UNIVERSITY OF TURIN

DOCTORAL SCHOOL OF

SCIENCE AND INNOVATIVE TECHNOLOGIES

PHD PROGRAMME IN
AGRICULTURE, FOREST AND FOOD SCIENCE

CICLO: XXXIV

INSECT POLLINATION IN AGROSYSTEMS: MANAGEMENT, EFFECT ON PRODUCTION, AND RESOURCES PROVISIONING FOR POLLINATORS

Luca Carisio

Supervisor:	**Coordinatore del Ciclo:**
Prof. Marco Porporato	**Prof. Domenico Bosco**

ANNI

2019 - 2021

Index

Summary

The animal pollination is one of the most ecologically important mutualist interaction between animals and plants. Most of angiosperm plants require animal pollination to reproduce themselves and support genetic diversity, which is important to preserve plant populations. Since around 75% of cultivated plants need animal pollination, the economic value of the pollination ecosystem service is considerable. An efficient and science-based approach management of pollination in agrosystem areas may support crop production and at the same time may reduce the use of agricultural inputs affecting environmental health. This thesis addressed different aspects of pollination in agrosystems.

Chapter 1 deal the importance of the placement and the density of compatible pollen source (pollinizer plants) in self-incompatible crops system as in apple cultivation which I used as study crop system. Nowadays, an intermixed orchard design, consisting in mixing pollinizer with production trees, is usually adopted. Moreover, it is possible that pollen dispersal could involve pollinizers placed in surrounding orchards; therefore, this should be taken into account in orchard design. In this study, I found that the density of pollinizer in main cv. tree surrounding agrosystems positively affected pollination efficiency. The outcomes of this study suggested that intermixed orchard generally assures uniform pollination across the orchard and that farmers should design new orchards with a specific focus on pollinizer availability in the surroundings.

In Chapter 2, I analysed the effect of pollination on fruit appearance developing an image analysis tool to test the hypothesis that pollination may affect the symmetry of apples. The hypothesis was tested for three commercial classes separately, to understand whether the pollination effect was consistent among classes. I also compared the performance of the image analysis tool with a

simplified symmetry measurement that has been previously used. The results showed that increasing pollination led to significant increase of apple symmetry. The image analysis tool resulted to be more precise to assess the pollination effect on fruit symmetry than the previously used symmetry measurement, leading to the conclusion that pollination effect on apple shape has been probably overlooked because of the use of oversimplified symmetry measures. The effect of pollination on apple symmetry was not consistent among commercial classes since it resulted significant for little and medium size apples but unimportant for large size apples. Our findings suggest that the effect of pollination on fruit production should be assessed taking into account different variables and different commercial classes of apple.

In Chapter 3, I analysed the quantity of nectar resources that are provided by two mass-flowering crop agrosystems. Despite its importance, the production of nectar by flowers has been mainly assessed through one sampling method, the nectar replenished in a 24h period. Yet, detailed knowledge of nectar dynamics is limited, especially for cultivated plants. The nectar production could be driven by insect probing and rate of visitation. Therefore, exploring the interaction between the nectar dynamics and insect visits could be particularly important to estimate nectar resources for insects. In this Chapter, I measured the nectar production dynamic of lavender (*Lavandula hybrida*) and fennel (*Foeniculum vulgare*) and evaluated the insect visitation rate on those crops. Then I developed a simulation model to identify how plausible different scenarios of visitation rates and insect strategies for flower selection may change the standing nectar and the daily nectar production of the two crops. Finally, I tested whether simulation estimates of daily nectar production differed from the commonly used 24h nectar production measurement. Lavender and fennel had equal nectar volume replenishment in 24h, but lavender replenished nectar volumes quicker than fennel. Flower preferences of visiting insects and the standing nectar indicated high exploitation of fennel nectar, probably because fennel also provided pollen and its flowers are easy to probe. The simulation models suggested that nectar production is insect-driven, but the effect of insect selection and visitation was different between the two crops. Finally, I found that the measurement of daily nectar production produced by the simulation models was always greater than the nectar replenished within 24h in flowers isolated from pollinators. This study demonstrates complex effects of plant/insect interactions on nectar production. Reliable estimates of nectar resources may be achieved when considering nectar dynamics and visitation rates.

In conclusion, the results and findings described in the chapters of this PhD thesis provide new insights on different aspects of pollination in agrosystems. The explored aspects can contribute to understand how pollination could be better managed in self-incompatible cultivation, providing clear information that farmer can easily adopt in the orchard design to enhance pollination. Still focusing on apple, the thesis provides original results on the effect of apple symmetry, which can affect the economic value of fruits. This will allow a more comprehensive evaluation of the effect and the impact of the pollination ecosystem service. Finally, the thesis highlights some caveats on the nowadays most adopted method to assess the nectar resources provided by agrosystems environments. This last result may greatly contribute to a better evaluation of food resources for insect and pollinators, considering that is one of the basis to understand how to support animal pollinator and their essential ecosystem service.

1 Introduction

1.1 Plant pollination

Plants can reproduce through two different strategies. The first one is the asexual reproduction that consists in a part of the plant that separates and originates a new individual which is a clone of its parent. This reproduction form is the most ancestral and, despite being efficient for some plants (mostly isolated, aquatic or alien plants), does not allow genetic diversity between individuals and thereby evolution (Silvertown, 2008). Plant genetic diversity is strongly linked to the resilience and the survival of plant populations to abiotic and biotic disturbances (Iriondo et al., 2008). For this reason, many plants evolved the capacity to reproduce sexually, sometimes exclusively. Among plants, angiosperms present flowers that produce pollen grains (male gametes) and ovules (female gametes). The process of the transfer of the pollen to the ovule performed by a carrier is called pollination. Pollen can be transported in different ways and by different carriers to the flower's receptive organ (stigma), such as wind by passive transfer, or animals by active transport. Carrier animals moving pollen from a plant to another are called pollinators, and can be insects, birds, or little mammals. The active transfer of pollen by pollinators is the most common as the proportion of angiosperms that require animal pollination range between 78% and 94% from temperate to tropical zones (Ollerton et al., 2011). Pollinators visit flowers to get nectar, pollen and water produced by plants. Nectar and pollen provided by flowers are both nutrition and attractants for the pollinator (Aronne et al., 2012; Quinlan et al., 2021). Therefore, pollination can be considered as a form of mutualism between plants and animals. In this mutual exchange plants enhance the reproduction success of plants, while pollinators receive food. Pollinators can be specialists, also called oligolectic, have been classified as both, pollinators that tend to probe only plants belonging to the same family (Bommarco et al., 2010) or to the same species or genera (Banaszak-Cibicka & Żmihorski, 2012). Generalist bee species are also called polylectic and they do not show any specific preference for a group of plants, being able to exploit nectar from a wide spectrum of plants (Michener 2020).

Pollinators, either generalist or specialist, are definitely more efficient pollen carriers than wind or water. The major advantages of a targeted pollination is that the interspecific pollen transfer is reduced and ultimately, the pollinators competition among plants is lessen (Morales & Traveset, 2008). Despite the higher efficiency of pollinators compared to different pollen dispersal mechanism, it has been estimated that animal pollination can successfully transfer only 1% of produced pollen to a stigma (Harder, 2000). The low rate of successful transfer is partially due to some biological mechanisms that plant have evolved, which reduce self-fertilization and increase cross-pollination (Devaux et al., 2019). An extreme example of these mechanisms is when plants have separated male and female flowers on separate plants (dioecious). Dioecious plants can only reproduce sexually through cross-pollination and a long transfer of the pollen to another plant individual is required. In this case the chances of the pollen not reaching a stigma are increased (Knight et al., 2005). Moreover, when female and male structures are together on the same plant (monoicous plants), it is common to have either available pollen to be collected from the male part or receptive stigmas in different times to avoid self-fertilization (dichogamy) and self-interference (Webb, 1986).

1.2 Plant fecundation and fruit development

When the pollen is successfully transferred to the stigma surface, the pollen grain is hydrated and can germinate, grow and reach the ovary, realizing the ovule fertilization. However, different factors can determine pollen grain growth failure even at this stage (Willmer, 2011). For instance, the pollen growth can be impeded by the competition between pollen grains in the stigma surface (Marshall et al., 1996). Another factor might be the physiological mechanism carried out by some plants that prevent self-fertilization or fecundation. An example of mechanism is the self-/nonself pollen recognition that some plants adopt to inhibit the growth of self-pollen tubes. Self-pollen recognition and growth inhibition, process are controlled by a single genetic S locus (Takayama & Isogai, 2005). The self-compatibility mechanism is common as it has been reported for 40% of angiosperms (Hendry & Grime, 1993), including in important crops (e.g. apple crops) (Ramírez & Lee, 2013).

When pollen growth or the ovule fecundation have been successful, different physiological processes involving phytohormones are triggered. Phytohormones as abscisic acid, auxine, cytokinins and gibberellins, are transferred to the fruit or produced by the fruits (Grant et al., 1989; Ruan et al., 2012). In the classical theory these hormones regulate the competition between seeds and fruits as in attracting plant resources (nutrients and water) (Chapman & Sadjadi, 1981). The alternative theory is that seed and fruit, through plant hormone mechanism, inhibit the development of the other close seeds and fruit (Bangerth, 2000). In any case, seeds play a pivotal role as source of phytohormones, and for this reason the number of seeds has a positive correlation with the fruit set and the seed abortion halts fruit maturation in different plant species (Ruan et al., 2012). The link between the number of seeds and the pollination is well known, so that different studies on pollination adopted the seed set as a proxy of the pollination intensity (Morandin & Winston, 2006; Steffan-Dewenter & Tscharntke, 2000). Therefore, in most of the cases pollination is the '*sine qua non*' condition for fruit development and sexual reproduction of most of wild and cultivated angiosperms. Nonetheless, especially in angiosperms, some plants can produce fruits without seed fertilization (parthenocarpy *sensu stricto*) (Picarella & Mazzucato, 2019). The number of parthenocarpic plants is limited since the consume of energy for the production of fruits that do not have seed, thus not producing offspring, is disadvantageous. The parthenocarpy could be useful only for wild plant that are subjected to fruit predatory pressure. Indeed, the availability of seed-less fruits may deceive the seeds/fruits predators (Traveset, 1993; Zangerl, Berenbaum, & Nitao, 1991). In the case of cultivated plants, farmers have shown interest in having seed-less fruits aiming to a fruit production less pollination dependent and because these fruits can be more pleasant to eat (Picarella & Mazzucato, 2019). For these reasons parthenocarpic cultivated plants are more frequent than wild ones, but still do not represent the majority of crops.

1.3 Importance of pollination in agriculture

Pollination is of paramount importance when dealing with agricultural systems. In fact, around 75% of crops relay on animal pollination for producing fruits (Klein et al., 2007), and insect pollination is needed for the production of 3 to 8% of global crop weight (Aizen et al., 2009). There have been different estimates of the economic value of pollination as ecosystem service (Hanley et al., 2015). Historically, such estimations were performed using different methods: 1) considering the value of pollination as input of crop production by means of functions accounting for the pollination dependence of the crop (Calderone, 2012). 2) Through econometric techniques that calculate losses due to lack of pollination (Gallai et al., 2009). 3) By estimating costs needed to compensate for the absence of pollinators (e.g. Allsopp et al., 2008). Nonetheless, the difference on the methods and on the parameter adopted in the estimation of pollination values have led to great differences between the studies. For example, in USA the overall economic value of pollination has been estimated to be 2.8 X 10^9 € by Losey & Vaughan (2006), 14.5 X 10^9 € by Morse & Calderone (2000) and between 3 and 10 X 10^9 € by Southwick & Southwick (1992). Even though estimates can be inaccurate, they are essential to illustrate the economic value of the benefits of pollination ecosystem service (Costanza et al., 1997). Secondly, estimates are important to build awareness of citizens and policy makers about the risk of losing the pollination ecosystem services (Abson & Termansen, 2011). Remarkably, decision-makers are currently aiming to integrate into their cost-benefits analysis the estimates of the pollination economic value. This type of analyses is of particular interest, especially when focused on a specific farming system, since they can provide information enabling farmers to improve their practices, or even their income.

In fact, pollination ecosystem services, as every other ecosystem service, can replace the benefits of farmer managed agricultural inputs, reducing their use and their related costs. At the same time, the reduction of agriculture inputs has been suggested as a way to enhance agricultural ecosystems and biodiversity. The concept of the replacement of agricultural inputs with ecosystem services for a sustainable crop production has been called "ecological intensification" (Bommarco et al., 2013).

1.4 Pollination service in the framework of ecological intensification

The maximum agriculture yield potential is not reached in most of agricultural systems (Lobell et al., 2009). The efforts to reach the maximum yield potential fail after a certain threshold when the addition of agriculture inputs has no further effects on production, making the costs of the production non-economical beneficial (Neumann et al., 2010). Ecological intensification consists in assessing how agricultural inputs and ecosystem services interact together in defining crop production and to exploit this knowledge in order to support production (Doré et al., 2011). Nowadays, in many agricultural context, the increase of farmer managed inputs does not increase the production, therefore, the ecological intensification can be a strategy to tackle the incoming challenge of the global increasing food demands (Bommarco et al., 2013). Indeed, the study of the interaction between ecosystem services and agriculture input can shed light on practices to fulfil the production gap. Theoretically, the interactions between anthropic and natural drivers of crop production can be additive, positive or negative (Garibaldi et al., 2018). The effect on production is additive if the two drivers benefit the crop production but the level of both drivers does not enhance nor decrease the benefit on the production of the other driver. Positive effects happen when the benefit on production of a driver at a defined level increases as the level of a second production driver changes. Finally, the relationship is negative when the benefit of a driver at fixed level decreases as the provisioning of second drivers increases, so that the second driver has a compensative effect on the second one.

Focusing on the pollination ecosystem service, there are many examples of studies that analyse whether pollination ecosystems services interact with agriculture inputs through additive, positive or negative mechanisms (Tamburini et al., 2019). For example, the pollination level, water and nutrient availability showed an additive effect of yield production of coffee plants, but no evidence reported the interaction between these production drivers (Boreux et al., 2013). Positive synergic effects were found in *Brassica napus*, with the maximum benefit of pollination realized at high fertilizing doses (Garratt et al., 2018). An example of negative interaction was described in common bean (*Phaseolus vulgaris*), for which high doses of nitrogen lead to cancel pollination benefits to the production (Ramos et al., 2018). Therefore, the study of pollination in agrosystems has the potential to unveil unknown mechanisms between production drivers and to enhance production, reducing at the same time the impacts on the environment.

1.5 Strategies to improve pollination and fruit set in agrosystems

The support to pollination and fruit set in agrosystems should be provided preventing pollen limitation and those conditions that can lead to fruit abortion. To pursue these objectives, different strategies can be adopted, and they can be divided into categories according to the subject of the strategy, as summarized in Table 1.

Table 1. Summary of the strategies that could be adopted to improve pollination in agrosystems.

Strategy subject	Strategy
Pollinators management	*Evaluating pollinator deficit according to visitation rate thresholds and resources provided by the agrosystems to pollinators* 1) Add managed pollinators and their distribution within the field 2) Support wild pollinator habitats (food, nesting sites)
Agricultural field design	*For self-compatible hermaphrodites or imperfect monoecious plants* 1) Interplanting distance according to pollinator foraging distance *For self-incompatible, dioicous dichogamies plants* 2) intermixed orchard to spread pollen donors across the orchard
Agricultural practices	*Agronomic factor management (water, nutrient, light) aiming to support pollination and fruit carrying capacity* 1) good management for supporting pollination 2) good management for supporting fruit development

1.5.1 *Pollinator management*

In agrosystems, the issue of pollen limitation has been typically overcome by managing the quantity of pollinators, for instance adding honey bee colonies close to the pollen dependent crops. In the frame of this strategy, different researchers provided indication of the number of honeybee colonies that should be placed to have a sufficient transfer of pollen (Free, 1970; Abbasi et al., 2021; de Ribeiro et al., 2015). However, the quantity of honey bee is not always linked to the amount of pollination service provisioning (Rollin et al., 2015).

This missing association between honey bee number and amount of pollination might be due to the unpredictable effect of bee dispersion that can highly vary depending on the landscape context (Lajos et al., 2021). When the dispersion is low and/or managed pollinator density is too high, the excessive visitation rate can damage the flowers and reduce crop production (Sáez et al., 2014; Rollin & Garibaldi, 2019). The honey bees, as other pollinators have a great dispersion capacity and this might be associated to the different flower composition that dilutes honey bee density in different landscapes (Holzschuh et al., 2016). As I highlighted in the third chapter of the thesis, mass-flowering crops can exert different attraction level for honey bee so that honey bee placed for the pollination of a specific crop can actually focus most of their visitation efforts on another close mass-flowering crop.

Recently, the assessment of a number of hives that should be placed in a crop has been indicated as unsuitable measure aiming to ensure sufficient level of pollination, therefore scientists suggested to use visitation rate thresholds, instead. A visitation rate threshold is the minimum number of pollinator visits that a flower should receive to be sure that the pollination level is sufficient (Rollin & Garibaldi, 2019). When these thresholds are not reached, managed pollinators should be added. Visitation rate thresholds are of particular interest, although for many pollen-dependent crops such data is still missing. The increase of pollinators and of pollinator services, can be achieved also through a good management of the bee colonies. In pear and apple orchard in Argentina, it was observed that the placement of densely populated honey bee colonies instead of lowly populated honey bee colony led to an 7000 $/ha increase of farmer’ gain due to more effective pollination services (Geslin et al., 2017).

Nevertheless, to reach a sufficient pollination level, the pollination service should not be planned only by means of managed bee. Different studies highlighted that wild bees and other flower-visiting insects can be more efficient pollinators than honeybees (Garibaldi et al., 2014; Garratt et al., 2016). The diversity of pollinator communities has shown benefits on the production of different crops (Greenleaf & Kremen, 2006; Schurr, Geslin, et al., 2021; Vergara & Badano, 2009). For this reason, one of the most suggested strategies to prevent pollination limitation in flowering crops is to improve habitats known to sustain pollinators. The improvement of pollinator habitat can be addressed by increasing the amount and the quality of food resources at landscape scale, namely pollen and nectar. These two food sources can be assessed in the environment in different ways aiming to evaluate whether food resources are sufficient to support a healthy and diverse pollinator community. The mainstream method for the assessment of pollen and nectar resources provided by flowers is to measure how much of them has been cumulated in a 24h period (Baude et al., 2016; Hicks et al., 2016; Timberlake, Vaughan, Baude, & Memmott, 2021). However, as I will show in chapter 3 of the thesis, this method has some limitations that should be addressed to develop a better understanding of pollinator flower resources. One of the most common methods to enhance food resources for pollinators is planting flower strips in cultivated or urban areas (Requier & Leonhardt, 2020). Flower strips in field edge has been shown to increase the species richness of butterflies and wild bees in a long-term study (Buhk et al., 2018). The positive effect of flower strips on wild bees can be explained by different observed mechanism as the increase of food provisioning efficiency, of offspring emerging rate and the reduction of parasitism rate (Ganser et al., 2021). Further strategies adopted to improve the habitats for pollinators include:

- providing nesting habitats such as artificial nests or patches of bare ground;
- conserving areas which provide non-floral resources such as resin leaf or honeydew, important as both, food and nesting material. (Requier & Leonhardt, 2020).

1.5.2 Agricultural field design

Pollen limitation comprehends two components, a quantitative and a qualitative one (Aizen & Harder, 2007). The quantitative component is given by the ratio between the number of pollen grains that reach the stigma and the number of ovules that can be fertilized (Sáez et al., 2018). Besides through pollinators managing, the quantitative component could be also influenced by the agricultural field design. In fact, low distances between crop plants increase the chance of cross-pollination (Kron et al., 2001). For crops which are self-compatible and can rely on self-reproduction, the distance between plant is of little importance, as the pollinator visit on the same plant can still lead to self-fertilization and fruit development. On the other hand, plant with xenogamy reproduction strategy, due to self-incompatibility, dioecy or dichogamy, require cross-pollination from close compatible plants. Such requirement has been called the qualitative component of pollination (Aizen & Harder, 2007). Crops that are affected by both, quantitative and qualitative components, need a well-planned agricultural field design for maximising pollination benefits on crop production. Agricultural field design consists in managing the placement of each other compatible plants and their reciprocal ratio. There are many crops of great economic interest for which the agricultural field design has been adopted, as method for managing quantitative and qualitative components of pollination; some examples are apple, kiwi, avocado and coffee (Dymond et al., 2021; Klein et al., 2003; Kron & Husband, 2006; Sáez, Negri, Viel, & Aizen, 2019). Overall, agricultural field design has been a neglected and understudied factor affecting pollination in agrosystems, thus farmers are still experimenting different approaches (Sáez et al., 2018). Chapter 1 of this thesis explore apple orchard design and how the placement and the density of pollinator plants which provide compatible pollen to the main cultivar plants affects the pollination. In this study, I found that the intermixed orchard design which consist in placing uniformly pollinator plants within the apple orchard provide the highest pollination efficiency.

1.5.3 Agricultural practices

Limitation or excess of the level of different agronomic factors can affect both, pollination and fruit growth process. For instance, not well-watered crops can suffer of drought stress, which can affect flower traits and pollinator attractiveness (Alqudah et al., 2011). In fact drought can reduce pollen availability, decrease the number of ovules and even alter some traits that are related to pollinator attraction such as nectar quantity and composition, or number of flowers (Descamps et al., 2021; Neumann Andersen et al., 2002; Sheoran & Saini, 1996). Nitrogen availability is another agronomic factor that has been linked to change of flower traits, nectar quantity and composition, so that changes of plant-pollinator interaction due to the nitrogen availability are expected (David et al., 2019). However, a comprehensive understanding of the effect of nitrogen availability on pollination in cropped areas still require further studies. The link between pollination provisioning and other agronomic factor can be also indirect. For example Pecenka et al. (2021) observed that in corn field, the reduction of pesticides such as neonicotinoid, which is used to reduce the risk of insect pest damages, increased pollinator visitation rates in the close watermelon field, resulting in 26% higher yield of watermelon and no significant negative impact on the yield of corn fields.

As highlighted by Bos et al. 2007 the evaluation of the benefits of pollination also requires a clear understanding of the fruit set carrying capacity that a plant can sustain. This carrying capacity is given by the level of photosynthesis, water, nutrients and pest control (Bos et al., 2007; Garratt et al., 2018; Tamburini et al., 2019). After the pollination, both water and nutrient availability for plant can affect the crop carrying capacity since the two factors might interact with the physiological processes involved in seed formation (Alqudah et al., 2011; Tamburini, Lami, & Marini, 2017). Thus, when agronomic factors are not well managed, benefits of the pollination can be reduced. As the response of pollination to different agronomic factors is complex, the effect of pollination on production is context-dependent and it is difficult to draw general conclusions (Tamburini et al., 2019).

A clear example of this complexity is given by the results of a recent study that compared the effect of pollination on apple across different countries and different cultivars (Garratt et al., 2021). This study suggested that the increase of pollination led to contrasting results between apple varieties and study areas.

Moreover, considering pollinator benefits focusing exclusively on crop yield could be misleading. As a matter of facts, the quality of crops of fruits are also pivotal on defining their commercial values, as demonstrated in different studies. For example, pollination was found to enhance fruit quality reducing the proportion of mishappen fruit for strawberries and apples (Klatt et al., 2013; Wu et al., 2021), increasing the oil content of oilseed rape and the vitamins in almonds (Bommarco et al., 2012; Brittain, Kremen, Garber, & Klein, 2014). In chapter 2, I evaluated how pollination can affect the symmetry of apple of different commercial grades exploring the link between pollination and production quality. The use of yield and quality measures to assess pollination effect on production can allow a more comprehensive understanding of pollination importance in enhancing food production. Further studies are required to identify which are the pollination needs of different crops and cultivar in different contexts. This specific knowledge will allow farmers and scientist to develop scientific-based agronomic strategies that will face the global challenge of increasing food demands.

1.6 Phd Thesis objectives

The Phd project was carried out within the Phd Programme in Agriculture, Forest and Food Science provided by the University of Torino (Unito). The Phd research project on “Insect pollination in agrosystems: management, effect on production, and resources provisioning for pollinators” aims to broaden the knowledge on pollination in mass-flowering crops areas to better understand the positive effects of pollination on crop production, how the pollination ecosystem service can be managed to maximise its positive effects on production, and finally how food resources for pollinator can be quantified in mass-flowering crops areas. The research activities explored such questions in two different crop systems. Pollination importance for the production and its management were explored in apple orchards in a research project developed in partnership with La Fondazione per la ricerca, l’innovazione e lo sviluppo tecnologico dell’agricoltura piemontese (Agrion). The research activity focused on the provisioning of food resources by mass-flowering crops areas was carried out in fennel and lavender cropped areas in France. This last activity was part of the project, led by Aix Marseille Université, focused on assessing the importance of pollination and pollinator on fennel crops. I participated to this project during a 3-months research period in France. The research activities granted the preparation of three manuscript, which are presented as individual chapter of this thesis.

- Chapter 1 **“Effects of pollinizer density and apple tree position on pollination efficiency in cv. Gala”**, a study assessing how density and position of apples trees placed in orchard affect the pollination and the production.
- Chapter 2 **“Influence of seed set on apple symmetry assessed by image analysis: an overlooked effect of pollination on fruit shape.”**

In this chapter, the effect of pollination on apple symmetry was analysed.

- Chapter 3 "**The interaction of nectar dynamic and insect visitation affects the estimation of nectar resources"**. In this study, a simulation model to predict the volume of nectar collected by flower-visiting insects was developed as new method to assess food resources for insects

The results achieved during the PhD, including some side projects, have been shared with the scientific community at national and international conferences, both as oral communication and posters. A comprehensive list of these activities is available in Annex I.

In addition to the scientific results of the PhD project, I contributed to research focused on the impact of two Hymenoptera alien species, *Megachile sculpturalis* and *Vespa velutina*. Specifically, the impact of the alien bee *Megachile sculpturalis* on nest of the native species *Osmia bicornis* was investigated. Focusing on *Vespa velutina*, I contributed to review its impact on beekeeping and on native species and I assessed the impact on native wasp species in field conditions. The link to the three manuscripts on the alien Hymenopterans is available in the Annex II.

