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Pollination effectiveness of different strawberry floral visitors in Ribatejo, Portugal: selection of potential pollinators. Part 2

S. Albano*[1], E. Salvado*, S. Duarte*, A. Mexia, P.A.V. Borges*****

* *Instituto Nacional dos Recursos Biológicos (INRB L./INIA), Unidade de Protecção das Plantas, Quinta do Marquês, Nova Oeiras, 2784-505 Oeiras, Portugal.*

** *Instituto Superior de Agronomia, Secção de Protecção Integrada (ISA - SAPI/DPPF), Tapada da Ajuda, Lisboa, Portugal.*

*** *Universidade dos Açores, Departamento de Ciências Agrárias (Azorean Biodiversity Group - CITA-A), Terra-Chã, 9701-851 Angra do Heroísmo, Portugal.*

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Abstract: This study was carried out in a strawberry (*Fragaria* × *ananassa* Duch.) field located in Ribatejo, Portugal, and aims to describe the qualitative component of the visits for three strawberry floral visitors, attaining the best results in a previous work. The main objectives were: (1) to assess the pollination rate (PR) resulting from a single visit of *Apis mellifera* L., Syrphidae and native bees, and (2) to characterize the foraging behaviour of each of these categories in order to select the potential pollinators of strawberry crops under open field conditions in the Ribatejo. All analysed categories were shown to be potentially useful pollinators, since there were no significant differences among them in the pollination rates, after a single visit. The observed distinct foraging behaviour among them did not result in significantly different pollination rates. Growers are recommended to take advantage of the several pollinators, either the honey bee or the native pollinators (Syrphidae and native bees). The importance of diversifying pollination sources, avoiding the dependence on a single specific group is stressed. This study also suggests measures which envisage the conservation, establishment and increase of native pollinators' populations in the typical agro-ecosystem of the Ribatejo region.

1. Introduction

Flowers of all the current commercial strawberry cultivars (*Fragaria* x *ananassa* Duch.) are hermaphrodite and self-fertile. However, these flowers may not be completely self-fertilizing (McGregor, 1976). Indeed, the stamens are positioned in such a way within the flower that, when anthers dehisce, pollen drops on many, but not necessarily all of the pistils (McGregor, 1976). The fertilized ovules (achenes), through auxin release, promote receptacle development (Nitsch, 1950). The achenes, resulting from fertilized ovules, are surrounded by a well-developed fleshy tissue, while receptacle zones containing non-fertilized ovules will not develop, originating a misshapen and smaller berry (Vincent *et al*., 1990). If there is no insect-transported pollen, the combined action of gravity and wind assures most of the pollination, even though the pollination rate of the achenes rarely surpasses 60% (Pion *et al*., 1980). There is a relationship between the number of fertilized ovules (achenes) and berry weight (Nitsch, 1950; de Oliveira *et al*., 1983; Chagnon *et al*., 1989; Albano *et al*., 2005 a).

Honey bees (*Apis mellifera* L.) are recognized as the main pollinator of the strawberry crop (Nye and Anderson, 1974; Goodman and Oldroyd, 1988; Chagnon *et al*., 1989; de Oliveira *et al*., 1991; Svensson, 1991; Free, 1993). Nonetheless, several recent studies have been carried out with the aim of extending the range of appropriate pollinators for this crop. For greenhouse conditions *Bombus* spp. is another widely used pollinator group (Paydas, 2000; Zaitoun *et al*., 2006). Several species of stingless bees have also been the subject of many studies. In Japan, *Nannotrigona testaceicornis* Lepeletier and *Trigona minangkabau* have been successful tested for strawberry pollination inside greenhouses (Maeta *et al*., 1992; Kakutani *et al*., 1993) and Malagodi-Braga and Kleinert (2004) in Brazil have shown that *Tetragonisca angustula* Latreille is an effective strawberry pollinator that can promote a significant increase in overall strawberry production. Some Megachilidae, such as *Osmia rufa* L., were also

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[1] Corresponding author: silviamalbano@yahoo.com

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found to be effective pollinators of this crop, applicable in plastic tunnels or greenhouses (Wilkaniec and Radajewska, 1997).

