACCA SELLOWIANA*), NUMBER OF FRUIT-FLY PUPAE, MEAN (±SEM) NUMBER OF PUPAE PER FRUIT, NUMBER OF EMERGED *ANASTREPHA FRATERCULUS*), NUMBER OF EMERGED *DORYCTOBRACON AREOLATUS* AND PARASITISM RATE IN ORCHARD. BOTH ORCHARDS WERE LOCATED IN RIO GRANDE DO SUL STATE, BRAZIL AND SAMPLED IN 2010.

Fruits	Number of fruits	Number of Pupae	Mean pupae/fruit ± SEM	Number of Emerged SAFF	Number of Emerged Parasitoids	Parasitism rate
CG	580	3,671	12.6 ± 0.23	2,638	239	8.3%
PG	1,360	3,531	5.2 ± 0.05	1,686	578	25.5%

SAFF is the South American Fruit fly, *Anastrepha fraterculus*.
CG is common guava (*Psidium guajava*), and PG is pineapple guava (*Acca sellowiana*).

TABLE 2. STATISTICS FOR HETEROGENEITY ANALYSIS OF THE COUNT DATA ON *ANASTREPHA FRATERCULUS* AND THE AS-SOCIATED PARASITOID, *DORYCTOBRACON AREOLATUS,* COLLECTED IN TREES OF A COMMON GUAVA ORCHARD AND IN TREES OF A PINEAPPLE GUAVA ORCHARD, BOTH LOCATED IN RIO GRANDE DO SUL STATE, BRAZIL, AND SAMPLED IN 2010.

				Heterogeneity analysis		
Plant	Insect	Date	Mean insects/tree	I[a]*	k[b]	I_δ[c]
CG	SAFF	6 Mar	76.3	11.8	0.007	1.14
		18Mar	16.5	31.5	0.001	2.78
	Par	6 Mar	6.0	1.9	0.262	1.16
		18 Mar	3.4	3.7	0.005	4.61
PG	SAFF	26 Feb	23.1	28.6	0.001	2.16
		11 Mar	10.7	14.38	0.005	2.21
	Par	26 Feb	6.5	7.7	0.016	2.01
		11 Mar	3.8	10.4	0.009	3.38

SAFF is the South American Fruit fly, *Anastrepha fraterculus*. Par is the parasitoid.
CG is common guava (*Psidium guajava*), and PG is pineapple guava (*Acca sellowiana*).
[a]Index of dispersion where * indicates significance of Chi-square test ($\sqrt{\chi^2}$),
[b]k values of fit of the data to a Negative Binomial distribution
[c]Morisita Index (I_δ)

parisons in both orchards, 3 in the common guava orchard and 2 in the pineapple guava orchard (Table 4). In general, significant spatial associations were detected between *A. fraterculus* and *D. areolatus*. Particularly, in the common guava orchard, the strongest evidence of spatial association ($I_a > 0.62$, $P < 0.001$) was found between *A. fraterculus* and *D. areolatus* at the second sampling date. The clustering maps show that patches and gaps for each species were more coincident in space (Figs. 1B and 1D). Modest evidence of significant spatial associations ($P = 0.04$ to 0.05) were found between the *D. areolatus* counts at the first and second sampling dates and the *A. fraterculus* and *D. areolatus* counts at the first sampling date (Table 4). In the pineapple guava orchard, evidence was strong for 2 significant spatial associations between *A. fraterculus* and *D. areolatus*, i.e., at both the first and the second sampling date. The clustering maps show that patches and gaps for both species were coincident in space for both the first (Figs. 2A & C) and the second sampling date (Figs. 2 B and D). However, the associations between these 2 insect species at different sampling dates were not significant.

