

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Spatio-temporal distribution, diversity and monitoring of fruit flies
(Diptera, Tephritidae) in papaya orchards in the state of Espírito Santo,
Brazil**

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Thesis presented to obtain the degree of Doctor in Science.
Area: Entomology

**Piracicaba
2021**

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Agronomic Engineering

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Tephritidae) in papaya orchards in the state of Espírito Santo, Brazil**

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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DEDICATION

With love

I dedicate

To my dear grandfather, Gabriel Maia Araújo (in memoriam).

Thank you for being the biggest supporter of my dreams and my great friend.

I offer

To my mother Maria Hilda and my brothers Maurício and Mauriane,

who are always with me in all my conquests.

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And to my ears came the voice of everything in heaven and on earth and under the earth and in the sea, and of all things which are in them, saying. To him who is seated on the high seat, and to the Lamb, may blessing and honor and glory and power be given forever and ever.

And the four beasts said, So be it. And the rulers went down on their faces and gave worship.

Revelation 5:13-14

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RESUMO

Distribuição espaço-temporal, diversidade e monitoramento de moscas-das-frutas (Diptera, Tephritidae) em pomares de mamão no estado do Espírito Santo, Brasil

A busca por informações sobre ecologia de moscas-das-frutas de importância econômica (Diptera, Tephritidae) em áreas agrícolas tem crescido nas últimas décadas em razão da biossegurança alimentar. O conhecimento sobre monitoramento a longo prazo desses tefritídeos em ecossistemas agrícolas e a influência de áreas vizinhas, têm sido alvo de investigação por diversos pesquisadores, entretanto, pesquisas que compilam essas informações em um só estudo ainda são escassas. Um sistema de manejo de moscas-das-frutas implementado há mais de 20 anos em pomares de mamão no estado do Espírito Santo, o systems approach, proporcionou a exportação do mamão para os Estados Unidos. Este cenário reúne oportunidades propícias para investigar aspectos comportamentais de moscas-das-frutas no espaço e no tempo. Neste sentido, o objetivo desta pesquisa foi conhecer a distribuição espaço-temporal e como está estruturada a comunidade de moscas-das-frutas em áreas produtoras de mamão no estado do Espírito Santo. Utilizou-se uma série temporal de 13 anos de monitoramento semanal de moscas-das-frutas, de diferentes pontos georreferenciados em cultivos de mamão. O levantamento das espécies foi realizado por meio de armadilhas McPhail e Jackson, utilizando 1 armadilha por hectare nas áreas. O estudo foi dividido em três seções. Na primeira, funções matemáticas, particionamento de diversidade e análise espectral foram utilizadas para descrever a relação espécie-área e padrões de riqueza e abundância de espécies ao longo do tempo, sob influência do systems approach e de diferentes distâncias de uma reserva de Mata Atlântica. A riqueza e a abundância foram influenciadas pelo tempo e pela distância, o particionamento de diversidade indicou mudanças nos valores de riqueza dentro da β -diversidade e a análise espectral projetou tendências de picos. Na segunda seção, análises de regressão e distribuição de frequência foram empregadas para caracterizar os padrões de agregação de três espécies de moscas-das-frutas economicamente importantes para o Brasil, em três áreas com diferentes distâncias da Mata Atlântica e em dois períodos do systems approach. Os resultados mostraram que áreas próximas à Mata Atlântica e o período de impacto do systems approach na abundância proporcionaram maior agregação das espécies. Por último, um modelo probabilístico de coocorrência de espécies foi usado para conhecer os padrões de interações existentes entre as espécies, bem como uma análise de força de interação foi aplicada, para saber o quão fortemente associadas as espécies estão entre si, comparando os diferentes cenários em estudo. Somente associações positivas e aleatórias foram observadas, o maior número de pares de espécies formados foi em pomares mais distantes da Mata Atlântica e no período de implementação contínua do systems approach. A força de interação para associação positiva apresentou leve mudança entre as áreas e ao longo dos anos. Em todas as seções, os resultados descrevem mudança nos padrões populacionais investigados em função do systems approach e influência da Mata Atlântica nas diferentes áreas delimitadas. As informações geradas neste estudo contribuem para o conhecimento sobre ecologia de moscas-das-frutas no Brasil e geram informações que poderão contribuir para a otimização de manejo desses tefritídeos dentro de uma perspectiva de grande área.

Palavras-chave: *Anastrepha*, *Ceratitis capitata*, Série temporal, Diversidade e Coocorrência

ABSTRACT

Spatio-temporal distribution, diversity and monitoring of fruit flies (Diptera, Tephritidae) in papaya orchards in the state of Espírito Santo, Brazil

The search for information on the ecology of fruit flies of economic importance (Diptera, Tephritidae) in agricultural areas has grown in recent decades due to food biosecurity. Knowledge about the long-term monitoring of these tephritids in agricultural ecosystems and the influence of neighboring areas have been the subject of an investigation by several researchers. However, research that compiles this information in only one study is still scarce. A system of management of fruit flies implemented more than 20 years ago in papaya orchards in the state of Espírito Santo, the systems approach, provided the export of papaya to the United States. This scenario provides favorable opportunities to investigate behavioral aspects of fruit flies in space and time. In this sense, the objective of this research was to know the spatio-temporal distribution and the community structure of fruit flies in areas of papaya production in the state of Espírito Santo. We used a time series of 13 years of weekly monitoring of the fruit flies of different georeferenced points in papaya crops. The species survey was carried out using McPhail and Jackson traps, using 1 trap per hectare in the areas. The study was divided into three sections. In the first, mathematical functions, partitioning of the diversity, and spectral analysis were used to describe the species-area relationship and patterns of species richness and abundance over time, under the influence of the systems approach and different distances from an Atlantic Rainforest. Species richness and abundance were influenced by time and distance, partitioning of diversity indicated changes in richness values within β -diversity and spectral analysis projected different peak trends. In the second section, regression and frequency distribution analyzes were used to characterize the aggregation existing patterns of three species of fruit flies economically important for Brazil, in three areas with different distances from the Atlantic Rainforest and in two periods of the systems approach. The results showed that areas near the Atlantic Rainforest and the period of impact of the systems approach on abundance provided greater aggregation of species. Finally, a probabilistic model of species co-occurrence was used to know the patterns of interactions existing between species, as well as an analysis of interaction strength, was applied, to know how strongly associated species are between itself, comparing the different scenarios under study. Only positive and random associations were observed, the largest number of species pairs formed was in orchards more distant from the Atlantic Rainforest and in the period of continuous implementation of the systems approach. The strength of interaction for the positive association has changed slightly between areas and over the years. In all sections, the results describe changes in the population patterns investigated due to the systems approach and the influence of the Atlantic Rainforest in the different delimited areas. The information generated in this study contributes to the knowledge about the ecology of fruit flies in Brazil and generates information that may contribute to the optimization management of these tephritids in a wide area perspective.

Keywords: *Anastrepha*, *Ceratitis capitata*, Time series, Diversity and Co-occurrence

1 GENERAL INTRODUCTION

Fruit flies (Diptera, Tephritidae) are insects of great economic importance for the world fruit trade (Aluja, 1994). These flies cause considerable losses in the commercialization and exportation of fruits, imposing high economic costs in the control and eradication of species (Dias et al., 2018; Enkerlin, 2005). For reasons of biosecurity, strict quarantine restrictions are imposed by importing countries of fruits to prevent the introduction of them, resulting in obstacles to the global fruit trade (Lanzavecchia et al., 2014; Aluja and Mangan, 2008). Brazil has great diversity of species (Zucchi and Moraes, 2012, 2008), of which several species are of economic importance (Zucchi, 2000). Among fruit flies, *Anastrepha* species Schiner, 1968 and *Ceratitis capitata* (Wiedemann, 1824) are the main pests causing serious harm to fruits in the Americas. *Anastrepha* is a genus with great diversity of known species, numbering over 300 (Norrbon et al., 2015). *Ceratitis capitata* is endemic to most African countries, in sub-Saharan Africa (Cab International, 2020) and can be found in practically all regions with tropical and temperate climate (Zucchi, 2000). It is an invasive species with great adaptive capacity and the only representative of the genus in Brazil, where it is widely distributed (Zucchi, 2015).

These species infest several fruits, including papaya (*Carica papaya* L.), an important product for agricultural export to developing countries. Brazil is the world's second largest papaya exporter (Barbosa et al., 2020). The infestation of papaya by fruit flies was first recorded in Brazil in crops cultivated in the state of Espírito Santo (Martins and Alves, 1988). Although papaya is not a potential host for these tephritids species, its export to the USA was interrupted for 13 years but resumed at the end of 1997, after implementation of the systems approach in the state of Espírito Santo. Systems approach is a set of management measures with strong biological and ecological basis, aiming to reduce the risks of papaya infestation by fruit flies (Martins and Malavasi, 2003a, 2003b). The result of these actions has placed papaya crops under low prevalence of fruit flies (Martins et al., 2011) and stimulated intense monitoring to prevent phytosanitary problems capable of disrupting continuous exports (Martins et al., 2012).

The areas of papaya crops cultivation in the state of Espírito Santo are located in a strategic region for production, close to a large Atlantic Rainforest area. Several factors can influence the presence and abundance of fruit flies in a region, such as climate, interspecific relationships, availability of hosts, presence of natural enemies, and the spatial configuration of landscapes (Aluja, 1994). Most studies which focused on the monitoring of fruit flies have emphasized the above mentioned topics with collections in areas of agricultural orchards, often monocultures. Few studies have evaluated the influence of natural vegetation on the dynamics of these insects in orchards (Querino et al., 2014; Araujo et al., 2019). In Atlantic Forest

environments, a great diversity of resources is expected, resulting in higher complexity of interactions than monocultures due to the space-time variation of host plants (Novotny et al., 2005, 2010). Orchards located in fragmented forest environments can suffer significant influence on the diversity, distribution, and abundance of species of fruit flies (Raghu et al., 2000).

In Brazil, several studies have been conducted in agricultural areas (Uramoto and Martins, 2006; Deus et al., 2016; Nicácio et al., 2019; Ramos et al., 2019). However, studies describing the ecological patterns of these insects in heterogeneous environments are still scarce. Also, regarding spatio-temporal distribution, there are still few studies addressing management in wide areas and with long-term monitoring data. These investigations are important because of the existence of several aspects not yet understood concerning the composition and structure of fruit fly communities, mainly in crops with an established management strategy.

Population dynamics studies are essential to analysis of the spatio-temporal distribution of pests, because it reveals ecological patterns important to investigation of population projection trends (Lima et al., 2009). However, this approach is only possible when time series data are available for long term evaluations. Usually, time series analysis aims to identify the nature of phenomena, particularly fluctuations expressing the time trajectory of fruit flies in the current study, which reflect a sequence of numerical observations (Crawley, 2013). In addition, time series are used to predict future values of the time series (Crawley, 2013). Although population dimension is a significant aspect of analyzing the population dynamics of pests, community structure is also important to understanding the diversity of species, as well as the use of food resources and therefore, the relationship between insect species and host plants. Understanding the diversity patterns of species is fundamental to explaining the composition of local fauna and making comparisons among different areas. A relevant question about this in this context would be why species richness varies across areas or regions and their respective sizes. Some studies have argued that species richness patterns among regions may be explained by ecological limits associated with different area sizes on diversity over time (Wiens, 2011).

The first issue analyzed in this thesis emphasized patterns of richness and abundance of *Anastrepha* species, associated with species infestation periods, critical areas and pest management. The analysis considered the fit of data from a long time series to mathematical functions, describing the relationship between species richness and area. Also, partitioning of diversity was employed to understand how different sizes of areas could explain the change in

alpha and beta diversity over time. Spectral analysis introduced a method of evaluating population peaks and its potential of recurrence in fruit flies.

The second part of this thesis presents the comparative aggregation patterns of three important fruit fly species, *C. capitata*, *A. fraterculus* and *A. obliqua* as well as the influence of the Atlantic Rainforest on the distribution of these species. This aspect involved regression analysis and frequency distribution, with results important to understanding how the species are spatially distributed and what this represents for pest management actions. The third study aimed to understand how *Anastrepha* species pairs interact with each other, describing the main patterns of species co-occurrence and interactions strength through probabilistic models and regressions. This knowledge is important to understand the potential for competition and/or coexistence between *Anastrepha* species.

These three aspects were analyzed considering a 13-year time series for monitoring of fruit flies in papaya orchards, considering areas with different distances from the Atlantic Rainforest, and different time periods consistent with the systems approach. In view of the contextualization presented and the need to analyze in detail populations dynamics, diversity, distribution, and interactions of tephritids, the present study aimed to investigate the spatio-temporal distribution and the community structure of fruit flies in areas of papaya production in the state of Espírito Santo, Brazil. The hypotheses tested in our study were based on the assumptions that:

- ✓ Time and distance from the Atlantic Rainforest influence the diversity and abundance of fruit flies.
- ✓ The abundance and diversity of fruit flies are influenced by the size of the area and management actions.
- ✓ The spatial distribution of the main species of fruit flies has aggregated patterns in relation to time, management actions, and different spatial delimitations in relation to the Atlantic Rainforest.
- ✓ Co-occurrence patterns are influenced by time, management actions, and spatial delimitations.

This study was divided into three chapters addressing the following issues:

- i. Long-term fruit fly monitoring in Brazil and impact of the systems approach on the richness and abundance of fruit flies (Diptera, Tephritidae, *Anastrepha*) (Chapter 2).
- ii. Aggregation and spatio-temporal dynamics of fruit flies (Diptera, Tephritidae) in papaya orchards associated with different area delimitations in Brazil (Chapter 3).

- iii.* Spatio-temporal co-occurrence of *Anastrepha* species (Diptera, Tephritidae) in papaya orchards in the state of Espírito Santo, Brazil (Chapter 4).

Chapters 2 and 3 of this thesis were written following the guidelines for manuscripts submission to *The Canadian Entomologist* and *Acta Scientiarum Agronomy*, respectively, accepted for publication.

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2 LONG-TERM FRUIT FLY MONITORING IN BRAZIL AND IMPACT OF THE SYSTEMS APPROACH ON RICHNESS AND ABUNDANCE OF FRUIT FLIES (DIPTERA, TEPHRITIDAE, *Anastrepha*)¹

Abstract

In this study we investigated ecological patterns of richness and abundance of *Anastrepha* fruit flies in papaya orchards at different distances from an Atlantic forest remnant in the state of Espírito Santo, Brazil. Based on a long-term series with a dataset from 1998 to 2010, mathematical functions, richness partitioning and spectral analysis were employed to compare richness and abundance in different time series associated with a systems-approach program, for exporting Brazilian papaya to the United States. Richness and abundance were influenced by time and distance, but to different extents. A spatio-temporal analysis of richness and abundance in fruit flies, taking into account the implementation of the systems approach, revealed clear effects of the pest management on fruit fly richness and abundance. The richness partitioning analysis indicated different distribution of richness values and predictions for richness within β -diversity, directly associated with implementation of the systems approach. The spectral analysis projected different trends for peaks, also associated with system management.

Keywords: richness, abundance, *Anastrepha*, models, systems approach, papaya orchards

2.1 Introduction

Papaya (*Carica papaya* L.) is an important tropical fruit marketed worldwide, with most of the production in a few countries, especially India and Brazil (Pathak et al., 2019). Although Brazil has extensive planted areas and a favorable climate for the production of papaya year-round, phytosanitary problems can limit the export of fruits, among which is the infestation of fruit flies (Diptera, Tephritidae). Among the main species of fruit fly in Brazil, 121 members of the genus *Anastrepha*, native to the Americas, occur in the country. Among the pests causing serious problems for papaya exports for quarantine reasons, in particular are *Anastrepha fraterculus*, a native polyphagous species, and *Ceratitis capitata*, an exotic species registered to Brazil in 1901 (Martins et al., 1993; Martins and Fornazier, 2014). A successful management program entitled “systems approach” was implemented in Brazil in the 1990s, in order to make it possible to export papaya to the USA (Martins and Malavasi, 2003a, 2003b). The systems approach can be viewed as the integration of actions during the pre- and post-harvest periods, in order to improve production, harvesting, packaging and transport of a commodity that meets

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the requirements for quarantine security (Martins and Malavasi, 2003b). Part essential of this type of program is the monitoring of fruit fly abundance and richness.

Knowledge of the abundance of fruit flies as well as the distribution patterns of species richness is essential for pest management programs, because analysis of this information allows evaluation of the period of pest infestation, identification of critical areas for planting and/or scenarios for the implementation of control measures, both by harvest management and by release of control agents or by management and conservation of the diversity of natural enemies of the fruit flies (Lundgren and Fausti, 2015). In this study, we investigated the patterns of richness and abundance of *Anastrepha* over time and space in papaya orchards located at different distances from an Atlantic forest remnant in the state of Espírito Santo, Brazil. The analyses were based on a full time series (1998 to 2010) and two partial series within the full series, to investigate the impact of the systems-approach program on the richness and abundance of fruit flies in commercial papaya production fields.

2.2 Material and methods

2.2.1 Dataset

The dataset used in this study is part of a project entitled “Survey of fruit flies in the state of Espírito Santo, Brazil”, coordinated by INCAPER – Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural. In this chapter we used only data from fruit flies of the *Anastrepha* genus, which were obtained from weekly monitoring using 1 McPhail trap (containing 5% hydrolyzed corn protein) per 2 ha at different georeferenced points (18°02'35.7" to 20°33'58.3" S and 39°13'00.7" to 41°09'16.1" W) in papaya orchards in commercial papaya production fields of Espírito Santo, Brazil (Fig. 1) from 1998 to 2010. This is the main papaya production for export in the state and is located close to an Atlantic forest complex, consisting of the Reserva Biológica de Sooretama, Reserva Natural Vale, Reserva Particular do Patrimônio Natural Mutum Preto, Recanto das Antas and adjacent private area.

We investigated the richness and abundance of a pool of species of *Anastrepha* collected in 168 areas with vary in size, from 2 to 275 ha. The distance between each orchard and forest remnant was established based on the same point in the forest (19°59'04" S and 39°55'24" W), a central area located in the Reserva Natural Vale, an Atlantic forest remnant, highly biodiverse forest with about 23,000 ha of protected area. Monthly data on rainfall, temperature and relative humidity from 1998 to 2010 were obtained from INCAPER in order to evaluate the influence of these factors on the richness and abundance of fruit flies.

The analyzes were carried out only with female *Anastrepha* specimens because the identification of the species is based mainly on the characteristics of the females (e.g., Zucchi, 2000). The voucher specimens are deposited at the Museum of Entomology Luiz de Queiroz (MELQ), Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo and at the Regional Museum of Entomology at the Federal University of Viçosa.

2.2.2 Time series and the systems approach

The time periods consisted of a full time series of fruit flies in papaya orchards from 1998 to 2010, and four partial time series, two of them with data since the beginning of the systems approach implementation and the other two with data showing the impact of the systems approach on abundance and diversity of fruit flies. The first partial time series, from 1998 to 2005, was analyzed for species richness. The second time series, from 1998 to 2003, was analysed for fruit fly abundance. Richness and abundance were analysed separately due to the impact of the system approach occurring at different times for abundance and richness of species. The third time series, from 2006 to 2010, was analysed for richness of fruit fly species and the fourth partial series, from 2004 to 2010, was analysed for abundance. For convenience we named the series the “continuous implementation of systems approach” as the period with management actions before observing any impact on richness and abundance for the series between 1998 and 2005, and “impact on richness” and “impact on abundance”, as the periods with visible impact on richness and abundance for the series between 2004 to 2010.

