

Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest

NATACHA P. CHACOFF* and MARCELO A. AIZEN†

*Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucumán, CC 34 Yerba Buena (4107) Tucumán, Argentina; and †Laboratorio Ecotono, Universidad Nacional del Comahue, Quintral 1250, Bariloche (8400) R'ío Negro, Argentina

Summary

1. Over the last decade, there has been much concern about the decline in pollinator abundance and diversity caused by different types of anthropogenic disturbances, including deforestation and habitat fragmentation. However, little empirical information exists documenting this decline and its consequences for cultivated flowering crops. We tested the hypothesis that remnants of natural habitats act as a source of flower-visiting insects for neighbourhood crops.

2. Over 3 consecutive years we evaluated flower-visiting insect diversity, visitation frequency and composition in four grapefruit *Citrus paradisi* Macf. plantations at increasing distances (edge, 10, 100, 500 and 1000 m) from remnants of subtropical premontane forest in NW Argentina.

3. The frequency of visits to grapefruit flowers decreased by more than twofold as distance to the forest increased and the flower-visiting fauna became more depauperate. Even the feral africanized honeybee *Apis mellifera*, the dominant flower visitor to grapefruit flowers, showed a decline at distances > 500 m from the forest edge. However, the greatest relative declines occurred among stingless and solitary bees as well as other native flower visitors, which were rarely seen a few hundred metres inside the plantations. In addition, flower-visiting insect faunas among plantations became more homogeneous as distance from the edge increased.

4. These trends were consistent over years and among plantations up to 50 km apart. Thus, we can conclude that negative forest edge effects on flower-visiting insects inside grapefruit plantations are widespread in the increasingly deforested landscape of NW Argentina.

5. *Synthesis and applications.* This study provides empirical evidence for considering remnants of natural habitats as a source of both native and alien flower-visiting insects that can be potential pollinators for agriculture. Increasing edge density in agricultural lands, through preservation and restoration of natural habitats, can foster stocks of diverse and abundant insect pollinators.

Key-words: africanized honeybees, agroecosystems, bees, *Citrus*, ecosystem service, pollinator diversity, Yungas

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Introduction

The destruction and fragmentation of natural habitats through agricultural intensification have been recog-

nized as being among the main causes of declines in local and global biodiversity (Debinsky & Holt 2000). Reductions in flower-visiting insect diversity and abundance have received much attention in recent years (Buchmann & Nabhan 1996), mainly because pollination represents a critical ecosystem service for both natural and agricultural ecosystems (Constanza *et al.* 1997).

Causes of a much publicized 'pollinator crisis' include not only habitat fragmentation and changes in

Correspondence: Natacha P. Chacoff, Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucumán, CC 34 Yerba Buena, (4107) Tucumán, Argentina (Fax: +54 3814253728; e-mail: natachachacoff@arnet.com.ar).

land use, but also pesticide and herbicide use, and introduction of non-native species (Kearns, Inouye & Waser 1998). Effects of habitat fragmentation for pollinators and pollination have now been reported for many ecosystems (e.g. Aizen & Feinsinger 1994a; Debinsky & Holt 2000; Cresswell & Osborne 2004). Although many of these effects could be mediated by the creation of abrupt boundaries between remnants of native habitats and their surrounding anthropogenic matrix, research on 'edge effects' on pollinators and pollination is still in its infancy (Tscharrntke *et al.* 2002; Jules & Shahani 2003). Here we study edge effects on the species diversity, abundance and composition of flower-visiting insects associated with grapefruit plantations bordering remnants of premontane subtropical forest in NW Argentina. From both conceptual and applied perspectives, studies of edge effects from habitat remnants into the matrix can be as important as studies focusing on more classical edge effects from the matrix into native habitat fragments (e.g. Cunningham 2000; Steffan-Dewenter, Munzenberg & Tscharrntke 2001). Remaining natural habitats surrounded by agricultural, pasture or urban development maintain some degree of terrestrial connectivity through the modified surrounding habitats. From a classical conservation perspective, this anthropogenic matrix has been usually treated as a sterile 'ocean', an area considered biologically uniform, inhospitable and thus ecologically irrelevant (Ricketts 2001; Jules & Shahani 2003). More recently, Murphy & Lovett-Doust (2004) reported that the matrix may strongly affect between-patch processes by (1) reducing or enhancing dispersal and colonization rates; (2) providing alternative, suboptimal habitat; and (3) acting as a source of novel invading species.

Pollinators, in particular, can be sensitive to the quality of the matrix. Although it can represent a poor breeding and nesting habitat it can also represent a plentiful source of pollen and nectar, for example when the matrix supports a massive-flowering crop (Westphal, Steffan-Dewenter & Tscharrntke 2003). One practical consequence of the preservation of these semipermeable edges and entire habitat mosaics is that remnants of native vegetation, such as natural forests, may represent an important source of pollinators for many pollination-dependent crops (e.g. Klein *et al.* 2003a,b; Kremen *et al.* 2004).

