

Project title: Cucurbit Pollination: Mechanisms and Management to Improve Field Quality and Quantity

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The results and conclusions in this report are based on an investigation conducted over a three-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

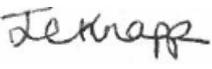
AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- Honeybees (*Apis mellifera*) and buff-tailed bumblebees (*Bombus terrestris*) were the most abundant pollinators of courgette. *B. terrestris* had a more equal preference for visiting male and female courgette flowers and carried more loose pollen grains than *A. mellifera*. Therefore *B. terrestris* is likely to be the most effective pollinator of courgette.
- Full (hand pollination) increased courgette yield by 39% when compared to the yield when pollination was excluded. However, there was no evidence of pollination limitation (by insect pollinators) on crop yields when compared to the hand/full pollination.
- There was no evidence that (i) that wild flowers 'distract' pollinators from courgette flowers and (ii) putting commercial colonies of *B. terrestris* increase yields, in this study.

Background

Negative impacts from intensifying agriculture have generated concerns that pollinator-dependent crop species, such as courgette *Cucurbita pepo* L., may be experiencing a pollination deficit. This project explores the extent to which pollination influences fruit set; how pollination could be improved; and how in doing so growers' profits and agricultural resilience could increase, using UK field-grown courgettes as a model system.

This study is the first to explore cucurbit pollination in the United Kingdom. Consequently, no information was available prior to this study about how effective UK species were at pollinating cucurbits. Likewise, no work had been done on the pollinator dependence of "Tosca", a popular variety of courgette in the UK and the principle variety used in this study; whether these plants were experiencing a pollination deficit, and indeed, the economic value of pollination to UK courgette production. Based on this information floral resources influencing the most effective pollinators were explored, and the effect of cucurbit nectar and pollen on bumblebee population dynamics, using computer simulations was explored for the first time.

Accordingly, this research directly addresses priorities on the Outdoor Cucurbit Research and Development Priority List: "Pollination for fruit quality: supporting pollinating insects", and the Agriculture and Horticulture Development Board's Field Vegetable Sector Priority List: "To supply consistent quality product and continuity and to achieve customer satisfaction", as well as: "making efficient use of resources to improve returns".

Therefore, by understanding courgette pollination dynamics within the context of UK growing conditions, the project aimed to give commercial growers practical management options that

would allow them to improve the quantity and quality of their yield, supporting sustainability and profitability. These findings are also relevant to hobbyist growers as well as the wider public by promoting the value of pollination to horticultural crop production.

Three key areas of courgette pollination are addressed in this project (highlighted with solid arrows in Figure 1). The first explores the mechanism, variability, and economic value of courgette pollination. The second looks at ways of improving pollination within courgette fields. Finally, the third explores the mutualism between courgette and its key pollinator species.

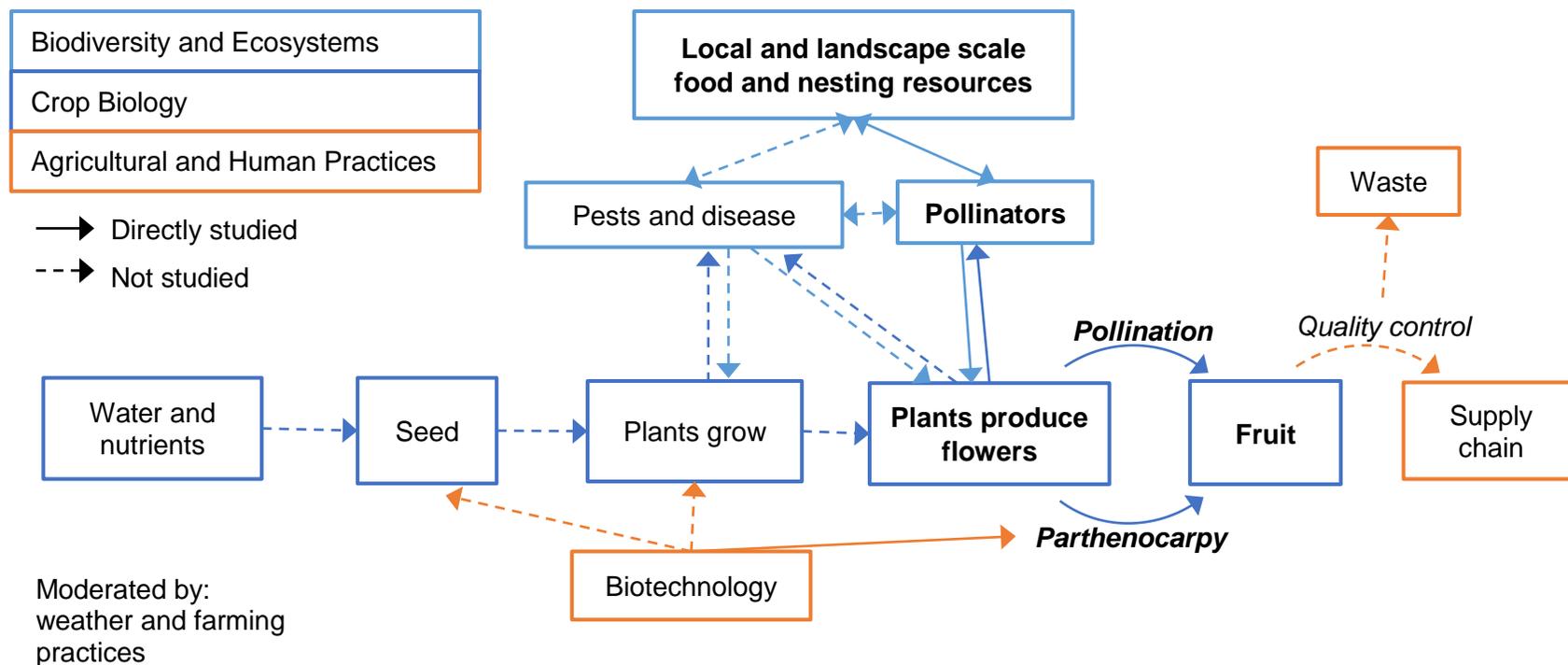


Figure 1 Conceptual framework of the different factors (broadly categorised into crop biology, agricultural and human practices, and biodiversity and ecosystems) which effect fruit set in pollinator-dependent crops. Solid lines and bold text show factors which were directly studied whilst dashed lines and plain text show factors which were considered but not directly studied in this project.

Summary

Funding Objective 1: What are the most effective wild pollinators of field-grown courgettes in South West UK in terms of visitation rate and pollen transfer efficiency?

Quantifying the effectiveness of individual pollinator species can help growers target their pollination management to species most likely to increase yields. Pollinator surveys were conducted throughout the project to determine which pollinator species were most abundant, had the highest visitation rate to male and female courgette flowers and transferred the most courgette pollen. Buff-tailed bumblebees (*B. terrestris*) and honeybees (*A. mellifera*) were the most abundant pollinator species in courgette fields and had the highest visitation rates to courgette flowers. Of these two species *B. terrestris* had a more equal preference to male and female courgette flowers and transferred the most pollen grains. Therefore, *B. terrestris* is likely to be the most effective pollinator of courgette in the South West of the UK.

Funding Objective 2: Does pollination deficit limit yield (number, quality of fruits)?

Courgette production in the UK is estimated to be worth £6.7 million. However, little is known about this crop's requirement for insect-mediated pollination (pollinator dependence) and if pollinator populations in the landscape can fulfil its pollination needs (pollination deficit). Consequently, pollination experiments were conducted to ask to what extent does pollination influence fruit set and if field grown courgettes were experiencing a pollination deficit? Results showed that full or hand pollination increased yield by 39% but there was no evidence of insect pollination limitation on crop yield. This was evidenced by a surprisingly low pollination deficit of just 3% between the hand pollinated and the open pollinated crop and there was no statistical difference in yield (length grown, circumference, and weight) between the open- and hand-pollinated crops. Nonetheless, the high economic value of courgettes means that reducing even the small pollination deficit could still increase profit by ~ £166/ha. Interestingly, 56% of fruit was able to reach marketable size and shape without any pollination – this finding led to the parthenocarpy meta-analysis presented in the science section. Results are discussed in the context of the economic value of pollination to courgette production in the UK.

Funding Objective 3: Does the introduction of commercial bumblebees improve yield?

Introducing commercial bumblebee colonies or honeybee hives can interrupt the damaging cycle of lower yields from a reduced diversity and abundance of wild pollinators, often caused by losses in (semi-) natural habitat. To explore if commercial colonies of *B. terrestris* improved courgette yield, colonies were placed into a field at the stocking density recommended by

Koppert Biological Systems. There was no difference in courgette yield when *B. terrestris* colonies were open, compared to when they were closed. This is likely due to the already high level of 'natural' pollination at study sites.

Funding Objective 4: Does the introduction of a flower strip between rows or along a field edge alter the effectiveness of pollination? If pollinators are supported with other floral resources do they visit the courgette flowers more (attraction) or less (distraction)?

Allocation of floral resources to increase pollinator abundance is the primary basis for pollinator-supportive land management; however, relatively little is known about how the scale of floral resources (field or farm) may affect different pollinator groups which may or may not pollinate the focal crop. Bumblebees were significantly more abundant on courgette flowers in fields with a greater species richness of wild flowers which would be viewed as weeds in the crop. No flower-strips were grown between the rows in this study. Although solitary bees were not observed to visit courgette flowers, their abundance and species richness in courgette fields were significantly greater with more semi-natural habitat and a greater species richness of wild flowers. For both honeybees and bumblebees, their abundance in field margins did not significantly reduce their abundance on courgette flowers and both species show a preference for courgette flowers in the morning when flowers are open, before 'switching' to wild flowers around the cropped area when courgette flowers are closed. These findings suggest that wild flowers do not compete with courgette flowers for pollination services. Indeed, wild flowers help to fulfil bees' nutritional requirements beyond the nectar and pollen provided by courgette.

Funding Objective 5: Is there an interaction between nitrogen and pollination levels?

Whilst several studies have explored the positive, mediating effect of forage availability on pollinator visitation and crop yield many overlook other factors which influence yield (pre or post pollination) such as, soil quality, water availability, weather conditions, and farming practices. Using the experimental design for funding objective 4, additional data were collected on the farming intensity (e.g. the type of farm machinery used during picking, the number of herbicide, fungicide and fertiliser applications and cropping practices), soil nutrients and yield. Results showed that region, field size and nutrients were not important predictors of courgette yield. Instead, bumblebee abundance on flowers in the field margin was the most important factor for increasing courgette yield (although this was not statistically significant). There was also no evidence of competition for soil resources between wild flowers and courgette flowers, with neither floral abundance nor floral species richness negatively affecting courgette yield.

Unfortunately, the limited sample size of eight fields meant that it was not possible to test the interaction of soil nutrients and pollination.

Funding Objective 6: Design and run a citizen science project to record courgette pollination within garden/ allotment systems in comparison to commercial systems.

This objective was not achieved because it was clear that it was not going to give useful results to the UK commercial growing industry. Instead a meta-analysis exploring parthenocarpy (fruit set in the absence of pollination) in horticultural crops was judged to be more important to growers. This meta-analysis is provided as supplementary information in the science section of the full report. Results showed genetic modification, hormone application and selective breeding for parthenocarpy were able to significantly increase fruit quantity and quality in 18 usually pollinator-dependent crop species. The effect of courgette nectar and pollen on wild bee populations was also thought to be more important - model simulations using Bumble-BEEHAVE showed that early season courgette increased the number of hibernating queens, colonies, and foragers.

Pollination workshop

Findings of this PhD project have been presented to outdoor cucurbit growers group (2015, 2016 and 2018), as well as within annual reports and the cucurbit pollination factsheet.

Whilst a single overarching workshop was not held, bespoke expert-advisory meetings were held with Riviera Produce, Southern England Farms, Trevaskis Farm, and the Hall-Hunter partnership on pollination of their crops. Meetings continue with growers and farmers across the South West UK to provide bespoke pollinator management advice under the BEE-STEWARD project. BEE-STEWARD combines the latest research on pollinators to create an environment that benefits pollinators, farmers, businesses and society. A demonstration and discussion of the BEE-STEWARD support tool can be found in the supplementary information at the end of this report.

Financial Benefits

Since 41% of courgette yield is dependent on pollination the total economic value of insect pollination to courgettes is estimated to be worth approximately £3,398/ha (Table 1). Due to high levels of open pollination observed in Cornwall, pollination deficit was estimated to be just 3%. Nevertheless, if pollination was maximised, the economic value of courgettes would increase by approximately £166/ha (Table 1).

Table 1 Calculation of the economic value of pollinators to courgette production at a hectare and national scale. P was 0.43 £/ha (DEFRA 2016). Total area of UK courgette production is 807.75 ha (British Growers Association, personal communication 22nd September 2016). D was 0.41 and D_{max} 0.43 calculated from experimental results for funding objective 2.

	Economic value (£)	
	Per ha	UK Value
Quantity Q (Kg)	19,274	-
Economic value EV (£)	8,288	6,694,632
Total economic value of insect pollination $IPEV$ (£)	3,398	2,744,735
Maximum economic value of pollination service $MaxEV$ (£)	3,564	2,878,821
Value of pollination deficit $PDef$ (£)	166	134,086

Action Points

Since the total economic value of insect pollination to courgette is estimated to be worth £3,398 per ha growers may wish to:

- 1) **Preserve their wild flowers within, and on the edge of fields as a way of attracting pollinators into courgette fields.** Wild flowers facilitate pollination services to courgette and support pollinator nutrition. There is no evidence that they 'distract' pollinators from courgette flowers.
- 2) **Use parthenocarpic varieties, in combination with other environmentally considerate practices to achieve stable pollination.** Parthenocarpy could be advantageous to all crops, whether they are experiencing a pollination deficit or not by improving fruit uniformity caused by stochastic poor pollination. In return, these parthenocarpic crops can continue to provide valuable nectar and pollen resources for wild and managed bees, and other flower-visiting insects
- 3) **Plant courgette near to previous courgette crops (within the season and over time) to utilise local bumblebee populations which may have been boosted by the availability of courgette nectar in the past.** However, bees will also need alternative floral resources to fulfil their nutritional requirements for pollen and nectar over space and time.

SCIENCE SECTION

Introduction



Figure 2 *B. terrestris* visiting a pistillate courgette flower for nectar. Photograph taken by Daphne Wong.

Optimising ecosystem services

Globally, agricultural land is continuing to expand and intensify to meet rising food demands (Bommarco et al., 2013). Although successful for increasing yields, the loss and/or simplification of natural habitats combined with the replacement of many biological functions with artificial inputs has negatively affected the resilience and productivity of agricultural systems (Bommarco et al., 2013; Hooper et al., 2005). Sustainable management of agricultural systems requires artificial inputs to be replaced or complemented with regulating and supporting ecosystem service management or 'environmentally-friendly' practices to enhance crop productivity (Bommarco et al., 2013; Tilman et al., 2002). In doing so, producers can improve yields (Bommarco et al., 2013) and minimise negative impacts from intensive agriculture such as: habitat loss, nutrient runoff, and pesticide poisoning of non-target species (Tilman et al. 2002; Zhang et al. 2007; Power 2010; Pretty & Bharucha 2014).

Insect-mediated pollination (the transfer of pollen within or between flowers via an insect) is a key regulating service for many crops and wild plants; with 75% of global crop plants requiring insect pollination for fruit set, a figure which contains some of the most nutritional and culturally

important components of human diet (Klein et al., 2007). Indeed worldwide pollination services are estimated to be worth \$153 billion, representing 9.5% of worldwide agricultural production used for human food (Gallai et al., 2009). However, observed losses of pollinator populations combined with our dependence on their contribution to food security, has led to a widespread concern that we are facing a 'pollinator crisis' (Steffan-Dewenter *et al.* 2005; Potts *et al.* 2010; although see Ghazoul 2005). This has been exacerbated by a disproportionate increase in the area of land producing pollinator-dependent crops (relative to nondependent crops); increasing the demand for pollination services (Aizen et al., 2008).

To this end crop producers frequently rely on managed pollinator species to fulfil their pollination needs (Mader et al., 2010). Increasing the abundance of species such as *Apis mellifera* L. (the Western honeybee) can interrupt the damaging cycle of lower yields resulting from a reduced abundance and species richness of wild pollinators, often caused by losses in (semi-) natural habitat (Garibaldi et al., 2011). This is because wild and managed bee populations are limited by the abundance, diversity, and proximity to food and nesting sites which can be provided in (semi-) natural habitat (Roulston and Goodell, 2011). At a field scale, floral resources can be enhanced by planting wild flower strips, allowing areas to be naturally colonised by wild flowers, and maintaining floriferous hedgerows. At a farm scale, proximity to, or quantity of natural and semi-natural habitat can increase pollinator abundance as they spill into crop areas (Garibaldi et al., 2011). Nonetheless, the effectiveness of pollinator-supporting practices are often variable and greatly depend on the complexity of the habitat surrounding a crop field, with more simplistic landscapes generally showing greater increases in pollinator abundance following management interventions than more complex landscapes (Batáry, Báldi, Kleijn, & Tschardtke, 2011; Scheper *et al.*, 2013; Herbertsson *et al.*, 2018).

Indeed, nectar and pollen from pollinator-dependent crop flowers (Figures 2 & 3) can also provide pollinators with a substantial source of food (Bailes et al., 2015; Holzschuh et al., 2016, 2013; Westphal et al., 2003). This mutualistic relationship means that crop flowers can directly influence their own pollination success. In the short term, pollinators may be transiently attracted into the crop, enhancing their densities at crop flowers (Holzschuh et al., 2016). Whilst in the longer term, phenological matching of crop flowering and pollinator activity could increase colony establishment and development (Bailes *et al.* 2015; although see Holzschuh *et al.* 2016). Interestingly, manipulating floral rewards to encourage pollinator visitation via selective breeding, has generally received little attention (Bailes et al., 2015).

Measuring pollination success

In order to target pollinator management to species most likely to increase yield, much research has focused on quantifying pollinator performance in crops (Rader et al., 2016). Broadly, there are two approaches for quantifying species-level pollinator performance: the first estimates pollinator behaviour and/ or pollen deposition on stigmas (funding objective 1), whilst the second estimates the pollinator's contribution to yield, usually measured as seed set or fruit weight (Ne'eman et al., 2010). Arguably, when other environmental factors which influence fruit production e.g. soil type and cultivation practices cannot be standardised, single visit pollen deposition may be the most direct measure of pollination success (Kremen et al., 2004). However, species-level effectiveness does not take into account the effectiveness of an entire pollinator community for a plant species in a given space or time (Willcox et al., 2017). Thus, in funding objective 2, the contribution of all species to courgette fruit set was studied. For example, Willcox *et al.* (2017) identify that competitive or facilitative pollinator interactions and/or conspecific or heterospecific pollen transfer, observed by studying community-level effectiveness, may separately (or in combination) affect a plant's reproductive success.

Cucurbits

Cucurbitaceae (Cucurbits or gourds) are a large plant family which include major food plants such as *Cucurbita* (squash, pumpkin, courgette), *Cucumis* (cucumber, melon), and *Citrullus* (watermelon) (Kumar, 2016). Over centuries cucurbits have been domesticated for their fleshy fruits, roots, leaves, shoots, seeds and flowers for food and commodity goods and are therefore, economically important crops (Bates et al., 1990). Cultivated cucurbits can be grown in a variety of agricultural environments; from widespread monocultures to small-scale, traditional garden systems and many are able to persist in environmental conditions usually considered marginal for agriculture (Bates et al., 1990).

From a biological viewpoint, cucurbits' co-evolution with insects has provided much scientific intrigue. For example, their ability to produce bitter cucurbitacins has led to research into whether or not these compounds can be used for biological control, particularly against beetles (Metcalf et al., 1982). Likewise, cucurbits' dependency on pollination (Free, 1993) means cucurbit flowers offer large quantities of nectar and pollen as floral rewards to visiting insects such as solitary bees, bumblebees and honeybees (Tepedino, 1981). In particular, the North American squash and gourd bees belonging to the genera *Peponapis* (Figure 3) and *Xenoglossa* are thought to rely exclusively on *Cucurbita* pollen to rear their offspring (Hurd, Linsley & Michelbacher 1974; Tepedino 1981).