2. CHAPTER 1

Effects of pollinizer density and apple tree position on pollination efficiency in cv. Gala

Luca Carisio*, Sara Straffon Díaz, Aulo Manino, Marco Porporato

Scientia Horticulturae, 273: 109629 (2020), doi:10.1016/j.scienta.2020.109629

*Corresponding author

Abstract: Pollen donor plants (pollinizers) availability and arrangement are considered fundamental to provide compatible pollen to main cultivar ensuring fruit production in apple orchards. Apple farmers manage pollination efficiency by setting the amount of pollinizer plants and the arrangement of pollinizer/main cv. tree within the orchard. Nowadays, an intermixed orchard design, consisting in mixing pollinizer with production trees, is usually adopted. Moreover, it is possible that pollen dispersal could involve pollinizers placed in surrounding orchards; therefore, this should be taken into account in orchard design. We sampled 'Gala' trees with different pollinizer densities combined with different positions within row in three orchards of North-West Italy assessing pollination efficiency. The density of pollinizer in main cv. tree surroundings affected positively pollination efficiency up to 30 m. In addition, only trees adjacent to pollinizers showed higher pollination level while trees differently positioned had lower and similar pollination efficiency. Our outcomes suggest intermixed orchard assures generally uniform pollination across the orchard and that farmers should design new orchards with a specific focus on pollinizer availability in the surroundings.

Keywords: *Malus domestica*, Management factor, Orchard design, Cross-pollination

2.1 Introduction

Agricultural production can be improved by increasing regulating ecosystem services, while the impacts of intensive agriculture should be reduced concurrently (Bommarco et al., 2013). Among the regulating services, insect pollination is particularly relevant given that 35 % of the annual agricultural production volume is obtained from crops dependent on such ecosystem service (Potts et al., 2016). Without regard to relevant synergies between pollination and other concomitant factor (Bommarco et al., 2013; Tamburini et al., 2019), such as plant growth and physiology (Knight et al., 2005), agronomic factors (Musacchi & Serra, 2018) and weather conditions (Sharma et al. 2003; Sheffield, 2014; Vicens & Bosch, 2000); insect pollination success depends on a complex network of interactions between different players: the plant that receives pollen, the plant that provides it, and the pollen vector who carries it between plants (Kremen et al., 2007). Farmers may adopt practices of ecological intensification by acting on these agents, in order to compensate for any pollination limitation and thereby to achieve better yields with less or equal impacts on the environment (Kovács-Hostyánszki et al., 2017). Pollinators in temperate areas are mainly represented by managed bees (the most important are *Apis mellifera*, *Bombus terrestris*, and *Osmia rufa*) and wild bees (Kremen et al., 2007). To date, the mainstream strategy adopted by producers to promote pollination service through pollinators management is to maintain high abundance of honey bees, especially where wild bees are absent or declining (Geslin et al., 2017). Within pollination networks, the relationship between pollen receptor plants and pollen-donor plants (pollinizer) is defined by two components, one quantitative and the other qualitative. The quantitative component is given by the ratio between available pollen grains and the ovules to be fertilized (Aizen & Harder, 2007); when ratio is unbalanced with more ovules than pollen, then pollen limitation is set up (Knight et al., 2005). In turn, pollen limitation could result in a qualitative and/or quantitative reduction of pollination-dependent crops productivity (Bartomeus et al., 2014). The qualitative component depends on the degree of genetic compatibility between pollen donor and receptor plants, along a gradient from self-compatible plants to those needing genetically distant pollen donors (Lloyd & Schoen, 1992). This component determines that pollination level relays on plant-pollinizer compatibility degree (quality component) in case of same amount of pollen reaching stigmas (Aizen & Harder, 2007). Among insect pollination-dependent crops, apple (*Malus domestica*) shows a self-incompatible fertilization system, with the exception of some self-compatible cv. (Ramírez & Davenport, 2013). Moreover, apple plays a major economic role being one of the four most consumed fruits worldwide (Musacchi & Serra, 2018). It has been shown that pollination represents a critical step affecting quantity and quality of apple production (Buccheri & Di Vaio, 2005; Garratt et al., 2014; Geslin et al., 2017; Matsumoto et al., 2008; Sheffield 2014). To ensure effective pollination in the apple orchard, it is necessary to supply two or more cultivars (one the main cv. and at least one pollinizer cv.) (Kron & Husband, 2006; Ramírez & Lee, 2013). The set down of a new apple orchard involves quantitative and qualitative components in two steps of farmer management: first, to decide the proportion between pollinizer plants and main cv. plants and secondly, to determine the distribution of the pollinizer trees. A high pollinizer/main cv. ratio increases the availability of compatible pollen resulting in higher production (Sharma et al., 2003). Apple growers widely prefer to maintain low pollen donor proportions since their presence in orchards makes fruit harvesting more complex and expensive (Free, 1962; Sáez et al., 2018). However, this practice could lead to a pollen limitation. In addition, the management of pollination service through the manipulation of pollinizer proportion at single orchard scale could be misleading. Indeed, the density of compatible plants surrounding a target plant has been demonstrated to impact at wide scales for some wild plant populations (Wagenius, 2019). In apple orchards, the highest pollen dispersal occurs at ranges from 35 to 86m (Blazek, 1975; Kron, Brian, Peter, & Kevan, 2001; Milutinović, Šurlan-Momirović, & Nikolić, 1996) which suggests that apple trees pollination near orchard margins could be greatly influenced by the pollinizer density of the neighbouring apple fields. In this case, pollination management perspective should come out of the single orchard. It has been observed that some growers laid out new orchard without including pollinizer trees, counting on the presence of adjacent compatible apple orchard, although this practice has no basis in current scientific literature. Once the density of pollinizer plants in the orchard is defined, the spatial distribution is not trivial. In the past, orchards were configured in blocks in which the pollinizer cv. trees were planted on few rows alongside several rows of main cv. (Kron, Brian, et al., 2001). Nowadays, instead of this orchard design, an intermixed orchard planting is used alternating pollinizers trees within main cv. rows. Several investigations suggested that the inter- mixed orchard should be the most effective design to enhance pollen flow (Free, 1962; Kron, Husband, et al., 2001; Quinet & Jacquemart, 2017; Sáez et al., 2018). While numerous studies have analysed pollen dispersal effect due to orchards block design on certain production parameters of apple (Free, 1970; Kron, Brian, et al., 2001; Kron, Husband, et al., 2001), only two studies have addressed the effect of intermixed orchard on pollen dispersal. One is based on modelling tools (Sáez et al., 2018) and the second used an experimental apple orchards with densities and conditions that were rather different from those normally adopted for highly intensive production purposes (Matsumoto et al., 2008). Therefore, it remains necessary to assess how pollination changes across productive intermixed orchard with low pollinizer/main cv. proportion in function of tree position within orchard row and pollinizer density in the surroundings. The aims of this study are: 1) to verify the effect of pollinizer density on pollination and the range in which density has effects; 2) to determine whether different focal positions of main cv. trees are associated with differential pollination levels; 3) to evaluate whether different conditions of pollinizer density and position show pollen limitation, through the use of supplementary pollination as reference of optimal pollination. Pollinizer density is expected to have a positive effect on pollination at a scale that exceed the size of a single orchard. Additionally, considering that selected positions of main focal trees have different arrangements in relation to the nearest pollinizer, we expect that the more a tree is isolated from pollinizers, the lower its pollination level is, as it has been observed by other authors (Blazek, 1975; Kron, Brian, et al., 2001; Milutinović et al., 1996). We consider that this knowledge is useful to maximize pollination through shaping the orchard design.

2.2 Materials and methods

2.2.1 *Study area*

The experiment was settled in 2019 in the intensive fruit growing area of Cuneo District, NW Italy. The climate is continental with cold winters and intermediate summers (Casasso & Sethi, 2017). Mean annual temperature is 12°C and mean annual rainfall is 800mm (Fick & Hijmans, 2017). In study area ∼18,000 ha are dedicated to apple, pear, peach and cherry farming. Apples orchards altogether extend to ∼6300 ha and produce the greatest amount of red variety apples in Italy (Vittone et al., 2019). Since apple orchards are the main farming landscape component, every orchard is usually surrounded by other apple orchards of same or different cultivars. Apple orchard management is highly specialized allowing to reach mean yields of 40−68 t/ha of apples that meet the quality standards defined by Council Regulation (EC) No 1234/2007 (European Comission, 2007). Such production is supported by an intensive farming strategy: high tree densities ranging between 1700 and 2700 tree/ha, use of different classes of pesticides and phytoregulators, and displacement of managed honey bee colonies to satisfy pollination needs since wild bees are scarce in the area. Experiment was carried out in three apple orchards located in Manta and Verzuolo municipalities (Table 1). Manta and Verzuolo landscape matrix is mainly agricultural, while natural or semi-natural areas are few and with limited surface. Orchards are composed of ‘Gala’ as main cultivar and ‘Granny Smith’ as pollinizer and trees are arranged

into parallel rows. ‘Granny Smith’ is the pollinizer usually adopted in ‘Gala’ intensive plantation since its pollen is fully compatible (Broothaerts, Van Nerum, & Keulemans, 2004). Orchards have similar tree densities and are characterized by an intermixed pollinizer design. This consists in rows in which single pollinizer trees are planted after a certain number of main cultivar trees. The inner orchard percentage of pollinizer is similar across experimental fields (Table 1).

Table 1. Experimental orchard features. AM = ‘Ambrosia’,FJ= ‘Fuji’, GS=‘Granny Smith’,RD = ‘Red Delicious’. (W) = west, (N) = north, (E) = east, (S) = south.

Orchard	Area (m^2)	Tree/ha	Inner orchard pollinizers (GS) (%)	Within/ Between row distance (m)	Compatible cultivars in the surroundings
1	15279	2180	6.0	1.00 x 3.90	AM (E)
2	14370	2660	8.1	0.95 x 3.40	RD (W), AM (NW), GS (N and S), AM (E), FJ (SW)
3	6870	2480	6.3	1.00 x 3.80	AM (W), GS (N and E)

Each selected orchard borders with at least another orchard where different apple cultivar are present. Such cultivar are fully compatible donors of ‘Gala’ according to literature (Broothaerts et al., 2004; Ramírez & Lee, 2013; Schneider, Stern, & Goldway, 2005) with the exception of ‘Ambrosia’, of unknown compatibility; however, local farmer technicians accredit ‘Ambrosia’ as compatible with ‘Gala’ basing on empirical knowledge. Blooming of apple trees inside experimental orchards overlapped with most of flowering period of compatible trees in surroundings. During blooming period, the main daily temperatures were 5.6–16.3 °C and monthly average rainfall was 67 mm. The three orchards are of similar age (~17 years) with similar agronomic management, but they differ in product and timing adopted for thinning treatment. In the area, pollination is provided by the distribution of 3 honeybee colonies/ha and pollinators visiting fruit flowers are mostly honeybees (~94 %); while other pollinators (bumblebees, wild bees and syrphid flies) are the minority (less than 3%) (Unpublished results).

2.2.2 *Sapling tree selection*

Every main cv. tree (‘Gala’) inside experimental orchards and every pollinizer tree within 45m from orchard edges were located. In order to select sampling trees among main cv. trees, firstly we quantified the number of pollinizer trees within a 30m buffer area per every main cv. tree placed inside experimental orchards using Qgis (version 3.2.3) (Fig. 1a).

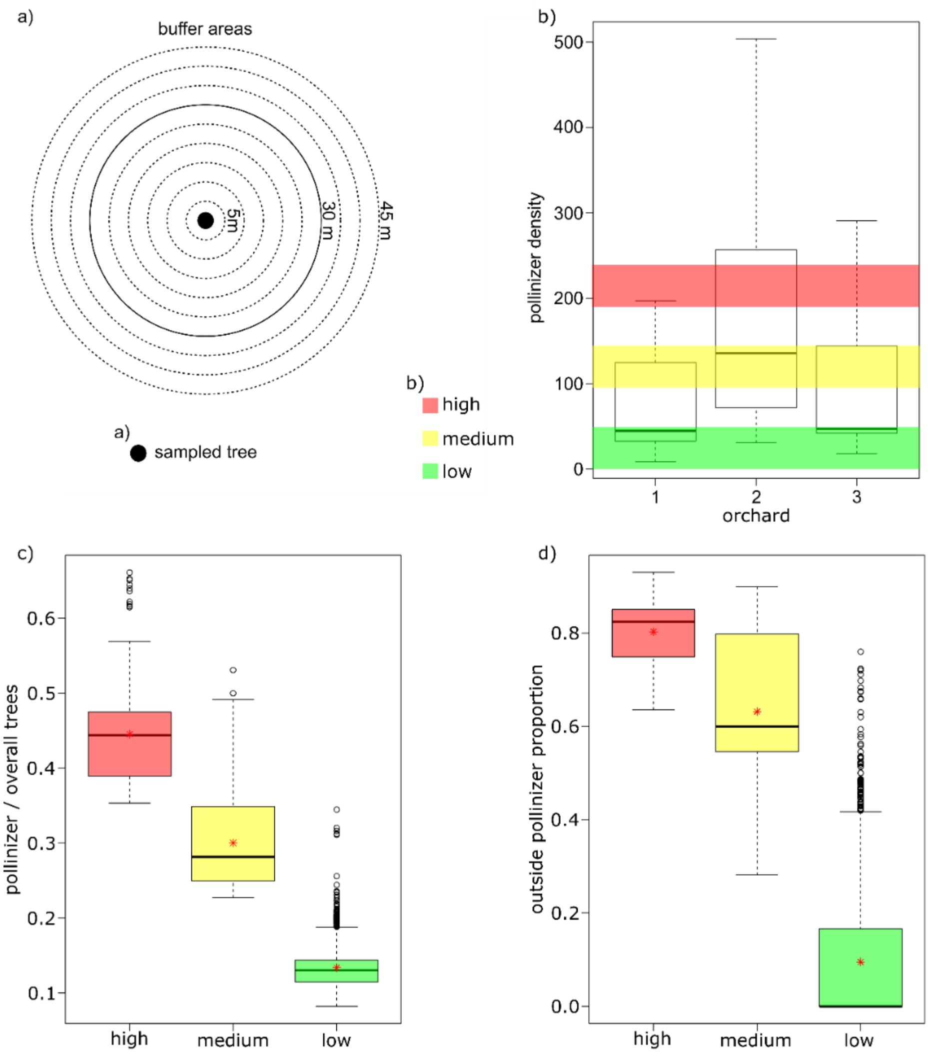


Fig. 1. Experimental structure. a) 30m buffer area used for sampling tree selection and 9 buffers (5-45 m) drawn from sampled trees in which density was defined (explanatory variable, section 2.3.); b) 30m pollinizer density; trees within the high (170-200), medium (95-125) and low (20-50) pollinizer density intervals were used as sampling pool; c) proportion of pollinizers among apple trees within 30m buffer area; d) proportion of pollinizers placed outside from experimental orchard. Red (*) indicates the mean (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Thirty m buffer area was chosen a priori by the reason that most of pollination should occur within this distance, according to lowest distances of maximum pollen dispersal previously reported (Blazek, 1975; Kron et al., 2001a; Milutinović et al., 1996). Since trees differed in their pollinizer densities regarding 30m buffer areas (Fig. 1b), we defined three density intervals (low, medium, high) applicable to all three orchards. 'Gala' trees settled into such density intervals were used as pool from which sampling trees were picked. Pollinizers among trees within the buffer area (Fig. 1c) and outside experimental orchard (Fig. 1d) were determined. Within the pool, we selected only trees placed in three focal main cv. tree positions (A, I, M). These positions should receive different amount of compatible pollen (explanation in section 2.2.3 and Fig. 2c).

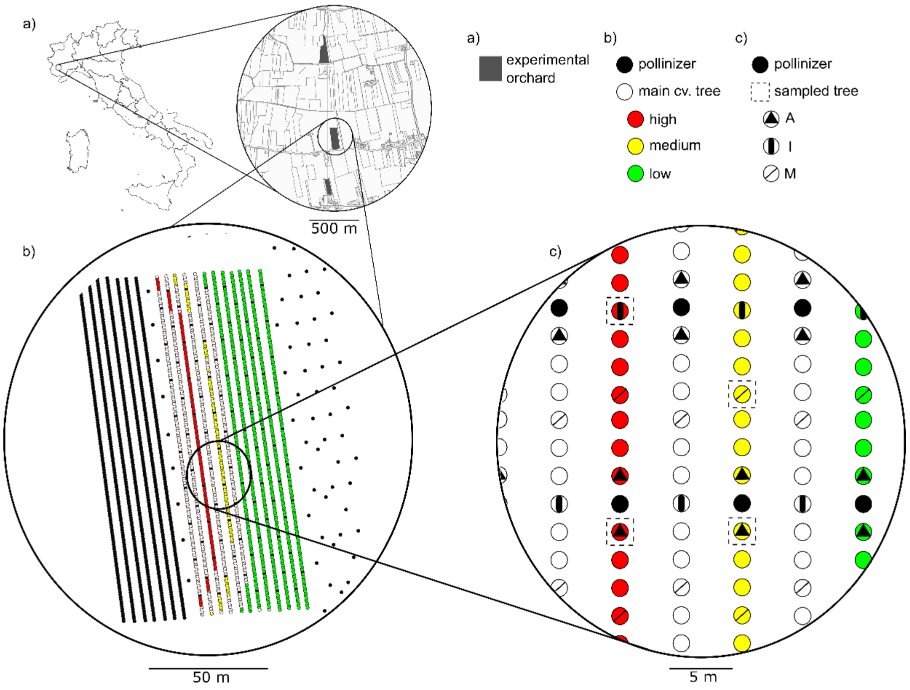


Fig. 2. a) study area with the 3 experimental orchards, b) arrangement of trees within high, medium and low density intervals and distribution of pollinizer trees (black dots),

c) detail on orchard design with arrangement of A (adjacent to pollinizer), I (pollinizers in neighbour rows) and M (no close pollinizer) focal tree positions combined with high, medium and low density intervals.
differed

Within this integrated pool of main cv. trees belonging to density intervals and simultaneously placed in focal positions, we chose 108 sampling trees following the nested structure position/density/orchard. The experimental structure was balanced selecting 4 trees per orchard with the same position/density combination (4 replicates X 3 positions X 3 density intervals X 3 orchards=108 sampling trees). For these 108 sampling trees, explanatory variables and fruit measurements were quantified.

2.2.3 *Explanatory variables*

Tree position: focal positions A, I and M were defined in relation to the closest pollinizers: A, position next to pollinizer in the same row; I, position in the middle between pollinizers of its own row and adjacent to two pollinizers on flanking rows; M, the most isolated position from pollinizers, located halfway between A and I positions (Fig. 2c). We expected differences among positions because: 1) pollinators are inclined to visit neighbouring trees (Vicens and Bosch, 2000b), usually. belonging to the same row (Sáez et al., 2018), and 2) there is a pollinator propensity to move along linear landscapes features (Cranmer et al., 2012) such as vegetation rows. Despite A and I positions have a direct flying path from pollinizers, pollinators should prefer A because it is the closest to pollinizer and arranged within the same row. Given that M position has not any neighbour pollinizer, it should implicate A > M and I > M. Therefore, positions were assumed to receive different amount of compatible pollen as A > I > M. Pollinizer density: in block design orchards, distance between pollinizer block and main cv. tree was used assessing pollen dispersal and pollination efficiency (Kron et al., 2001a, 2001b). In intermixed orchards, there is not a single distance from compatible trees since pollinizers are at various distances; thus we propose pollinizer density, which has not been tested before, as possible proxy alternative to evaluate compatible pollen availability. Pollinizer density was quantified around each sampled tree using Qgis within 9 buffers of 5 m, 10 m, 15 m, 20 m, 25 m, 30 m, 35 m, 40 m, and 45m (Fig. 1a). This count defines a density continuous variable per each buffer. We used continuous density to evaluate how its effect on fruit measurement changed across spatial scales. Scenarios: previously defined density intervals (low, medium and high, see Section 2..2.2 and Fig. 2c) were combined with tree position setting nine scenarios: M_low, M_medium, M_high, I_low, I_medium, I_high, A_low, A_medium, A_high. Pollination levels per each position-density scenario were compared with supplementary pollination treatment (see Section 2.2.4).

2.2.4 Pollination treatment

We selected 10 trees within one central row per each orchard with at least one not sampled tree between them and minimum distance of 27m from orchard edges. This sampling distribution was previously adopted, since it allows for a good representation of treatment effects and it reduces edge effects (Garratt et al., 2014). Before flowering, we selected one branch per tree at 1.2–1.8 m above the ground on the same row side, to avoid influence due to microclimate. During flowering (early April), flowers of the selected branches were treated with supplementary pollination applying compatible pollen to stigmas with a paintbrush. Pollen application was repeated per each flower at least three times, each one separated by 2–3 days. Compatible pollen was collected from apple trees of ‘Red Delicious’ and ‘Granny Smith’ in neighbouring orchards (see Table 1). Treated branches were left open to insect pollination across the whole flowering period. The addition of insect pollination with hand supplementary pollination is considered as a reference of the highest pollination level reachable in apple orchards (Knight et al., 2005). Every treated branch was labelled in order to be identified at fruit setting and at harvest time.

2.2.5 Fruit measurements

Fruit set: when flowering peak was reached, we counted flowers of selected trees on 1–3 branches positioned at 1.2–1.8m above the ground. On 25th July all fruits on these branches were counted and fruit set, as ratio between fruits and flowers, was determined. To prevent overestimation, the count was done after the effect of natural and chemical thinning had occurred. Seed number and seed distribution: when apple fruit diameters averaged 10 mm, one fruit per corymb on each sampled branch was taken to laboratory and frozen (2013 fruits to evaluate position and density effects and 332 to define supplementary pollination level). We recorded minimum and maximum height per apple. Fruits were cut and seeds were counted (Seed number) in each apple reporting presence/absence of seeds for individual carpel. Apples with a continuous sequence of three or more seed-filled carpels were assigned to the category “optimal seed distribution”; otherwise to the category “inefficient seed distribution”, since these categories are linked to symmetry in shape of ripe apples (Sheffield et al., 2014). Therefore, we defined a binary variable “seed distribution” (optimal vs. inefficient). In our experiment, we consider seed set and seed distribution as two proxies of pollination level. Since fruits sampled for seed counting were collected before thinning has occurred, we repeated seed count on 604 fruits that fell down due to thinning. This test was done as preliminary test to evaluate whether seed number and the optimal seed distribution were affected by the chemical thinning treatment; test results suggest that pre/post thinning samples were not different between them (p value of generalized linear model not significant). Fruit shape measurements: during the week before harvest (last week of August), we recorded minimum and maximum values of diameter and height of 7–11 apple per each sampled tree (589 apple in total) using an electronic calibre. Through these measurements, we calculated the fruit shape similarly to Sheffield (2014): 1) mean diameter as average between fruit minimum diameter and maximum diameter, 2) mean height as average between fruit minimum height and maximum height, 3) the ratio mean-diameter/mean-height and 4) the symmetry index as the ratio between maximum fruit height /minimum fruit height. Fruit and branch measurement replicates and their division among explanatory variable levels and orchards are indicated in Appendix S1.

2.2.6 Data analysis

Generalized linear mixed-effects models (GLMM) were used to investigate position and density effects on fruit set, seed number and seed distribution. Poisson error was used to analyse seed number, while binomial error was adopted to test fruit set and seed distribution. Fruit shape measurement were normally distributed with the exception of the symmetry index that was arcsine transformed before analysis (Sokal & Rohlf, 1995). Thus, position and density effects on fruit shape were evaluated by linear mixed effects models. The models included the nested structure branch\tree\row\field as random factor. Fruit set models had the nested structure tree\row\field as random factor. To evaluate density effect and its scale range, continuous densities were used as fixed effect. Each density scale, after scaling, was tested separately avoiding correlation between fixed factors. The model with lowest Akaike’s information criterion (AIC) was considered as the most explanatory one (Symonds & Moussalli, 2011). Position (discrete variable with three levels: A, I and M) was used individually as fixed factor. A variable composed of position/density scenarios and the supplementary pollination treatment was used as fixed factor to evaluate the differences in seed set and seed distribution between scenarios and if any scenario was pollen limited. Per each model that used a discrete variable as fixed effect marginal means were estimated and Bonferroni's post hoc test was performed when the fixed effects were significant. All analyses were carried out in R software (version 3.5.1); leme4 (Bates, Mächler, Bolker, & Walker, 2015) and emmeans (Lenth, Singmann, Love, Buerkner, & Herve, 2018) packages were used respectively for GLMMs and marginal means.

2.3 Results

2.3.1 Density effect

In agreement with our first hypothesis, we found that pollinizer density had a positive effect on seed production and the probability of optimal seed distribution inside a scale range of 10–30m (Table 2).

Tab. 2 Results from GLMMs testing pollinizer density effect at different buffer area scale on seed set and the probability of optimal seed distribution. (-) indicates models that did not converge.

	Seed set n=2013				Seed distribution n=2013			
Buffer (m)	Intercept	Estimates	AIC	*P*	Intercept	Estimates	AIC	*P*
5	1.665	-0.011	9105	0.505	1.772	-0.110	1713	0.2249
10	1.663	0.059	9093	<0.0001	1.781	0.331	1706	0.0052
15	1.667	0.065	9092	<0.0001	1.800	0.389	1703	0.0005
20	1.669	0.073	9088	<0.0001	1.824	0.464	1695	<0.0001
25	1.67	0.063	9093	<0.0001	1.821	0.384	1700	<0.0001
30	1.669	0.049	9098	0.004	1.808	0.281	1706	0.0017
35	1.668	0.036	9102	0.051	1.795	0.184	1711	0.0539
40	1.667	0.025	9104	0.216	-	-	-	-
45	1.665	0.01	9105	0.659	-	-	-	-

Instead, fruit set, mean width, mean height, height/width and symmetry index were not affected (P value>0.05) (Table 3).

Tab. 3 Means of variables that were not significantly affected by position and density (P value>0.05 in GLMMs).

	Fruit set (25 Jul)	Mean width (mm)	Mean height (mm)	height/width	Symmetry index
Replicates	n=191	n=589	n=589	n=589	n=589
Position					
A	13%	64.7	59.4	0.92	0.95
I	11%	64.9	59.9	0.92	0.94
M	13%	65.6	61.2	0.93	0.95
Density					
high	12%	65.2	60.3	0.92	0.95
medium	14%	65.4	60.3	0.92	0.95
low	11%	64.4	59.9	0.93	0.95
Mean	12%	65.1	60.2	0.92	0.95
Range	0-55%	45.7-78.4	36.2-76.4	0.72-1.08	0.76-0.99

seed distribution response variables (lowest AIC=9088 for seed set and AIC=1695 for seed distribution). The effect of density as continuous variable across buffer scales, when significant, was always positive both on seed set (Fig. 3a) and the probability of optimal seed distribution (Fig. 3b).