The accelerated destruction of the subtropical mountain forest of NW Argentina and southern Bolivia (the Yungas) represents a major conservation issue (Dinerstein *et al.* 1995). Nearly 50% of the highly diverse premontane forest belt of the Yungas that originally occupied approximately 2.1 million of hectares has been converted to agricultural land (Brown & Malizia 2004). This has led to a landscape of small and altered forest fragments surrounded by sugarcane *Saccharum officinarum* L., soybean *Glycine soja* Siebold & Zucc and citrus (*Citrus* spp.) plantations, among other crops. With few exceptions (e.g. sugarcane), most of these crops are pollinator-dependent or benefit from insect

pollination. Present agricultural practices not only reduce natural or seminatural habitat in the landscape but also increase the mean distance from the crops to remnant forest patches.

In this study, we tested the hypothesis that Yungas forest patches act as a source of pollinators for grapefruit (*Citrus paradisi* Macf.), one of the most important export and cash crops from the region. *Citrus* are generally characterized by a lack of strong pollination requirements (McGregor 1976; Roubik 1995), but grapefruit varieties cultivated in NW Argentina are highly dependent on insect-mediated pollen transfer for fruit production (N. Chacoff & M.A. Aizen, unpublished data). In particular, we asked the following questions: (1) do visitation diversity and frequency of flower-visiting insects to grapefruit change with increasing distance to the forest; (2) does the feral alien honeybee *Apis mellifera* L., the most frequent visitor to citrus flowers (Free 1993), show the same distance response as native visitors; and (3) do the composition and structure of pollinator assemblages change as distance to the forest increases?

Materials and methods

STUDY AREA

In Argentina, the Yungas extend along discontinuous mountain ranges between 22° to 29°S, and include three altitudinal forest belts: premontane lowland, lower montane and upper montane forest (Cabrera 1976). The northern premontane lowland forest type is the most species-rich as well as the most endangered because of clearcutting (Grau & Brown 2000; Brown *et al.* 2001). This study took place in grapefruit plantations adjacent to altered premontane lowland forest in the upper Bermejo River Basin, near Orán city (23°08' S; 64°20' W) in Salta province.

In this region, there is an annual deforestation rate of 2.3% (Brown & Malizia 2004). In the northern sector of the Argentine Yungas, near Orán, citrus plantations occupy 13.500 ha (Danza 2001). Most of them are grapefruit (57%) and represent more than 30% of the national grapefruit production, which is mainly exported (Danza 2001).

STUDY SITES AND EXPERIMENTAL DESIGN

We considered five 'distance treatments' to the forest edge at each of four different grapefruit plantations: 0 m or 'edge' (the first grapefruit tree row in the plantation from the edge), 10 m (the third row), 100, 500 and 1000 m. Grapefruit in these plantations were of the red and very red seedless varieties (Foster Seedless, at Peña Colorada, Rio Red at Citrusalta and Rouge La Toma at Manero and La Toma). At the beginning of the 2000 flowering season, 20 focal plants were selected at each distance for the visit frequency censuses across the whole study. Flower visitors of each plant were

observed over 3 consecutive years (2000–02) and throughout each flowering season (middle August–early October). The plantations were embedded within a citrus agricultural matrix (either grapefruit or orange), and were all adjacent to the forest on one side and adjacent to other citrus plantations on the side furthest from the forest.

None of the study citrus plantations had domestic beekeeping activity during the study period (personal communication of the owners; N. Chacoff, personal observation). Thus, we inferred that all the *A. mellifera* visiting grapefruit flowers were feral africanized honeybees (Schneider, DeGrandi-Hoffman & Smith 2004). Over the 3 years of this study, we found no bee tree nests and only one ground nest of *Bombus atratus* Franklin found in just one plantation. Thus, we inferred that most bees observed in the plantation were nesting in the nearby forest. Heavy use of herbicides in these plantations ensured that citrus flowers were almost the exclusive and dominant source of nectar and pollen present in the plantations. Distances between plantations range from 5 to 50 km, which exceeded the expected flight distances of most foraging bees and other invertebrate flower-visiting taxa (Osborne *et al.* 1999; Walther-Hellwig & Frankl 2000; Gathmann & Tscharrntke 2002; Steffan-Dewenter & Kuhn 2003; Klein, Steffan-Dewenter & Tscharrntke 2004). Thus, the four plantations included in this study were considered true replicates (Hulbert 1984).