The use of managed species (*A. mellifera*, *Bombus* spp. and others) as pollinators may be vital in: i) large monocultural crops, where a great pollination effort is required; ii) ecosystems where populations of natural pollinators are reduced due to lack of adequate habitat and the use of pesticides; iii) enclosed crops such as in greenhouses; and iv) seasonal crops that precede the annual activity of pollinator insects (Teixeira and Branco, 2006). However, the problems that beehives have been facing in recent years (parasites, africanisation and others) and the consequent decline of their number, have resulted in the decrease of the available colonies for pollination and the rise of rental prices (Delaplane and Mayer, 2000). This, together with threats to native bee abundance and diversity, has contributed to the increase in research efforts on the role of native bee species in pollination of agricultural crops (Stubbs and Drummond, 2001). Furthermore "Colony Collapse Disorder" (CCD) has recently created a very serious problem for beekeepers and could threaten the pollination industry (Johnson, 2008).

Native pollinators are especially appealing because they are more adapted to regional conditions and may assure pollination of strawberry flowers even when climatic conditions do not favour honey bee activity (de Oliveira *et al*., 1991). On certain crops, some native bee species were shown to possess a pollination efficiency that is equivalent, or higher, to that of *A. mellifera* (Freitas and Paxton, 1998; Canto-Aguilar and Parra-Tabla, 2000). In the particular case of strawberry crops, there is evidence for a complementary effect of native bees and honey bees visits on flower pollination (Chagnon *et al*., 1993; Malagodi-Braga and Kleinert, 2007). Chagnon *et al*. (1993) also suggest that the introduction of beehives may be questionable in sites where population densities of natural pollinators are high.

Several methods have been used to compare the efficiency and effectiveness of flower-visiting insects as pollinators in strawberry crop. Nye and Anderson (1974) estimated pollination efficiency of different visiting-insect categories by attributing scores based on factors such as the amount of loose pollen carried on the body of the insect, body size, hairiness, and degree of activity. Abrol (1989) compared the efficiency of different insect pollinators on the basis of their field behaviour, nectar-pollen carrying capacity and ability to pollinate flowers per unit of time. Chang *et al*. (2001) and Zaitoun *et al*. (2006) performed comparative studies with *A. mellifera* and *Apis cerana* Fabr., and with bumble bees and honey bees, respectively, analysing several pollination effects in factors like fruit weight and percentage of malformed fruits. Other works, such as Chagnon *et al*. (1993), measured the effect of the number and length of visits (honey bees and native bees) on the pollination rate. Kakutani *et al*. (1993) compared *A. mellifera* and *T. minangkabau* in different aspects, and used a model that described the percentage of fertilized achenes in a single berry as a function of the number of bee visits to a flower. Malagodi-Braga and Kleinert (2007) based their analysis on several aspects such as fruit weight, number of achenes, fertilization rates, and length of visits, either by a single or four visits by several species of insects, in order to compare their relative efficiency.

In this study, the pollination effectiveness of a strawberry floral visitor was measured through the determination of pollination rates resulting from a single visit (the ratio of fertilized ovules to total ovules, or ovule fertilization efficiency per visit, in accordance with Inouye *et al*., 1994), similar to the procedure carried out in other studies on this crop (Chagnon *et al*., 1989, 1993).

The quantitative and qualitative components of several insect visits should be analysed (Herrera, 1987) to select the potential pollinators for strawberry crop. Since the quantitative component of several insect visits under open field conditions in the Ribatejo region has been already analysed in Albano *et al*. (2009), this particular study aims to assess the qualitative component of visits by measuring the pollination rate (PR) resulting from a single visit of *A. mellifera*, Syrphidae and native bees as these three insect categories were shown in the above mentioned study, to possess higher Visitation Rate Indexes (I_{VR}). In addition, the present work aims to characterize the foraging behaviour of each of these categories in order to select the potential pollinators of strawberry crop under the open field conditions in the Ribatejo.

2. Materials and Methods

Field work

The work was carried out in a strawberry field situated in the locality of Lamarosa (Coruche), in the Ribatejo region, which is one of the main regions for the production of strawberries in Portugal (Costa *et al*., 2008). With a total of 2.5 ha of 'Camarosa' strawberry in open field conditions, this field was located on a large farm composed of 600 ha of cork oak forest, managed for cork harvesting and cattle grazing. In this field, Integrated Pest Management (IPM) procedures were adopted for crop protection; planting occurred in the second week of October 2005, with a plant density of approximately 60,000 plants/ha; plants were planted, in double-rows, at a distance of 30 cm from each other, growing on black plastic mulching; a drip irrigation system was used.

