1 2 9 0

UNIVERSIDADE DE
COIMBRA

Tiago Miguel Mota Parreira

QUANTIFYING FLORAL RESOURCES FOR HONEYBEES (*APIS MELLIFERA*) IN PORTUGUESE LANDSCAPES

Dissertação no âmbito do Mestrado em Ecologia orientada pelo Professor Doutor José Paulo Filipe Afonso de Sousa e pela Professora Doutora Sílvia Raquel Cardoso Castro Loureiro e apresentada ao Departamento de Ciências da Vida.

Outubro de 2021

Agradecimentos

Por estes dois anos de mestrado e pela ajuda para realizar este trabalho, gostaria de agradecer:

Aos meus orientadores, Professor Doutor José Paulo Sousa e Professora Doutora Sílvia Castro, pela ajuda e orientação durante este período.

À Sara, pela ajuda no laboratório e pelos conselhos dados em relação à dissertação.

À minha família, especialmente ao meu pai, à Isabel, à tia Helinha, à avó Luísa e à minha Filipa. Uns por terem ido para o campo comigo em tempo de necessidade, todos por terem ouvido os meus queixumes. Espero que pelo menos tenham gostado das aulas de “plantas”.

Aos meus amigos da terra, Rui, João, Tiago, Fred, Beninho, Ruca, Rufino, Chico e Bibita, porque ninguém me ouviu reclamar tanto quando alguma coisa não dava logo certo como vocês.

Aos meus amigos da universidade, Gonçalo, Franco, Humberto, e Beatriz, muito aguentaram as minhas insatisfações e angústias. Sem vocês não teria conseguido.

Table of Contents

Resumo

A polinização é um serviço de ecossistema crucial para persistência e reprodução das plantas com flores, sendo vital para a manutenção das comunidades de plantas silvestres e da produtividade agrícola.

As abelhas-do-mel são polinizadores-chave, fornecendo serviços de polinização para as principais culturas do mundo. Além disso, as abelhas-do-mel têm preferência nas plantas utilizadas para forrageamento, uma vez que o néctar é convertido em mel, que é a principal fonte de energia, enquanto o pólen é a fonte de lípidos e proteínas. Diferentes espécies de plantas produzem diferentes quantidades e composições de recompensas florais (pólen e néctar) para os visitantes florais.

Polinizadores, como as abelhas-do-mel, usam traços florais como preditores de recompensas. Por exemplo, apenas plantas de alta viabilidade podem pagar os custos de produção de flores maiores e providenciarem recompensas para os polinizadores.

Neste estudo, foram caracterizados traços florais, valores de interesse apícola e valores de "bee-friendliness", e recompensas florais obtidas em Idanha-a-Nova e Lousã. Para isso, foram feitas pesquisas bibliográficas de recompensas florais e valores de interesse apícola, bem como quantificações do volume do néctar, concentrações de açúcar e produção de pólen tanto em campo como em laboratório. Embora a apicultura seja uma atividade económica importante nessas paisagens, existe pouca ou nenhuma informação sobre valores de recompensas e interesse apícola.

Os resultados deste estudo mostram que, entre todas as características florais estudadas, apenas tamanhos médios de unidades reprodutivas podem ser relacionados com valores de interesse apícola. Recursos florais na forma de néctar são bons preditores de valores de interesse apícola em paisagens portuguesas. Os resultados também mostram que o valor de “bee-friendliness” é suportado por valores de néctar.

O conhecimento da associação de características florais e valores de interesse apícola é fundamental para auxiliar os apicultores a melhorar a aptidão das suas colónias.

Palavras-chave: Traços florais, Interesse apícola, Polinização, Síndromes de polinização, Comunidade vegetal

Abstract

Pollination is an ecosystem service that is crucial to the persistence and reproduction of flowering plants, being vital to the maintenance of both wild plant communities and agricultural productivity.

Honeybees are key managed pollinators, providing pollination services to leading crops worldwide. Furthermore, honeybees have preferences in the plants used for foraging since nectar is converted to honey, which is the major source of energy, while pollen is the source of lipids and proteins. Different plant species produce different amounts and compositions of floral rewards (pollen and nectar) for floral visitors.

Foraging pollinators, such as honeybees, use floral traits as predictors of rewards. For instance, only plants of high viability can pay the costs of producing large flowers and providing pollinator rewards.

In this study, floral traits, apicultural interest values, and bee-friendliness values were characterized, and floral rewards were accessed in Idanha-a-Nova and Lousã. For that, bibliographic research of flower rewards and apicultural interest values was made, and quantification of nectar volume, sugar concentration, and pollen production were both made in the field and laboratory. Even though apiculture is an important economic activity in those landscapes, little to no information on reward values and apicultural interest was known.

The results of this study show that, among all studied floral traits, only mean reproductive unit sizes are related with apicultural interest values. Floral rewards in the form of nectar are a good predictor of apicultural interest values in Portuguese landscapes. The results also show that the bee-friendliness value is supported by nectar values.

The knowledge of the association of floral traits and apicultural interest values is fundamental to helping beekeepers improve the fitness of their colonies.

Keywords: Floral traits, Apicultural interest, Pollination, Pollination syndromes, Plant community

Introduction

Pollination

Pollination is a transference of pollen grains from anthers (part of a stamen that contains pollen) to the stigma (receptive surface of the pistil). That can occur either between stigma and anther from the same flower or different flowers from the same individual plant (self-pollination) or between anthers and stigmas from different plants (cross-pollination). Once pollen touches the stigma, pollen tube germination occurs, which results in the fertilization of ovules. Pollination is an ecosystem service that is crucial to the persistence and reproduction of flowering plants, being vital to the maintenance of both wild plant communities and agricultural productivity.

The great majority of flowering plants cannot set seeds or fruits without fertilization (IPBES, 2016; Potts et al., 2010) (Fig. 1).

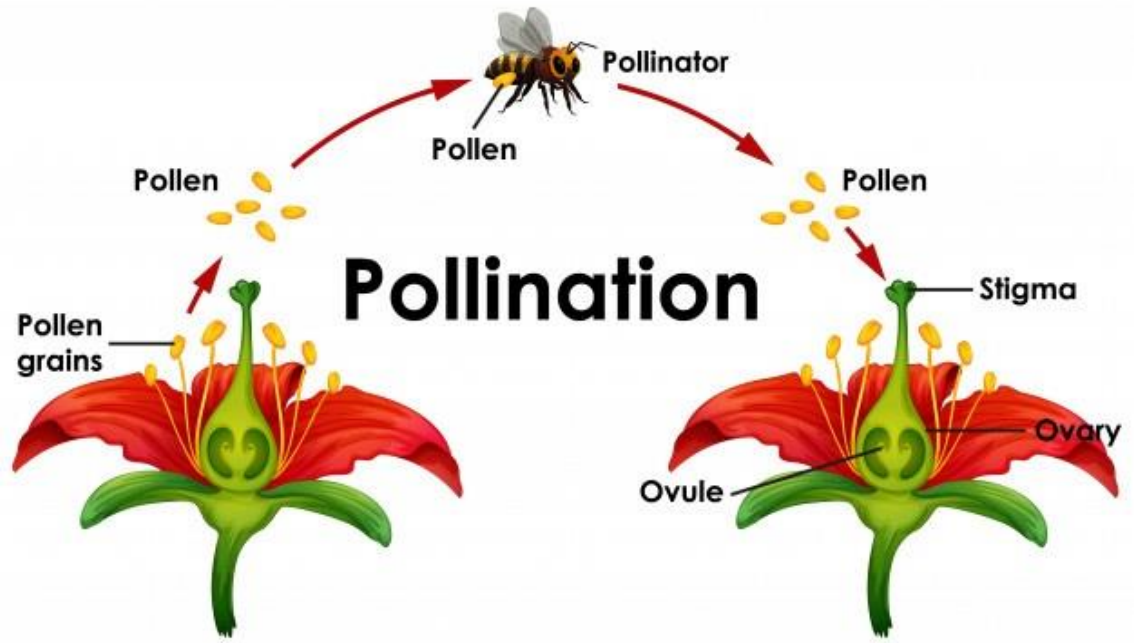


Fig. 1 - Pollination.

Worldwide, there are many animals responsible for pollination, such as insects (*e.g.*, bees, wasps, butterflies, moths, beetles, some flies, thrips), birds, bats, and other vertebrates. The main mode of pollination across the planet is entomophily or insect pollination, and bees are the most efficient pollinators. In natural habitats, and as a consequence of honeybees dissemination due to the beekeeping increase, honeybees are the most frequent visitor in 13% of plant species and the only one in 5% of them (Hung et al., 2018). About 87.5% of all flowering plant species depend on animal pollination (Ollerton et al., 2011); also, more than 70% of world crops depend on some level of pollination (Klein et al., 2007). Without pollination, it would be expected that crop production and diversity had a reduction of 5 to 8% (Aizen et al., 2009). In 2015, pollination services were evaluated to be worth worldwide between 235 to 577 billion US dollars. Furthermore, in Europe, pollination by honeybees is worth approximately € 4.25 billion, and pollination by other taxa is worth approximately € 0.75 billion, with *Apis mellifera* L. being the most economically valuable pollinator of crop monocultures worldwide (Klein et al., 2007; Potts et al., 2006). There is clear evidence of the decline of pollinators (Potts et al., 2010). Besides, a diverse range of pollinators is necessary to ensure that declines in honeybees are buffered (Shuler et al., 2005; Wratten et al., 2012). That loss of pollinators leads to a great economic loss and is caused mainly by alterations in the landscape. With the increase in agriculture, there is an increased demand for pollination services. So due to the loss of pollinators an increasing investment in managed insects, such as *Apis*.

Drivers of insect pollinator declines

Pollinators are affected by various stressors such as pests and diseases, predators, climate change, landscape intensification, shortage of food and nesting resources, and crop management practices (Paudel et al., 2015; POLL-OLE-GI, 2019). The decline in pollinator abundance and diversity can lead to a decline in pollination services for wild plant communities, affecting populations of animal-pollinated plants and potentially reducing floral resources for the pollinators (Potts et al., 2010). Insect pollinators' stressors can be divided into three categories: biological, environmental, and chemical.

Biological stressors are constituted by parasites, pathogens (including viruses), and predators. For instance, honeybees parasites, varroa mites, feed on the bee’s fat tissue (Ramsey et al., 2019), and act as a reservoir and incubator of viruses. The hornet *Vespa velutina nigrithorax* Lepeltier, is an important pest, originally from Asia, that spread to France, Italy, and Portugal, because they have a fast expansion rate and feed on honeybees.

Environmental stressors include climate change, habitat fragmentation, lack of flower resources, and monocultures (Steinhauer et al., 2018). Habitat loss at some extreme spatial scale of patch size and isolation is deleterious to both communities of insect pollinators and the sexual reproduction of plants (Cane, 2001). Habitat fragmentation is one of the leading causes of species endangerment (Xiao et al., 2016) which is noticeable through changes in species richness and abundance (Winfree, 2010), pollen limitation (Thompson et al., 2010), and changes in pollinator assemblages (Rands & Whitney, 2011). Plant species may represent important food resources for pollinators, and pollinators are important for the reproduction of some plant species. Therefore, the changes in species richness and abundance of pollinators and plants derived from habitat fragmentation would change the resource availability and disrupt plant-pollinator interactions (Xiao et al., 2016).