Figure 3 *P. pruinosa* visiting a staminate courgette flower for pollen in California, USA.

From an agricultural viewpoint, various mechanisms have been explored to improve cucurbit yield such as eliminating dioecy (Boualem et al., 2015), improving sex expression of flowers (Rodriguez-Granados et al., 2017), and producing F₁ hybrid seed (Robinson, 2000). Indeed, the yield per hectare of cucurbit crops has steadily increased over the last 50 years particularly in Asia where pioneering technological advancements and genetic improvements, especially with seedless varieties, have advanced cucurbit production worldwide (Figure 4) (McCreight et al., 2013). Likewise, and most relevant to this project, cucurbit yield can also be increased by improving the level of pollination (Hoehn et al., 2008; Kouonon et al., 2009).

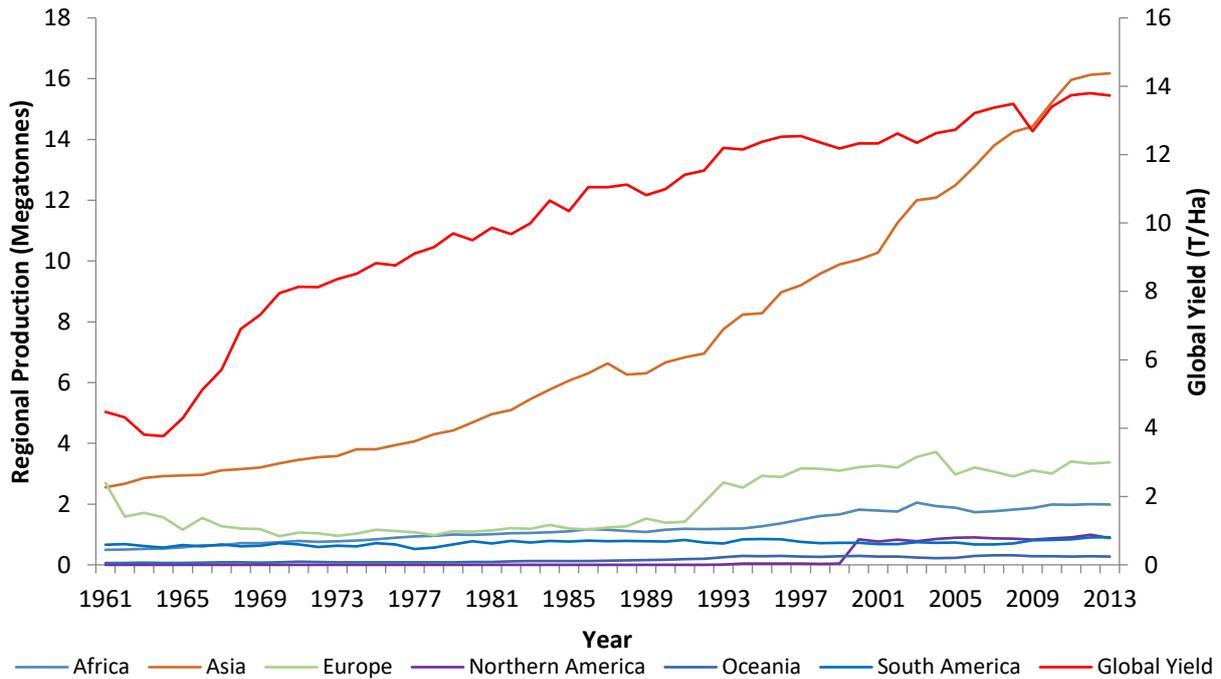


Figure 4 Regional production (primary y axis) and average global yield (secondary y axis) of *Cucurbita* species from 1961 to 2013. Data source: FAOSTAT (Aggregate, may include official, semi-official, estimated or calculated data).

Mechanism of cucurbit pollination

Pollinators vary in the way that they travel to collect nectar in staminate and pistillate flowers because the nectar is in different places (Figure 5). For example, in staminate flowers, bees are forced into a vertical position to gather nectar which means that pollen adheres to the bee's backs. Once out of the staminate flower, bees tend to sit on a flower or leaf and clean excess pollen grains from themselves using their back legs (see also Figure 45). This usually happens in the first hour of anthesis when pollen grains are plentiful. Nonetheless, many pollen grains will remain on the bees. Once inside a pistillate flower, bees unload their pollen grains on to the stigma as they make their way to the base of the corolla. Here, two to three bees may collect nectar at the same time and continue to release more pollen grains as they move symmetrically around the whole circumference of the corolla (Figure 5).

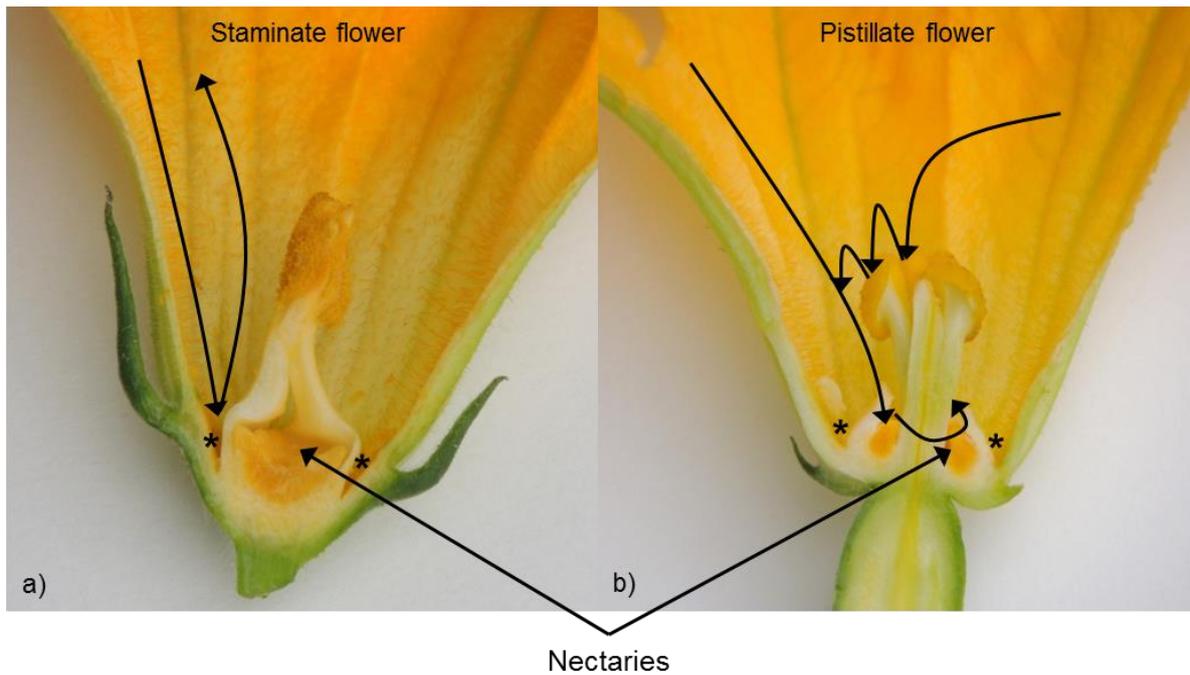


Figure 5 Morphology of staminate (a) and pistillate (b) flowers. The nectaries are shown and arrows show the path of bees collecting nectar. Asterisks (*) show where pollen accumulates. Diagram modified from (Nepi, Massimo and Pacini, 1993).

Pollinator dependency

Cucurbits are described as having an 'essential need' for insect-mediated pollination (Free, 1993; Klein et al., 2007). This assertion is based on previous research which has shown that seed number (Roldán-Serrano and Guerra-Sanz, 2005) and fruit set of courgette (Roldán-Serrano and Guerra-Sanz, 2005) and summer squash (*Cucurbita pepo* L., Vidal *et al.* 2010) and fruit set of cucumber (*Cucumis sativus* L., Gingras, Gingras & DeOliveira 1999; Walters 2005) are positively correlated to the number of pollinator visits. Similarly, fruit has been shown to abort in the absence of pollination in cucumber (Motzke et al., 2015), melon (*Cucumis melo* L., Kouonon *et al.* 2009), pumpkin (*Cucurbita moschata* Duch. ex Poir., Hoehn *et al.* 2008), and courgette (*Cucurbita pepo*, Martínez *et al.* 2014). Interestingly, fruit development of small-sized summer squash varieties was not as influenced as larger ones by the addition of honey bee colonies; suggesting that some smaller varieties may be able to set fruit with a smaller pollen load (Walters and Taylor, 2006).

However, under certain conditions some cucurbit varieties may be able to set without fertilisation, and therefore without pollination, via a process called parthenocarpy. The ability of a plant to set fruit without pollination has long been recognised as a desirable characteristic for greenhouse-grown cucumber which has led to an extensive selective breeding program for this genetic trait (Robinson and Reiners, 1999). Whilst other cucurbit species have generally received less attention, several varieties of summer squash have been observed to

set fruit without any pollination (Kurtar, 2003; Martínez et al., 2013; Robinson and Reiners, 1999). However, evidence suggests that parthenocarpic varieties may still produce a greater quantity and quality, including a higher sugar content (Shin et al., 2007) of fruits when pollinated by insects (Martínez *et al.* 2013; Robinson & Reiners 1999; Nicodemo *et al.* 2013).

Pollinator efficiency

In North America research has shown *Peponapis* spp. to be highly abundant (Tepedino, 1981), effective pollinators of *Cucurbita* crops, depositing more pollen grains per stigma and visiting crop flowers more frequently than *A. mellifera* (Canto-aguilar and Veterinaria, 2000). Likewise, Artz and Nault (2011) observed *Bombus impatiens* C. (another North American species) to be a highly effective pollinator in summer squash, depositing more than three times the number of pollen grains per stigma and nearly always contacting the stigma compared to *A. mellifera* and *Peponapis pruinosa* S.. Consequently, in small fields (0.5 ha) with managed *B. impatiens* colonies present, fruit yield was nearly twice that of non-supplemented fields (Table 2). *B. impatiens* have also been observed to set more seeds per fruit than *A. mellifera* in watermelon (Stanghellini et al., 1998). Nonetheless, *A. mellifera* has been observed to spend two to three times' longer handling summer squash flowers than *B. impatiens* and *P. pruinosa* respectively (Artz and Nault, 2011). However, *A. mellifera* appears to significantly favour pistillate cucurbit flowers (Artz et al., 2011; Phillips and Gardiner, 2015; Tepedino, 1981), unlike species such as *P. pruinosa* whose proportion of visits more closely resemble the natural sex ratio of cucurbit flowers (Artz et al., 2011; Phillips and Gardiner, 2015).

Although these studies demonstrate that several species can be highly effective pollinators, some evidence suggests that a diverse assemblage of species is required for optimum fruit set. For example, a diverse assemblage of pollinators have been observed to visit melon, watermelon (Ali et al., 2015), and summer squash (Ali et al., 2014) and species diversity has been shown to increase seed set in pumpkin (Hoehn et al., 2008). Likewise, Pisanty *et al.* (2015) observed spatial and temporal variation in pollinator visitation to watermelon, suggesting niche complementarity.

Management for pollination in cucurbit crops

Pollination deficit

Although some researchers have addressed the extent of cucurbits' dependency on pollinators and pollinator efficiency in cucurbits, relatively few, and none outside of the United States, have looked at if cucurbit crops are experiencing a pollination, i.e. if crop quality and quantity could be improved with more pollination (Garratt *et al.*, 2013).

Walters and Taylor (2006) showed that adding colonies of *A. mellifera* to fields of winter squash increased fruit weight (per hectare) by 100%. However, similar studies have shown that supplementing fields with *B. impatiens* and *A. mellifera* did not increase pollinator visitation to, or yield of summer squash (Petersen et al., 2013). Likewise, introduced *B. impatiens* did not influence fruit weight, seed set and visitation in summer squash and there was no difference in yield between open- and hand-pollinated flowers (Petersen et al., 2014). In addition, several studies have found no evidence of increased *A. mellifera* visitation to *Cucurbita* species (Shuler et al., 2005), despite fields having *A. mellifera* colonies added.

These results (summarised Table 3) are most likely due to already high levels of open pollination at study sites, evidenced by high yields in control plots (Artz et al., 2011; Petersen et al., 2014, 2013). Since pollinator visitation positively influences yield, fruit set is directly dependent on pollinators and the ecosystems which support their populations. Therefore, these types of results are highly dependent on the spatial and temporal context of the landscape surrounding each crop field. Nonetheless, determining if a study site is experiencing a pollination deficit should be a vital step before implementing any management interventions to promote pollinator populations. This was demonstrated by Julier & Roulston (2009) who combined pollinator visitation data at their study sites with published data on pollination requirements of summer squash to determine that wild bee densities at their study sites were sufficient to fulfil their pollination needs. Pollination deficit in UK courgette crops is determined in this project.

Table 2 Effect of supplemented commercial pollinators on cucurbit yield and pollinator visits to cucurbit flowers.

Citation	Species	Commercial Pollinator	Effect	Dependent variable
Artz and Nault. 2011	<i>Cucurbita pepo</i>	<i>A. mellifera</i>	Positive effect	<i>A. mellifera</i> visits per flower
Artz and Nault. 2011	<i>Cucurbita pepo</i>	<i>A. mellifera</i>	No effect	<i>B. impatiens</i> visits per flower
Artz and Nault. 2011	<i>Cucurbita pepo</i>	<i>A. mellifera</i>	Negative effect	<i>P. pruinosa</i> visits per flower
Peterson <i>et al.</i> 2013	<i>Cucurbita pepo</i>	<i>A. mellifera</i>	No effect	Yield (fruit weight)
Walters <i>et al.</i> 2006	<i>Cucurbita pepo</i>	<i>A. mellifera</i>	No effect	Yield (fruit weight)
Walters <i>et al.</i> 2006	<i>Cucurbita moschata</i>	<i>A. mellifera</i>	Positive effect	Yield (fruit weight)
Walters <i>et al.</i> 2006	<i>Cucurbita maxima</i>	<i>A. mellifera</i>	Positive effect	Yield (fruit weight)
Peterson <i>et al.</i> 2014	<i>Cucurbita pepo</i>	<i>B. impatiens</i>	No effect	<i>B. impatiens</i> visits per flower
Peterson <i>et al.</i> 2013	<i>Cucurbita pepo</i>	<i>B. impatiens</i>	No effect	Yield (fruit weight)
Peterson <i>et al.</i> 2014	<i>Cucurbita pepo</i>	<i>B. impatiens</i>	No effect	Yield (fruit weight)
Artz and Nault. 2011	<i>Cucurbita pepo</i>	<i>B. impatiens</i>	Positive effect	Yield (number of fruits)
Peterson <i>et al.</i> 2014	<i>Cucurbita pepo</i>	<i>B. impatiens</i>	No effect	Yield (number of seeds)

Spatial scales of pollination management

Whilst managed species such as *A. mellifera* and *B. impatiens* can greatly enhance cucurbit yield, cucurbits could also experience greater yields in more diverse habitats; where increased species richness and abundance of wild pollinators can improve pollination services (Garibaldi et al., 2011; Hoehn et al., 2008) and provide insurance against any pollinator loss (Shuler et al., 2005). Improving the quantity and quality of pollen and nectar resources available for pollinators, and allowing areas to remain undisturbed for nesting, mating, and hibernation could benefit pollinator populations and therefore reduce pollination deficits (Bommarco et al., 2013). At a field scale (Table 3), wild flowers co-flowering with crops have been shown to increase solitary bee abundance in muskmelon and watermelon (Winfree et al. 2008). Since *P. pruinosa* preferentially lay their eggs in crop areas at depths around 12 to 30 cm (Julier & Roulston 2009; Hurd et al. 1974), no-tillage farms have been shown to have an almost three-fold increase in *P. pruinosa* density (Shuler et al., 2005). However this evidence is conflicted by other studies which have observed no effect of tillage on *P. pruinosa* abundance (Julier & Roulston 2009), most likely due to different phenology of study crops, which were surveyed mid-August in Julier & Roulston (2009) compared to July (Shuler et al., 2005). Further, *P. pruinosa* have been observed to emerge from heavily tilled and disturbed areas (Minter & Bessin 2014). The effectiveness of field scale pollinator-supporting practices are often variable and greatly depend on the complexity of the habitat surrounding a crop field, with more simplistic landscapes generally showing greater increases in pollinator species richness after interventions than in complex landscapes (Batáry, Báldi, Kleijn, & Tschamntke, 2011; Scheper et al., 2013).

At a farm scale (Table 4) smaller crop fields and increased proximity to natural habitats can increase forage and nesting opportunities nearer to crops and is likely to be why species known for their longer flight distances such as *A. mellifera* are found in high abundance in large, intensively farmed fields (Osborne, Martin, Carreck, et al. 2008). Consequently, Kremen et al. (2004) found that pollination by native bees in watermelon was strongly associated with the proportion of natural habitat within a 1 to 2.5 km radius of a farm sites. This relationship was strong enough for the authors to suggest that based on the area of natural habitat, pollination services to a given area could be estimated (Kremen et al., 2004). Although organic farming was shown to increase pollinator abundance, and therefore, reduce pollination deficit in oilseed rape (Morandin and Winston, 2005), similar findings have not been observed in cucurbits (Kremen et al. 2004; Winfree et al. 2008, Table 4). For example, organic (versus conventional) farming has been shown to be less important than the amount of natural habitat surrounding a study site for predicting pollen deposition (Kremen et al., 2004), pollinator

abundance (Kremen et al., 2004; Winfree et al., 2008), and species richness (Winfree et al., 2008) in watermelon. At a farm scale, the area of mass-flowering crops, including the cucurbit itself, may 'dilute' pollinator densities if large, or 'concentrate' pollinator densities if small (Holzschuh *et al.* 2016). This will be especially pronounced if additional food and nesting sites are not provided, meaning that pollinators move transiently between available forage rather than increasing their population size (Holzschuh *et al.* 2016, see also work additional to funding objectives). The complexity of field and farm-scale resources becomes further complicated when species-level responses are taken into consideration. Increasing the proximity of forage to suitable nesting sites may be more important for 'door step foragers' such as *Bombus muscorum* L., *B. pascuorum* Sc. and *B. lapidarius* L. which are known to forage close to their nests (Osborne *et al.*, 2008). Other species with longer flight distances (such as *B. terrestris* L. and *Apis mellifera* L.) are found in high abundance in large, intensively farmed fields, far away from available nest sites (Osborne *et al.*, 2008). Since previous research has shown that *B. terrestris* and *A. mellifera* can fulfil the pollination requirements of courgette (in UK: Knapp and Osborne 2017) and *B. impatiens* and *A. mellifera* of pumpkin (in USA: Petersen *et al.* 2013), increasing the proximity of forage to suitable nesting sites may be less important to cucurbit crops because they are primarily serviced by long range, generalist pollinators. This highlights the need to match pollinator-supportive management practices with crops' individual requirements for pollination, since an increase in pollinator species richness may not necessarily be required for yield to be improved (Kleijn *et al.*, 2015; Winfree *et al.*, 2015).