The field observations were made during the 2005 blooming period (from late March until late May). Five beehives of *A. mellifera* were set up in the vicinity of the field. In this experiment only primary flowers were used because they are the first to open in the inflorescence, they are larger, and produce the most commercially valuable berries (Chagnon *et al*., 1989). Despite the duration of stigma receptivity, which can last up to seven days after flower opening (Moore, 1964), the best period for pollination occurs between the first and fourth day (McGregor, 1976). For this reason, we only used flowers aged one or two days.

Every day, several closed flower buds were enclosed in small tulle bags in order to exclude all insect visits. When a new virgin flower opened, its stem was tagged well below the flower to avoid affecting normal insect visitation. The flowers were exposed to insect visits during the period of highest activity of insect visitors (from 10:00 to 16:00). For each observed visit, the category of the insect, the date of the visit and the behaviour adopted by the insect during the visit were registered using a voice recorder. After receiving a visit, each flower was enclosed again to avoid further insect visits. Simultaneously, throughout the observation period, 60 flower buds chosen at random were marked. Thirty were enclosed in tulle and formed the sample for the "Unpollinated" treatment. The remaining were kept open for the "Naturally pollinated" treatment.

The exclusion bags permitted airflow around the flowers and allowed wind to move the flower. The bags were removed when the flowers senesced. At the moment of ripeness normally considered for commercial purposes, the fruits were picked and their weight recorded. Then, in the laboratory, each fruit was divided horizontally in two halves (basal - peduncle side; apical side), and for each half, the number of well developed achenes, malformed or aborted achenes, and the number of undeveloped ovules were counted. The total pollination rate (PR_{total}) of each berry was determined by the proportion of fertilized ovules (achenes), according to Chagnon *et al*. (1989, 1993). The basal (PR_{basal}) and apical (PR_{apical}) pollination rates were also calculated for each collected fruit.

The analysed parameters were only computed for the three categories of visitors that showed the highest I_{VR} in a previous study carried out in the same region: Syrphidae (Diptera), *A. mellifera* (Hymenoptera) and native bees (Hymenoptera) (Albano *et al*., 2009). To increase the probability of observing visits from the categories apart from *A. mellifera*, the observations were initiated in late March and were concentrated on the most frequent groups for each category: *Eristalis* spp. (Diptera, Syrphidae), and Halictidae family (Hymenoptera, Halictidae) (Albano *et al*., 2009).

Data analysis

Due to the non-normality of the data relating to the pollination rate (PR) parameter, comparison of PR_{total}, PR_{basal} and PR_{apical} for the distinct treatments was made by applying the non-parametric Kruskall-Wallis test. Multiple comparison tests were performed to verify which of the treatments differed. To examine the relationships between the number of fertilized ovules (including the well developed and the malformed achenes) and the fruit weight, Pearsons correlation was used for the total of the data. To study this correlation separately for March/April and May, 40 fruits were selected, at random, for each period. The significance level used for all the tests was 5%. Statistical tests were performed with STATISTICA software (StatSoft, Inc., 2004), version 7.0.

3. Results

Foraging behaviour

Differences were found between the behaviour of *A. mellifera* and native bees during their visits to strawberry flowers. While *A. mellifera* always landed on the top of flowers, native bees landed on the stamen zone, and occasionally even on the petal zone. During visits, both *A. mellifera* and native bees performed a circular movement around the flower, allowing contact with basal pistils (which are close to the stamens), but while *A. mellifera* ensured a contact with the head as its proboscis was inserted into nectaries, native bees made that contact using the whole body, amid the rows of stamens, alternating between pollen and nectar collection. While foraging for nectar, the body size of *A. mellifera* allowed permanent contact of its thorax and abdomen with the apical pistils, promoting the transport and deposit of pollen within this flower region. However native bees, due to their smaller body size, rarely contacted the apical region, restricting most of their action to the basal zone. It should be noted that this description referred to the whole sample of observed individual bees of the Halictidae family. In post study observations, other native bees, such as those belonging to the Andrenidae and Megachilidae families, with larger body sizes, were occasionally observed on flowers, showing a similar behaviour to that of *A. mellifera*.

Eristalis spp. (Diptera, Syrphidae) were mostly observed performing a circular movement around flower stamen rows, reaching the nectaries with their proboscis and probing the anthers, one by one. During their visits, these indigenous syrphid flies allowed permanent contact of their bodies not only with the basal but also with the apical pistils, due to their large body sizes. Conversely, other members of Syrphidae, such as *Syritta* spp. and *Sphaerophoria* spp., which were occasionally observed on flowers not selected for the experiment, exhibited a behaviour that did not ensure a permanent contact of their bodies with most flower stigmas.