TABLE 3. SADIE STATISTICS FOR THE SPATIAL ANALYSIS OF COUNTS OF *ANASTREPHA FRATERCULUS* AND THE ASSOCIAT-ED PARASITOID, *DORYCTOBRACON AREOLATUS*, ASSESSED OVER 2 CONSECUTIVE ASSESSMENT TIMES IN 2010 ON TREES IN A COMMON GUAVA ORCHARD AND ON TREES IN A PINEAPPLE GUAVA ORCHARD, BOTH LOCATED IN RIO GRANDE DO SUL STATE, BRAZIL.

			Index of aggregation[a]		Index of clustering[b]			
Plant	Insect	Date	I_a	P_a	v_i	v_j	$P(v_i)$	$P(v_j)$
CG	SAFF	6/3	1.001	0.426	0.9610	-0.9220	0.527	0.631
		18/3	**1.352**	0.032	1.2640	**-1.3370**	0.053	0.034
	Par	6/3	0.950	0.553	1.0020	-0.8930	0.422	0.729
		18/3	1.103	0.233	1.223	-1.0764	0.092	0.284
PG	SAFF	26/2	1.056	0.327	1.0050	-1.0300	0.375	0.362
		11/3	0.890	0.553	1.0500	0.084	0.315	0.653
	Par	26/2	1.001	0.388	0.8500	-0.9260	0.656	0.488
		11/3	1.214	0.199	1.185	-1.12	0.200	0.274

SAFF is the South American Fruit fly, *Anastrepha fraterculus*. Par is the parasitoid.
CG is common guava (*Psidium guajava*), and PG is pineapple guava (*Acca sellowiana*).