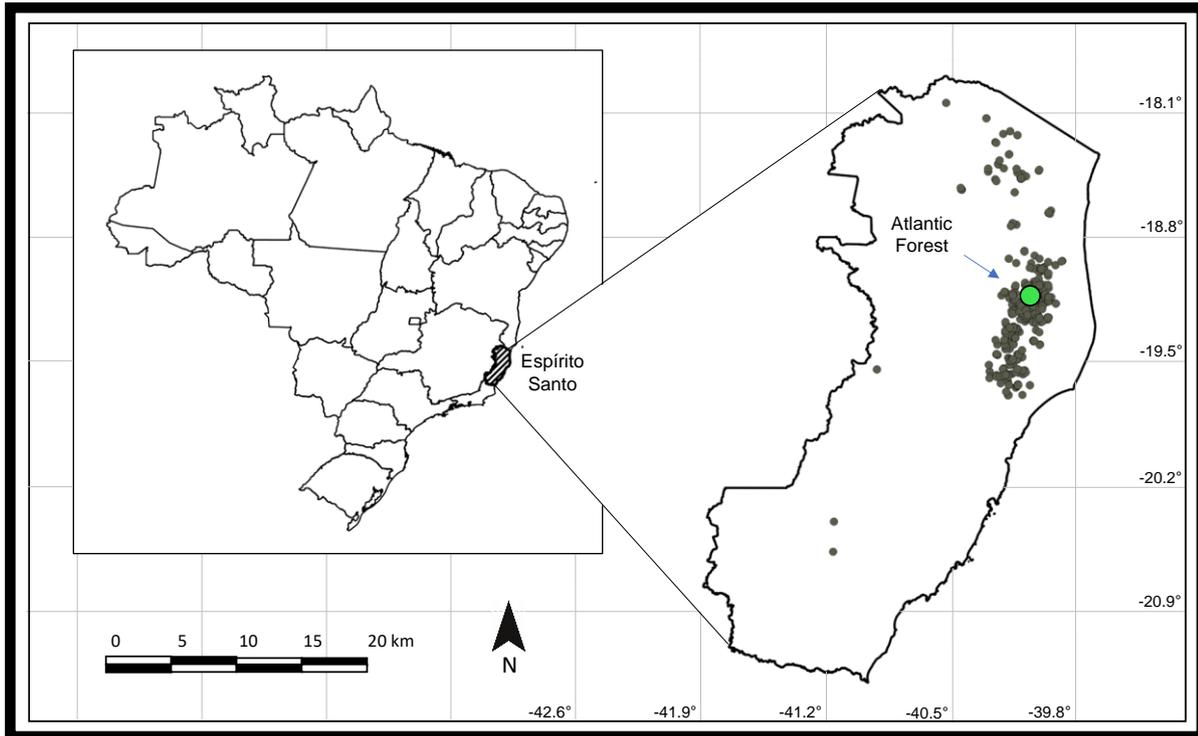


Fig 1. Maps of fruit fly collection sites in the state of Espírito Santo, Brazil, from 1998 to 2010.

2.2.3 Data analysis

The results indicated that the relationship between richness or abundance and rainfall, temperature and humidity followed a normal distribution. Then, multiple regression was employed to analyze the significance of the relationship between richness or abundance and the above environmental variables. The distribution of richness and abundance at time or distance between the papaya orchards and the Atlantic forest remnant did not follow a normal distribution. Therefore, generalized linear models with Poisson errors were used to analyze the relationship between fruit fly richness or abundance and time or distance between papaya orchards and the Atlantic forest remnant.

2.2.4 Nonlinear models fitted to the data

We used the R package ‘sars’ to fit 20 SAR models describing convex and sigmoid shapes (Matthews et al., 2019), using non-linear and linear regression (CRAN i. e. 1.1.1; R Core Team) in order to find the best model shape to describe the relationship between fruit-fly richness and area, by comparing information criterion weights (ICW) using a barplot. For the models showing the best shape for the dataset, nonlinear regressions were performed to test the significance of the relationship between richness and area, and the Akaike Information Criterion (AIC) criterion was used to compare the best fit of the models to the data.

2.2.5 Richness partitioning analysis

In this study, β -diversity was partitioned in order to understand the degree to which different area sizes could explain the changes in richness in different time periods, and also to compare the impact of the systems approach on the richness and abundance of fruit flies. The total number of species in all areas of the study was described as γ . The mean richness of the areas was represented by $\bar{\alpha}$ and was associated with the area size. Then, the reason why there were species not captured in specific areas could be associated with area size. β_{area} was an estimate of the mean difference between $\bar{\alpha}$ and the diversity predicted for the largest area. Generally, β contains both β_{area} and $\beta_{replace}$, and this second term determines the mean number of missing species due to factors other than area size (Henry and Stevens, 2009). For the context of this study, other regions, such as $\beta_{ecoregion}$ and $\beta_{geogr.region}$ could also belong to β . “Ecoregion” is a biogeographic regionalization of the terrestrial biodiversity, and “geographic region” is defined as a biodiversity hotspot characterized by high species diversity (Reid, 1998). The partitioning can then be described as $\beta = \beta_{area} + \beta_{replace}$ where $\beta_{replace}$ is the mean number of missing species that are not explained by patch size. Then, in the context investigated here, the full partition can be viewed as $\gamma = \bar{\alpha} + \beta_{area} + \beta_{ecoregion} + \beta_{geogr.region}$ (Henry and Stevens, 2009), where $\beta_{replace} = \beta_{ecoregion} + \beta_{geogr.region}$.

2.2.6 Time series

Periodograms were developed to evaluate the presence of potentially recurring peaks, based on comparisons among spectrum values. The spectrum value is useful when comparing the potential for new population peaks after a previous occurrence, based on frequencies rather than fluctuations of numbers, producing a periodogram (Crawley, 2013). For specific cases such as the dynamic series, ten-year cycles would have a frequency of 0.1 per year. The result of the analysis is a plot on log scale, in units of decibels, with the frequency (x axis) indicating the bandwidth and 95% confidence interval in decibels shown by a vertical bar in the top right-hand corner (Crawley, 2013). The interpretation of the result indicates that at the maximum value where the spectrum takes place, cycles could occur. For example, if the value is 0.1, the result would show cycles with a period of $1/0.1 = 10$ years. Longer cycles for frequency 0.05 would result in cycles of length $1/0.05 = 20$ years.

2.3 Results

A total of 34 species was obtained during the 13-year series, with *Anastrepha fraterculus* (Wiedemann, 1830) and *Anastrepha obliqua* (Macquart, 1835), showing the highest abundance, 54.48% and 16% of the specimens obtained, respectively (Table 1). Among the variables (rainfall, temperature and relative humidity) evaluated with respect to their influence on richness and abundance, only relative humidity showed a positive relationship with richness ($t = 2.826$, $p = 0.0199$). Our results indicate an initial increase of richness and abundance during the period of continuous implementation of the systems approach, followed by a decrease after 2005, a period characterized by the impact on richness and on abundance, when observing the dispersal of points (Fig. 2A, B). Fruit fly richness and abundance varied significantly over time, from 1998 to 2010 (Table 2). Table 2 also shows the variation in richness and abundance in response to distance between the papaya orchards and the Atlantic forest remnant, with significant variation only in the abundance. The subseries 1998 to 2005 and 1998 to 2003, respectively analyzing richness and abundance over time, indicated a significant relationship for abundance but not for richness (Tables 3). The same result was observed for the distance between papaya orchards and Atlantic forest remnant (Table 3). The subseries 2006 to 2010 and 2004 to 2010, respectively for richness and abundance, indicated a significant relationship with time for both richness and abundance (Table 4), but with respect to distance, only abundance showed a significant relationship (Table 4).

Table 1

Relative abundance of fruit fly species from 1998 to 2010 in papaya orchards in the state of Espírito Santo, Brazil.

Species	Relative abundance (%)	Species	Relative abundance (%)	Species	Relative abundance (%)
<i>A. fraterculus</i>	54.48	<i>A. antunesi</i>	0.30	<i>A. consobrina</i>	0.04
<i>A. obliqua</i>	16.00	<i>A. paralela</i>	0.30	<i>A. grandis</i>	0.04
<i>A. distincta</i>	8.91	<i>A. leptozona</i>	0.19	<i>A. minensis</i>	0.04
<i>A. serpentina</i>	6.77	<i>A. sororcula</i>	0.19	<i>A. sagittifera</i>	0.04
<i>A. pickeli</i>	6.57	<i>A. nascimento</i>	0.15	<i>A. sp. 2</i>	0.04
<i>A. pseudoparalela</i>	2.08	<i>A. bondari</i>	0.13	<i>A. dissimilis</i>	0.02
<i>A. bahiensis</i>	1.82	<i>A. sp. 1</i>	0.13	<i>A. furcata</i>	0.02
<i>A. barbiellini</i>	1.10	<i>A. atlantica</i>	0.09	<i>A. lanceola</i>	0.02
<i>A. manihoti</i>	0.71	<i>A. bezzii</i>	0.09	<i>A. mixta</i>	0.02
<i>A. zenildae</i>	0.61	<i>A. zernyi</i>	0.09	<i>A. tumida</i>	0.02
<i>A. montei</i>	0.41	<i>A. amita</i>	0.06		
<i>A. bivittata</i>	0.37	<i>A. quararibea</i>	0.06		

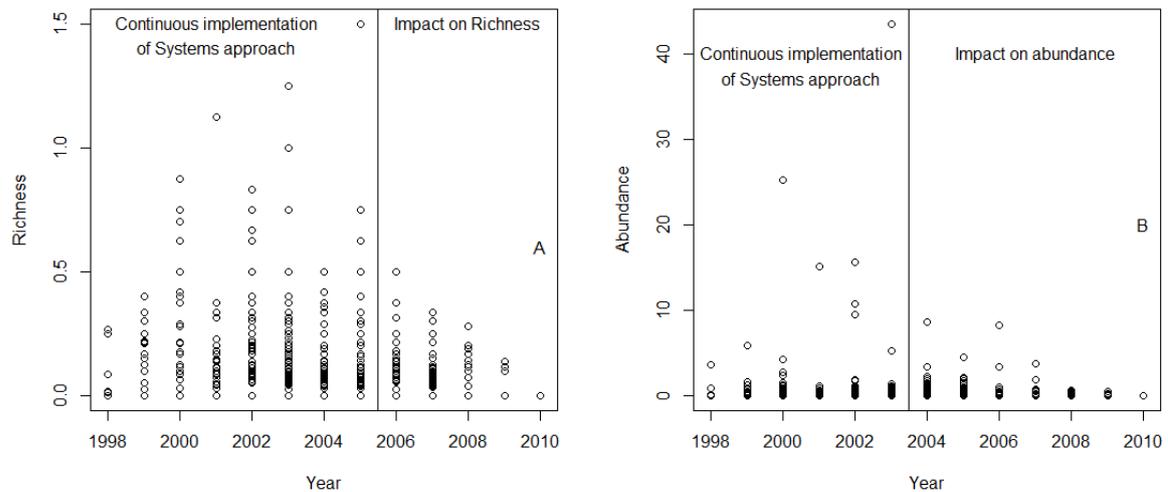


Fig. 2. Richness (A) and abundance (B) of fruit flies per ha from 1998 to 2010 in papaya orchards, taking into account the effect of the systems approach and its impact on the richness and abundance of flies.

Table 2

Regression with Poisson errors taking into account *Anastrepha* richness and abundance variation in response to time and distance between papaya orchards and Atlantic forest remnant.

Analysis of richness variation from 1998 to 2010 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	322.94	39.88	8.098	5.59e-16
Year	-0.16	0.02	-8.12	4.68e-16
Analysis of abundance variation from 1998 to 2010 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	502.25	21.29	23.59	<2e-16
Year	-0.25	0.01	-23.57	<2e-16
Analysis of richness variation in response to distance (1998 to 2010)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-0.82	0.15	-5.37	7.9e-18
Distance	-0.001	0.001	-0.92	0.35
Analysis of abundance variation in response to distance (1998 to 2010)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	0.77	0.09	8.6	<2e-16
Distance	-0.004	0.0009	-4.73	2.17e-06

Table 3

Regression with Poisson errors taking into account *Anastrepha* richness and abundance variation in response to time and distance between papaya orchards and Forest Atlantic remnant.

(to be continued)

Analysis of richness variation from 1998 to 2005 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	94.63	78.28	1.2	0.23
Year	-0.05	0.04	-1.21	0.23
Analysis of abundance variation from 1998 to 2003 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	223.5	44.97	4.96	6.82e-07
Year	-0.11	0.022	-4.96	7.59e-07

Table 3

Regression with Poisson errors taking into account *Anastrepha* richness and abundance variation in response to time and distance between papaya orchards and Forest Atlantic remnant.

(conclusion)				
Analysis of richness variation in response to distance (1998 to 2005)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-0.51	0.17	-3.05	0.002
Distance	-0.002	0.001	-1.09	0.27
Analysis of abundance variation in response to distance (1998 to 2003)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	1.75	0.10	16.65	< 2e-16
Distance	-0.009	0.001	-7.84	4.29e-15

The ICWs were investigated for 20 mathematical functions describing model curve shapes. The barplot for the full series (1998–2010) with respect to species richness per area shows the performance of the functions by comparing their respective ICW (Fig. 3). The Gompertz model was the function showing the highest ICW (Fig. 3) with the lowest AIC, 921, and the Weibull4 model showed the next highest ICW, with AIC = 927. The distribution of richness values in response to area sizes indicated that higher richness frequency was observed in areas below 50 ha, and 6.55 was the theoretical estimate of richness with the Gompertz function (Fig. 4).

Table 4

Regression with Poisson errors, taking into account *Anastrepha* richness and abundance variation in response to time and distance between papaya orchards and Forest Atlantic remnant.

Analysis of richness variation from 2006 to 2010 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	994.6	168.2	5.9	3.40e-09
Year	-0.49	0.008	-5.9	3.25e-09
Analysis of abundance variation from 2004 to 2010 in response to time				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	948.2	103.63	9.15	<2e-16
Year	-0.47	0.05	-9.15	<2e-16
Analysis of richness variation in response to distance (2006 to 2010)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-2.3	0.45	-5.4	6.35e-08
Distance	0.005	0.004	1.33	0.18
Analysis of abundance variation in response to distance (2004 to 2010)				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-0.78	0.15	-5.2	2.03e-07
Distance	0.005	0.001	3.61	3e-04

The barplot for the subseries 1998 to 2005 (period of continuous implementation of the systems approach) shows the performance of the functions by comparing their respective ICWs. The Gompertz model also showed the highest ICW and AIC = 722, a lower value than the

Asymptotic model, with $AIC = 727$, and the Persistence function 1, with $AIC = 728$ (Fig. 5). The distribution of richness values in response to area sizes indicates that higher richness was also observed in areas below 50 ha, and the Gompertz model resulted in a theoretical richness of 6.39 (Fig. 6).

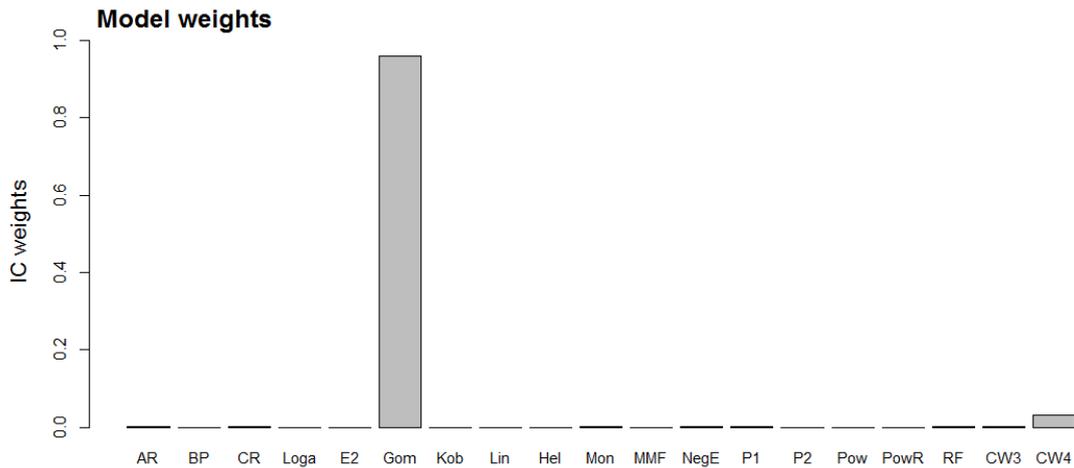


Fig. 3. Barplot of the IC weights of 19 different models for the series (1998–2010), emphasizing species richness per area size, with Gompertz model (Gom) showing the highest ICW. (AR) Asymptotic, (BP) Beta-P, (CR) Chapman–Richards, (Loga) Logarithmic, (E2) Extended Power 2, (Gom) Gompertz, (Kob) Kobayashi, (Lin) Linear, (Hel) Logistic, (Mon) Monod, (MMF) Morgan–Mercer–Flodin, (NegE) Negative Exponential, (P1) Persistence Function 1, (P2) Persistence Function 2, (Pow) Power, (PowR) Power Rosenzweig, (RF) Rational, (CW3) Weibull-3, (CW4) Weibull-4.

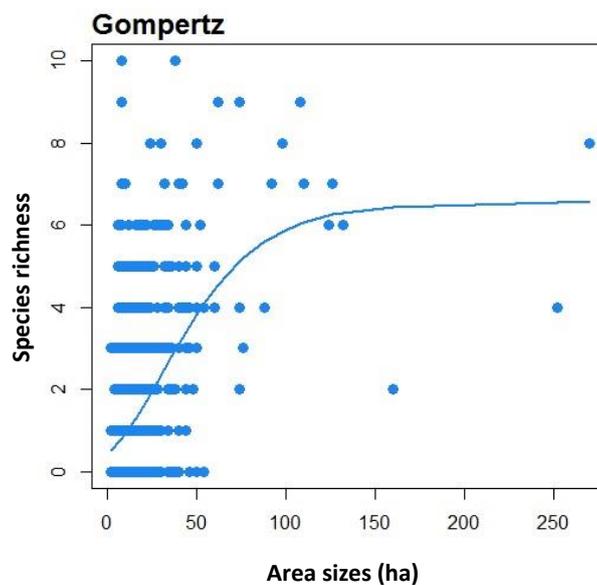


Fig. 4. The fit (solid line) of the Gompertz model to data for fruit fly richness (solid circles) in different area sizes in the state of Espírito Santo, from 1998–2010.