Anthropogenic changes in environmental limiting factors are likely to cause significant loss of plant diversity, leaving niches empty and creating plant communities dominated by weedier species (poor competitors but good dispersers) (Tilman & Lehman, 2001). Moreover, climate warming and an expected reduction of precipitation could decrease nectar secretions, notably in Mediterranean areas. The decrease of nectar secretions, together with shifts in flower phenology, due to temperature, can impact plant-pollinator interactions (Takkis et al., 2018). Agriculture and monocultures are a major problem because they promote changes in land use, and loss and fragmentation of habitat, therefore reducing the diversity of wildflowers. The removal of weeds that provide forage for pollinators is also a major factor in the decline of pollinators in the agroecosystems. Habitats that used to offer resources to pollinators, such as grasslands, field margins, and hedgerows (*i.e.* green infrastructures) are becoming increasingly limited (Nicholls &

Altieri, 2013). The loss of green infrastructures has been related to one of the main causes of pollinator decline (Ricketts et al., 2008).

The use of pesticides in agriculture is a part of the chemical stressors and is a major problem for insect pollinators' fitness. For instance, in France, the use of imidacloprid has been suspended because of concerns that it may have a drastic effect on bee populations, causing loss of honeybees and weakening hives (Aliouane et al., 2009). Also, the use of herbicides can affect non-target plants (Cedergreen et al., 2007), therefore, killing plants that are important for honeybees. Furthermore, sub-lethal effects may affect various stages of bee development, from the immune system to the effects on learning and orientation (Desneux et al., 2007). Negative effects have also been documented in queens. When the queens were fed with contaminated food during larvae development, they showed a reduction of immunocompetence that can affect their resistance to diseases and had fewer mattings, leading to a lower genetic diversity (Brandt et al., 2017; Forfert et al., 2017). Interactions between livestock grazing management and phosphorus-based fertilizers, *i.e.* soil phosphorus, used in agriculture, were found to reduce native plant richness and an increase in exotic plant richness when fertilizers were applied (Dorrough et al., 2006). Therefore, promoting a loss of natural areas and biodiversity in plants. Loss of plant diversity is considered to be the major cause of loss of bee diversity in agricultural habitats (Le Féon et al., 2010). For instance, nutritional stress, due to poor quality or lack of floral resources can reduce bee tolerance to diseases and pesticides (Nicolson, 2011).

Ecological restoration

A method to mitigate the effects of environmental stressors, such as agriculture, is the creation of green infrastructures. Green infrastructures are a set of natural, semi-natural, and artificial networks of multifunctional ecological systems that can be promoted within, around and between agricultural and urban areas with the purpose to preserve and increase the habitat, species, and gene diversity in ecosystems (Tzoulas et al., 2007).

Implementing wildflower strips is a beneficial mitigation measure for various insect groups via increased plant diversity and flower abundance (Korpela et al., 2013). Those wildflower strips can be promoted in various sites, such as road verges, railway embankments, and hedgerows of agricultural fields (Albrecht et al., 2020; Tikka et al., 2001). In intensively managed agroecosystems, the establishment of wildflower strips or hedgerows is the most applied measure to improve crop pollination (Kremen et al., 2019; Martin et al., 2019; Scheper et al., 2015). Wildflower strips and hedgerows, established along field edges, provide green infrastructure to pollinators, offering diverse food resources, nesting sites, and overwintering opportunities (Albrecht et al., 2020; Holland et al., 2016). These wildflower strips must take into account the phenological succession throughout the day and the season and the nutritional needs of pollinators (Vaudo et al., 2015a).

Apis mellifera L.

As stated above, honeybees are, currently, key managed pollinators, providing pollination services to leading crops worldwide (Fig. 2). Honeybees are social insects with a complex and highly optimized colony arrangement. The honeybee colony is headed by a single queen, the only fertile female of the colony. Queen bees, after being born, take a nuptial flight where they mate with up to 50 drones, *i.e.* males (Withrow & Tarpy, 2018), accumulating their sperm in the spermatheca, using it accordingly to the colony needs. The drones are haploid, and their main function is reproduction. The workers are diploid, and take care of young larvae and the queen, attack intruders, and forage for resources for the colony.

Fig. 2 - *Apis mellifera*.

Honeybees have preferences in the plants used for foraging since nectar is converted to honey, which is the major source of energy, while pollen is the source of lipids and proteins (Crailsheim, 1992). Furthermore, honeybees are selective in the choice of floral resources, preferring, *e.g.*, the nectar with higher concentrations of proline (Bertazzini et al., 2010) and also selecting nectar with sucrose over other sugars (Bachman & Waller, 1977). Moreover, honeybees rarely collect pollen and nectar simultaneously and usually direct their visits to flower species with a predominance of pollen or nectar (Pankiw & Page, 2000; Robinson & Page, 1989; Rollin et al., 2016). Also, the study of Talavera et al. (1988), in Western Andalusia, says that *Rubus ulmifolius* Schott, *E. globulus* Labill. were species of major beekeeping interest in terms of both nectar and pollen; *Calluna vulgaris* (L.) Hull, *Erica australis* L., *E. umbellata* L., *Lavandula stoechas* L., *Rosmarinus* *officinalis* L. were species of major beekeeping interest in terms of nectar; *Genista tridentata* L., *Ulex parviflorus* Pourr., *U. minor* Roth were species of major beekeeping interest in terms of pollen.

The survival and development of honeybee colonies are influenced by the regularity, quality, and quantity of nectar and pollen: (1) after over-wintering for the replacement of workers; (2) during spring and summer when the population has peaked, and; (3) in autumn for the storage of winter food (Wratten et al., 2012). Honeybees have an energy requirement of resting adults is 11 mg honey/day and for nurses, it is 53.42 mg honey/day and an average colony has an annual pollen requirement of 20 kg (EFSA, 2016; Wratten et al., 2012). Therefore, both larvae and adults are highly dependent on colony food stores, and adult honeybees may adapt their foraging or brood-care strategies according to the respective need and supply of carbohydrates and proteins (Brodschneider & Crailsheim, 2010). In honeybees, colonies can regulate foraging nectar accordingly to nectar quality and location, and colony needs; furthermore, honeybees can also regulate pollen foraging based on colony demand and food availability (Kitaoka & Nieh, 2009).

Foraging ranges are determined by landscape characteristics and honeybee’s fitness (Abou-Shaara, 2014). The flight distance is one of the biggest costs in terms of energy and time for honeybees and the workers must weigh that cost against the gain of the food (Seeley, 1994). Honeybees usually forage from a range of 500 m to 3 km from the colony but can go to greater distances (up to 7 km) (Couvillon et al., 2015). For foraging purposes, honeybee workers have developed an interesting system to recruit more bees if needed: the performance of a waggle dance to inform other workers where to find the resources. Those waggle dances are influenced by the sun where bees change positions even though it is the same location, whilst the duration of the dance informs the distance between the colony and the feeding site (Frisch, 2014).

Healthy honeybee populations and profitable beekeeping practices depend therefore on landscapes with ample and nutritious sources of pollen and nectar from the flowering vegetation present in the landscape (Decourtye et al., 2010).

Flower rewards

Plants offer rewards to pollinators to persuade them to repeatedly visit their flowers and transfer pollen to other flowers (Faegri & Van der Pijl, 1979; Knuth, 1906). The nectar produced by flowers has the only purpose to be used as a reward for pollinators since nectar is the principal source of carbohydrates for most bee species (Cohen & Shmida, 1993; Vaudo et al., 2015a). The energy content of a flower's nectar depends on its volume and sugar concentration (Corbet, 2003). Older flowers can have a reduced proportion of sucrose in nectar (Nicolson, 2011). Although honeybees prefer nectar concentrations of 30-50%, they have a much wider range: 15-65%; and honeybee sucking rates sharply decreased when nectar sugar concentrations exceeded 50-60% (Betts, 1920; Nicolson, Nepi, & Pacini, 2007). Moreover, honeybees cannot taste sugars in solutions with concentrations below 10%, which is a safeguard against net energy loss (Kevan & Baker, 1983). Nectarless plants are less visited by pollinators since the only reward they give is pollen. Although female flowers usually do not produce pollen, some can produce sterile pollen to ensure visitation from pollinators (Kawagoe & Suzuki, 2003).

Some trees, such as *Acacia* spp., are also important for honeybees because honeydew is produced directly from extrafloral nectaries. Although the chemical composition of honeydew differs in enzyme and sugar content from floral honey it still has the same nutritional value (Moncur et al., 1995).

Different plant species produce different amounts and compositions of floral rewards (pollen and nectar) for floral visitors. Flower rewards may also be different in natural ecosystems and agro-ecosystems. On one hand, natural ecosystems have an abundance of wildflowers which frequently offer nectar production as a reward. On the other hand, agroecosystems such as arable lands often have a predominance of species that are poor in nectar, and honeybees are rewarded with pollen (Rollin et al., 2016). Besides, variability in nectar production also exists among flowers within a plant. Nectar production may vary with flower age, stage of development, and flower size (Scoble & Clarke, 2006).

Crop fields like cereals, when intensely managed, usually do not offer wild floral resources (Shuler et al., 2009). Despite that, some crops have an important role as pollen sources, including white and red clover (*Trifolium repens* L. and *T. pratense* L.), corn (*Zea mays* L.), rape (*Brassica napus* L.), and sunflowers (*Helianthus* sp.) (Keller et al., 2005). Although flowering crops can provide highly rewarding resources during some periods of the year, they do not provide them in a continuous manner (Westphal et al., 2003). Le Féon et al. (2010) found a positive relationship between bee species richness and the proportion of semi-natural habitats, *e.g.*, margins of crop fields, suggesting that those remnants of semi-natural habitats are of great importance for bee conservation.

Pollen is one of the prime nutrient resources used for bee larva development. It consists mostly of lipids (including phytosterol), proteins, amino acids, sugars, and vitamins (Somme et al., 2015). Lipids, contained in pollen, are important to honeybees mainly as a source of energy but also involved in the synthesis of reserve fat and glycogen, and membrane structure cells. Therefore, lipid components such as fatty acids and sterols are important in honeybee reproduction, development, and nutrition (Manning, 2001). In honeybees, pollen consumption is higher in young adults, allowing their hypopharyngeal glands to produce jelly for feeding larvae (Nicolson, 2011). At two weeks, after transitioning to foraging, they consume mainly carbohydrates (Brodschneider & Crailsheim, 2010). In contrast, foraging workers consume little to no pollen, suggesting that they have reduced need for protein, excluding proline for their in-flight metabolism (Nicolson, 2011). Low pollen diversity might represent a major limiting factor for honeybees' development (Alaux et al., 2010). Bees may produce lower quality offspring when larvae are reared on the pollen of less preferred host plants or may fail to produce offspring altogether (Scheper et al., 2014). Furthermore, polyfloral diets enhanced some immune functions compared with monofloral diets, meaning that the diversity in floral resources confers bees with better in-hive anti-septic protection (Alaux et al., 2010). Moreover, lipids in pollen, such as decanoic (capric), dodecanoic (lauric), myristic, linoleic, and linolenic acids, have antimicrobial properties and may also have antifungal activity (Manning, 2001). However, a polyfloral blend is not necessarily better than monofloral pollen of good nutritional value (EFSA, 2017).

Floral traits

Foraging pollinators use floral traits as predictors of rewards. For instance, only plants of high viability can pay the costs of producing large flowers and providing pollinator rewards (Møller & Eriksson, 1995). Floral traits that are commonly recorded include flower size, symmetry, the timing of anthesis, overall corolla shape, color, and reward type; nectar, color, flower size, and anthesis patterns were reported as important traits most often

(Dellinger, 2020). Flower size is the most honest signal to pollinators since bigger flowers produce more nectar, and higher pollen volume and pollen grain number (Ortiz et al., 2020). Nevertheless, species that produce small flowers have survived, proving that even small flowers can attract pollinators (Dafni et al., 2007).