[a]I_a is the overall index of aggregation and P_a is the proportion of the 2028 random cases larger than D_r. Significant aggregation (**bold numbers**) is indicated when $P_a < 0.05$
[b]vj and vi correspond to the average values of the indices of clustering v_I (patch) and v_J (gap). P values correspond to the proportion of randomized I or J that exceed the observed values. Significant clustering is indicated when P < 0.05.
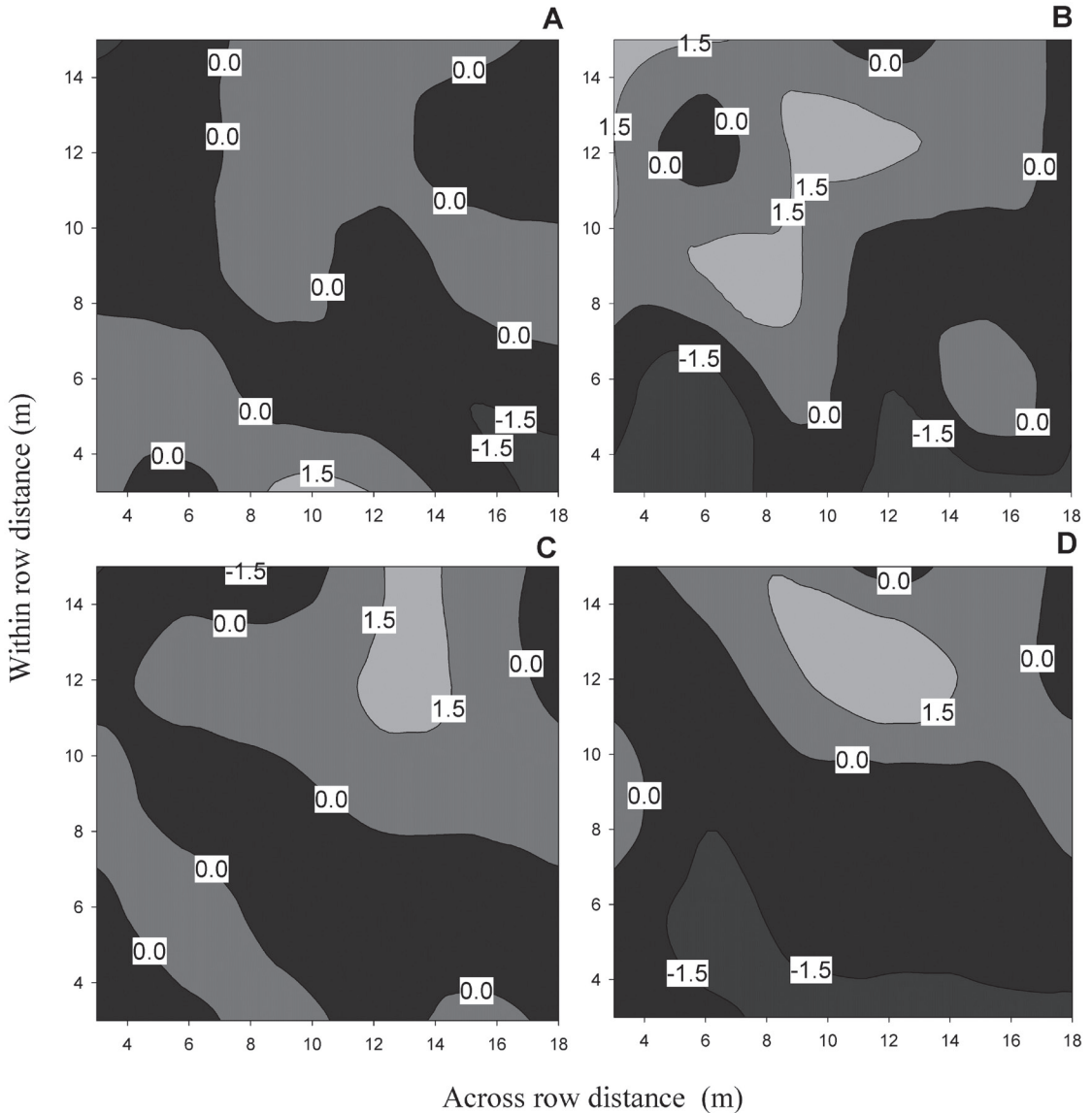
Fig. 1. Maps of clustering indices estimated using the spatial analysis of distance indices (SADIE) for *Anastrepha fraterculus* counts assessed on Mar 6 (A) and Mar 18 (B), and its parasitoid, *Doryctobracon areolatus*, counts assessed on Mar 6 (C) and Mar 18 (D) in a common guava (*Psidium guajava*) orchard. Axes show distance in sampling units (trees) with y-axis in the direction of rows of the trees. Areas within white contours (≥ 1.5) indicate strong clustering as patches, those within dark gray contours (≤ -1.5) strong clustering as gaps in distribution.