SAMPLING OF FLOWER VISITORS

Insect visits to flowers were observed between 0930 and 1700 hours under typical weather conditions, i.e. sunny and slightly cloudy days with low wind velocity. Different sites were sampled on different days. Censuses of visit frequencies were restricted to grapefruit trees in full bloom (i.e. > 50% of their flowers opened). For each sampling day and distance, three censuses were made on different trees at least 30 m apart and observation times were randomized among distances. Each census consisted of a 15-min observation period to a flowering branch or group of branches, so a similar number of flowers (mean \pm 1 SD = 10 ± 5 flowers observed per census) was observed on each sample tree. After counting the number of open flowers, we carefully recorded the number of visitors and identified them to the lowest taxonomic level possible. However, some native visitors, particularly some congeneric bee species, could not be identified from our observation posts; thus, for diversity estimations they were recorded only as morphospecies. These censuses were carried out during 8–10 days per plantation and per year distributed throughout the flowering season. In total, 941 censuses were performed over 3 consecutive years in the four grapefruit plantations.

During the 2002 flowering season, insects visiting grapefruit flowers were also recorded along transects to assess edge effects on the composition of the flower-vis-

iting assemblage. These transects consisted of 10-min walks at a given distance (i.e. 0, 10, 100, 500 or 1000 m) along which all presumed legitimate flower visitors were identified to the lowest taxonomic level possible. On a given sampling day, two transects per distance were made and different distances were surveyed in a random order. Transect surveys were conducted during a total of 20 days and over a total of 33 hours.

After frequency censuses or along transects, flower-visiting insects were collected with sweep-nets or with an entomological aspirator for taxonomic determination. Because we collected for vouchering, only new species were captured. Bees and syrphid flies, the dominant grapefruit flower visitors, were identified to the species or at least to the genus level. Specimens of some Halictidae from the genera *Augochlora*, *Dialictus* and *Augochloropsis* and some Andrenidae could not be identified to the species level and were classified into morphospecies.

DATA ANALYSIS

From censuses of visit frequencies, we estimated the following four variables: (1) species richness; (2) total visit frequency; (3) visit frequency by honeybees only; and (4) visit frequency by other visitors. Variable 1 was expressed as no. morphospecies/census and variables 2–4 were expressed as no. visits \times 15 min⁻¹ \times flower⁻¹. Only insect visitors actually or potentially performing pollination (i.e. those contacting anther and/or stigma) have been included in the analysis.

The effect of distance to the forest edge was assessed using general (variables 2 and 3) and generalized (variables 1 and 4) linear mixed models (Littell, Stroup & Freund 2002). The latter models are used when variables cannot achieve normality even after transformation. In all models, distance was considered as a fixed effect and site, year and two- and three-way interactions as random effects. As this study focused on the effect of distance to the forest edge, we did not consider significance tests associated with random effects. However, variance and covariance estimates > 0 are provided for comparison purposes (Appendix 1).

To analyse variables 2 and 3 we used the MIXED procedure in SAS (SAS 1999) that implements a generalization of the standard linear model allowing the incorporation of random effects (for further details see Littell *et al.* 1996). To analyse variables 1 and 4, we used the macro program GLIMMIX (available at <http://ftpsas.com/techsup/download/stat/glmm800.html>) that iteratively calls SAS procedure MIXED (SAS 1999). In all cases Satterthwaite's approximation method was used to estimate degrees of freedom of the models because our data sets were not completely balanced (Littell *et al.* 1996). We assessed further the relationship between variables 1–4 and distance using a linear and quadratic regression approach applying contrasts. A summary of all fitted models to the census data is shown in Table 1.

Table 1. Type of general and generalized linear models applied to the different dependent variables estimated from the census data (see Data analysis) and results of the models for the distance (fixed) effect including linear and quadratic contrasts. For each model, information on the transformation of the dependent variable, distribution of the error term and link function are included

Dependent variable	Measurement units	Transformation	Error distribution	Link function	General linear model		Linear contrast		Quadratic contrast	
					d.f.§	F	d.f.	F	d.f.	F
1 Species richness	No. morphospecies/census	None	Poisson	Log	4,9.4	1.60	1,10.6	5.64**	1,10.0	0.04
2 Total visit frequency	No. visits \times 15 min ⁻¹ \times flower ⁻¹	Square root	Normal	4,11.8	3.17*	1,12.1	11.68***	1,11.9	0.60	
3 Visits by <i>Apis mellifera</i>	No. visits \times 15 min ⁻¹ \times flower ⁻¹	Square root	Normal	4,11.9	2.18	1,12.2	7.61**	1,12	0.97	
4 Visits by other visitors	No. visits \times 15 min ⁻¹ \times flower ⁻¹	None	Binomial	Logit	4,7.69	5.71**	1,12.2	17.16***	1,12.2	2.04

§Numerator, denominator degrees of freedom. * $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$.