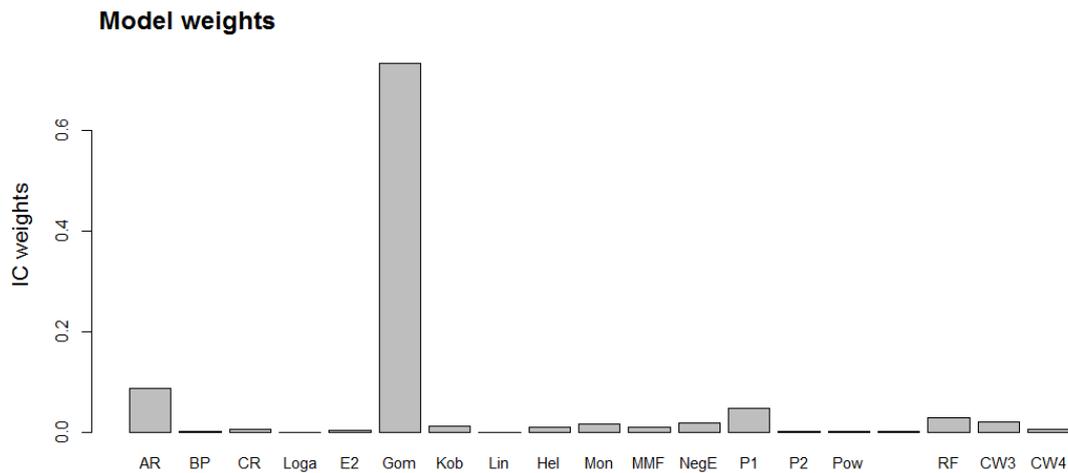


Fig. 5. Barplot of the IC weights of 19 different models for the years 1998 to 2005, emphasizing species richness per area size in the state of Espírito Santo, with Gompertz model (Gom) showing the highest ICW. (AR) Asymptotic, (BP) Beta-P, (CR) Chapman–Richards, (Loga) Logarithmic, (E2) Extended Power 2, (Gom) Gompertz, (Kob) Kobayashi, (Lin) Linear, (Hel) Logistic, (Mon) Monod, (MMF) Morgan–Mercer–Flodin, (NegE) Negative Exponential, (P1) Persistence Function 1, (P2) Persistence Function 2, (Pow) Power, (PowR) Power Rosenzweig, (RF) Rational, (CW3) Weibull-3, (CW4) Weibull-4.

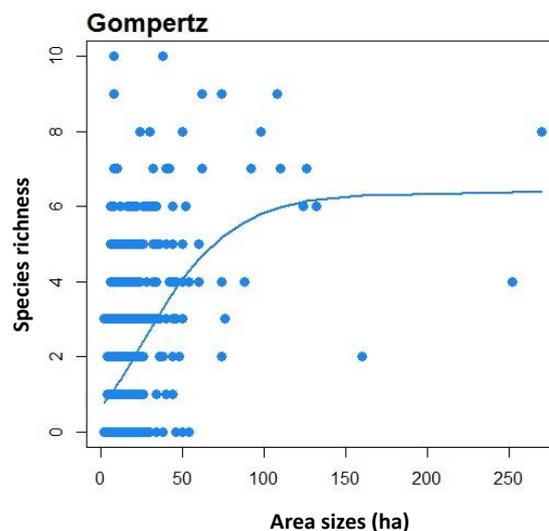


Fig. 6. The fit (solid line) of the Gompertz model to data for fruit fly richness (solid circles) in different area sizes in the state of Espírito Santo, from 1998 to 2005.

The barplot for the subseries 2006 to 2010 did not clearly indicate a specific model to fit the curve to these data as observed in the previous series, although Weibull3 (CW3) showed the highest ICW (Fig. 7). The AIC values were close to each other, showing the following values for the four models with the highest ICW: Weibull3 AIC = -2.75 , Persistence Function 1 (P1) AIC = -2.69 , Weibull4 (CW4) AIC = -2.53 and Gompertz (Gom) AIC = -2.34 . Fig. 9 shows the fits for these functions, except Persistence Function 1, because its model shape is

convex and not sigmoid as in Weibull3, Weibull4 and Gompertz. In addition, the Persistence function 1 exhibited theoretical richness < 0.5 , a value much lower than the other models, probably in response to its convex shape.

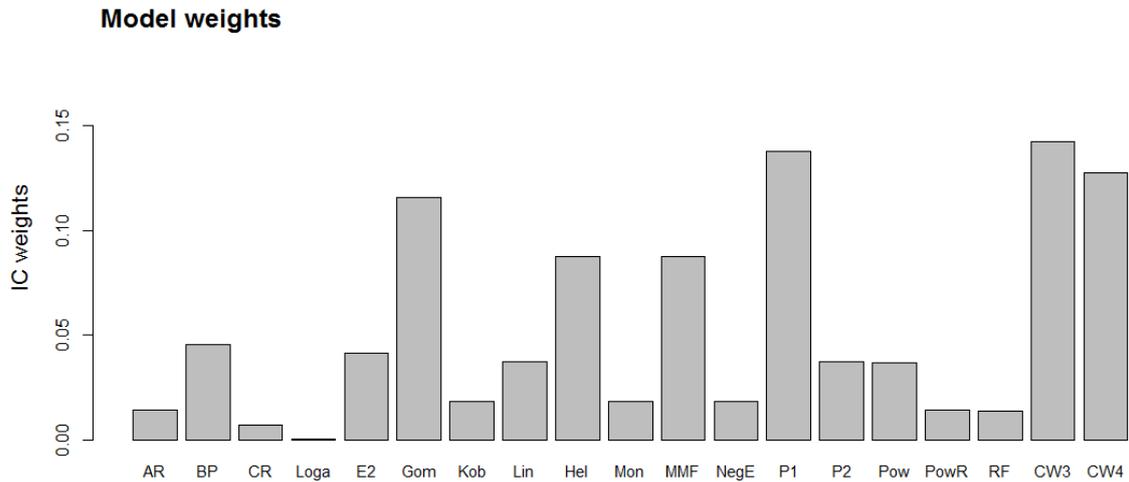


Fig. 7. Barplot of the IC weights of 19 different models for the years 2006 to 2010, emphasizing species richness per area, with no specific indication for the highest ICW. (AR) Asymptotic, (BP) Beta-P, (CR) Chapman–Richards, (Loga) Logarithmic, (E2) Extended Power 2, (Gom) Gompertz, (Kob) Kobayashi, (Lin) Linear, (Hel) Logistic, (Mon) Monod, (MMF) Morgan–Mercer–Flodin, (NegE) Negative Exponential, (P1) Persistence Function 1, (P2) Persistence Function 2, (Pow) Power, (PowR) Power Rosenzweig, (RF) Rational, (CW3) Weibull-3, (CW4) Weibull-4.

The diversity partitioning was investigated by analyzing the distribution of richness in fruit flies according to the Beta diversity types (Figs. 8A, B). Most of the richness values were within the interval above 1 and below 10, the β -area. The α -area contained the second-highest concentration of richness values, followed by β -eco with a few richness values close to the line between β -area and β -eco (Figs. 8A, B). No richness value was observed in β -geo (Figs. 8A, B). Figures 8A also shows the Gompertz curve fitted to the full series and 8B the same curve fitted to the data influenced by the continuous implementation of the systems approach. Comparing the distribution of richness values in figures 8 and 9, a clear difference between the figures can be observed, with the predicted richness decreasing from 6.39 to 1.57 (Weibull3), 1.51 (Weibull4) and 1.83 (Gompertz), and the decrease occurring from the continuous implementation of the systems approach to the impact on richness subseries (Figs. 8B and 9).

A comparison among the series, full series (1998 to 2010), continuous implementation of the systems approach (1998–2005) and impact on richness (2006–2010) also shows that the highest fruit fly predicted richness occurred from 1998 to 2010 (Figs. 8A). However, the distribution of richness values was almost the same for the full series and the series for the

continuous implementation of the systems approach, with most of the points in β_{area} (Figs. 8A and B). A slight difference in terms of point distribution can be observed exactly on the dotted line close to 10. In Fig. 8A, the points are distributed above or below this line, but in Figure 8B a couple of points fall on the dotted line. Only in the impact on richness series were the points distributed in a narrower range, with most of the richness values scattered between 0 and 5 for all fitted models (Fig. 9). For this scenario, the β -area had a narrower vertical range than in the previous scenarios, and this characteristic led to the points also being distributed in β -eco.

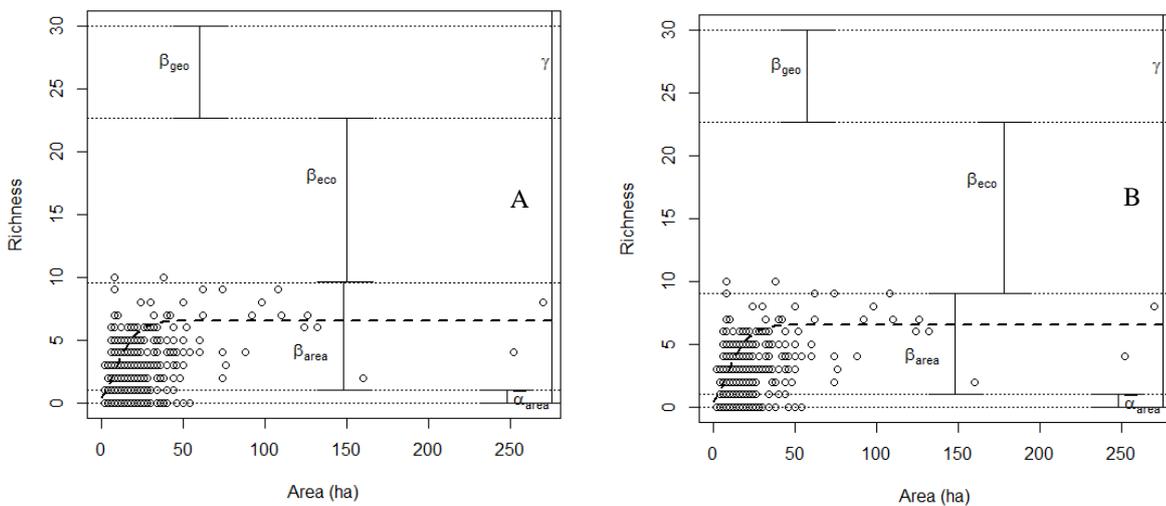


Fig. 8. Additive diversity partitioning to analyze the distribution of fruit fly species (A) from 1998 to 2010 (full series), in areas with different sizes, characterizing different types of beta diversity, with the Gompertz curve fitted. Predicted richness for the maximum area = 6.55. (B) Partitioning from 1998 to 2005 (continuous implementation of the systems approach), in areas with different sizes characterizing different types of Beta diversity, with the Gompertz curve fitted. Predicted richness for the maximum area = 6.39.

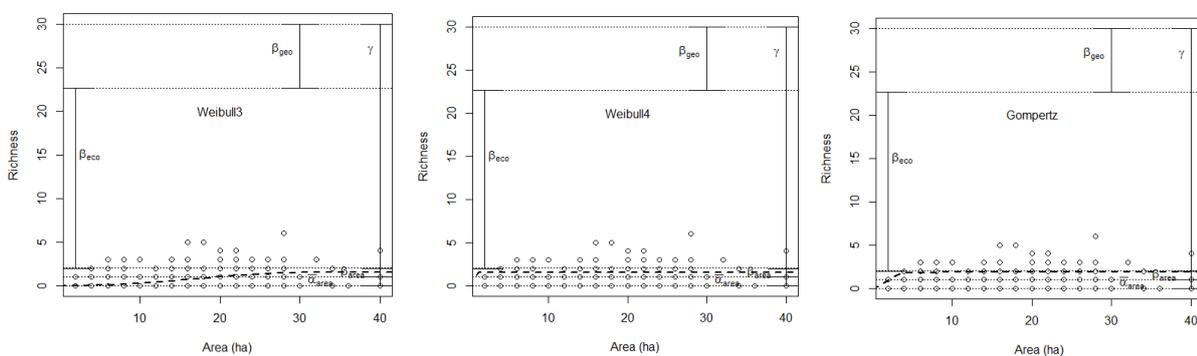


Fig. 9. Additive diversity partitioning to analyze the distribution of fruit fly species from 2006 to 2010 (impact on richness), in areas with different sizes, characterizing different types of beta diversity, with the Weibull3, Weibull4 and Gompertz curves fitted. Predicted richness for the maximum value = 1.57 (Weibull3), 1.51 (Weibull4) and 1.83 (Gompertz).

The time-series analysis for both the full series and the two subseries, the continuous implementation of the systems approach and the impact on abundance, indicated clear

differences in terms of peaks, mainly between the continuous implementation of the systems approach and the impact on abundance series, with respect both to peak magnitude (fly axis) and to the time of occurrence (time axis) (Fig. 10 A, B, C). A comparative spectral analysis revealed a maximum spectrum value of 1295 for the full series, 2798 for the series for the continuous implementation of the systems approach, and a maximum spectrum value of 367 for the series for impact on abundance (Fig. 11A, B, C). The difference between the three spectrum values reflects the series analyzed, and the lowest value (367) occurred for the impact on abundance series. Comparing the potential peak production among the three scenarios, the full time series (1998-2010) and the subseries for the continuous implementation of the systems approach (1998-2005) would show a maximum peak with a period of $1/0.16 = 6.25$ years. However, the impact on abundance series (2004-2010) would show a maximum peak with a period of $1/0.0008 = 125$ years.

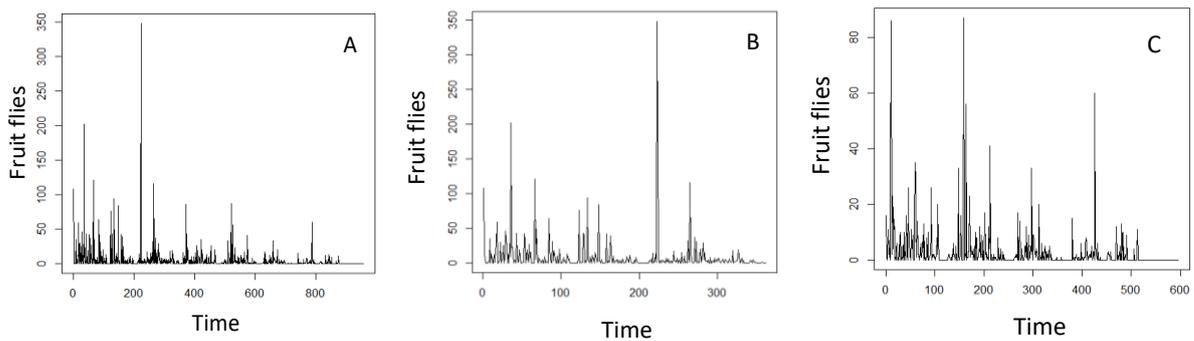


Fig. 10. (A) Full time series (1998–2010), (B) continuous implementations of the systems approach subseries and (C) impact on abundance subseries, as times series object in R language.

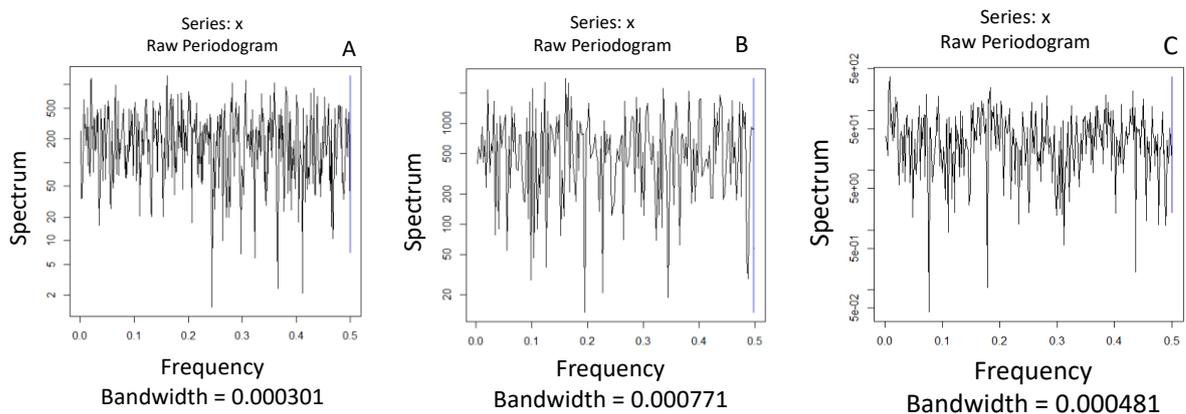


Fig. 11. **A)** Spectral analysis for full series (1998–2010) with maximum spectrum of 1295 and frequency of 0.16, projecting a period of $\frac{1}{0.16} = 6.25$ years for a new peak of this size to occur, **B)** continuous implementation of the systems approach (1998–2005) with maximum spectrum of 2798 and frequency of 0.16, also projecting a period of $\frac{1}{0.16} = 6.25$ years; and **C)** impact on abundance (2004–2010) with maximum spectrum of 367 and frequency of 0.008, projecting a period of $\frac{1}{0.008} = 125$ years between peaks.

2.4 Discussion

Of the *Anastrepha* species obtained in the data series from 1998 to 2010, a high percentage (87%) were previously recorded for the state of Espírito Santo (39 species) (Zucchi and Moraes, 2008). This number of species was observed for the total data set. However, for the analyses, the species richness per area was used. The maximum value for richness per area was 10 species. *Anastrepha fraterculus* and *A. obliqua* were the most abundant species in the data set. Both have wide geographical distributions and are highly polyphagous. *A. fraterculus* is the species with the largest number of host plants in Brazil (116), followed by *A. obliqua* (51) (Zucchi and Moraes, 2008). Dominance of these species has been observed in several Brazilian states (Aguiar-Menezes et al., 2008; Garcia et al., 2003; Lopes et al., 2015; Monteiro et al., 2019; Oliveira et al., 2019). Commonly, in commercial orchards one or two species of fruit flies are dominant, comprising a significant part of the total of captured specimens (Aluja et al., 1996). An array of biotic and abiotic factors including host plants may simultaneously contribute to the establishment of fruit flies as dominant and codominant species in specific areas (Aluja et al., 1996, Rwomushana et al., 2009). In addition, both *A. fraterculus* and *A. obliqua* are considered a complex of cryptic species *sensu lato*, difficult to identify morphologically.

Our results suggest that only relative humidity positively influenced richness of *Anastrepha*, and that abundance was not influenced by any environmental variable. Environmental variables, particularly rainfall and soil moisture, can affect fruit-fly life stages in different ways (Montoya et al., 2008; Quesada-Moraga et al., 2012). Generally, rainfall, temperature and relative humidity affect both the diversity and abundance of fruit flies (Garcia et al., 2003; Gnanvossou et al., 2017; Nascimento et al., 1982; Trassato et al., 2015), but the correlation coefficients of the present works are usually low and some of them are not statistically significant. The little of effects of temperature and rainfall on richness and abundance found here is probably a function of a local aspect, i.e., an area with orchards that are not far from each other and are all influenced by the same conditions, resulting in an apparent stability of values during the observation period.

Richness and abundance of fruit flies varied significantly from 1998 to 2010. The two subseries investigated, the continuous implementation of the systems approach and the impact on richness and abundance, showed different periods; the continuous implementation of the systems approach subset described an increasing dispersal of points for both richness and abundance of flies, whereas the impact on richness and abundance subset showed the exact opposite. The high abundance and richness of fruit flies before 2006 probably occurred in

response to the little effect of pest management in previous years. Even with the implementation of the systems approach before 2006, the abundance and richness of flies require some time to show the effect of previous control actions. However, the implementation of pest management did result in a lower richness and abundance of species of *Anastrepha* after a few years. The relationship between richness and abundance is generally associated with commonness and rarity, which are usually cited as dependent on the heterogeneity of resources (Verberk, 2011). Agroecosystems tend to contain habitats with a limited supply of resources, the prime example being monocultures, compared to natural environments or even to polycultures or intercropping, where the diversity of species can be higher (Ratnadass et al., 2012). In this study, the fruit fly richness reached its maximum value at 10 species per area, and decreased in response to a set of actions, resulting in successful pest management. The data analyzed here were obtained mainly from papaya orchards, although probably with some influence from urban and Atlantic forest remnant areas. The maximum richness found here is coherent with fruit fly surveys in tropical areas, which generally do not find richness levels higher than those estimated in this study (Almeida et al., 2019; Marsaro Júnior et al., 2012). The abundance of flies also changed during the study period. The most pronounced change in the number of flies occurred after 2005, probably as part of the actions taken in the systems approach (Martins et al., 2012).

