Spatial pattern of parasitism in *Diatraea saccharalis* Fab. (Lep., Crambidae) populations at two different spatial scales in sugarcane fields in Brazil

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Abstract: The biological control of *Diatraea saccharalis* is regarded as one of the best examples of successful classical biological control in Brazil. Since the introduction of the exotic parasitoid, *Cotesia flavipes*, the decrease in *D. saccharalis* infestation in sugarcane fields has been attributed to the effectiveness of this agent. Native Tachinidae fly parasitoids (*Lydella minense* and *Paratheresia claripalpis*) have also been implicated in the success. Quantitative data confirming the actual contribution of these agents to the control of *D. saccharalis* are, however, rather scant. The purpose of this study was to investigate the spatial pattern of parasitism of these parasitoids in *D. saccharalis* populations at two large spatial scales (fields and zones). To investigate this subject, a large data set comprising information collected from a sugarcane mill located in the state of São Paulo, Brazil (São João sugarcane mill) was analysed. When regressions between the proportion parasitism against host density were computed, the percentage of significant regressions with either a positive or a negative slope was very small at both spatial scales for both parasitoid species. Regressing the densities of tachinid-parasitized hosts against host densities per field showed that these parasitoids presented a 'moderate aggregative' response to host densities, as 53.33% of the regressions were positively significant. *Cotesia flavipes* was 'weakly aggregated' on host densities at the field level, because only 33.33% of the regressions were positively significant. At the zone level, neither aggregative nor spatial proportion parasitism responses were evident for either parasitoid species due to the small percentage of significant regressions computed.

1 Introduction

Successful use of parasitoids in biological control programmes requires a better understanding of spatial relationships between host density and rates of parasitism, as spatial density-dependent parasitism (direct and inverse) may contribute to host regulation and to stability in parasitoid-host systems (Chesson and Murdoch, 1986; Hassell, 1986, 2000). Thus, it is important to determine under what conditions non-random spatial patterns of parasitism are common in nature.

Heads and Lawton (1983) were the first to test empirically the hypothesis of aggregative responses by parasitoids at different spatial scales, specifically in the leafminer, *Phytomyza ilicis* Curtis (Dipt., Agromyzidae), where they found that spatially density-dependent mortality imposed by the larval parasitoid *Chrysocharis gemma* Walker (Hym., Eulophidae) was most strongly detected at the smallest spatial scale. Addicott et al. (1987) pointed out that the correct spatial scale of investigation depends on the movement patterns of the organisms involved. However, studying two species of parasitoids with widely differing dispersal abilities,

Stiling et al. (1991) showed no density dependence relationship at any spatial scale. In more extensive reviews, Stiling (1987) found spatial density dependence in only 27% of 137 interactions between parasitoids and their hosts, while Walde and Murdoch (1988) found density dependence in 13 of 57 field studies.

Although several studies have failed to demonstrate spatial density dependence (Morrison and Strong, 1980; Weis, 1983; Murdoch et al., 1985; Reeve and Murdoch, 1985; Smith and Maelzer, 1986), in the last decade a large number of studies have demonstrated it (either, inverse or direct) in many specific systems and spatial scales (Freeman and Smith, 1990; Rothman and Darling, 1990, 1991; Fowler et al., 1991; Hopper et al., 1991; Hails and Crawley, 1992; Zhang and Sanderson, 1993; Kuhlmann, 1996; Sperber and Collevatti, 1996; Lill, 1998). Nevertheless, few studies have investigated the stability mechanisms of these populations with sufficiently long-lasting empirical data sets from biological control programmes. Biological control partitioners may examine the density-dependent process because population regulation is expected to be

density-dependent (Turchin, 1995), hence testing the applicability of the ecological theory to the practice of biological control (Murdoch and Briggs, 1996).