For insect data from transect surveys we conducted a non-metric multidimensional scaling (NMDS) ordination using Pc-Ord version 4.0 (McCune & Mefford 1999). The rows of this input matrix represented the morphospecies (50 rows), and the columns the five distances for each of the four sites (20 columns). To standardize observations by sampling effort (sample sizes varied between four and 16, with an average of 9.5 transect surveys per distance and site), for each cell of the matrix the number of times each pollinator species was observed was divided by the total number of transects made at each distance in each site.

We ran two separate analyses: one using the complete matrix (50 \times 20) and the other using a reduced matrix (28 \times 20) in which rare species (species that appear only once in the census) were deleted. The NMDS ordination was based on a Bray–Curtis distance coefficient matrix (Legendre & Legendre 1998). We used a two-dimensional configuration in both because the final stress (19.74 and 24.96, respectively) did not decrease significantly in the three-dimensional configuration (McCune & Mefford 1999). Because the results of some ordination methods can be affected spuriously by unequal sample sizes despite standardization (Legendre & Legendre 1998), we also rarefied samples by the minimum number of transects surveyed (four) using ECOSIM (Gotelli & Entsminger 2001). Here we report results from the analysis of the complete matrix only, because they were very similar to those of either the reduced and rarefied matrices.

To assess the relative importance of the plantation (= site) factor and the distance to the edge factor on species composition we conducted a permutation test following Vazquez & Simberloff (2003). To conduct this test, we constructed two additional matrices with the same dimensions as the Bray–Curtis matrix (i.e. 20 \times 20). In the first matrix, pairs of distances surveyed in the same site were represented by zeros whereas pairs of distances surveyed in different sites were represented by the number 1, independently of the distance to the forest edge. In the second matrix, pairs of the same distance to the edge (in the same or different plantations) were represented by zeros whereas pairs of different

distances to the edge were represented by 0.25, 0.5, 0.75 and 1, according to how many distance treatments separated a given pair. We then calculated the standardized Mantel correlation statistics (r_m) to measure the independence of the entries of the dissimilarity matrix from those of the other two. We randomly permuted the elements of one of the matrices, and recalculated the statistics 1000 times to estimate a P -value for each observed statistic.

To investigate further whether, within each plantation and for the whole data set, poorer species assemblages (usually those far from the forest edge; see Results) represent non-random, nested subsets of those present near the edge; presence/absence matrices were assembled by listing distances as rows and insect species as columns for each site separately and for all sites together. A nestedness index was computed for each matrix with the Nestedness Calculator software (AICS Research, University Park, MN, USA; Atmar & Patterson 1993). The estimated metric measures the disorder of the matrix using the distribution of unexpected presences and absences of species compared to those in a perfectly nested matrix (Atmar & Patterson 1993). This measure of disorder is called temperature (T) and ranges between 0 and 100°. The level of nestedness is defined as $n = (100 - T)/100$, with values ranging from 0 and 1, maximum nestedness being 1 (Bascompte *et al.* 2003). For each matrix, Nestedness Calculator generates a population of $n = 1000$ random matrices and estimates P , the probability of a random replicate being more or equally nested than the observed matrix. It has been argued, however, that Nestedness Calculator may overestimate the degree of nestedness and its statistical significance, because the null model assumes that all species are equally common (Fischer & Lindenmayer 2002). In spite of this potential drawback, all significant results presented in this paper (but one) are highly nested at the level of $P \leq 0.005$; thus, they are unlikely to be the result of a type I error. Nestedness indexes were estimated for the complete insect assemblage and for bee species only (superfamily Apoidea), as bees represented the most diverse and functionally important component of the flower-visiting insect assemblage.

Results

VISITOR SPECIES RICHNESS AND FREQUENCY OF VISITS

During the 3 years of this study, we observed a total of 2976 insects visiting flowers.

The number of morphospecies per census was two to four times higher at the forest edge than at 1000 m inside the plantations (Fig. 1). The average number of visitor taxa per census of visit frequency decreased monotonically with distance to the forest edge ($y = 0.8273 - 0.0003x$; Table 1). The effect of distance to the edge on the richness of flower visitors was relatively consistent among years (Fig. 1).

Our results showed strong negative edge effects on visit frequency. The total frequency of visits (square-root transformed) declined linearly from the forest edge to the interior of the plantations ($y = 0.6823 - 0.0003x$; Table 1), which represented a decrease of about threefold from 0 to 1000 m. Total visit frequency varied over years (Appendix 1) but the sign and magnitude of edge effects were similar over the 3 years of this study (Fig. 1). Variation among sites was much lower than among years. Also, the variation attributed to the site–distance interaction was relatively small (Appendix 1), indicating similar responses of total visit frequency with distance to the edge in different plantations.