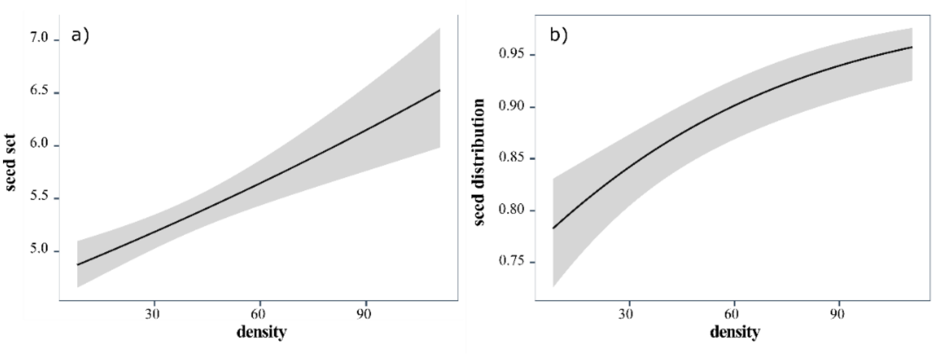


Fig. 3 Density effect on seed set (a) and the probability of optimal seed distribution (b) at 20m buffer scale. Grey area indicates 95 % confidence intervals.

2.3.2 Position effect

Tree position had a significant effect on seed set and the probability of optimal seed distribution (both P value<0.001) (Fig. 4) whereas fruit set, mean width, mean height, height/width and symmetry index were not affected (P value>0.05) (Table 3). Apple trees in A position produced on average significantly more seeds (6.00 seeds) and they had significantly higher probability of optimal seed distribution (0.907) than apple trees in I and M positions (respectively seed set 5.14 and 4.83; probability of optimal seed distribution 0.828 and 0.806). I and M positions did not have a significantly different amount of seed per apple and different probability of optimal seed distribution.

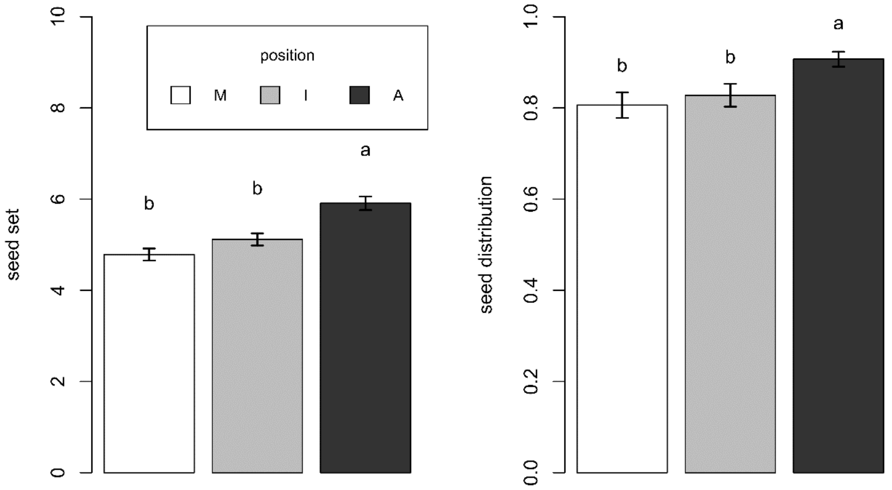


Fig. 4 Seed set a) and the probability of optimal seed distribution b) marginal means and 95 % confidence intervals by post hoc test on GLMM using tree position as fixed

factor. Bars with different letters are significantly different according to Bonferroni's post hoc test.

Density showed a positive trend on seed set within tree position (A, I, M). However, scenarios characterized by same position were not different between them (Fig. 5). Seed set was found significantly different between trees in M position, under low and medium densities (scenarios M_low and M_medium with seed set of 4.4 and 4.6 respectively), and trees in A position under medium and high densities (A_medium=5.9, A_high=6.3). These last two scenarios were also significantly different from I_low scenario (4.6). From seed distribution perspective (Fig. 6), density had a staggered positive trend that never caused different optimal seed distribution probabilities within positions. The highest optimal seed distributions were reached by A_medium and A_high scenarios (0.931 and 0.916 respectively). A_medium had significantly higher probability of optimal seed distribution compared with scenarios M_low, M_medium and I_low (0.741, 0.782 and 0.771 optimal seed distribution respectively), whereas the scenario A_low (0.866) had significantly different optimal seed distribution probability from M_low scenario only.

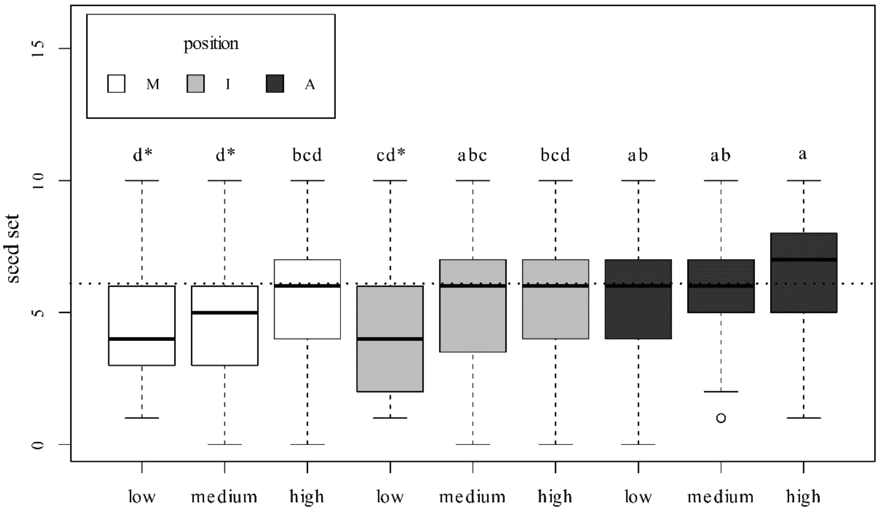


Fig. 5 Box-plot of seed set for nine scenarios (scenarios description in Section 2.3.). Boxes sharing letters are not significant different (Bonferroni's post hoc test). The horizontal dotted line indicates mean seed number obtained with hand supplemented pollination (6.03). (*) Significant differences comparing scenarios with supplemented pollination treatment.

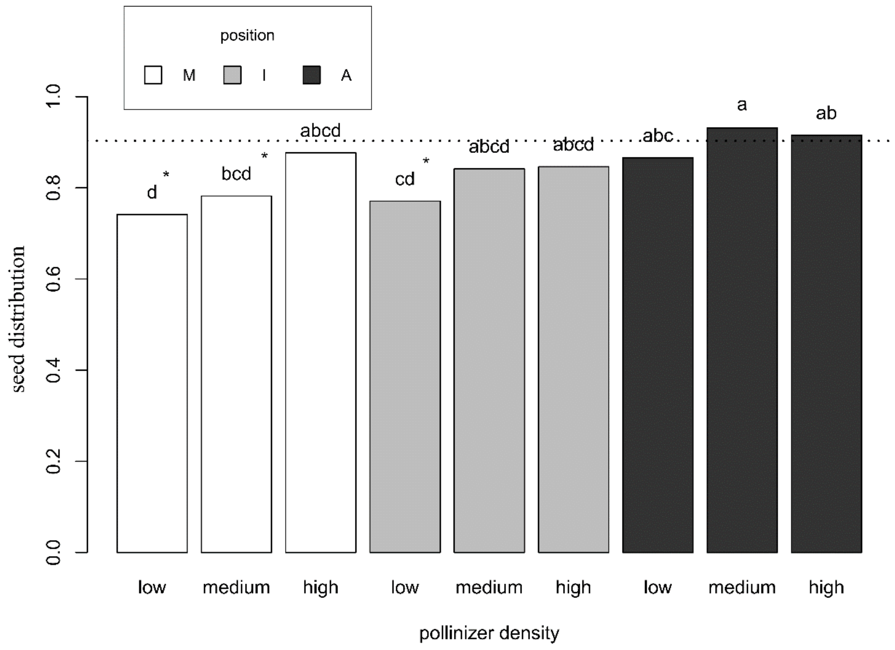


Fig. 6 Occurrence of optimal seed distribution in nine scenarios (scenarios description in Section 2.3.). Bars sharing letters have not significant different probability of optimal seed distribution (Bonferroni's post hoc test). The horizontal dotted line indicates occurrence of optimal seed distribution obtained through hand- supplemented pollination (0.903). (*) Significant differences in probability of optimal seed distribution between scenarios and supplemented pollination treatment.

2.3.3 Pollen limitation

Scenarios M_low, M_medium and I_low were significantly different from supplementary pollination both for seed set and the probability of optimal seed distribution. The rest of scenarios reached same pollination level of supplementary pollination and high-density scenarios never suffered of pollen limitation (Fig. 5 and 6). Trees within high- density interval (Fig. 1b), which should not suffer of pollen limitation, showed a mean ratio pollinizer/trees of 22 % inside 20m buffer areas. Considering all main cultivar trees within experimental orchard, only 25 % reached the 22 % pollinizer/trees ratio (Fig. 7). Moreover, such condition was attained only when there was high availability of pollinizers outside from experimental orchards.

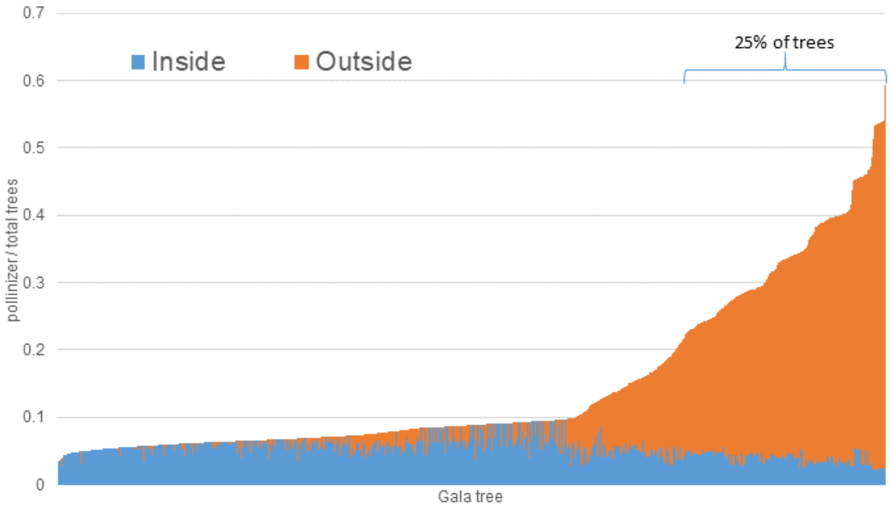


Fig. 7 Pollinizer/total trees ratio per each main cultivar tree placed inside experimental orchard (8240 in total) within 20 m buffer area. Histograms are ordered from the lowest ratio to the highest. Each histogram shows also the proportion of pollinizers that were placed inside/outside experimental orchards.

2.4 Discussion

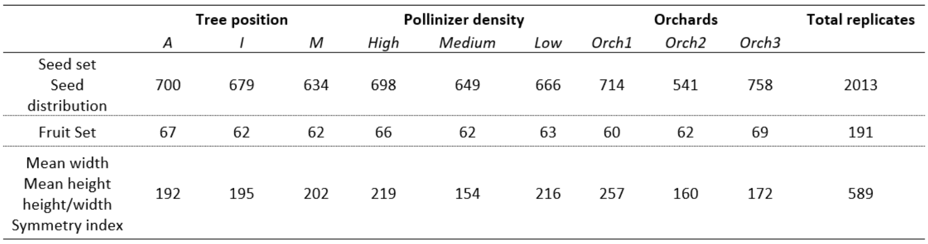
Pollinizer density effect on pollination occurred within 30m from the apple tree. In addition, we found the strongest effect within 20 m. These results are consistent with the ~15m range in which the majority of the pollen is dispersed (Kron, Brian, et al., 2001). The placement of pollinizer plants in apple orchards is a consolidated practice adopted by farmers to ensure pollination and therefore, an adequate production. This practice is coherent with suggestions that have been made by several authors across time (Free, 1970; Kron, Husband, et al., 2001; Sáez et al., 2018), although it is not free of controversies: 1) it lowers the number of producing plants within orchard and 2) it makes harvesting more difficult and expensive (Sáez et al., 2018). To overcome these issues, a common practice in apple farming is to remove pollinizer plants, relying on pollination provided by adjacent apple orchards composed by compatible pollinizers. Our findings on the range of pollinizers effect have strong implications for the orchard design: pollinizers in orchard interior have a fundamental role for pollination success and they should not be removed. However, removal of interior pollinizer up to 20m from orchard edges might be considered when neighbour orchards composed by compatible pollinizers are present. In this assessment, we showed that producing trees had few differences in pollination level because of the focal position they have in orchard row. Contrary to our expectations, in the context of intermixed orchards, a different isolation degree from close pollinizers did not affect seed set and seed distribution of producing plant. Pollination of producing plants with pollinizers in neighbour rows was equal to pollination of apple trees without any close pollinizer. Only apple trees adjacent to pollinizer had higher pollination level. This position was probably favoured by the pollinators mobility and their tendency to fly between adjacent trees in the same row (Cranmer, McCollin, & Ollerton, 2012; Free, 1962; Quinet & Jacquemart, 2017; Vicens & Bosch, 2000). Trees adjacent to pollinizers are only a small fraction of the amount of producing plants, while all the remaining trees are more isolated from pollinizers, suggesting uniformity in pollination level among the majority of apple trees. Therefore, we can assert that intermixed orchard is the most effective one to achieve homogeneous spatial distribution of pollen. Intermixed design has been already suggested for pear (Quinet & Jacquemart, 2017) and apple cultivation, although in apple farming such recommendation was merely based on simulation models and experimental orchards (Matsumoto et al., 2008; Sáez et al., 2018). To our knowledge, this is the first study assessing intermixed orchard performance in intensive farming. In this study, pollination of hand-pollinated trees was compared with pollination reached under different condition of pollinizer density and position. The results indicated that under low pollinizer density, apple trees were pollen limited with the exception of trees adjacent to a pollinizer tree. Whereas, different tree positions did not lead to pollen limitation when pollinizer density was high. These findings suggest that orchard design should target high pollinizer density to prevent pollen limitations and the potentially related production loss. In our experiment, high pollinizer density was mostly localized next to the orchard edges and it was predominantly due to pollinizers belonging to sur- rounding orchards. Therefore, the inner orchard pollinizer/main cv. ratio seen in this experiment was not sufficient to reach high pollinizer density and to avert pollen limitation. A potential solution might be suggested by our results, adopting a ratio pollinizer/main cv. trees of 1:4 in orchard interior. Nevertheless, the suggested ratio would increase harvesting complications associated with intermixed orchard, thus farmers should find the best compromise between pollination needs and management practices (Quinet & Jacquemart, 2017). Apple quality parameters and fruit set of Gala cv. were not affected by pollinizer density and apple tree position as was found for seed set and seed distribution. Pollination efficiency has been habitually measured using the seed set; in addition this measure is positively correlated with the size and the weight of different apple cv. included Gala (Buccheri & Di Vaio, 2005; Garratt et al., 2014; Matsumoto, Soejima, & Maejima, 2012). Seed distribution has been adopted as complementary pollination efficiency measure since it is related to apple fruit shape (Dražeta et al., 2015; Sheffield et al., 2014). Although, the positive relationship between pollination efficiency and apple quality seems not supported by our results. This contradiction might be partially explained by the narrow differences in pollination efficiency that were found across the pollinizer density range and between apple tree positions. Despite we identified discrepancies in pollination levels due to orchard design, these were probably balanced by manifold agronomic factors that were overlooked in these trials.

2.5 Conclusions

Our study evidenced that pollinizer density and tree position of trees from pollinizer should be considered to avoid pollen limitation. These results highlight that managing pollination through orchard design is an effective option to face the issue of growing pollination service demands. In order to maximize pollination, new orchard design should not only consider intermixed orchard planting, but also compatible cultivars and pollinizers placed in surrounding orchards. Shaping pollination service on the basis of orchard improvements is allied to ecological intensification strategies. In this study we did not focus on pollinator communities, environmental and farm management factors, that commonly affect apple fruit quality. Future researches on the interaction between pollination and other crucial factors should be undertaken aiming to develop production stability.

2.6 Supplementary materials

Tab S1 – Replicates of fruit measurements



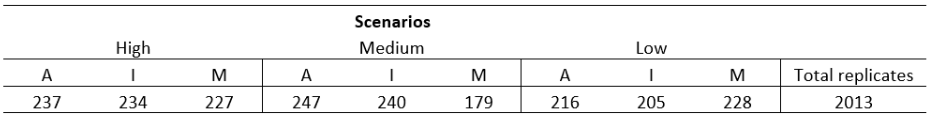
	Tree position			**Pollinizer density**			**Orchards**			**Total replicates**
	A	*I*	*M*	*High*	*Medium*	*Low*	*Orch1*	*Orch2*	*Orch3*	
Seed set Seed distribution	700	679	634	698	649	666	714	541	758	2013
Fruit Set	67	62	62	66	62	63	60	62	69	191
Mean width Mean height height/width Symmetry index	192	195	202	219	154	216	257	160	172	589

Tab S2 – Replicates of seed set and seed distribution measurement in apples developed from supplementary pollinated flowers

Orchards

	Orch1	Orch2	Orch3	Total replicates
Seed set Seed distribution	124	107	101	332

Tab S3 – Replicates of seed set and seed distribution measurement in apples divided by scenario



Scenarios									
High			Medium			Low			
A	I	M	A	I	M	A	I	M	Total replicates
237	234	227	247	240	179	216	205	228	2013

3. CHAPTER 2

Influence of seed set on apple symmetry assessed by image analysis: an overlooked effect of pollination on fruit shape

Luca Carisio*, Sara Straffon Díaz, Aulo Manino, Marco Porporato

Preprint, 273: doi: 10.31220/agriRxiv.2021.00060

*Corresponding author

ABSTRACT: The role of pollination ecosystem service on crop yield and some quality parameters have been largely studied. Nevertheless, the effect of pollination on fruit appearance is still a poorly explored field and it has been assessed by means of oversimplified measurements. Here, we developed an image analysis tool to test the hypothesis that pollination greatly affects the symmetry of one of the most consumed fruits worldwide: the apple. The hypothesis was tested for three commercial classes separately, to understand whether the pollination effect was consistent among classes. We also compared the performance of the image analysis tool with a simplified symmetry measurement that has been previously used. Finally, the effect of pollination on apple symmetry was compared with the effect on weight. We quantified the amount of non-symmetrical area of harvested Gala cultivar apples using two-dimension images of fruits sectioned along the longitudinal plane. We showed that increasing pollination led to significant increase of apple symmetry. The image analysis tool resulted more precise to assess the pollination effect on fruit symmetry than the previously used symmetry measurement, leading to the conclusion that pollination effect on apple shape has been probably overlooked because of the use of oversimplified symmetry measures. The effect of pollination on apple symmetry was not consistent among commercial classes since it resulted significant for little and medium size apples but unimportant for large size apples. Differently from the symmetry, apple weight appeared as not influenced by the pollination. Our findings suggest that the effect of pollination on fruit production should be assessed taking into account different variables and different commercial classes of apple.

Keywords: Fruit growth, *Malus domestica*, Gala, fruit quality, fruit shape, axi-symmetric fruit

Abbreviations: ES: ecosystem services, SDR: Symmetry difference ratio

3.1 Introduction

Intensive agriculture has reached high crop production by suppling a heavy amount of inputs (e.g. fertilizers, water, pesticides) (Pretty, 2008; Tilman, Cassman, Matson, Naylor, & Polasky, 2002). However, the challenge of increasing yields to satisfy growing food demand, should be matched with the urgent need to minimize the impact on the environment and on human health due to the excessive use of such agricultural inputs (Godfray et al., 2010; Pretty, 2008). This challenge can be addressed by an “ecological intensification”, that means maintaining and integrating ecosystem services (ES) (e.g. soil fertility, water retention, pollination, etc.) to support crop production (Bommarco et al., 2013). ES can replace or even boost benefits from agricultural inputs (Blaauw & Isaacs, 2014; Davis, Hill, Chase, Johanns, & Liebman, 2012). To promote more sustainable agricultural practices, broadening knowledge on ES effects on production is needed. This issue is more effectively addressed by means of commercial variables (e.g. fruit grading, colour, quality etc.) that are straightforward and thus they are capable to convince farmers of ES benefits (Kleijn et al., 2019). Focusing on the horticultural sector, one important parameter is the fruit appearance. Fruit size, shape and colour are features that influence consumer choice (Djekic, Radivojevic, & Milivojevic, 2019; Loebnitz

& Grunert, 2018; Wendin et al., 2019), and are increasingly taken into account by farmers when they plan agricultural management. Indeed, a good fruit appearance may reduce the amount of fruits excluded from sale and thus, food waste.

Among ES, pollination supports the production in about 75% of most important crops (Garibaldi et al., 2013; Klein et al., 2007). On the other hand, pollen limitation has been linked to yield loss and quality reduction of different crops (Bommarco et al., 2012; Garratt et al., 2014; Klatt et al., 2013; Tamburini, Berti, Morari, & Marini, 2016). Worldwide, the expansion of pollination-dependent crops has accelerated (Aizen et al., 2019); simultaneously, the overall amount of pollination ES has dropped because of the decline of pollinators, particularly the bees (Herrera, 2020; Potts et al., 2010; Zattara & Aizen, 2021). This mismatch between pollination demands and pollination ES provisions has raised general concern on effects on global food production (Potts, Imperatriz-Fonseca, et al., 2016; Vanbergen & Initiative, 2013). However, the relative importance of pollination ES in ensuring yield and quality of pollinator-dependent crops is not ubiquitous, but it depends on crop and cultivar traits (Garibaldi et al., 2013; Geslin et al., 2017) and on the interaction between pollination and other agronomic and abiotic factors that contribute to modify plant physiology (Tamburini et al., 2019).

As pollination ES is among the critical factors regulating fruit grown, it affects also fruit appearance and its marketability. Traditionally, pollination effect on fruit appearance has been evaluated as fruit size that is usually strongly positively correlated with fruit weight (e.g. Garratt et al., 2014 for apple; Ryder et al., 2020 for cherry; Quinet and Jacquemart., 2017 for pear and Sáez et al., 2019 for kiwifruit). Nevertheless, few studies considered fruit shape and malformations as pollination-dependent parameters (e.g. Andersson et al., 2012 for strawberry; Xiao et al., 2009 for tomato and Sheffield, 2014 for apple).

Apple is a pollination-dependent crop cultivated worldwide, and it is one of the four most-consumed fruits (Musacchi & Serra, 2018). It is also widely used as a model in studies focused on fruit development (Eccher, Ferrero, Populin, Colombo, & Botton, 2014). Apple seed fertilisation through cross-pollination is needed for fruit development, as parthenocarpy and self-fertilization are unusual (Galimba, Bullock, Dardick, Liu, & Callahan, 2019; Musacchi & Serra, 2018). The number of fertilized seeds per apple is a measure of pollination ES extent. Fertilized seeds are producers of phytohormones involved in fruit abscission (Ferrero et al., 2015), and they are also associated with fruit growth. Apple fruit growth is a complex process in which many endogenous and exogenous factors are involved (Musacchi & Serra, 2018). Despite this complexity, several studies pointed out a positive effect of pollination on apple weight (Buccheri & Di Vaio, 2005; Webber et al., 2020) and on apple appearance (Buccheri & Di Vaio, 2005; Garratt et al., 2014). The majority of the studies focusing on the pollination effect on apple appearance have explored the differences between deformed and regular fruits (Buccheri & Di Vaio, 2005; Elsysy, Serra, Schwallier, Musacchi, & Einhorn, 2019; Herrmann, Beye, de la Broise, Hartlep, & Diekötter, 2019; Matsumoto et al., 2012; Wu et al., 2021), whereas quantitative measures to assess the regularity of fruit shapes have been rarely adopted (Carisio, Díaz, Ponso, Manino, & Porporato, 2020; Cory S Sheffield et al., 2014). In these last cases, fruit shape measurements were oversimplified (e.g. ratio between maximum and minimum fruit heights), thus they may be not capable to account for overall irregularities of fruit shape. In addition, the pollination effect, either on apple weight or on apple appearance, has been seldom analysed among commercial classes (but see Webber et al., 2020). From farmer’s perspective, it is pivotal to understand if pollination may be a relevant factor to obtain apples fitting the attributes of the most valuable commercial class.

A more precise tool to analyse fruit shape is image analysis. Image analysis have been developed to measure agricultural products (Sabliov, Boldor, Keener, & Farkas, 2002). Focusing on fruits, it has been adopted to measure volume and estimate mass (Vivek Venkatesh, Iqbal, Gopal, & Ganesan, 2015), to characterize the cultivars of different crops (e.g. common bean (Kara, Sayinci, Elkoca, Öztürk, & Özmen, 2013), cherry (Beyer, Hahn, Peschel, Harz, & Knoche, 2002),) including apple (Currie et al., 2000; Malladi, Battapady, Hampton, & Jing, 2020) and also to investigate apple growth process (Jing & Malladi, 2020). However, to our knowledge, image analysis has never been used to explore the pollination effect on apple appearance. Moreover, most of the analysis on fruit image have been developed by the use of sophisticated equipment or implementing not-open-access software code. These factors can be considered serious obstacles to reproducing results and replicating experiments in agricultural science.

In this study, we implemented an image analysis tool using R language and R libraries by means of scanner pictures, with the aim of exploring the effect of pollination, intended as number of seeds per apple, on apple symmetry. Notably, we tested for the following hypothesis:

H1) Pollination has a positive effect on fruit symmetry, thus we expect that the number of seeds increases with fruit symmetry, and we tested the hypothesis between different commercial classes to understand whether the pollination effect is consistent among classes.

H2) The image analysis is more precise to assess pollination effect on symmetry than biometric measurements feasible in field data recording and we tested this hypothesis comparing our symmetry measurement with a Symmetry index used by (Sheffield, 2014) which is based on calliper measurements.

H3) The strength of pollination effect on symmetry is different from that on fruit weight, thus we expect that the pollination effect on symmetry is stronger and more evident than that on weight.

3.2 Materials and methods

3.2.1 *Pollination and fruit samples*

We used apples of Gala cultivar which has been one of the most adopted in apple pollination studies (Carisio et al., 2020; Elsysy et al., 2019; Garratt et al., 2014; Webber et al., 2020). The number of seed per fruit, namely the seed set, has been largely used as proxy of pollination efficiency. Accordingly, from now on, we will refer to a higher or lower pollination as equal to a greater or lower number of seed per apple.

We used for the analysis apples of three commercial classes: 104 apples of waste class (diameter < 65 mm, low colour intensity), this class is destined to juice industry, 133 of medium class (70 mm < diameter < 75 mm, good colour intensity), and 88 of premium class (85 mm < diameter < 90 mm, good colour intensity). The three commercial classes have respectively the lowest, the intermediate and highest price in the Italian market. Fruits were provided in November 2020 by a large-scale apple distribution industry and stored in a refrigerated room until analysis, which was carried out in the last two weeks of November. Before performing the fruit image analysis, all apples were weighted.