Pollination syndrome is defined as a set of floral traits, adapted to their single most efficient functional pollinator group, associated with the attraction of those functional groups of pollinators (Faegri & Van der Pijl, 1979; Fenster et al., 2004; Rosas-Guerrero et al., 2014; Dellinger, 2020). A functional pollinator group is a group of pollinators that select the same floral traits combinations, and different functional groups select different combinations of traits (Dellinger, 2020). Although pollination syndromes are supposed to reflect adaptation to primary pollinators, less efficient pollinators, *i.e.*, secondary pollinators, may also play a role in floral evolution (Rosas-Guerrero et al., 2014).

In animal perception, corolla symmetry may be an indicator of phenotypic and genotypic quality (Møller, 1993). Relative asymmetry was compared in radial and bilateral flowers, and it was determined that bilateral flowers demonstrate significantly lower levels of corolla asymmetry (Neal et al., 1998). Furthermore, Kevan & Baker (1983) demonstrated that the flowers visited in apoidean (which includes honeybees) pollination systems are usually zygomorphic, *i.e.* with bilateral symmetry, with hidden rewards. However, some tubular flowers with radial symmetry are also considered “bee flowers”.

Flowering length, *i.e.*, time of anthesis, blossom cover, and flower shape are also traits with ecological importance. Time of anthesis is a method to access the duration of resource availability; blossom cover gives us the perception of the number of flowers available per plot and allows the flowers to be seen at longer distances (Fornoff et al., 2017). Regarding flower shape, Appanah (1990) demonstrates that larger bees (such as honeybees) prefer stereomorphic, deep, three-dimensional flowers.

Objectives

The motivation of this study is that Portuguese landscapes are very diverse and although beekeeping is a very important economic sector in many regions, *e.g.,* Lousã, not much is known about the main floral resources and their importance for honeybees. Therefore, information about reward availability in the landscape is very important when studying managed bee populations as they directly affect the fitness of the colonies. The existing databases with nectar and pollen production per species are nonetheless incomplete and none of these has focused on the Portuguese flora. The information for plant species, present in Portuguese landscapes, is scattered and data gathered under distinctive climatic conditions.

Recently the B-GOOD project was financed and has the principal objective to explore the various socio-economic and ecological factors beyond bee health, and test, standardize and validate methods for measuring and reporting selected indicators affecting bee health, in which my study is being developed. The Portuguese team has several apiaries installed in two different landscape windows (Lousã and Idanha-a-Nova) and is studying the evolution of colonies and the distribution of resources in the surrounding landscape. Remains to study the value of each plant species from the perspective of food resources.

The main goals of this MSc thesis are (1) the development of a database for the Portuguese flora reward production, complemented with field and laboratory analysis to evaluate their quality, and bibliographic research; (2) to find a relationship between beekeepers apicultural classification (0, 1, 2, and 3 indicating no, low, medium, and high apicultural interest, respectively), floral rewards (nectar amount, concentration and energetic gain, and pollen amount) and floral traits (*e.g.*, flower/inflorescence size, corolla tube length, color, symmetry, flower shape, anthesis, blossom cover, anthers exerted) to a) characterize floral types associated with apicultural interest categories, b) explain floral rewards with floral traits and if it can be inferred for species with no nectar/pollen/apicultural value based on floral traits, and c) combine the information to infer apicultural interest of plant species; and finally (3) the validation of the index described in B-GOOD Deliverable 3.1 (bee-friendliness value), using floral rewards. The main questions are (1) if in Portuguese flora is also applicable that higher-quality floral rewards can be predicted using certain floral traits, and therefore if floral traits can be associated with apicultural interest categories and predict the quality of floral rewards; (2) if the quality of floral rewards can be associated with apicultural interest categories; and (3) if the bee-friendliness value can be a good predictor of apicultural interest.

It is hypothesized that floral traits are capable to predict the quality of floral reward and the apicultural interest of plant species. It is also hypothesized that the bee-friendliness value is a good predictor of apicultural interest.

For that, nectar and pollen samples will be collected from the main plant species visited by bees, and thus with beekeeping interest, their production quantified, and their composition characterized. The database will be a key element to develop models of honeybee colony performance (EFSA and B-GOOD project). This work assists the characterization of the landscapes' resource potential and correlates with honeybee exploitation areas and colony performance, and together with surveys of flower abundance and distribution, it will allow the development of spatial and temporal maps of the available resources.

Materials and methods

Study sites

This study was developed in two different landscapes, Idanha-a-Nova and Lousã. These landscapes differ in diversity and flowering patterns.

Idanha-a-Nova (Fig. 3) is an agricultural landscape used as cattle farms, constituted majorly by arable land and with crops used as fodder with a dominance of permanent and temporary pastures, oak forests, leguminous crops, cereal fields, and some scrubland areas. Beekeeping season starts early in the year (beginning of March) and the honey production is focused on the *Lavandula* sp. flowering period. There is a great amount of flower diversity at the beginning of the season, such as *Echium plantagineum* L., *Rubus idaeus* L., *R. ulmifolius* Schott, and *Trifolium* sp., helping the normal development of the colony. Nonetheless, despite this diversified offer, the relevant flowering period ends in July, leaving colonies exposed to a lack of flower resources.

Fig. 3 - Idanha-a-Nova.

Lousã (Fig. 4) is a mountainous region constituted mainly of shrubland, broadleaf, and coniferous trees. The forested area is composed of several softwood and hardwood species, such as *Castanea sativa* Mill., Eucalyptus *globulus* Labill., *Pinus pinaster* Aiton, and *Quercus robur* L. During the cold weather, most beekeepers transport the colonies to warmer locations during winter and move them back in April, when the beekeeping season starts. Honey production is focused on *Erica* sp. flowering (mainly *Erica arborea* L. and *Erica umbellata* L.) and later *Castanea sativa* Mill. forests. After July (honey harvest) the colonies still have some available resources to prepare for winter (*e.g.*, *Calluna vulgaris* (L.) Hull). Resources are available from March onwards (*Ulex* sp., *Erica* sp., *Genista* sp.), which can sustain the colonies even during the cold weather in early spring. It has a high range of altitude that ranges from 200 m to 1000 m, with hilltops and deep valleys.

Fig. 4 - Lousã.

Quantification of nectar and nectar sugar per flower per day

Flowers needed to be bagged for 24 h before extracting nectar to assure the absence of pollinators. I measured nectar production with a capillary micropipette and determined sugar concentration with a portable refractometer as suggested by Castro *et al.* (2008). Bibliographic research was also made to complement the database of nectar production.

Flower bagging

First, flowers were cut to observe if nectar or nectaries were present. Then, I marked flowers to be sampled by covering them with a bag made of nylon 1x1 mm mesh to exclude insect visitors (Fig. 5). Each bag was closed around the plant with string (Baude et al., 2016; Hicks et al., 2016).

Fig. 5 - Flowers bagged

Nectar sampling

For those species in which visible nectar could be obtained, I sampled nectar directly using microcapillary tubes, starting with a 0.5 µL microcapillary, and used larger volumes where available nectar allows. For each flower, were used as many capillaries as necessary to empty the flower (Hicks et al., 2016) (Fig. 6). Nectar was sampled between 9 A.M. to 11 A.M., and always avoiding direct sun to prevent evaporation.

Fig, 6 - Nectar collecting.

Nectar measurement (volume, sugar content, and energy)

(i) Nectar volume

The total volume of nectar or rinse from each flower was calculated by using calipers to measure the length of the nectar columns in the microcapillary tubes. Total volume/flower was obtained using (total measured nectar column length/length of a single microcapillary) x microcapillary unit of volume.

(ii) Sugar concentration

Sugar concentration was measured using a sucrose refractometer (percentage by weight) (Castro et al., 2008). After the nectar was collected in the microcapillary tube and the nectar column length was measured, I quickly placed the nectar on the refractometer prism for the sucrose measurement. Between each measurement, I cleaned the refractometer prism with distilled water and optical paper (Hicks et al., 2016).

(iii) Nectar energetics

First, sugar concentration was transformed in sugar weight per flower using the following formula (Galetto & Bernardello, 2005):

$$SW = \left(0.00226 + (0.00937 * SC) + (0.0000585 * SC^2)\right) * N,$$

SW – Sugar Weight
SC – Sugar Concentration
N – Nectar Volume

Then sugar weight was transformed into calories using the following formula (Galetto & Bernardello, 2005):

$$NE = SW * 4,$$

NE – Nectar Energy

Quantification of floral pollen volume per flower

The number of pollen grains per flower was estimated in all the anthers of flower buds that are about to open from distinct individuals of each population. In cases of flowers with an indefinite number of anthers (>10), five random anthers were selected. In Asteraceae, where anthers could not be seen with a magnifying microscope, one flower was removed and processed as an anther. Flower buds were placed in 70% ethanol for return to the lab. Bibliographic research was also made to complement the database of pollen production.

I examined the flowers under a magnifying microscope and guaranteed that all anthers were closed. Then, I extracted all the anthers from the flower to an Eppendorf tube with a known volume (e.g., 0.5 µl) of 70% ethanol and pressed slightly with a needle to open the anthers. Later, I agitated the Eppendorf tubes to release the pollen from the anthers. Afterward, I inspected a few anthers under the microscope to guarantee that all pollen grains have been released from the anther, otherwise, repeated the previous step. The pollen was then evenly dispersed in 70% ethanol using a vortex, and with a pipette, I removed 40 µl solution. Afterward, with the coverslip already on the Neubauer chamber, I slowly expelled the solution from the pipette into the V-cut on both sides of the Neubauer chamber. Then, I viewed the Neubauer chamber through a compound microscope (amplification x100) and counted the number of pollen grains in each corner of 1 mm x 1mm regions on both sides of the Neubauer chamber (8 counts total) (Fig. 3). Pollen grains that touch the top and left outside edges of the 1 mm x 1 mm region were also counted. Then, I calculated the average number of pollen grains per unit volume (= $25x10^{-5}$). Finally, I extrapolated the total number of pollen grains in the original solution.

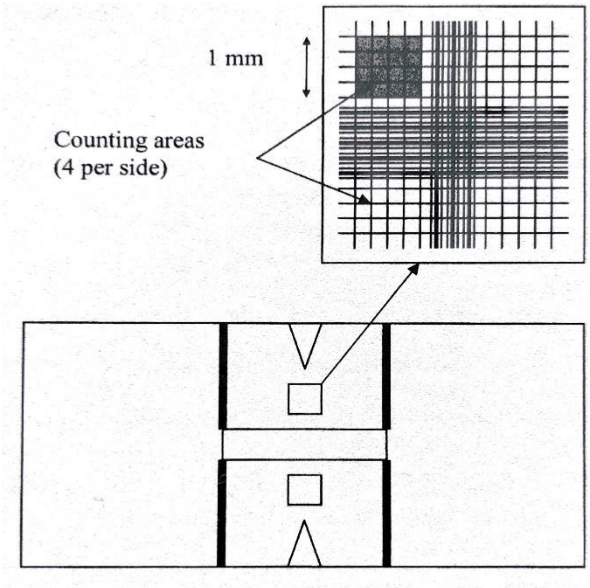


Fig. 7 - Neubauer chamber grid showing the 1 mm x 1 mm area used for pollen counting.

Floral traits

Flower size, color, symmetry, flower shape, anthesis, and blossom cover were the selected traits for this study. The parameters for the categories of flower size, color, symmetry, and flower shape are obtained from Castroviejo (1986).

Flower size is expressed as the length-width average of the exposed surface of the corolla in a 2D projection. In plant taxa with circular flowers, the floral size equals the flower diameter. In cases of strongly compact inflorescences, *e.g.*, Asteraceae, floral size is considered as the size of the inflorescence. Flower size was divided into three categories: small (1-10 mm), medium (10-20 mm), and large (> 20 mm). If the range of floral size for a taxon was between two categories, the category that overlaps more with the taxon flower size was chosen, giving preference for the larger category.