Generally, fruit flies do not infest papaya, but the *Papaya meleira virus* (PMeV) reduces the resistance of fruits to fruit flies, enabling them to be infested while still unripe (Martins et al., 2012). When papaya fruits are harvested in early stages of maturity, the risk of infestation by fruit flies is low in response to the toxicity of benzyl-isothiocyanate, present in the latex of the fruit (Martins et al., 2012; Tang, 1973). Analysis of the data from 2006 to 2010 provided strong evidence of the positive effect of fruit harvest management at the beginning of ripening. The eradication of fruits at advanced maturation stage and of plants with PMeV, as well as the set of actions taken in the systems approach, resulted in a significant decrease in both the richness and abundance of fruit flies. Our results also indicated that the fruit fly richness showed no significant variation in response to distance of the papaya orchards from the Atlantic forest remnant. However, fruit fly abundance changed significantly in response to this factor for the full data series, and for both subseries, the continuous implementation of the systems approach and the impact on abundance. It is possible that the distance analysed was not far enough to show richness changes, but was far enough to affect the fly abundance. Some authors have reported that native vegetation acts as a reservoir for economically important *Anastrepha* species (Garcia et al., 2017; Uchôa and Nicácio, 2010; Uramoto et al., 2008). However, few have assessed the influence of nearby vegetation on the fruit fly community of orchards. A

pioneer study in the state of Minas Gerais, Brazil, showed that guava orchards adjacent to fragments of native vegetation harbor fruit fly species in common (Querino et al., 2014). The abundance of *Anastrepha* tends to be higher near the preserved native forest, due to the availability of hosts for the flies.

Comparison of data for the two subseries (continuous implementation of the systems approach and impact on richness) showed a clear change in species richness (Fig. 2A). The richness of flies was perceptibly higher from 1998 to 2005 than from 2006 to 2010; this decrease was probably due to implementation of the system approach (Martins, 2011). Generally, crop environments have lower insect diversity than natural areas, in response to the lower diversity of host plants (Aluja, 1999; Gurr et al., 2003). However, the richness analysis discussed here emphasizes another aspect, involving host plants and the diversity of species exploiting the same resource, since some species of *Anastrepha* are among the main pests of fruit grown in Brazil (Araujo et al., 2019). The diversity of tephritids in fruits depend essentially on the association of each fly species with its host plants. Certainly, the attractiveness of different host plants may differently influence the presence of species in host plants, impacting the community structure of insects visiting the fruits (Almeida et al., 2019).

The information criterion weights (ICW) differed among the periods investigated, especially when comparing the continuous implementation of the systems approach with the impact on richness subseries, indicating that the Gompertz model usually best described the nonlinear shape between species richness and area in each period. The ecological patterns produced by the theoretical curves are very similar to each other for the full series and the continuous implementation of the systems approach subseries. These results were expected, because the continuous implementation of the systems approach period is part of the full series. However, the impact on richness period clearly shows very different richness values from the continuous implementation of the systems approach period, indicating a strong effect of the previously implemented management actions (Fig. 2A).

The sigmoid pattern observed in the relationship here investigated, described by the Gompertz model, takes place generally in more complex systems, differing from the patterns frequently observed in studies of this nature, including insects (Lomolino, 2001). In this study, we tested 20 models providing a wide range of possibilities for describing the relationship between area and species. We found that the Gompertz function provided the best shape for the full series and the continuous implementation of the systems approach subseries (Figs. 3 and 5). For the impact on richness subseries, no specific model described its trajectory, probably in response to the low values of the series resulting from the papaya pest management. According

to the ICW criterion, the best shape for impact on richness data was the Weibull3 model, with the lowest AIC value of all. Actually, the three functions, Weibull3, Weibull4 and Gompertz resulted in sigmoid curves; then, we fitted the three functions to the impact on richness subseries and found a sigmoidal pattern similar to those previously observed in the full series and continuous implementation of the systems approach subseries, but with lower theoretical richness values.

The analysis of relationship between richness values and area size indicates that higher richness was predominantly observed in areas below 50 ha. This result can be associated with inter habitat differences and influences, especially with respect to size and proximity of other areas such as municipalities or forests, as observed in different taxonomic groups (Lomolino, 2001; Rico-Gray and Oliveira, 2007). The papaya orchards analysed exhibited different sizes in areas not so distant from each other, with predominance of aggregated areas varying their sizes and these conditions may have influenced the concentration of values below 50 ha.

The diversity partitioning analysis indicated that most of the richness values were within β_{area} (an estimate of the mean difference between $\bar{\alpha}$ and the diversity predicted for the largest area) for the full and continuous implementation of the systems approach series. Despite the similarity in terms of richness for flies within these series, comparison of all the series showed a significant decrease in fruit fly richness when comparing the full or continuous implementations of the systems approach series with the impact on richness subseries. This decrease was probably influenced by the effects also coming from the systems approach. There is an apparent similarity between figures 8A and 8B, because the full and continuous implementation of the systems approach series are the same except for the time periods. However, the distribution of points is slightly different with respect to the asymptote. The dotted line close to 10 shows a trend toward change in the point distribution, as a transition between the continuous implementation of the systems approach and the impact on richness periods. In the impact on richness period, the distribution of points is completely different from the continuous implementation of the systems approach. The decrease in numbers from the systems approach affected the width of β_{area} . The parametric space for β_{area} was narrower in the impact on richness period than in the continuous implementation of the systems approach, probably in response to the decrease of number of fly species. The changes in beta diversity of insects living in Atlantic forest remnants, including fruit flies, were analyzed by Novotny et al. (2007). These authors emphasized the relationship between species-area, but also discussed the role of host plant diversity in the relationship between richness and area size in tropical areas, with results indicating a low rate of change in beta diversity for tropical forests.

Although preference for host plants is decisive in defining the presence of several species in different environments, the movement of species, especially polyphagous species, also is highly important in the configuration of insect communities (Mazzi and Dorn, 2012). The movement of insect pests from one place to another is, on the one hand, associated with their origin, abundance and spatio-temporal distribution. But this movement is also capable of influencing species richness at other localities at different scales, because of the relationships to different environments and to other species, since resource availability significantly influences the balance of intra- and interspecific relationships (Mazzi and Dorn, 2012; Senger et al., 2009). The significant decrease in species investigated here is certainly a response to the systems approach, differently from natural systems, where diversity rates tend to be more stable than in systems with strong interventions (Novotny et al., 2007). Orchards are systems with low stability for species diversity, even for fruit flies, particularly when pest management is implemented systematically, as seen in this study.

The richness change in β_{area} in the context of diversity partitioning may also have important implications for expansions of fruit fly populations and species distributions, because part of the insects affected by pest-management techniques may attempt to establish in other locations, including new orchards. This movement dynamic may be important with respect to new infestations, mainly if the species dispersing are polyphagous and have the potential to exploit areas with different climatic conditions. Scenarios such as this require well-established management programs such as the systems approach, particularly because of its combination of preventive and monitoring actions. The maximum richness clearly decreased when comparing the continuous implementation of the systems approach and the impact on richness periods, with values decreasing from 6.55 to 1.83. This result highlights the importance of using richness partitioning analysis for problems in agriculture, mainly when species richness is involved. The richness of fruit fly species depends directly on their host plant preference, but this association is also influenced by the landscape, particularly when orchards are scattered in different areas, including Atlantic forest remnant (Monteiro et al., 2019).

The time series investigated here showed several peaks, occurring in different time periods and with different population sizes, especially comparing the full series or continuous implementation of the systems approach with the impact on abundance subseries. Peaks may occasionally be associated with cycles in insect populations, with different explanations for their occurrence. Immigrating populations may show substantially increased growth rates in habitats that are closely connected to each other, reducing the time period between peaks in situations with high connectivity between habitats. This condition could result in more rapid

changes from peaks to valleys than in isolated populations, depending on the population density (Johnson et al., 2006).

Cycles in insects that are susceptible to population peaks have been studied in the laboratory and field, focusing on population dynamics and emphasizing the occurrence of unpredictable outbreaks in response to changes in demographic parameters (Desharnais, 2005; Estay et al., 2012). Historically, discussions of population cycles in dipterans have dealt with the classic experiments with blowflies (Nicholson, 1954), and cycles have been reported in laboratory populations of *Drosophila melanogaster* (Meigem, 1830) (Prout and McChesney, 1985) or by food pulses produced experimentally in *C. capitata* (Carey et al., 2002). Cycles have been analyzed much more often in laboratory conditions, with different controlled variables such as temperature, humidity or photoperiod (Prout and McChesney, 1985). The existence of cycles or observation of recurrent peaks in natural conditions is not as common, but in insects has particular importance for agricultural landscapes, because of the risk of outbreaks in crops. For this reason, spectral analyses provides important information, and also in cases of strong interactions, such as in host-parasitoid systems. In these cases, peaks can be taken into account in decisions regarding interventions in agricultural systems (Ferreira and Godoy, 2014).

The spectral analysis yielded useful results, indicating that the time series for the full and continuous implementation of the systems approach would show peaks with a period of 6.25 years, and the impact on abundance subseries would show peaks with a period of 125 years. These results are important because they show that pest management as used in the systems approach can delay the repetition of peaks to a large extent. This result confirms the good potential of the pest-management strategies employed in papaya orchards, prioritizing harvesting of unripe papayas and pest monitoring. An 11-year series with *Anastrepha* species monitoring in orchards located in Veracruz, Mexico, showed populations exhibiting relatively stable dynamics over time, characterized by a density-dependent effect but also with annual peaks corresponding to the fruiting phenology of their primary hosts (Aluja et al., 2012). However, despite the stability observed in Mexican orchards, the authors suggested that a good year in terms of fruit production is also capable of producing fruit fly outbreaks when pest management is neglected (Aluja et al., 2012).

This study combined three analyses in an attempt to better understand the ecological patterns inherent in a long-term series, and also projected trends of potential infestations of fruit flies, focusing on a context of monitoring and papaya harvesting management. The analyses essentially complemented each other, because they were capable of evaluating the relationships

among time periods, distance from a Atlantic forest remnant, harvesting and pest management based on the richness and abundance of *Anastrepha* fruit flies, which are economically important for Brazilian fruit growers.

2.5 Conclusion

Richness and abundance of fruit flies were significantly influenced by time and distance, but abundance was more sensitive than richness to changes of values. The systems approach significantly influenced the decrease in richness and abundance, results that made possible the export of papaya to the USA. The richness partitioning analysis indicated different predictions for richness within β -diversity in response to the systems approach and the spectral analysis projected different trends for peaks, also associated with system management.

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3 AGGREGATION AND SPATIO-TEMPORAL DYNAMICS OF FRUIT FLIES (DIPTERA, TEPHRITIDAE) IN PAPAYA ORCHARDS ASSOCIATED WITH DIFFERENT AREA DELIMITATIONS IN BRAZIL²

Abstract

We investigated aggregation patterns in three fruit fly species economically important in Brazil, namely *Ceratitis capitata*, *Anastrepha fraterculus*, and *A. obliqua*. The study was carried out in a buffer zone and two neighborhoods by comparing two-time series associated with the management strategy of fruit flies (systems approach). The abundance of these three species significantly decreased over the years with a negative binomial regression model describing the relationship between abundance and time in the full area, buffer zone, and their neighborhoods. In addition, the negative binomial model was also well fitted to the frequency distribution data of fruit flies in all analyzed scenarios. *Anastrepha obliqua* showed the highest aggregation degree, considering both the full area and time series. *A. fraterculus* exhibited the lowest aggregation level, and *C. capitata* showed an intermediate degree. The buffer zone exhibited the highest aggregation degree for all species, and neighborhood 2 exhibited the lowest aggregation degree. The aggregation degree was higher in the time series impacted by the systems approach than the series in the first years of its implementation.

Keywords: *Ceratitis capitata*, *Anastrepha fraterculus*, *Anastrepha obliqua*, buffer zone, regression models, frequency distribution

3.1 Introduction

The main tephritid species distributed across the Neotropical Region include two taxonomic groups, *Anastrepha*, which comprises over 300 species native to the Americas and occurs in all regions of Brazil, and *Ceratitis capitata* (Wiedemann, 1824), a species with worldwide distribution (Barr et al., 2018; De Meyer et al., 2002). The genus *Anastrepha* is distributed from a latitude of 35 °N to 35 °S, in different environments, from the sea level to an altitude of 3,000 m or more. *Anastrepha* spp. are classified in several infrageneric groups (Norrbom et al., 1999), among which the *fraterculus* group has several economically important species. To understand the distribution of fruit flies, it is essential to associate them with their host plants, emphasizing the fruit-growing regions, utilizing a biogeographical criterion (Aluja & Liedo, 1986). A significant diversity of fruits is generally infested by fruit flies, including papaya, a fruit with a high production on a commercial scale (Barr et al., 2018; Façanha et al., 2019).

The papaya-producing areas in Brazil contribute significantly to the international market, owing to the annual production capacity and for currently being in areas considered to

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have a low prevalence of fruit flies (Martins et al., 2011). Papaya exports have been possible due to the implementation of an export program in the USA. The program, recognized as “systems approach”, includes a set of actions during the pre- and post-harvest periods of papaya for the management of fruit flies (Martins & Malavasi, 2003b, 2003a). It started approximately 25 years ago in the state of Espírito Santo, Brazil, following the requirements for quarantine security.

In Brazil, some species of *Anastrepha* and *C. capitata* are the most economically important flies because they attack various commercial orchards and are widely distributed in the country (Zucchi, 2000). The geographical distribution of these species is strongly linked to the distribution of their hosts (Selivon, 2000). However, the degree of association between host plants and fruit flies varies between regions (Malavasi et al., 1980). *Anastrepha fraterculus* (Wiedemann, 1830) and *A. obliqua* (Macquart, 1835) exhibit the highest degree of polyphagy (116 and 51 associated hosts, respectively) in Brazil (Zucchi & Moraes, 2008). *Ceratitis capitata*, an exotic species, also has a wide range of hosts, being associated with 94 plant species in Brazil (Zucchi & Moraes, 2012).

Recent studies have been conducted with fruit flies to better understand their spatial distribution in several areas of Brazil (Frighetto et al., 2019; Garcia et al., 2017; Santos et al., 2020). These studies have taken into account landscape arrangement as one of the factors determining species distribution in different parts of the world (Flores et al., 2016; Gutierrez & Ponti, 2011; Kounatidis et al., 2008; Meyer et al., 2007). In general, changes in natural ecosystems, when converting them into agroecosystems, have intensified questions on how to better understand the community structure of pests and their natural enemies as well as investigating the processes determining the abundance of species in different areas (Altieri & Nicholls, 2004; Vandermeer, 1995). Therefore, studies on the spatio-temporal distribution pattern associated with environmental conditions are of high relevance in fruit fly management (Aluja et al., 2003; Aluja & Mangan, 2008; Rull et al., 2018).

Spatio-temporal patterns of distribution in fruit flies have been investigated using different statistical tools such as geostatistics and exponential family models to investigate the frequency distribution of species and populations (Garcia et al., 2017; Nicácio et al., 2019; Oliveira et al., 2019). These include Poisson and negative binomial functions, usually employed to investigate random and clumped ecological distribution patterns. Insects, in general, particularly fruit flies, tend to exhibit clumped distribution because of the common aggregation of larvae in fruits (Deus et al., 2016). Understanding the aggregation patterns of insects may be useful for pest management in critical areas, such as orchards infested by fruit flies. As for fruit

flies in Brazil, this knowledge is still scarce, especially when it involves a time series reflecting real conditions in orchards.

In this study, a long-term time series of *C. capitata*, *A. fraterculus*, and *A. obliqua* was analyzed to investigate the aggregation patterns of fruit fly species, taking into account spatio-temporal distribution in different area delimitations and a time series associated with a management strategy of fruit flies (systems approach). We aim to compare the time series of the three fruit fly species in different spatial delimitations, namely a buffer zone and neighborhoods 1 and 2, to verify the spatio-temporal distribution patterns in the areas. Therefore, we analyzed the influence of the native forest on the distribution of fruit fly species by comparing the abundance distribution for the three species as a function of time, and under different spatial delimitations in a distance gradient from the Atlantic rainforest remnant. Although previous studies have investigated aspects of population dynamics in fruit flies (Aluja et al., 2012; Galli et al., 2019; Raga et al., 2017), a comparative spatio-temporal distribution, taking into account the distance from the native forest as well as pest management, has yet to be performed, especially on three abundant fruit fly species.

3.2 Material and Methods

3.2.1 Data set

The data set was obtained from the monitoring of fruit flies in commercial papaya crops in the State of Espírito Santo, Brazil. This monitoring is part of a set of measures adopted for the management of fruit flies in papaya orchards, the systems approach, which aims to maintain crops with a low prevalence of tephritids, i.e., below seven fruit flies per trap per week (FTD), thus enabling the export of fruits to the USA (Martins & Malavasi, 2003b; Martins et al., 2011).

The study region is located in the vicinity of the municipality of Linhares, with an altitude of 33 m, and it is considered a production center for papaya in the state of Espírito Santo, with a mean production of 50 t/ha year (INCAPER, 2020). In the region, Ombrophilous Dense Forest, also known as Tableland Forest, is prevalent and the predominant climate is warm tropical, and humid, with a rainy season in summer and dry season in winter, reaching a mean annual temperature of 23 °C (ICMBio, 2020).

The fruit flies were collected weekly using McPhail traps, with 300 mL of an attractive solution of hydrolyzed corn protein (5%) for collection of *Anastrepha* specimens, and Jackson traps with the sex pheromone Trimedlure were used to capture males of *C. capitata*. The traps were distributed at 1 trap per 1 ha of cultivated area, using 50% McPhail traps and 50% Jackson traps, interspersed, at different georeferenced points (18°02' 35.7" to 20°33' 58.3" S and 39°13'

00.7" to 41°09' 16.1" W) in commercial papaya production fields in Espírito Santo State (Figure 1). INCAPER – Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural provided us with a time series from 1998 to 2010, containing abundant data for *C. capitata*, *A. fraterculus*, and *A. obliqua*. The nominal *A. fraterculus* is used here in sensu lato, as it comprises a complex of cryptic species.