The main flower visitor at all sites and years was *A. mellifera*, which accounted for nearly 95% of all visits to grapefruit flowers. The visit frequency of honeybees (square-root transformed) also showed a linear decrease with distance ($y = 0.6316 - 0.0002x$; Table 1) of about 50% from the forest edge to 1000 m. Among-year variation in honeybee visit frequency was nearly three times higher than among sites (Appendix 1). In addition, edge effects on honeybee visits were relatively consistent over time and space (Fig. 1).

Stingless and solitary bees were the second most frequent visitor group to grapefruit flowers after honeybees, accounting for 3.5% of all the visits, followed by wasps (0.8%), syrphid flies (0.3%), butterflies (0.2%) and beetles (0.1%). Flower visitors other than honey-

bees showed collectively the strongest relative decline from the forest edge to the interior of the plantation ($y = 0.0310 - 0.00003x$; Table 1), decreasing about six times from the forest edge to 500 m and nearly eight times to 1000 m. The relative visit frequency of these other visitors represented 14% of all visits at the forest edge and only 2% at 1000 m. Site had the highest variability associated with a random factor. Also, a relatively large covariance associated with the site \times year–distance interaction indicated that the magnitude of the decline in visitation by other visitors with distance to the forest edge depended on the particular site \times year combination (Appendix 1). The year, site \times distance, and site \times year factors accounted for no independent variation in visit frequency of these other flower visitors (variances and covariances = 0).

ASSEMBLAGE DIVERSITY AND COMPOSITION

We identified a total of 50 insect species on grapefruit flowers during our transect surveys, including 22 (44%) species from six families of bees (Apidae, Andrenidae, Anthophoridae, Chrysididae, Halictidae and Megachilidae), 11 (22%) of butterflies, seven (14%) of wasps, five (10%) of syrphid flies, two (4%) of beetles and two other diptera and one bug (see Appendix S1 in Supplementary material). In the surveys, we recorded the presence of other social bees besides *A. mellifera*, including *B. atratus* and six different species of stingless bees (Meliponinae). Among solitary bees, the most diverse families were Anthophoridae and Halictidae with six and five species, respectively (Appendix S1).

We recorded 32 insect species visiting flowers at the forest edge and only 14 species at 1000 m inside the plantations (Fig. 2). Among social bees, *A. mellifera* and *B. atratus* were present at all distances from the forest edge, but only 40% of all the species of stingless bees found at the forest edge were also present at 1000 m. Among solitary bees, only one of the five species of Anthophoridae present at the edge was also present at 1000 m. In general, six bee species were recorded exclusively at the edge, but none was exclusive to distances far from the edge (500 and 1000 m). Diversity of syrphid flies also decreased with distance to the forest edge

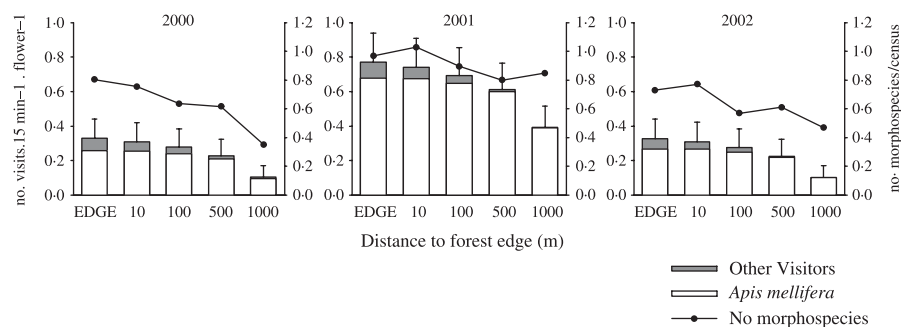


Fig. 1. Frequency of visits by all insects, by *Apis mellifera* only, by other insect visitors, and species richness of visitors for each of the 3 studied years. Means correspond to best linear unbiased predictors. Error bars correspond to ± 1 SE of total frequency of visits. Dots represent mean species richness per 15-min census.

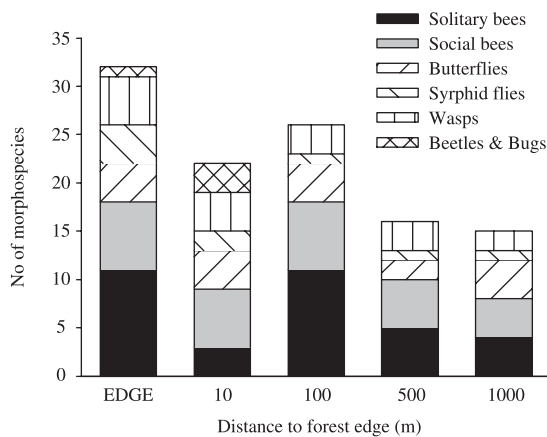


Fig. 2. Number of insect morphospecies sampled along transects (see Methods) at increasing distances from the forest edge.

but butterflies and wasps were apparently less affected (Fig. 2 and Appendix S1).