Corolla color was divided into five categories: white; yellow; violet, purple, red, pink, brown; blue; and green. In the case of two or more concurrent colors, the dominant (> 50% of flower surface) was selected, and in taxa with individuals bearing flowers of different colors, the most frequent was selected.

Taxa were assigned to two levels based on the number of floral symmetry axes: radial symmetry and bilateral symmetry. In the case of compact inflorescences, *e.g.*, Asteraceae, the symmetry of the whole inflorescence was considered and not the individual flowers.

Anthesis, *i.e.*, flowering season, was divided into four seasons: spring, summer, autumn, and winter. If the flowering period of a taxon was within two seasons, the season with the most overlap was chosen.

Blossom cover was divided into three categories: low (1-15%), medium (16-30%), and high (> 31%). If the blossom cover percentage of a taxon overlapped two categories, the higher category was chosen.

Taxa were assigned to eight categories based on flower shape: disk, a shallow flower with petals more or less spread out in a flat circle; disk-tube, a flower with a flattened part abruptly arising on a tubular stalk; funnel, an upward-facing funnel-shaped flower that the insects enter with much of or the entire body; bell, a downward-facing bell-shaped flower that the insects enter with much of or the entire body; tube, a tubular flower; gullet, a flower with a lip serving as a landing platform for insects to insert their head or whole body into the corolla tube; flag, the "butterfly"-shaped flower of the Fabaceae and Polygalaceae; head, a densely-packed flower aggregation with more or less flat or spherical appearance; and brush, single flowers or aggregations with numerous well-protruding anthers that form a surface brush.

Statistical analysis

Differences between apicultural categories in floral traits (number of flowers per inflorescence, mean reproductive unit size, inflorescence size, blossom cover, color, symmetry, anthers exerted, flower shape) were evaluated using a Kruskal-Wallis test, due to a lack of normality of the data and homogeneity of variances, even after logarithmic, square root, and other transformations. Thereafter, post-hoc Mann-Whitney tests were performed between apicultural categories where significant differences were found previously.

A Categorical Principal Component Analysis (CATPCA), based on a correlation matrix, was performed with floral traits categories (reproductive unit size, color, symmetry, anthers exerted, anthesis, floral shape), with objects labeled as apicultural interest categories. Missing apicultural interest was categorized as 0.

Due to a lack of normality of the data and homogeneity of variances, even after logarithmic, square root, and other transformations, a Spearman correlation was performed between Bee-Friendliness value and floral rewards (nectar production, sugar concentration and energy, and pollen production per flower and reproductive unit).

All analyses were performed using IBM SPSS 27.0 software (IBM Corp., 2020). Although, boxplots were made using *Statistica 7* software (StatSoft, Inc., 2004).

Results

Characterization of floral traits associated with the apicultural classification

A Kruskal-Wallis test showed that the mean reproductive unit size significantly affects the apicultural classification, H (2) = 7,214, p = 0,027 (Tab. 1), while for the remaining floral traits no significant differences were obtained between classes of apicultural interest. Boxplots further illustrating the differences in mean reproductive unit size are shown in Figure 8.

Table 1 – Kruskal-Wallis test relating apicultural interest with floral traits (mean reproductive unit size, inflorescence size, blossom cover, number of flowers per inflorescence, color, symmetry, floral shape, time of anthesis, and anthers exerted).

	Mean reproductive unit size	Inflorescence size	Blossom cover	Nº flowers	Color
Kruskal-Wallis's H	7,214	,532	,389	1,160	1,305
df	2	2	2	2	2
Sig.	,027	,766	,823	,560	,521

	Symmetry	Floral shape	Anthesis	Anthers exerted
Kruskal-Wallis's H	1,691	2,946	4,421	1,981
df	2	2	2	2
Sig.	,429	,229	,110	,371

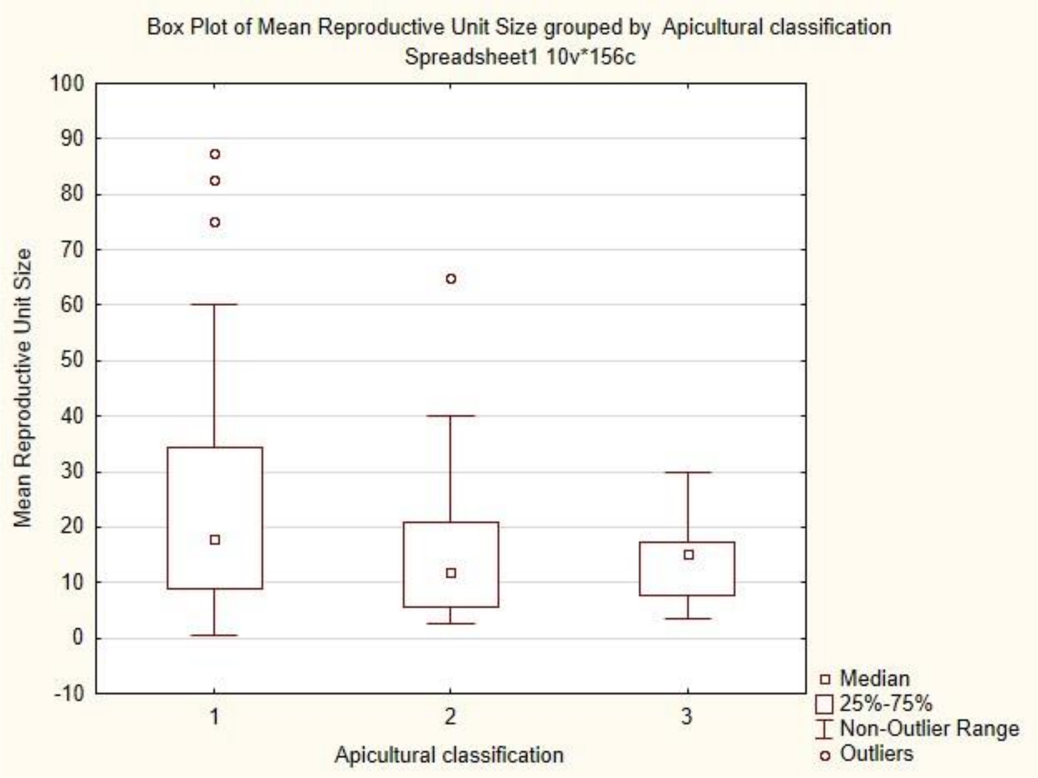


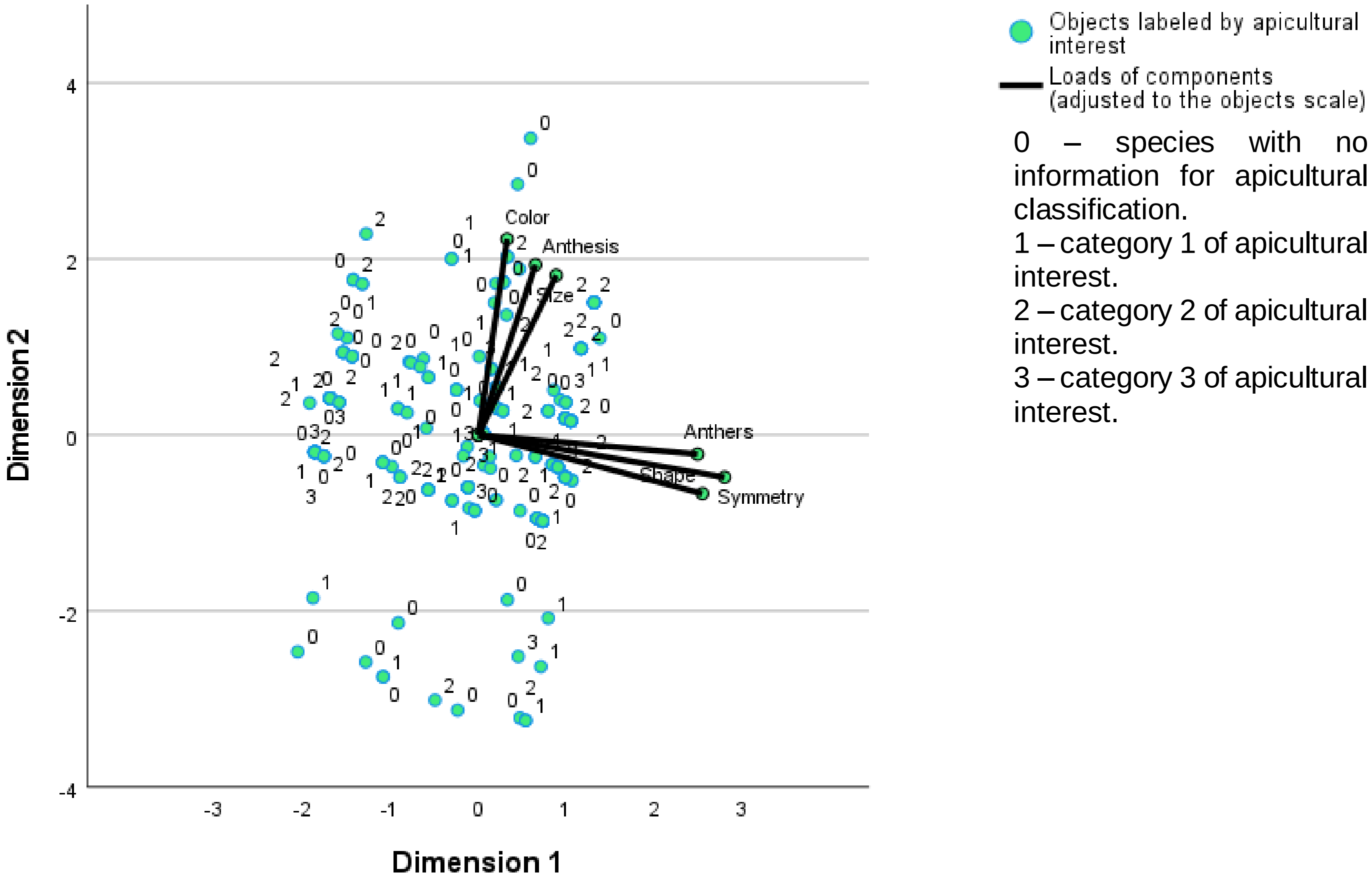
Fig. 8 - Boxplot between mean reproductive unit size and apicultural classification.

Mean reproductive unit size mainly affects categories 1 and 2 of apicultural classification. Post-hoc Mann-Whitney tests were used to compare all pairs of groups. The difference between category 1 and category 2 was significant, $U\ (N_1 = 72, N_2 = 64) = 1719{,}50, z = -2{,}549, p = 0{,}011$ (Tab. 2), with category 1 having bigger reproductive unit sizes than category 2, and with category 3 having intermediate sizes not differing from category 1 and 2.

Table 2 – Post-hoc Mann-Whitney test between groups (category 1 and category 2) of apicultural classification.

	Mean Reproductive Unit Size
Mann-Whitney' U	1719,500
Wilcoxon W	3799,500
Z	-2,549
Sig. (2-tailed)	,011

A CATPCA analysis showed a trend to gather color, anthesis, and size. Although objects of different apicultural categories are very scattered, these variables show a cluster of apicultural interest values of 1. For example, larger reproductive unit sizes have lower apicultural interest values (Fig. 9).



0 – species with no information for apicultural classification.
1 – category 1 of apicultural interest.
2 – category 2 of apicultural interest.
3 – category 3 of apicultural interest.

Fig. 9 - Biplot with clusters of species labeled by apicultural interest.

Characterization of floral rewards associated with the apicultural classification

A Kruskal-Wallis test showed that the apicultural classification significantly affects nectar production, sugar concentration, and energetic gain (Tab. 3), while for the remaining floral rewards no significant differences were obtained between classes of apicultural interest. Boxplots further illustrating the differences in nectar production, sugar concentration, and energetic gain are shown in Figure 10, Figure 11, and Figure 12, respectively.