Diatraea saccharalis Fab. (Lep., Crambidae) is reported to be the most important sugarcane pest in the southeast region of Brazil (Gallo et al., 1988). The sugarcane borer builds internal galleries in the sugarcane plants causing direct damages resulting in apical bud death, weight loss and atrophy. Indirect damage also occurs through contamination by yeasts which cause red rot in the stalks causing contamination or inverting the sugar, with increasing yield losses in both sugar and alcohol (Macedo and Botelho, 1988). These pest populations have apparently been controlled by the larval parasitoid *Cotesia flavipes* Cam. (Hym., Braconidae) (strains from India and Pakistan) which has reduced the Infestation Intensity (II) of this pest in sugarcane fields in the state of São Paulo (Macedo et al., 1993; Botelho, 1998). Two Tachinidae parasitoid species, *Lydella minense* Towns. and *Paratheresia claripalpis* Wulp., native to South America, were initially reared and released into sugarcane fields with the purpose of controlling this pest. A large data set has been stored in some sugarcane mills in Brazil (mainly in the south-east region). This data set has only been analysed by correlation between the rate of parasitism and the II through time (Macedo et al., 1993; Botelho, 1998) instead of considering a more reliable ecological approach.

Here, we present host–parasitoid spatial analysis of the *D. saccharalis* biological control programme by larval parasitoids existing in sugarcane fields in the state of São Paulo, Brazil, looking for spatial aggregative responses of parasitism at two large spatial scales, and questioning the potential of these biocontrol agents in promoting regulation and stability in *D. saccharalis* populations in sugarcane fields.

2 Materials and Methods

2.1 Data sources

The data set used to support this study was provided by the São João sugarcane mill, which is located in the state of São Paulo, Brazil (see table 1 for more detailed information). In

Table 1. *Basic information about the São João sugarcane mill data and location from May 1982 to December 1996*

Location	São Paulo, Brazil Latitude: 22 °25′30″S Longitude: 47 °21′34″W
Approximate size	85000 ha
Months with missing data (without samples)	7/1982, 7/1985, 8/1989, 4–6/1990, 9–10/1990, 5/1992, 6/1993, 8/1993, 10–12/1994*
Smallest spatial level	Field†
Number of fields sampled per month	Maximum, 113; minimum, 2

* 1995 and 1996 were completely eliminated because of a different sampling scheme and outlying data, respectively.

† Fields and zones are variable, ranging from 10 to 30 ha and from 20 to 60 ha, respectively (approximately).

order to control production, sugarcane mill managers concentrate on small areas. These areas are known as 'fields' and 'zones' (groups of fields separated by roads). *Diatraea saccharalis* and its parasitoids were sampled at the field levels. Daily, a group of approximately five workers entered the sugarcane fields and sampled *D. saccharalis* populations and released parasitoids. One person per field walked between rows looking for damaged plants. When damaged plants were found, larvae were removed and placed on plastic dishes containing an artificial diet, and were then taken to the laboratory. *Diatraea saccharalis* larvae and the emerged parasitoids were recorded to estimate the percentage of parasitism. Field samples were recorded as man-hours. Although samples were taken monthly, often they were not continuous, either temporally (at the smallest spatial scales) or spatially because of unplanned demand for field workers. However, this sampling scheme has not been recently adopted and this description is valid only for the period of time during which this sampling programme was used. A more detailed description of this data set is shown in table 1.

This data set contains monthly information concerning the number of unparasitized and parasitized *D. saccharalis* in each sample per field.

2.2 Preliminary considerations for analysis

First, we considered both Tachinidae fly species as a single species due to frequent zero values in the samples (lack of insects sampled). Thus, the term 'species' refers to the *D. saccharalis*, *C. flavipes* and combined Tachinidae flies (*L. minense* density + *P. claripalpis* density, prior to transformation).

The information concerning insect densities were $\log(x_t + 1)$ transformed, with x_t being the density estimated per field. For analysis computed considering the zone spatial scale, the proportion parasitism was calculated as follows (further explanations are found in the subsequent item):

$$\text{PP} = \log(P) - \log(P + H), \qquad (1)$$

where P is the parasitized *D. saccharalis* density, H the unparasitized *D. saccharalis* density, and PP the proportion parasitism. Total parasitoid density was calculated by summing *C. flavipes* and tachinid densities prior to $\log(x_t + 1)$ transformation. The above equation is a simple calculation of proportion parasitism when log-transformed data are adopted instead of raw data.