3.2.2 Fruit image and photo processing

Apples were sectioned along the longitudinal plane. The vertical plane incision passed through the sepal-end cavity and the pedicel-joint (Fig. 1a). We counted total amount of seeds per apple (between 0 and 10 in Gala cultivar). Each apple sections was placed, separately, on an HP 4520 printer and scanned. The image was saved in jpg format with 300 dpi resolution. After preliminary analysis, it was verified inconsistency between pixel counts of the same apple section (see 2.3 for the details on measurements), due to section position over the scanner surface. To adjust scanner measurements, we used a calibrated blue square of known area that was pinned under the apple cut surface before scansion (Fig. 1a). In this way, pixel counts of the same apple section became irrelevant, regardless its position on the scanner. Extraneous objects present in scansion, such as dust, were removed by the function “subtract background” of ImageJ (1.53e). Then per each image, we added two green dots, one over the pedicel-joint and the second on the sepal-end (Fig. 1a). These were aimed to adjust apple rotation and to identify apple central axis in the image analyses process. A mismatched fruit orientation would affect measurement of apple symmetry. After obtaining two images per apple, we performed apple image analysis by means of R language code (Carisio, Straffon Díaz, Manino, & Porporato, 2021)(see Carisio, 2021 for analysis code). The analysis was executed in R 4.0.3, using the packages OpenImageR (Mouselimis, Machine, Johanne, Mohammad, & Achanta, 2020) for the photo editing (crop and rotate) and countcolor (Weller, 2019) to isolate the pixels of the scanned objects (apple and the reference square). The image analysis steps are graphically summarized in Fig 1 as follows: 1) photo was rotated to have the y-axis passing across the green dots. 2) Pixels belonging to apple image and blue square were selected through the identification of specific RGB colour frequencies ranges. (Fig 1b). All selected pixels were counted. The measure of apple surface in cm^2 was calculated by the formula:

$$S = (k * Pa + Ps) / Ps$$

Where k is the known area of the attached square (4 cm^2), Pa and Ps are the number of pixels that belong to apple and to the square surfaces respectively. 3) We identified apple central axis as the line between the green dots (Fig 1a). The photo was cropped on the apple central axis and the right image cut was flipped in order to have the two photos with the same orientation on x and y axes (Fig 1c) 4) Apple pixels were transformed in data points (a pixel for each data point) of x and y coordinates for both images and stored in two different databases (Fig 1d). The height of each of apple halves was measured by the difference of y axis values between the highest and the lowest point of the database. This procedure simulates the measure of apple heights by means of a calliper 5) We contrasted the two databases of right and left halves and we isolated all points which were unique for one of the databases (Fig 1e). These selected points correspond to the no-overlapping area between the left and the right apple halves. The amount of no-overlapping points were counted and transformed in a cm^2 measure by the formula:

$$OV = (k * Po) / Ps$$

Where k is the known-measure of the attached square, Po and Ps are the number of pixels of the no-overlapping area and of the square surfaces respectively.

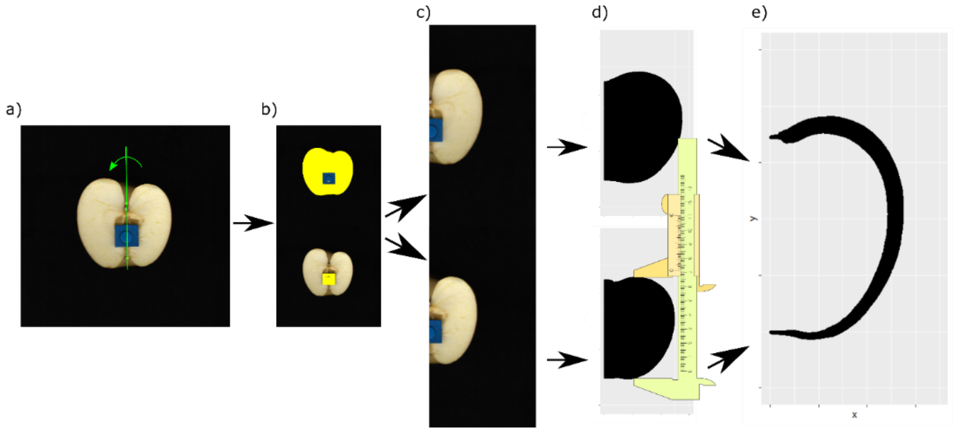


Fig 1. Photo analysis process: a) rotation of apple; b) identified pixel (yellow colour) of the apple and of the square respectively; c) apple photo cropping and flipping to same axis orientation; d) transformation of pixels to data points and measurement of apple height simulating calliper measurement; e) isolation of no-overlapping area between the apple halves.

3.2.3 Variables and Statistical analysis

To assess the effect of the seed set on symmetry and the difference in symmetry between apple classes we considered three variables: the symmetry index according to Sheffield (2014), the no-overlapping area and the symmetry difference ratio (SDR). The Sheffield's symmetry index is calculated as the ratio between minimum and maximum apple height. This index has been proved as the most correlated to seed number and it is easy to measure in field experiments since is based on calliper measurements (Sheffield, 2014). The second variable is the measure in cm^2 of the no-overlapping area between the left and the right apple halves; the third variable, SDR, is calculated as the percentage ratio between the no-overlapping area and the total scanned apple surface. A 0% SDR indicates a perfect symmetry and correspondence between apple halves, otherwise, a value of 100% means total miss-match between halves. This last variable was adopted in order to normalise the measure of symmetry between apple classes despite their differences in volume. Since we measured the three variables for both sections of each apple, we used for the analysis the averaged measures between the two observations. This allows to reduce the errors due to apple cut impreciseness. To test the effect of pollination on symmetry and if the effect is different between apple commercial classes, we used generalized mixed models (glm) with Gaussian distribution and logarithmic link function. In these, SDR was used as explanatory variable and seed set as fixed effect. At first, model was implemented using all data, and then we implemented a model per each apple class. To test the differences in SDR, no-overlapping area and seed set between apple commercial classes, we adopted a glm with Gaussian distribution and logarithmic link function except for seed set which was analysed using a Poisson distribution. The difference between classes were validated by a Tuckey post-hoc test. The effect of seed set on the symmetry index and the relationship between the symmetry index and SDR were tested by a glm with Gamma distribution and logarithmic link function. The effect of seed set on apple weight was analysed by a linear model per each apple class. The diagnostic of all models was performed checking for patterns in model residuals. All statistical analyses were performed with R 4.0.3 (see Carisio, 2021 for statistical analysis code).

3.3 Results

Overall, the pollination affected apple symmetry as we found a negative correlation between SDR and seed set (Tab1 a, Fig. 4a). However, when we distinguished the effect by each apple class, we found that seed set had a significant effect on the waste and the medium classes, whereas it was not influential in premium class (Fig 2, Tab1.). The pollination effect was more evident in the waste class in which the increase of the number of seeds caused an estimated reduction of SDR from 18.50 % to 4.88 % and less pronounced for medium class, as SDR decrease ranged from 9.32 % to 4.88 %.

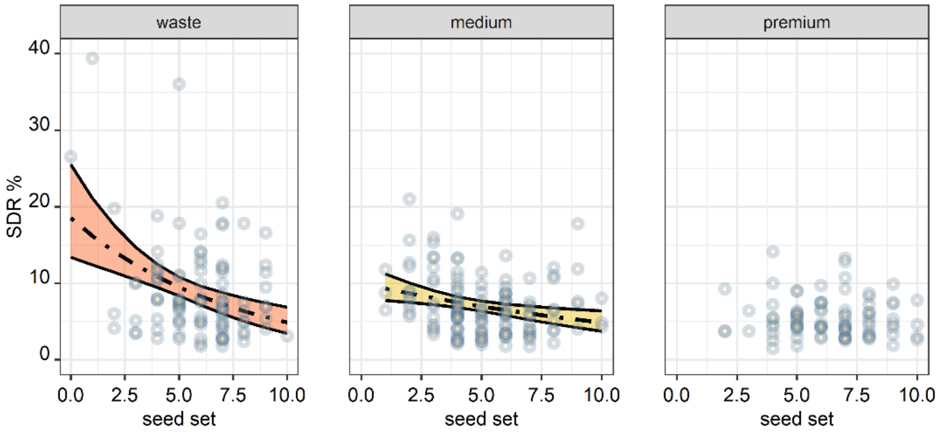


Fig 2. Scatterplot between seed set and SDR in waste, medium and premium apple classes. For significant relationships (waste and medium), the marginal effect was added. Coloured areas indicate 95 % confidence intervals.

Tab1. Generalized mixed model outputs for model with all data a), and models with only Premium b), Medium c) and Waste d) apple class data.

	Estimate	Std. Error	t value	*P*	R^2
a) Overall data					0.700
Intercept	2.4166	0.093	25.88	<0.0001	
seed set	-0.0818	0.017	-4.72	<0.0001	
b) Premium					0.015
Intercept	1.770	0.174	10.15	<0.0001	
seed set	-0.012	0.026	-0.43	0.664	
c) Medium					0.578
Intercept	2.305	0.115	20.06	<0.0001	
seed set	-0.072	0.023	-3.06	0.0027	
d) Waste					0.986

Intercept	2.918	0.164	17.74	<0.0001
seed set	-0.133	0.031	-4.25	<0.0001

The SDR showed significant differences between apple classes (Fig. 3a): waste apple class had the greatest SDR (mean ± sd = 8.89 ± 6.41 %) followed by the medium class (mean ± sd = 7.02 ± 3.78 %) and by the premium class (mean ± sd = 5.46 ± 2.59 %). The no-overlapping area was similar between the three classes (respectively mean ± sd = 2.89 ± 1.40 cm2, 2.39 ± 1.27 cm2, 2.41 ± 1.75 cm2), and only the difference between medium and premium class was slightly significant (Fig. 3b). Seed set was higher for premium class apples (mean ± sd = 6.35 ± 1.91) and it was significantly different (Fig. 3c) from seed set of medium class apples (mean ± sd = 5.06 ± 2.05). Waste class apples had greater seed set (mean ± sd = 5.88 ± 1.92) than the medium class, but did not differ from the premium class.

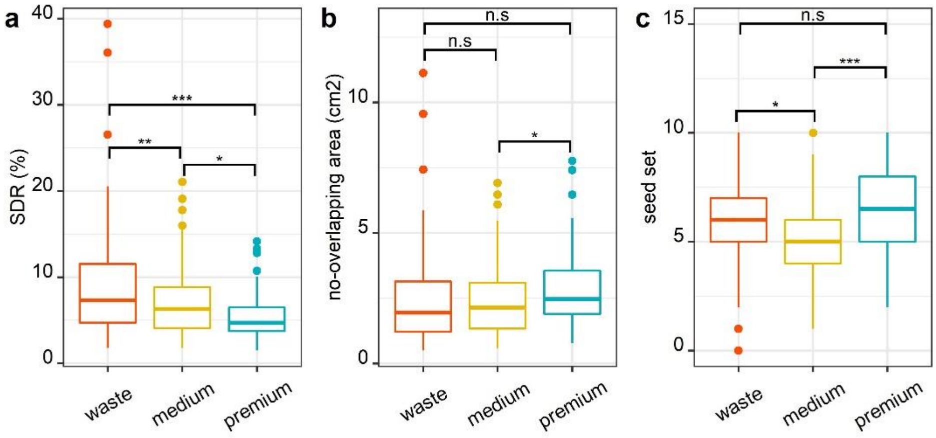


Fig 3. Differences between commercial classes for the variables: a) SDR, b) no overlapping area and c) the total amount of seeds per apple.

The symmetry index was significantly affected by the seed set (β = 0.007 ± 0.001, p value < 0.0001, Fig. 4b). The relationship between the symmetry index and SDR was strong ($\beta = 0.012 \pm 0.000$, p value < 0.0001, $R^2 = 0.899$ Fig 4c). However, the model that adopted SDR as symmetry measure was more explanatory than the model that used the symmetry index ($R^2 = 0 .700$ for SDR and $R^2 = 0 .053$ for symmetry index).

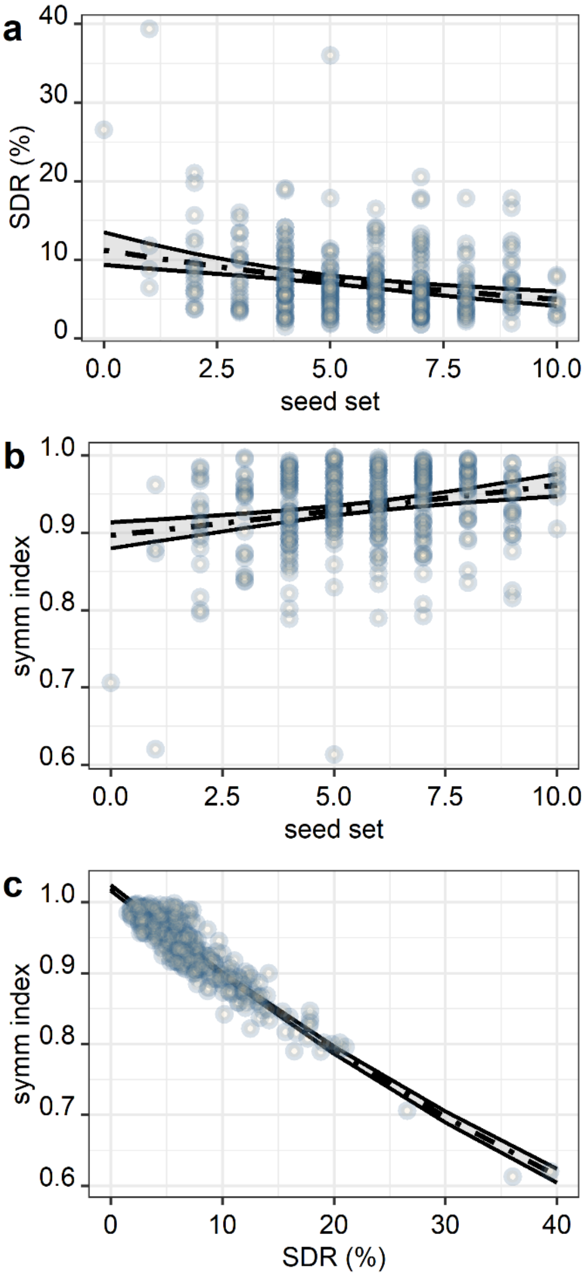


Fig4. Marginal effect of seed set a) over SDR and b) over the symmetry index using all data. c) Relationship between the symmetry index and SDR.

Apple weight was not significantly influenced by seed set for all the three apple classes (Tab. S2, Fig S1).

3.4 Discussion

Image analysis has been employed for measurement of many agricultural products (Sabliov et al., 2002; Vivek Venkatesh et al., 2015). This study constitutes the first attempt to assess the effect of pollination ecosystem services in fruit symmetry by means of an image analysis tool. This tool was implemented aiming to provide an open access software code that allows to potentially reproduce the symmetry analysis on a wide range of axi-symmetric fruits. The advancement of a low-impact farming trough ecological intensification needs a better understanding of the positive effects of ecosystem services on agricultural production. Our study demonstrated a strong effect of pollination on fruit symmetry. In particular, we found that an increasing number of fertilized seeds led to significant increase of apple symmetry. When seed number was maximum, apples had in average 5% of asymmetrical scanned surface, whereas under low pollination, the non-symmetrical area was about the double. This result aligns with previous studies that have identified a positive effect of seed number and seed distribution in apple symmetry for ‘McIntosh’, ‘Cortland’ (Sheffield, 2014) and ‘Fuji’ (Wu et al., 2021), although the effects detected by the two studies were slight. In addition, our result differs from a previous report of lack of pollination effect on ‘Gala’ symmetry (Carisio et al., 2020). Due to either low or null effect, these existing studies suggest that pollination affects fruit symmetry minimally. In contrast, our study proved a strong effect of seed set on ‘Gala’ apples symmetry, leading to the conclusion that the role of pollination on defining apple shape has been probably overlooked. The concept of an overlooked effect of pollination is supported by the comparison between our image analysis measurement and the ratio between minimum and maxim apple height (symmetry index), which has been employed as symmetry measurement on previous studies (Carisio et al., 2020; Sheffield, 2014). In particular, we found that the correlation between the image analysis measurement with seed set was greater than that between the symmetry index and the seed set. This result indicates that the symmetry index is inaccurate to assess pollination effect on fruit symmetry. Although it has not been tested, it is likely a lack of reliability for further apple symmetry measurements, as for example the subjective distinction between deformed and regular apples that have been employed in several studies on apple pollination (Matsumoto et al., 2012; Wu et al., 2021). Despite the impreciseness in assessing fruit symmetry, the symmetry index was found lightly correlated with seed set so it could still be a valuable approach for field measurements.

Surprisingly, we found that the positive effect of the seed set on fruit symmetry was not consistent among the three commercial classes employed for the analysis. All except one, the greatest size commercial class (here called premium), had the symmetry influenced by the seed set. Premium apple class showed also an average standardized non-symmetrical area significantly lower than the other two commercial classes. We advance two hypotheses to explain such a lack of effect of seed set on the premium commercial class. The first one is that premium class apples have been not influenced because they had an extraordinarily low rate of apple with few seeds. Premium apples showed in average a greater number of seed than medium size apple; in addition, 94% of premium apples had more than three seeds. Therefore, in premium class, low seed set apples were rare, as well as in non-symmetric apples. The second hypothesis is that the premium class had a growing process less influenced by pollination. Pollination benefits on yield and on fruit growth may be additive, synergic, or replaced by the effect of other fruit growth factors (Tamburini et al., 2019). In the case of premium apple, it is likely that a trade-off between pollination benefits and benefits due to other growth factors has occurred, and therefore pollination resulted as not influential. For example, the flower position as growing factor may neutralize pollination effect: central fruitlet within the 4:6-fruitlets corymb cluster, has shown to dominate the lateral fruitlets in resources intake, regardless the number of fertilized seeds (Bangerth, 2000; Jakopic et al., 2015). Furthermore, another aspect of apple growth process might have been influential. After seeds fertilization, apple developing is exponential with intensive cells production (Bain & Robertson, 1951; Jing & Malladi, 2020). Then, the growth slows down and most of the apple development is due to cell expansion (Malladi & Johnson, 2011). However, the cell expansion can reach a plateau since it continues until space between cells is still available. As it has been observed by Bain and Robertson (1954), larger apples differing each other in volume, had the same space between cells. This means that in case of larger size fruit, such as the premium class, a cell packing threshold is reached. Therefore, we speculate that premium apples, that were non-symmetrical in the first step of growth process, became symmetrical during cell expansion because of a compensation process: higher cell expansion in apple parts where cell-packing was not reached.

In our study, we found that the average amount of the non-symmetrical surface between commercial classes was either not different or lightly contrasting, although differences between classes were significant when the symmetry measurement was normalized with overall apple scanned surface. The lowest-size apples had the highest average amount of normalized non-symmetric surface. Furthermore, this commercial class had fruit symmetry most affected by seed set. For lowest-size class, a symmetric shape is less important from a commercial standpoint, since it is destined to juice industry.

We are aware that observed symmetry differences between apple commercial classes might be the result of a sampling bias and that our data do not allow to understand which were the biological processes that led to the symmetry differences. However, our results revealed potential differences in pollination effect on fruit growth process between different sizes of apples. In addition, they suggest that pollination is important for having symmetric low-medium size apples, whereas it is not fundamental regarding larger size apples. We believe that an approach distinguishing between commercial classes is pivotal to provide clear indications to farmers on the benefits of pollination ecosystem service.

In our study, we did not find an effect of seed set on apple weight. A positive effect of pollination on apple weight was found in several studies, some of which focused on ‘Gala’ (Buccheri & Di Vaio, 2005; Sheffield, 2014; Webber et al., 2020), whereas other did not find such a positive effect (Carisio et al., 2020; Wu et al., 2021). This confirms that seed set has and indirect link with apple weight. Indeed, seed set affects the crop load and thus growth resource distribution by regulating the fruitlet abscission, which usually targets fruitlets that were poorly pollinated (Ferree & Warrington, 2003). In addition, many low seed number apples are likely of being removed through the season by hand thinning because they should be more subjected to poor development (Garratt et al., 2014). As a result, the weight of harvested apples appears as marginally affected by seed set because most of low seed number apples were previously discarded. This leads to a pollination effect on apple weight that it is usually, as in our study, not detectable. This result underlines that a communication to farmer on pollination benefit only focused on weight gain could be misleading, since the effect on harvested fruits is not commonly evident. We suggest including in to-farmer communications also other variables such as quality, grading and shape fruit parameters which contribute in determining crop production value (Fijen, Scheper, Vogel, van Ruijven, & Kleijn, 2020). Although apple commercial classes in Europe do not take into account fruit shape and symmetry, in grading process deformed fruits are usually excluded from market (Musacchi & Serra, 2018). Our results highlight that pollination is a critical pre-harvest factor in fruit development and it should be properly managed to reduce production losses.

3.5 Conclusions

The challenge to maintain/increase fruit production, and at the same time to reduce agricultural negative impacts, requires an extensive understanding of the benefits given by ecosystem services. The benefits of pollination ecosystem service to yield and quality of different fruits have been well studied. However, focusing on the quality parameter of apple symmetry, the effect of pollination has been tested by simple measurement or visual assessments. Here, we showed through a precise and simple image analysis approach that pollination greatly affects the symmetry of Gala apples and that the effect was not consistent across different commercial classes. The image analysis tool developed as an open-access resource within this study could be applied on a wide range of axi-symmetric fruits testing different hypotheses. The biological reason that has led to an effect of pollination on apple symmetry that changed according to commercial classes should be investigated in future researches. Moreover, we encourage the research community to evaluate ecosystem service benefits taking into account a broad number of fruit quality variables and distinguishing the effects between different commercial classes. This approach could allow a more practical understanding of ecosystem service that it may be more effectively transferred to farmers.

3.6 SUPPLEMENTARY MATERIALS

Tab. 2 Linear model outputs testing the relationship between weight and seed set

	Weight models			
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
a) Premium				
Intercept	280.519	5.632	50.82	>0.0001
seed set	-0.536	0.828	-0.65	0.53
c) Medium				
Intercept	155.291	1.130	137.49	>0.0001
seed set	0.110	0.207	0.53	0.596
d) Waste				
Intercept	117.585	0.939	125.20	>0.0001
seed set	-0.060	0.152	-0.39	0.696

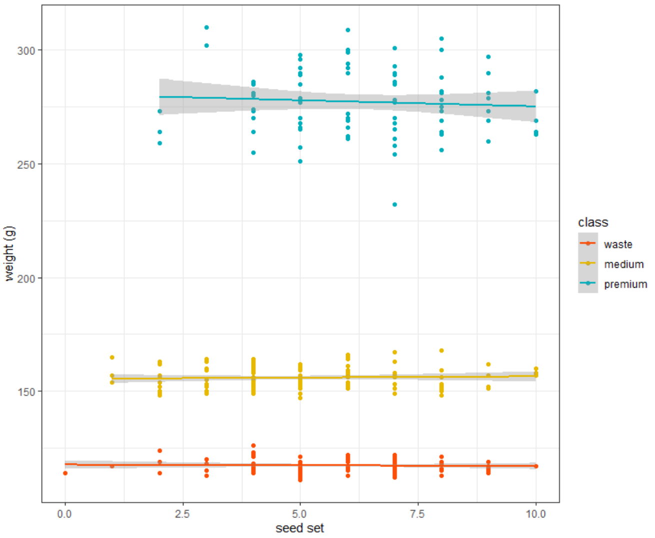


Fig. 5 Effect of seed set on apple weight per each apple commercial class

4. CHAPTER 3

The interaction of nectar dynamic and insect visitation affects the accurate estimation of nectar resources

Luca Carisio*, Lucie Schurr, Véronique Masotti, Marco Porporato,

Gabriel Nève, Laurence Affre, Sophie Gachet & Benoît Geslin

Submitted to Functional Ecology

*Corresponding author

ABSTRACT

1. Nectar is a key resource for many insects. Despite its importance, the production of nectar by flowers has mainly been assessed with one sampling method, in which nectar is replenished over a 24 h period. However, detailed knowledge of nectar dynamics is limited, especially in cultivated plants. Nectar production can be driven by insect probing and visitation rate. This means that exploring the interaction between nectar dynamics and insect visits could be particularly important for estimating insect nectar resources.
2. We measured the nectar production dynamics of lavender (*Lavandula hybrida*) and fennel (*Foeniculum vulgare*) and evaluated the insect visitation rate of these crops. We developed a simulation model to identify how different scenarios of visitation rates (average and maximum) and insect strategies for flower selection (random selection or rewarding flower selection) may change the standing nectar and daily nectar production of the two crops. We then tested whether simulated

estimates of daily nectar production differed from the commonly used 24 h nectar production measurements.

3. Lavender and fennel had equal nectar volume replenishment over 24 h, but lavender replenished its nectar quicker than fennel. Flower preferences of visiting insects and the standing nectar indicated high rates of fennel nectar exploitation, likely because fennel also provides pollen and its flowers are easy to probe. The simulation models suggested that nectar production is insect-driven, but the effects of insect selection and visitation differed between the two crops. Finally, we found that the measurement of daily nectar production produced by the simulation models was always greater than that of the nectar replenished within 24 h in flowers isolated from pollinators.
4. This study is the first to develop a simulation model to predict daily nectar production. The model demonstrated the complex effects of plant/insect interactions on nectar production. Reliable estimates of nectar resources may be achieved when nectar dynamics and visitation rates are considered. In contrast, estimates based on the measurement of nectar replenished over 24 h may be skewed, especially when flower-visiting insects are abundant.

Key words: mass-flowering crops, floral resources, pollen, flower traits, mass-flowering crops, nectar rewards, insect foraging, plant–insect interactions

4.1 Introduction

Pollinator richness and abundance are directly linked to the diversity, quality, and quantity of plant resources, specifically pollen and nectar (Donkersley et al., 2014; Goulson et al., 2015; Roulston & Goodell, 2011). Therefore, it is vital to develop pollinator conservation strategies that use extensive knowledge of how pollinator floral choices are driven by floral resources, and specifically which natural and cultivated areas provide substantial food resources (Aronne et al., 2012; Baude et al., 2016; Quinlan et al., 2021). Several studies have addressed the complex issue of food resources by estimating the quantity of nectar produced in different environments, along with historical or seasonal variations (Baude et al., 2016; Guezen & Forrest, 2021; Hicks et al., 2016; Timberlake et al., 2019). These estimates used nectar produced over 24 h by flowers that were isolated from flower-visiting insects by a mesh bag as a proxy for plant nectar production performance. This assumes that 1) the amount and frequency of nectar removal by flower-visiting insects should not affect the total nectar produced daily and 2) there are no physiological or physical mechanisms that might slow down or accelerate nectar production, or lead to nectar re-absorption. Hence, these two assumptions are only valid if flowers present a linear nectar production dynamic (defined in supplementary materials). However, linear nectar dynamics are unlikely. Studies on nectar production of some pant species suggest a non-linear dynamic as, for some plant species, when the flowers were visited by foraging animals several times, the nectar produced was either more or less than flowers visited a single time (Biella et al., 2021; Castellanos et al., 2002; Luo et al., 2014; Ordano & Ornelas, 2004; Ornelas & Lara, 2009; Stahl et al., 2012; Ye et al., 2017). Moreover, some insect-pollinated plants have been found to fully replenish their nectar within a few hours before interrupting this replenishment (Castellanos et al., 2002; Luo et al., 2014). Other plant species have also displayed nectar reabsorption (Burquez & Corbet, 1991; Pacini & Nepi, 2007; Parachnowitsch et al., 2019). Despite the importance of nectar dynamics in estimating flower-visiting insect resources, studies have focused mostly on wild angiosperms, whereas, few examples exist for cultivated plants (e.g., *Brassica napus* and *Borrago officinalis*) (Burquez & Corbet, 1991; Chabert et al., 2018).