Table 3 – Kruskal-Wallis test relating apicultural classification with floral rewards (nectar production, sugar concentration, energetic gain, pollen production, and pollen production per reproductive unit).

	Nectar production	Sugar concentration	Energetic Gain	Pollen production	Pollen production per reproductive unit
Kruskal-Wallis' H	13,856	28,016	6,738	1,351	,007
df	2	2	2	2	1
Sig.	<,001	<,001	,034	,509	,934

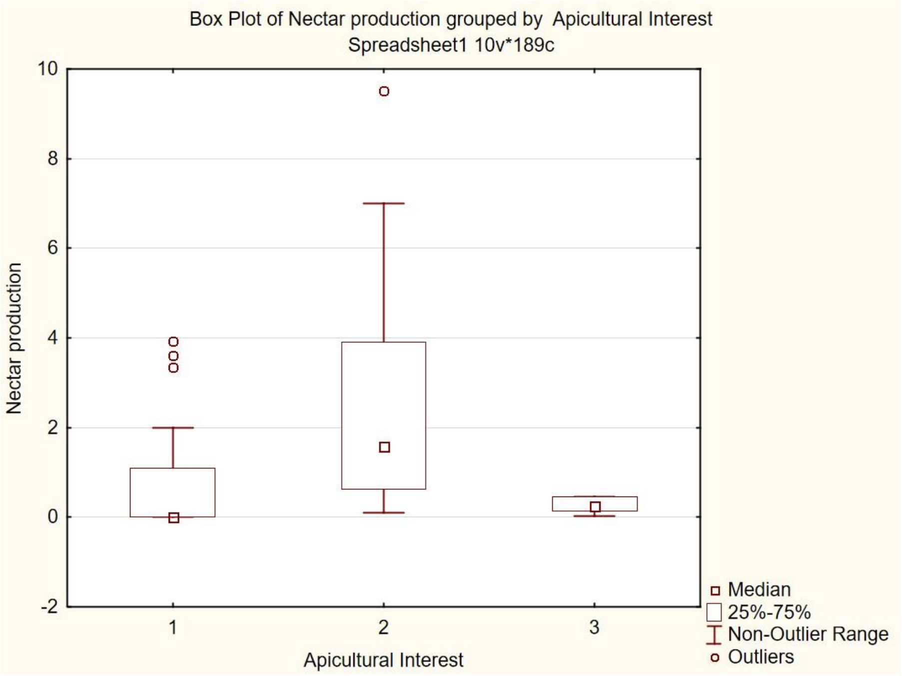


Fig. 10 - Boxplot between nectar production and apicultural classification.

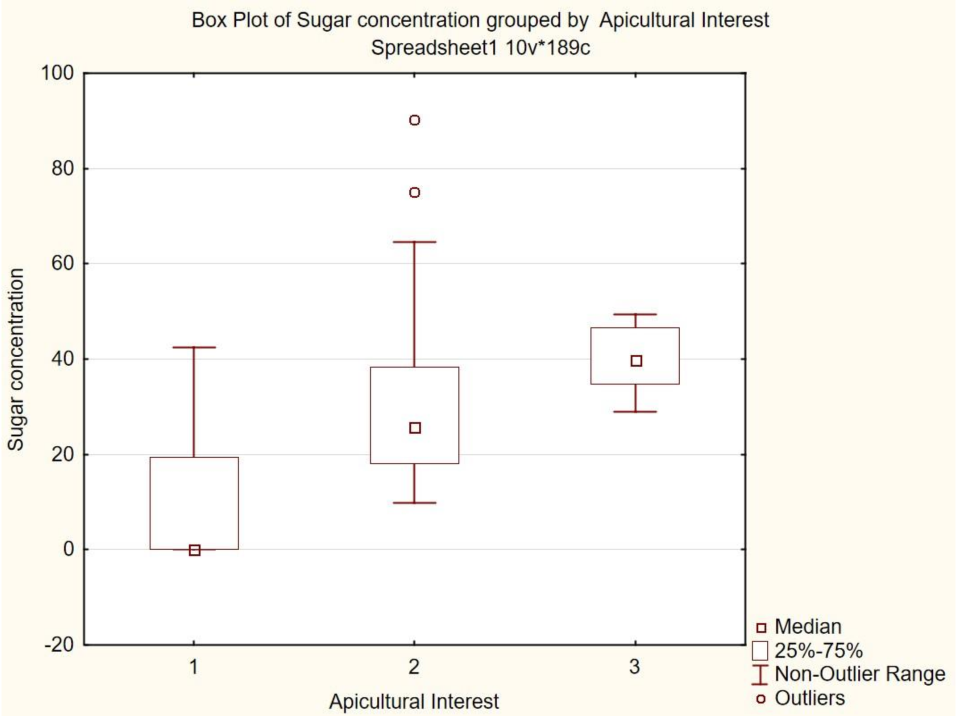


Fig. 11 - Boxplot between sugar concentration and apicultural classification.

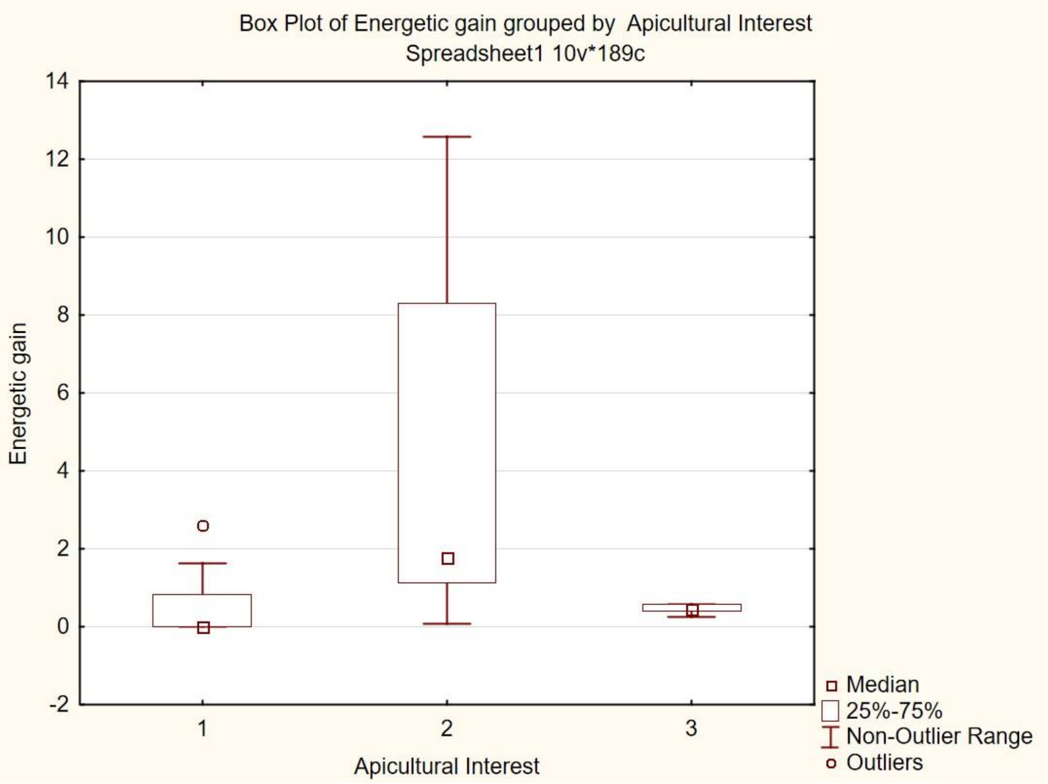


Fig. 12 - Boxplot between energetic gain and apicultural classification.

Apicultural classification category 1 and category 2 affect nectar production and sugar concentration. The difference between category 1 and category 2 was significant, $\mathrm{U}_{\text{nectar production}}\ (N_1 = 38, N_2 = 20) = 168{,}500, Z = -3{,}533, p < 0{,}001$; $U_{sugar\ concentration}(N_1 = 32, N_2 = 20) = 101{,}000, Z = -4{,}241, p < 0{,}001$ (Tab. 4), with category 2 having bigger values of nectar volume and sugar concentration than category 1, and with category 1 not differing from category 2 for energetic gain values.

Table 4 - Post-hoc Mann-Whitney test between groups (category 1 and category 2) of apicultural classification.

	Nectar production	Sugar concentration	Energetic Gain
Mann-Whitney' U	168,500	101,000	138,000
Wilcoxon W	909,500	629,000	348,000
Z	-3,533	-4,241	-,722
Sig. (2-tailed)	<,001	<,001	,470

Apicultural classification category 1 and category 3 affect energetic gain and sugar concentration. The difference between category 1 and category 3 was significant, $\mathrm{U}_{\text{energetic gain}}\ (N_1 = 20, N_2 = 6) = 24{,}000, Z = -2{,}384, p = 0{,}017$; $U_{sugar\ concentration}(N_1 = 32, N_2 = 8) = 10{,}000, Z = -4{,}265, p < 0{,}001$ (Tab. 5), with category 3 having bigger values of sugar concentration than category 1, with category 1 having bigger values of energetic gain than category 3, and with category 1 not differing from category 3 for nectar volume values.

Table 5 - Post-hoc Mann-Whitney test between groups (category 1 and category 3) of apicultural classification.

	Nectar production	Sugar concentration	Energetic Gain
Mann-Whitney's U	88,500	10,000	24,000
Wilcoxon W	829,500	538,000	45,000
Z	-1,459	-4,265	-2,384
Sig. (2-tailed)	,145	<,001	,017
	,167	,000	,028

Apicultural classification category 2 and category 3 affect nectar production, sugar concentration, and energetic gain. The difference between category 2 and category 3 was significant, $\mathrm{U}_{\text{nectar production}}\ (N_1 = 20, N_2 = 7) = 33{,}500, Z = -2{,}020,\ p = 0{,}043$; $U_{sugar\ concentration}(N_1 = 20, N_2 = 8) = 42{,}000, Z = -1{,}932, p = 0{,}053$; $\mathrm{U}_{\text{energetic gain}}\ (N_1 = 16, N_2 = 6) = 18{,}000, Z = -2{,}415, p = 0{,}016$ (Tab. 6), with category 2 having bigger nectar volume and energetic gain values than category 3, and with category 3 having bigger sugar concentration values than category 2.

Table 6 - Post-hoc Mann-Whitney test between groups (category 2 and category 3) of apicultural classification.

	Nectar production	Sugar concentration	Energetic Gain
Mann-Whitney's U	33,500	42,000	18,000
Wilcoxon W	61,500	252,000	39,000
Z	-2,020	-1,932	-2,415
Sig. (2-tailed)	,043	,053	,016

<u>Characterization of Bee-Friendliness value with floral rewards</u>

Among all variables explored for correlation with bee-friendliness value (BF value), I found a significant and positive correlation between BF value and nectar production, $r\ (54) = 0.444, p <\ 0.001$ (Tab. 7). I also found a significant and positive correlation between BF value and sugar concentration, $r\ (52) = 0.444, p <\ 0.001$ (Tab. 7). No significant correlations were found for any of the other floral rewards.

Table 7 – Correlation between BF value and nectar production, pollen production, pollen per reproductive unit, and energetic gain.