As many agricultural systems are isolated from natural habitats, crop producers may need to provide floral resources and nesting sites suitable for pollinators. In the UK, farm stewardship schemes provide guidance on hedgerow and field margin management, particularly favoured by bumblebee species (Carvell *et al.*, 2015; Dicks *et al.*, 2015; Osborne *et al.*, 2008b; Wood *et al.*, 2015a). Alternatively, costs can be directly offset by increased profit from improved quality and quantity of yields. For example, the economic benefit of improved blueberry yields following wild flower establishment has been shown to exceed the original cost of wild flower establishment (Blaauw and Isaacs, 2014). As evidenced by these accounts of cucurbit growing, understanding a crop's requirement for pollination and, in turn, how pollinators vary spatially and temporally in the landscape is essential to design and deliver optimum crop management. The economic value of pollination can be included in cost-benefit analyses to inform decision making at a farm and policy level (Hanley *et al.*, 2014). This is because valuation based on a crop's dependence for pollination will show the detrimental impact that a decline in pollinator populations may have, and valuation based on the pollination deficit will show the potential that increasing pollinator populations may have. Consequently, quantifying

the economics of pollination is a fundamental way for growers to understand the implications that changes in pollinator populations may have on their yield and economic return. Despite the economic importance of many cucurbit species and their 'dependence' on pollination, no studies have calculated the economic value of pollination to cucurbit crops. In other high value crops such as apple, economic valuations have shown that maximising pollination could increase UK output by £5.7 million per year (Garratt et al., 2013).

The positive, mediating effect of forage availability on pollinator visitation and crop yield has been shown in pumpkin (Petersen and Nault 2014), strawberry (Connelly *et al.* 2015), poppy (Hardman *et al.* 2016), and coffee (Saturni *et al.* 2016). Likewise, several studies have looked at forage availability at different spatial scales, in terms of patch size (Heard et al., 2007), and field versus farm scale allocation of resources (Hardman et al., 2016). However, these analyses do not differentiate between pollinator visitors to the focal crop and all other pollinator species, thus increases in pollinator numbers may not necessarily benefit crop pollination.

Table 3 Effect of field-scale pollinator management practices on pollinator visits to, and pollen deposition on cucurbit flowers.

Citation	Species	Agricultural Practice	Effect	Dependent variable
Phillips and Gardiner 2015	<i>Cucurbita pepo</i>	Floral strips	No effect	“Bee visitation frequency” per flower
Phillips and Gardiner 2015	<i>Cucurbita pepo</i>	Floral strips	No effect	Pollen deposition per flower
Shuler <i>et al.</i> 2005	<i>Cucurbita pepo</i>	Pesticide use	No effect	<i>Bombus</i> spp. visits per flower
Shuler <i>et al.</i> 2005	<i>Cucurbita pepo</i>	Pesticide use	No effect	<i>P. pruinosa</i> visits per flower
Julier and Roulston. 2009	<i>Cucurbita pepo</i>	Soil clay content	Negative effect	<i>P. pruinosa</i> abundance
Shuler <i>et al.</i> 2005	<i>Cucurbita pepo</i>	Tillage	No effect	<i>B. impatiens</i> visits per flower
Julier and Roulston. 2009	<i>Cucurbita pepo</i>	Tillage	No effect	<i>P. pruinosa</i> abundance
Shuler <i>et al.</i> 2005	<i>Cucurbita pepo</i>	Tillage	Positive effect	<i>P. pruinosa</i> visits per flower

Table 4 Effect of farm-scale pollinator management practices on cucurbit yield, pollinator visits to, and pollen deposition on cucurbit flowers.

Citation	Species	Landscape complexity	Effect	Dependent variable
Kremen <i>et al.</i> 2004	<i>Citrullus lanatus</i>	Organic farming	No effect	Pollen deposition per flower
Winfree <i>et al.</i> 2008	<i>Citrullus lanatus</i>	Organic farming	No effect	Bee abundance/ flower/ time
Winfree <i>et al.</i> 2008	<i>Cucumis melo</i>	Organic farming	No effect	Bee abundance/ flower/ time
Winfree <i>et al.</i> 2008	<i>Citrullus lanatus</i>	Woodland	Positive effect	Bee abundance/ flower/ time
Winfree <i>et al.</i> 2008	<i>Cucumis melo</i>	Woodland	No effect	Bee abundance/ flower/ time
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Landscape diversity	Positive effect	<i>A. mellifera</i> visitation frequency
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Landscape diversity	Positive effect	<i>B. impatiens</i> visitation frequency
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Landscape diversity	Positive effect	Yield (fruit weight)
Phillips and Gardiner 2015	<i>Cucurbita pepo</i>	Landscape diversity	Positive effect	“Bee visitation frequency”
Phillips and Gardiner 2015	<i>Cucurbita pepo</i>	Landscape diversity	Positive effect	Pollen deposition per flower
Julier and Roulston. 2009	<i>Cucurbita pepo</i>	Natural habitat	No effect	<i>P. pruinosa</i> abundance
Kremen <i>et al.</i> 2004	<i>Citrullus lanatus</i>	Natural habitat	Positive effect	Pollen deposition per flower
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Grassland	No effect	<i>A. mellifera</i> abundance per flower
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Grassland	Positive effect	<i>B. impatiens</i> abundance per flower
Peterson and Nault. 2014	<i>Cucurbita pepo</i>	Grassland	Positive effect	Yield (fruit weight)
Artz and Nault. 2011	<i>Cucurbita pepo</i>	Field size	No effect (but positively interacted with <i>A. mellifera</i> supplementation)	<i>B. impatiens</i> , <i>A. mellifera</i> , and <i>P. pruinosa</i> abundance
Kremen <i>et al.</i> 2004	<i>Citrullus lanatus</i>	Field size	No effect	Pollen deposition per flower

Nutritional value of courgette for bees

Loss of floral resources due to changes in land management is generally thought to be the primary driver of reported declines in pollinator populations (Baude et al., 2016; Brown and Paxton, 2009; Potts et al., 2010). This is because generalist flower visitors such as bumblebees (*Bombus* spp.) rely on an abundant and diverse selection of floral resources for nectar and pollen to meet their energy requirements: nectar is rich in sugars, a source fuel, and pollen is rich in protein which is essential for growth and development (Rotheray et al., 2017).

Whilst mass-flowering crops may enhance pollinator densities (Westphal et al., 2003) it is largely unknown if this is due to a transient movement of bees between patches of forage or due to an actual increase in colony growth (Holzschuh et al., 2016). This is because mass-flowering crops only provide temporary pulses of nectar and pollen unlike natural areas (with higher floral species richness) which are able to provide resources that are more stable over time (Montero-Castaño et al., 2016). Nonetheless, intense flowering periods and large area of mass-flowering crops in the landscape may still benefit pollinators spatially and temporally, potentially boosting bee populations (and not just forager numbers) *and* pollination.

Since accurately studying bumblebee colony development in a field setting can be difficult (Westphal et al., 2009; Wood et al., 2015b), this study uses an *in-silico* approach to simulate the population dynamics of the buff-tailed bumblebee, *Bombus terrestris* L. in landscapes with and without courgette fields using the agent-based model *Bumble-BEEHAVE* (Becher & Twiston-Davies *et al.* 2018). Although other Bumblebee models exist (Crone and Williams, 2016; Häussler et al., 2017; Olsson et al., 2015), *Bumble-BEEHAVE* is uniquely able to simulate the effects of multifactorial stressors on bumblebee survival at individual, colony and population levels on a daily basis, based on nectar and pollen sources which are approximated from real landscape maps of study sites.

Simulations were run in BEE-STEWARD (www.beehave-model.net), a software tool that combines in a user-friendly way the bumblebee model *Bumble-BEEHAVE* and the landscape defining features of BEESCOUT (Becher et al., 2016). BEESCOUT was developed as the landscape module for the honeybee model BEEHAVE (Becher et al., 2014) and for *Bumble-BEEHAVE* (Becher and Twiston-Davies *et al.* 2018), and creates input files from images of landscape maps. These input files define the number and specification of food sources such as, nectar and pollen, flowering phenology etc. and therefore represent landscapes in the BEEHAVE and *Bumble-BEEHAVE* models. BEE-STEWARDS' interface also enables users

to simulate the effects that different management options, such as changing crop types will have on bumblebee population dynamics.

Pollination in context

Agricultural expansion and intensification are thought to be responsible for reduced ecological functioning of farmland ecosystems. Sustainable management of these systems requires beneficial services such as pollination, pest control and nutrient cycling to be optimised whilst minimising any negative effects that they may have on other services. For example, effective biological weed control may reduce the availability of forage for pollinators and natural enemies, whilst areas planted for forage may compete with crop species for abiotic and biotic resources (Bretagnolle and Gaba, 2015). These factors are of particular concern to growers of pollinator-dependent crop species who must manage the complex relationship between pollinators, their habitat requirements and other ecosystem services important for determining yield (Bommarco et al., 2013).

Whilst pollination clearly affects cucurbit yield, there are many other environmental factors which contribute and interact with each other to influence fruit set such as soil quality, water availability and weather conditions (Boreux et al., 2013; Klein et al., 2014; Motzke et al., 2015). For example, soil nitrogen has been shown to increase the number, weight, and viability of pollen grains, with pollen from plants grown in higher nitrogen environments observed to produce courgettes with more seeds (Lau Tak-Cheung and Stephenson, 1993). Likewise, Motzke *et al.* (2015) showed that weeds control and fertilisation were able to reduce the yield gap of cucumbers by 45% and 18% respectively, however, these factors, even in combination, were unable to account for a total absence of pollination (increased yield gap of 75%).

Therefore, the productivity of pollinator-dependent crops relies on the presence of high functioning ecosystems to support pollinator populations, regulate disease, purify and cycle water and nutrients. Any impact (particularly anthropogenic (Winfree et al., 2009)) on the wider ecosystem will have a detrimental impact on crop yields and farmers profits (Goulson et al., 2015; Potts et al., 2010; Steffan-Dewenter et al., 2005). Thus, whilst this project is principally concerned with the mechanism of cucurbit pollination and management for this service, other factors which may affect cucurbit yield, although not directly studied, were still taken into account.

Commercial study sites

Courgettes were grown outdoors, in the field at a density of 13,585 plants per hectare. Each field (average field size of 5.2 ± 1.3 ha (SE)) was situated more than 2km apart to ensure independent pollinator communities between fields (Vaissière, 2010) and was conventionally managed with minimum chemical input other than fungicidal sprays (P.E. Simmons and Son, personal communication). Little or no herbicide is used on courgette fields due to the short picking intervals of the crop, which results in a high abundance and diversity of flowering weeds within the crop. Work was undertaken as follows:

- In 2015 and 2016, the pollination conditions of Courgette (var. 'Tosca') were manipulated at 7 sites across Cornwall, UK.
- In 2016, pollinators and wild flowers were surveyed at 9 sites, in three key courgette growing regions of England: Cornwall (5 sites), Worcestershire (2 sites) and Cambridgeshire (2 sites). In addition, nutrients and yield were additionally surveyed in these areas.
- In 2016 and 2017, pollinator efficiency was surveyed at 19 sites across Cornwall, UK.
- In 2017, the effect of managed colonies of *B. terrestris* on courgette pollination were explored at 14 sites, and on courgette yield at 1 site in Cornwall, UK.

Funding Objective 1: What are the most effective wild pollinators of field-grown courgettes in South West UK in terms of visitation rate and pollen transfer efficiency?

Quantifying the effectiveness of individual pollinator species can help growers target their pollination management to species most likely to increase yields. Pollinator surveys were conducted to determine which pollinator species were most abundant, had the highest visitation rate to male and female courgette flowers and transferred the most courgette pollen (Figure 6).



Figure 6 *B. terrestris* covered in courgette pollen after visiting a staminate flower for nectar.

Research Questions

- 1) What pollinator has the highest visitation rate to courgette flowers and is there a difference between 0m and 50m from the edge of the crop?
- 2) Do different pollinator species show a preference to male or female courgette flowers?
- 3) What pollinator transfers the most pollen grains of courgette?

Methods

Pollinator Surveys

To quantify *B. terrestris* abundance at courgette flowers and therefore, their potential pollination efficiency, four 50 m transects were established within the crop (from the edge of the crop to the centre, 25m apart). Transects were walked at a steady pace (~5 minutes each) with observations made 1 m either side and in front of the recorder (over a length of 50 m). This was done three times during the blooming period for each site in 2016 and 2017, resulting in a total of 228 transects surveyed over the two years.

To observe bee visitation at 0m and 50m from the edge of the crop, three flowers (staminate and pistillate) (on the first day of anthesis) were randomly selected at each of these locations. This method was used (rather than sampling a unit area) because it was the best way of observing multiple flowers simultaneously. Bee visitors were recorded over two 15-minute periods, at each field and location within the crop (0 m and 50 m from the edge), totalling 4 observational periods per field. Pollinator visitation rate was calculated as the number of visits per minute per flower summed across the two surveys for each of the two distances from the edge of the crop.

Swabbing *B. terrestris* for pollen grains

To quantify the number of courgette pollen grains the two most abundant pollinators, *B. terrestris* (n = 17) and *Apis mellifera* (n = 4) were randomly collected from courgette flowers, placed in sample pots and swabbed for pollen. Bees were gently cooled under ice packs, and their entire body swabbed with small cubes of glycerine jelly (with fuchsin dye) positioned on the end of cocktail sticks, before they were released. In the laboratory, microscope slides were prepared by melting the piece of glycerine jelly under a cover slip. The numbers of pollen grains were then calculated under a 20 X magnification (Kremen et al., 2002).

Results

Visitation to courgette

A. mellifera and *B. terrestris* were the most abundant pollinator species observed visiting courgette flowers across the two years of this study (although commercial colonies of *B. terrestris* were added to fields in 2017). Figure 7 Proportion of nectar visits to staminate and pistillate flowers for *A. mellifera*, *B. terrestris/ lucorum*, *B. pratorum*, *B. hypnorum* and *B. lapidarius* recorded on pollinator transects in 2016 and 2017, as well as the proportion of staminate and pistillate flowers. *B. terrestris* showed equal preference to staminate and pistillate flowers, which may affect pollination efficiency.

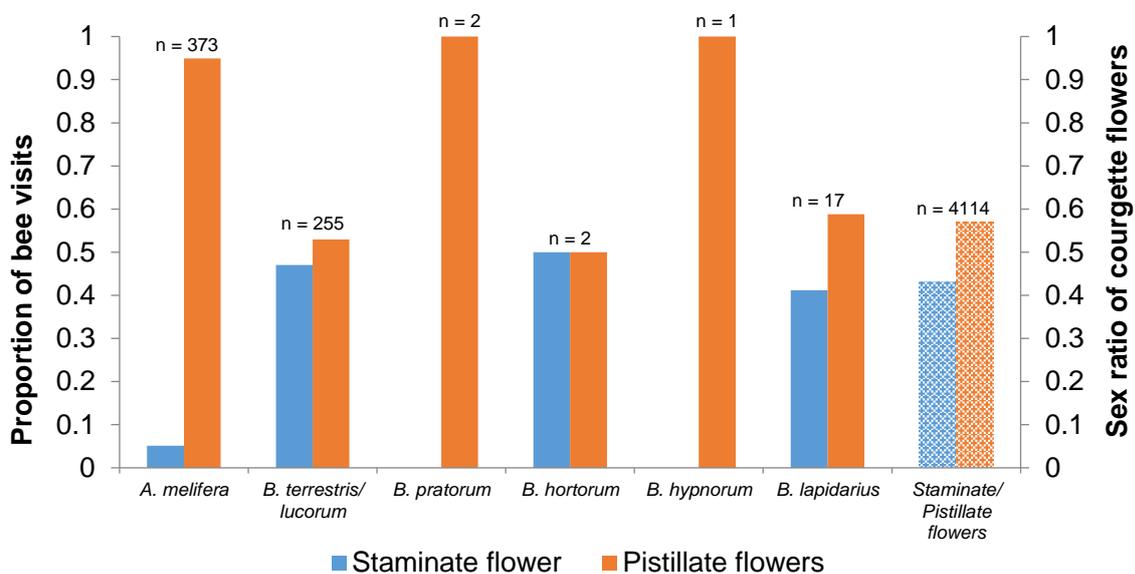


Figure 7 Proportion of nectar visits to staminate and pistillate flowers for *A. mellifera*, *B. terrestris/ lucorum*, *B. pratorum*, *B. hypnorum* and *B. lapidarius* recorded on pollinator transects in 2016 and 2017, as well as the proportion of staminate and pistillate flowers.

Overall, there was no change in pollinator visitation rate with distance from the edge of the crop (contrast estimate 0.04 ± 0.05 SE, $T = 0.72$, $P = 0.47$). However, *Bombus* spp. were more abundant at the edge of the crop, unlike *Apis mellifera* which were more abundant within the crop (Figure 8).

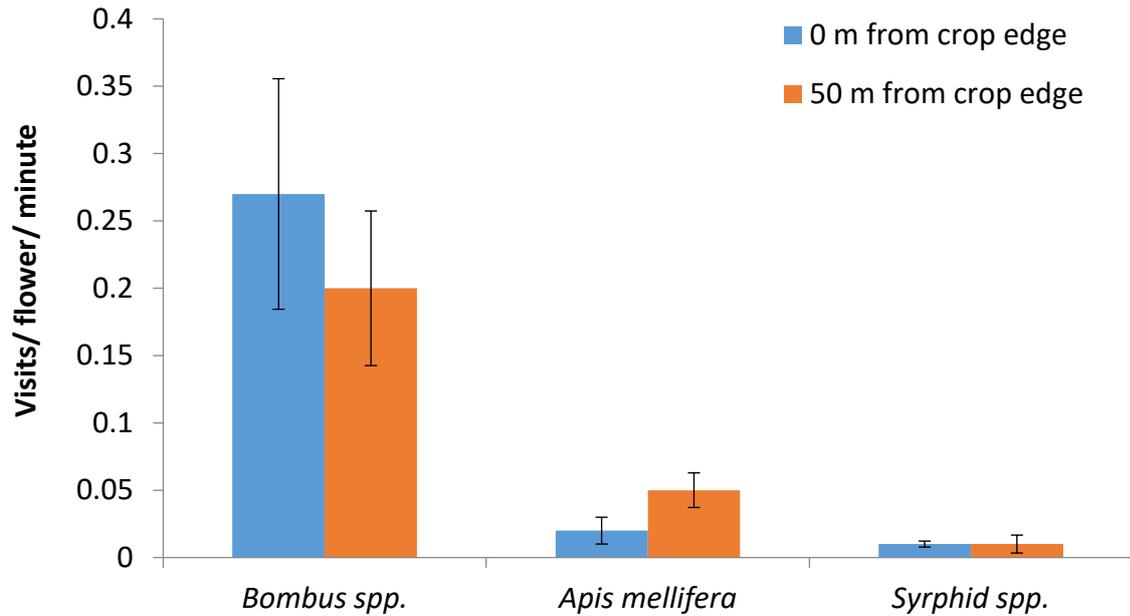


Figure 8 Flower visitation rate for *Bombus* spp. (*B. terrestris/ lucorum*, *B. pascuorum* and *B. hortorum* combined), *Apis mellifera* and *Syrphid* spp at 0 m and 50 m from the edge of courgette fields in 2016. Mean \pm SE (n = 10). There was no change in pollinator visit

Pollen Transfer

B. terrestris carried an average of 1866 ± 476 (n = 13) pollen grains on their bodies, more than *A. mellifera* which carried an average of 122 ± 39 (n = 4) pollen grains on their bodies.

Discussion

A high abundance, but not diversity, of pollinators were recorded visiting courgette flowers during this project. This highlights that only a few abundant species, rather than high species richness (contrary to a previous study on pumpkins (Hoehn et al., 2008) and watermelons (Kremen et al., 2002)), can deliver pollination services to a whole crop (Kleijn et al., 2015; Winfree et al., 2015). However, any loss of these functionally important species could greatly reduce pollination services (Larsen et al., 2005). Fortunately, these species are generally widespread, resilient to agricultural expansion and can be encouraged through simple conservation measures (Kleijn et al., 2015). Observations of pollinator visitation and yield also show that the pollination requirements of courgette can be fulfilled without squash and gourd bees (belonging to the genera *Peponapis* and *Xenoglossa*) which have previously been regarded as the most important pollinators of *Cucurbita* crops in North America (Hurd et al., 1974).