2.3 Parasitoid aggregation in relation to *D. saccharalis* density and spatial distribution of proportion parasitism

The spatial relationship between parasitized *D. saccharalis* density and total *D. saccharalis* density (parasitoid aggregation), and the relationship between proportion parasitism and total *D. saccharalis* density (spatial distribution of proportion parasitism) were examined at two spatial scales: field and zone.

Regression analysis of total *D. saccharalis* density against parasitized *D. saccharalis* densities per field and zone were computed (both spatial scales considered) for both parasitoid species, and the same procedure was adopted when the proportion parasitism was considered as the dependent variable instead of parasitized *D. saccharalis* density. For the field spatial scale, the proportion parasitism was calculated using raw data and was arcsin square-root transformed (Sokal and Rohlf, 1995).

As the number of fields per zone was variable, an average density between the fields was calculated for each zone

instead of a sum (at least two fields per zone were considered). In this case, as the average was taken into account, the proportion parasitism was computed as equation 1 instead of using calculation from the raw data. The aggregative response for the total number of parasitoids per field and zone was not considered here because we were only interested in aggregative responses considering each parasitoid species; however, the total proportion parasitism was investigated because it provides information about possible additive effects of the parasitoids in promoting host regulation and stability (HASSELL, 2000).

2.4 Manipulation of maps for analysis

As the São João sugarcane mill contains a large spatial area (thousands of hectares) and the samples were not spatially continuous, only the nearest areas (fields or zones) were considered for analysis. As zones were numbered on maps (in order to control the local production of sugarcane) these numbers were used as an identification of their geographical positions. The maps were provided by the sugarcane mill managers and were constantly checked. Groups of zones around the 2000 number were geographically located in a particular region in the sugarcane mill. Groups of zones around the 5000 number were geographically located in another spatial region, and this was also the case for groups of zones around the 6000 and 7000 numbers. Each of these groups was called 'replicate'; thus four replicates were present. The month with the highest number of samples in each year was chosen for analysis, hence 13 different months (13 years of data; see table 1) were the focus of analysis. A total of 45 regression analyses were computed for the field spatial scale instead of 52 (13 months × 4 replicates), as insect density information from the four replicates was not always present in each chosen month. A small number of regressions were computed for the zone spatial level when compared with the field level because some replicates had less than three zones available (a total of 35 regression analyses were computed at this spatial resolution level).

To better clarify this explanation, we give the following hypothetical example. Suppose that in a chosen month, 25 zones were sampled considering the whole sugarcane mill, and six, seven, five and seven (a total of 25) of these were located around the 2000, 5000, 6000 and 7000 numbers, respectively. Thus, four regressions ($n = 6, 7, 5$ and 7, respectively) between parasitoids and host densities would be computed for this month considering the zone spatial scale. However, as zones are formed by groups of fields, let us suppose that each zone is composed of four fields (this is fictitious because the number of fields per zone was variable, as mentioned previously). Thus, 24, 28, 20 and 28 fields (multiplying the number of zones per four fields each) were available for analysis in each geographical area. As before, four regression analyses ($n = 24, 28, 20$ and 28, respectively) would be computed, but now considering the field spatial scale. The entire procedure was followed for each chosen month. Analysis in spatial scales larger than zones were impracticable due small sample size.

The spatial variation in *D. saccharalis* densities at each spatial scale was assessed by calculating the variance/mean ratio of the raw data densities for each replicate at each spatial level. Dispersion of each sample was tested by a chi-square test (SOUTHWOOD, 1978).

3 Results

Mean values and variances of host density per field and zone for each replicate (fig. 1) demonstrated significant >1 ($P < 0.05$) variance/mean ratios indicating that host populations were highly clumped among fields and zones.