To acknowledge the effect of insect visitation on daily nectar production, Castellanos et al. (2002) asked, “How much nectar might have been produced if the flowers had been emptied at a realistic rate?”. Answering such a question is fundamental in determining the number of flower-visiting insects that can rely on nectar resources provided by a given environment. Specifically, this question is relevant in agricultural lands where the density of managed insects, such as *Apis mellifera*, may be extremely high; therefore, a high visitation rate may strongly affect daily nectar production and standing nectar stock (Corbet et al., 2001; Geslin, Aizen, et al., 2017; Sáez et al., 2017; Torné-Noguera et al., 2016; Wignall et al., 2020). Hives are often installed near crops, either for pollination services or to produce a specific type of honey. In some cases, the overabundance of *A. mellifera* can lead to either intraspecific or interspecific exploitative competition owing to resource shortages (Angelella et al., 2021; Balfour et al., 2013; Lindström et al., 2016; Ropars et al., 2019; Torné-Noguera et al., 2016). Thus, accurate estimation of the nectar production of crops and agricultural landscapes is crucial as it can a) help to estimate the maximal load of beehives in agricultural areas, b) design accurate conservation practices for wild insects, and c) avoid potential exploitative competition for flower resources between managed and wild insects (Angelella et al., 2021; Herbertsson et al., 2016; Lindström et al., 2016; Torné-Noguera et al., 2016).

In this study, we first assessed the potential food resources provided to flower-visiting insects of two mass-flowering crops, fennel (*Foeniculum vulgare*) and lavender (*Lavandula hybrida*), by estimating nectar dynamics and pollen production. We also evaluated the visitation rate of insects foraging on these crops and characterized their resource preferences. We then developed a stochastic simulation by means of the measured nectar dynamic and field insect visitation rate in order to understand how plausible scenarios of insect visitation rates and insect strategies for selecting flowers may change the standing nectar and daily nectar production of the two crops. We hypothesized that i) the standing nectar and daily nectar production would be insect-driven, and ii) simulation estimates of daily nectar production would differ from the commonly used 24 h nectar production measurement.

4.2 Materials and methods

4.2.1 Study area

The experiment was carried out in the Mediterranean area of “Plateau de Valensole” (Alpes-de-Haute-Provence, South of France, Figure S1). The area is a mosaic of truffle oak groves, grain crops (durum and soft wheat or barley), and mass-flowering-crops (*Lavandula hybrida*, *Salvia sclarea*, *Foeniculum vulgare*, and *Helichrysum italicum*). These crops are cultivated for essential oils that are used in the pharmaceutical, cosmetic, and beverage industries. Among them, we studied lavender (*L. hybrida)*, which is important for both economic reasons (medicinal, cosmetic, and honey production) and tourism (Provence’s emblematic plant), and fennel (*F. vulgare)*, which is used to flavor aniseed drinks. *Lavandula hybrida*, also called lavandin, is a hybrid of *L. angustifolia* and *L. latifolia*. Like many hybrids, lavender is sterile, does not produce any pollen, and is known to be a good nectar producer (Dussaubat et al., 2021; Escriche et al., 2017). This species measures up to one meter high and produces numerous blue flowers organized in dense spikes. The flower morphology is tubular (7 mm long and 1-2 mm wide) with nectaries located deep at the bottom of the flower. The fennel variety used was ‘Jupiter’ (developed by Pernod-Ricard® company), for which the nectar and pollen production potential is currently unknown. This plant can grow to 2.5 m in height and forms numerous small, yellow flowers organized in large, flat inflorescences called umbels (Piccaglia & Marotti, 2001). Each flower contains five stamens. Fennel nectaries are located on the stigma surfaces and are easily accessible to flower-visiting insects. The flowering period of lavender in the Valensole area extends from mid-June to the end of July. Fennel crops have two blooms, therefore, the flowering period extends from mid-June to mid-September. Fennel and lavender have a bloom overlap between three and five weeks.

This area has an intense beekeeping industry, which means that honeybees (*Apis mellifera*) are the most abundant flower-visiting insects (Schurr, Geslin, et al., 2021). In the study area, fennel flowers are visited by a wide range of insects (Hymenoptera, Lepidoptera, Coleoptera, and Diptera) (Schurr, Geslin, et al., 2021) and the same was expected for lavender, as its flowers are known to be probed by various insects (Benachour, 2017; Herrera, 1990).

4.2.2 Field measurements of nectar and pollen

Nectar measurements were taken between the end of June and the end of July 2019, 2020, and 2021, when the fennel and lavender flowering periods overlapped. All 652 measurements were recorded in good weather conditions (sunny days and light wind, temperature between 17 and 35 C°) in 17 different fields (Table S1, Figure S1). We collected nectar produced by fennel and lavender using three methods: a) standing nectar volume, b) replenished nectar volume, and c) nectar volume replenished over 24 h.

a) Standing nectar is the volume of nectar available in randomly selected open flowers. This represents the reward that an insect can obtain by visiting a random flower at a given time (Corbet, 2003; Parachnowitsch et al., 2019). For each plant (Table S1), we measured the nectar volume from an average of 11 flowers (between 4 and 25) and then calculated the standing nectar as the average volume between the sampled flowers per plant. Standing nectar volume was recorded between 09:30 and 14:30 for lavender and between 09:15 and 16:45 for fennel. There was a minimum of 20 m between each sampled plant, which were at least 5 m from the border of the field.

b) The replenished nectar volume is the amount of nectar accumulated by the flower over a defined time span. For each plant (Table S1), we selected one inflorescence at the active flowering stage. Active inflorescences were those that had most flowers open without any browning, indicating flower senescence (Guitton et al., 2010). In addition to standardize the measurement between plants of the same species due to spatial process or different flower senescence, we excluded lavender inflorescences at the top or the lowest part of the plant. For fennel, we selected inflorescences in secondary branches at a standard height (approximately 1.6 ± 0.2 m from the ground) and with an average width of 10 cm. We considered active fennel umbels with completely yellow flowers, at least 50 % of flowers open, and only peripheral flowers withered (Schurr et al., 2021). Active flowers are those are after drainage, the inflorescence was enclosed in a mesh bag to prevent insect visits for five different time spans: 30, 60, 120, 210, and 360 min. Then, inflorescences were unbagged and the nectar volume of 10 randomly selected flowers was measured and the mean was calculated. To account for potential variation due to the time of the day, we distributed the treatments across the day, except for the 360 min treatment due to time constraints. Measures of the replenished nectar volume were used to estimate the average nectar dynamics per species.

c) The nectar volume replenished over 24 h (Table S1) was assessed similarly to the protocol adopted for replenished nectar volume, but inflorescences were not drained prior to bagging. This is the standard measurement method for nectar production (Baude et al., 2016; Hicks et al., 2016; Timberlake et al., 2019).

For all three methods, nectar volume was measured by extracting the nectar accumulated in flowers by means of microcapillaries of 0.5 µl or 1 µl (HIRSCHMANN®, minicaps). The sugar concentration was measured for the replenished nectar, and the nectar was replenished over 24 h using a refractometer (Bellingham Stanley) (g sucrose per 100 g solution and expressed as brix %).

The number of pollen grains was estimated for fennel only, as the lavender hybrid does not produce pollen (Barbier, 1963). We quantified the number of pollen grains produced in one fennel flower (protocol in the supplementary materials).

4.2.2 Visitation rate

The total visits by insects to flowers in a 5 min period were counted in a 0.36 m^2 plot. Plots were delimited by a quadrat measuring 0.6 x 0.6 m. This size was chosen after trial observations found it to be the most appropriate for viewing all flowers at once in the dense crops. A visit was recorded when an insect touched the reproductive parts of a flower to gather resources (i.e., foraging behavior; a simple landing was not counted as a visit). Insect visit was counted also when the same insect visited different flowers of the same plot during the same observation round. The identification of each insect was recorded according to two categories (*Apis mellifera* vs. other insects). The number of flowers in the plot was also systematically estimated following the methods described by Schurr, Masotti, et al. (2021), excluding non-active inflorescences, as indicated for the replenished nectar measurements.

4.2.3 Simulation

Simulation model overview

We developed a stochastic simulation model of the nectar rewards collected by insects (RCI) across the day, of the daily nectar produced by a flower (DNF), and of simulated standing nectar. The aim of the simulation was to assess the effect of the flower selection performed by insects on RCI, DNF, and standing nectar. In the simulation, flower selection was defined by insect visitation rates and the insects' capacity to select between rewarding and non-rewarding flowers. The simulation was developed using plant and insect variables extracted from field measurements following the steps highlighted in Figure 1.

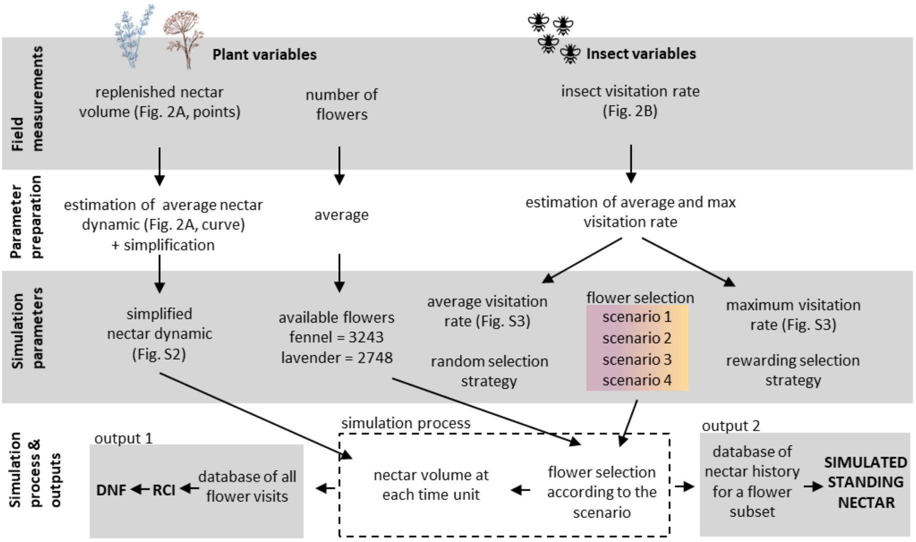


Fig. 1 Flowchart of the simulation of the Daily Nectar produced by a Flower (DNF), the nectar Rewards Collected by Insects (RCI) across the day and simulated standing nectar for fennel and lavender

Simulation parameters

To obtain the simulation parameters, preparation was carried out consisting of the estimation and simplification of the field measurements. The simulation parameters are as follows:1) nectar dynamic, 2) available flowers, and 3) flower selection scenarios.

The nectar dynamic is the average kinetic curve of replenished nectar within a defined time span. The nectar dynamic curve was estimated using field-replenished nectar volume data (see statistical analysis for details). Because the nectar dynamics were unknown for the longer observations (see Figure 2A and Discussion), nectar dynamics as a parameter were simplified by maintaining a constant nectar value when the estimation reached the peak (Figure S2). At the peak, the flowers were considered full, with neither more nectar production nor nectar re-absorption/evaporation. The simulation assumed that flowers repeat the same nectar dynamics after an insect visit, without changes in the nectar dynamic due to the potential stimulation/depression effects linked to insect visits. Despite nectar dynamic is likely to change depending on several factors, the methods employed for estimating the nectar dynamic should ensure a reliable average estimate. The available flower parameters were estimated by calculating the average number of flowers present in the visitation rate observation plots (see visitation rate). The effect of different numbers of available flowers that could be selected by flower-visiting insects was not the focus of this study and it was therefore kept constant in all simulations.

The flower selection parameters were organized into four scenarios. Each scenario is a combination of two levels of visitation rate (average/maximum) and two levels of insect selection strategy (random/rewarding). Visitation rates were measured in the field (see visitation rate). The average visitation rate was estimated between 06:00 and 20:00, when flower-visiting insects were active (see the statistical analysis and Figure S3). Due to logistic constrains, we did not observe visitations earlier than 08:30 or later than 18:30. Therefore, for earlier and later estimates, we assigned the first and last actual estimates, respectively. The maximum visitation rate was also considered and set to be constant across all simulations, and equal to the maximum visitation rate value recorded for each plant species (Figure S3). Although a constant visitation rate is unlikely under field conditions, the maximum level allows the simulation of the highest insect nectar demands. Flower-visiting insects can also select between rewarding and not rewarding flowers, which can affect the timing and frequency of flower visits (Goulson et al., 2001; Knauer et al., 2021). In the simulation, insects that did not select between rewarding and non-rewarding flowers had a random selection strategy (random level), whereas insects with selection capability preferentially adopted a rewarding selection strategy (rewarding level). At the random level, all flowers had the same probability of being visited. At the reward level, flowers should be visited at longer and more regular periods, since insects should avoid flowers without rewards. To simulate the latter condition at the reward level, the probability of a flower being visited by an insect was set to increase proportionally as the time since the last visit increased. Specifically, within the available flowers that insects can select, the flowers most recently visited have a probability of insect visits close to 0, whereas, the flowers visited the longest time ago have the maximum probability of being visited.

Simulation process and outputs

The simulation process reproduced plant/insect interactions in an area of 0.36 m^2. The simulation was modeled for 14 h, starting at 06:00, when flower-visiting insects generally begin their foraging activity, and ending at 20:00. We divided the 14 h of simulation into units of 5 min and assigned an identification to each available flower. Every five minutes the simulation process defined which of the available flowers was visited according to the scenario. Then, the simulation was extrapolated from the nectar dynamic parameter, the nectar volume of each flower at each time unit, according to the time elapsed since the last insect visit. To calculate nectar volume, it was assumed that the insects collected all available nectar at each visit. This assumption was validated in the field prior to data collection, as we had tested using a microcap immediately after visits (10 observations per crop), that visiting insects collected all nectar. The process of calculating the nectar volume every five minutes allowed quantification of the nectar volume that had been collected by insects at each visit, producing the simulation output of the RCI. Using the RCI data, we determined DNF as the sum of the rewards supplied to flower-visiting insects throughout the day. The complete flower history, which is the measure of nectar volume across time considering insect visits (see the example in Figure S4), was recorded for a random subset of 50 flowers per simulation. From the flower histories, the simulation calculated the simulated standing nectar as the average volume of nectar contained by flowers over time. The simulated standing nectar was visually compared with the field standing nectar to evaluate the simulation reliability and to identify which insect selection scenario produced standing nectar trends closest to the true values. The simulation was repeated 10 times per plant for each scenario (2 plants × 4 scenarios × 10 simulations), producing 80 simulations in total. All simulation data were aggregated to assess the differences in RCI, DNF, simulated standing nectar, and time between consecutive visits between scenarios. The parameters used for the simulation could be influenced by different seasonal or climatic factors, however, this was not accounted for as it was beyond the scope of this study. The simulations were carried out with R 4.0.2 (see Carisio, 2021 for the simulation code). The average RCI and standing nectar for the simulation scenario were plotted using the geom_smooth function of ggplot2 (Wickham, 2011).

Nectar resources at landscape level

For each crop, we calculated the daily sugar production per flower (g) using the formula described by Baude et al. (2016), first using the average DNF between scenarios and the average sugar concentration recorded from replenished nectar measurements, and then using the 24h replenished nectar volume and its average sugar concentration. We then estimated the daily nectar production at the landscape level (g ha^{-2} day^{-1}) by multiplying sugar production by the average estimated number of flowers per hectare. These calculations allow the comparison of nectar production at the landscape level assessed by means of nectar dynamic and insect-flower interaction data, with the nectar dynamic assessed through the measurement of nectar replenished over 24 h.

4.2.4 Statistical analysis

We used generalized additive mixed models (GAMM) to test the effect of time and plant species on replenished nectar volume and sugar concentration (Wood, 2017). The time since nectar draining was continuous and modelled with cubic spline smoothing. The estimate of the GAMM for replenished nectar volume corresponds to species nectar dynamics. A GAMM model was also implemented to estimate the average visitation rate across the day using plant species, number of flowers, and time of day as fixed factors. The latter two variables were modelled using cubic spline smoothers. The average predictions of GAMMs for replenished nectar and visitation rates were used to implement the simulation parameters. The effects of plant species on the volume of nectar replenished over 24 h and on its sugar concentration were analyzed using two generalized mixed models (GLMMs). Both models had plant species as a fixed factor, but the first adopted a gamma distribution and logarithmic link function, while the second adopted a binomial distribution. To test the differences in the visitation rates and proportion of honeybees compared to other flower-visiting insects between the two crops, we developed a GLMM model with a Poisson error distribution for the first variable and a binomial error distribution for the second variable. In these two models, plant species were used as a fixed factor and field as a random factor. All significant differences in GLMM models between groups were validated using Tukey’s post-hoc tests. The normal distribution of residuals in each test was routinely checked. All analyses were carried out with R 4.0.2 (R Core Team, 2000), using the packages lme4, emmeans, car for GLMM models, and the mgcv package for GAMM modelling (Bates et al., 2015; Lenth et al., 2018; Wood & Wood, 2015).

4.3 Results

4.3.1 Field experiment results

Fennel and lavender flowers showed non-linear nectar dynamics affected by species and time (GAMM, F = 61.921, edf = 5.192, $P < 0.001$ for plant species and time, R–sq (adj) = 62 %) (Figure 2A). Two hours after draining, the lavender flowers had, on average, a greater replenished nectar volume than fennel flowers (Figure 2A). The sugar concentration of replenished nectar in 6 h was not correlated with the time for either fennel or lavender (non-predictive GAMM, R–sq (adj) = 6 %). Sugar concentration was on average 56.25 ± 7.45 % for fennel (n = 48) and 53.39 ± 14.01 % for lavender (n = 56) (mean ± SD). The replenished nectar volume and corresponding sugar concentration over 24 h were not different between the two crops (non-predictive GLMM) (volume per flower: 0.061 ± 0.042 μl and 0.062 ± 0.036 μl; concentration per flower: 66.09 ± 13.33 % and 67.48 ± 6.75 %, respectively, for fennel and lavender) (mean ± SD) (Figure 2B). The median fennel pollen count was 1,200 pollen grains per flower. The visitation rate was significantly higher for fennel than for lavender (GLMM, $X^2 = 436.34$, Df = 1, $P < 0.001$) (Figure 2C), and the daily visitation pattern changed between fields, with one or two peaks in the day (Figure S5). For both crops, the most abundant flower-visiting insect was the honeybee; this was especially pronounced for lavender (GLMM, $X^2 = 8.537$, Df = 1, , $P < 0.003$) (Figure 2D). The proportion of honeybees to other insects was 0.86 ± 0.30 for lavender and 0.62 ± 0.36 for fennel (mean ± SD).

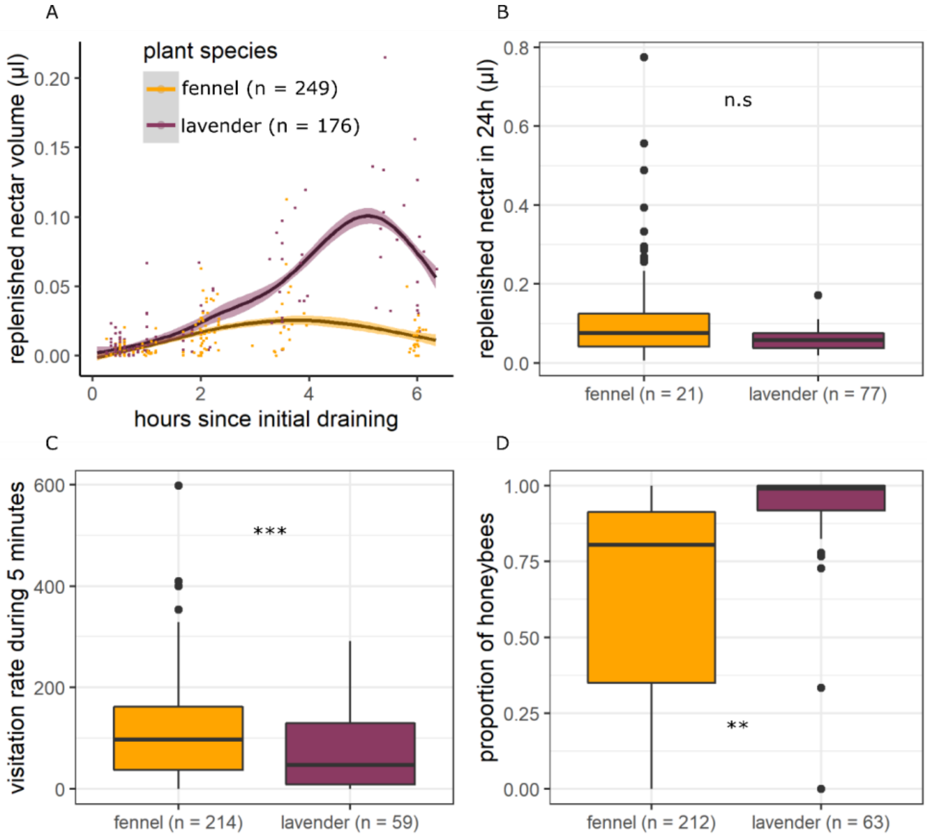


Fig. 2. Nectar replenishing potential of fennel and lavender and results on flower-visiting insects. (A) Nectar dynamic in six hours since flower draining; solid lines are the GAM model estimates, shaded areas are confidence intervals, points are the replenished nectar measurements; (B) Replenished nectar in 24 hours. (C) Insect visitation rate; (D) Proportion of honeybees (*Apis mellifera*) in five minute-intervals. Orange and purple points, smoothlines and boxplot refer to fennel and lavender respectively. The asterisks indicate significant differences according to Tukey test following a GLMM (n.s = not significant difference, ** = $P < 0.005$, *** = $P < 0.0001$)

Fennel flowers were always empty throughout the day except in the morning (standing nectar: 0.001 ± 0.007 µl, n = 81) (mean ± SD) (Figure 3A). Lavender flowers provided a standing nectar, which fluctuated somewhat (mean = 0.06 ± 0.05 µl, n = 48) without any particular pattern throughout the day (Figure 3A).

4.3.1 Simulation results

The average standing nectar predicted by the simulation differed between the species and scenarios (Figure 3B and 3C). The scenario with the average visitation rate and random insect selection was the most similar to the field average standing nectar of lavender (Figure 3B). For fennel, the high visitation rate scenario was most similar to the average field standing (Figure 3C). Simulation scenarios differed in average RCI for both plant species, although the RCI appeared to be mostly affected by visitation rate intensity rather than insect selection (Figure 3B and 3C).

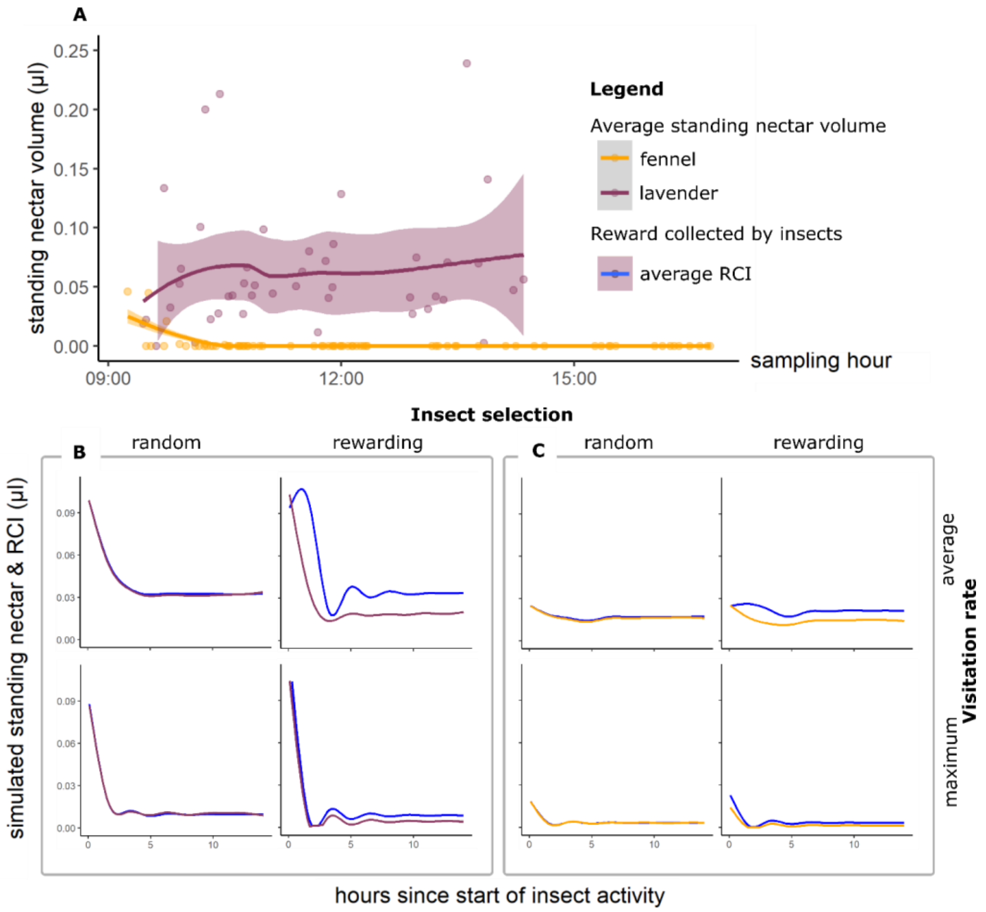


Fig. 3. (A) Standing nectar measured in the field for lavender and fennel between 09:00 and 16:45. (B and C) Simulated standing nectar and RCI at each visit estimated by four different flower selection simulation scenarios (insect visitation rate average/maximum X insect selection of flower random/rewarding) for lavender (B) and for fennel (C), since the start of the simulation (at 06:00). Solid lines are the estimates for standing nectar (orange for fennel and purple for lavender) or the estimates for the average reward collected by flower-visiting insects (blue line), shaded areas are confidence intervals and dots are field measurements of standing nectar.