Pollination rate (PR)

The initial sample was slightly reduced as some fruits could not be included in the analysis because they had problems (for example, *Botrytis*) that could influence their weight (final sample sizes: Unpollinated n= 30; Naturally Pollinated n= 30; One visit of *A.*

mellifera n= 29; One visit of native bee (Halictidae) n= 18; One visit of Syrphidae (*Eristalis* spp.) n= 14).

The PR $_{total}$ parameter differed significantly between the several treatments (H (4, 121) =53.27; p <0.0001). The "Unpollinated" treatment showed a PR $_{total}$ significantly lower than other modalities (Fig. 1). For this treatment the fruits only achieved a PR $_{total}$ of approximatelly 53%.

The three categories of insects analysed did not reveal significant differences among them. The PR $_{total}$ of the "Naturally pollinated" flowers did not differ significantly from the modalities which received one visit from *A. mellifera* or from native bees, but was significantly lower with regard to the flowers assigned to the Syrphidae treatment.

Regarding the PR $_{apical}$ and PR $_{basal}$ parameters, significant differences were found between the several modalities (PR $_{apical}$: H (4,121) =65.35; p < 0.0001; PR $_{basal}$: H (4,121) =25.59; p <0.0001) (Fig. 1).

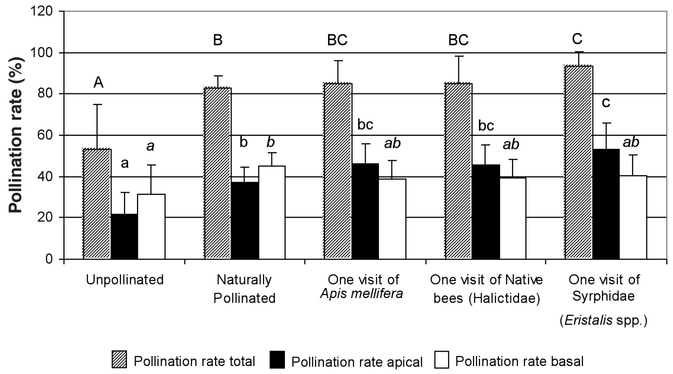

Fig. 1 - Means (±SE) for Pollination rate total, Pollination rate apical and Pollination rate basal for each treatment (Bars of the same Pollination rate with different letters are significantly different to p<0.05).

The PR $_{apical}$ for the "Unpollinated" treatment was significantly lower than all the others. The PR $_{basal}$ for the "Unpollinated" treatment was only statistically lower only than the "Naturally pollinated" treatment. All the treatments involving one insect visit did not reveal significant differences among them for both the PR.

A statistically significant positive correlation was found between the number of fertilized ovules (achenes) and the fruit weight (r= 0.60; p< 0.0001; n= 121). When analysing the fruits collected in March/April, distinct from the ones collected during May, it was verified that, despite observing a positive correlation in the two periods, the correlation index was higher for the first period (March/April: r= 0.82; p < 0.0001; n= 40; May: r = 0.47; p < 0.01; n= 40).

4. Discussion and Conclusions

The comparison of results between the "Unpollinated" treatment and others revealed a strong contribution of insect visits for the pollination of strawberry crops, in accordance with other studies (Chagnon *et al*., 1989, 1993; López-Medina, 2002; Malagodi-Braga, 2002; Albano *et al*., 2005 a, b; López-Medina *et al*., 2006). It was shown that a single insect visit was able to promote, in comparison to the "Unpollinated" treatment, improvements both on PR $_{basal}$ and PR $_{apical}$ (in this latter case, statistically significant). This result may indicate that the apical pistils, located in a region where, possibly, the isolated action of wind and gravity is not sufficient to promote an adequate flower pollination, will significantly benefit from insect action even with a single visit.

Our results indicate that the three analysed categories of flower-visiting insects are statistically equivalent in terms of PR $_{total}$. For all insect categories, high values of PR $_{total}$ (on average, higher than 84%) were achieved after a single visit. However, it was shown in another work on 'Veestar' strawberry that a single visit by *A. mellifera* produced higher PR values than those obtained by native bees (Chagnon *et al*., 1993). Malagodi-Braga and Kleinert (2007) also found higher fertilization rates after single visits by *A. mellifera* than after single visits of the native bee *Dialictus* sp. (Halictidae) on 'Oso Grande' strawberry.