DISCUSSION

Among the Tephritidae species reported in fruit orchards in Rio Grande do Sul state, *A. fraterculus* is the dominant and often the only species occurring on Myrtaceae (Reyes et al. 2012). The parasitism rate observed in our study for these 2 species of fruit crops is similar to that in another study in the same geographic region (Nunes et al.

2011), and to our previous observations in common guava and pineapple guava (Pereira-Rego et al. 2013).

Heterogeneity analysis suggested that the *A. fraterculus* population was aggregated at the sampling unit level, here an individual tree, agreeing with other studies using insect trap data. Females of different species of *Anastrepha* and other fruit fly species were aggregatedly distributed in
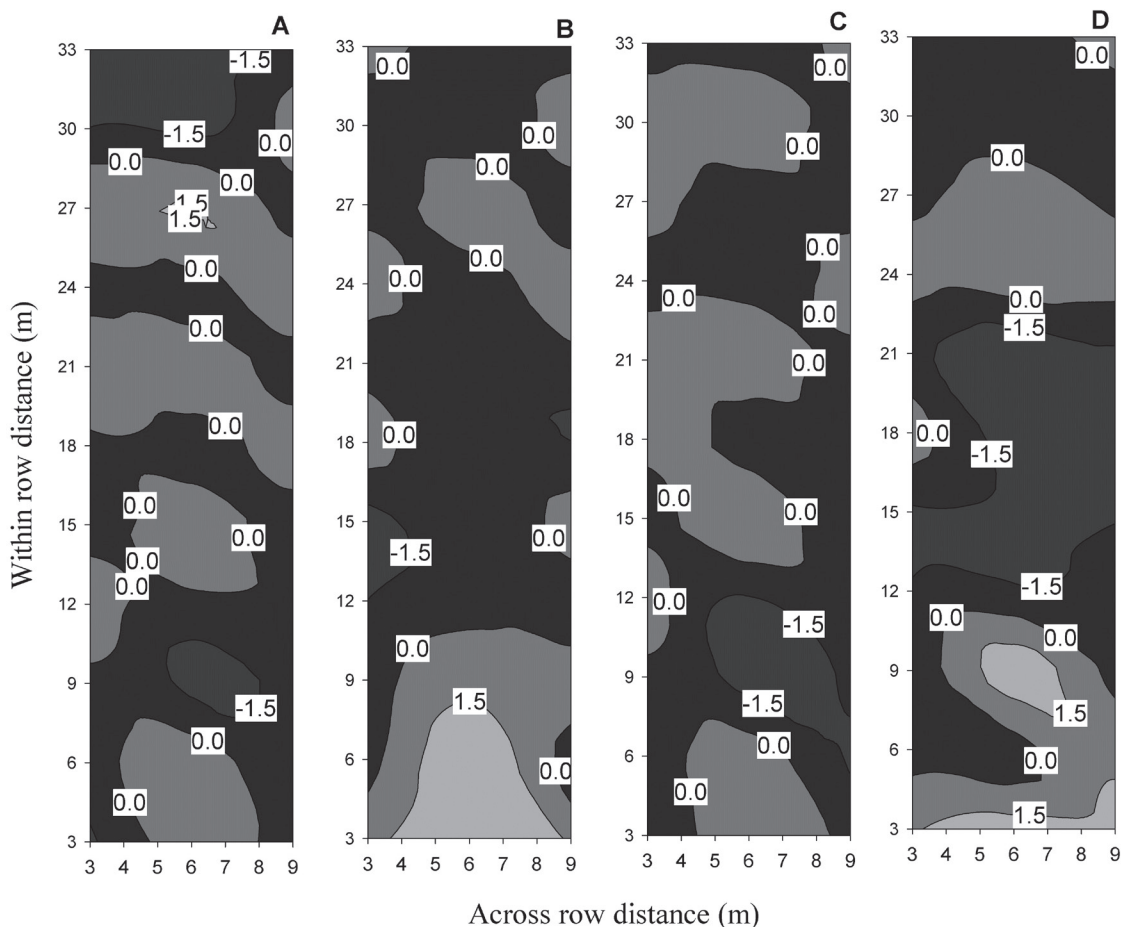
Fig. 2. Maps of clustering indices estimated using the spatial analysis of distance indices (SADIE) for *Anastrepha fraterculus* counts assessed on Mar 6 (A) and Mar 18 (B) and for its parasitoid, *Doryctobracon areolatus*, counts assessed on Mar 6 (C) and Mar 18 (D) in a pineapple guava (*Acca sellowiana*) orchard. Axes show distance in sampling units (trees) with y-axis in the direction of rows of the trees. Areas within white contours (≥ 1.5) indicate strong clustering as patches, those within dark gray contours (≤ -1.5) strong clustering as gaps in distribution.

traps placed in an orchard with numerous host tree species (Uramoto et al. 2005). Aggregation was also reported for *Bactrocera* species affecting several crops (Dimou et al. 2003; Soemargono et al. 2011). The attraction of the traps may lead to overestimating the size of local populations due to migration of insects attracted from adjacent areas (Jenkins et al. 2011). Count data of larvae from infested fruit, as conducted in our study, may more realistically provide evidence of search and host selection behavior of the female fruit fly.

