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Short communication

Soybean crops may benefit from forest pollinators

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ABSTRACT

Increasing evidence indicates that pollinator diversity and pollination services are highly threatened by the destruction and fragmentation of natural habitats and the intensification of agricultural landscapes. Here we analyze the bee visiting ensemble on soybean flowers and the effects of pollinator visits on soybean reproductive success, within a fragmented Chaco forest landscape embedded in a soybean matrix in central Argentina. We assessed visitation rates in relation to distance from the forest (5, 50 and 100 m) compared soybean bee assemblages with those on wild flowers in the nearby forest fragments, and carried out an exclosure experiment in order to assess the contribution of insect visits to soybean reproductive success. We also analyzed the relationship between visitor body size and the distance from the forest to the visited flower. Five species belonging to two families of bees were observed visiting soybean flowers. The bee species observed on soybean were well represented in the forest, and *Apis mellifera* was the most abundant species, visiting soybean flowers at all studied distances from the forest. Instead, wild visitors displayed a turnover of species throughout those distances, with smaller species being restricted to the forest proximity and replaced by larger ones toward the interior of the crop. Total visitation rates were significantly and negatively affected by distance to the forest. All plant productivity variables measured in the exclosure experiments were significantly improved in exposed flowers, duplicating the values observed without pollinators. The present study offers preliminary evidence linking forest proximity to higher visitation rates and presence of wild pollinators on soybean flowers thus providing for the first time, evidence of the forest role as pollinator donor for the soybean crop. It also shows that pollinator activity matters for this crop, leading to increased soybean yield. Further research on this topic is necessary in order to provide informed guidelines to enhance soybean production while simultaneously promoting natural habitat conservation.

1. Introduction

Forests and other natural habitats are being converted into agricultural lands at extraordinarily high rates, with dramatic effects on biodiversity and, ultimately, on ecosystem services (Palmer et al., 2004). Pollination is a crucial ecosystem service, with nearly 90% of the world's wild plants (Ollerton et al., 2011), over 70% of the major crops and at least one third of the global food production (Klein et al., 2007) depending on animal pollination. Moreover, although many animal-pollinated wild plants can self-pollinate to some degree, all rely on pollinators in the long term for genetic exchange among individuals (Winfree et al., 2011).

Increasing evidence indicates that pollinator diversity and pollination services are highly threatened by the destruction and fragmentation of natural habitats and the intensification of agricultural landscapes (Potts et al., 2010). Forests and other natural areas near agricultural systems usually support a variety of wild pollinators, with an important and well documented movement toward the cultivated land (see review in Blitzer et al., 2012). The exchange of insects between crops and natural environments has received much attention in fragmented habitats, where the increased extent of edges facilitates dispersal of organisms across habitats. The “spillover” of insects to either side of the edges can strongly affect the dynamics of ecological processes in such environments (Rand et al., 2006). Moreover, pollinator richness, visitation rates and pollination on crops have been shown to decline with increasing distance from natural

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habitats (Ricketts et al., 2008; Carvalheiro et al., 2010). Given the mounting environmental conflict between conservation and agriculture, the consequences of pollinator spill-over between natural and managed systems need to be better understood (Blitzer et al., 2012), in order to predict future changes in pollinators, animal-pollinated plants and resulting pollination services (Potts et al., 2010).

Soybean (*Glycine max* L. Merril) is currently the most important seed legume worldwide, contributing 25% of the global edible oil and about two-thirds of protein concentrate for livestock feeding (Agarwal et al., 2013). Despite its relevance, information on soybean pollination ecology across countries and varieties is still scarce (Chacoff, 2010). Although primarily self-compatible, soybean flowers display anatomic traits predicted in insect pollinated plants, such as nectar guides and floral nectaries (Palmer et al., 2009). It increasingly appears that the impact of flower-visiting insects has been underestimated, with evidence that insects such as honey bees contribute to cross pollination (Yoshimura, 2011). With a "modest dependence" on pollinators (Klein et al., 2007), soybean production has been shown to increase in presence of foraging *Apis mellifera* (Moreti et al., 1998; Chiari et al., 2005, 2008).

On the other hand, although soybean flowers offer low-quality nectar and may, thus, not be particularly attractive for bees (Erickson, 1975), their high density in crop fields may attract pollinators from nearby natural environments (Chiari et al., 2005). In fact, soybean flowers have proved the main source of pollen (Free, 1993) and nectar resources (Santos et al., 2013) for some beehives.