The two-dimensional space defined by the two first axes of the NMDS explained 69% of the variance and showed that different distances to the edge were grouped according to site (Fig. 3a). Mantel's test also confirmed an association between similarity in the insect fauna and site ($r_m = 0.25$, $P = 0.001$). Thus, site was an important determinant of the insect visitor fauna independent of distance to the edge. However, there was also an association between similarity in the insect fauna and shared distance to the edge ($r_m = 0.18$, $P = 0.03$), suggesting that edge effects also influence the similarity in insect faunas independent of site. In particular, we found an increase in mean similarity in the insect fauna among different sites as distance to the edge increased (Spearman's $r = 1$, $n = 5$, $P < 0.0001$), demonstrating a convergence among sites in the composition of the insect faunas towards the interior of the plantations (Fig. 3b). This trend towards higher homogeneity is depicted in the NMDS ordination, where insect assemblages sampled far from the forest edge are clustered together in the centre of the two-dimensional graph (Fig. 3a).

The average nestedness was $0.65 \pm \text{SE } 0.06$ for the complete insect assemblage and 0.73 ± 0.08 for the bee assemblage alone. We detected significant or marginal nestedness in one site (La Toma) for the complete insect assemblage and in two sites (La Toma and Citrusalta) when analysis was restricted to the bee component. For both the complete and bee assemblages we found significant nestedness when faunas were pooled across sites (Table 2).

Discussion

In the last decade, there has been much concern about the decline in pollinator abundance and diversity caused by different types of anthropogenic disturbances (e.g.

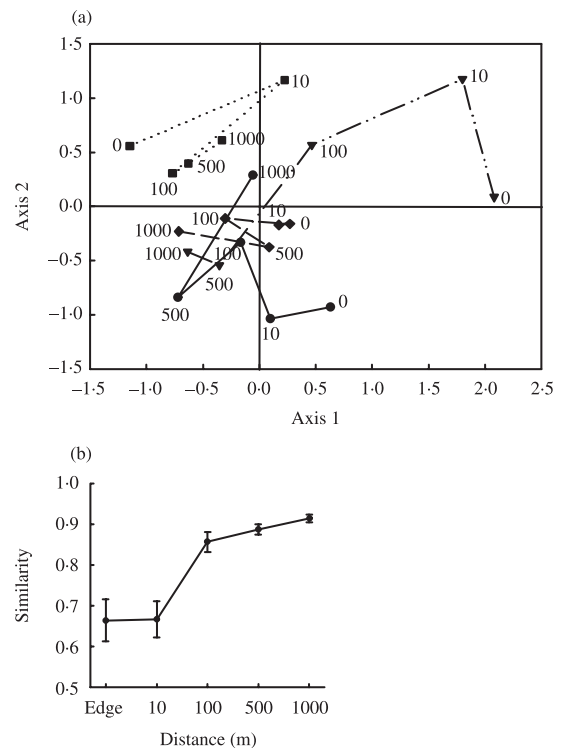


Fig. 3. (a) Non-metric multidimensional scaling (NMDS) ordination of the sites and distance based on the presence of pollinator's species; axes 1 and 2 explain 43.7% and 25.3% of total variance, respectively. The NMDS is based on the complete matrix of 50 species per 20 site \times distance classes. Line segments link flower-visiting faunas at increasing distances from the forest edge within the same site. Different plantations are represented by different symbols: Citrusalta ●; Manero ◆; Pc ■ and La Toma ▼. (b) Mean (± 1 SE) similarity (averaged over sites) vs. distance to the edge.

Rathcke & Jules 1993; Buchmann & Nabhan 1996; Murcia 1996; Kearns, Inouye & Waser 1998). However, little empirical information exists either documenting this decline or reporting how different pollination services are affected at both local and global scales. The main objective of this study was to assess the hypothesis that remnants of natural habitats can be seen as a source of pollinators for neighbourhood crops and our results clearly support this proposition. We found that not only the frequency of visits to grapefruit flowers decreased as distance to the forest increased, but also the flower-visiting fauna became more depauperate. Even the feral, africanized honeybees, the dominant flower visitor to grapefruit flowers, showed a decline at distances > 500 m from the forest edge. The most dramatic decline occurred among stingless and solitary bees as well as other native flower visitors, which were rarely seen at a few hundred metres inside the plantations. Because these trends were consistent over years and among plantations up to 50 km apart, we can conclude that negative forest edge effects on insect flower visitors inside grapefruit plantations are widespread in the increasingly deforested landscape of NW Argentina.