The DNF varied between scenarios, with lavender having the highest DNF in the average visitation rate and rewarding selection scenario (0.260 ± 0.003 µl) and the lowest in the maximum visitation rate and rewarding selection scenario

(0.233 ± 0.006 μl) (Figure 4A). Fennel DNF was highest in the maximum visitation rate and rewarding selection scenario (0.111 ± 0.004 μl) and lowest in the average visitation rate and random selection scenario (0.073 ± 0.025 μl) (mean ± SD) (Figure 4B). In all the scenarios and for both species, the average DNFs were higher than the average nectar production over 24 h. Both for fennel and for lavender, the average time between two consecutive flower visits was always shorter than the time needed for the flower to reach the peak of replenished nectar (highest average time span between visits 3.12 ± 1.82 h and 2.33 ± 1.22 h respectively for fennel and lavender) (Figure 4C and Figure 4D). In the maximum visitation rate scenario, flowers were always visited before the nectar peak was replenished. The estimated daily sugar production at the landscape level by our simulation was 5,797 g ha^{-2} day^{-1} for fennel and 12,798 g ha^{-2} day^{-1} for lavender, while the estimation over 24 h of measurement was 4,839 g ha^{-2} day^{-1} and 4,231 g ha^{-2} day^{-1} for fennel and lavender, respectively.

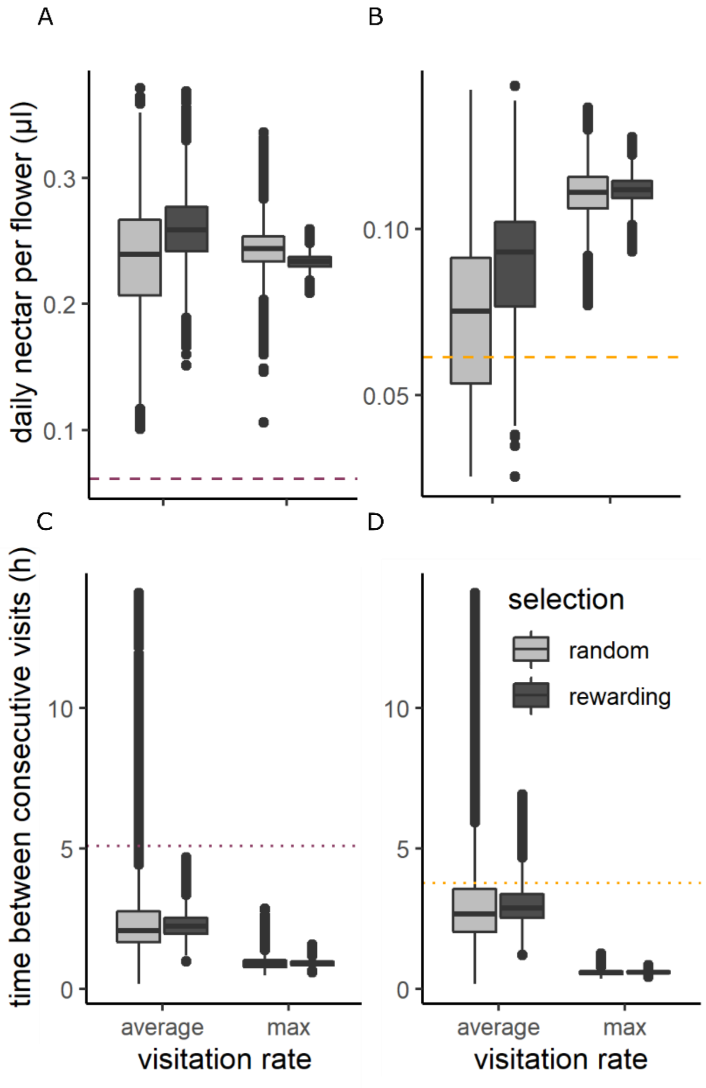


Fig. 4. Daily nectar volume for each flower (DNF) estimated by the simulation scenarios (insect visitation rate average/maximum X insect selection of flower random/rewarding) for lavender (A) and for fennel (B). Dashed lines indicate the average nectar volume replenished by flowers isolated from insects for 24h. Prediction of the time between two consecutive visits at the same flower according to the simulation scenario for lavender (C) and for fennel (B). Dotted lines indicate the time required for flowers to reach nectar dynamic peak.

4.4 Discussion

Our study analyzed a) the nectar production potential of two important mass-flowering crops, fennel (*Foeniculum vulgare*) and lavender (*Lavandula hybrida*), b) the preferences of flower-visiting insects for these crops, and c) the potential effect of insect-plant interactions on nectar production. Using field data, we developed a simulation model that estimated the daily nectar production of the two crops, accounting for the effects of insect visitation rates and insect capacity to select between rewarding and non-rewarding flowers. Our field results, together with the simulation model, indicate that although fennel had lower nectar production performance than lavender and close to null standing nectar in the field, flower-visiting insects exploited fennel flowers at a higher rate. This is likely because the fennel flowers also provided pollen and because the open structure of the flowers can be probed by a wider spectrum of insects. As hypothesized, our study showed that when nectar production dynamics are non-linear, the daily nectar production of a flower can be insect-driven and can change through interactions with flower-visiting insects. Interestingly, our simulation indicated that the effects of different insect visitation rates on nectar production were inconsistent between the two crops. The daily nectar volume estimated by our simulation was always higher than that of the nectar replenished over 24 h. This led us to conclude that in a mass-flowering crop context, estimating nectar resources by means of the nectar replenished over 24 h may be skewed and lead to underestimation of nectar production. Therefore, the future attempts to estimate the amount of nectar provided by a landscape should consider insect interactions with flowers.

4.4.1 Food resources produced by lavender and fennel for flower visitors

We found that lavender flowers produced nectar quickly. The lavender nectar dynamics showed that the replenished nectar peaked at 5 h and within 3.75 h they exceeded the average replenished nectar of flowers isolated for 24 h. This result suggests that lavender flowers may reabsorb nectar when it is not exploited for long periods. Signs of nectar reabsorption have previously been found in *Lavandula pubescens* (Nuru et al., 2015), but not for *Lavandula hybrida*, so this remains to be confirmed through dedicated analyses. As hypothesized for other plants, a re-absorption mechanism might reduce the energy costs to attract the pollinators needed to ensure seed set (Burquez & Corbet, 1991; Nepi & Stpiczyńska, 2008; Pacini & Nepi, 2007). Regarding fennel, the estimated nectar dynamics peaked before 4 h, but the peak was lower than the nectar replenished in flowers isolated for 24 h. This difference suggests that fennel flowers replenished at a slow rate and that nectar dynamics are likely to peak after a longer time than was considered in this study. Nectar replenishment data were remarkably dispersed over long periods in both species, which may have led to an imprecise estimate of nectar dynamic peaks. Dispersed nectar data seem to be the ‘rule’ in studies evaluating nectar dynamics, as they are greatly affected by individual flower and plant variation (Castellanos et al., 2002; Luo et al., 2014; Nicolson & Nepi, 2005), as well as by exogenous factors (Chabert et al., 2018). For example, *Carum carvi* (Apiaceae) plants of the same variety grown under the same controlled conditions showed fourfold difference in the replenished nectar between anthesis and fertilization (Langenberger & Davis, 2002). Therefore, the nectar dynamics of lavender and fennel should be considered as rough estimates of the average replenished nectar that may greatly change. Despite this limitation, we believe that at the landscape level, the nectar dynamic estimate is more accurate in assessing pollinator food resources than the measure of nectar replenished over 24 h. Indeed, the two crops showed no significant differences in the volume and sugar concentration of the nectar replenished over 24 h, thus they would appear equally valuable as pollinator food resources. However, if we compare the nectar dynamics of the two species over six hours, lavender was more productive than fennel, leading to the conclusion that lavender may be a more important nectar resource for flower-visiting insects, especially in areas where flowers are visited frequently.

4.4.2 Standing nectar and flower-visiting insect preferences

We found that the rate of insect flower visitation was higher in fennel than lavender. Honeybees were the dominant flower visitors for both crops, although the dominance was less pronounced in fennel. This was likely because of the numerous managed honeybee colonies placed in the study area for honey production. When measured in an area where flowers were exploited by a similar flower-visiting insect community, the standing nectar was constant and high for lavender, whereas for fennel, the standing nectar was close to zero from the first hour onwards. These results suggest that the nectar produced by fennel is immediately consumed by insects. At the same time, flower visitors seemed to neglect lavender flowers, despite their larger nectar rewards.

Focusing on the dominant flower-visiting insect, the honeybee, the exploitation of fennel flowers seems to contrast with the finding that honeybees preferentially choose flowers with a high nectar reward (Balfour et al., 2015; Duffield et al., 1993). In contrast, fennel flowers may also be visited for pollen reward. Indeed, several studies have found that the abundance or quality of pollen rewards is a major driver of honeybee flower choice (Aronne et al., 2012; Quinlan et al., 2021). In addition, according to optimal foraging theory, flower-visiting insects should adopt a foraging strategy that maximizes energy intake (Stephens & Krebs, 1987). The latter can be assessed as the difference between the energy provided by the floral food resource (nectar and pollen) and the sum of energy costs due to flower choice and flower probing (Balfour et al., 2015). We speculate that fennel may have a lower foraging cost because (1) the open structure of flowers and inflorescences allows flower-visiting insects to rapidly quantify and collect the offered nectar resources, and movements between flowers require less energy expenditure. In contrast, in lavender, quantification cues are prevented, and flower handling is more complicated because of the narrow flower morphology and dense spikes. The diversity of pollinators reported in fennel suggests that fennel flower traits do not constrain insect visits (Schurr, Geslin, et al., 2021; Smith-Ramírez et al., 2005; Thompson, 2001). In contrast, lavender has shown a tighter spectrum of flower-visiting insects in this and previous studies (Balfour et al., 2013; Benachour, 2017; Herrera, 1990). For both plants, the flower-visiting insects exploited multiple flowers within the same inflorescence. There are on average 381 flowers per fennel umbel, which is much higher than the 19 flowers per lavender spike (Schurr, Masotti, et al., 2021). Therefore, a single foraging flight may allow flower-visiting insects to exploit a greater number of flowers in fennel than in lavender plants.

4.4.3 Simulation model results

To our knowledge, this study constitutes the first attempt to estimate the daily nectar production, standing nectar, and average reward collected by flower-visiting insects across a daily period, considering the effects of non-linear nectar dynamics, insect visitation rates, and insect selection strategy. The simulation produced standing nectar trends similar to those observed from the field data, suggesting that the model can provide reliable estimates. The average and maximum visitation rate scenarios were consistent with the lavender and fennel observed in standing nectar. This result supports the hypothesis that fennel nectar was overexploited by flower-visiting insects (especially honeybees) and is in accordance with previous findings of low-standing nectar due to high insect exploitation (Corbet et al., 2001; Geslin, Gauzens, et al., 2017; Sáez et al., 2017; Torné-Noguera et al., 2016; Wignall et al., 2020).

As expected, our simulation showed that daily nectar production varied among the scenarios, and identical scenarios produced either increasing or decreasing production in the two investigated crops. Therefore, a generalizable effect of insects on daily nectar production among plants is missing. The lack of a general pattern is due to the complexity of interactions between plants and flower-visiting insects that are not ‘a priori’ predictable. Previous studies have found that insect visits have numerous effects on nectar production (Castellanos et al., 2002; Luo et al., 2014; Ordano & Ornelas, 2004; Ornelas & Lara, 2009; Ye et al., 2017). Nevertheless, we found a consistent pattern of a higher daily nectar volume than has been previously found for replenished in flowers isolated from insects for 24 h. Indeed, the estimated daily sugar production at the landscape level by our simulation was always greater than the estimation produced by the 24 h measurement. The explanation for this difference is that in an environment such as the one in our study, flower-visiting insects probed more often than the time required for the flower to fully replenish, and consequently, the rate of nectar replenishment will be higher on average. This explanation is consistent with previous studies showing that flowers can replenish nectar fully within a few hours, rather than requiring a whole day (Castellanos et al., 2002; Luo et al., 2014). Moreover, our results highlight that previous studies may have underestimated the total amount of nectar resources through the use of the 24 h replenished nectar proxy (Baude et al., 2016; Hicks et al., 2016; Timberlake et al., 2019), which could lead to incorrect conclusions that plants produce equivalent total nectar supplies. This underestimation may be particularly prominent in environments where honeybees are intensely managed meaning that visitation rates to mass-flowering crops are particularly high. We suggest that a better understanding of nectar resources at the landscape level might be achieved by estimating nectar production over different time spans and by defining the density and visitation frequency of insects.

4.5 Supplementary materials

Data availability statement

The R code of the simulation models, field data and generated data are available in the OSF repository at the link: DOI 10.17605/OSF.IO/AHGVU

Terms and Definitions

Replenished nectar volume: nectar volume accumulated by one flower within a given time period. During this period flowers are isolated from insects to prevent nectar removal. Unit measure in this study is μl. Replenished nectar volume measures are the dots on Fig 2A.

Nectar dynamic (or nectar kinetic): the estimate of average nectar replenished by one flower in a defined period calculated using the measures of replenished nectar volume. In the present study, nectar dynamic has been calculated by replenished nectar volume data separately for each species (GAM model). Unit measure is μl. Nectar dynamics are the two curves in Fig 2A.

Nectar volume replenished in 24h: nectar volume cumulated by one flower in a 24h of isolation from pollinators. The nectar volume is the most usual measurement in studies assessing nectar production potential of different plants. Unit measure in the present study is μl.

Standing nectar: The standing nectar is the volume of nectar available in randomly selected open flowers. This measure represents the reward that a flower-visiting insect could obtain by visiting a random flower at a given time. Since there is a high among flowers variation, each measurement is the average between numerous flowers (4-25 flowers) measured at the same time (one minute to collect all the nectar from multiple flowers). Unit measure in the present study is μl. Standing nectar measures are the points in Fig 3A.

Average standing nectar: is the average standing nectar across the time calculated using standing nectar measures. It was calculated separately for each species. Average Standing nectar measures are the curves in Fig 3A.

Visitation rate: total number of flowers visited within a plot by flower-visiting insects in a five minute period.

Nectar reward collected by insect (RCI): is the nectar reward in volume collected by insect in a single flower visit. Flower-visiting insects could select between rewarding vs. not rewarding. In this case RCI is higher than the standing nectar measured in the field. When insects are not capable of selecting flowers according to the reward, and they select them randomly, RCI and standing nectar are similar. The average RCI across the day calculated by the simulation are the blue line in Fig2B & 2C.

Daily Nectar production by a Flower (DNF): is the total volume produced by a flower and is calculated as the sum of rewards collected by insects.

Protocol for pollen measurements

In summer 2020, five flower buds ready to open were harvested on three umbels per plant, on three plants, in two fennel fields (N = 90 flowers). We let flowers dry in open air in 2 mL Eppendorf tubes, one flower per tube. We then extracted the pollen grains following Suehs et al. 2006, directly from the whole flower (i.e. from the five stamens). To destroy plant tissues (except pollen grains), we put 225 µL of sulphuric acid in each tube and let it degrade for one night. We then added 375 µL of Triton solution (a mix of 2 mL of Triton x100 and 100 mL of distilled water) before crushing flower in it with glass rods (subsequently rinsed with 750 µL of Triton water to not lose any pollen grain). Samples were then centrifuged during five minutes at 2000 rpm and 20 °C. The centrifugation was set to stop slowly to be sure that pollen grains stay in the bottom of the tube. The supernatant was then sucked and removed, and the pollen grains were re-suspended with 1.5 mL 96% Ethanol. Samples were centrifuged again, then the maximum volume of supernatant were removed, and pollen were finally left to dry under a hood. Then, 20 to 30 µL of counting solution (20 mL of glycerol + 30 g of saccharose + 80 mL of distilled water for 100 mL of counting solution) was added in each sample. Tubes were then placed in an ultrasonic bath for five to ten minutes to dislocate the pile of pollen grains and facilitate the count. The solution was put in a Malassez counting cell for counting pollen grains. To have the total number of grains contained in the tube, i.e. produced in one flower, we multiplied the number of pollen grains found on the slide by the volume of counting solution added.

Reference for pollen measurement

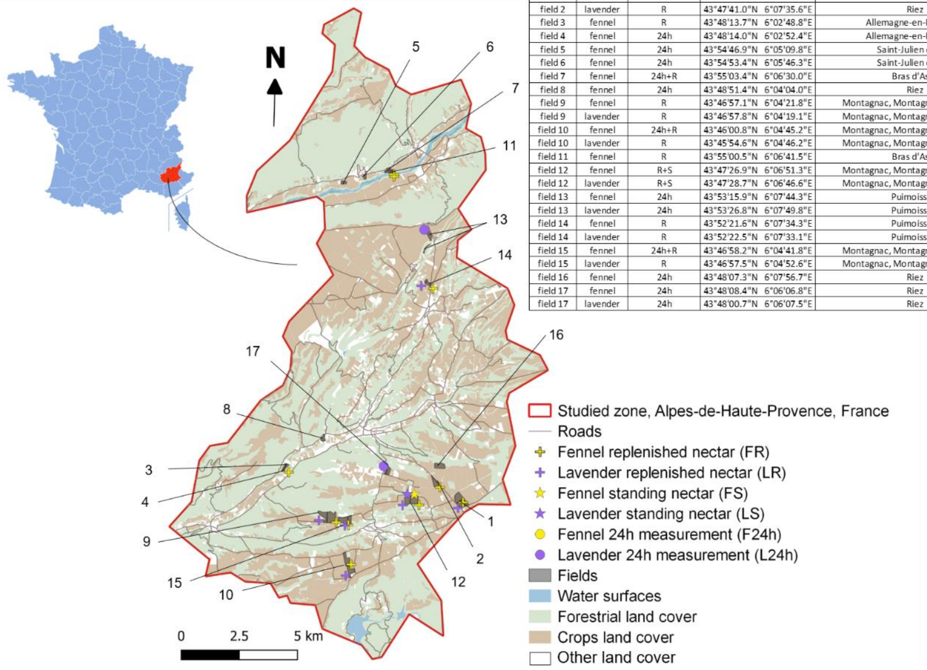
Suehs, C. M., Charpentier, S., Affre, L., & Médail, F. (2006). The evolutionary potential of invasive Carpobrotus (Aizoaceae) taxa: Are pollen-mediated gene flow potential and hybrid vigor levels connected? Evolutionary Ecology, 20(5), 447–463. doi: 10.1007/s10682-006-0013-0

Supplementary table

Tab. S1 – Number of observations per each nectar measurement method divided by field.

	Standing		Replenished		24h	
field	fennel	lavender	fennel	lavender	fennel	lavender
field 1			18	18		
field 2			18	18		
field 3			15			
field 4					12	
field 5					13	
field 6						
field 7			19			
field 8						
field 9			21	21		
field 10			18	15		
field 11			38			
field 12	81	48	57	64		
field 13					12	11
field 14			21	19		
field 15			24	21	16	
field 16					12	
field 17					12	10

Supplementary figures



Field	Crops	Sample method	GPS coordinates	Location in Alpes-de-Haute-Provence, France	Elevation (m)
field 1	fennel	24h+R	43°47'23.5"N 6°08'22.1"E	Montagnac, Montagnac-Montpezat	654
field 1	lavender	R	43°47'20.5"N 6°08'28.0"E	Montagnac, Montagnac-Montpezat	654
field 2	fennel	24h+R	43°47'43.4"N 6°07'43.4"E	Riez	650
field 2	lavender	R	43°47'41.0"N 6°07'35.6"E	Riez	650
field 3	fennel	R	43°48'13.7"N 6°02'48.8"E	Allemagne-en-Provence	466
field 4	fennel	24h	43°48'14.0"N 6°02'52.4"E	Allemagne-en-Provence	465
field 5	fennel	24h	43°54'46.9"N 6°05'09.8"E	Saint-Julien d'Asse	450
field 6	fennel	24h	43°54'53.4"N 6°05'46.3"E	Saint-Julien d'Asse	454
field 7	fennel	24h+R	43°55'03.4"N 6°06'30.0"E	Bras d'Asse	462
field 8	fennel	24h	43°48'51.4"N 6°04'04.0"E	Riez	496
field 9	fennel	R	43°46'57.1"N 6°04'21.8"E	Montagnac, Montagnac-Montpezat	604
field 9	lavender	R	43°46'57.8"N 6°04'19.1"E	Montagnac, Montagnac-Montpezat	604
field 10	fennel	24h+R	43°46'00.8"N 6°04'45.2"E	Montagnac, Montagnac-Montpezat	605
field 10	lavender	R	43°45'54.6"N 6°04'46.2"E	Montagnac, Montagnac-Montpezat	605
field 11	fennel	R	43°55'00.5"N 6°06'41.5"E	Bras d'Asse	460
field 12	fennel	R+S	43°47'26.9"N 6°06'51.3"E	Montagnac, Montagnac-Montpezat	640
field 12	lavender	R+S	43°47'28.7"N 6°06'46.6"E	Montagnac, Montagnac-Montpezat	640
field 13	fennel	24h	43°53'15.9"N 6°07'44.3"E	Puimoisson	726
field 13	lavender	24h	43°53'26.8"N 6°07'49.8"E	Puimoisson	726
field 14	fennel	R	43°52'21.6"N 6°07'34.3"E	Puimoisson	704
field 14	lavender	R	43°52'22.5"N 6°07'33.1"E	Puimoisson	704
field 15	fennel	24h+R	43°46'58.2"N 6°04'41.8"E	Montagnac, Montagnac-Montpezat	611
field 15	lavender	R	43°46'57.5"N 6°04'52.6"E	Montagnac, Montagnac-Montpezat	611
field 16	fennel	24h	43°48'07.3"N 6°07'56.7"E	Riez	657
field 17	fennel	24h	43°48'08.4"N 6°06'06.8"E	Riez	636
field 17	lavender	24h	43°48'00.7"N 6°06'07.5"E	Riez	636

Fig. S1 Map of the study area

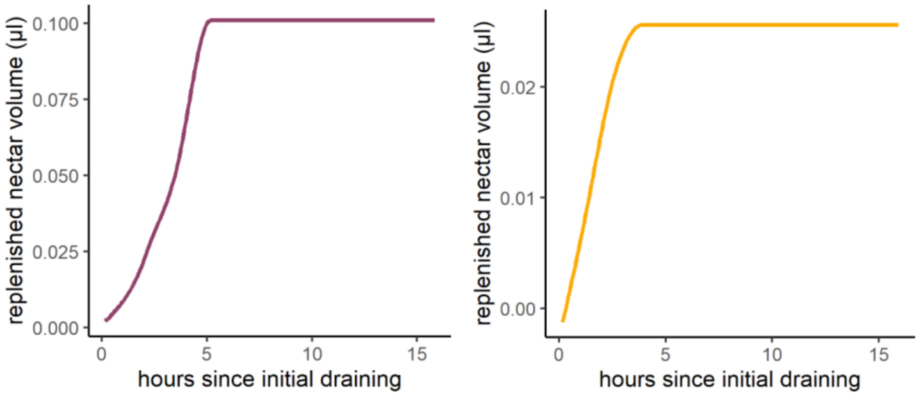


Fig. S2 Simplified nectar dynamic used as simulation parameter for lavender (purple) and fennel (orange)

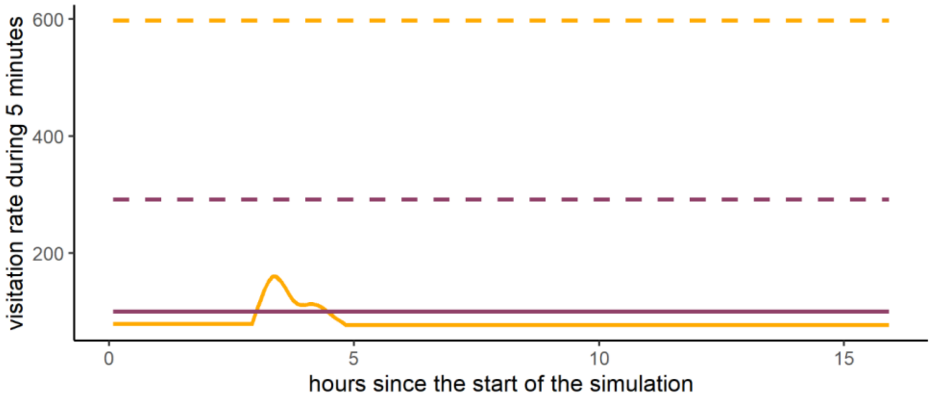


Fig. S3 Visitation rate parameters across the time for lavender (purple) and fennel (orange). Solid lines are average visitation rate parameters, dashed lines are maximum visitation rate parameters.

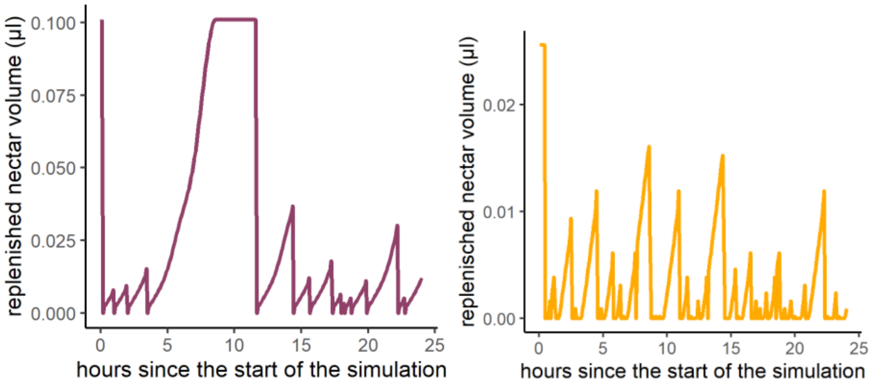


Fig. S4 Single flower visitation history examples for lavender in average and random scenario (purple) and for fennel in maximum random scenario.