There was no difference in bee visitation at 0 m and 50 m from the crop edge (Figure 8). This may be because 50 m from the crop edge is not far enough from natural or semi-natural habitat (such as hedgerows). This is to be expected given that even 'door step foragers' such as *B.*

muscorum, *B. pascuorum* and *B. lapidarius* are known to forage at distances greater than this (Darvill et al., 2004; Knight et al., 2005; Walther-Hellwig and Frankl, 2000). Distance from the edge of the crop is unlikely to be a problem for the majority of cucurbit fields in Cornwall, where the average distance to the centre of a field is around 100 m (average field size of 5.2 ± 1.3 ha (SE)) but could be more likely for cucurbit fields in Cambridgeshire where the average distance to the centre of a crop is around 200 m (average field size of 16.5 ± 3.1 ha). Likewise, spatial and temporal variation in the landscape surrounding each field may influence the level of open pollination. For example, other studies have demonstrated that sites situated nearer to natural and semi-natural habitat are more likely to have a greater species richness of pollinators and higher pollination rate (Garibaldi et al., 2011; Kremen et al., 2004; Morandin and Winston, 2006). Studies have also shown that larger fields (particularly towards the centre) are more likely to have lower species richness and reduced pollination rate (Artz et al., 2011; Garibaldi et al., 2016).

Whilst *B. terrestris* and *A. mellifera* showed a strong fidelity to courgette, visiting crop flowers more often than wild flowers in the hedgerows, in the morning when courgette flowers were open (see funding objective 4), *B. terrestris* had a more equal preference to staminate and pistillate courgette flowers than *A. mellifera* (Figure 7). In addition, *B. terrestris* was observed to carry more loose pollen grains on their body and therefore, have a higher pollination potential than *A. mellifera*. Combined, these results suggest that *B. terrestris* is the most effective pollinator of courgette crops in the South West of the UK.

This work shows that allowing uncultivated areas around the crop to be colonised by species-rich wild flowers may be an effective way of boosting the abundance of bumblebees - important pollinators of courgette, as well as the abundance and species richness of solitary bees which are important pollinators of other crop and wild flower species. Providing floral resources may benefit pollination services and pollinator conservation.

Funding Objective 2: Does pollination deficit limit yield (number, quality of fruits)?

Systematically reviewing the literature for this project showed how little information is available on pollinator dependence within, and between crop species. Since there was no data available on field-grown Tosca, the courgette variety used in this study (Figure 9), I conducted pollination experiments to estimate pollinator dependency and to see if this variety was experiencing a pollination deficit at field sites in Cornwall. Since pollinator dependency and pollination deficit combine to directly influence yield I used economic valuations to clearly demonstrate to growers the importance of conserving pollination services for courgette production in the UK



Figure 9 Developing courgette fruit. Photograph by Daphne Wong.

Research Questions

To understand whether the dynamics of pollination are affecting yield quality or quantity and to improve guidance to growers for obtaining productive and sustainable yields, we sought answers to the questions:

- 1) Does pollination influence growth rate, quality and quantity of fruits?
- 2) Are courgettes experiencing a pollination deficit and does this increase with distance from the edge of a field?
- 3) What is the estimated economic value of pollinators and their potential profitability to courgette production in the UK?

Methods

Pollination treatments

In 2015 and 2016, the pollination conditions of Courgette (var. 'Tosca') were manipulated in seven fields across Cornwall, UK. In 2015, 180 flowers were manipulated at two fields and in 2016, 300 flowers at five fields, totalling 480 flowers over the course of the study ().

As courgette is monoecious, each pistillate flower was assigned to one of the following treatments: hand pollination (n = 60), open pollination (n = 60), or no pollination (n = 60) in 2015 and hand pollination (n = 100), open pollination (n = 100), or no pollination (n = 100) in 2016. Hand pollinated flowers were treated on the first day of anthesis around 08:00 in the morning, with pollen from a staminate donor flower (from a neighbouring plant) using a paint brush. Open pollinated flowers were left to be pollinated naturally by insects visiting the fields. The no pollination treatment was initiated the day before expected anthesis by securing PVC mesh bags with wire ties to pistillate flowers. Bags had a mesh size of 0.2mm, designed to be permeable to wind and rain yet exclude any pollinators. To the best of our knowledge no commercially reared *B. terrestris* L. or *A. mellifera* colonies had been introduced within a 1 km radius of each farm. The level of pollinator dependence (the difference between open- or hand- and no pollination) can be interpreted as courgette's 'demand' for pollen, whilst the pollination deficit (the difference between hand- and open-pollinated crops) indicates the 'supply' of pollen in the landscape relative to maximal pollination.

All experimental flowers were individually identified with marker pen written on pieces of flagging tape, tied to the base of each fruit. To avoid the confounding effect of a plant investing in additional fruits from un-monitored pollination events, only one fruit per plant was studied (Avila-Sakar et al., 2001; Stephenson et al., 1988).

Quantity and quality measures

In 2015 and 2016 fruits were harvested 10 days post-anthesis, weighed on scales, measured using a tape measure (length and circumference (circumference only in 2015)), and their sugar content (°Brix) recorded (only in 2016). °Brix is considered to be a simple and objective measure which can be used by growers to assess fruit quality, since sweetness is appreciated by consumers (Kleinhenz and Bumgarner, 2013). °Brix was measured on a hand-held refractometer (Bellingham-Stanley, range 0 - 50%) by taking a homogenised value from three 1cm² pieces of fruit (middle and either end).

Experimental fruits were classed as 'aborted' if they did not meet minimum commercial standards, i.e. if they were less than 14 cm long, 30 mm wide (at the mid-point), and over 5° in curvature, or showed any obvious signs of bacterial damage, such as blossom end rot. Therefore, fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) is also a measure of fruit quality. As fruit set was measured over 10 days courgettes were generally larger than commercial standards. Since these experiments were conducted at a commercial farm some fruits were accidentally removed by pickers. Consequently, final sample sizes were less than the number initiated and are not completely balanced between treatments (hand pollination n = 151, open pollination n = 157, no pollination n = 153).

Effect of pollination over time

In 2015, 180 of the experimental pistillate flowers were measured at two fields (hand pollination (n=60), open pollination (n=60) and no pollination (n=60)) (Figure 10). Fruit length was measured daily from the first day of anthesis to 10 days post-anthesis to explore the effect of pollination treatment on fruit length over time. All pollination treatments were conducted simultaneously within each field to minimise environmental variation between treatments.

Pollination with distance into a crop

In 2016, a total of 100 experimental flowers were left to be pollinated naturally in five different fields at 0 m (n = 50) and 50 m (n = 50) into the crop from the field edge (10 flowers per field and location into the crop) (Figure 10). In each field, the edge of the crop was a hedgerow. Therefore, 0 m into the crop was closer to semi-natural habitat than 50 m in the crop.

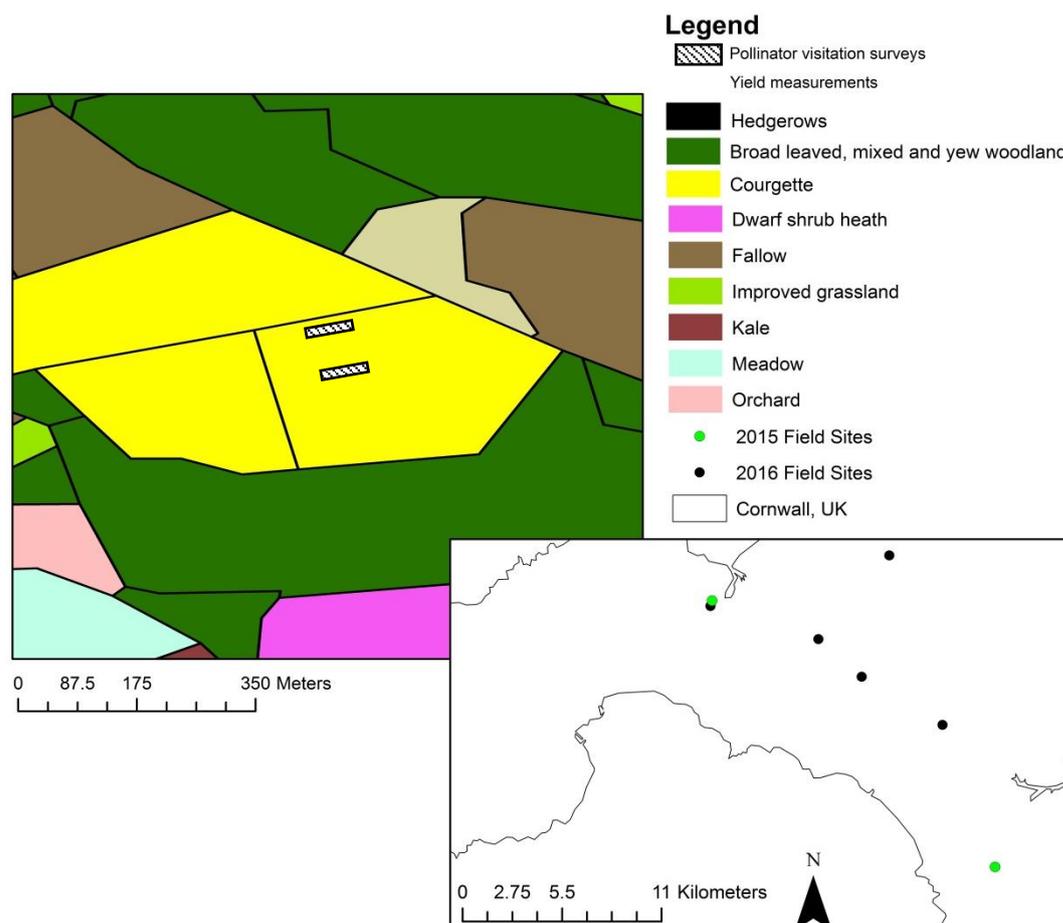


Figure 10 General design of a field site showing the location of pollinator visitation surveys and yield measurements at 0 m and 50 m from the edge of the crop (2016). In 2015 yield measurements were only conducted 0m from the edge of the crop. The location of all field sites, two sites in 2015 and five sites in 2016 are also shown in the context of Cornwall, UK.

Economic value of pollination

It is often assumed that a loss of pollinators will decrease the value of horticultural crops however; yield is also dependent on variety, management practices, and environmental conditions (Boreux et al., 2013; Bos et al., 2007; Klein et al., 2014; Motzke et al., 2015). As these inputs improve, fruit quantity (fruits produced per plant over a season) and fruit quality (size and shape) will increase, improving the grower's economic advantage. Based on Melathopoulos *et al.*, (2015) the economic value of these combined factors (under open pollination conditions) can be broadly estimated as:

$$EV = P \times Q \quad (1)$$

Where EV (£/Ha or £ for UK) is the total economic value/ unit area, P is the price (£/kg), and Q is the quantity of crop grown (Kg/Ha or Kg in UK). To estimate the economic value of courgettes for the UK, and the proportion which depends on insect pollination, we have used national statistics and local data. P was calculated as the average weekly price (£/kg) of all courgette varieties (data were unavailable for individual varieties) from June to September

2016 (DEFRA, 2016). Q was the average yield (kg/ha) of one courgette variety, Tosca, at the 2015 study site in Cornwall (P.E. Simmons and Son, personal communication 29th June 2016).

Using the pollination manipulations in this study, a coefficient of pollinator dependency (D) can be calculated as the fruit set as a result of open pollination (f_p) compared to pollinator exclusion (f_{pe}). D relates to pollinator dependency in particular conditions, whereas D_{max} is the maximum dependency of a crop on pollinators. D_{max} is calculated as the fruit set as a result of hand pollination (f_{pmax}) compared to pollinator exclusion (f_{pe}). These can be used to determine the extent to which fruit set would increase or decrease if pollination was improved or removed.

$$D(\text{or } D_{max}) = 1 - \frac{f_{pe}}{f_p \text{ (or } f_{pmax})} \quad (2)$$

To calculate the economic value of pollination ($IPEV$), i.e. the proportion of the crop's value that would be lost if all pollinators were removed, the total value of the crop (per hectare) is multiplied by D .

$$IPEV = EV \times D \quad (3)$$

On the other hand, if pollination was maximised (equivalent to hand pollination) then the maximum economic value ($MaxEV$) of courgettes would be:

$$MaxEV = EV \times D_{max} \quad (4)$$

Subtracting $IPEV$ from $MaxEV$ reveals the pollination deficit ($PDef$) at a particular location. This is the potential profitability that pollinators could provide under maximal pollination conditions.

$$PDef = MaxEV - IPEV \quad (5)$$

[For further explanation of these equations, see Melathopoulos *et al.* (2015)].

EV , $IPEV$, $MaxEV$ and $PDef$ were all calculated for courgettes and then multiplied by the total area of courgette production (for all varieties) in the UK (British Growers Association, personal communication 22nd September 2016)) to calculate values for UK production. Owing to a lack of data (in this study and the wider literature) on pollinator dependence and the area of different courgette varieties in the UK, figures are only based on one courgette variety (Tosca) for D and all varieties for P and Q .

Statistical analysis

All analyses were performed in the R package lme4 (Bates *et al.*, 2015). Error distributions were Gaussian unless otherwise stated and residual plots were used to check for normality

and heteroscedasticity. *Post hoc* Tukey tests were calculated using the multcomp package (Hothorn et al., 2008).

Pollination treatment

The effect of pollination treatment (fixed effect) on fruit growth (length 10 days after anthesis) (2015 and 2016 data combined), weight (2015 and 2016 data combined), circumference (2015 data only) and °Brix (2016 data only) was tested with field specified as a random effect.

Fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) was modelled using a GLM with a binomial error distribution with field and pollination treatment as fixed effects.

Pollination with distance into the crop

Fruit set (with a binomial error distribution), fruit growth (length after 10 days), weight, and °Brix under open pollination conditions were assessed in relation to distance from the edge of the crop, pollinator visitation rate (visits per minute per flower, summed across the two surveys for each of the two distances from the edge of the crop) and their interaction as fixed effects and field was specified as a random effect. Pollinator visitation rate was assessed in relation to distance from the edge of the crop with field specified as a random effect.

Results

Pollination deficit and pollinator dependence

Fruit set of Tosca (in 2015 and 2016) significantly increased with hand- and open-pollination compared to no-pollination conditions however, there was no significant difference between hand- and open-pollination (Table 5). Overall fruit set was 98% for hand pollinated flowers, 95% for open pollinated flowers and 56% under no-pollination conditions. Over half of the experimental flowers subjected to the no pollination treatment were able to set fruit to marketable size and weight. However, fruit length, weight and circumference (not °Brix) for non-pollinated flowers were significantly decreased compared to hand- and open- pollinated flowers.

Table 5 Results from the LMMs and GLM on the effect of pollination treatment (hand pollination, open pollination and no pollination) on field grown courgette quality and quantity measures (mean \pm standard error). N = the number of fruits analysed. *Post hoc* Tukey tests used to test for differences in pollination treatment are shown.

Measure	Hand pollinated (mean \pm SE (n))	Open pollinated (mean \pm SE (n))	Pollinator exclusion (mean \pm SE (n))	Tukey <i>post hoc</i> tests		
				Contrast estimate \pm SE	Test statistic (z-value)	P-value
Fruit set (%)	98 \pm 2.2 (151)	95 \pm 2.9 (157)	56 \pm 10.9 (153)	HP - NP: 2.71 \pm 0.82	3.31	0.003
				OP - NP: 2.35 \pm 0.77	3.07	0.006
				HP - OP: 0.35 \pm 0.84	0.42	0.91
Fruit growth (length in cm after 10 days)	22.8 \pm 0.5 (148)	22.0 \pm 0.5 (149)	16.5 \pm 0.8 (86)	HP - NP: 7.16 \pm 0.68	10.56	<0.0001
				OP - NP: 6.26 \pm 0.67	9.26	<0.0001
				HP - OP: 0.9 \pm 0.57	1.56	0.26
Fruit weight (g)	829.9 \pm 35.1 (148)	768.3 \pm 33.2 (149)	520.1 \pm 41.6 (86)	HP - NP: 362.6 \pm 42.38	8.56	<0.0001
				OP - NP: 298.16 \pm 42.27	7.05	<0.0001
				HP - OP: 64.44 \pm 35.8	1.8	0.17
Fruit circumference (cm)	17.4 \pm 0.5 (60)	18.5 \pm 0.7 (60)	15.0 \pm 0.5 (60)	HP - NP: 7.43 \pm 0.75	9.96	<0.0001
				OP - NP: 6.73 \pm 0.74	0.94	<0.0001
				HP - OP: 0.7 \pm 0.74	9.09	0.62
Brix	3.8 \pm 0.04 (88)	3.8 \pm 0.04 (89)	3.8 \pm 0.08 (54)	HP - NP: 0.002 \pm 0.08	0.03	1.0
				OP - NP: 0.07 \pm 0.07	1.03	0.67
				HP - OP: 0.06 \pm 0.08	0.86	0.56

Effect of pollination over time

Despite fruit length remaining similar for the first four days (just before fruits achieve a minimum marketable weight), non-pollinated fruits did not grow as long in length as open- and hand-pollinated fruits (Figure 11).

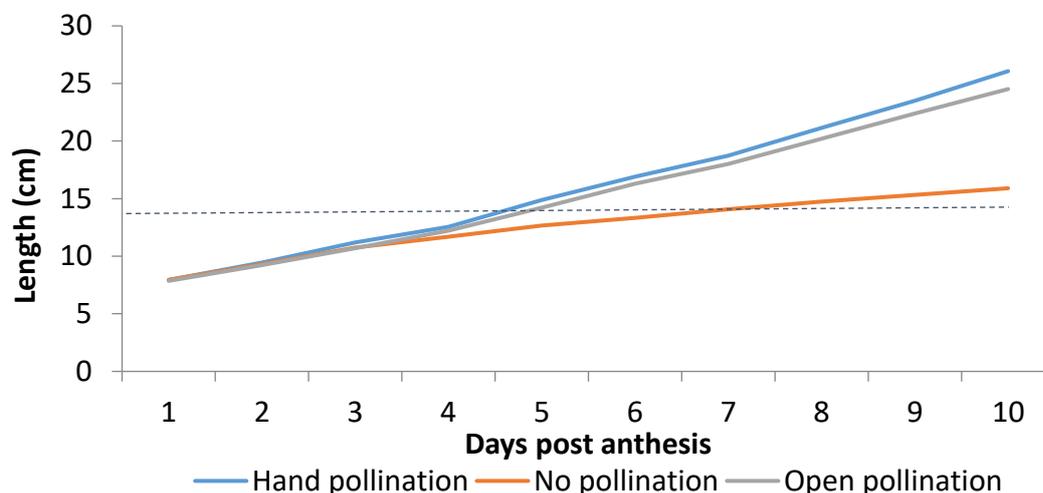


Figure 11 Average daily length (y axis) of field grown courgettes subject to pollination treatments (hand pollination, open pollination and no pollination) over 10 days (x axis). The dashed line shows the minimum length required for commercial courgettes.

Pollination with distance into a crop

Distance from the edge of the crop had no effect on percentage fruit set, fruit growth, weight and °Brix of open-pollinated plants (Table 6).

Table 6 Results from the GLMMs and LMMs on the effect of distance from the crop edge on field grown courgette quality and quantity measures (mean \pm standard error). N = the number of fruits analysed.