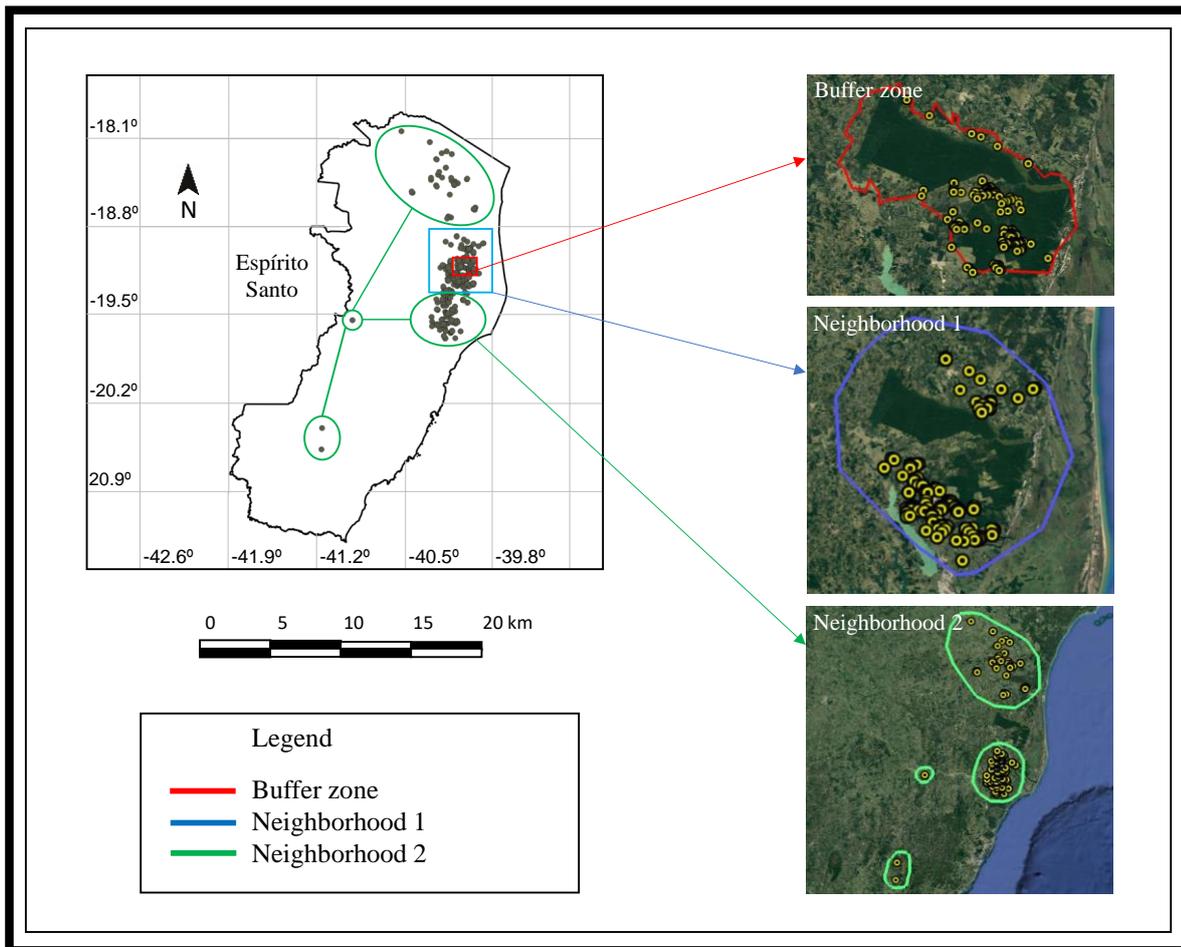


Figure 1. Maps of fruit fly collection sites in the state of Espírito Santo, Brazil, from 1998 to 2010.

3.2.2 Time series with different area delimitations

The dataset was analyzed in different area delimitations to investigate the relationship between abundance and time. Additionally, the frequency distribution patterns of fruit fly species were analyzed in papaya orchards close and distant from the Reserva Natural Vale, an Atlantic forest remnant that is highly biodiverse with approximately 23,000 ha of protected area (Kierulff et al., 2014). The area delimitations were classified as a buffer zone, neighborhood 1, and neighborhood 2 (Figure 1). The buffer zone is a delimitation of the Ministry of Environment of Brazil, an area established around a conservation unit to filter the negative impacts of

activities occurring outside it, such as pollution, invasive species, and the advancement of human occupation, especially in units close to intensely occupied areas. Neighborhood 1 borders the buffer zone and neighborhood 2 is the pool of all areas further from the buffer zone.

As the present study aimed to investigate temporal space aggregation over a wide area regarding landscape composition, the delimited regions were investigated concerning the distance from the Atlantic forest remnant because it is the main element of the landscape capable of significantly influencing the distribution of fruit flies. Considering the center of the Atlantic Forest Reserve, the distances to the buffer zone, neighborhood 1, and neighborhood 2 are 9 km, 17 km, and 50 km, respectively.

The papaya orchards are randomly distributed in these regions with sizes between 2 and 275 ha, with a density of 1,100 to 1,500 plants/ha (Alves, 2003). A total of 332 orchards were studied in the buffer zone, 251 in neighborhood 1, and 378 in neighborhood 2, from 1998 to 2010.

3.2.3 Time series with different periods

The full-time series (1998-2010) was subdivided into two subseries, 1998 to 2003 and 2004 to 2010, to document and compare the evolution of the systems approach, and their effects on the abundance of *C. capitata*, *A. fraterculus*, and *A. obliqua*. The systems approach is a set of actions during the pre- and post-harvest periods, implemented in 1990s to improve production, harvesting, packaging, and transport of a commodity that meets the requirements for quarantine security (Malavasi & Martins, 2005; Martins & Malavasi, 2003b). For convenience, we named the time series as “continuous implementation of the systems approach” for the series between 1998 and 2003, and “impact on abundance” for the series between 2004 and 2010. Continuous implementation of the systems approach determines sequential, permanent, and uninterrupted management actions since the beginning of its implementation. The impact on abundance describes years after which abundance was visibly influenced by the systems approach.

3.2.4 Data analysis

The Shapiro-Wilk test was used to evaluate data normality, with results indicating that the relationship between abundance and rainfall, temperature, and humidity did not follow a normal distribution. Then, Spearman’s correlation was applied to investigate the correlation between the variables mentioned above. Generalized linear models (GLMs) with Poisson and negative binomial errors were used to analyze the relationship between fruit fly abundance and

time, according to the series and subseries mentioned above. The Akaike information criterion (AIC) was used to select the best GLM model to fit the fruit fly dataset.

Based on the results obtained with the GLM models, the frequency distribution of fruit flies was fitted to the negative binomial distribution models to confirm whether the spatio-temporal distribution of *C. capitata*, *A. fraterculus*, and *A. obliqua* followed a clumped pattern, and also to compare the degree of aggregation among areas and time subseries. The number of flies per sampling unit was summarized as a frequency distribution, described as the area delimitations with 0, 1, 2, 3, ...*n* fruit flies. The *k* parameter in the negative binomial distribution was used to estimate the spatial aggregation degree of flies. The value of *k* indicates the highest aggregation with *k* tending to zero. *K* was calculated by computing the maximum likelihood estimate for aggregation, and the fit of the negative binomial model was tested using the Pearson's χ^2 statistic (Crawley, 2007).

3.3 Results

A total of 13,889 adults of *C. capitata* (FTD = 0.007), 2,999 of *A. fraterculus* (FTD = 0.006), and 797 of *A. obliqua* (FTD = 0.006) were collected in papaya orchards in the State of Espírito Santo, during a 13-year monitoring period. The three-time series for the three fruit fly species from 1998 to 2010 described the population dynamics of *C. capitata*, *A. fraterculus*, and *A. obliqua*, indicating a decreasing trend in abundance for the species, particularly visible after 2003 (Figure 2). Regression analyses with Poisson and negative binomial errors were performed for the three datasets showing a significant relationship between time (years) and abundance when data were fitted to the two functions (Table 1). However, the AIC value indicated that the negative binomial model was the best regression function to explain the abundance variation in response to the time series for *C. capitata*, *A. fraterculus*, and *A. obliqua*.

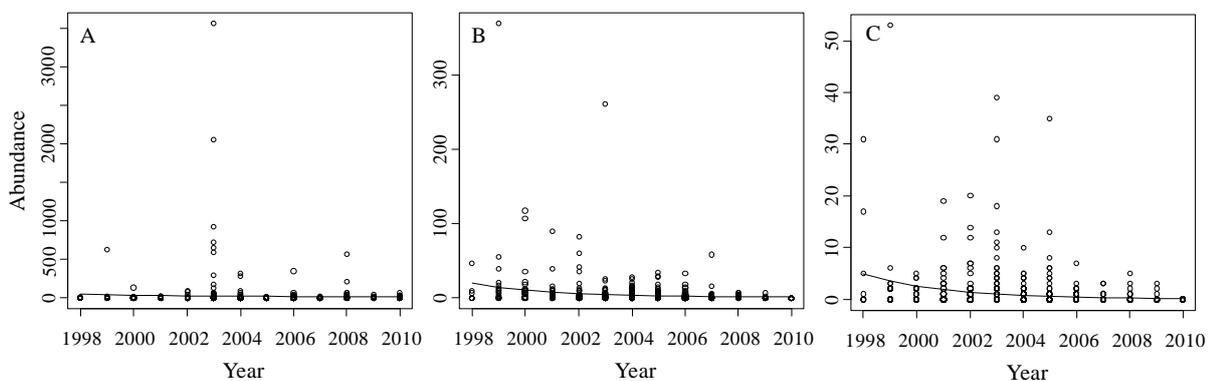


Figure 2. Time series for *C. capitata* (A), *A. fraterculus* (B) and *A. obliqua* (C) describing the variation of abundance in response to time.

Table 1. Regressions with Poisson and Negative binomial errors taking into account fruit fly abundance variation in response to time (1998-2010).

Regression with Poisson errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	287.22	6.44	44.61	<2e-16
Time	-0.14	0.003	-44.19	<2e-16
AIC 101672				
Regression with negative binomial errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	406.30	87.83	44.61	3.73e-06
Time	-0.20	0.043	-4.59	4.29e-06
AIC 3426*				
Regression with Poisson errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	672.09	14.83	45.31	<2e-16
Time	-0.33	0.007	-45.21	<2e-16
AIC 12297				
Regression with negative binomial errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	687.59	58.33	11.79	<2e-16
Time	-0.34	0.03	-11.77	<2e-16
AIC 3189*				
Regression with Poisson errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	579.42	28.25	20.51	<2e-16
Time	-0.28	0.01	-20.50	<2e-16
AIC 3593				
Regression with negative binomial errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	650.86	71.26	9.13	<2e-16
Time	-0.32	0.03	-9.13	<2e-16
AIC 1848*				

*Best model

The abundance variation of the three species in response to time was also significant when fly abundances were compared within the full area from 1998 to 2010 (Table 1). This result was also observed in the delimited areas, buffer zone (Table 2), neighborhood 1 (Table 3), and neighborhood 2 (Table 4).

Table 2. Regressions with Poisson and negative binomial errors taking into account fruit fly abundance variation in response to time (1998-2010) in the buffer zone.

Regression with Poisson errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	243.43	14.78	16.48	<2e-16
Time	-0.12	0.007	-16.36	<2e-16
AIC 13010				
Regression with negative binomial errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	584.97	143.39	4.08	4.51e-05
Time	-0.29	0.07	-4.07	4.70e-05
AIC 848*				
Regression with Poisson errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	904.25	24.80	36.46	<2e-16
Time	-0.45	0.01	-34.38	<2e-16
AIC 4293				
Regression with negative binomial errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	776.93	99.14	7.84	4.64e-15
Time	-0.39	0.05	-7.83	4.83e-15
AIC 910*				
Regression with Poisson errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	940.10	72.56	12.96	<2e-16
Time	-0.47	0.04	-12.96	<2e-16
AIC 651				
Regression with negative binomial errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	757.53	138.27	5.48	4.28e-08
Time	-0.37	0.07	-5.48	4.07e-08
AIC 349 *				

*Best model

Table 3. Regressions with Poisson and negative binomial errors taking into account fruit fly abundance variation in response to time (1998-2010) in the neighborhood 1.

Regression with Poisson errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-399.76	24.28	-16.47	<2e-16
Time	0.20	0.01	16.53	<2e-16
AIC 5942				
Regression with negative binomial errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	-511.19	149.91	-3.41	6.5e-04
Time	0.25	0.07	3.42	6.3e-04
AIC 804*				
Regression with Poisson errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	813.32	25.47	31.93	<2e-16
Time	-0.40	0.01	-31.85	<2e-16
AIC 4263				
Regression with negative binomial errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	826.54	117.16	7.05	1.73e-12
Time	-0.41	0.06	-7.04	1.85e-12
AIC 923*				
Regression with Poisson errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	589.71	50.69	11.63	<2e-16
Time	-0.29	0.02	-11.63	<2e-16
AIC 1170				
Regression with negative binomial errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	607.87	143.33	4.24	2.22e-05
Time	-0.30	0.07	-4.24	2.22e-05
AIC 538*				

*Best model

Table 4. Regressions with Poisson and negative binomial errors taking into account fruit fly abundance variation in response to time (1998-2010) in the neighborhood 2.

Regression with Poisson errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	183.04	9.02	20.29	<2e-16
Time	-0.09	0.004	-19.91	<2e-16
AIC 74733				
Regression with negative binomial errors for <i>C. capitata</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	873.57	168.30	5.19	2.10e-07
Time	-0.43	0.08	-5.17	2.33e-07
AIC 1690*				
Regression with Poisson errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	250.70	32.04	7.82	5.14e-15
Time	-0.12	0.02	-7.79	6.36e-15
AIC 2866				
Regression with negative binomial errors for <i>A. fraterculus</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	326.10	102.06	3.19	1.40e-03
Time	-0.16	0.05	-3.18	1.44e-03
AIC 1335*				
Regression with Poisson errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	439.62	46.08	9.54	<2e-16
Time	-0.22	0.02	-9.54	<2e-16
AIC 1627				
Regression with negative binomial errors for <i>A. obliqua</i>				
Coefficients	Estimate	Standard error	z value	Pr(> z)
Intercept	541.18	117.12	4.62	3.82e-06
Time	-0.27	0.06	-4.62	3.82e-06
AIC 934*				

*Best model

Spearman's correlation analysis between the abundance of fruit flies and the variables temperature, humidity, rainfall, and time (1998-2010) indicated a single negative significant correlation ($\rho = -0.14$ $p < 0.001$), i.e., between abundances of *C. capitata* and humidity.

The frequency distribution analysis (Table 5) shows significant clumped patterns for the three fruit fly species in all the areas studied. When studying the influence on each area, the

three species were more aggregated in the buffer zone. Observing each species separately, it was noticed that the aggregation degree was practically the same for *C. capitata* in the full area, neighborhoods 1, and 2, but the buffer zone exhibited its highest aggregation, reaching $k = 0.08$ (Table 5). *Anastrepha fraterculus* also exhibited the maximum aggregation degree in the buffer zone, but with $k = 0.13$, and in neighborhood 2, it showed its lowest aggregation degree, with $k = 0.25$. *Anastrepha obliqua* exhibited its maximum aggregation in the buffer zone, with $k = 0.03$, and its lowest aggregation level in neighborhood 2.

Table 5. Fits of the negative binomial distribution model to the frequency of *C. capitata*, *A. fraterculus* and *A. obliqua* in full area, buffer zone, neighborhood 1 and neighborhood 2 from 1998-2010.

Full area					
	\bar{x}	s^2	k	$\chi^{2(*)}$	df
<i>C. capitata</i>	7.18	309.21	0.14	6.00	60
<i>A. fraterculus</i>	2.71	100.61	0.15	3.07	117
<i>A. obliqua</i>	0.83	11.26	0.13	5.23	53
Buffer zone					
	\bar{x}	s^2	k	$\chi^{2(*)}$	df
<i>C. capitata</i>	2.31	73.88	0.08	0.01	60
<i>A. fraterculus</i>	1.93	48.43	0.13	0.05	58
<i>A. obliqua</i>	0.37	8.90	0.03	0.80	53
Neighborhood 1					
	\bar{x}	s^2	k	$\chi^{2(*)}$	df
<i>C. capitata</i>	2.35	67.47	0.13	0.03	65
<i>A. fraterculus</i>	4.12	194.80	0.17	0.24	91
<i>A. obliqua</i>	1.06	16.98	0.13	0.01	39
Neighborhood 2					
	\bar{x}	s^2	k	$\chi^{2(*)}$	df
<i>C. capitata</i>	5.97	319.20	0.14	0.10	88
<i>A. fraterculus</i>	2.30	27.90	0.25	5.54	41
<i>A. obliqua</i>	1.08	9.34	0.20	1.28	31

* $p < 0.05$

The comparison between the continuous implementation of the systems approach and the impact on abundance also revealed that the two time series exhibited clumped frequency distribution for the three species analyzed (Table 6). The species showed more aggregated patterns during the impact on abundance when the full area was evaluated. Further evidence of an increase in aggregation can be observed when comparing the *C. capitata* time series in the

buffer zone, with $k = 0.13$ for the series between 1998 and 2003, $k = 0.07$ for the series between 2004 and 2010, and *A. fraterculus* when the k value decreased from 0.2 (1998-2003) to 0.09 (2004-2010) (Table 6). Although *A. obliqua* has exhibited a clear trend to increase its aggregation in the full area, in the buffer zone, and neighborhoods 2, its aggregation degree trended to decrease. In both areas, a loss of aggregation was observed, with k values increasing from the 1998-2003 series to the 2004-2010 series. An increase in aggregation was only observed for the full area and neighborhood 1.

Table 6. Fits of the negative binomial distribution model to the frequency of *C. capitata*, *A. fraterculus* and *A. obliqua* in continuous implementation of Systems approach (1998-2003) and impact on abundance (2004-2010).

(to be continued)

Full area 1998-2003					
	\bar{x}	s^2	k	$\chi^{2(*)}$	Df
<i>C. capitata</i>	5.56	291.55	0.13	0.07	86
<i>A. fraterculus</i>	4.21	167.36	0.20	0.45	91
<i>A. obliqua</i>	1.37	18.77	0.20	0.04	53
Full area 2004-2010					
	\bar{x}	s^2	k	$\chi^{2(*)}$	Df
<i>C. capitata</i>	2.88	123.58	0.10	0.06	88
<i>A. fraterculus</i>	1.55	20.57	0.18	4.39	58
<i>A. obliqua</i>	0.40	3.28	0.11	3.67	35
Buffer zone 1998-2003					
	\bar{x}	s^2	k	$\chi^{2(*)}$	Df
<i>C. capitata</i>	1.70	20.36	0.13	0.0008	22
<i>A. fraterculus</i>	3.83	75.22	0.2	0.003	40
<i>A. obliqua</i>	1.04	30.36	0.07	0.29	54
Buffer zone 2004-2010					
	\bar{x}	s^2	k	$\chi^{2(*)}$	Df
<i>C. capitata</i>	2.08	65.66	0.07	0.005	59
<i>A. fraterculus</i>	0.94	19.86	0.09	1.01	58
<i>A. oblique</i>	0.23	0.48	0.16	4.51	03
Neighborhood 1 1998-2003					
	\bar{x}	s^2	k	$\chi^{2(*)}$	Df
<i>C. capitata</i>	1.28	7.76	0.21	0.03	16
<i>A. fraterculus</i>	8.58	469.11	0.21	0.0001	91
<i>A. oblique</i>	1.96	33.01	0.25	0.07	39

Table 6. Fits of the negative binomial distribution model to the frequency of *C. capitata*, *A. fraterculus* and *A. obliqua* in continuous implementation of Systems approach (1998-2003) and impact on abundance (2004-2010). (conclusion)

Neighborhood 1 2004-2010					
	\bar{x}	s^2	k	$\chi^2(*)$	df
<i>C. capitata</i>	3.01	111.39	0.12	0.04	66
<i>A. fraterculus</i>	1.60	15.60	0.21	0.01	34
<i>A. obliqua</i>	0.65	9.31	0.08	0.001	35
Neighborhood 2 1998-2003					
	\bar{x}	s^2	k	$\chi^2(*)$	df
<i>C. capitata</i>	8.44	444.72	0.15	0.06	86
<i>A. fraterculus</i>	2.37	32.03	0.26	0.12	41
<i>A. obliqua</i>	1.6	16.35	0.21	0.02	31
Neighborhood 2 2004-2010					
	\bar{x}	s^2	k	$\chi^2(*)$	df
<i>C. capitata</i>	3.23	152.54	0.13	0.04	88
<i>A. fraterculus</i>	2.18	23.72	0.24	0.015	29
<i>A. obliqua</i>	0.58	1.71	0.28	0.097	08

* $p < 0.05$

3.4 Discussion

Among the species of Tephritidae reported in agricultural orchards in Brazil, *C. capitata*, *A. fraterculus*, and *A. obliqua* have been dominant (Duarte et al., 2016; Lopes et al., 2015; Querino et al., 2014; Rosa et al., 2018). These species are widely distributed in the Brazilian territory, are highly polyphagous, and have a wide range of hosts already registered (Zucchi & Moraes, 2008, 2012). As observed in surveys of fruit flies in commercial plantations (Aluja et al., 1996), also in the areas of commercial production of papaya in the state of Espírito Santo, few species of fruit flies are common.