Regression of proportion parasitism against host density showed that the percentage of significant regressions with either a positive or a negative slope was very small at both spatial scales for both parasitoid species (table 2). The lack of significant regressions at both spatial scales may indicate that the spatial distribution of the proportion parasitism for both parasitoid species is independent of host density at both spatial levels. For the total number of parasitoids (proportion parasitism), an independent relationship to spatial host densities at both spatial scales was also observed (table 2). When the densities of tachinid-parasitized hosts were regressed against host densities per field, these parasitoids presented a 'moderate aggregative' response on host densities as 53.33% of the regressions were significant with a positive slope (table 2). *Cotesia flavipes* was 'weakly aggregated' on host densities at the field level, because only 33.33% of the regressions were significant with a positive slope. At the zone level, neither aggregative nor spatial proportion parasitism responses were evident for either parasitoid species due to the small percentage of significant regressions computed (table 2). Sample size at the zone level was considerably smaller than at the field level and could interfere with the regressions.
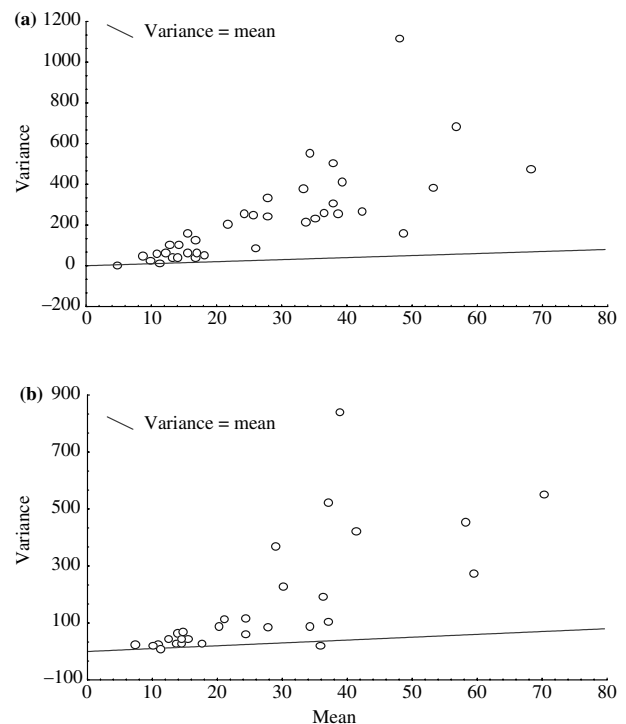
Fig. 1. Mean values and variances of Diatraea saccharalis *densities per field (a) and zone (b). Forty-five and 35 replicates were considered for field and zone spatial levels, respectively. The lines represent equal mean values and variances*

Table 2. *Regressions involving the density and the proportion of parasitized* Diatraea saccharalis *larvae vs. the total* D. saccharalis *density per field and zone. Analyses were computed for* Cotesia flavipes, *tachinids and the total number of parasitoids. The parasitoid and host densities were* $\log(x_t + 1)$ *transformed*

Spatial level		Parasitoids				
		C. flavipes		Tachinids		
		Density	%	Density	%	Total of parasitoids (%)
Field	$r^2 \pm$ SD	0.39 ± 0.13	0.24 ± 0.09	0.46 ± 0.20	0.29 ± 0.12	0.32 ± 0.12
	$b \pm$ SD	0.68 ± 0.20	−0.02 ± 0.44	0.72 ± 0.19	−0.32 ± 0.44	−0.43 ± 0.33
	N	45	45	45	45	45
	+ SIG (%)	33.33	6.67	53.33	4.44	2.22
	− SIG (%)	0	6.67	0	13.33	20
	n	13–39	21–30	7–39	18–37	18–37
Zone	$r^2 \pm$ SD	0.82 ± 0.14	0.84 ± 0.14	0.78 ± 0.18	0.88 ± 0.07	0.72 ± 0.24
	$b \pm$ SD	1.04 ± 0.45	−1.00 ± 0.38	1.16 ± 0.54	−0.57 ± 1.34	−0.66 ± 0.38
	N	35	35	35	35	35
	+ SIG (%)	14.71	0	11.76	2.94	0
	− SIG (%)	0	23.53	0	5.88	11.76
	n	4–7	4–9	4–9	4–6	6–11