Measure	0 m from the crop edge (mean \pm SE (n))	50 m from the crop edge (mean \pm SE (n))	Contrast estimate \pm SE	Test statistic	P-value
Fruit set (%)	92 \pm 5.8 (5)	97.8 \pm 2.2 (5)	0m - 50m: 0.95 \pm 1.64	Z = 0.576	0.56
Fruit growth (length in cm after 10 days)	26.3 \pm 0.7 (45)	24.3 \pm 0.9 (44)	0m - 50m: 2.65 \pm 2.39	T = 1.106	0.27
Fruit weight (g)	1009.3 \pm 53.3 (45)	923.1 \pm 61.7 (44)	0m - 50m: 147.51 \pm 167.14	T = 0.883	0.38
Brix	3.8 \pm 0.1 (45)	3.9 \pm 0.1 (44)	0m - 50m: 0.12 \pm 0.20	T = 0.615	0.54

Economic value of pollinators

Courgettes are grown over 808 ha in the UK, which is not a large area compared to other crops, but each hectare of courgettes is worth over £8,000 to the grower in market value (Table 7). The current economic value (EV) of courgettes in the UK is therefore estimated to be £6,694,632. Our pollination experiments demonstrate that the crops studied had a D of 0.41 i.e. 41% of fruit set was dependent on natural pollination (f_p compared to f_{pe}). This means that, if all UK crops are pollinated as well as they are in Cornwall, then pollinators contribute £2,744,735 to the total economic value of courgettes in the UK ($IPEV$). The maximum dependency on pollinators under maximal pollination conditions (f_{pe} compared to f_{pmax}) was 0.43. Therefore, if the pollination deficit observed from our pollination experiments (although not significantly different from open pollination) is assumed to be similar across the UK, then there is scope to improve crop pollination by just 3% which will increase the value of courgettes in the UK by £134,086 (Table 7).

Table 7 Calculation of the economic value of pollinators to courgette production at a hectare and national scale. P was 0.43 £/ha (DEFRA 2016). Total area of UK courgette production is 807.75 ha (British Growers Association, personal communication 22nd September 2016). D was 0.41 and D_{max} 0.43 calculated from experimental results in Error! Reference source not found..

	Economic value (£)	
	Per ha	UK Value
Quantity Q (Kg)	19,274	-
Economic value EV (£)	8,288	6,694,632
Total economic value of insect pollination $IPEV$ (£)	3,398	2,744,735
Maximum economic value of pollination service $MaxEV$ (£)	3,564	2,878,821
Value of pollination deficit $PDef$ (£)	166	134,086

Discussion

The importance of pollinators to courgettes is demonstrated through a significant reduction in fruit size and weight under no pollination conditions. Consequently, percentage fruit set, the size and weight, but not sugar content, of courgettes were significantly increased with pollination. As all flowers within a field experienced the same environmental conditions, the observed reduction in fruit set (for non-pollinated and open pollinated flowers) was due to the absence of pollen. The relatively high fruit set of hand pollinated flowers (98%) suggests that

resources (such as nutrient and water availability) were unlikely to be limiting courgette growth and fruit set and demonstrates the quality and quantity of courgettes under optimal pollination conditions. Unfortunately, it was impossible to identify any differences in pollinator dependence between courgette varieties as data from this study are only available for one courgette variety.

Nonetheless, it is of industrial and ecological interest that 56% of non-pollinated flowers were still able to reach marketable size and shape without any pollination at all. This is due to the natural parthenocarpic tendency of courgettes, previously observed in Tosca (Martínez *et al.*, 2013) and other varieties (Robinson and Reiners, 1999). However, Martínez *et al.*, (2013) concluded that Tosca was not truly parthenocarpic as fruits consistently showed a burst in ethylene around three days after anthesis which is thought to cause early fruit abortion in non-pollinated flowers. This may explain the slower growth rate around three days post anthesis (Figure 11) and reduced fruit set in non-pollinated flowers (Table 5). The effect of parthenocarpy appeared to have no effect of sugar content in courgettes, unlike observations in melon (Hayata *et al.*, 2000; Shin *et al.*, 2007).

Since courgette yield is dependent on pollination ($D = 0.41$) the total economic value of insect pollination to courgettes is estimated to be worth approximately £3,398/ha and is consequently a significant proportion of the total economic value of courgettes (Table 7). Due to high levels of open pollination observed in Cornwall, pollination deficit was estimated to be just 3%. Nevertheless, if pollination was maximised, the economic value of courgettes would increase by approximately £166/ha. This is similar to the apple variety 'Cox' which has an estimated pollination deficit of £146/ha in the UK (Garratt *et al.*, 2013). Interestingly, this was partly due to no significant difference between the yield of open pollinated and pollinator excluded flowers which demonstrate the ability of the Cox variety to set fruit in the absence of pollinators. However, the same study showed that the Gala variety had a much higher pollination deficit of £6,459/ha, due to an increased dependency of this variety on pollination and higher yield from hand-pollinated flowers. This demonstrates how important it is to include different pollinator dependency ratios based on inter-variety differences when performing economic valuations.

The price of courgettes used in this valuation (despite being a seasonal average) is likely to vary in response to the supply and demand of courgettes on the open market (Garratt *et al.*, 2013; Melathopoulos, Cutler and Tyedmers, 2015). Consequently, the economic value of insect pollination to courgettes presented in this study, tells us our actual and potential dependency on pollinators at this current time, rather than an absolute value. If pollinator populations were to decline in the UK, the supply of courgettes would decrease, which would

increase demand (especially if alternative countries were also unable to meet demands). This would raise the price of courgettes on the open market and increase the total economic value of insect pollination.

Despite the relatively small pollination deficit in this study, spatial and temporal fluctuations in pollinator populations mean that it may still be beneficial for growers to improve pollination services, even if pollination deficits are due to natural variation in yield. A relatively quick and simple way of doing this is to use commercial bee species which are known to be effective pollinators of cucurbit crops (Artz and Nault, 2011; Petersen et al., 2014) although this is not certain for in this project commercial colonies did not have an impact on yield and quality.

A longer-term but more sustainable option could be to enhance floral resources, a significant limiting factor in bee populations (Roulston and Goodell, 2011). Increased floral resources can attract pollinators to a site and provide resources for both managed and wild bees beyond that of the focal crop (Carvell et al., 2007) (supplementary info: nutritional value of courgette for bees). Generally the effectiveness of these measures is moderated more by the surrounding landscape, rather than the size of the area planted (Batáry et al., 2011; Heard et al., 2007; Herbertsson et al., 2018), with more simplistic landscapes showing greater yield increases than ones which already have good floral resources. As Cornwall already benefits from biodiverse hedgerows and generally smaller field sizes, availability of floral resources may be strongly influencing the high pollination rates observed in this study. This would support the findings of Garratt *et al.*, (2017) which showed hedgerows to be a valuable source habitat for pollinators and natural enemies. Therefore, these findings provide a clear incentive for growers in this region to maintain and protect these habitats to ensure high and stable pollination services in the future. Growers may also benefit from using crop varieties which have been selectively bred to be fully parthenocarpic (currently not done by commercial growers of courgette), especially in combination with pollinator-supportive practices (Knapp *et al.*, 2016).

Although confined to a single geographic region and variety, this study highlights the importance of pollination for improving yields, even when over half of the fruit set can still be achieved via parthenocarpy. Understanding a crop's demand for pollinators can help growers choose what varieties to use. In areas with lower visitation rates, potentially due to large fields or less natural habitat, growers may wish to increase the supply of pollinators. In doing so they may increase their agricultural resilience and further their economic advantage.

Realistic estimates of the amount of insect pollination required for optimum fruit set need to account for not only the variability in pollination deficit that might result from variable pollinator densities and environmental conditions, but also the variability in pollinator dependence between varieties of single crop species, for which there is currently little good evidence

(Knapp *et al.*, 2016; Melathopoulos *et al.*, 2015, although see Garratt *et al.* 2014). In the wider context, discussion and strategies for improving horticultural crop production need to incorporate costs and benefits associated with different methods of maximising pollination, whilst remembering that factors other than pollination also contribute to fruit set.

Funding Objective 3: Does the introduction of commercial bumblebees improve courgette yield?

Supplementing crop fields with managed bee species (Figure 12) can bolster pollinator numbers to stabilise yields. Managed colonies of *B. terrestris* were placed into a courgette field at the recommended stocking density and resulting yield measurements were taken.



Figure 12 Commercial bumblebee colony introduced for pollination services.

Research Question

- 1) Does the introduction of commercial bumblebees improve courgette yield?

Methods

Managed bumblebee colonies

Bombus terrestris was the focus of this study because of their natural abundance at study sites and availability as commercial colonies (Biobest Biological Systems, Belgium). Colonies

were placed in each field (with sugar water but no additional pollen) at a density of three colonies per field.

Yield

The effect of supplementing colonies of *B. terrestris* was investigated by comparing the yield of courgettes when colonies were open to when colonies were closed. One field (2.58 Ha) in Cornwall was stocked with six commercially reared *B. terrestris* colonies (Biobest Biological Systems, Belgium), near to the recommended stocking density for courgette (Koppert Biological Systems, Netherlands).

Colonies were opened or closed on alternate sampling days from the 1st August to the 7th September. On days where colonies were open bees were free to forage, but on days where colonies were closed, bees were contained within their colony. The afternoon before bees were contained the standard entrance was changed to one with a one-way valve so that any foragers already outside the colony could return, whilst preventing any additional bees from leaving the colony. Each colony contained one reproductive queen and around 80 workers and was provided 1.4 litres of sugar syrup for additional nutrition, as recommended by Biobest. Since colonies were only closed for one day there was no need to provide additional pollen. Colonies were insulated and waterproofed with polystyrene and placed inside metal cages which were pegged to the ground to reduce the risk of predation from badgers.

Since courgette flowers are monoecious, pistillate flowers were individually monitored (identified with marker pen written on pieces of flagging tape, tied to the base of each fruit) to quantify yield from plots randomly located within the field. This was done with *B. terrestris* colonies open (n = 100) and closed (n = 100) over 10 sampling days (20 flowers per day). Fruits were then harvested 10 days post-anthesis, weighed on scales and measured using a tape to determine their length. To avoid the confounding effect of a plant investing in additional fruits from un-monitored pollination events, only one fruit per plant was studied (Stephenson *et al.* 1988, Avila-Sakar *et al.* 2001). Experimental fruits were classed as 'aborted' if they did not meet minimum commercial standards, i.e. they were less than 14 cm long, 30 mm wide (at the mid-point), and over 5° in curvature, or showed any obvious signs of bacterial damage, such as blossom end rot. Therefore, fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) is also a measure of fruit quality. As fruit set was measured over 10 days courgettes were generally larger than commercial standards. Since these experiments were conducted at a commercial farm some fruits were accidentally removed by pickers. Consequently, final sample sizes were less than the number initiated and

are not completely balanced between treatments (with *B. terrestris* n = 97, without *B. terrestris* n = 96).

Results

There was no difference in courgette yield when *B. terrestris* colonies were open, compared to when they were closed (Figure 13).

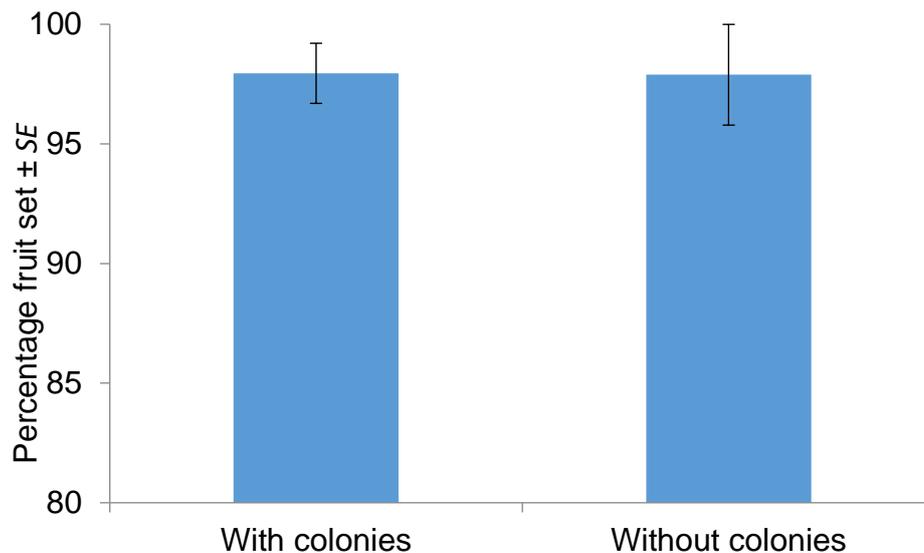


Figure 13 Percentage fruit set with and without commercial *B. terrestris* colonies open. Data were combined for the 5 days when colonies were open and the 5 days when colonies were closed.

Discussion

The degree to which managed pollinator species will improve yield depends on the pollination deficit at a given site. Results from this study showed that fields stocked with managed *B. terrestris* colonies (Biobest Biological Systems, Belgium) at the recommended stocking density for courgette (Koppert Biological Systems, Netherlands) was unable to significantly increase. Therefore, the recommendation of funding objective 2; to use managed bumblebee colonies to maximise yields (to hand pollination levels), are likely unfeasible in areas with a very low pollination deficit. Indeed managed colonies of *B. impatiens* were also unable to increase pumpkin yield, owing to already high levels of pollination at study sites in New York State (Petersen et al., 2014, 2013).

Nonetheless, areas with a greater pollination deficit may benefit from using managed pollinator species. For example, Artz, Hsu & Nault (2011) observed a significant increase in pumpkin yield following the addition of managed *B. impatiens* colonies at study sites which were also in New York State. This demonstrates the potential for spatial and temporal fluctuations in wild pollinator communities within a region, which may or may not result in pollination deficits. However, growers should be cautious not to rely on single species to fulfil their pollination needs, since cucurbits could also experience greater yields in more diverse habitats; where increased species richness and abundance of wild pollinators could improve spatial and temporal fluctuations in pollination services (Garibaldi et al., 2011; Hoehn et al., 2008), provide insurance against any pollinator loss (Shuler et al., 2005) and reduce the spread of disease and pathogens (Kremen and Miles, 2012). Therefore, promoting effective wild pollinators of courgette, through sustainable pollinator-supportive practices, could be the best way to increase grower's agricultural resilience for pollination services.

Funding Objective 4: Does the introduction of a flower strip between rows or along a field edge alter the effectiveness of pollination? If pollinators are supported with other floral resources do they visit the courgette flowers more (attraction) or less (distraction)?

Whilst 56% of fruit was able to reach marketable size and shape without any pollination, pollination significantly increased yield at an estimated worth of £3,398 per ha. Since floral resources are a significant limiting factor in bee populations, growers may wish to boost the floral resources available to pollinators as a way of mitigating potential fluctuations in pollinator populations which may adversely affect their yield. Results are discussed in the context of management decisions which can be made to improve either crop pollination, or species conservation; or both.

All study sites throughout this project were conventionally managed, however, the short picking interval of the crop means that little or no herbicide is used, resulting in a high abundance and diversity of wild flowers within and around the crop. No flower strips were therefore grown within the commercial studied crops. The abundance of wild flowers so close to crop flowers led to a study on how floral resources at different spatial scales, i.e. field scale: within the crop, around the crop, the whole field, and farm-scale: mass-flowering crops and semi-natural habitat may affect pollinator visitation to courgette (Figure 14).



Figure 14 Courgette co-flowering with wild flowers in Cornwall, UK.

Research Questions

This study focuses on pollinator visitation to mass-flowering courgette (*Cucurbita pepo* L.) and co-flowering wild flowers to further understand the extent to which available forage (at different spatial scales) may improve pollination and/or pollinator conservation in crop fields. To do this we ask:

- 1) Which pollinator species visit courgette flowers and which ones visit co-flowering wild flowers in crop fields?
- 2) How does forage availability and pollinator presence in field margins affect pollinator visitation to courgette flowers?
- 3) How does forage availability affect the abundance and species richness of pollinators which do not visit courgette?
- 4) Do wild flowers 'attract' or 'distract' pollinators to and from courgette flowers?

Methods

In 2016, nine courgette (var. Tosca) fields were surveyed in three key courgette growing regions of England in Cornwall (five sites), Worcestershire (two sites) and Cambridgeshire (two sites), to represent a range of climatic variables. Each field was visited three times between 1st June and 31st July, during courgette blooming. Eight 50 m transects per field were established; four along each field margin and four within the crop (from the edge of the crop to the centre, 25 m apart).

Pollinator surveys

Transects were walked at a steady pace (~5 minutes each) with observations made 1 m either side and in front of the recorder (over a length of 50 m). Halfway along each transect, one pan trap (15 cm diameter plastic bowl sprayed with yellow UV paint) was placed 20 cm off the ground (average height of courgette flowers) for 24 hours immediately after conducting a pollinator transect, to prevent any reduction in transect numbers by pan traps. Traps were filled with dilute scentless soapy water. All bees and hoverflies were identified to species level in the laboratory. Pollinator transects, and pan traps were analysed separately (transects were used to calculate the number of pollinators on courgette flowers and pan traps were used to estimate the abundance and species richness of solitary bees (non-courgette pollinators)). Solitary bees were chosen for further analysis over hoverflies due to their effectiveness at pollinating other crop species such as oilseed rape (Garratt et al., 2014). Using this combination of survey techniques gave a better representation of overall species richness and an insight into plant-pollinator interactions (Westphal et al., 2008). In addition, network diagrams of pollinator/ flower interactions in courgette fields, divided into field margins and the cropped area itself were constructed.

In 2017, additional transects in the crop and the field margins were simultaneously surveyed by two observers from 08:15 to 15:30 h at ten sites, resulting in an additional 640 transects. This was to capture pollinator activity in the four hours either side of courgette senescence, which occurs around 12:00 h. Since colonies of *B. terrestris* were added to all fields in 2017, foragers from these colonies are highly likely to have been recorded on pollinator transects.

Floral surveys

Flowering plant surveys were conducted at the same time and location as the pollinator transects. To calculate the availability of non-crop floral resources (wild flowers), the numbers of open flowers or floral units (in the case of composites) of insect rewarding plant species (defined in Hardman et al. (2016)) were recorded.

Landscape variables

Since semi-natural habitat and mass-flowering crops can increase pollinator populations (Kremen, Williams, Bugg, Fay, & Thorp, 2004; Westphal, Steffan-Dewenter, & Tscharntke, 2003), the total area of semi-natural habitat (woodlands and heathland) and mass-flowering crops (courgette, oilseed rape, and field beans) were calculated in 500 m and 2000 m radii of each field (m²) using CEH Land Cover® plus: Crops (for information on annual crop types) and Land Cover 2007 (for information on habitat types (Centre for Ecology and Hydrology, 2011, 2016) ArcGIS 10.2.2.

Statistical analysis

All analyses were carried out using R (R Core Team, 2017). Linear mixed effect models (Bates, Mächler, Bolker, & Walker, 2015) were used to investigate how wild plant abundance and species richness at different spatial scales influenced 1) the abundance of bee species observed visiting courgette flowers (honeybees and bumblebees) and 2) the abundance and species richness of other bee species (solitary bees) which were not observed visiting courgette flowers.

For each response variable, a set of candidate models were constructed using rescaled (between 0 and 1) predictor variables. For courgette visitors (honeybees or bumblebees), the full fixed effects model contained the abundance of the same species on field margins (either honeybees or bumblebees depending on the response variable), the overall abundance of either honeybees or bumblebees (opposite to the response variable as a measure of competition), abundance of wild flowers per crop transect, species richness of flowers per crop transect, total number of wild flowers per field (sum of wild flowers in the margins and cropped area), area of semi-natural habitat (m² in 500 m and 2000 m radii), area of mass-flowering crops (m² in 500 m and 2000 m radii), field size and the interaction between mass-flowering crops or semi-natural habitat and wild flowers per crop transect and wild flowers per field. Region and field size were entered as random effects, and a null model including random effects only included in the candidate model set. Abundance and species richness of floral resources were always tested in separate models due to collinearity, as were semi-natural habitat and mass-flowering crops (at 500 m and 2000 m radii).

Non-courgette visitors (solitary bees) were analysed in similar models but included data from both margins and cropped area (per transect) in the response variable. Transect location (margin or cropped area) was added as a fixed effect.

For each stage of the analysis, all possible combinations of the full model (all fixed effects) were compared to the null intercept-only model using Akaike's An Information Criterion for small sample sizes (AICc) (Barton, 2017), with the lowest AICc score defining the model that best describes the data (Symonds & Moussalli, 2011).