Ceratitis capitata was more abundant than the other two species of *Anastrepha* in papaya orchards in the State of Espírito Santo. Studies indicate that *C. capitata* prefers agricultural and/or anthropized environments when compared to *Anastrepha* species that prefer environments with native vegetation (Alvarenga et al., 2010; Querino et al., 2014). The collection methods used in this study may also have influenced the abundance of fruit flies, mainly because *C. capitata* was captured with a trap using a sexual pheromone as bait, and *Anastrepha* species were collected using food attractive traps; however, both methods are usually used for fruit fly monitoring (Silva et al., 2011). Additionally, the eventual dominance

of *C. capitata* in papaya orchards in Espírito Santo can also be interpreted from results showing temporal segregation between *C. capitata* and *A. obliqua*, as previously documented in loquat orchards (Lopes et al., 2015). There are also results showing high dominance of *C. capitata* when co-occurring with *A. fraterculus* in different localities of Argentina (Segura et al., 2006).

The decrease in the abundance of fruit flies, statistically confirmed with Poisson and negative binomial regressions, was potentially due to the impact of the systems approach applied in the studied area. Comparing the abundance values during the years, the decrease in population size is easily noticeable for *A. fraterculus* and *A. obliqua*. However, although *C. capitata* also exhibited a significant decrease in abundance in response to time, it was not as visible as observed in the other species, potentially due to its high variation in abundance values. In 2003, *C. capitata* exhibited a significantly higher abundance than *A. fraterculus* and *A. obliqua*, alongside its abundance in other years. There is no specific reason for this outbreak, although *C. capitata* outbreaks have commonly been noticed in other parts of the world where outbreaks frequently occur (Montoya et al., 2005; Barnes et al., 2006; Carey, 2010).

In the systems approach, papaya fruits are harvested at stages 1 and 2 of ripeness (MAPA, 2008; Martins & Malavasi, 2003b). In these stages, the fruits have high concentrations of benzyl-isothiocyanate (BITC), a natural chemical compound present in fruit latex, and its concentration decreases as the fruit ripen (Nascimento et al., 2003; Seo et al., 1983). BITC acts as an ovicide, inhibiting oviposition, and killing the initial larvae of fruit flies (Seo & Tang, 1982). There is no risk of infestation by these insects when the papaya is harvested in the early stages of maturation.

Despite having two models to describe significantly the fruit fly dynamics for the three species, the AIC values indicated that the negative binomial model was the best regression function to explain the relationship between time and fly abundance. In addition, the analysis taking into account the frequency distribution of the fruit flies confirmed the suitability of the negative binomial model for all scenarios analyzed (different periods: 1998 to 2003, 2004 to 2010, and different areas: full area, buffer zone, and neighborhoods 1 and 2). Insect count data are generally not distributed (Sileshi, 2006), and there is evidence showing that the frequency distribution in this taxonomic group is ecologically described by a negative binomial distribution (Deus et al., 2016; Maldonado et al., 2016; Nicácio et al., 2019). This function characterized by variance significantly higher than the mean (Crawley, 2013), was observed in our results.

Landscape heterogeneity in the buffer zone may have contributed to the greater aggregation of species because it contains a large fragment of preserved natural vegetation

exhibiting large quantities of resources available for fruit fly maintenance, which may condense the concentration of fruit flies in the same area. Fruit flies in agricultural areas tend to be influenced by the presence of fragments of adjacent native vegetation (Aluja et al., 1996; Uchôa & Nicácio, 2010). Forest fragments have contributed to the presence of *Anastrepha* species in adjacent guava orchards (Querino et al., 2014). According to Meats (2007), the conformation of the landscape is a relevant factor in the distribution of fruit flies. Natural environments may result in a more aggregated distribution of flies than other types of environments. In all the scenarios analyzed, *A. obliqua* exhibited abundances significantly lower than those of the other species, and under this condition, any movement of individuals among sampling units may proportionally influence the spatial pattern of distribution. Furthermore, this can result in a decrease in empty units, making the system prone to random occupancy, characterized by k parameter exhibiting values more distant from zero (Reigada & Godoy, 2005).

In neighborhood 1, the aggregation degree of *C. capitata* and *A. obliqua* increased from the first series to the second series, while *A. fraterculus* maintained the same k value. This result, when compared to the buffer zone, allows us to conclude that the population aggregation behavior may change in response to different local conditions, and this is potentially intrinsic to each species, with responses including intense variation regarding distribution among areas. The aggregation trend from the first to the second series was also maintained in neighborhood 2, but similarly to the buffer zone, *A. obliqua* decreased its aggregation from the first to the second series.

At the global scale, it is usual to observe changes in aggregation in insects described by outbreaks over time, mainly in response to climatic changes, with important implications for pest management (Williams & Liebhold, 1995). However, at the local scale, the spectrum of variable values changes, such as temperature and others generally investigated, is much more restricted, making the influence of temperature or other variables less impactful, as observed in our correlation tests. Therefore, at the local scale, there are potentially other factors capable of influencing the spatial distribution of insects. Changes in the spatial distribution patterns of host plants can influence the intensity of ecological interactions between plants and herbivorous insects (Hakes et al., 2018). Papaya orchards in the investigated areas were not distributed significantly far from each other. This condition may be associated with the general aggregation pattern discussed here. However, distance and size are not homogeneous among the orchards, and these factors certainly influenced each species differently, explaining the small differences in aggregation observed in the three fruit fly species, with particular emphasis on *A. obliqua*.

Comparing the two periods in the full area, a slight aggregation trend was found for the three species in the series 2004-2010. This result may be due to the management actions implemented in the papaya orchards in Espírito Santo State (Martins & Malavasi, 2003b), mainly because the fruit fly species aggregation is implied in several areas with zero individuals. The decrease in fruit flies in response to the systems approach implementation resulted in more empty areas when compared with the previous condition. This information is relevant, mainly for when sequential sampling plans are implemented to monitor critical population sizes, with direct implications for decisions based on economic threshold and/or prediction of pest outbreaks (Ferreira & Godoy, 2014; Nicácio et al., 2019).

Our results can also be useful as subsidies to optimize monitoring plans and risk mapping in orchards. In this sense, studies should be carried out to discuss the possibility of sampling plans suitable to local realities and critical areas to minimize the costs of installing traps and/or enhancing pest control in locations with a higher risk of infestation.

3.5 Conclusion

The aggregation pattern of fruit fly species was significantly influenced by area and time. The buffer zone and the impact on the abundance period provided a maximum aggregation of populations of *C. capitata*, *A. fraterculus*, and *A. obliqua*, indicating a significant association between aggregation degrees and a decrease in the abundance of fruit flies.

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4 SPATIO-TEMPORAL CO-OCCURRENCE OF *Anastrepha* SPECIES (DIPTERA, TEPHRITIDAE) IN PAPAYA ORCHARDS IN THE STATE OF ESPÍRITO SANTO, BRAZIL

Abstract

The co-occurrence of the ecological patterns of *Anastrepha* species in papaya orchards was investigated in areas at different distances from a native forest in Espírito Santo, Brazil. By using a long term series, with dataset from 1998 to 2010, we investigated the co-occurrence of fruit flies in three delimited areas at different distances from the Atlantic Rainforest. The co-occurrence was also investigated by comparing two periods characterized by the implementation of a special pest management (from 1998 to 2003), named systems approach, and its impact on fruit fly diversity (from 2004 to 2010). The interaction strength for positive associations was also evaluated. The results indicated the occurrence of positive and random associations between species pairs. No negative association was observed in the investigated pairs. The influence of time and different spatial delimitations was discussed, as well as the interaction strength for positive association of species pairs. The pattern of co-occurrence was different between areas and over time, with the interaction strength varying significantly over time.

Keywords: interspecific interaction, fruit flies, systems approach, time series.

4.1 Introduction

Fruit flies (Diptera, Tephritidae) are of great economic importance because several species attack fruit orchards in tropical and subtropical areas (White et al., 2000). In Brazil, there are 121 fruit fly species of the genus *Anastrepha* (Zucchi and Moraes, 2008), some of which are economically important and may cause loss of papaya exports for quarantine reasons (Martins and Fornazier, 2014). The presence of these pests influences the production of papaya, leading to a considerable increase in production costs and significant losses in productivity by limiting the export of fresh fruits (Martins et al., 1993; Martins et al., 2012).

Several fruit fly species exploit discrete resources, mainly fruits, commonly present in commercial orchards (Sousa et al., 2019; Soares et al., 2020), but also frequently use natural forest host plants (Garcia et al., 2008; Lampert et al., 2020). Several aspects of pest management have been intensely investigated in fruit flies (Jessup et al., 2007; Phillips, 2013; Dias et al., 2018). However, ecological aspects, such as co-occurrence or coexistence of species have not yet been systematically analyzed. Interspecific interaction is an important issue for insect community structure, and important studies have documented significant aspects of this process, mainly with respect to dispersal and also in the context of biological invasions (Denno et al., 1995; Malacrida et al., 2007; Papadoulos, 2014).

There is significant importance in interspecific interactions of fruit flies (Denno et al., 1995), mainly because they may use similar food resources, despite particular differences in choosing and exploiting different fruits. The geographical movement of tephritids have been discussed in the context of competition and displacement of species, with relevance to the local community structure of fruit flies (Denno et al., 1995, Papadoulos, 2014). The introduction of tephritids in areas already occupied by local species may result in an important decline of native species with implications for the structure of the local fauna, resulting in changes in the interaction strength among fruit flies and their natural enemies (Segura et al., 2006). The way species handle the use of resources, when it is shared, may determine if the mere time co-occurrence of species, i. e., species found simultaneously in the same place, could result in a coexistence process (Duyck et al., 2008).

Species coexistence depends on intensive success over several generations, demanding active interaction between species and studies to prove its real consolidation, which are essentially founded on the ways in which species co-occur (HilleRisLambers et al., 2012; Staples et al., 2016). The time of co-occurrence and the response of each interacting species may reveal the potential for competitive exclusion or the coexistence level. At some extension, species can be positively, negatively or randomly associated with each other (Gotelli & Ulrich, 2010; Veech, 2013; Griffith et al., 2016). Therefore, co-occurrence can be interpreted as a measurable event, characterized by the study of a pair of species.

In this study, we investigated the co-occurrence of the ecological patterns of *Anastrepha* species in papaya orchards, distributed in areas at different distances from a native forest in Espírito Santo, Brazil. By using a long-term series, with a dataset from 1998 to 2010 we studied the co-occurrence of fruit flies in three delimited areas at different distances from the Atlantic Rainforest. The co-occurrence was also investigated comparing two periods, from 1998 to 2003 and from 2004 to 2010, characterized by the implementation of special pest management, named systems approach, and its impact on the fruit fly diversity. The interaction strength between pairs of species was also analyzed.

4.2 Materials and Methods

4.2.1 Dataset

Data on the co-occurrence of *Anastrepha* were obtained from the monitoring of fruit flies in commercial papaya orchards in Espírito Santo state, Brazil, with collections made by Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER). The

monitoring is part of a fruit fly management program (systems approach), in order to make possible the export of papaya to the USA (Martins and Malavasi, 2003).

Fruit flies were collected weekly using McPhail traps containing 5% hydrolyzed corn protein, from 1998 to 2010. One trap per 2 ha was used in georeferenced points in papaya orchards (18° 02'35.7" to 20° 33'58.3" S and 39° 13'00.7" to 41° 09'16.1" W) (Fig. 1).

Our study was based exclusively on the collected females, since the identification of *Anastrepha* species is based mainly on aculeus tip (e.g., Zucchi, 2000). The nominal *A. fraterculus* is being used herein in lato sensu as it comprises a complex of cryptic species. The voucher specimens are deposited at the Museum of Entomology Luiz de Queiroz –(MELQ), Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo and at the Regional Museum of Entomology at the Federal University of Viçosa.

4.2.2 Collection area

Presence/absence dataset was investigated from a pool of *Anastrepha* species widely distributed in orchards. Dataset was analysed for the full papaya area and for three spatial delimitations namely the buffer zone, neighbourhood 1 and neighbourhood 2, in order to compare the distribution of fruit flies in growing distances from the Atlantic rainforest, a protected area (23,000 ha) with high biodiversity (Fig. 1). The buffer zone is a delimitation bordering the protected rainforest created to filter negative impacts, such as pollution, invader species and human activities. Neighbourhood 1 is the area between the buffer zone and neighbourhood 2, a set of all areas distant from the Atlantic rainforest (Fig. 1).

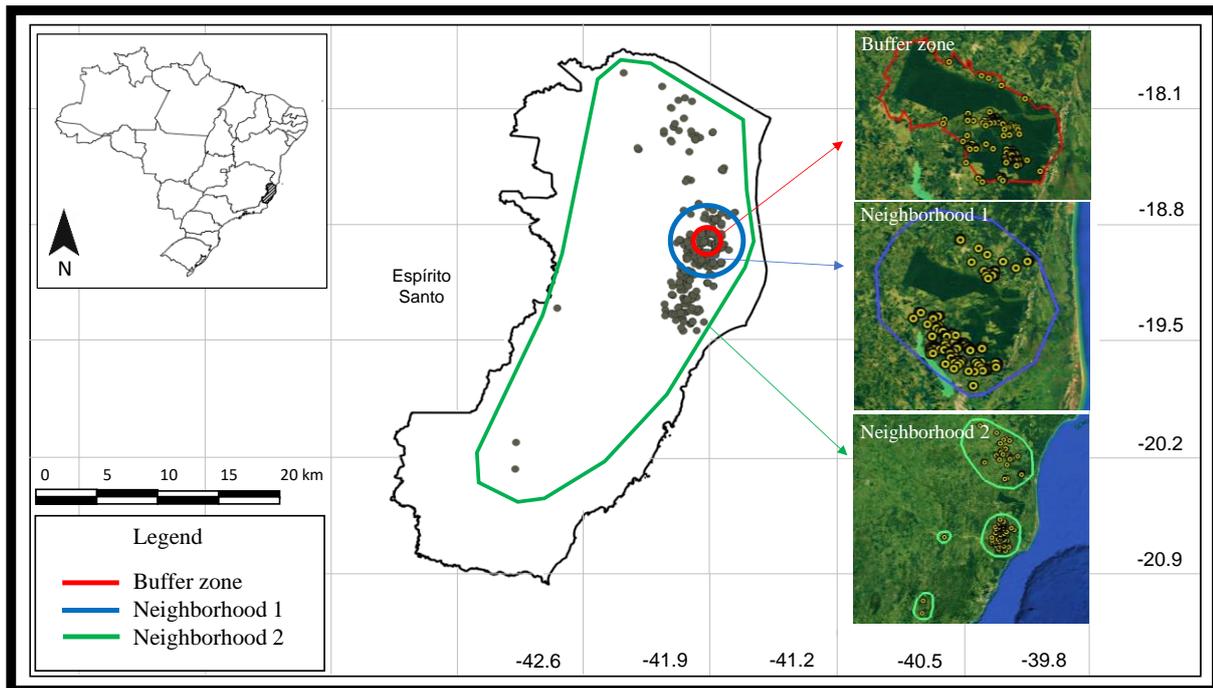


Figure 1. Collection areas in papaya orchards in the state of Espírito Santo, Brazil (modified from Araujo et al. [in press])

The buffer zone, and neighbourhoods 1 and 2 are respectively at distances of 9, 17 and 50 km, from the center of the Atlantic Rainforest. The papaya orchards, randomly distributed in these areas, vary in size, from 2 to 275 ha.

4.2.3 Time series and systems approach

The full time series were subdivided into two, named continuous implementation of systems approach (1998-2003) and systems approach impact on diversity (2004-2010). This subdivision was proposed to compare the co-occurrence of fruit fly species in the two periods, 1998-2003 and 2004-2010. The systems approach is a management program implemented in the 1990s in papaya orchards in the state of Espírito Santo, which includes a set of measures that aim to reduce the incidence of fruit flies in the orchards, with the objective to meet quarantine requirements for export (Martins and Malavasi, 2003).

4.2.4 Co-occurrence analysis

A recent proposition to model the co-occurrence between species has been made by Veech (2013), to investigate the probability that species co-occur with frequencies higher or lower than the observed frequency. The probabilistic formalism considers that $P_{1,2}$ is the probability that species 1 and 2 will co-occur according to a frequency either lower P_{low} or

higher P_{high} than the observed one. Then, when the probability of species 1 co-occurring with species 2 is higher than the observed frequency the association is considered negative and the opposite condition determines a positive association. The association is said to be random when the expected and observed frequencies are approximately the same (Veech, 2013). Species co-occurrence involves the movement of species towards the same location, with scenarios similar to habitat selection. Assuming that in a specific patch, species 1 and 2 tend to be present at the same time, the probability that both species co-occur exactly at $n(p)$ patches is given by,

$$P_{n(p)} = \frac{\binom{s_1}{n(p)} \binom{s-s_1}{s_2-n(p)}}{\binom{s}{s_2}}$$

In this equation for $n(p) = 1$ to s_1 sites (or patches), s_1 = number of patches where species 1 occurs, s_2 = number of patches where species 2 occurs, and s = total number of patches where both species could occur. The term $\binom{s_1}{n(p)}$ determines the number of ways of selecting $\binom{s_1}{n(p)}$ patches containing species 1, given that there are s_1 of such patches in the set of all patches. The term $\binom{s-s_1}{s_2-n(p)}$ is the number of ways of selecting $s_2 - n(p)$ patches that have species 2 but not species 1 given that there are $s - s_1$ of such patches. Multiplying these two quantities together (the numerator) gives the total number of ways of selecting $n(p)$ patches with species 1 and 2. The term $\binom{s}{s_2}$ determines the total number of ways that s_2 number of sites could be obtained out of a total of s sites. Thus, the equation gives the proportion of s_2 patches that also have species 1, under the condition that the two species co-occur at $n(p)$ habitats (Veech, 2013; Griffith et al., 2016).

The analyses were performed using the dataset of *Anastrepha* species (n=961) organized in areas classified as buffer zone (n=332), neighbourhood 1 (n=251) and neighbourhood 2 (n=378) between 1998 and 2010, with the two sub series continuous implementation of systems approach (1998-2003) and systems approach impact on diversity (2004-2010). Presence and absence matrices were built based on species observed in the areas mentioned above using a probabilistic model proposed by Veech (2013) to investigate significant paired co-occurrences. The package used (“co-occur”) is written in R computation language (Griffith et al., 2016). This model calculates the theoretical frequency of co-occurrence for each pair of species based on random distribution and independent of other species distributions (Griffith et al., 2014), comparing it with observed frequency to allow estimation of the probability of species co-

occurrence. A 5% significance level was used to consider a significant frequency, with positive ($p_{gt} < \alpha$), negative ($p_{lt} < \alpha$) and non-significant co-occurrence.