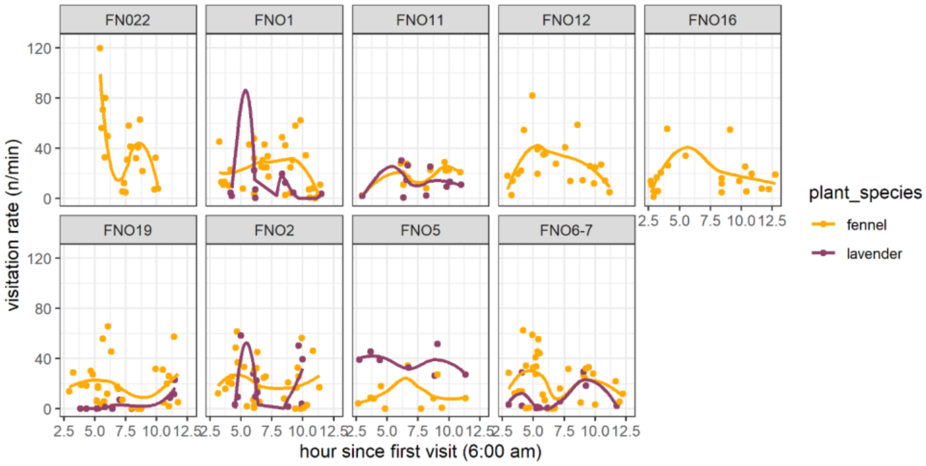


Fig. S5 Visitation rate observations per each field across hours since flower-visiting insects start to forage. An average visitation rate per plant species was added through the geom_smooth function of ggplot2.

5. CONCLUSIONS

The results and findings described in the chapters of this PhD thesis provide important knowledge on different aspects of pollination in agrosystems. The study on the effects of density and position of pollinizer trees in apple orchards highlights how pollen limitations for self-incompatible crops could be prevented through a well-planned orchard design. The study was the first in assessing the effect on pollination of an intermixed orchard, where main cultivar and pollen donor cultivar are uniformly mixed. Therefore, the results provide clear indications of one of the strategies that farmers could adopt in apple orchard. The identification of strategies that are easy to implement is a critical step for developing more efficient and less environmental impacting agriculture. I also found that the farmer practice of reducing density of pollinizer plant within orchards can jeopardize the pollination service into the inner orchard part and therefore, this practice should be avoided.

In Chapter 2, I explored the benefits of pollination on the fruit quality, specifically on the symmetry of apples. While the effect of pollination on yield have been widely studied, data on the effect of various quality parameter are still missing. A new image tool method was developed, allowing the measurement of the effect of pollination on the quality parameter of fruit symmetry.

The results indicated that probably the classical visual assessment of fruit shape adopted to explore the effect of pollination on fruit symmetry tends to overlook the pollination effect. The new image analysis method was implemented and shared as open access programming code. Therefore, the code could be easily adopted to other pollination-dependent fruits for which knowledge on pollination effect on fruit shape are still missing. In general, the results provided by the studies performed on apple during this PhD provide a novel practical understanding of ecosystem service that may be more effectively transferred to farmers.

In Chapter 3, I evaluated the resources that can be provided by agrosystems to pollinators. As it was previously highlighted, the abundance and the diversity of pollinators is crucial to support the pollination ecosystem services. However, the study showed that our understanding of the resources provided by pollinator is still limited since the method adopted to quality food resources does not consider the effect of plant/insect interaction on nectar and pollen production. The thesis fills this gap developing a simulation model allowing the estimation of the daily nectar production of crops including the effect on the production of insects exploitation of the nectar cumulated.

The novel simulation is a promising new tool to overcome the limitation of the method used nowadays for measuring nectar resources. Even in this case, the simulation was developed as open access code a could be easily applied to other agrosystems. Moreover, the same method could be used to answer important unsolved questions regarding the interaction between flower-visiting insects, as for example how beekeeping activity can exert food competition pressure on wild pollinators.

In conclusion, the combination of the thesis findings may contribute to improve pollination in agrosystems and may give new strategies to support crop production through an ecological intensification process.

6. REFERENCES

Abbasi, K. H., Jamal, M., Ahmad, S., Ghramh, H. A., Khanum, S., Khan, K. A., … Zulfiqar, B. (2021). Standardization of managed honey bee (Apis mellifera) hives for pollination of Sunflower (Helianthus annuus) crop. *Journal of King Saud University - Science*, *33*(8), 101608. doi: 10.1016/j.jksus.2021.101608

Abson, D. J., & Termansen, M. (2011). Valoración de los Servicios del Ecosistema en Términos de Riesgos y Beneficios Ecológicos. *Conservation Biology*, *25*(2), 250–258. doi: 10.1111/j.1523-1739.2010.01623.x

Aizen, M. A., Aguiar, S., Biesmeijer, J. C., Garibaldi, L. A., Inouye, D. W., Jung, C., … Seymour, C. L. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biology*, *25*(10), 3516–3527. doi: 10.1111/gcb.14736

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. doi: 10.1093/aob/mcp076

Aizen, M. A., & Harder, L. D. (2007). EXPANDING THE LIMITS OF THE POLLEN-LIMITATION CONCEPT: EFFECTS OF POLLEN QUANTITY AND QUALITY. *Ecology*, *88*(2), 271–281. doi: 10.1890/06-1017

Alqudah, A. M., Samarah, N. H., & Mullen, R. E. (2011). Drought Stress Effect on Crop Pollination, Seed Set, Yield and Quality. In *Alternative Farming Systems, Biotechnology, Drought Stress and Ecological Fertilisation* (pp. 193–213). Dordrecht: Springer Netherlands. doi: 10.1007/978-94-007-0186-1_6

Angelella, G. M., McCullough, C. T., & O’Rourke, M. E. (2021). Honey bee hives decrease wild bee abundance, species richness, and fruit count on farms regardless of wildflower strips. *Scientific Reports*, *11*(1), 3202. doi: 10.1038/s41598-021-81967-1

Aronne, G., Giovanetti, M., Guarracino, M. R., & de Micco, V. (2012). Foraging rules of flower selection applied by colonies of Apis mellifera: Ranking and associations of floral sources. *Functional Ecology*, *26*(5), 1186–1196. doi: 10.1111/j.1365-2435.2012.02017.x

Bain, J. M., & Robertson, R. N. (1951). The physiology of growth in apple fruits. I. Cell size, cell number, and fruit development. *Australian Journal of Scientific Research. Ser. B: Biological Sciences*, *4*(2), 75–107. doi: 10.1071/bi9510075

Balfour, N. J., Gandy, S., & Ratnieks, F. L. W. (2015). Exploitative competition alters bee foraging and flower choice. *Behavioral Ecology and Sociobiology*, *69*(10), 1731–1738. doi: 10.1007/s00265-015-1985-y

Balfour, N. J., Garbuzov, M., & Ratnieks, F. L. W. (2013). Longer tongues and swifter handling: Why do more bumble bees (Bombus spp.) than honey bees (Apis mellifera) forage on lavender (Lavandula spp.)? *Ecological Entomology*, *38*(4), 323–329. doi: 10.1111/een.12019

Banaszak-Cibicka, W., & Żmihorski, M. (2012). Wild bees along an urban gradient: Winners and losers. *Journal of Insect Conservation*, *16*(3), 331–343. doi: 10.1007/s10841-011-9419-2

Bangerth, F. (2000). Abscission and thinning of young fruit and their regulation by plant hormones and bioregulators. *Plant Growth Regulation*, *31*(1–2), 43–59. doi: 10.1023/a:1006398513703

Barbier, E. (1963). Les lavandes et l’apiculture dans le sud-est de la france. *Annales de l’Abeille*, *6*(2), 85–159.

Bartomeus, I., Potts, S. G., Steffan-Dewenter, I., Vaissière, B. E., Woyciechowski, M., Krewenka, K. M., … Bommarco, R. (2014). Contribution of insect pollinators to crop yield and quality varies with agricultural intensification. *PeerJ*, *2014*(1), 1–20. doi: 10.7717/peerj.328

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*. doi: 10.18637/jss.v067.i01

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. doi: 10.1038/nature16532

Benachour, K. (2017). Insect Visitors of Lavender (Lavandula officinalis L.): Comparison of Quantitative and Qualitative Interactions of the Plant with Its Main Pollinators. *African Entomology*, *25*(2), 435–444. doi: 10.4001/003.025.0435

Beyer, M., Hahn, R., Peschel, S., Harz, M., & Knoche, M. (2002). Analysing fruit shape in sweet cherry (Prunus avium L.). *Scientia Horticulturae*, *96*(1–4), 139–150. doi: 10.1016/S0304-4238(02)00123-1

Biella, P., Akter, A., Muñoz-Pajares, A. J., Federici, G., Galimberti, A., Jersáková, J., … Mangili, L. (2021). Investigating pollination strategies in disturbed habitats: the case of the narrow-endemic toadflax Linaria tonzigii (Plantaginaceae) on mountain screes. *Plant Ecology*, *222*(4), 511–523. doi: 10.1007/s11258-021-01123-7

Blaauw, B. R., & Isaacs, R. (2014). Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *Journal of Applied Ecology*, *51*(4), 890–898. doi: 10.1111/1365-2664.12257

Blazek, J. (1975). Pollination in variety blocks of apple trees. *Proceedings of the III International Symposium on Pollination, 1974*.

Bommarco, R., Biesmeijer, J. C., Meyer, B., Potts, S. G., Pöyry, J., Roberts, S. P. M., … Ockinger, E. (2010). Dispersal capacity and diet breadth modify the response of wild bees to habitat loss. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1690), 2075–2082. doi: 10.1098/rspb.2009.2221

Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: Harnessing ecosystem services for food security. *Trends in Ecology and Evolution*, *28*(4), 230–238. doi: 10.1016/j.tree.2012.10.012

Bommarco, R., Marini, L., & Vaissière, B. E. (2012). Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia*, *169*(4), 1025–1032. doi: 10.1007/s00442-012-2271-6

Boreux, V., Kushalappa, C. G., Vaast, P., & Ghazoul, J. (2013). Interactive effects among ecosystem services and management practices on crop production: Pollination in coffee agroforestry systems. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(21), 8387–8392. doi: 10.1073/pnas.1210590110

Bos, M. M., Veddeler, D., Bogdanski, A. K., Klein, A. M., Tscharntke, T., Steffan-Dewenter, I., & Tylianakis, J. M. (2007). Caveats to quantifying ecosystem services: Fruit abortion blurs benefits from crop pollination. *Ecological Applications*, *17*(6), 1841–1849. doi: 10.1890/06-1763.1

Brittain, C., Kremen, C., Garber, A., & Klein, A. M. (2014). Pollination and plant resources change the nutritional quality of almonds for human health. *PLoS ONE*, *9*(2). doi: 10.1371/journal.pone.0090082

Broothaerts, W., Van Nerum, I., & Keulemans, J. (2004). Update on and Review of the Incompatibility (S-) Genotypes of Apple Cultivars. *HortScience*, *39*(5), 943–947. doi: 10.21273/HORTSCI.39.5.943

Buccheri, M., & Di Vaio, C. (2005). Relationship Among Seed Number, Quality, and Calcium Content in Apple Fruits. *Journal of Plant Nutrition*, *27*(10), 1735–1746. doi: 10.1081/PLN-200026409

Buhk, C., Oppermann, R., Schanowski, A., Bleil, R., Lüdemann, J., & Maus, C. (2018). Flower strip networks offer promising long term effects on pollinator species richness in intensively cultivated agricultural areas. *BMC Ecology*, *18*(1), 1–13. doi: 10.1186/s12898-018-0210-z

Burquez, A. A., Corbet, S. A., Burquez, A., & Corbettt, S. A. (1991). Do Flowers Reabsorb Nectar ? *Functional Ecology*, *5*(3), 369–379.

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. *PLoS ONE*, *7*(5), 24–28. doi: 10.1371/journal.pone.0037235

Carisio, L., Díaz, S. S., Ponso, S., Manino, A., & Porporato, M. (2020). Effects of pollinizer density and apple tree position on pollination efficiency in cv. Gala. *Scientia Horticulturae*. doi: 10.1016/j.scienta.2020.109629

Carisio, L., Straffon Díaz, S., Manino, A., & Porporato, M. (2021). Influence of seed set on apple symmetry assessed by image analysis: an overlooked effect of pollination on fruit shape. *Agrirxiv*. doi: https://10.31220/agriRxiv.2021.00060

Casasso, A., & Sethi, R. (2017). Assessment and mapping of the shallow geothermal potential in the province of Cuneo (Piedmont , NW Italy). *Renewable Energy*, *102*, 306–315. doi: 10.1016/j.renene.2016.10.045

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2002). Dynamic nectar replenishment in flowers of Penstemon (Scrophulariaceae). *American Journal of Botany*, *89*(1), 111–118. doi: 10.3732/ajb.89.1.111

Chabert, S., Lemoine, T., Raimond Cagnato, M., Morison, N., & Vaissière, B. E. (2018). Flower age expressed in thermal time: is nectar secretion synchronous with pistil receptivity in oilseed rape (Brassica napus L.)? *Environmental and Experimental Botany*, *155*(November), 628–640. doi: 10.1016/j.envexpbot.2018.08.004

Chapman, G. P., & Sadjadi, A. S. (1981). Exogenous Growth Substances and Internal Competition in Vicia faba L. *Zeitschrift Für Pflanzenphysiologie*, *104*(3), 265–273. doi: 10.1016/s0044-328x(81)80121-3

Corbet, S. A. (2003). Nectar sugar content: estimating crop and secretion rate in the field. *Apidologie*, *2003*(34), 1–18. doi: 10.1051/apido

Corbet, S. A., Bee, J., Dasmahapatra, K., Gale, S., Gorringe, E., La Ferla, B., … Vorontsova, M. (2001). Native or Exotic? Double or Single? Evaluating Plants for Pollinator-friendly Gardens. *Annals of Botany*, *87*(2), 219–232. doi: 10.1006/anbo.2000.1322

Costanza, R., D'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., … van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. LK - https://royalroads.on.worldcat.org/oclc/4592801201. *Nature TA - TT -*, *387*(6630), 253–260. Retrieved from https://www-nature-com.ezproxy.royalroads.ca/articles/387253a0.pdf

Cranmer, L., McCollin, D., & Ollerton, J. (2012). Landscape structure influences pollinator movements and directly affects plant reproductive success. *Oikos*, *121*(4), 562–568. doi: 10.1111/j.1600-0706.2011.19704.x

Culley, T. M., Weller, S. G., Sakai, A. K., Culley, T. M., Weller, S. G., & Sakai, A. K. (2002). The evolution of wind pollination in angiosperms. *Trends in Ecology & Evolution*, *17*(10), 491. doi: 10.1016/s0169-5347(02)02600-9

Currie, A. J., Ganeshanandam, S., Noiton, D. A., Garrick, D., Shelbourne, C. J. A., & Oraguzie, N. (2000). Quantitative evaluation of apple (Malus x domestica Borkh.) fruit shape by principal component analysis of Fourier descriptors. *Euphytica*, *111*(3), 219–227. doi: 10.1270/jsbbs.52.243

David, T. I., Storkey, J., & Stevens, C. J. (2019). Understanding how changing soil nitrogen affects plant–pollinator interactions. *Arthropod-Plant Interactions*, *13*(5), 671–684. doi: 10.1007/s11829-019-09714-y

Davis, A. S., Hill, J. D., Chase, C. A., Johanns, A. M., & Liebman, M. (2012). Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health. *PLoS ONE*, *7*(10), e47149. doi: 10.1371/journal.pone.0047149

de Ribeiro, M. F., da Silva, E. M. S., de Lima Júnior, I. O., & Kiill, L. H. P. (2015). Visita de abelhas melíferas (Apis mellifera) em flores de melão amarelo (Cucumis melo) usando diferente número de colmeias. *Ciencia Rural*, *45*(10), 1768–1773.

Descamps, C., Quinet, M., & Jacquemart, A. L. (2021). The effects of drought on plant–pollinator interactions: What to expect? *Environmental and Experimental Botany*, *182*(June 2020), 104297. doi: 10.1016/j.envexpbot.2020.104297

Devaux, C., Porcher, E., & Lande, R. (2019). Mating systems and avoidance of inbreeding depression as evolutionary drivers of pollen limitation in animal-pollinated self-compatible plants. *Annals of Botany*, *123*(2), 327–336. doi: 10.1093/aob/mcy181

Djekic, I., Radivojevic, D., & Milivojevic, J. (2019). Quality perception throughout the apple fruit chain. *Journal of Food Measurement and Characterization*, *13*(4), 3106–3118. doi: 10.1007/s11694-019-00233-1

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., & Wilson, K. (2014). Honeybee nutrition is linked to landscape composition. *Ecology and Evolution*, *4*(21), 4195–4206. doi: 10.1002/ece3.1293

Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M., & Tittonell, P. (2011). Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. *European Journal of Agronomy*, *34*(4), 197–210. doi: 10.1016/j.eja.2011.02.006

Dražeta, L., Lang, A., Hall, A., Volz, R., Jameson, P., Dražeta, L., … Jameson,

P. E. (2015). *Modelling the influence of seed set on fruit shape in apple Modelling the influence of seed set on fruit shape in apple*. *0316*. doi: 10.1080/14620316.2004.11511755

Duffield, G. E., Gibson, R. C., Gilhooly, P. M., Hesse, A. J., Inkley, C. R., Gilbert, F. S., & Barnard, C. J. (1993). Choice of flowers by foraging honey bees (Apis mellifera): possible morphological cues. *Ecological Entomology*, *18*(3), 191–197. doi: 10.1111/j.1365-2311.1993.tb01089.x

Dussaubat, C., Maisonnasse, A., Belzunces, L. P., Brunet, J. L., & Kretzschmar, A. (2021). Preliminary report of honeybee physiological changes pre- and post-hybrid lavender season in high and low weight gain colonies. *Apidologie*, *52*(2), 463–472. doi: 10.1007/s13592-020-00835-5

Dymond, K., Celis-Diez, J. L., Potts, S. G., Howlett, B. G., Willcox, B. K., & Garratt, M. P. D. (2021). The role of insect pollinators in avocado production: A global review. *Journal of Applied Entomology*, *145*(5), 369–383. doi: 10.1111/jen.12869

Eccher, G., Ferrero, S., Populin, F., Colombo, L., & Botton, A. (2014). Apple (Malus domestica L. Borkh) as an emerging model for fruit development. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, *148*(1), 157–168. doi: 10.1080/11263504.2013.870254

Elsysy, M., Serra, S., Schwallier, P., Musacchi, S., & Einhorn, T. (2019). Net enclosure of ‘honeycrisp’ and ‘Gala’ apple trees at different bloom stages affects fruit set and alters seed production. *Agronomy*, *9*(9). doi: 10.3390/agronomy9090478

Escriche, I., Sobrino-Gregorio, L., Conchado, A., & Juan-Borrás, M. (2017). Volatile profile in the accurate labelling of monofloral honey. The case of lavender and thyme honey. *Food Chemistry*, *226*, 61–68. doi: 10.1016/j.foodchem.2017.01.051

Ferree, D. C., & Warrington, I. J. (2003). *Apples: botany, production, and uses*.

CABI.

Ferrero, S., Carretero-Paulet, L., Mendes, M. A., Botton, A., Eccher, G., Masiero, S., & Colombo, L. (2015). Transcriptomic signatures in seeds of apple (Malus domestica L. Borkh) during fruitlet abscission. *PLoS ONE*, *10*(3). doi: 10.1371/journal.pone.0120503

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*. doi: 10.1002/joc.5086

Fijen, T. P. M., Scheper, J. A., Vogel, C., van Ruijven, J., & Kleijn, D. (2020). Insect pollination is the weakest link in the production of a hybrid seed crop. *Agriculture, Ecosystems and Environment*, *290*(May 2019), 106743. doi: 10.1016/j.agee.2019.106743

Free, J. B. (1962). The behaviour of honeybees visiting field beans (Vicia faba). *The Journal of Animal Ecology*, 497–502.

Free, J. B. (1970). Insect pollination of crops. *Insect Pollination of Crops.*

Galimba, K. D., Bullock, D. G., Dardick, C., Liu, Z., & Callahan, A. M. (2019). Gibberellic acid induced parthenocarpic ‘Honeycrisp’ apples (Malus domestica) exhibit reduced ovary width and lower acidity. *Horticulture Research*, *6*(1). doi: 10.1038/s41438-019-0124-8

Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, *68*(3), 810–821. doi: 10.1016/j.ecolecon.2008.06.014

Ganser, D., Albrecht, M., & Knop, E. (2021). Wildflower strips enhance wild bee reproductive success. *Journal of Applied Ecology*, *58*(3), 486–495. doi: 10.1111/1365-2664.13778

Garibaldi, L. A., Andersson, G. K. S., Requier, F., Fijen, T. P. M., Hipólito, J., Kleijn, D., … Rollin, O. (2018). Complementarity and synergisms among ecosystem services supporting crop yield. *Global Food Security*, *17*(December 2017), 38–47. doi: 10.1016/j.gfs.2018.03.006

Garibaldi, L. A., Steffan-dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., … Carvalheiro, L. G. (2014). Honey Bee Abundance. *Science*, *339*(May), 1608–1611. doi: 10.1126/science.1230200

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., … Klein, A. M. (2013). Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. *Science*, *339*(6127), 1608–1611. doi: 10.1126/science.1230200

Garratt, M. P.D., Breeze, T. D., Jenner, N., Polce, C., Biesmeijer, J. C., & Potts, S. G. (2014). Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. *Agriculture, Ecosystems and Environment*, *184*, 34–40. doi: 10.1016/j.agee.2013.10.032

Garratt, M P D, Breeze, T. D., Boreux, V., Fountain, M. T., & Mckerchar, M. (2016). *Apple Pollination : Demand Depends on Variety and Supply Depends on Pollinator Identity*. 1–15. doi: 10.1371/journal.pone.0153889

Garratt, Michael P.D., Bishop, J., Degani, E., Potts, S. G., Shaw, R. F., Shi, A., & Roy, S. (2018). Insect pollination as an agronomic input: Strategies for oilseed rape production. *Journal of Applied Ecology*, *55*(6), 2834–2842. doi: 10.1111/1365-2664.13153

Garratt, Michael P.D., de Groot, G. A., Albrecht, M., Bosch, J., Breeze, T. D., Fountain, M. T., … Zhusupbaeva, A. (2021). Opportunities to reduce pollination deficits and address production shortfalls in an important insect-pollinated crop. *Ecological Applications*, *31*(8), 1–12. doi: 10.1002/eap.2445

Geslin, B, Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., … Vereecken, N. (2017). Massively Introduced Managed Species and Their

Consequences for Plant–Pollinator Interactions. *Advances in Ecological Research*, *57*, 147–199. doi: 10.1016/bs.aecr.2016.10.007

Geslin, Benoît, Aizen, M. A., Garcia, N., Pereira, A. J., Vaissière, B. E., & Garibaldi, L. A. (2017). The impact of honey bee colony quality on crop yield and farmers' profit in apples and pears. *Agriculture, Ecosystems and Environment*, *248*(July), 153–161. doi: 10.1016/j.agee.2017.07.035

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., … Toulmin, C. (2010). Food Security: The Challenge of Feeding 9 Billion People. *Science*, *327*(5967), 812–818. doi: 10.1126/science.1185383

Goulson, D., Chapman, J. W., & Hughes, W. O. H. (2001). Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *Journal of Insect Behavior*, *14*(5), 669–678. doi: 10.1023/A:1012231419067

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined Stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229). doi: 10.1126/science.1255957

Grant, M. C., Doust, J. L., & Doust, L. L. (1989). Plant Reproductive Ecology: Patterns and Strategies. In *Evolution* (Vol. 43). doi: 10.2307/2409595

Greenleaf, S. S., & Kremen, C. (2006). *Wild bees enhance honey bees ' pollination of hybrid sunflower*. (Track II).

Guezen, J. M., & Forrest, J. R. K. (2021). Seasonality of floral resources in relation to bee activity in agroecosystems. *Ecology and Evolution*, *11*(7), 3130–3147. doi: 10.1002/ece3.7260

Guitton, Y., Nicolè, F., Moja, S., Benabdelkader, T., Valot, N., Legrand, S., … Legendre, L. (2010). Lavender inflorescence: A model to study regulation of terpenes synthesis. *Plant Signaling and Behavior*, *5*(6), 749–751. doi: 10.4161/psb.5.6.11704

Hanley, N., Breeze, T. D., Ellis, C., & Goulson, D. (2015). Measuring the economic value of pollination services: Principles, evidence and knowledge gaps. *Ecosystem Services, 14*, 124–132. doi: 10.1016/j.ecoser.2014.09.013

Harder, L. D. (2000). Pollen dispersal and the floral diversity of monocotyledons. *Monocots: Systematics and Evolution. CSIRO, Melbourne*, 243–257.

Hendry, G. A., & Grime, J. P. (1993). *Methods in comparative plant ecology: a laboratory manual*. Springer Science \& Business Media.

Herbertsson, L., Lindström, S. A. M., Rundlöf, M., Bommarco, R., & Smith, H. G. (2016). Competition between managed honeybees and wild bumblebees depends on landscape context. *Basic and Applied Ecology*, *17*(7), 609–616. doi: 10.1016/j.baae.2016.05.001

Herrera, C. M. (1990). Daily Patterns of Pollinator Activity , Differential Pollinating Effectiveness , and Floral Resource Availability , in a Summer-Flowering Mediterranean Shrub Author (s): Carlos M . Herrera Published by : Wiley on behalf of Nordic Society Oikos Stable UR. *Oikos*, *58*(3), 277–288.

Herrera, C. M. (2020). Gradual replacement of wild bees by honeybees in flowers of the Mediterranean Basin over the last 50 years. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1921), 16–20. doi: 10.1098/rspb.2019.2657

Herrmann, J. D., Beye, H., de la Broise, C., Hartlep, H., & Diekötter, T. (2019). Positive effects of the pollinators Osmia cornuta (Megachilidae) and Lucilia sericata (Calliphoridae) on strawberry quality. *Arthropod-Plant Interactions*, *13*(1), 71–77. doi: 10.1007/s11829-018-9636-7

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), 1–37. doi: 10.1371/journal.pone.0158117

Holzschuh, A., Dainese, M., González-Varo, J. P., Mudri-Stojnić, S., Riedinger, V., Rundlöf, M., … Steffan-Dewenter, I. (2016). Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecology Letters*, *19*(10), 1228–1236. doi: 10.1111/ele.12657

Iriondo, J. M., Maxted, N., & Dulloo, M. E. (2008). *Conserving plant genetic diversity in protected areas: population management of crop wild relatives*. CABI.