Models with a change in AICc of 2 or less when compared to the best fitting model were examined (model averaging was not carried out due to correlated predictor variables) and the most parsimonious of these (the one with the lowest AICc) further checked for multicollinearity using variance inflation factors (VIFs) (Fox & Weisberg, 2011). All covariates had VIFs <5. The full and best fitting models were visually checked to meet model assumptions (homogeneity of variance, normal distribution of residuals and the presence of influential

values). The significance of fixed effects in the best fitting model were tested using summary t and p -values (Kuznetsova, Brockhoff, & Christensen, 2016).

Results

Pollinator community

Overall, a total of 958 pollinators of 53 different species were recorded during this study. All 53 species were recorded in pan traps and 9 species were recorded in pollinator transects (in the margin and cropped area of courgette fields). Pollinators caught in pan traps included 57% hoverflies, 31% solitary bees and 12% social bees (honeybees or bumblebees), while only social bees (84%) and hoverflies (16%) were observed during transect surveys. Nonetheless, three bee species and one hoverfly species accounted for 77% of all pollinator records: *Apis mellifera* ($n = 379$), *Bombus terrestris/ lucorum* ($n=196$), *Bombus lapidarius* ($n = 55$), and *Eupeodes corollae* ($n = 110$).

An average of 46% of pollinator species occurred in both the margin and within the crop, which included 6 bumblebee, 8 solitary bee, and 9 hoverfly species. Honeybees (and to a lesser extent bumblebees) were more abundant in the cropped area, whilst solitary bees were more abundant on field margins (Figure 15a). Courgette was the most visited floral resource with 52% of all flower visits recorded (including margins) being to male or female courgette flowers. The next most frequently visited flower species were *Sinapsis arvensis* and *Cirsium palustre* which received just 8% and 6% of pollinator visits respectively.

Floral resources

Across all regions floral species richness was greater in field margins than in the cropped area (mean margin = 29 ± 4 SE, crop = 12 ± 2 SE). Likewise, the number of flowers was generally greater in field margins than in the cropped area (mean margin = $23,273 \pm 5,365$ SE, crop = $10,529 \pm 4,838$ SE) (summed per transect) (Figure 15). The amount of semi-natural habitat varied from 0 - 13% (median = 3%) in a 500 m buffer and 1 - 17% (median = 9%) in a 2000m buffer. The percentage area of mass-flowering crops varied from 14 - 37% (median = 23%) in a 500m radius and 2 - 13% (median = 3%) in a 2000 m radius. The majority of mass-flowering crop in the landscape (94%) was courgette and was therefore flowering simultaneously with courgette at the study sites.

Figure 15 shows (i) boxplots of honeybee, bumblebee, and solitary bee abundance (ii) species richness of wild flowers, and (iii) the abundance of wild flowers within courgette fields (light grey) and in the field margins (dark grey). (i) shows summed values from pan traps and transects, while (ii) and (iii) show summed values from transects, all from the three survey

dates per season. Boxplots represent the median, interquartile range, and maximum and minimum recorded values.

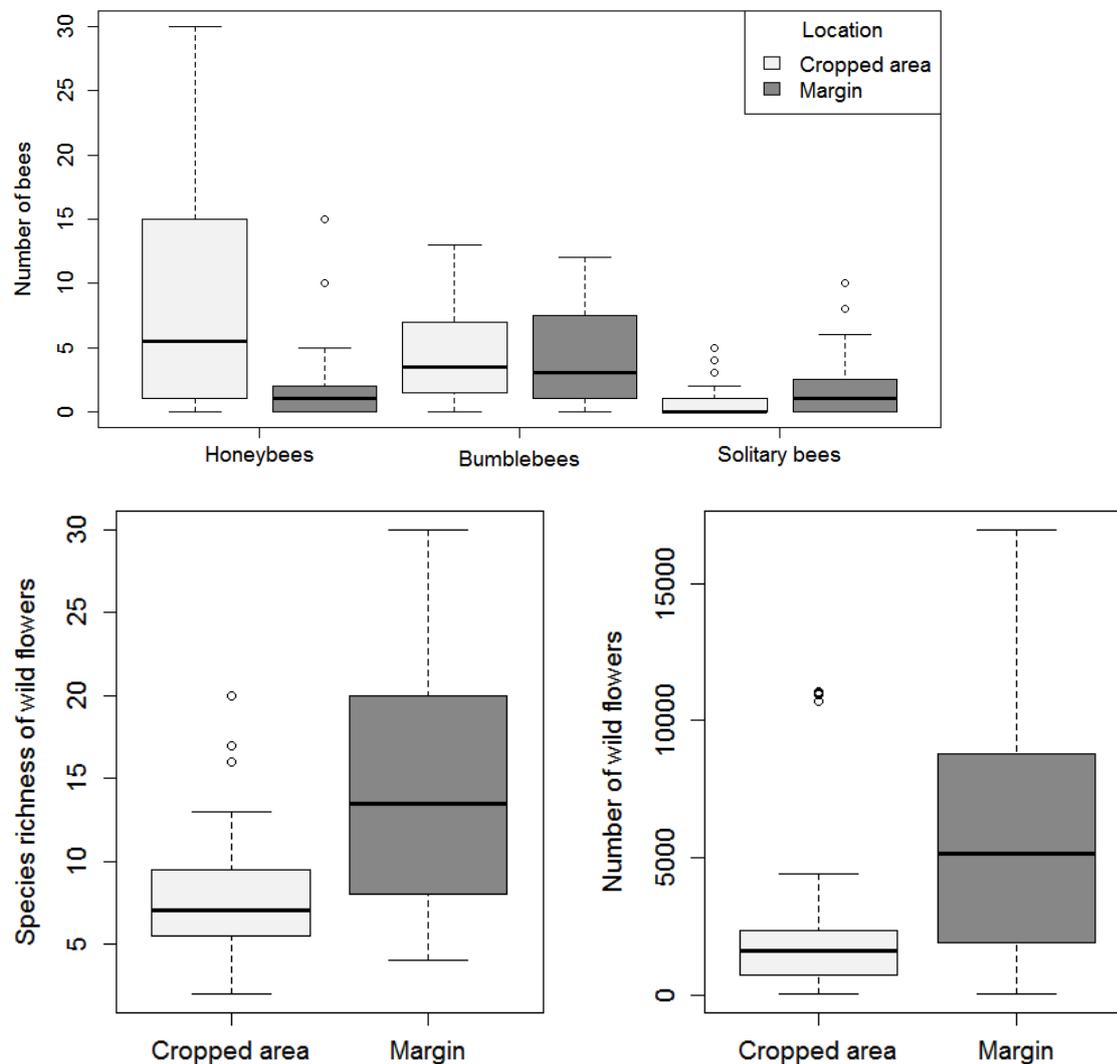


Figure 15: boxplots of honeybee, bumblebee, and solitary bee abundance; species richness of wild flowers and; the abundance of wild flowers within courgette fields

Figure 16 shows pollinator communities of courgette fields divided into field margins (green) and the cropped area (yellow). Pollinator communities for field margins are upside-down for easy comparison with pollinator communities in the cropped area. The width of bars indicates the proportion of species which were recorded during pollinator transects feeding on a particular plant species. Full plant species names for labels are: *Angelica sylvestris*, *Crepis vesicaria*, *Digitalis purpurea*, *Geranium dissectum*, *Geranium robertianum*, *Sonchus asper*, *Stachys sylvatica*, *Trifolium repens*, *Viccia cracca*.

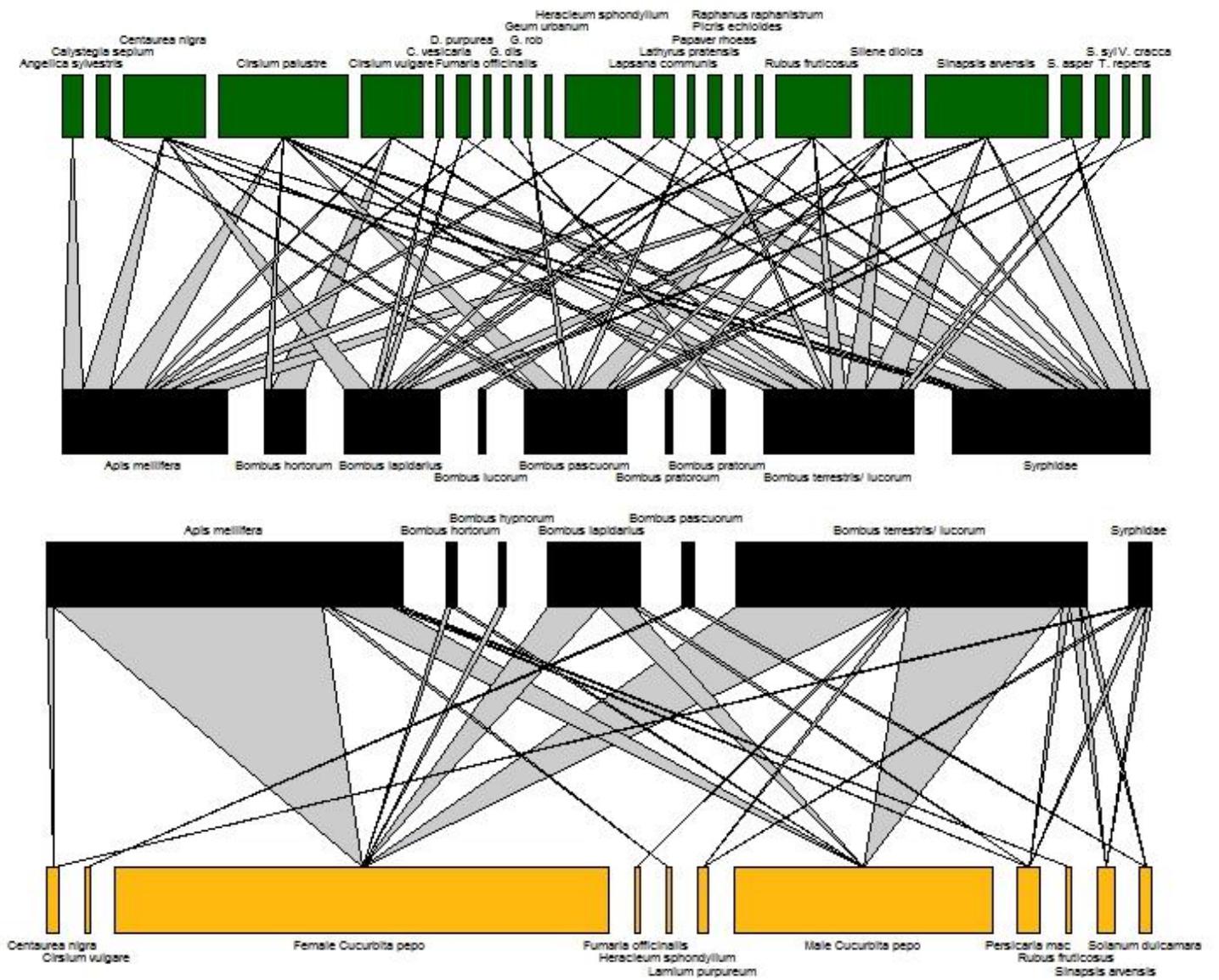


Figure 16: pollinator communities of courgette fields divided into field margins (green) and the cropped area

Effect of floral resources on courgette pollinators

Honeybee abundance on courgette flowers was negatively correlated to the quantity of semi-natural habitat in a 2000 m radius (m^2) ($\beta = -0.10 \pm 0.13$, $t = -7.44$, $p = 0.023$) (Figure 17a), as well as a decrease in species richness of wild flowers in the cropped area (summed per transect), although this was not statistically significant ($\beta = -0.22 \pm 0.1$, $t = -1.83$, $p = 0.08$) (Table 8).

Bumblebee abundance on courgette flowers was positively correlated with an increase in species richness of wild flowers in the cropped area (summed per transect) ($\beta = 0.73 \pm 0.2$, $t = 33.44$, $p = 0.002$) (Figure 17b).

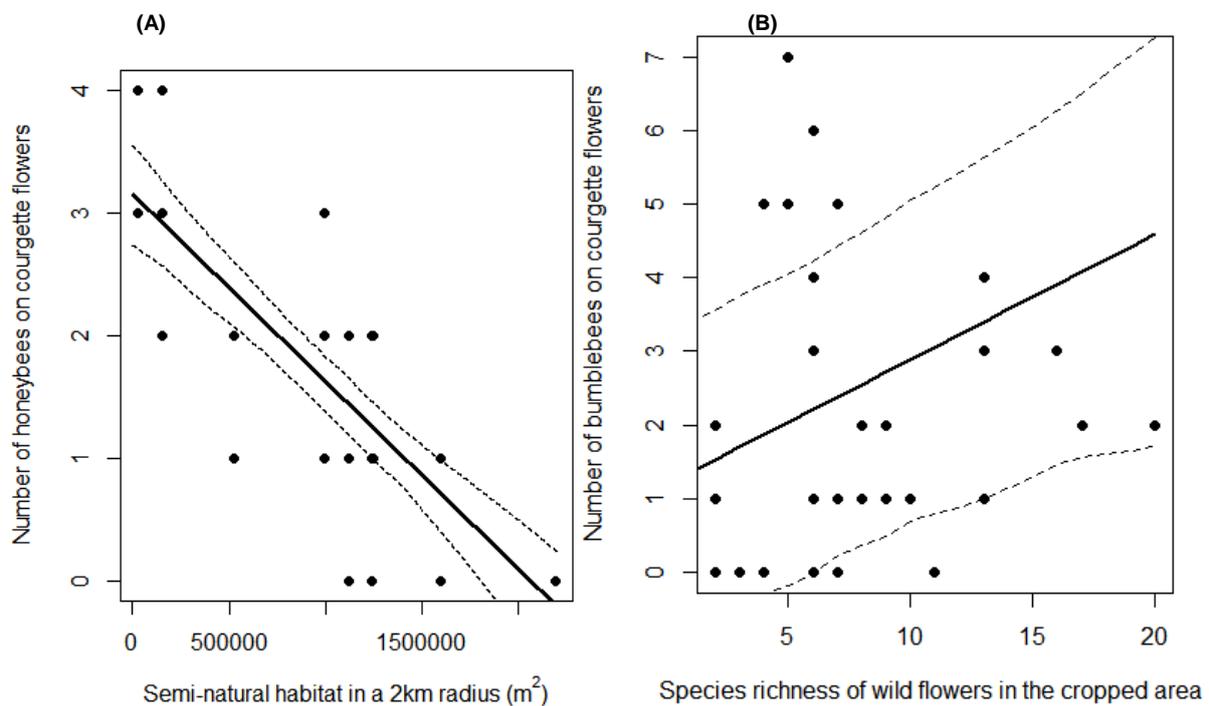


Figure 17 Significant effects from best fitting models (lowest AICc) of (A) honeybee abundance on courgette flowers in relation to semi-natural habitat (SNH) in a 2000 m radius (m^2) surrounding courgette fields, and (B) bumblebee abundance on courgette flowers in relation to the species richness of wild flowers in the cropped area. Solid lines are predicted values from linear models and dashed lines are 95% confidence intervals calculated via bootstrapping of 200 simulations.

Both *A. mellifera* and *B. terrestris* show a preference for courgette flowers in the morning when they are open (Figure 18).

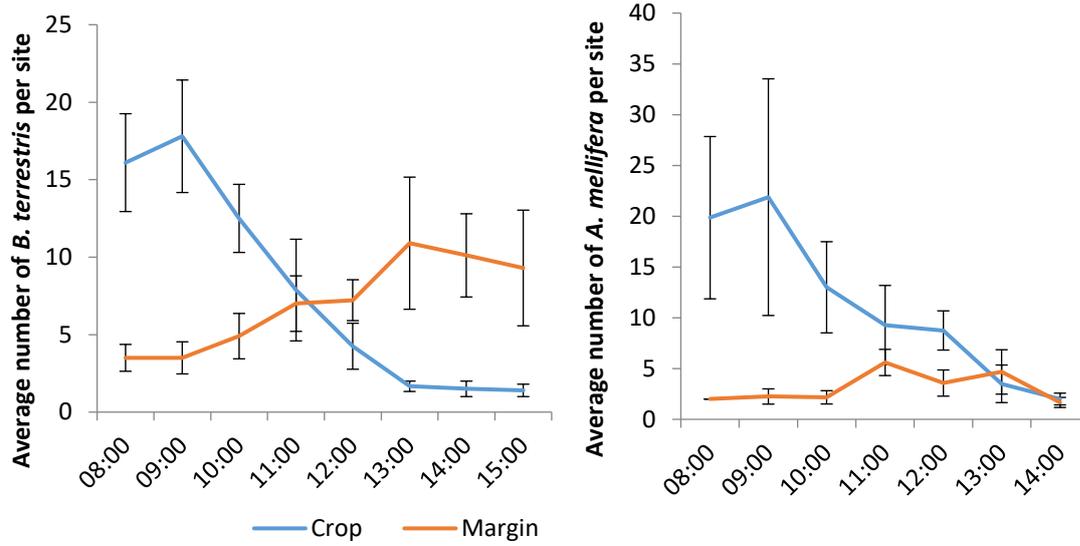


Figure 18 (A) average number of *B. terrestris* and (B) *A. mellifera* in the crop and on the margin over time, data were summed per transect in either the crop or on the margin and averaged by site (n = 10), all sites contained commercial colonies of *B. terrestris*.

Effect of floral resources on solitary bees

The best fitting model for solitary bee abundance included transect location (margin or crop), an increase in field size, species richness of wild flowers in fields, semi-natural habitat in a 2000 m radius and the interaction between wild flower species richness and semi-natural habitat (Table 8). Solitary bee abundance was positively related to wild flower species richness (summed per transect) ($\beta = 0.93 \pm 0.33$, $t = 2.78$, $p = 0.007$) and there was a significant interaction between floral species richness (summed per transect) and semi-natural habitat in a 2000m radius (m^2) ($\beta = -1.46 \pm 0.35$, $t = -4.24$, $p < 0.001$), with an increase in wildflower species richness positively correlated with solitary bee numbers in landscapes with low semi-natural habitat (Figure 19a). For all other predictors in the best fitting model the relationship was not significant (margin transect location ($\beta = 0.76 \pm 0.5$, $t = 1.50$, $p = 0.14$); field size (Ha) $-\beta = 0.72 \pm 0.4$, $t = 1.63$, $p = 0.11$); semi-natural habitat in a 2000m radius (m^2) $\beta = -0.23 \pm 0.37$, $t = -0.61$, $p = 0.55$).

The best fitting model for solitary bee species richness included location (margin or crop), species richness of wild flowers in fields, semi-natural habitat in a 2000 m radius and the interaction between wild flower species richness and semi-natural habitat (Table 8). Solitary bee species richness was significantly greater in field margins than in the cropped area ($\beta = 0.74 \pm 0.32$, $t = 2.35$, $p = 0.02$) (Figure 19b) and wildflower species richness had a positive relationship with solitary bee species richness in areas with low or medium levels of semi-natural habitat (overall interaction effect size $\beta = -0.57 \pm 0.2$, $t = -2.35$, $p = 0.02$, Fig. 3B). There was no significant relationship with wild flower species richness (summed per transect) ($\beta = -0.33 \pm 1.9$, $t = 1.88$, $p = 0.07$), or semi-natural habitat in a 2000 m radius (m^2) ($\beta = -0.33 \pm 0.19$, $t = -1.69$, $p = 0.12$).