Here, we analyze the bee visiting ensemble on soybean flowers and the effects of pollinator visits on soybean reproductive success, within a fragmented Chaco forest landscape in Central Argentina. Soybean is the main export from Argentina, which is the third largest soybean exporter worldwide (Calvo et al., 2011). By analysing visit rates in relation to distance from the forest, and by comparing soybean bee assemblages with those on wild flowers in the nearby forest fragments, we hoped to provide for the first time, evidence of the forest role as pollinator donor for the soybean crop. We expected bee richness and visitation rates to decrease and to be restricted to larger species (body size being a good estimator of bee foraging distance, e.g., Greenleaf et al., 2007), on flowers located further away from the forest. Moreover, we expected lower reproductive success on soybean flowers experimentally isolated from pollinators.

2. Materials and methods

2.1. Study area

The Chaco Serrano district belongs to the most extensive dry forest in South America, with 750 mm annual rainfall concentrated mostly in the warm season (October–April), and mean temperatures between 26 °C (maximum) and 10 °C (minimum) (Luti et al., 1979). The native vegetation comprises an open tree stratum (up to 15 m high), 1–3 m high shrubs covering 10–80% of the ground, a herbaceous layer (up to 95% cover), and many vines and epiphytes (Cabido et al., 1991). This vegetation is currently restricted to isolated patches of native vegetation within an intensely managed agricultural matrix, largely dominated by wheat in winter and soy in summer. Within a fragmented Chaco Serrano landscape in Central Argentina (31°10′ – 31°30′ S and 64°00′ – 64°30′ W) we selected from satellite images (QuickBird, October 2010), nine landscapes circles (500 m diameter) including on average 32.66% (SE = 7.33) natural vegetation cover. At the time of the study, the transgenic soybean variety ALM 3830 was cultivated in the area.

During December 2010–January 2011, hymenopteran visits to soybean flowers were observed on each landscape circle (further referred to as "sites") at three distances from the forest edge: 5, 50 and 100 m. At each distance, all visits by hymenopteran insects were recorded during a 10-min interval in two linear plots, interval in each of two lineal plots 50 cm in length along a row of soybean plants, i.e., one hour per site and sampling date. Each site (with two plots at three distances from the forest) was observed four times during the flowering period (total 4 h per site), under similar weather conditions (moderately windy and sunny days). Attending to possible differential preferences in visitation time across bee species half of the observations on each site was made in the morning (8:30–12:30 h) and the other half in the afternoon (14:00–18:00 h). Moreover, insects visiting soybean flowers were collected and taken to the laboratory, where they were identified, and their wing and body length were measured with a calibrated ocular micrometer at 2.5× (±0,01 mm).

The pollinator ensemble observed on soybean, was compared with a regional database compiling flower-visitor records from 45 h of observation per site in nine forest areas (Musicante, 2013) in order to corroborate the species occurrence in the forest. The number of other *Fabaceae* species available in the forest, provided also by the same database, was considered to detect possible interaction preferences.

Visitation rate was estimated as: [(visitor number/open flowers in the patch)/observation time] × 1000, as widely used in the relevant literature contributions (Vázquez et al., 2005). Visitation rates were calculated: (a) for each pollinator species in soybean (this study) and in the forest (data from Musicante, 2013); (b) for soybean plants at each distance to the forest in each site, summing up all visiting pollinator species.

2.2 Exclosure experiment

This experiment was carried out in order to assess the contribution of insect visits to soybean reproductive success. Ten plants, at least 10 m apart from each other and at 5 m from the forest edge, were randomly selected in each site. On each plant, 6 floral buds were marked and on three of them pollinators were excluded by enclosing the buds in voile bags. After a month, all mature fruits developed from marked flowers were harvested and carried to the laboratory to be measured and weighed, and to estimate variables indicative of plant productivity: percentage of aborted flowers, number of seeds per fruit, weight of fruits and seeds and reproductive success (Dafni et al., 2005). Relative reproductive success was estimated as (no. fruits/no. flowers) × (mean no. seeds per fruit/mean no. ovules per flower). The mean number of ovules per flower was previously estimated by counting ovules in ten randomly selected soybean flowers per site, which were excised before opening and conserved in alcohol 70%.

2.3. Data analysis

Variations in pollinator richness and visitation rates were analyzed by means of linear mixed models (LME) with distance to forest as fixed factor and site as random. Prior to these analyses, Mantel tests were performed to check for spatial effects of site location on the response variables. Euclidean distance matrices of visitation rates and visitor species richness were compared with the geographic distance matrix (latitude and longitude at the site center point).

Visitation rates displayed by the visitor species observed in soybean and in the forest were compared by means of *G*-test, in order to check for differential activity in relation to habitat type. Spearman's rank correlations between femur or wing length of each native visitor and the distance from the forest to the flower it visited were also performed, in order to explore possible dispersal limitations for forest species to visit soybean flowers.