Table 2. Nestedness indexes for the complete grapefruit visitor assemblage and for bee species only in each site and for all the sites together. The nestedness index used (see Data analysis) ranges from 0 to 1 (maximum nestedness). The number of species used to calculate matrix nestedness and the probability of a non-nested assemblage are also given

Sites	Nestedness index	No. of species	<i>P</i>
	All insects/bees	All insects/bees	All insects/bees
Citrusalta	0.63/0.85	21/10	0.40/0.06
Manero	0.58/0.59	22/11	0.50/0.58
PC	0.57/0.59	23/12	0.58/0.69
La Toma	0.83/0.91	24/13	0.005/0.004
All sites together	0.77/0.83	50/21	< 0.00001/< 0.00001

Statistically ($P < 0.05$) and marginally significant ($0.05 < P < 0.10$) differences are shown in bold type.

EDGE EFFECTS ON HONEYBEES

The feral africanized honeybee *A. mellifera* contributed more than 90% of the visits to grapefruit flowers and represents the main pollinator of *Citrus* worldwide, as well as of a great diversity of subtropical and tropical crops in the Neotropics, mainly because of its capability to persist in agricultural landscapes (Roubik 1995, 2002). This bee has a great invasion ability, can colonize and persist in many different habitat types, nest under a vast variety of conditions and forage on a great diversity of both native and alien flowers (Schneider, DeGrandi-Hoffman & Smith 2004; Villanueva & Roubik 2004). Because feral *A. mellifera* colonies are reported to thrive in highly disturbed habitats (e.g. Aizen & Feinsinger 1994b; Morales & Aizen 2002; Steffan-Dewenter & Kuhn 2003), we could not anticipate the relatively strong edge effect found from the forest into the plantations. We found that at 1000 m from the forest edge the frequency of visits to grapefruit flowers by honeybees decrease to nearly 50%.

During the 3 years of this study we did not find any honeybee nest in the study grapefruit plantations. In addition, no beekeeping activity is possible in the lowlands of NW Argentina because of rapid africanization of domestic hives (Schneider, DeGrandi-Hoffman & Smith 2004). Thus, we infer that (1) most if not all feral honeybees visiting grapefruit flowers dwell in the neighbouring forest and (2) these plantations provide poor nesting conditions because of either low habitat quality or active management.

Assuming that grapefruit plantations provide spatially homogeneous pollen and nectar rewards, the strong decrease in honeybee visit rates observed at distances of about 1000 m from the forest edge (where they monopolized grapefruit flowers) could be attributed to flight costs rather than to any other constraint. Although honeybees can exploit food resources > 10 km away from their colony (Seeley 1984), they forage more typically to maximum distances < 2000 m (Beekman & Ratnieks 2000; Steffan-Dewenter & Kuhn 2003; Beekman *et al.* 2004).

Visit frequency is an important quantitative component affecting pollination success, particularly in relatively simple pollinator assemblages dominated by just

one species (Bond 1994; Herrera 2000). Therefore, the decline in total visitation to grapefruit flowers found at increasing distances from the edge could compromise pollen transfer and eventually limit fruit production.

EDGE EFFECTS ON POTENTIAL ALTERNATIVE POLLINATORS

Visitation by all the native pollinators as a group exhibited a much greater relative decline with the distance from the edge. Non-honeybee visitors accounted for nearly 15% of all visits to grapefruit near the forest edge but their visitation almost ceased beyond 500 m. Among the most impaired groups were some stingless and solitary bees (see also Liow, Sodhi & Elmqvist 2001; Klein *et al.* 2002). In the absence of honeybees, some of these visitors might prove alternative efficient pollinators (Parker, Batra & Tepedino 1987; Heard 1999). However, this study provides evidence that in the absence of artificial beekeeping, pollination by these bees is linked tightly to the preservation of fine-grained mosaics of plantations and forest remnants.

The decrease in species richness of insects visiting grapefruit flowers far from the edge may not be important for grapefruit pollination, given the presumed high degree of self-compatibility exhibited by this crop (Cameron & Frost 1968; Gmitter 1995) and the dominance of *A. mellifera* as its almost exclusive pollinator (Roubik 1995). However, diverse pollinator guilds in other pollination systems can be linked to genetically diverse pollen loads, high fruit and seed set and vigorous offspring (Herrera 1987; Waser *et al.* 1996). Declines in pollinator diversity also means low chances of pollination reassurance, particularly in crops which rely heavily in just one pollinator species such as *A. mellifera*.

Similar strong edge effects of forest remnants into agricultural matrix have been found in the pollination of coffee (Klein *et al.* 2003a,b; Ricketts 2004). Steffan-Dewenter & Tscharntke (1999) also reported decreasing bee abundance and diversity with increasing distance to seminatural grassland fragments. Based on this limited sample of edge effect studies but a wider literature of fragmentation effects on pollinator assemblages (reviewed in Debinsky & Holt 2000; Aizen, Ashworth & Galetto 2002), we can now affirm that habitat

fragmentation leads to a decrease in pollinator diversity and a simplification of pollination systems in agricultural landscapes.