According to our study, the behavioural differences between native bees (Halictidae) and *A. mellifera*, as described by other authors (Chagnon *et al*., 1993; Malagodi-Braga and Kleinert, 2007), did not result in significant differences on PR $_{total}$, PR $_{apical}$ and PR $_{basal}$ between these categories. Results suggest that cultivar Camarosa, used in this study, is less dependent on insect pollinators than the cultivars used in the above mentioned studies. In this regard, Connor and Martin (1973) showed that there are significant variations in the ratio of stamen height to receptacle height among strawberry cultivars, and cultivars with shorter stamens have an advantage in insect pollination. Floral morphology (size and shape) is one of the factors that influence pollen deposit and dispersal during single visits by pollinators (Harder *et al*., 2001) and may be the origin of the differences found between cultivar ´Camarosa` and others. It is possible that the behaviour of native bees (belonging to the family Halictidae), which acted mostly in the region of basal pistils (close to the anthers), may have resulted in promoting the displacement of pollen from the anthers and its dispersion by the wind within the flower, possibly to the apical region. Similar results were obtained by Cane *et al*. (1992) and Shuang-Quan *et al*. (2002) regarding bee action in typically wind-pollinated plant species. *Eristalis* spp. had a very similar flower-visiting behaviour to *A. mellifera* and, thus, the lack of significant differences on the PR values between these two groups was expected. Results from other studies (Nye and Anderson, 1974) already suggested that *Eristalis* spp., including *E. tenax,* would be as efficient as *A. mellifera* in strawberry crop.

The comparison of the PR $_{total}$ between the "Naturally pollinated" treatment and the single insect visit treatments suggests that the visitation rate in the observed field was relatively low and the open flowers had, on average, one or fewer insect visits. It should be noted that, among all visiting exposure treatments, the "Naturally pollinated" was the only one for which the PR $_{apical}$ was lower than the PR $_{basal}$, which further supports the hypothesis of a low deposit on the apical region of these flowers which is typically favoured by insect visits.

Although it is not the aim of this study to determine the appropriate number of visits to obtain fruits of high commercial value, the results showed that, after single insect visits (of any kind of insect category analysed), high pollination rates were obtained. In other studies, using other cultivars, pollination rates of about 85% were found only after three visits by honey bees or indigenous bees (Chagnon *et al*., 1993). The same authors, assessing the 'Veestar' cultivar, concluded that the total length of honey bee visits necessary for adequate pollination was, approximately, 40 s, which corresponds approximately to four honey bee visits. Skrebtsova (1957 *in* Chagnon *et al*., 1989) estimated that an adequate fruit set requires 16 to 19 visits, and an optimal fruit set needs 20 to 25 visits. The comparison of results between these and the present study suggests, again, that cultivar 'Camarosa' has a higher self-pollination rate than the cultivars used in such studies and possibly it will require a lower number of visits for high commercial value fruits to be obtained.

The positive correlation found in this study between the number of fertilized ovules (achenes) and fruit weight has also been demonstrated in previous works on this crop (Nitsch, 1950; de Oliveira *et al*., 1983; Chagnon *et al*., 1989; Albano *et al*., 2005 a). The higher correlation level found in March/April, in comparison to that found in May, may indicate that fruit weight is more dependent on the pollination success during the first period than during the second, suggesting that, as the blooming period progresses, the weight is increasingly more dependent on other factors, such as the competition for resources by developing fruits. Other authors (Salvado *et al*., unpublished), in a study on tomato crop, also found that the influence of seeds on fruit weight in upper clusters, in late blooming, is less important, probably due to the action of other factors. All this suggests that the start of blooming is probably the most important period with regard to pollination.

For cultivar Camarosa in the Ribatejo region, under open field conditions, the start of blooming varies from late January to early February, depending on the year. The start of the blooming period is typically characterized by low temperatures, low light intensity levels, which limit pollen production and germination (Thompson, 1971; Risser, 1997), and high relative humidity levels that limit pollen transport. These conditions emphasize the importance of insect pollinators during this period. In Spain, a study carried out with the same cultivar under tunnel conditions showed that the period from November until January was the most problematic in terms of pollination (López-Medina, 2002).