4.2.5 Interaction strength for positive associations

Since no negative association was detected, just the interaction strength for positive associations was estimated for all species pairs established with the co-occurrence analysis. GLM with Poisson errors was used to analyze the influence of each pair of species on each other. The objective of this analysis was to investigate a bidirectional relationship, evaluating both the influence of species 1 on species 2 and the reverse influence. Then, to investigate the positive co-abundance, species pairs were established both as dependent and independent variables. Statistical significance was evaluated at $p < 0.05$. The regression coefficient (slope) was used to estimate the positive interaction strength between species. Regression coefficient has regularly been used as competition coefficient in several studies emphasizing interspecific competition, evidencing the interaction abilities of species pairs (Hallett and Pimm, 1979; Luo et al., 1998; Hart et al., 2018). The interaction strength between species pairs was compared between time and spatial delimitations with linear regression and overlap of standard deviations. All analyses were performed in the language R (Crawley, 2013).

4.3 Results

4.3.1 *Anastrepha* species

A total of 5,278 females, belonging to 35 species of *Anastrepha*, were recorded in papaya orchards during a monitoring of 13 years. The highest number of fruit flies was observed in neighbourhood 2, with 27 species. The second highest number of fruit flies was observed in neighbourhood 1, with 25 species and the third one was recorded in the buffer zone, with 23 species. Seventeen species were shared in the three areas, and *A. fraterculus* and *A. obliqua* were the most abundant species (Table 1).

Table 1. Relative abundance of fruit fly species from 1998 to 2010 in papaya orchards in the state of Espírito Santo, Brazil

<i>Anastrepha</i> species	Full Area (%)	Buffer Zone (%)	Neighbourhood 1 (%)	Neighbourhood 2 (%)
<i>A. fraterculus</i> *	56.82	75.83	60.91	41.18
<i>A. obliqua</i> *	15.10	9.24	14.21	19.59
<i>A. distincta</i> *	7.96	3.03	6.97	11.94
<i>A. serpentina</i> *	5.95	1.97	4.99	9.32
<i>A. pickeli</i> *	5.85	4.09	5.95	6.88
<i>A. pseudoparallela</i> *	1.82	1.06	1.02	3.01
<i>A. bahiensis</i> *	1.59	0.83	1.29	2.34
<i>A. barbiellini</i> *	1.08	0.83	0.64	1.62
<i>A. manihoti</i> *	0.63	0.08	1.45	0.24
<i>A. zenildae</i> *	0.53	0.53	0.59	0.48
<i>A. montei</i>	0.36	0.15	0.43	0.00
<i>A. bivittata</i> *	0.32	0.15	0.32	0.43
<i>A. antunesi</i> *	0.27	0.08	0.05	0.57
<i>A. parallela</i> *	0.27	0.68	0.11	0.14
<i>A. leptozona</i> *	0.19	0.38	0.05	0.19
<i>A. sororcula</i> *	0.17	0.15	0.21	0.14
<i>A. bondari</i>	0.13	0.15	0.00	0.24
<i>A. nascimentoi</i> *	0.13	0.15	0.11	0.14
<i>A. sp. 1</i>	0.11	0.00	0.00	0.29
<i>A. martinsi</i> *	0.09	0.15	0.16	0.43
<i>A. atlantica</i>	0.08	0.00	0.00	0.19
<i>A. bezzii</i>	0.08	0.00	0.05	0.14
<i>A. zernyi</i>	0.08	0.00	0.00	0.19
<i>A. amita</i>	0.06	0.00	0.16	0.00
<i>A. quararibae</i>	0.06	0.00	0.05	0.10
<i>A. consonbrina</i>	0.04	0.00	0.05	0.05
<i>A. grandis</i>	0.04	0.08	0.00	0.05
<i>A. minensis</i>	0.04	0.15	0.00	0.00
<i>A. sagittifera</i>	0.04	0.00	0.11	0.00
<i>A. sp. 2</i>	0.04	0.15	0.00	0.00
<i>A. dissimilis</i>	0.02	0.08	0.00	0.00
<i>A. furcata</i>	0.02	0.00	0.05	0.00
<i>A. lanceola</i>	0.02	0.00	0.00	0.05
<i>A. mixta</i>	0.02	0.00	0.05	0.00
<i>A. tumida</i>	0.02	0.00	0.00	0.05

*common species between areas

4.3.2 Co-occurrence

The co-occurrence between 79 species pairs was evaluated because these pairs had co-occurrence greater than or equal to 1. However, when the three areas were separately analyzed the number of pairs recorded was significantly different among them and also with respect to the total area. In the buffer zone, 17 pairs were observed, 34 pairs were recorded in the neighbourhood 1 and 60 in the neighbourhood 2. In all scenarios, only positive and random associations were observed and most species pairs were positive, which was higher in orchards more distant from the forest.

The main species which contributed to the observed probabilistic patterns were *A. fraterculus* and *A. obliqua*, which exhibited positive co-occurrence with most of the species (Fig. 2).

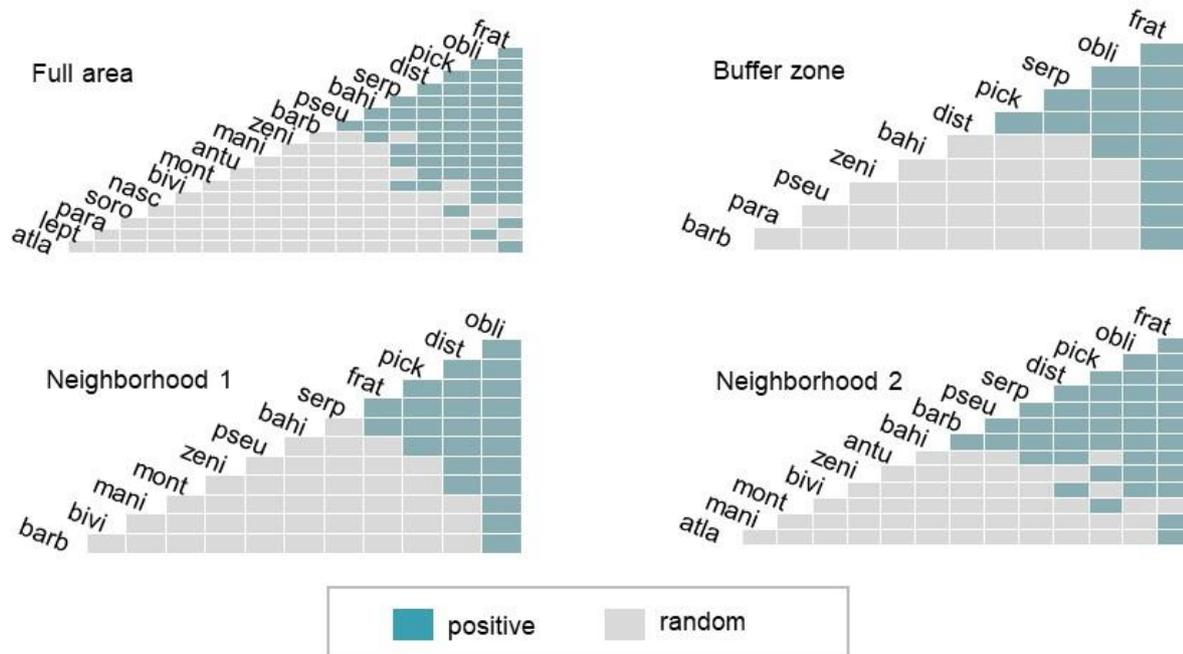


Figure 2. Co-occurrence matrix for *Anastrepha* species in papaya orchards in the state of Espírito Santo, Brazil (frat. *A. fraterculus*, obli. *A. obliqua*, dist. *A. distincta*, serp. *A. serpentina*, pick. *A. pickeli*, pseu. *A. pseudoparallela*, bahi. *A. bahiensis*, barb. *A. barbiellinii*, mani. *A. manihoti*, zeni. *A. zenilda*, mont. *A. montei*, bivi. *A. bivittata*, antu. *A. antunesi*, para. *A. parallela*, lept. *A. leptozona*, soro. *A. sororcula*, nasc. *A. nascimentoi*, atla. *A. atlantica*)

In order to understand the individual contribution of each species to the establishment of positive and random pairs, a pairing profile was created (Fig. 3). The pairing profile was used to measure the percentage of significant interactions per species. Comparing the full area with the buffer zone and neighbourhoods 1 and 2, it is possible to observe some similarities in the full area, as well as neighbourhoods 1 and 2. In these positive and random scenarios, co-occurrences occupy similar percentages, but in the buffer zone there is a clear prevalence of positive pairing (Fig. 3).

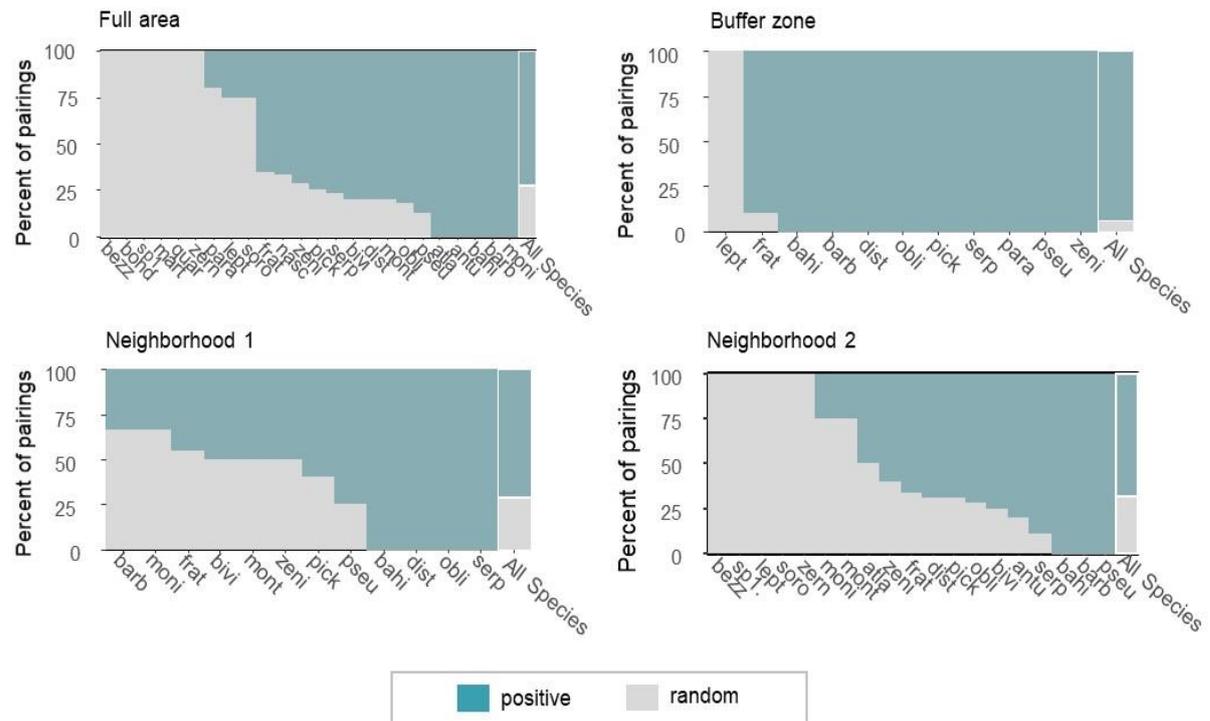


Figure 3. Percentage of contribution of each *Anastrepha* species in different areas for positive and random establishment of species pairs in papaya orchards in the state of Espírito Santo, Brazil from 1998 to 2010 (frat. *A. fraterculus*, obli. *A. obliqua*, dist. *A. distincta*, serp. *A. serpentina*, pick. *A. pickeli*, pseu. *A. pseudoparallela*, bahi. *A. bahiensis*, barb. *A. barbiellinii*, mani. *A. manihoti*, zen. *A. zenildae*, mont. *A. montei*, bivi. *A. bivittata*, antu. *A. antunesi*, para. *A. parallela*, lept. *A. leptozona*, soro. *A. sororcula*, bond. *A. bondari*, nasc. *A. nacementoi*, mart. *A. martinsi*, atla. *A. atlantica*, bezz. *A. bezzii*, quar. *A. quararibae*).

In the full area, 24 species of *Anastrepha* exhibited significant interactions with 18 species contributing to the establishment of at least a positive pair. *Anastrepha atlantica*, *A. antunesi*, *A. bahiensis*, *A. barbiellinii* and *A. manihoti* presented only positive associations (Fig. 3). In the buffer area, 9 of the 11 species established only positive pairs. In addition, *A. fraterculus* presented 90% of positive associations, and for *A. leptozona* 100% of associations were random (Fig. 3). In neighbourhood 1, all species exhibited at least 1 positive association with other species, with *A. bahiensis*, *A. distincta*, *A. obliqua* and *A. serpentina* showing exclusively positive pairs (Fig. 3). Neighbourhood 2, exhibited a similar pattern compared with the full area, with more than 50% of species significantly contributing to positive associations, with *A. bahiensis* and *A. barbiellinii* showing 100% of positive pairs (Fig. 3).

4.3.3 Systems approach

The pattern of co-occurrence in the continuous implementation of systems approach (1998-2003) and the systems of impact on diversity (2004-2010) showed that the number of positive and random pairs was lower in the first series compared to the second time series. No negative pair was recorded. Neighbourhood 2 exhibited higher positive pairs than the buffer zone and neighbourhood 1. Positive pairs were more frequent in *A. fraterculus*, *A. obliqua*, *A. serpentina*, *A. pickeli* and *A. distincta* (Fig. 4).

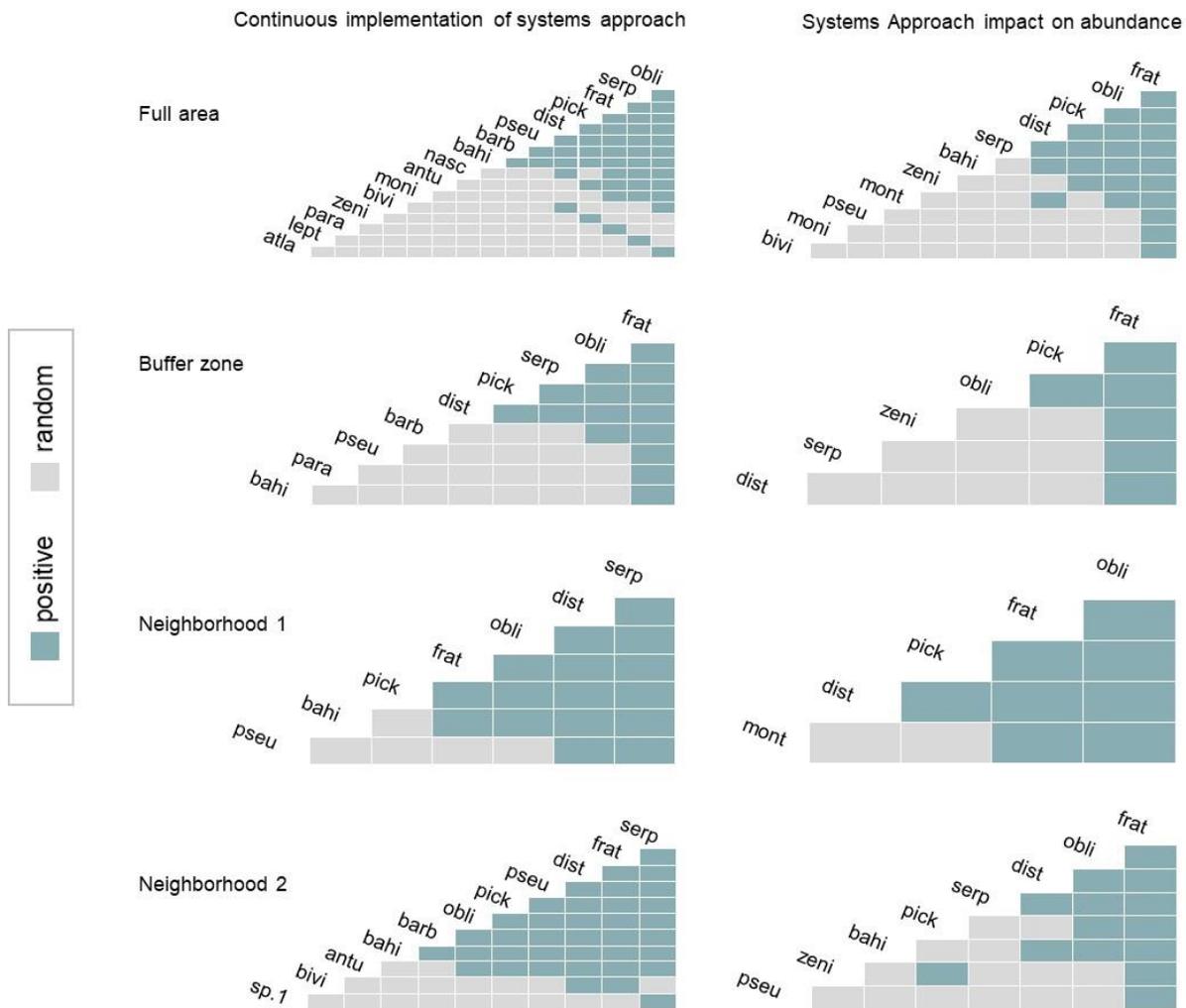


Figure 4. Co-occurrence matrix for *Anastrepha* species comparing the first series, 1998-2003, with the second series 2004-2010 (frat. *A. fraterculus*, obli. *A. obliqua*, dist. *A. distincta*, serp. *A. serpentina*, pick. *A. pickeli*, pseu. *A. pseudoparallela*, bahi. *A. bahiensis*, barb. *A. barbiellinii*, mani. *A. manihoti*, zen. *A. zenildae*, mont. *A. montei*, bivi. *A. bivittata*, antu. *A. antunesi*, para. *A. parallela*, lept. *A. leptozona*, soro. *A. sororcula*, nasc. *A. nascimentoi*, atla. *A. atlantica*).

The highest percentage of positive pairs was observed in the buffer zone and the lowest percentage of positive pairs was found in neighbourhood 1 (Fig. 5). Comparing the two series in the full area, there was no expressive difference of positive pairs frequency for all study areas (Fig. 5).