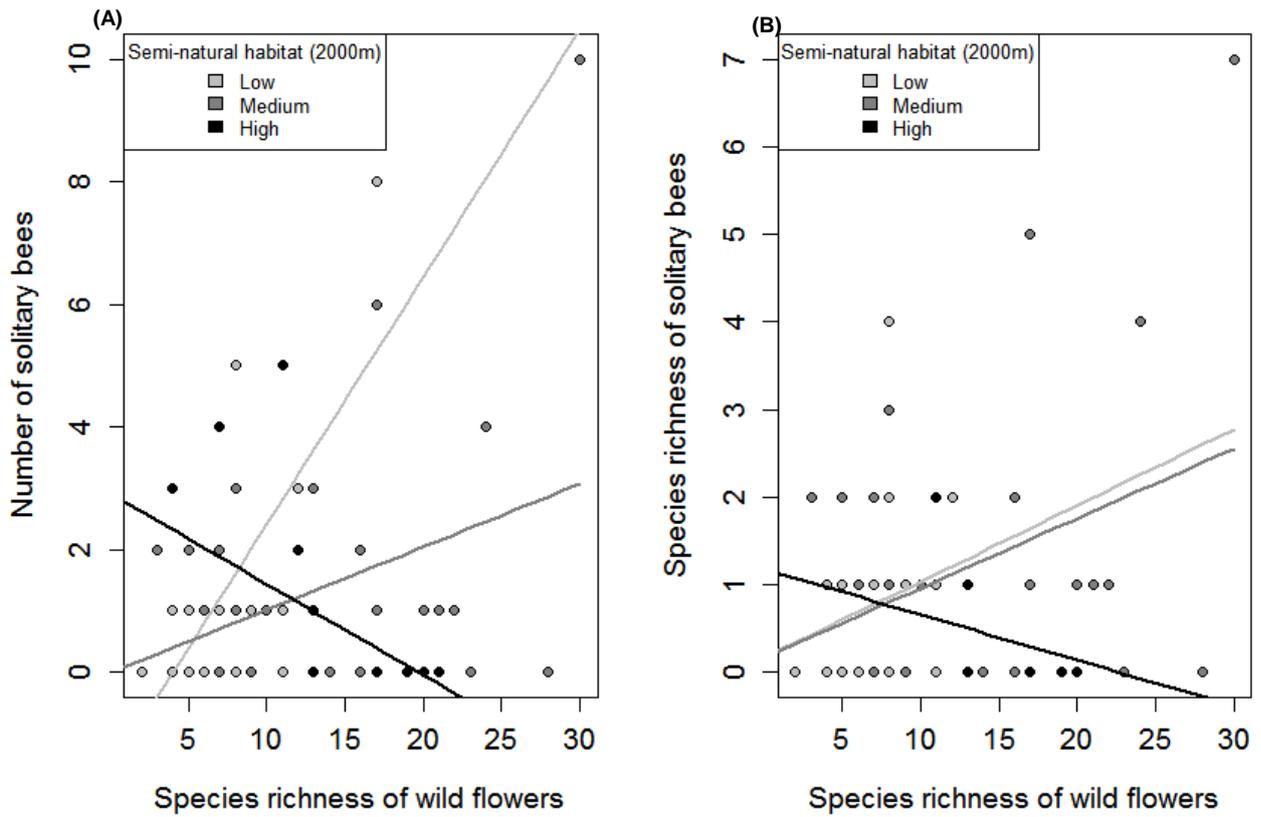


Figure 19 Significant interaction effects from best fitting models (lowest AICc) of (A) solitary bee abundance, and (B) solitary bee species richness in courgette fields in relation to the species richness of wild flowers in courgette fields and semi-natural habitat (SNH) in a 2000 m radius (m²) surrounding courgette fields. Solid lines are predicted values from linear models.

Table 8 Top (maximum of three) best fitting models (with $\Delta AICc < 2$) describing the effect of field size and floral resources (at a local, and landscape scale) for 1) honeybees, and 2) bumblebees which were observed visiting courgette flowers, 3) solitary bee abundance, and 4) solitary bee species richness which were recorded over the whole courgette field. Only coefficient estimates for continuous fixed effects which occur in the best fitting models are presented in the table. Global models contained for 1 and 2: Field size + (Abundance of wild flowers in the cropped area + Species richness of wild flowers in the cropped area) + Total number of wild flowers in the field + Margin honeybee abundance or Margin bumblebee abundance + Overall honeybee abundance or Overall bumblebee abundance + (Semi-natural Habitat (SNH) 500m + Semi-natural Habitat 2000m) + (Mass-flowering Crops (MFC) 500m + Mass-flowering Crops 2000m) + *all measures of wild flowers* * MFC/SNH. For 3 and 4 the global models were the same but included wild flowers over the whole field, transect location, and did not include honeybee or bumblebee abundance. Preceding brackets indicate where due to multicollinearity only one predictor variable for local floral resources and one variable for semi-natural habitat and mass-flowering crops were entered into the model. The R^2 (a measure of overall model fit) is divided into marginal R^2 (R^2_m) and conditional R^2 (R^2_c) following Nakagawa and Schielzeth (2013).

	Intercept	Field size	SR wild flowers (cropped)	Abundance of wild flowers	SR wild flowers	Margin bumblebee abundance	Margin honeybee abundance	SNH 2000m	Location	SNH 2000m* SR wild flowers	df	AICc	$\Delta AICc$	Weight	R^2_m	R^2_c
1) Honeybee abundance on courgette flowers	1.61		-0.22			-		-1.01	-		6	86.60	0.00	0.13	0.73	0.73
	1.72					-	-0.06	-1.04	-		6	87.23	0.63	0.10	0.15	0.65
	1.61					-		-1.06	-		5	87.27	0.68	0.09	0.70	0.71
									-							
2) Bumblebee abundance on courgette flowers	1.53		3.06						-		5	127.50	0.00	0.15	0.09	0.80
	1.79		2.91			-0.06			-		5	128.84	1.35	0.08	0.09	0.80
3) Solitary bee abundance in courgette field	-3.53	2.36	-		13.43	-	-		+ Margin	-20.96	9	294.79	0.00	0.39	0.31	0.31
	-1.49		-		11.42	-	-		+ Margin	-19.80	8	295.19	0.40	0.32	0.28	0.28
4) Solitary bee SR in courgette field	-0.45		-		5.31	-	-		+ Margin	-8.28	8	234.17	0.00	0.07	0.21	0.21
	0.50		-			-	-		+ Margin		5	234.53	0.35	0.06	0.11	0.16
	0.36		-	0.97		-	-		+ Margin		6	234.65	0.48	0.05	0.14	0.20

SR = Species richness

Abundance and SR of wild flowers refer to a whole courgette field, unless otherwise stated.

Discussion

Enhancement of floral resources to increase pollinator populations is the primary basis for pollinator-supportive land management; however, less is known about how the scale of floral resources (field or farm) affects different crop pollinators. By understanding the dynamics more fully, pollinator-supportive land management can be targeted at *either* pollinator species which are known to visit the focal crop, with the aim of improving crop pollination, *or* at other pollinator species more generally, with the aim of improving species conservation; or both.

Community networks of pollinators in courgette fields show that whilst field margins support a greater number of pollinator species, many of these species also occur within the cropped area, suggesting movement between the two areas. Of these species, *A. mellifera* and *B. terrestris* were the most abundant and occur in their greatest numbers within the cropped area. These two species also show a preference for courgette flowers, with *B. terrestris* showing a more equal preference than *A. mellifera* for male and female courgette flowers which may affect their relative effectiveness as pollinators (also see funding objective 1) (Artz, Hsu, & Nault, 2011; Ne'eman, Jürgens, Newstrom-Lloyd, Potts, & Dafni, 2010). These networks also show that several widespread, resilient species (rather than species-rich communities) are the main pollinator visitors to courgette (see also Knapp & Osborne (2017)). Although these species respond positively to increased provision of floral resources their populations can be increased by beekeepers/ farmers introducing managed colonies to an area. Honeybee density on crop flowers may also be intensified by their *en masse* recruitment to areas of good forage (von Frisch, 1967) and beekeepers may have placed more honeybee colonies into areas with more mass-flowering crop.

In the morning, *B. terrestris* and *A. mellifera* show a preference for courgette flowers, compared to hedgerow flowers, when courgette flowers are open, suggesting that neither species are 'distracted' by wild flowers on the margin. These results also highlight the importance of maintaining wild flowers to fulfil bees' requirements for nectar and pollen beyond that of the focal crop. Indeed flower rich areas have been shown to increase colony density (Wood et al., 2015b) and food supplementation shown to increase colony development, particularly of queen and male bumblebees (Pelletier and McNeil, 2003). However, the extent to which pollinators are attracted into mass-flowering crops will vary depending on the relative quality and quantity of floral resources in the mass-flowering crop and nearby semi-natural habitat. In this study it appears that providing additional floral resources to mass-flowering courgette facilitates pollination services to courgette, supporting bee nutrition without distracting bees from courgette flowers.

Bumblebee abundance on courgette flowers was correlated with an increase in species richness of wild flowers in the cropped area. As bumblebees have been observed to stay constant to an area of good forage (Osborne et al., 1999) it is likely that they are foraging between wild and crop flowers (due to their phenology) at a specific locality. Importantly, the abundance of honeybees or bumblebees on the field margin did not influence their abundance on courgette flowers, suggesting that wild flowers are not competing with crop flowers for pollinator visitation, supporting findings from almond orchards (Lundin et al., 2017). Nonetheless, honeybee foragers were more abundant on courgette flowers in areas with less semi-natural habitat; and where there was lower species richness of wild flowers in the cropped area, suggesting that courgette may compete with co-flowering wild flowers for honeybee visits, although this was not statistically significant. Whilst in the short-term wild flowers within the crop may compete with courgette for pollination services, these wild flowers may improve pollinator populations and thus crop pollination in the long term (Mitchell et al., 2009). Unfortunately, no information is available on the abundance of honeybee hives or the location of apiaries within 10 km of the study sites. Therefore, it is impossible to determine if honeybee forager abundance is moderated by the amount of semi-natural habitat in the landscape or beekeeping practices. The overall abundance of honeybee foragers at a site did not influence bumblebee abundance at courgette flowers and vice versa, nor interact with other landscape features, suggesting that these species are not in competition with each other for common resources and that the surrounding landscape is not moderating any potential competition between species (Herbertsson, Lindstrom, Rundlof, Bommarco, & Smith, 2016).

Floral species richness was more important for predicting solitary bee abundance and species richness (non-courgette visitors) in sites with less semi-natural habitat. This suggests that a higher species richness of wild flowers in courgette fields is needed if they are located in areas with less semi-natural habitat. Likewise, the abundance and species richness of solitary bees were higher in the margin (where there is a higher abundance and species richness of wild flowers) than the cropped area. These findings are likely due to solitary bees generally having broader dietary and nesting requirements, and much smaller foraging ranges (150 - 600 m) than honeybees and *B. terrestris* (the predominant bumblebee species) (Gathmann & Tschamntke, 2002; Greenleaf, Williams, Winfree, & Kremen, 2007). Wild flowers have been shown to be more effective at increasing pollinator abundance and species richness in simple landscapes (with less semi-natural habitat) compared to complex landscapes (Scheper et al., 2013). Since Scheper et al. (2013) and this study only analysed foraging individuals it is impossible to determine if differences in pollinator abundance and species richness between study sites were due to the transient movement of species between areas of forage, or due to an actual population increase. Floral resources on the field margin also increased the

abundance of *Eupeodes corollae*, a hoverfly species whose larval stage is a natural predator of soft-bodied arthropods such as aphids (Gomez-Polo et al. 2014). Therefore, provisioning floral resources at the field scale may increase pollinator abundance, species richness, and natural enemies of pests (Blaauw & Isaacs, 2014; Wood, Holland, Hughes, & Goulson, 2015). This study shows that it is important to know which pollinator species visit crop or wild flowers so that management decisions can be made to improve either crop pollination, or species conservation; or both (Kleijn et al., 2015). These findings demonstrate that allowing uncultivated areas around the crop to be colonised by species-rich wild flowers is an effective way of boosting the abundance of bumblebees - important pollinators of courgette, as well as the abundance and species richness of solitary bees – important pollinators of other crop and wild flower species which may be co-flowering in the landscape. Thus, provisioning floral resources may benefit pollination services and pollinator conservation.

Funding Objective 5: Is there an interaction between water use (or nitrogen use) and pollination levels?

Whilst several studies have explored the positive, mediating effect of forage availability on pollinator visitation and crop yield many overlook other factors which influence yield (pre- or post-pollination) such as, soil quality, water availability, weather conditions, and farming practices. Using the experimental design for funding objective 4, additional data were collected on the farming intensity, soil nutrients and yields of courgette at eight sites in Cornwall, Worcestershire and Cambridgeshire.



Figure 20 Staminate courgette flower. Photograph by Daphne Wong.

Research Question

Using the experimental design from funding objective 4, additional data were collected to ask:

- 1) What is the most important environmental factor for influencing courgette yield?

Methods

Additional environmental variables

The nine fields were managed by five horticultural growing companies: with similar farming practices checked using questionnaires. No foliar insecticides were applied at any fields since courgette experiences little pest damage in the UK. Whilst all fields were subject to the same tillage regime (ploughed and harrowed), one site in Cornwall was consistently tilled at depths 4 cm less than other fields. Each field differed slightly in the number of herbicide (0-5 applications, median = 2.5), fungicide (0-4 applications, median = 2) and fertiliser applications (1-5 applications, median = 2.5). All fields were planted with the same density of courgette and none were stocked with commercial pollinators, however, the density of managed pollinator colonies in the surrounding landscape was unknown.

As nitrogen, pH, phosphorous and potassium are important indicators of plant growth, a single homogenised soil sample was collected from four locations (halfway along pollinator transects) within the crop (2 - 5cm deep). This was done three times within the blooming period, for each field, totalling 27 soil samples. All soil analyses were conducted using a professional agriculture field test kit (Hanna Instruments, Rhode Island, US) which used colourmetric tests for nitrogen, pH, and phosphorous and a turbidimetric test for potassium.

Yield

At the end of the season, each farmer (apart from one) provided data on their total marketable yield (kg) per field. This was then calculated as yield/Ha/day based on the area of the field and the number of days which it was cropped. Due to one farmer not providing data the final sample size of this stage of the analysis was 8.

Statistical analysis

Linear models using lme4 (Bates et al. 2015) were used to determine the importance of *Apis*, *Bombus*, and solitary bee abundance (on the margin or within the crop) and site variables for influencing (3) courgette yield (per Ha per day). The full model contained region, field size, *Apis*, *Bombus*, and solitary bee abundance (on the margin or within the crop), crop floral abundance, and a measure of either; nitrogen, phosphorous or potassium. Due to the limited sample size of 8 sites, no interaction terms were specified.

For each stage of the analysis, all possible combinations of the full model (all fixed effects) were compared to the null intercept-only model using Akaike's Information Criterion for small sample sizes (AICc) using the MuMin package (Barton 2017), with the lowest AICc score defining the model that best describes the data (Symonds and Moussalli 2011).

Results

On average yield was greatest, but most varied in Cornwall, then Cambridgeshire, then Gloucestershire (Figure 21). Across all sites, the average values for nutrients were: nitrogen 3.2 ± 0.3 (SE), phosphorous 2.5 ± 0.3 , and potassium 2.5 ± 0.2 .

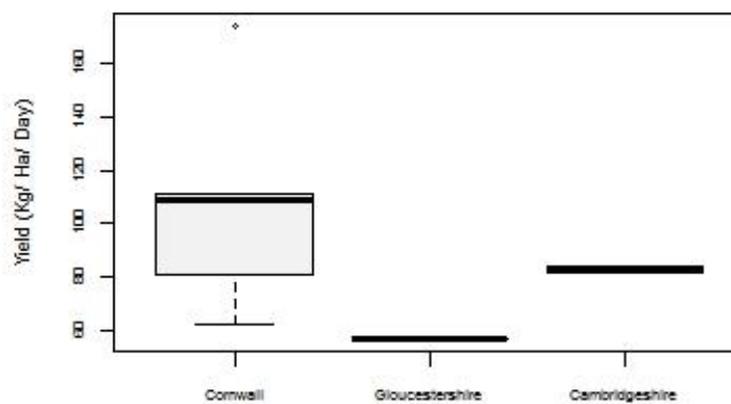


Figure 21 Average marketable yield (Kg) per day for each region.

Yield was best predicted by an increase in *Bombus* abundance per field margin ($R^2 = 0.29$) (

Table 9, Figure 21). The next best fitting model (an increase in crop solitary bee abundance) explained less of the overall variance ($R^2 = 0.23$) (

Table 9).

Table 9 Top three best fitting models (AICc $\Delta < 2$) describing the impact of field size, region, nutrients, pollinators (on field margins and within the crop) and floral resources (at a local, and landscape scale) for 3) courgette yield. Global model: Field size + Region + (Combined nutrients + Nitrogen + Potassium + Phosphorous) + Crop Floral Abundance + (*Apis mellifera* per field + *Bombus* on field margins per field + solitary bees on field margins per field + *Apis* in the crop + *Bombus* in the crop + solitary bees in the crop). Brackets indicate where due to multicollinearity, only one predictor variable for pollinators was entered into the model. Model results only show coefficient estimates for continuous fixed effects which occur in the best fitting models.

Intercept	Total <i>Bombus</i> in field margins	Total solitary bees in the crop	Nitrogen	df	AICc	Δ AICc	Weight	R ²
74.19	51.63			3	88.44	0.00	0.16	0.29
78.16		50.58		3	89.05	0.62	0.12	0.23
60.85			49.97	3	89.63	1.20	0.09	0.18

Discussion

Pollinator abundance and species richness within the crop did not affect yield. Nonetheless, pollinator abundance on the edge of the field appeared to have a positive effect on yield in the most predictive model, but this was not statistically significant. *Bombus* abundance on the edge of the field was also more important than overall pollinator abundance/ species richness (across the whole field) and *Bombus* and *Apis* abundance/ species richness. However, this relationship was much weaker than has been observed in pumpkins (Petersen and Nault 2014) and berry crops (Blaauw and Isaacs 2014). These results support findings from oilseed rape which have shown a correlation between pollinator visitation and yield, but no evidence of yield declining with distance from the crop edge (Woodcock *et al.* 2016). Edge pollinator abundance was also more important than species richness for improving yield, supporting previous findings in courgette (Knapp and Osborne 2017).

The yield models also showed that region, field size and nutrients were not important predictors of courgette yield. However, the limited sample size of eight fields meant that it was not possible to test the interaction of these predictors with pollinator abundance/ species richness, although it is known that nutrient availability can interact with pollination to influence yield (Klein *et al.* 2014, Marini *et al.* 2015). There was also no evidence of competition for soil resources and pollinators between wild flowers and courgette flowers, with neither floral abundance nor floral species richness negatively effecting courgette yield. This supports work in other species which have shown no negative effect of non-crop flowers on crop yield (Cierjacks *et al.* 2016). In this analysis, the weaker signal for yield is likely due to the way the analysis was structured; having single values for yield, per field, over the whole season, rather than measures of yield which directly followed the survey rounds at each site.

Additional work: Re-evaluating strategies for pollinator-dependent crops: how useful is parthenocarp?

Preliminary pollination experiments unexpectedly showed that courgette was able to set fruit without any pollination, a process known as parthenocarp (funding objective 2). Therefore, to improve estimates of pollinator dependence in crops and to improve guidance to farmers for improving fruit production, I conducted a meta-analysis of studies examining the extent and effectiveness of parthenocarp-promoting techniques (genetic modification, hormone application and selective breeding) currently being used commercially, or experimentally, on pollinator-dependent crops in different test environments (no pollination, hand pollination, open pollination). All techniques significantly increased fruit quantity and quality in 18 pollinator-dependent crop species (not including seed and nut crops as parthenocarp causes seedlessness). The degree to which plants experienced pollen limitation in the different test environments could not be ascertained, so the absolute effect of parthenocarp relative to optimal pollination could not be determined. Parthenocarp has the potential to lower a crop's demand for pollinators, whilst extending current geographic and climatic ranges of production. Thus, growers may wish to use parthenocarpic crop plants, in combination with other environmentally considerate practices, to improve food security and their economic prospects.