Along with decreases in pollinator diversity with distance to the forest, we also found predictable changes in pollinator species composition. Pollinator assemblages varied among plantations, but they tended to become homogeneous far from the forest edge. Besides *A. mellifera*, the pollinator fauna of the interior of the plantations was characterized by *B. atratus*, some halictid bees and a few wasps. We also found evidence that flower visitor species, particularly bees, sampled in the interior of the plantations were non-random, nested subsets of those found near to the edge. Recently, Kremen (2004) reported that pollinator communities may become disassembled in a strongly non-random order, with the functionally more important species tending to be more sensitive to agricultural intensification. From our results, we can assemble a picture in which a relatively idiosyncratic, heterogeneous pollinator fauna is 'spilled' outside forest edges, but only the same subset of species is able to persist and forage inside plantations, on crop flowers, a few hundred metres distant from these edges. Among those species that can tolerate or thrive in agricultural landscapes there are some light-tolerant pollinators (Liow, Sodhi & Elmqvist 2001) and large body-sized bees, a trait that is related commonly to high flight capabilities and extensive foraging ranges (Steffan-Dewenter & Tscharntke 1999; Gathmann & Tscharntke 2002). Overall, our results show that conversion of land use to agriculture brings about not only a depauperation, but also a homogenization of the pollinator fauna.

Large fluctuations in insect populations, including pollinators, are common (Williams, Minckley & Silveira 2001). Throughout the 3 years of this study, we found consistent trends with the richness and abundance of flower visitors declining as the distance to the edge increased. Visits performed by *Apis mellifera* varied greatly between years, but the visitation rate of other pollinators taken together, even representing a low proportion of all visits, did not change so abruptly. A diverse pollinator assemblage may buffer spatial and temporal heterogeneity of feral populations of *A. mellifera*, thus reducing the dependence on a single exotic pollinator and thus increasing the stability of pollination services provided by natural habitats.

AGRICULTURAL AND CONSERVATION IMPLICATIONS

A much-promoted pollination crisis has direct implications for cultivated plants (Roubik 1995). However, our knowledge is so limited that even for many common crops we do not know the identity of all floral visitors, or how efficiently they pollinate the flowers (Richards 2001). More generally, there is still a lack of empirical studies quantifying the contribution of wild pollinators to crop yields or the effects of a reduction

in pollinator diversity and abundance to agriculture. Among the few exceptions are studies showing a contribution of native bees to coffee pollination (Klein *et al.* 2003a; De Marco & Monteiro Coelho 2004; Ricketts 2004). In addition, native bee assemblages can provide pollination service for watermelon when honeybees are absent (Kremen, Williams & Thorp 2002). However, the pollination service provided by native bees depends on the proportion on natural habitats surrounding farms (Kremen, Williams & Thorp 2002; Kremen *et al.* 2004). Contrary to expectations based on a labile breeding system (Cameron & Frost 1968), we found that grapefruit production in NW Argentina is severely pollen-limited and the presence of an insect vector that transfers pollen is essential to fruit set (N. Chacoff & M. A. Aizen, unpublished data).

Whereas forests may represent a source of pollinators for many crops, massive-flowering crops may provide alternative, albeit temporary, pollen and nectar resources for many pollinators (Banaszak 1992; Westphal, Steffan-Dewenter & Tscharntke 2003). This will increase the abundance of many key pollinators such as bumblebees, and also the connectivity among remnant patches of native habitats (Ricketts 2001; Jules & Shahani 2003). However, most agricultural lands are not suitable nesting habitats for the majority of solitary and social bees, including feral honeybees. This deficiency can be supplied by remnants of native habitats that can provide nesting and floral resources throughout the entire year (Lagerlof, Stark & Svensson 1992). Thus, increasing edge density in agricultural lands, through preservation and restoration, can foster stocks of diverse and abundant pollinators.

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Supplementary material

The following supplementary material is available as part of the online article (full text) from <http://www.blackwell-synergy.com>

Appendix S1. List of insect visitors taxonomic and group identity, distance to the forest where each morphospecies was found and the number of times each morphospecies was observed during transect censuses.

Appendix 1

Variance and covariance parameters > 0 associated with random effects in the analysis of frequency of visits and species richness

Dependent variable†	Source of variation					
	Year	Site	Site × distance	Year × distance	Site × year	Site × year × distance
1. Species richness	–	0.011	–	0.034	–	–
2. Total visit frequency	0.009	0.002	0.001	–	0.011	–
3. Visits by <i>Apis mellifera</i>	0.009	0.003	0.001	–	0.002	–
4. Visits by other visitors	–	0.271	–	0.133	–	–0.837

†Numbers correspond to analysis column in Table 1.