Based on SADIE analysis, which takes into account the position of the trees, the patterns of both *A. fraterculus* and *D. areolatus* were mostly random in the 2 orchards and at various sampling dates. This means that trees with high or low insect counts were not concentrated at specific regions within the orchard, thus not forming

significantly large patches or gaps, as depicted by the clustering indices. The only exception was at the second sampling of *A. fraterculus* in common guava, which may relate to the higher attractiveness of matured fruit or to temporal pest dynamics of the pest that leads to higher numbers of individuals towards the end of the season at specific sites of the orchard (Matrangolo 1998). We hypothesize that the *A. fraterculus* populations become established randomly in sites of the orchard, and tended to build in numbers and to aggregate within a tree due to site-specific factors. Thus, the high heterogeneity in the number of individuals among the trees may be due to specific factors such as number of the initial population infesting the tree and those related to the microenvironments and insect behavioral responses to them (Taylor 1984).

TABLE 4. SPATIAL ASSOCIATION MEASURED BY SPATIAL ANALYSIS OF DISTANCE INDICES (SADIE) FOR PAIRWISE DATA-SETS OF SOUTH AMERICAN FRUIT FLY (SAFF, *ANASTREPHA FRATERCULUS*) AND AN ASSOCIATED PARASITOID (PAR, *DORYCTOBRACON AREOLATUS*) ASSESSED OVER 2 ASSESSMENT TIMES IN TREES OF A COMMON GUAVA (CG, *PSIDIUM GUAJAVA*) ORCHARD AND ON TREES OF PINEAPPLE GUAVA (PG, *ACCA SELLOWIANA*) ORCHARD, BOTH LOCATED IN RIO GRANDE DO SUL STATE, BRAZIL.

		Common guava		Pineapple guava	
Dataset 1	Dataset 2	I_a[a]	*P*-value	I_a	*P*-value
SAFF - 1st date	SAFF - 2nd date	-0.0066	0.51	0.0708	0.36
Par - 1st date	Par - 2nd date	**0.365**	0.042	-0.134	0.77
SAFF - 1st date	Par -1st date	**0.324**	0.048	**0.4838**	0.005
SAFF - 2nd date	Par - 2nd date	**0.624**	0.001	**0.6291**	0.001
SAFF - 1st date	Par - 2nd date	0.1786	0.22	-0.2219	0.89
SAFF - 2nd date	Par - 1st date	0.021	0.4671	-0.0118	0.52

[a]Index of association calculated by SADIE and associated probability for the association (positive values) or dissociation (negative values) ($P < 0.05$). Data are from an individual tree as sampling unit. Bold numbers indicate significant association/dissociation.

It is well known that the heterogeneity of the habitat is the most obvious cause of aggregation (Poole 1974). Abiotic factors such as light, temperature, wind, humidity and other ecological factors such as competition for food, predation and sexual stimuli, may influence the patterns of aggregation of individuals in a population (Farias et al. 2001; Jahnke et al. 2008). In our case, a random spatial arrangement of the insect counts in one tree related to other trees may be related to a combination of factors such as the nutritional status of the plants, the availability of fruits and their nutritional quality, as well as characteristics of the surroundings of these orchards.

This is the first study on spatial patterns of insect pests affecting guava species using SADIE and to document spatial association of *A. fraterculus* and a parasitoid in organic orchards. Other studies using this technique to analyze spatial patterns of pest (Ciccadellidae) and parasitoid count data revealed aggregated patterns, but the key differences between those studies and our study are the much larger sizes of their orchards and their use of insect traps across the areas (Park et al. 2006; Thomson & Hoffmann 2013).

The spatial association of fruit flies and their parasitoids co-occurring at same time reflects both a temporal and spatial synchrony of these species, as previously reported for other pest parasitoid spatial interactions, also using SADIE analysis (Ferguson et al. 2003). This is supporting evidence that a parasitoid tends to follow its host and builds up its population in the same patches as the host (Wajnberg 2006). Such association may also be related to the optimal foraging effect (Nick et al. 2008), which is a key factor for the success of pest suppression by the parasitoid.

An improved understanding of the spatial distribution pattern of *A. fraterculus* and its parasitoid(s) can assist in the optimization of integrated pest management in orchards, both to increase effectiveness of the release of parasitoids for biological control, and to minimize unwanted environmental impacts of chemical control practices.

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