N, number of regressions; + SIG, number of significant regressions at $P < 0.05$ with a positive slope; − SIG, number of significant regressions at $P < 0.05$ with a negative slope; $r^2 \pm$ SD, average of the significant coefficient of determinations and its respective standard deviations; $b \pm$ SD, average of the slopes of the significant regressions and its respective standard deviations; n, sample size in each replicate; Density, regressions between parasitized host density vs. total host density (aggregative response); %, regressions between the proportion parasitism vs. total host density (aggregative response of proportion parasitism). For the field spatial level the proportion parasitism was transformed to arcsin √prop., and for the zone spatial level it was calculated as equation (1) (see methods).

4 Discussion

Every month a huge amount of parasitoids is released into sugarcane fields (MACEDO et al., 1993; BOTELHO, 1998) with the aim of controlling *D. saccharalis* populations in the state of São Paulo. As previously shown, such hosts are unevenly distributed between zones and fields (fig. 1). Considering these facts, in a supposedly well elaborated parasitoid release strategy, we should expect high levels of parasitism in areas with high host densities as more parasitoids should be released in areas exhibiting high levels of pest infestation. However, this predictable pattern was only observed in half of the cases, with 53.3% being the highest proportion of significant regressions with positive slopes (table 2) considering an aggregative response among hosts and parasitoids. Curiously, this highest spatial aggregative response was achieved by the tachinids, which stopped being released at the beginning of the 1990s, and not by *C. flavipes*, which is still intensively released into the fields.

Unfortunately, we could not go deeper into spatial parasitism aggregation analysis at smaller spatial scales than those studied here, because numerically the highest parasitoid response to host density was present at the smaller spatial scale (field level) (table 2).

As the effectiveness of multiple introductions of parasitoid species has been questioned (FERGUSON and STILING, 1996), the real economic benefits from the introduction of tachinids plus *C. flavipes* should be carefully studied as the parasitoids as a whole exhibited an independent proportion parasitism relationship to spatial host distribution at both spatial scales (table 2).

As has been reported (MACEDO et al., 1993; BOTELHO, 1998), *D. saccharalis* densities have decreased over the last two decades in the state of São Paulo, although the II has been used as *D. saccharalis* density estimation (this differs from the density estimation adopted here). Sugarcane is a complex agricultural system where different varieties have been cultivated over the last two decades, combining susceptible and resistant varieties through time (MACEDO and BOTELHO, 1988). Sugarcane plants can be cut five or six times (approximately) until the crop is replanted. Even when plants are from the same variety, they can support different cuts, or be destined for irrigation or seedling production, and all these factors can lead to different *D. saccharalis* infestation levels, either spatially or temporally (MACEDO and BOTELHO, 1988). Furthermore, in the state of São Paulo, the extent of cultivated sugarcane area has increased considerably from the early 1980s to the late 1990s. Another complexity factor added to this system is the harvest strategy adopted by the mills, extending over approximately 5 months per year. In other words, plants would not be of the same age across the smaller spatial scales during the growing period. Although several factors can potentially lead to different spatial host distribution in sugarcane fields, according to our results, *C. flavipes* and the tachinids were unable to aggregate in fields and zones containing high host densities.

Regarding the fact that the biological control of *D. saccharalis* is one of the best examples of successful biological control programmes in Brazil (BOTELHO, 1998), more studies concerning the spatial and temporal patterns of parasitism in smaller spatial scales than those studied here are encouraged, providing clearer information about the real contribution of *C. flavipes* and Tachinidae flies to the promotion of stability and regulation of *D. saccharalis* populations.

Acknowledgements

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