Selection and management of potential pollinators

The results presented in this study indicate the existence of a set of potentially useful pollinators, with equivalent effectiveness levels, which includes both native pollinators (Syrphidae and native bees) and domesticated honey bees. However, results regarding the Syrphidae group should be interpreted with caution since they were based on a small sample. However, the information gathered in this study, supplemented with the data made available in Albano *et al*. (2009), may provide useful information for further discussion on management issues concerning these insects, seeking to maximise pollination in strawberry crops.

During the early blooming phases, when the number of indigenous insects (native bees and Syrphidae) are relatively low (Albano *et al*., 2009), it is important to advise growers to install beehives of *A. mellifera*, in order to increase the probability of honey bee visits to compensate the lack of native pollinators. As stated in Albano *et al*. (2009), the success of using honey bee colonies may require the use of certain management practices that seek to maintain and enhance the number of foragers throughout the blooming season (Currie, 1997; Ohishi, 1999). It is important to recognize the competition faced by strawberry flowers, either by other crops or natural vegetation, since this type of crop is not especially attractive to pollinators (Darrow, 1966; McGregor, 1976). Furthermore, strawberry cultivars vary in their attractiveness to pollinators (Vincent *et al*., 1990; Abrol, 1992). These factors may explain the unexpected relatively low PR found for the "Naturally pollinated" treatment.

As strawberry blooming progresses in the Ribatejo region, indigenous insects, such as Syrphidae and native bees, become increasingly more abundant (Albano *et al*., 2009). These insects have smaller foraging ranges compared to those of *A. mellifera*. As a result, problems of competition with other crops are less relevant. Flight range for many bee species is no more than 200 m (Eickwort and Ginsberg, 1980), while honey bees frequently forage several kilometres away from the nest (Visscher and Seeley, 1982; Beekman and Ratnieks, 2000). Despite their unquestionable value, it is probable that the population density of indigenous pollinators is not satisfactory on its own to ensure the pollination of the many hectares of different horticultural crops (including strawberry) that occur in this region. Furthermore, several works indicate that pollinator species abundance, in particular bee species abundance and diversity, are decreasing worldwide (e.g. Batra, 1984; Michener, 2000; Stubbs and Drummond, 2001). Some of the most important factors that can explain the declines in generalist native pollinators and bee communities are increases in pesticide use, increasing monoculture agricultural production systems, and the removal of hedgerows and other uncultivated open areas that provide wild flowering plants and nesting sites (Kevan, 1975; Banaszak, 1986; Kevan *et al*., 1990; Free 1993). For this reason, efforts should be made to increase their populations.

Faced with these circumstances, strawberry growers from the Ribatejo region are advised to take advantage of several pollinators, either the honey bee or the native pollinators (Syrphidae and native bees) shown here to be potentially useful. As mentioned in Albano *et al*. (2009) and in the present study, each category of pollinator shows some advantages and limitations. The best option for growers may be to increase diversification of pollination sources, avoiding the dependence of a single specific group.

The installation of beehives needs to conform to certain recommendations in order to increase the success of their use in this crop in the long term. The monitoring of these beehives by professional apiculture technicians should be considered. Simultaneously, measures that envisage the conservation, establishment and increase of native pollinators' populations in the typical agro-ecosystem of the Ribatejo region should be implemented. Some examples of such measures could be: (1) adopting long term environmentally-friendly management of crop programs (Steffan-Dewenter and Leschke, 2003) like Integrated Pest Management (IPM) or organic agriculture that take into account the beneficial contribution of pollinators (Vincent *et al*., 1990); using insecticides which are the least toxic to pollinators, spraying them when pollinator activity is lower, typically in the evening; (2) preserving or restoring natural land surrounding crops which will contribute to the abundance and diversity of natural pollinators' populations (Klein *et al*., 2003; Kremen *et al*., 2004; Ricketts, 2004; Kleijn and Van Langevelde, 2006); (3) providing artificial nesting sites or managing the fields to fit nesting requirements that encourage native pollinators (Loose *et al*., 2005); (4) minimising the use of herbicides, assuring diverse nectar and pollen resources are accessible all year round (Klein *et al*., 2003); and (5) research in the development or domestication of alternative pollinators (non-*Apis*) (Allen-Wardell *et al*., 1998). Implementing these measures may be extremely useful as they contribute to the increase of the diversity and abundance of native pollinators, and this allows increasing and stabilizing natural pollination performance, decreasing the dependency of a single introduced pollinator and promoting, at the same time, the establishment of other beneficial insects. As a consequence, these measures will contribute to sustainable agriculture practices.

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