			BF Value
Spearman's rho	BF Value	Correlation Coefficient	1,000
		Sig. (2-tailed)	.
		N	176
	Nectar production	Correlation Coefficient	,444**
		Sig. (2-tailed)	<,001
		N	56
	Sugar concentration	Correlation Coefficient	,444**
		Sig. (2-tailed)	<,001
		N	54

	Nectar energetics	Correlation Coefficient	,047
		Sig. (2-tailed)	,757
		N	46
	Pollen production per flower	Correlation Coefficient	,126
		Sig. (2-tailed)	,287
		N	73
	Pollen production per reproductive unit	Correlation Coefficient	,040
		Sig. (2-tailed)	,765
		N	57

The positive correlation found between BF value and nectar production can be depicted by the following equation and is represented in Fig. 13:

$$y = 0.99 + 0.25x$$

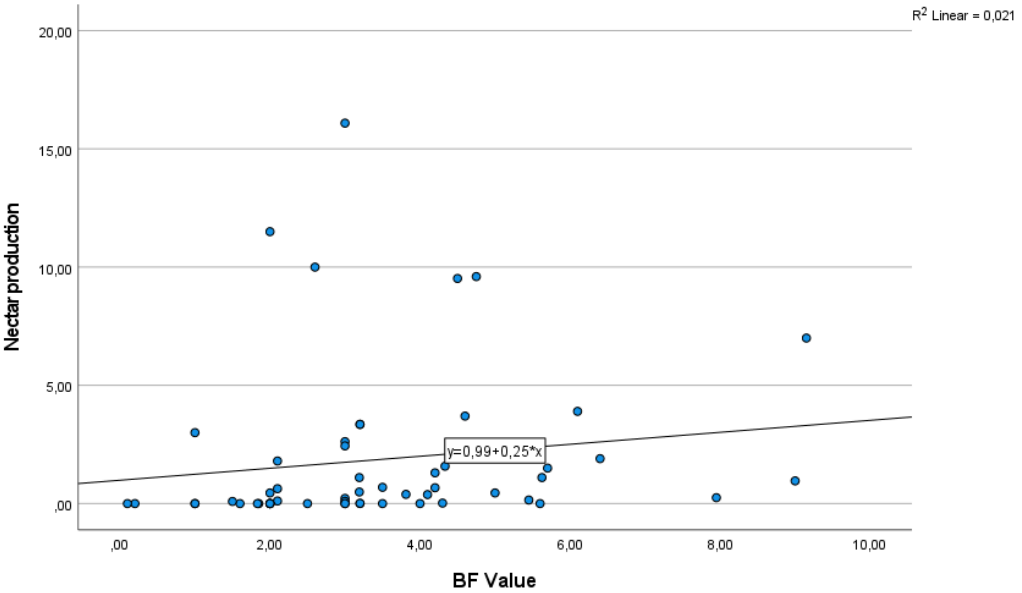


Fig. 13 - Correlation between BF value and nectar production.

The positive correlation found between BF value and sugar concentration can be depicted by the following equation and is represented in Fig. 14:

$$y = 9 + 3.76x$$

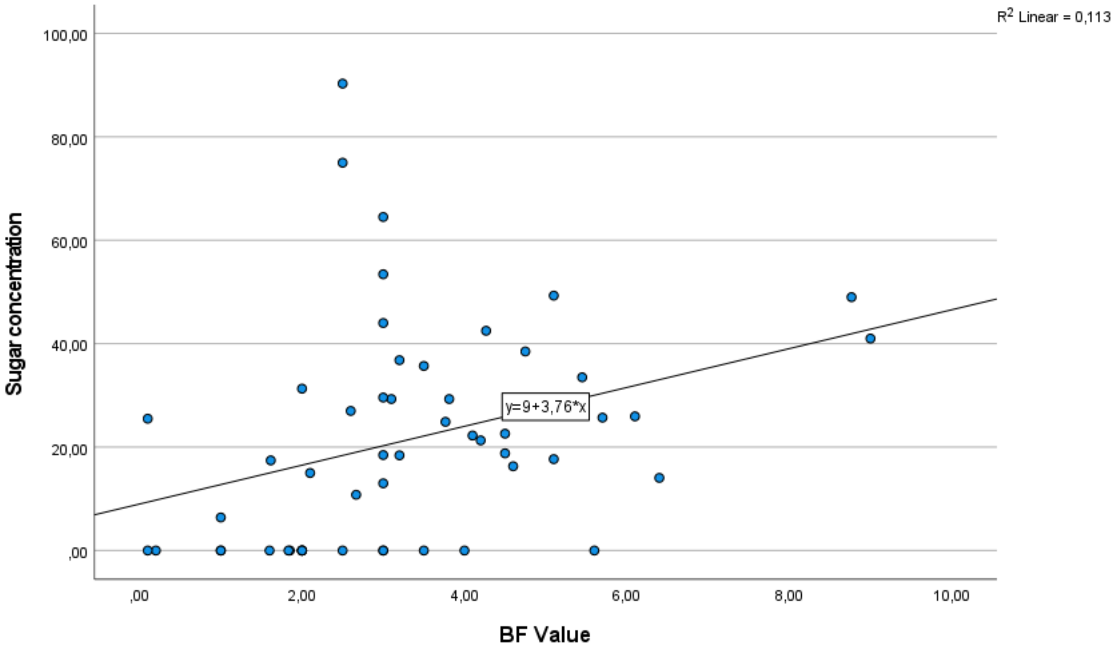


Fig. 14 - Correlation between BF value and sugar concentration.

<u>Important resources in the studied landscapes</u>

Whether in Idanha-a-Nova or Lousã, species with high blossom cover have lower apicultural interest, e.g., *Quercus rotundifolia* Lam. (52,5%) and *Quercus suber* L. (50%). Species with high apicultural interest have medium to low blossom cover, e.g., *Lavandula pedunculata* (Mill.) Cav. (27,17%) and *Verbena officinalis* L. (3%) (Tab. 8). Furthermore, in Lousã, there are 36, 34, and 10 species in categories 1, 2, and 3 of apicultural interest, respectively, and 59 species with no information of apicultural interest; and in Idanha-a-Nova, there are 56, 48, and 14 species in categories 1, 2, and 3 of apicultural interest, respectively, and 75 species with no information of apicultural interest.

Yet, both landscapes have a diverse set of species that are important resources for honeybees.

Table 8 – Summary table relating blossom cover with apicultural interest.

Idanha

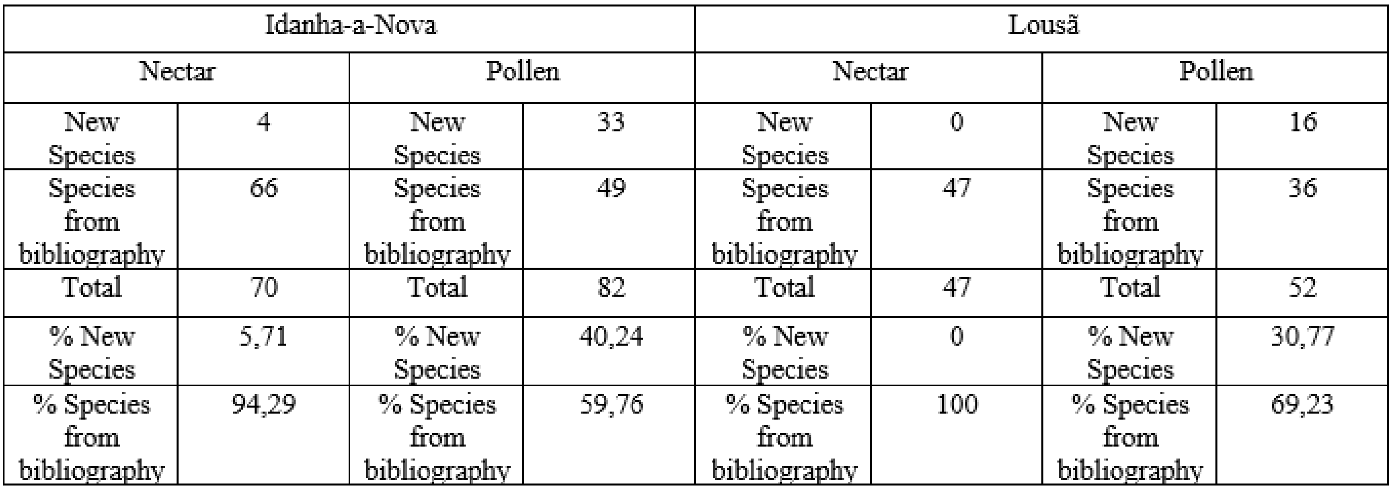
Species	Blossom cover (%)	Apicultural Interest	Species	Blossom cover (%)	Apicultural Interest
Quercus rotundifolia Lam.	52,5	1	*Lavandula pedunculata* (Mill.) Cav.	27,17	3
Retama sphaerocarpa (L.) Heywood	52,5	2	*Lythrum salicaria* L.	20	3
Cytisus striatus (Hill) Rothm.	45,84	1	*Trifolium repens* L.	20	3
Salix salviifolia Brot.	40		*Daucus carota* L.	17,08	3
Anthemis arvensis L.	38,54	1	*Salix atrocinerea* Brot	15	3
Rosa micrantha Borrer ex Sm.	35,83	2	*Prunus persica* (L.) Batsch	10	3
Spergularia purpurea (Pers.) G. Don	34,79		*Carduus tenuiflorus* Curtis	9,63	3
Echium plantagineum L.	34,57	2	*Trifolium pratense* L.	5,5	3
Cladanthus mixtus (L.) Oberpr. & Vogt	33		*Dittrichia viscosa* (L.) Greuter	3	3
Rubus ulmifolius Schott	32,67	2	*Mentha suaveolens* Ehrh.	3	3

Lousã

Species	Blossom cover (%)	Apicultural Interest	Species	Blossom cover (%)	Apicultural Interest
Quercus suber L.	50	1	*Eucalyptus globulus* Labill.	18,13	3
Genista tridentata L.	41,83	1	*Salix atrocinerea* Brot	15	3
Erica arborea L.	28,75	2	*Daucus carota* L.	10	3
Ulex minor Roth	24,25	1	*Prunus persica* (L.) Batsch	5,5	3
Erica australis L.	22,08	1	*Acer pseudoplatanus* L.	5	3
Rubus ulmifolius Schott	21,96	2	*Cirsium vulgare* (Savi) Ten.	3,25	3
Ulex micranthus Lange	21,71		*Verbena officinalis* L.	3	3
Erica cinerea L.	19,93	2	*Carduus defloratus subsp. glaucus* (Baumg.) Nyman	1	3
Eucalyptus globulus Labill.	18,13	3	*Cirsium filipendulum* Lange	1	3
Ulex parviflorus subsp. jussiaei (Webb) D.A.Webb	18	1	*Sedum arenarium* Brot.	1	3

For the plant species used in this study, I assessed 5,71% of new information for nectar and 40,24% of new information for pollen in Idanha-a-Nova, and 30,77% of new information of pollen in Lousã (Tab. 9).

Table 9 – Summary table of floral rewards for species obtained from bibliography versus new species (with no previous information) in Idanha-a-Nova and Lousã.



Idanha-a-Nova				Lousã			
Nectar		Pollen		Nectar		Pollen	
New Species	4	New Species	33	New Species	0	New Species	16
Species from bibliography	66	Species from bibliography	49	Species from bibliography	47	Species from bibliography	36
Total	70	Total	82	Total	47	Total	52
% New Species	5,71	% New Species	40,24	% New Species	0	% New Species	30,77
% Species from bibliography	94,29	% Species from bibliography	59,76	% Species from bibliography	100	% Species from bibliography	69,23

Discussion

The results support the hypothesis that floral traits and bee-friendliness values can predict the apicultural interest of plant species in Portuguese landscapes. Apicultural interest of plant species was significantly impacted by mean reproductive unit size although the trend was not completely expected. Low (1) and high (3) apicultural values group the species with bigger reproductive structures. Moreover, the production of rewards in the form of nectar were important predicting the apicultural value of plant species for honeybees. Finally, bee-friendliness value can predict the apicultural interest.