Jakopic, J., Zupan, A., Eler, K., Schmitzer, V., Stampar, F., & Veberic, R. (2015). It's great to be the King: Apple fruit development affected by the position in the cluster. *Scientia Horticulturae*, *194*, 18–25. doi: 10.1016/j.scienta.2015.08.003

Jing, S., & Malladi, A. (2020). Higher growth of the apple (Malus × domestica Borkh.) fruit cortex is supported by resource intensive metabolism during early development. *BMC Plant Biology*, *20*(1), 75. doi: 10.1186/s12870-020-2280-2

Kara, M., Sayinci, B., Elkoca, E., Öztürk, I., & Özmen, T. B. (2013). Seed size and shape analysis of registered common bean (Phaseolus vulgaris L.) cultivars in Turkey using digital photography. *Tarim Bilimleri Dergisi*, *19*(3), 219–234. doi: 10.1501/tarimbil_0000001247

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2013). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1775). doi: 10.1098/rspb.2013.2440

Kleijn, D., Bommarco, R., Fijen, T. P. M., Garibaldi, L. A., Potts, S. G., & van der Putten, W. H. (2019). Ecological Intensification: Bridging the Gap between Science and Practice. *Trends in Ecology and Evolution*, *34*(2), 154–166. doi: 10.1016/j.tree.2018.11.002

Klein, A. M., Steffan-Dewenter, I., & Tscharntke, T. (2003). Bee pollination and fruit set of Coffea arabica and C. canephora (Rubiaceae). *American Journal of Botany*, *90*(1), 153–157. doi: 10.3732/ajb.90.1.153

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*, Vol. 274, pp. 303–313. doi: 10.1098/rspb.2006.3721

Knauer, A. C., Kokko, H., & Schiestl, F. P. (2021). Pollinator behaviour and resource limitation maintain honest floral signalling. *Functional Ecology*, *35*(11), 2536–2549. doi: 10.1111/1365-2435.13905

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., … Ashman, T. L. (2005). Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 467–497. doi: 10.1146/annurev.ecolsys.36.102403.115320

Kovács-Hostyánszki, A., Espíndola, A., Vanbergen, A. J., Settele, J., Kremen, C., & Dicks, L. V. (2017). Ecological intensification to mitigate impacts of conventional intensive land use on pollinators and pollination. *Ecology Letters*, *20*(5), 673–689. doi: 10.1111/ele.12762

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., … Ricketts, T. H. (2007). Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. *Ecology Letters*, *10*(4), 299–314. doi: 10.1111/j.1461-0248.2007.01018.x

Kron, P., Brian, C., Peter, G., & Kevan, G. (2001). Across- and along-row pollen dispersal in high-density apple orchards: Insights from allozyme markers. *The Journal of Horticultural Science and Biotechnology*, *76*(3), 286–294. doi: 10.1080/14620316.2001.11511365

Kron, P., & Husband, Æ. B. C. (2006). *The effects of pollen diversity on plant reproduction : insights from apple*. 125–131. doi: 10.1007/s00497-006-0028-2

Kron, P., Husband, B. C., Kevan, P. G., & Belaoussoff, S. (2001). *Factors Affecting Pollen Dispersal in High-density Apple Orchards*. *36*(6), 1039–1046.

Lajos, K., Samu, F., Bihaly, Á. D., Fülöp, D., & Sárospataki, M. (2021). Landscape structure affects the sunflower visiting frequency of insect pollinators. *Scientific Reports*, *11*(1), 1–11. doi: 10.1038/s41598-021-87650-9

Langenberger, M. W., & Davis, A. R. (2002). Temporal changes in floral nectar production, reabsorption, and composition associated with dichogamy in annual caraway (Carum carvi; Apiaceae). *American Journal of Botany*, *89*(10), 1588–1598. doi: 10.3732/ajb.89.10.1588

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Package "emmeans." *Mran.Microsoft.Com*. doi: 10.1080/00031305.1980.10483031>.License

Lloyd, D. G., & Schoen, D. J. (1992). Self- and Cross-Fertilization in Plants. I. Functional Dimensions. *International Journal of Plant Sciences*, *153*(3, Part 1), 358–369. doi: 10.1086/297040

Lobell, D. B., Cassman, K. G., & Field, C. B. (2009). Crop yield gaps: Their importance, magnitudes, and causes. *Annual Review of Environment and Resources*, *34*, 179–204. doi: 10.1146/annurev.environ.041008.093740

Loebnitz, N., & Grunert, K. G. (2018). The impact of abnormally shaped vegetables on consumers' risk perception. *Food Quality and Preference*, *63*(August 2017), 80–87. doi: 10.1016/j.foodqual.2017.08.004

Losey, J. E., & Vaughan, M. (2006). The economic value of ecological services provided by insects. *BioScience*, *56*(4), 311–323. doi: 10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2

Luo, E. Y., Ogilvie, J. E., & Thompson, J. D. (2014). Stimulation of flower nectar replenishment by removal: A survey of eleven animal-pollinated plant species. *Journal of Pollination Ecology*, *12*(7), 52–62. doi: 10.26786/1920-7603(2014)2

Malladi, A., Battapady, H., Hampton, R., & Jing, S. (2020). Determination of cortex and pith contributions to fruit morphology in apple (Malus×domestica Borkh.) using image analysis. *Scientia Horticulturae*, *271*(May), 109474. doi: 10.1016/j.scienta.2020.109474

Malladi, A., & Johnson, L. K. (2011). Expression profiling of cell cycle genes reveals key facilitators of cell production during carpel development, fruit set, and fruit growth in apple (Malus×domestica Borkh.). *Journal of Experimental Botany*, *62*(1), 205–219. doi: 10.1093/jxb/erq258

Marshall, D. L., Folsom, M. W., Hatfield, C., & Bennett, T. (1996). Does interference competition among pollen grains occur in wild radish? *Evolution*, *50*(5), 1842–1848. doi: 10.1111/j.1558-5646.1996.tb03570.x

Matsumoto, S., Eguchi, T., Maejima, T., & Komatsu, H. (2008). Effect of distance from early flowering pollinizers ‘Maypole’ and ‘Dolgo’ on ‘Fiji’ fruit set. *Scientia Horticulturae*, *117*(2), 151–159. doi: 10.1016/j.scienta.2008.03.025

Matsumoto, S., Soejima, J., & Maejima, T. (2012). Influence of repeated pollination on seed number and fruit shape of ‘ Fuji ’ apples. *Scientia Horticulturae*, *137,* 131–137. doi: 10.1016/j.scienta.2012.01.033

Michener, C. D. (2000). *The bees of the world* (Vol. 1). JHU press.

Milutinović, M., Šurlan-Momirović, G., & Nikolić, D. (1996). Relationship between pollinizer distance and fruit set in apple. *Acta Horticulturae*. doi: 10.17660/ActaHortic.1996.423.11

Morales, C. L., & Traveset, A. (2008). Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, *27*(4), 221–238. doi: 10.1080/07352680802205631

Morandin, L. A., & Winston, M. L. (2006). Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agriculture, Ecosystems and Environment*, *116*(3–4), 289–292. doi: 10.1016/j.agee.2006.02.012

Morse, R. A., & Calderone, N. W. (2000). The value of honey bees as pollinators of US crops in 2000. *Bee Culture*, *128*(3), 1–15.

Mouselimis, L., Machine, S., Johanne, B., Mohammad, H., & Achanta, R. (2020). CRAN - Package OpenImageR. Retrieved March 11, 2021, from CRAN R website: https://cran.r-project.org/web/packages/OpenImageR/index.html

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*, *234*(February), 409–430. doi: 10.1016/j.scienta.2017.12.057

Nepi, M., & Stpiczyńska, M. (2008). The complexity of nectar: Secretion and resorption dynamically regulate nectar features. *Naturwissenschaften*, *95*(3), 177–184. doi: 10.1007/s00114-007-0307-2

Neumann Andersen, M., Asch, F., Wu, Y., Richardt Jensen, C., Næsted, H., Overgaard Mogensen, V., & Elaine Koch, K. (2002). Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. *Plant Physiology*, *130*(2), 591–604. doi: 10.1104/pp.005637

Neumann, K., Verburg, P. H., Stehfest, E., & Müller, C. (2010). The yield gap of global grain production: A spatial analysis. *Agricultural Systems*, *103*(5), 316–326. doi: 10.1016/j.agsy.2010.02.004

Nicolson, S. W., & Nepi, M. (2005). Dilute nectar in dry atmospheres: Nectar secretion patterns in Aloe castanea (Asphodelaceae). *International Journal of Plant Sciences*, *166*(2), 227–233. doi: 10.1086/427616

Nuru, A., Al-Ghamdi, A. A., Tena, Y. T., Shenkut, A. G., Ansari, M. J., & Al-Maktary, A. (2015). Floral phenology, nectar secretion dynamics, and honey production potential, of two lavender species (Lavandula dentata, and l. pubescens) in southwestern saudi arabia. *Journal of Apicultural Science*, *59*(2), 135–144. doi: 10.1515/JAS-2015-0028

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

Ordano, M., & Ornelas, J. F. (2004). Generous-like flowers: Nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia*, *140*(3), 495–505. doi: 10.1007/s00442-004-1597-0

Ornelas, J. F., & Lara, C. (2009). Nectar replenishment and pollen receipt interact in their effects on seed production of Penstemon roseus. *Oecologia*, *160*(4), 675–685. doi: 10.1007/s00442-009-1337-6

Pacini, E., & Nepi, M. (2007). Chapter 4 NECTAR PRODUCTION AND PRESENTATION. *Knowledge Creation Diffusion Utilization*, 167–214.

Parachnowitsch, A. L., Manson, J. S., & Sletvold, N. (2019). Evolutionary ecology of nectar. *Annals of Botany*, *123*(2), 247–261. doi: 10.1093/aob/mcy132

Pecenka, J. R., Ingwell, L. L., Foster, R. E., Krupke, C. H., & Kaplan, I. (2021). IPM reduces insecticide applications by 95% while maintaining or enhancing crop yields through wild pollinator conservation. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(44). doi: 10.1073/pnas.2108429118

Picarella, M. E., & Mazzucato, A. (2019). The occurrence of seedlessness in higher plants; insights on roles and mechanisms of parthenocarpy.

Frontiers in Plant Science, *9*(January), 1–11. doi: 10.3389/fpls.2018.01997

Piccaglia, R., & Marotti, M. (2001). Characterization of some Italian types of wild fennel (Foeniculum vulgare mill.). *Journal of Agricultural and Food Chemistry*, *49*(1), 239–244. doi: 10.1021/jf000636+

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 & Evolution*, *25*(6), 345–353. doi: 10.1016/j.tree.2010.01.007

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., … Vanbergen, A. J. (2016, December 8). Safeguarding pollinators and their values to human well-being. *Nature*, Vol. 540, pp. 220–229. Nature Publishing Group. doi: 10.1038/nature20588

Potts, S. G., Imperatriz Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L., … others. (2016). Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production.

Pretty, J. (2008, February 12). Agricultural sustainability: Concepts, principles and evidence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol. 363, pp. 447–465. Royal Society. doi: 10.1098/rstb.2007.2163

Quinet, M., & Jacquemart., A.-L. (2017). Cultivar placement affects pollination efficiency and fruit production in European pear (Pyrus communis) orchards. *European Journal of Agronomy*, *91*(January), 84–92. doi: 10.1016/j.eja.2017.09.015

Quinlan, G., Milbrath, M., Otto, C., Smart, A., Iwanowicz, D., Cornman, R. S., & Isaacs, R. (2021). Honey bee foraged pollen reveals temporal changes in pollen protein content and changes in forager choice for abundant versus high protein flowers. *Agriculture, Ecosystems and Environment*, *322*(August), 107645. doi: 10.1016/j.agee.2021.107645

Ramírez, F., & Davenport, T. L. (2013). Apple pollination: A review. *Scientia Horticulturae*, *162*, 188–203. doi: 10.1016/j.scienta.2013.08.007

Ramos, D. de L., Bustamante, M. M. C., Da Silva E Silva, F. D., & Carvalheiro, L. G. (2018). Crop fertilization affects pollination service provision – Common bean as a case study. *PLoS ONE*, *13*(11), 1–16. doi: 10.1371/journal.pone.0204460

Requier, F., & Leonhardt, S. D. (2020). Beyond flowers: including non-floral resources in bee conservation schemes. *Journal of Insect Conservation*, *24*(1), 5–16. doi: 10.1007/s10841-019-00206-1

Rollin, O., Bretagnolle, V., Fortel, L., Guilbaud, L., & Henry, M. (2015). Habitat, spatial and temporal drivers of diversity patterns in a wild bee assemblage. *Biodiversity and Conservation*, *24*(5), 1195–1214. doi: 10.1007/s10531-014-0852-x

Rollin, O., & Garibaldi, L. A. (2019). Impacts of honeybee density on crop yield: A meta-analysis. *Journal of Applied Ecology*, *56*(5), 1152–1163. doi: 10.1111/1365-2664.13355

Ropars, L., Dajoz, I., Fontaine, C., Muratet, A., & Geslin, B. (2019). Wild pollinator activities negatively related to honey bee colony densities in urban context. *PloS One*, 1–16. doi: 10.1101/667725

Roulston, T. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual Review of Entomology*, *56*, 293–312. doi: 10.1146/annurev-ento-120709-144802

Ruan, Y. L., Patrick, J. W., Bouzayen, M., Osorio, S., & Fernie, A. R. (2012). Molecular regulation of seed and fruit set. *Trends in Plant Science*, *17*(11), 656–665. doi: 10.1016/j.tplants.2012.06.005

Sabliov, C. M., Boldor, D., Keener, K. M., & Farkas, B. E. (2002). Image processing method to determine surface area and volume of axi-symmetric agricultural products. *International Journal of Food Properties*, *5*(3), 641–653. doi: 10.1081/JFP-120015498

Sáez, A., di Virgilio, A., Tiribelli, F., & Geslin, B. (2018). Simulation models to predict pollination success in apple orchards: a useful tool to test management practices. *Apidologie*, *49*(5), 551–561. doi: 10.1007/s13592-018-0582-2

Sáez, A., Morales, C. L., Garibaldi, L. A., & Aizen, M. A. (2017). Invasive bumble bees reduce nectar availability for honey bees by robbing raspberry flower buds. *Basic and Applied Ecology*, *19*, 26–35. doi: 10.1016/j.baae.2017.01.001

Sáez, A., Morales, C. L., Ramos, L. Y., & Aizen, M. A. (2014). Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology*, *51*(6), 1603–1612. doi: 10.1111/1365-2664.12325

Sáez, A., Negri, P., Viel, M., & Aizen, M. A. (2019). Pollination efficiency of artificial and bee pollination practices in kiwifruit. *Scientia Horticulturae*, *246*(August 2018), 1017–1021. doi: 10.1016/j.scienta.2018.11.072

Schneider, D., Stern, R. A., & Goldway, M. (2005). A comparison between semi- and fully compatible apple pollinators grown under suboptimal pollination conditions. *HortScience*, *40*(5), 1280–1282.

Schurr, L., Geslin, B., Affre, L., Gachet, S., Delobeau, M., Brugger, M., … Masotti, V. (2021). Landscape and Local Drivers Affecting Flying Insects along Fennel Crops (Foeniculum vulgare, Apiaceae) and Implications for Its Yield. *Insects*, *12*(5), 404. doi: 10.3390/insects12050404

Schurr, L., Masotti, V., Geslin, B., Gachet, S., Mahe, P., Jeannerod, L., & Affre, L. (2021). Is Fennel Crop Dependent on Insect Pollination? *SSRN*

Electronic Journal. doi: 10.2139/ssrn.3953077

Sharma, H. K., Gupta, J. K., & Thakur, J. R. (2003). Effect of bee pollination and polliniser proportion on apple productivity. *VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics 662*, 451–454.

Sheffield, C.S. (2014). Pollination, seed set and fruit quality in apple: studies with Osmia lignaria (Hymenoptera: Megachilidae) in the Annapolis Valley, Nova Scotia, Canada. *Journal of Pollination Ecology*, *12*(13), 120–128. doi: 10.26786/1920-7603(2014)11

Sheoran, I. S., & Saini, H. S. (1996). Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. *Sexual Plant Reproduction*, *9*(3), 161–169.

Silvertown, J. (2008). The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences*, *169*(1), 157–168. doi: 10.1086/523357

Smith-Ramírez, C., Martinez, P., Nuñez, M., González, C., & Armesto, J. J. (2005). Diversity, flower visitation frequency and generalism of pollinators in temperate rain forests of Chiloé Island, Chile. *Botanical Journal of the Linnean Society*, *147*(4), 399–416. doi: 10.1111/j.1095-8339.2005.00388.x

Sokal, R. R., & Rohlf, F. J. (1995). Biometry. Third edition. In *Biometry Third edition*.

Southwick, E. E., & Southwick, L. (1992). Estimating the Economic Value of Honey Bees (Hymenoptera: Apidae) as Agricultural Pollinators in the United States. *Journal of Economic Entomology*, *85*(3), 621–633. doi: 10.1093/jee/85.3.621

Stahl, J. M., Nepi, M., Galetto, L., Guimarães, E., & MacHado, S. R. (2012). Functional aspects of floral nectar secretion of Ananas ananassoides, an ornithophilous bromeliad from the Brazilian savanna. *Annals of Botany, 109*(7), 1243–1252. doi: 10.1093/aob/mcs053

Steffan-Dewenter, I., & Tscharntke, T. (2000). Resource overlap and possible competition between honey bees and wild bees in central Europe. *Oecologia*, *122*(2), 288–296. doi: 10.1007/s004420050034

Stephens, D. W., & Krebs, J. R. (1987). Foraging Theory. In *Princeton UniversityPress, Princeton, NJ*. Princeton University Press. doi: 10.1515/9780691206790

Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, *65*(1), 13–21. doi: 10.1007/s00265-010-1037-6

Takayama, S., & Isogai, A. (2005). Self-incompatibility in plants. *Annual Review of Plant Biology*, *56*, 467–489. doi: 10.1146/annurev.arplant.56.032604.144249

Tamburini, G., Berti, A., Morari, F., & Marini, L. (2016). Degradation of soil fertility can cancel pollination benefits in sunflower. *Oecologia*, *180*(2), 581–587. doi: 10.1007/s00442-015-3493-1

Tamburini, G., Bommarco, R., Kleijn, D., van der Putten, W. H., & Marini, L. (2019). Pollination contribution to crop yield is often context-dependent: A review of experimental evidence. *Agriculture, Ecosystems and Environment*, *280*(April), 16–23. doi: 10.1016/j.agee.2019.04.022

Tamburini, G., Lami, F., & Marini, L. (2017). Pollination benefits are maximized at intermediate nutrient levels. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1860). doi: 10.1098/rspb.2017.0729

Thompson, J. D. (2001). How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia*, *126*(3), 386–394. doi: 10.1007/s004420000531

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002, August 8). Agricultural sustainability and intensive production practices. *Nature*, Vol. 418, pp. 671–677. Nature Publishing Group. doi: 10.1038/nature01014

Timberlake, T. P., Vaughan, I. P., Baude, M., & Memmott, J. (2021). Bumblebee colony density on farmland is influenced by late-summer nectar supply and garden cover. *Journal of Applied Ecology*, *58*(5), 1006–1016. doi: 10.1111/1365-2664.13826

Timberlake, T. P., Vaughan, I. P., & Memmott, J. (2019). Phenology of farmland floral resources reveals seasonal gaps in nectar availability for bumblebees. *Journal of Applied Ecology*, *56*(7), 1585–1596. doi: 10.1111/1365-2664.13403

Torné-Noguera, A., Rodrigo, A., Osorio, S., & Bosch, J. (2016). Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. *Basic and Applied Ecology*, *17*(3), 199–209. doi: 10.1016/j.baae.2015.11.004

Traveset, A. (1993). Deceptive fruits reduce seed predation by insects in Pistacia terebinthus L. (Anacardiaceae). *Evolutionary Ecology*, *7*(4), 357–361. doi: 10.1007/BF01237867

Vanbergen, A. J., & Initiative, the I. P. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, *11*(5), 251–259. doi: 10.1890/120126

Vergara, C. H., & Badano, E. I. (2009). Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. *Agriculture, Ecosystems and Environment*, *129*(1–3), 117–123. doi: 10.1016/j.agee.2008.08.001

Vicens, N., & Bosch, J. (2000). Weather-dependent pollinator activity in an apple orchard, with special reference to Osmia cornuta and Apis mellifera (Hymenoptera: Megachilidae and Apidae). *Environmental Entomology*, *29*(3), 413–420. doi: 10.1603/0046-225X-29.3.413

Vivek Venkatesh, G., Iqbal, S. M., Gopal, A., & Ganesan, D. (2015). Estimation of volume and mass of axi-symmetric fruits using image processing technique. *International Journal of Food Properties*, *18*(3), 608–626. doi: 10.1080/10942912.2013.831444

Wagenius, S. (2019). Scale Dependence of Reproductive Failure in Fragmented Echinacea Populations. Published by : Wiley on behalf of the Ecological Society of America Stable URL *: https://www.jstor.org/stable/20069023 REFERENCES Linked references . 87*(4), 931–941.

Webb, C. J. (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. dichogamy. *New Zealand Journal of Botany*, *24*(1), 135–162. doi: 10.1080/0028825X.1986.10409725

Webber, S. M., Garratt, M. P. D., Lukac, M., Bailey, A. P., Huxley, T., & Potts, S. G. (2020). Quantifying crop pollinator-dependence and pollination deficits: The effects of experimental scale on yield and quality assessments. *Agriculture, Ecosystems & Environment*, *304*(January), 107106. doi: 10.1016/j.agee.2020.107106

Weller, H. (2019). Package countcolors. *CRAN R*. Retrieved from https://cran.r-project.org/web/packages/countcolors/index.html

Wendin, K., Egan, P. A., Olsson, V., Forsberg, S., Nilsson, A., & Stenberg, J. A. (2019). Is there a best woodland strawberry? A consumer survey of preferred sensory properties and cultivation characteristics. *International Journal of Gastronomy and Food Science*, *16*(January), 100151. doi: 10.1016/j.ijgfs.2019.100151

Wignall, V. R., Brolly, M., Uthoff, C., Norton, K. E., Chipperfield, H. M., Balfour, N. J., & Ratnieks, F. L. W. (2020). Exploitative competition and displacement mediated by eusocial bees: experimental evidence in a wild pollinator community. *Behavioral Ecology and Sociobiology*, *74*(12). doi: 10.1007/s00265-020-02924-y

Wignall, V. R., Campbell Harry, I., Davies, N. L., Kenny, S. D., McMinn, J. K., & Ratnieks, F. L. W. (2020). Seasonal variation in exploitative competition between honeybees and bumblebees. *Oecologia*, *192*(2), 351–361. doi: 10.1007/s00442-019-04576-w

Willmer, P. (2011). *Pollination and floral ecology* (P. U. Press, Ed.). Princeton.

Wood, S. N. (2017). *Generalized additive models: an introduction with R*. CRC press.

Wood, S., & Wood, M. S. (2015). Package 'mgcv.' *R Package Version*, *1*, 29.

Wu, P., Tscharntke, T., Westphal, C., Wang, M., Olhnuud, A., Xu, H., … Liu, Y. (2021). Bee abundance and soil nitrogen availability interactively modulate apple quality and quantity in intensive agricultural landscapes of China. *Agriculture, Ecosystems and Environment*, *305*(February 2020), 107168. doi: 10.1016/j.agee.2020.107168

Ye, Z. M., Jin, X. F., Wang, Q. F., Yang, C. F., & Inouye, D. W. (2017). Nectar replenishment maintains the neutral effects of nectar robbing on female reproductive success of Salvia przewalskii (Lamiaceae), a plant pollinated and robbed by bumble bees. *Annals of Botany*, *119*(6), 1053–1059. doi: 10.1093/aob/mcw285

Zangerl, A. R., Berenbaum, M. R., & Nitao, J. K. (1991). Parthenocarpic fruits in wild parsnip: Decoy defence against a specialist herbivore. *Evolutionary Ecology*, *5*(2), 136–145. doi: 10.1007/BF02270830

Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. *One Earth*, *4*(1), 114–123. doi: 10.1016/j.oneear.2020.12.005

7. ANNEX 1

Conference presentations: (*speaker)

*Díaz, S. S., **Carisio**, L., Manino, A., Biella, P., & Porporato, M. (2021). Interazioni tra l'ape gigante della resina introdotta in Europa e la fauna locale. Congresso Nazionale Italiano di Entomologia, 10 june 2021, Torino (Italia).

*Díaz, S. S., **Carisio**, L., Ponso, S., Manino, A., & Porporato, M. (2020). Effetto della configurazione del campo e della densità di "pollinizer" sul servizio di impollinazione in meleto. Agroinsecta, 26 sebtember 2019, Rivalta Scrivia (Italia).

***Carisio** L., Bianchi E., Lioy S., Manino L., Porporato M. (2019). Does *Vespa velutina* impact on native insects?. Vespa velutina and other invasive invertebrates species. LIFE STOPVESPA meeting, 22 march 2019, Torino (Italia).

Carisio L., Lioy S., Porporato M., *Manino A., (2018). Survey of wild bee communities threatened by Vespa velutina. EurBee 8° Congress of Apidology, 18 sebtember, Gent (Belgio).

Posters:

Carisio, L., Schurr, L., Geslin, B. (2021). Daily nectar productions of fennel (*Foeniculum vulgare*) and lavender (*Lavandula hybrida*) are affected by nectar dynamics and pollinator visitations, Ecology across borders, 12-15 december 2021, Liverpool (UK).

Carisio, L., Cerri, J., Lioy, S., Bianchi, E., Bertolino, S., & Porporato, M. (2021). Impatto di *Vespa velutina* sui Vespinae nativi in Liguria, Congresso Nazionale Italiano di Entomologia, 10 june 2021, Torino (Italia).

Carisio L., Bianchi E., Lioy S., Manino L., Porporato M., (2019). Wild bee communities
across Vespa velutina invasion gradient. Vespa velutina and other invasive invertebrates
species. LIFE STOPVESPA meeting, 22 march 2019, Torino (Italia).

Laurino D., **Carisio** L., Lioy S., Manino A., Bianchi E., Porporato M. (2019). Impact of *Vespa velutina* on honeybees and other pollinators. Apimondia International Apicultural Congress, 8-12 september, Montreal (Canada)

Carisio L., Manino A., Lioy S., Sottostanti G., Porporato M. (2018). Is *Vespa velutina* a threat to wild bee communities and pollination ecosystem service?. XI European Congress of Entomology, 2-6 july 2019, Napoli (Italia)

8. ANNEX 2

Published papers from project outside the Phd objectives:

Díaz, S. S., **Carisio**, L., Manino, A., Biella, P., & Porporato, M. (2021). Nesting, Sex Ratio and Natural Enemies of the Giant Resin Bee in Relation to Native Species in Europe. *Insects*, *12*(6), 545.

Rubiola, S., Moroni, B., **Carisio**, L., Rossi, L., Chiesa, F., Martano, G., ... & Rambozzi, L. (2021). Risk Factors for Bovine Cysticercosis in North-West Italy: A Multi-Year Case-Control Study. *Animals*, *11*(11), 3049.

Laurino, D., Lioy, S., **Carisio**, L., Manino, A., & Porporato, M. (2020). Vespa velutina: An alien driver of honey bee colony losses. *Diversity*, *12*(1), 5.

Preprint:

Carisio, L., Cerri, J., Lioy, S., Bianchi, E., Bertolino, S., & Porporato, M. (2020). Introduced Vespa velutina does not replace native Vespa crabro and Vespula species. EcoEvoRViX