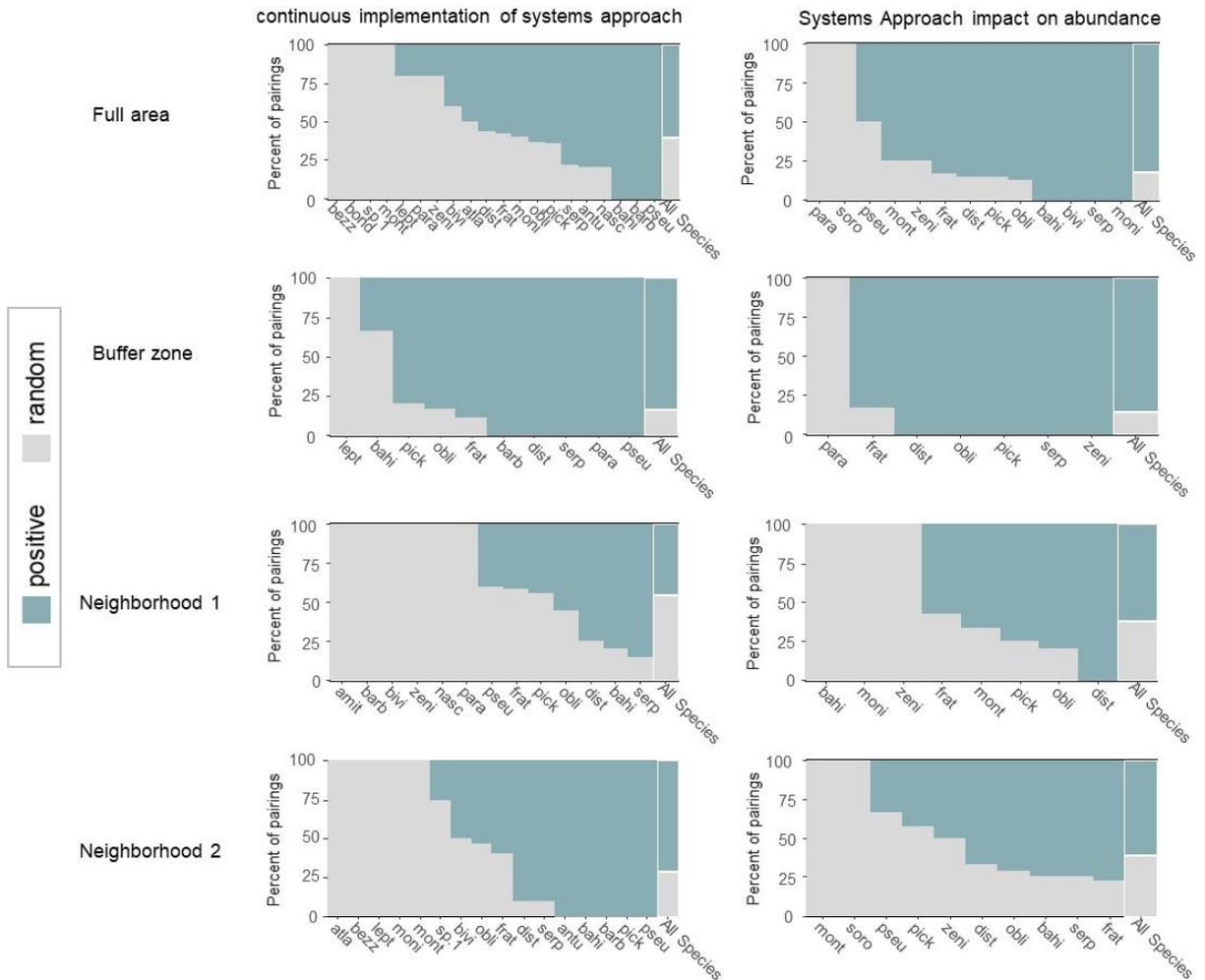


Figure 5. Percentage of individual contribution of *Anastrepha* species in all study areas for positive and random pair establishment in papaya orchards comparing the first series (1998-2003) with the second one (2004-2010) (frat. *A. fraterculus*, obli. *A. obliqua*, dist. *A. distincta*, serp. *A. serpentina*, pick. *A. pickeli*, pseu. *A. pseudoparallela*, bahi. *A. bahiensis*, barb. *A. barbiellinii*, mani. *A. manihoti*, zeni. *A. zenilda*, mont. *A. montei*, bivi. *A. bivittata*, antu. *A. antunesi*, para. *A. parallela*, lept. *A. leptozona*, soro. *A. sororcula*, bond. *A. bondari*, nasc. *A. nascimentoi*, atla. *A. atlantica*, bezz. *A. bezzii*, amit. *A. amita*).

The interaction strength values for positive association between species pairs exhibited a slight change when comparing the full area with the three delimited areas namely: buffer zone,

neighbourhood 1 and neighbourhood 2. The buffer zone exhibited the lowest value for interaction strength. However, the comparison among area values was characterized by a clear overlap among areas (Fig. 6).

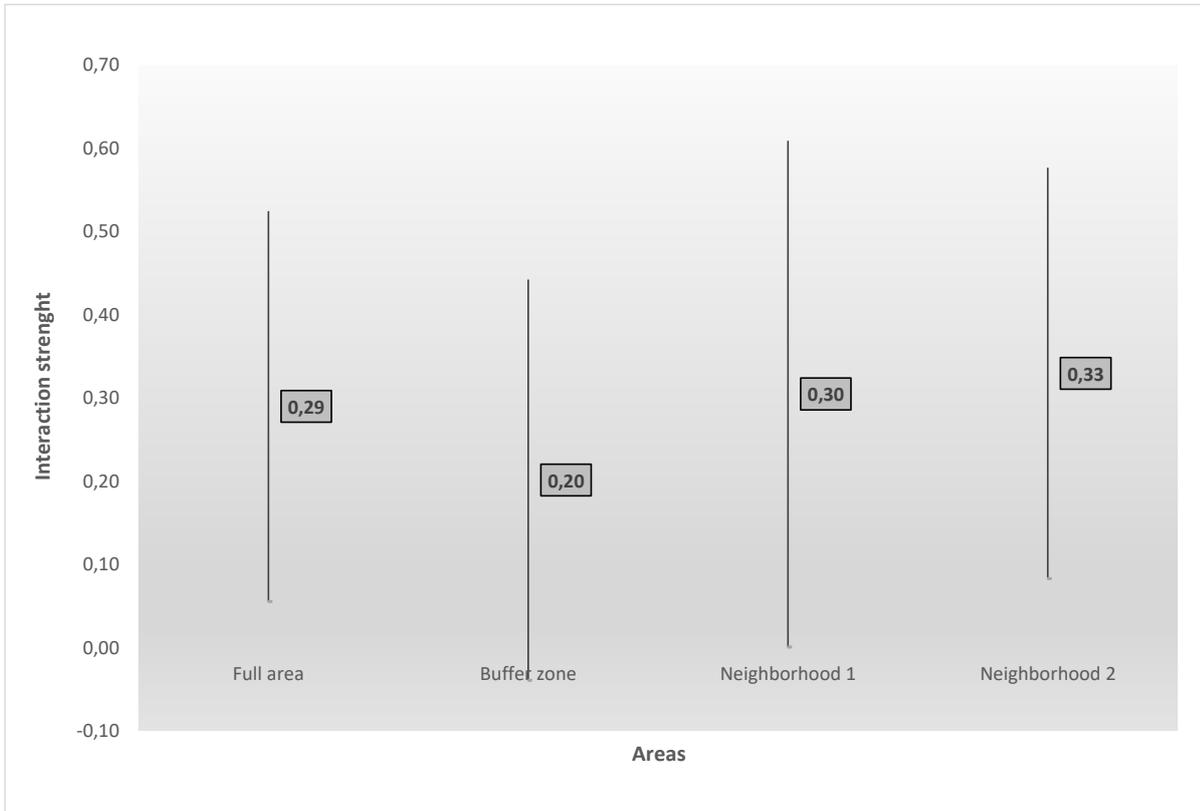


Figure 6. Comparison of interaction strength for positive associations in three delimited areas of papaya orchards in the state of Espírito Santo, Brazil from 1998 to 2010. Values in boxes determine the mean, and vertical lines show the standard deviation.

There was a slight but significant change in the interaction strength values during the years, with respect to the full area, evaluated by a linear regression ($F = 6.6$, $p = 0.01$) (Figure 7). However, this parameter was not strongly influenced when comparing the values within the two periods of the systems approach, but it was by examining the complete period. The trend line shows the increase in interaction strength. The absence of values for some years reflects no establishment of species pairs with positive associations. The relationship between time and interaction strength was also significant for the buffer zone ($F = 7.8$, $p = 0.03$), although the number of species pairs obtained was very low in this spatial delimitation, with pairs established only in the years 1999, 2003 and 2005. On the other hand, the relationship between time and interaction strength was not significant for neighbourhood 1 ($p = 0.08$) and marginally

significant for neighbourhood 2 ($F = 3.8$, $p = 0.054$), but with practically no variation in the interaction strength values in response to time.

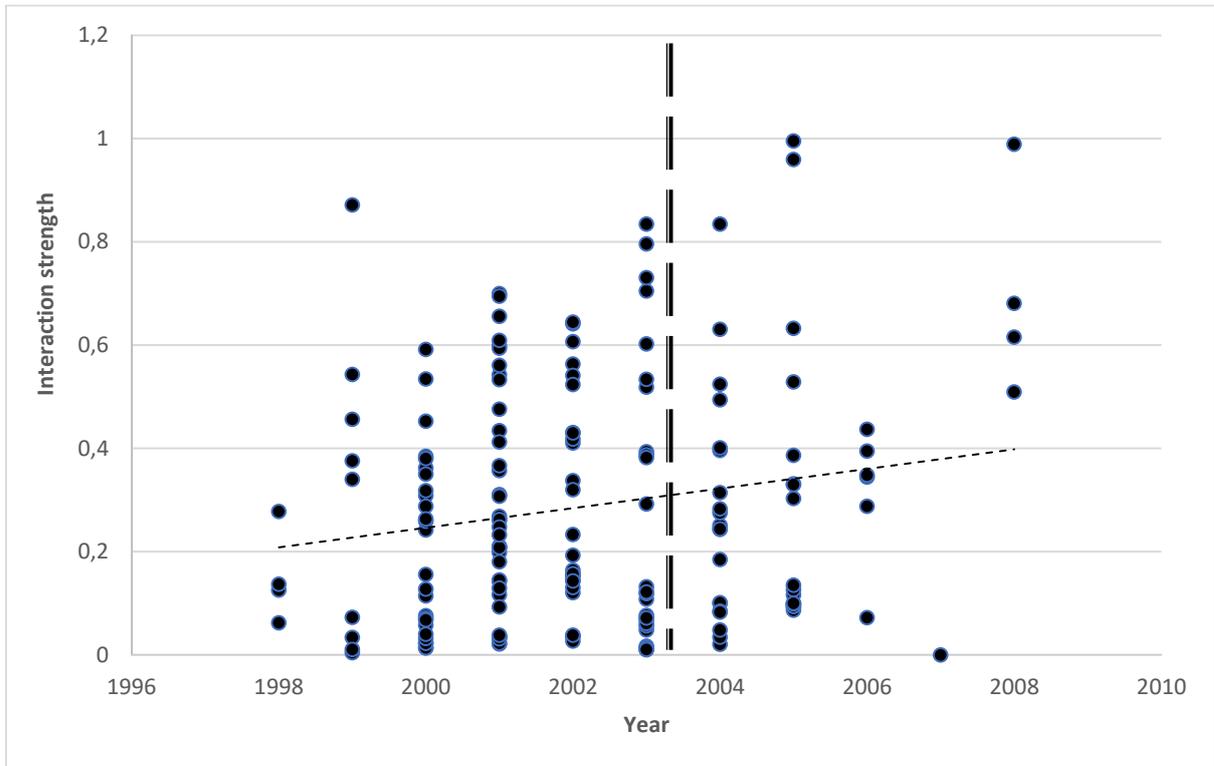


Figure 7. Comparison of interaction strength for positive associations over time in relation to papaya orchards in the state of Espírito Santo, Brazil from 1998 to 2010. Values to the left of the dashed vertical line represent continuous implementation of systems approach (1998-2003). Values to the right of the dashed vertical line represent systems approach impact on diversity (2004-2010).

4.4 Discussion

The state of Espírito Santo showed higher diversity of *Anastrepha* species in comparison with the other Brazilian states, totalling 39 species recorded (Zucchi and Moraes, 2008), with 35 of them observed in the current study. For each delimited area, more than 20 species were obtained while monitoring papaya orchards in Espírito Santo, the number of species was higher than normally collected in previous fruit fly surveys performed in other Brazilian states. The diversity of *Anastrepha* species is due to extensive fruit fly surveys carried out mostly in the last two decades in the state. In addition, the species richness is influenced by Atlantic Rainforest, which contributes to the conservation of species (Monteiro et al. 2019). The buffer zone showed the lowest richness of *Anastrepha* species (23), but its values is very close to those

in neighbourhood 1 and 2 (respectively 25 and 27 species). Most of the species were common to the three areas, probably because of predominant of some species of fruit trees. In addition, the richness of fruit fly species is mostly influenced by availability of food resources in the areas, and in the current study, papaya was predominant, making the habitats very similar to each other.

Anastrepha fraterculus and *A. obliqua* were the most abundant species in both the full area and the three areas. The dominance of few fruit fly species is generally common in surveys in an agricultural environment (Aluja et al., 1996). These species have been recorded as dominant in commercial orchards in Brazil (Dutra et al., 2009; Marsaro Junior et al., 2013; Oliveira et al., 2017). These species are part of a complex of cryptic species with a wide range of hosts (Zucchi and Moraes, 2008).

Our results showed that *Anastrepha* species co-occur in papaya orchards in Espírito Santo, exhibiting positive and random associations. No negative association was detected with the co-occurrence probability model. The simultaneous presence of fruit flies in papaya orchards is not implied in symbiotic associations. This co-occurrence serves as an evidence that some species possibly use similar food resources from host plants or other plants that occur nearby (Gotelli and Ulrich, 2010; Veech, 2013). Traps distributed throughout the area are capable of collecting several fruit fly species not always living in the areas sampled. The absence of negative associations suggests *a priori* nonexistence of competitive interactions in *Anastrepha* species (Gotelli and Ulrich, 2010, Lopes et al., 2015). Positive associations are certainly evidences of co-occurrence, but are also an indication of interspecific coexistence (Gotelli and Ulrich, 2010; Veech, 2013).

Papaya orchards are not considered as primary hosts for fruit flies and perhaps this is the reason why our results did not indicate negative association among species, and consequently possible absence of segregation of species, as inversely observed in orchards of several other fruits (Lopes et al., 2015). Studies have reported that weak competitive interactions allow the coexistence of species in several communities due to the availability of different resources and ability to exploit them (Neill et al., 2020). With this perspective, our hypothesis to support the prevalence of positive associations comes from the availability of diversified resources found in the Atlantic forest and their fragments near and/or randomly distributed among orchards, as also observed by other authors (Lopes et al., 2015; Araujo et al., 2019). A study conducted in guava orchards in Brazil, revealed that the diversity of fruit flies is strongly influenced by fragments of native vegetation present around the orchards (Querino et al., 2014).

Spatial distribution is another aspect capable of explaining co-occurrence patterns in fruit flies, as observed in the co-infestation patterns of *A. fraterculus* and *C. capitata* in Argentina (Devescovi et al., 2015). Understanding spatial distribution in different scales is essential to analyzing the ecological patterns of insect distribution. Different ecological processes may act at different scales in insect populations producing results, which are not always visible on a single scale (Wildemeersch et al., 2019). Aggregation and segregation spatial patterns may emerge at different scales in fruit flies, explaining different spatial configurations for species living in different habitats, geographical regions and time periods (Lopes et al., 2015; Deus et al., 2016).

Area size is another important aspect determining distribution patterns, which are capable of explaining, at least to some extent, intra (Canuto et al., 2019) and interspecific interactions (Shea and Chesson, 2002; Byers and Noonburg, 2003; Kaplan and Denno, 2007). Fruit flies use refuge areas and niche partitioning for coexistence in a regional scale (Deus et al., 2016). Also, climatic factors certainly act on the establishment and coexistence of species (Duyck et al., 2004). In Brazil, co-occurrence in fruit flies shows both positive and negative associations under specific circumstances, mainly in a local scale (Lopes et al., 2015; Amaral et al., 2017). Guava, loquat and peach orchards were significantly colonized by fruit flies (Lopes et al., 2015). Amaral et al. (2017) also described positive and negative co-occurrence of fruit flies in areas characterized by anthropogenic influences. Landscape heterogeneity is directly or indirectly related to the occurrence of interactions, but generally negative associations seem much more common in local scales (McGarvey and Veech, 2018).

In our study, positively and randomly co-occurring species pairs increased from the buffer zone to neighbourhood 2, but there was no clear evidence for this result. However, fruit flies usually migrate to new habitats searching for food resources when preferential or essential hosts are not unavailable (Silva et al., 2012). This behavior could be associated with the decline in species richness with the remoteness of the forest. Far from the forest, the diversity of host plants decreases, but the increase in positive and random pairs could be associated with the presence of fruit flies coming from small forest fragments near orchards (Araujo et al., 2019).

In all areas, species more intensely contributing with establishment of positive pairs were *A. fraterculus* and *A. obliqua*, which are recognized as polyphagous species. This characteristic allows the insects to be more flexible in terms of food resource use, thereby increasing the chances of co-occurrence in a long-term scale (Denno et al., 2003). *Anastrepha fraterculus* and *A. obliqua* showed the ability to positively interact with other fruit fly species in different areas (Amaral et al., 2017). Although many positive interactions have been observed

in papaya orchards in Espírito Santo, a significant percentual of random associations have also been recorded. This result suggests some variations with respect to the response of fruit fly communities facing distribution of host plants, distance from forest and also influence of micro climatic differences in the study areas (Duyck et al., 2006).

Both the co-occurrence and strength interaction were influenced by the decrease in species abundance over the years. Due to management implementations in the areas, the systems approach, fewer resources have been available over time for *Anastrepha* species within orchards. Also, this implementation probably made possible the dispersal of species to new habitats, consequently decreasing their co-occurrence in the orchards, resulting in a lower number of co-occurring pairs over the years, but with increased interaction strength. The interaction strength among species tends to increase with the decrease in diversity because the encounter rate under high species diversity dissipates the interactions (McCann, 2000; Wootton & Emmerson, 2005). Also, the patterns of co-occurrence between species of fruit flies are probably influenced by the seasonality of host plants and the temporal variation in temperature of orchards, as observed in guava, loquat, and peach (Lopes et al., 2015).

In the full area and buffer zone, the interaction strength for positive associations was slightly influenced by time. Although the systems approach have influenced the interaction strength over the years, it was not strongly influenced when analyzed within the continuous implementation period of the systems approach and its impact on diversity. Positive co-abundance is generally characterized by the ability of species to become capable of sharing resources with no negative impact on the species pairs. In this study, positive co-abundance was determined by the positive slope value obtained from the Poisson regression. Random co-abundance species were also observed herein and can be interpreted as species totally independent of each other, which is determined by non-significance between the species abundances. Positive interactions are reflected by species using the same resources, especially food items, and may result in benefits such as survival for at least one species belonging to the pair (Morin, 1999; Stiling, 1999). Nevertheless, this type of interaction may also influence species diversity providing scenarios for direct or indirect alliances between species, creating conditions capable of allowing interspecific coexistence, with benefits such as pollination, predator defense, reduction of physical stress and dispersal (Morin, 1999; Stiling, 1999).

In fruit flies, particularly, it is important to understand co-occurrence patterns, especially positive pairing, because pairs of species may indirectly influence the success of natural enemies. Generalist natural enemies may take advantage of species co-occurring, because they can exploit more than one species. This scenario could increase the chances of success of natural

enemies (predators or parasitoids), when prey or hosts are not advantageous in terms of nutritional conditions or offer behavioural resistance. This scenario may also characterize apparent competition, increasing the probability of success of natural enemies in biological control or integrated pest management (van Veen et al., 2006). This ability may push co-occurring species closer to some population size threshold, guaranteeing at the same time stochastic fluctuations capable of driving them to local extinction (Courchamp et al., 2000) and the persistence of natural enemies.

4.5 Conclusions

The patterns of co-occurrence and interaction of *Anastrepha* species were influenced by areas and time. In all scenarios (spatial and temporal) only positive and random associations were observed, with *A. fraterculus* and *A. obliqua* contributing significantly to the largest number of positive pairs.

Co-occurrence was greater in areas further from the reserve. However, the strength of the interaction between the areas showed little variation. The impact period of the systems approach (2004-2010) provided a lower number of positive co-current pairs, but with greater strength of the interaction over time.

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