Only one of the floral traits studied had a significant influence on the prediction of apicultural interest. Plant species with bigger reproductive unit size were representative of lower apicultural interest. Plant species with bigger reproductive unit size were expected to have a higher apicultural interest because honeybees tend to maximize the energy efficiency while foraging and the energy content of nectar is dependent on its volume and sugar concentration (Corbet, 2003; Schmid-Hempel & Schmid-Hempel, 1987). Furthermore, flower size is considered the most honest signal to pollinators since bigger flowers produce a higher pollen volume, pollen grain number, and sucrose amount (Ortiz et al., 2020; Scoble & Clarke, 2006). The apicultural value of plants is related to the daily volume of nectar secreted, the abundance of flowers, the concentration of sugar in nectar, and low competition (Wiese, 1987, as cited in Santos do Nascimento et al., 2014). Thus, further studies should address how competition and the abundance of flowers can affect this relation.

Apicultural interest values can be explained by the number of rewards produced by flowers in terms of nectar production (volume, sugar concentration, and energetic gain) as well as pollen production. In this study, pollen production, whether per flower or reproductive unit, did not differed significantly between apicultural categories. Thus, my results suggest that the amount of pollen grains produced per flower might not relate so straightforward with the apicultural values. I could hypothesize that differences would be found, with pollen production, if chemical analyses of proteins were made, since the quantity of pollen grains may not represent their quality. Wiese, 1987, as cited in Santos do Nascimento et al., 2014, showed that apicultural interest is significantly and positively related to nectar production and sugar concentration, thus supporting the results stated above. Nectar production is a factor, although not simple to quantify in some species, that may allow to obtain good references about apicultural interest values. For instance, larger nectar glands are related with higher nectar production, which is related with higher visitation rates (Castro, Silveira, &

Navarro, 2009). In addition, some authors use a separate value of apicultural interest for pollen and nectar, several others use a common value.

The bee-friendliness value is an index obtained through extrapolations from bibliography and lacks a quantitative validation. My data allow to do one of the first validations and is crucial for the utilization of this index in European projects, such as B-GOOD. Furthermore, bee-friendliness value is supported by nectar production and sugar concentration. Although the apicultural interest value is supported by energy gain, the bee-friendliness value is a scale, i.e., can better characterize the melliferous interest of a plant species. Moreover, the relation between bee-friendliness value and floral rewards can also be improved with chemical analyses of proteins, vitamins, and other nutrients and more data from species beyond the ones considered in the studied landscapes.

My results suggest that for Portuguese landscapes, nectar volume and sugar concentration are good predictors of apicultural value, although not supporting the assumption that larger flowers have higher apicultural values. My results also suggest that the bee-friendliness value is a convenient method to access the apicultural interest of plant species in Portuguese landscapes.

Considering the experimental design applied, some factors restricted the generalization of my results. For instance, only plant species from two regions of the country were studied. Although, several species have a significant distribution across the country.

Future perspectives

In this study, only energetic gain for honeybees was considered. Future studies should address honeybees’ protein, vitamin, and other nutrient needs, where chemical analyses of pollen should be conducted. Additionally, more plant species from other landscapes or with higher distribution should be used to better assess the relation between floral traits, floral rewards, and apicultural interest in Portuguese landscapes.

Conclusions

1. Mean reproductive unit size is related with apicultural interest. Low (1) and high (3) apicultural values group the species with bigger reproductive structures.
2. Floral rewards in the form of nectar are significantly and positively related with apicultural interest for Portuguese landscapes.
3. The bee-friendliness value is supported by nectar production and sugar concentration.

References

Abou-Shaara, H. F. (2014). The foraging behaviour of honey bees, Apis mellifera: A review. *Veterinarni Medicina* https://doi.org/10.17221/7240-VETMED

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., & Klein, A. M. (2009). How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany*, *103*(9), 1579–1588. https://doi.org/10.1093/aob/mcp076

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, *6*(4), 562–565. https://doi.org/10.1098/rsbl.2009.0986

Albrecht, M., Kleijn, D., Williams, N., Laidlaw, H. H. J., Tschumi, M., Blaauw, B., Sutter, L. (2020). Global synthesis of the effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield. *(In Press)*, 6–82.

Aliouane, Y., El Hassani, A. K., Gary, V., Armengaud, C., Lambin, M., & Gauthier, M. (2009). Subchronic exposure of honeybees to sublethal doses of pesticides: Effects on behavior. *Environmental Toxicology and Chemistry*, *28*(1), 113–122. https://doi.org/10.1897/08-110.1

Appanah, S. (1990). Plant-pollinator interactions in malaysian rain forests. *Man and the Biosphere Series, Vol. 7. Reproductive Ecology of Tropical Forest Plants*, *7*, 85–102.

Bachman, W. W., & Waller, G. (1977). Honeybee responses to sugar solutions of different compositions. *Journal of Apicultural Research*, *16*(4), 165–169. https://doi.org/10.1080/00218839.1977.11099882

Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K., Memmott, J. (2016). Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature*, *530*(7588), 85–88. https://doi.org/10.1038/nature16532

Bertazzini, M., Medrzycki, P., Bortolotti, L., Maistrello, L., & Forlani, G. (2010). Amino acid content and nectar choice by forager honeybees (Apis mellifera L.). *Amino Acids*, *39*(1), 315–318. https://doi.org/10.1007/s00726-010-0474-x

Betts, A. D. (1920). The Constancy of the Pollen-collecting Bee. *Bee World*, *2*(1–4), 10–11. https://doi.org/10.1080/0005772X.1920.11094543

Brandt, A., Grikscheit, K., Siede, R., Grosse, R., Meixner, M. D., & Büchler, R. (2017). Immunosuppression in Honeybee Queens by the Neonicotinoids Thiacloprid and Clothianidin. *Scientific Reports*, *7*(1), 4673. https://doi.org/10.1038/s41598-017-04734-1

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, *41*(3), 278–294. https://doi.org/10.1051/apido/2010012

Cane, J. H. (2001). Habitat fragmentation and native bees: A premature verdict? *Conservation Ecology*, *5*(1), art3. https://doi.org/10.5751/ES-00265-050103

Castro, S., Silveira, P., & Navarro, L. (2008). How flower biology and breeding system affect the reproductive success of the narrow endemic Polygala vayredae Costa (Polygalaceae). *Botanical Journal of the Linnean Society*, *157*(1), 67–81. https://doi.org/10.1111/j.1095-8339.2008.00784.x

Castro, S., Silveira, P., & Navarro, L. (2009). Floral traits variation, legitimate pollination, and nectar robbing in Polygala vayredae (Polygalaceae). *Ecological Research*, *24*(1), 47–55. https://doi.org/10.1007/s11284-008-0481-5

Castroviejo, S. et al. (Eds) Flora Iberica vol, 1, 1986 (LIV + 575 pp) and vol. 2, 1990 (LII + 897 pp.)-Real Jardin Botanico C. S. I. C. Madrid

Cedergreen, N., Kudsk, P., Mathiassen, S. K., & Streibig, J. C. (2007). Combination effects of herbicides on plants and algae: Do species and test systems matter? *Pest Management Science*, *63*(3), 282–295. https://doi.org/10.1002/ps.1353

Cohen, D., & Shmida, A. (1993). The evolution of flower display and reward. In M. K. Hecht, R. J. MacIntyre, & M. T. Clegg (Eds.), *Evol.Biol.* (Vol. 27, pp. 197–243). https://doi.org/10.1007/978-1-4615-2878-4_6

Corbet, S. A. (2003). Nectar sugar content: Estimating standing crop and secretion rate in the field. *Apidologie*, *34*(1), 1–10. https://doi.org/10.1051/apido:2002049

Couvillon, M. J., Riddell Pearce, F. C., Accleton, C., Fensome, K. A., Quah, S. K. L., Taylor, E. L., & Ratnieks, F. L. W. (2015). Honey bee foraging distance depends on month and forage type. *Apidologie*, *46*(1), 61–70. https://doi.org/10.1007/s13592-014-0302-5

Crailsheim, K. (1992). The flow of jelly within a honeybee colony. *Journal of Comparative Physiology B*, *162*(8), 681–689. https://doi.org/10.1007/BF00301617

Dafni, A., Lehrer, M., & Keyan, P. G. (2007). Spatial Flower Parameters and Insect Spatial Vision. *Biological Reviews*, *72*(2), 239–282. https://doi.org/10.1111/j.1469-185x.1997.tb00014.x

Decourtye, A., Mader, E., & Desneux, N. (2010). Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie*, *41*(3), 264–277. https://doi.org/10.1051/apido/2010024

Dellinger, A. S. (2020). Pollination syndromes in the 21st century: where do we stand and where may we go? *New Phytologist*, *228*(4), 1193–

1213. https://doi.org/10.1111/nph.16793

Desneux, N., Decourtye, A., & Delpuech, J.-M. (2007). The Sublethal Effects of Pesticides on Beneficial Arthropods. *Annual Review of Entomology, 52*(1), 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dorrough, J., Moxham, C., Turner, V., & Sutter, G. (2006). Soil phosphorus and tree cover modify the effects of livestock grazing on plant species richness in Australian grassy woodland. *Biological Conservation*, *130*(3), 394–405. https://doi.org/10.1016/j.biocon.2005.12.032

EFSA. (2016). Statement on the suitability of the BEEHAVE model for its potential use in a regulatory context and for the risk assessment of multiple stressors in honeybees at the landscape level. *EFSA Journal, 13*(6). https://doi.org/10.2903/j.efsa.2015.4125

EFSA. (2017). Specifications for field data collection contributing to honey bee model corroboration and verification. *EFSA Supporting Publications*, *14*(5). https://doi.org/10.2903/sp.efsa.2017.en-1234

Faegri, K., & Van der Pijl, L. (1979). *The Principles of Pollination Ecology. 3rd edn. Pergamom*. oxford.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 375–403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Forfert, N., Troxler, A., Retschnig, G., Gauthier, L., Straub, L., Moritz, R. F. A., Williams, G. R. (2017). Neonicotinoid pesticides can reduce honeybee colony genetic diversity. *PLoS ONE*, *12*(10), e0186109–e0186109. https://doi.org/10.1371/journal.pone.0186109

Fornoff, F., Klein, A. M., Hartig, F., Benadi, G., Venjakob, C., Schaefer, H. M., & Ebeling, A. (2017). Functional flower traits and their diversity drive pollinator visitation. *Oikos*, *126*(7), 1020–1030. https://doi.org/10.1111/oik.03869

Frisch, K. von. (2014). The Dance Language and Orientation of Bees. In *The Dance Language and Orientation of Bees*. https://doi.org/10.4159/harvard.9780674418776

Galetto, L., & Bernardello, G. (2005). Nectar energetics. *Practical Pollination Biology*.

Hicks, D. M., Ouvrard, P., Baldock, K. C. R., Baude, M., Goddard, M. A., Kunin, W. E., Stone, G. N. (2016). Food for pollinators: Quantifying the nectar and pollen resources of urban flower meadows. *PLoS ONE, 11*(6), e0158117–e0158117. https://doi.org/10.1371/journal.pone.0158117

Holland, J. M., Bianchi, F. J., Entling, M. H., Moonen, A.-C., Smith, B.

M., & Jeanneret, P. (2016). Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. *Pest Management Science*, *72*(9), 1638–1651. https://doi.org/10.1002/ps.4318

Hung, K. L. J., Kingston, J. M., Albrecht, M., Holway, D. A., & Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1870), 20172140. https://doi.org/10.1098/rspb.2017.2140

IBM Corp. (2020). *IBM SPSS Statistics for Windows, Version 27.0*. Armonk, NY: IBM Corp.

IPBES. (2016). The assessment report on pollinators, pollination and food production. In *Science* (Vol. 325). https://doi.org/10.1109/22.989993

Kawagoe, T., & Suzuki, N. (2003). Flower-size dimorphism avoids geitonogamous pollination in a nectarless monoecious plant Akebia quinata. *International Journal of Plant Sciences*, *164*(6), 893–897. https://doi.org/10.1086/378659

Keller, I., Fluri, P., & Imdorf, A. (2005). Pollen nutrition and colony development in honey bees: Part I. *Bee World*, *86*(1), 3–10. https://doi.org/10.1080/0005772X.2005.11099641

Kevan, P. G., & Baker, H. G. (1983). Insects as Flower Visitors and Pollinators. *Annual Review of Entomology*, *28*(1), 407–453. https://doi.org/10.1146/annurev.en.28.010183.002203

Kitaoka, T. K., & Nieh, J. C. (2009). Manuscript in preparation for Behavioral Ecology and Sociobiology Bumble bee pollen foraging regulation: Role of pollen quality, storage levels, and odor. *Behavioral Ecology and Sociobiology*, *63*(4), 501–510. https://doi.org/10.1007/s00265-008-0684-3

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Knuth, P. (1906). *Handbook of floral biology*. Clarendon Press, Oxford.

Korpela, E. L., Hyvönen, T., Lindgren, S., & Kuussaari, M. (2013). Can pollination services, species diversity and conservation be simultaneously promoted by sown wildflower strips on farmland? *Agriculture, Ecosystems and Environment*, *179*, 18–24. https://doi.org/10.1016/j.agee.2013.07.001

Kremen, C., Albrech, M., & Ponisio, L. C. (2019). Restoring Pollinator Communities and Pollination Services in Hedgerows in Intensively-Managed Agricultural Landscapes. In *The Ecology of Hedgerows and*

Field Margins (pp. 163–185). Routledge.

Le Féon, V., Schermann-Legionnet, A., Delettre, Y., Aviron, S., Billeter, R., Bugter, R., Burel, F. (2010). Intensification of agriculture, landscape composition and wild bee communities: A large scale study in four European countries. *Agriculture, Ecosystems and Environment*, *137*(1–2), 143–150. https://doi.org/10.1016/j.agee.2010.01.015

Manning, R. (2001). Fatty acids in pollen: A review of their importance for honey bees. *Bee World*, *82*(2), 60–75. https://doi.org/10.1080/0005772X.2001.11099504

Martin, E. A., Dainese, M., Clough, Y., Báldi, A., Bommarco, R., Gagic, V., Steffan-Dewenter, I. (2019). The interplay of landscape composition and configuration: new pathways to manage functional biodiversity and agroecosystem services across Europe. *Ecology Letters*, *22*(7), 1083–1094. https://doi.org/10.1111/ele.13265

Møller, A. P. (1993). Patterns of fluctuating asymmetry in sexual ornaments predict female choice. *Journal of Evolutionary Biology*, *6*(4), 481–491. https://doi.org/10.1046/j.1420-9101.1993.6040481.x

Møller, A. P., & Eriksson, M. (1995). Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos*, *73*(1), 15. https://doi.org/10.2307/3545720

Moncur, M. W., Mitchell, A., Fripp, Y., & Kleinschmidt, G. J. (1995). The role of honey bees (Apis mellifera) in eucalypt and acacia seed production areas. *Commonwealth Forestry Review*, *74*(4), 350–354. Retrieved from http://www.jstor.org/stable/42608329

Neal, P. R., Dafni, A., & Giurfa, M. (1998). Floral symmetry and its role in plant-pollinator systems: Terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics*, *29*(1), 345–373. https://doi.org/10.1146/annurev.ecolsys.29.1.345

Nicholls, C. I., & Altieri, M. A. (2013). Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. *Agronomy for Sustainable Development*, *33*(2), 257–274. https://doi.org/10.1007/s13593-012-0092-y

Nicolson, S. W. (2011). Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology*, *46*(2), 197–204. https://doi.org/10.1080/15627020.2011.11407495

Nicolson, S. W., Nepi, M., & Pacini, E. (2007). Nectaries and nectar. In *Nectaries and Nectar*. https://doi.org/10.1007/978-1-4020-5937-7

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Ortiz, P. L., Fernández-Díaz, P., Pareja, D., Escudero, M., & Arista, M. (2020). Do visual traits honestly signal floral rewards at community level? *Functional Ecology*. https://doi.org/10.1111/1365-2435.13709

Pankiw, T., & Page, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behavioral Ecology and Sociobiology*, *47*(4), 265–267. https://doi.org/10.1007/s002650050664

Paudel, Y. P., Mackereth, R., Hanley, R., & Qin, W. (2015). Honey Bees (Apis mellifera L.) and Pollination Issues: Current status, impacts and potential drivers of decline. *Journal of Agricultural Science*, *7*(6). https://doi.org/10.5539/jas.v7n6p93

POLL-OLE-GI. (2019). *Rural Green Infrastructures for Pollinator Protection. Technical Guide*. Retrieved from www.pollolegi.eu

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, *25*(6), 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Potts, S. G., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A., & Willmer, P. (2006). Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape. *Biological Conservation*, *129*(4), 519–529. https://doi.org/10.1016/j.biocon.2005.11.019

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., … Van Engelsdorp, D. (2019). Varroa destructor feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(5), 1792–1801. https://doi.org/10.1073/pnas.1818371116

Rands, S. A., & Whitney, H. M. (2011). Field margins, foraging distances and their impacts on nesting pollinator success. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0025971

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., … Viana, B. F. (2008). Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, *11*(5), 499–515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Robinson, G. E., & Page, R. E. (1989). Genetic determination of nectar foraging, pollen foraging, and nest-site scouting in honey bee colonies. *Behavioral Ecology and Sociobiology*, *24*(5), 317–323. https://doi.org/10.1007/BF00290908

Rollin, O., Benelli, G., Benvenuti, S., Decourtye, A., Wratten, S. D., Canale, A., & Desneux, N. (2016). Weed-insect pollinator networks as bio-indicators of ecological sustainability in agriculture. A review. *Agronomy for Sustainable Development*, *36*(1), 1–22. https://doi.org/10.1007/s13593-015-0342-x

Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezaraiza-Mikel, M., Bastida, J. M., & Quesada, M. (2014). A quantitative review of pollination syndromes: Do floral traits predict effective pollinators? *Ecology Letters*, *17*(3), 388–400. https://doi.org/10.1111/ele.12224

Santos do Nascimento, A., Lopes de Carvalho, C. A., & Lacerda Lopes Martins, M. (2014). Plants Visited by Apis mellifera L. (Hymenoptera: Apidae) in Recôncavo Baiano, State of Bahia, Brazil. *Brazilian Journal of Agriculture - Revista de Agricultura*, *89*(2), 97. https://doi.org/10.37856/bja.v89i2.123

Scheper, J., Bommarco, R., Holzschuh, A., Potts, S. G., Riedinger, V., Roberts, S. P. M., Kleijn, D. (2015). Local and landscape-level floral resources explain effects of wildflower strips on wild bees across four European countries. *Journal of Applied Ecology*, *52*(5), 1165–1175. https://doi.org/10.1111/1365-2664.12479

Scheper, J., Reemer, M., Van Kats, R., Ozinga, W. A., Van Der Linden, G. T. J., Schaminée, J. H. J., Kleijn, D. (2014). Museum specimens reveal loss of pollen host plants as key factor driving wild bee decline in the Netherlands. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(49), 17552–17557. https://doi.org/10.1073/pnas.1412973111

Schmid-Hempel, P., & Schmid-Hempel, R. (1987). Efficient Nectar-Collecting by Honeybees II . Response to Factors Determining Nectar Availability. *Journal of Animal Ecology*, *56*(1), 219–227.

Scoble, J., & Clarke, M. F. (2006). Nectar availability and flower choice by eastern spinebills foraging on mountain correa. *Animal Behaviour*, *72*(6), 1387–1394. https://doi.org/10.1016/j.anbehav.2006.03.024

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, *34*(1), 51–62. https://doi.org/10.1007/BF00175458

Shuler, R. E., Roulston, T. H., & Farris, G. E. (2005). Farming Practices Influence Wild Pollinator Populations on Squash and Pumpkin. *Journal of Economic Entomology*, *98*(3), 790–795. https://doi.org/10.1603/0022-0493-98.3.790

Somme, L., Vanderplanck, M., Michez, D., Lombaerde, I., Moerman, R., Wathelet, B., … Jacquemart, A. L. (2015). Pollen and nectar quality drive the major and minor floral choices of bumble bees. *Apidologie*, *46*(1), 92–106. https://doi.org/10.1007/s13592-014-0307-0

StatSoft, I. (2004). *STATISTICA (data analysis software system)*. Retrieved from www.statsoft.com

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M. P., & vanEngelsdorp, D. (2018). Drivers of colony losses. *Current Opinion in Insect Science*, *26*, 142–148. https://doi.org/10.1016/j.cois.2018.02.004

Takkis, K., Tscheulin, T., & Petanidou, T. (2018). Differential effects of climate warming on the nectar secretion of early-and late-flowering mediterranean plants. *Frontiers in Plant Science*, *9*(June), 1–13. https://doi.org/10.3389/fpls.2018.00874

Talavera, S., Herrera, J., Arroyo Marín, J., Devesa Alcaraz, J., & Ortiz, P. (1988). Estudio de la flora apícola de Andalucia Occidental. *Lagascalia*, *15*(1), 567–592.

Thompson, J. N., Laine, A.-L., & Thompson, J. F. (2010). Retention of mutualism in a geographically diverging interaction. *Ecology Letters*, *13*(11), 1368–1377. https://doi.org/10.1111/j.1461-0248.2010.01529.x

Tikka, P. M., Högmander, H., & Koski, P. S. (2001). Road and railway verges serve as dispersal corridors for grassland plants. *Landscape Ecology*, *16*(7), 659–666. https://doi.org/10.1023/A:1013120529382

Tilman, D., & Lehman, C. (2001). Human-caused environmental change: Impacts on plant diversity and evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(10), 5433–5440. https://doi.org/10.1073/pnas.091093198

Tzoulas, K., Korpela, K., Venn, S., Yli-Pelkonen, V., Kaźmierczak, A., Niemela, J., & James, P. (2007, June 20). Promoting ecosystem and human health in urban areas using Green Infrastructure: A literature review. *Landscape and Urban Planning*, Vol. 81, pp. 167–178. https://doi.org/10.1016/j.landurbplan.2007.02.001

Vaudo, A. D., Tooker, J. F., Grozinger, C. M., & Patch, H. M. (2015). Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*. https://doi.org/10.1016/j.cois.2015.05.008

Westphal, C., Steffan-Dewenter, I., & Tscharntke, T. (2003). Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*, *6*(11), 961–965. https://doi.org/10.1046/j.1461-0248.2003.00523.x

Wiese, H. (1987). Nova Apicultura. *Porto Alegre . RS: Agropecuária*, (20), 428.

Winfree, R. (2010). The conservation and restoration of wild bees. *Annals of the New York Academy of Sciences*, *1195*, 169–197. https://doi.org/10.1111/j.1749-6632.2010.05449.x

Withrow, J. M., & Tarpy, D. R. (2018). Cryptic “royal” subfamilies in honey bee (Apis mellifera) colonies. *PLoS ONE*, *13*(7). https://doi.org/10.1371/journal.pone.0199124

Wratten, S. D., Gillespie, M., Decourtye, A., Mader, E., & Desneux, N. (2012). Pollinator habitat enhancement: Benefits to other ecosystem services. *Agriculture, Ecosystems and Environment*, *159*, 112–122.

https://doi.org/10.1016/j.agee.2012.06.020

Xiao, Y., Li, X., Cao, Y., & Dong, M. (2016). The diverse effects of habitat fragmentation on plant–pollinator interactions. *Plant Ecology*, *217*(7), 857–868. https://doi.org/10.1007/s11258-016-0608-7