Figure 22 Courgette fruit set following a 'no pollination treatment', initiated the day before expected anthesis by securing PVC mesh bags with wire ties to pistillate flowers. Results of this pollination experiment were explored for funding objective 2.

Additional work: Nutritional value of courgette for bees

Pollinator dependence and pollination deficit (funding objective 2) and factors that may improve pollinator visitation (funding objective 4) are primarily concerned with what pollinators can do for courgette. However, courgette flowers may also help pollinators by providing abundant sources of nectar and pollen in the landscape which can boost population growth. *B. terrestris* was the focal pollinator species for this study due to its natural abundance in courgette fields, preference for courgette flowers, and contribution toward courgette yield (all funding objectives). By combining empirical data on nectar and pollen availability with model simulations (using the novel bumblebee model Bumble-BEEHAVE) we were able to quantify and simulate for the first time, the importance of courgette as a mass-flowering forage resource for bumblebees. Courgette provides vast quantities of nectar and pollen which ensures a high visitation rate and pollination potential of *B. terrestris*. Courgette pollen was not found in any pollen loads from returning foragers. Nonetheless, model simulations showed that early season courgette increased the number of hibernating queens, colonies, and adult workers (in the modelled landscape), but not colony size or nectar or pollen stores per bee. Courgette has the potential to improve bumblebee population dynamics however, bees can only benefit from this transient nectar source if alternative floral resources (particularly pollen) are also available to fulfil bees' nutritional requirements in space and time. Therefore, providing additional forage resources could simultaneously improve pollination services and bumblebee populations.



Figure 23 Sampling pollen loads from returning foragers caught in 'forager trap modules' (Osborne, Martin, Carreck, *et al.*, 2008) which were placed onto commercial colonies of *B. terrestris* positioned in courgette fields. Two years of surveying prior to this study had frequently shown *B. terrestris* collecting courgette nectar, but never courgette pollen. Photograph by Daphne Wong.

Project Discussion

Each funding objective of this project has drawn on different techniques and methodologies to determine the extent to which pollination influences fruit set (the mechanism), how pollination could be improved (the management), and how in doing so, growers' profits and agricultural resilience could be increased (the outcomes) (Figure 24). Where 'agricultural resilience' refers to a grower's ability to produce sufficient, nutritious food during periods of adverse environmental conditions, and therefore stable yields over time (Bullock et al., 2017).

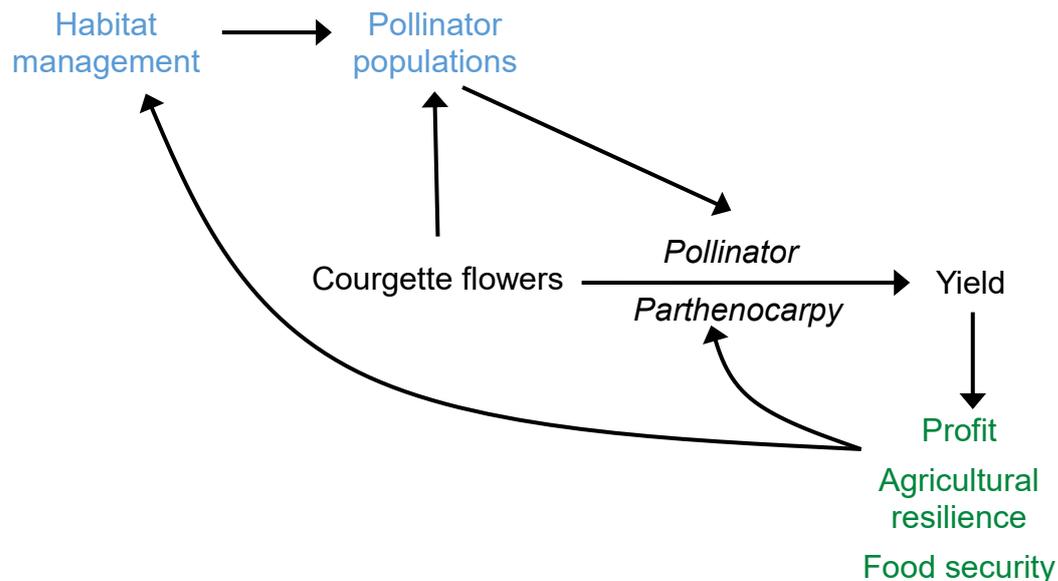


Figure 24 Mechanisms of (black text), management for (blue text), and outcomes of (green text) cucurbit pollination, investigated in this thesis using UK field-grown courgettes as a model system.

Can courgette pollination be improved?

Pollination experiments in the courgette variety 'Tosca' showed that insect pollination increased yield by 39% and that there was no evidence of pollination limitation on crop yield, evidenced by a low pollination deficit of just 3% (funding objective 2). Unexpectedly, 56% of fruit was able to reach marketable size and shape without any pollination, resulting in further experiments in a controlled temperature room with no pollinating insects on three additional varieties: 'Parthenon' (selectively bred to be parthenocarpic but not currently used by commercial growers), 'Greco' and 'Tosca', to compare to field, no-pollination experiments (Figure 25). However, after two attempts this experiment was ended as it was difficult to ensure that soil was not contaminated with insects and that plants were healthy enough to not affect fruit set. Growing different varieties in the Controlled Temperature room was of particular interest given that plastic pollination bags (used for funding objective 2) may elevate levels of ethylene and therefore influence fruit set. Likewise, different varieties are likely to vary in their level of pollinator dependence. Nonetheless, evidence of parthenocarpy is rarely discussed in

agroecology and inspired a meta-analysis (supplementary information) to quantify the extent and effectiveness of parthenocarpy in other crop species, which like courgette are also considered to be 'pollinator-dependent'.



Figure 25 CT room experiment exploring the pollination requirements of three courgette varieties 'Parthenon' (selectively bred parthenocarpic variety, not currently used by commercial growers), 'Greco', and 'Tosca'.

Whilst the yield of 'Tosca' was less dependent on pollination than expected, the total economic value of insect pollination to courgette production was still estimated to be worth approximately £3,398/ha. However, this economic valuation was based on the pollinator dependency and pollination deficit of just one courgette variety 'Tosca', thus inter-variety differences in pollinator dependence, or site-specific levels of pollination deficit may increase or decrease this economic value. For example, Garratt *et al.* (2014) estimated the apple variety 'Cox' to have a pollination deficit of £146/ha, compared to the variety 'Gala' which had a much higher pollination deficit of £6,459/ha. This was due to 'Gala' being more pollinator-dependent and having a larger pollination deficit (higher yield from hand-pollinated flowers) compared to 'Cox'. Whilst economic valuations are based on relatively simple estimates of pollinator dependence, pollination levels, and growing practices (which may not be representative of a larger spatial scale), they do clearly demonstrate the importance of pollinators to crop production (Gallai *et al.*, 2009).

Since growers may not know the level of pollination and therefore their potential pollination deficit, it would be useful to develop a predictive model which could determine if managed pollinators are required and/ or if longer term pollinator habitat creation is required. Although other pollination service models exist (e.g. Olsson *et al.* 2015), none have the capabilities of

BEE-STEWARD (www.beehave-model.net) which has an interface which already enables users to simulate the effects that different management options, such as wild flower strips will have on forager numbers and therefore, pollination potential. Thus, BEE-STEWARD would only need to be parametrised with crop/ variety's pollination requirements for bespoke simulations to be run for growers based on their level of pollinator dependence and likely abundances of bees (based on landscape maps).

How can courgette pollination be improved?

Whilst courgette is grown at different sites each year, *A. mellifera* and *B. terrestris* were the most abundant pollinators of courgette, occurring in their greatest numbers within the cropped area, at all field sites over the three field seasons of this thesis. Whilst both species show a preference for courgette flowers, funding objective 1 showed *B. terrestris* had a more equal preference for staminate and pistillate courgette flowers and carried more loose pollen grains (desirable for optimum pollen transfer) than *A. mellifera*. These findings also demonstrate that several widespread, resilient species (rather than species-rich communities) can fulfil the pollination requirements of courgette and that in the UK maximal yields can still be achieved without *Peponapis* and *Xenoglossa* bee species. A full species list of pollinator species recorded in courgette fields in 2016 and 2017 are in the supplementary information.

Although these species respond positively to increased provision of floral their populations can also be artificially increased by beekeepers and growers through the introduction of managed colonies. However, the degree to which managed pollinator species will improve yield depends on the pollination deficit at a given site. For example, results from the pollinator supplementation experiment (funding objective 3) at field sites in Cornwall (3% pollination deficit observed the year prior) showed that a field stocked with managed *B. terrestris* colonies (Biobest Biological Systems, Belgium) at the recommended stocking density for courgette (Koppert Biological Systems, Netherlands) was unable to significantly increase yield. Therefore, the recommendation of funding objective 2; to use managed bumblebee colonies to maximise yields (to hand pollination levels), are likely unfeasible in areas with a very low pollination deficit. Indeed managed colonies of *B. impatiens* were also unable to increase pumpkin yield, owing to already high levels of pollination at study sites in New York State (Petersen et al., 2014, 2013).

Nonetheless, areas with a greater pollination deficit may benefit from using managed pollinator species. For example, Artz, Hsu & Nault (2011) observed a significant increase in pumpkin yield following the addition of managed *B. impatiens* colonies at study sites which were also in New York State. This demonstrates the potential for spatial and temporal fluctuations in wild pollinator communities within a region, which may or may not result in pollination deficits. However, growers should be cautious not to rely on single species to fulfil their pollination

needs, since cucurbits could also experience greater yields in more diverse habitats; where increased species richness and abundance of wild pollinators could improve spatial and temporal fluctuations in pollination services (Garibaldi et al., 2011; Hoehn et al., 2008), provide insurance against any pollinator loss (Shuler et al., 2005) and reduce the spread of disease and pathogens (Kremen and Miles, 2012). Therefore, promoting effective wild pollinators of courgette, through sustainable pollinator-supportive practices, could be the best way to increase grower's agricultural resilience for pollination services.

To make recommendations on how growers could maintain or promote the high level of pollination observed for funding objective 2, it was necessary to determine the relationship between floral resources (of both courgette and wild flowers) and pollinators in courgette fields. Therefore, following an advert in AHDB's news bulletin for growers of 'Tosca', four more study sites (two in Worcestershire and two in Cambridgeshire) were selected to achieve a natural gradient of pollinators and floral resources at study sites. All sites had naturally occurring wild flowers in and around courgette flowers which provided an excellent opportunity to understand the extent to which floral resources mediate pollinator visitation to courgette flowers and courgette fields, at different spatial scales: field scale (in margins, and in the cropped area) and farm scale (500 m and 2000 m radii). Data were also collected at each site on soil pH, nitrogen, phosphorous, and potassium, as well as courgette yield at the end of the season (from farmers), in order to link the positive, mediating effect of forage availability on pollinator visitation and crop yield (although this has previously been shown in pumpkin in the US (Petersen, Jessica and Nault, 2014). However, having single values for yield, per field, over the whole season, rather than measures of yield which directly followed the survey rounds at each site, meant that the sample size was too small to detect any significant effects (funding objective 5). However, the methodology for this experiment is in place should it ever be repeated at a larger spatial scale.

Nonetheless, combining pollinator transects with pan trapping meant that the overall pollinator species richness of courgette fields was better represented, allowing management decisions to be formulated which could improve crop pollination, *or* species conservation; or both (Kleijn et al., 2015). Naturally occurring wild flowers (e.g. agricultural weeds and hedgerow flowers) are frequently overlooked floral resources for pollinators (Bretagnolle and Gaba, 2015) despite being free and sustainable, and thus more likely to be implemented by growers. Fully understanding the relationship and possible trade-offs between pollination and resource competition between courgette and co-flowering wildflowers was beyond the scope of this project. However, growers' concerns are likely to be minimal since many courgettes in the UK are grown in black plastic to suppress 'weeds' from growing immediately around crop plants (Figure 26). Indeed, wild flowers within the crop may have the additional advantage for

growers of improving soil structure which is vital for courgette fields picked daily using large tractors and rigs, especially during periods of heavy rainfall (Figure 26).



Figure 26 Damage to the soil following a period of heavy rainfall; two tractors were being used in this photograph to tow the rig. The use of black plastic to suppress ‘weed’ growth immediately around courgettes is also shown.

Data from funding objectives 4 and 5 did, however, show that wild flowers are unlikely to be competing with crop flowers for pollination services. For example, funding objective 4 showed *B. terrestris* visiting crop flowers more often than wild flowers in the hedgerows, in the morning when courgette flowers were open, before ‘switching’ to hedgerow flowers after courgette senescence, providing the first evidence of bee fidelity to a *Cucurbita* crop (Petersen et al., 2013). This was also supported by data from funding objective 4 which showed that the abundance of honeybees and bumblebees in field margins did not significantly reduce their abundance on courgette flowers.

Although solitary bees were not observed to visit courgette flowers, their abundance and species richness in courgette fields were significantly greater with more wild flower species and semi-natural habitat surrounding a site. Therefore, allowing uncultivated areas around the crop to be colonised by wild flowers is an effective way of boosting the abundance of bumblebees - important visitors to courgette flowers, as well as the abundance and species richness of solitary bees - benefitting pollinator conservation.

Whilst many studies have focused on how floral resources may increase pollinator abundance, it is generally unknown if observed increases in abundance are due to a transient movement of bees between patches of forage or due to an actual increase in colony development

(Holzschuh et al., 2016). Likewise, mass-flowering crops are frequently overlooked as a nutritional resource for pollinators despite being the intended forage resource for growers wishing to obtain pollination services. Therefore, by combining empirical data on courgette nectar and pollen with model simulations (using the novel bumblebee model *Bumble-BEEHAVE*), I quantified and simulated for the first time, the importance of courgette as a mass-flowering forage resource for bumblebees (work additional to funding objectives: supplementary information).

Whilst *B. terrestris* showed a strong fidelity to courgette flowers' bountiful nectar, no pollen loads from returning foragers were courgette pollen. Despite courgette being relatively high in protein (Petersen et al., 2013), its large sticky grains may make it difficult for the bees to collect (Vaissière and Vinson, 1994) and whilst *B. terrestris* has been observed to collect *Cucurbita* pollen in flight cages (Vaissière and Vinson, 1994), no studies have observed *B. terrestris* collecting cucurbit pollen in open fields. Therefore, *B. terrestris* may avoid collecting *Cucurbita* pollen, since as a generalist species it can visit alternative, more easily obtainable pollen (in open field settings). Nonetheless, early season courgette was shown to increase the number of hibernating queens, colonies, and adult workers, but not colony size or nectar or pollen stores per bee. Courgette has the potential to improve bumblebee population dynamics however, bees can only benefit from this transient nectar source if alternative floral resources (particularly pollen) are also available to fulfil bees' nutritional requirements in space and time. Wild flowers could also attract bumblebees into courgette fields as they were shown to be the most important predictor of bumblebee abundance at courgette flowers. As bumblebees have been observed to stay constant to an area of good forage (Osborne et al., 1999) it is likely that they were foraging between wild and crop flowers (due to their phenology) at a specific locality, thus supporting further work which showed *B. terrestris* 'switching' to wild flowers. Therefore, courgette fields with additional wild flowers may simultaneously improve pollination services and bumblebee populations.

Can we improve yields of other pollinator-dependent crops?

Promoting growers' agricultural resilience by understanding crops' pollination requirements is essential for pollinator-dependent food security. Indeed, discovering that courgette was less dependent on pollination than previously thought led to a systematic review and meta-analysis on studies inducing parthenocarpy in crop species, which like courgette are also believed to be pollinator-dependent (work additional to funding objectives: supplementary information). Indeed, whilst many ecological studies advocate increasing the 'supply' of pollinators (wild or managed) to improve crop yields (all funding objectives), there has been little focus on altering a crop's 'demand' for pollinators.

The systematic review identified 161 studies on parthenocarpy taking place on 40% of crops for which an increase in the supply of pollinators is the only approach suggested by ecologists to improve crop yields. Even single successes presented in the meta-analysis could lead to profound changes in production of certain crops, for example nearly all bananas on the global market are of the Cavendish variety, selectively bred to be parthenocarpic. Indeed, three species included in this study occur in the top twenty crops for global production (Mt/yr in Klein *et al.* 2007) (tomato #12; watermelon #15; apple #19) and are therefore highly likely to have biotechnological routes explored to increase their yields.

The results of this meta-analysis support the conclusions of Klein *et al.* (2007) and Melathopoulos, Cutler & Tyedmers (2015), that to get a more complete picture, varietal information is required – both in terms of pollinator dependence, but also in terms of choices that farmers are making. Realistically the best way of obtaining this information is if the pollination requirements of each variety are tested by institutes conducting variety trials and that this information, alongside quantities sold, are made freely available.

Parthenocarpic crop species could ensure food security in the face of pollinator decline or changing pollinator distributions, since the need for pollination is removed. Therefore, producers could extend their growing seasons in otherwise adverse climatic and environmental conditions, furthering their economic advantage and agricultural resilience, which will ultimately improve food security. However, parthenocarpy should not be used as a panacea for agricultural success especially since biodiversity decline in agricultural landscapes is often an indicator of poor ecosystem health, which can also cause poor fruit set.

Indeed parthenocarpy may further the ‘pollinator crisis’ as the imperative for pollinator conservation is removed as our dependence on pollinators is reduced (Brown *et al.*, 2016). This could affect pollination of non-parthenocarpic pollinator-dependent crops as well as wild plants. Whilst parthenocarpy may be contentious for some, environmental and technological solutions should be used in tandem to ensure that the best possible crop yields can be obtained in regions where they are needed most. Likewise, evidence of the effectiveness of different approaches to improve pollinator-dependent crop yields must be brought together for accurate valuations of ecosystem services and to ensure that decision-making is not skewed by one-sided arguments simply based on natural capital alone.

Project Summary

This project highlights the importance of pollination for improving yields, even when over half of fruit set can be achieved via parthenocarpy. The high abundance and pollination efficiency of *B. terrestris* (alongside other pollinator species) in field grown courgette was enough to fulfil its pollination requirements. However, since the total economic value of insect pollination to courgette is estimated to be worth £3,398 per ha, growers may wish to preserve their wild flowers within, and on the edge of fields as a way of attracting pollinators into courgette fields. In doing so growers will also support pollinators' nutritional requirements beyond those already provided by courgette. Indeed, courgette fields studied in this project, with abundant and diverse wild flowers can simultaneously improve pollination services to courgette and bumblebee populations in the surrounding landscape which will benefit courgette production in subsequent years. Nonetheless, for food security to be maximised, parthenocarpic varieties should be used in combination with these pollinator supportive practices, to ensure that the best possible yields can be obtained.

Knowledge Transfer

Publications

1. Knapp, J. L. J. L. & Osborne, J. L. Courgette Production: Pollination Demand, Supply, and Value. *J. Econ. Entomol.* 110, 1973–1979 (2017).
2. Knapp, J. L. J. L., Bartlett, L. J. L. J. & Osborne, J. L. Re-evaluating strategies for pollinator-dependent crops: How useful is parthenocarpy? *J. Appl. Ecol.* 54, 1–9 (2016).
3. Knapp, J. L., Shaw, R. & Osborne, J. L. Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation. *Basic Appl. Ecol.* (2018).
4. Knapp, J. L., Becher, M. A. Rankin, C. C. & Osborne, J. L. *Bombus terrestris* in a mass-flowering pollinator-dependent crop: A mutualistic relationship? *Ecology and Evolution.* - Under Rev. (2018).

Presentations and outreach

2018

- Williams' Lab (Prof N. Williams, University California Davis, USA) – Invited presentation
- Kremen Lab (Prof C. Kremen, University California Berkeley, USA) – Invited presentation
- Isaacs' Lab (Prof. R. Isaacs, Michigan State University, USA) - Invited presentation
- Met with cucurbit growers and visited sites in Michigan and California USA.
- Bumblebee Working Group Meeting (CEH, UK) – Contributed presentation
- Contributed to a case study on courgette pollination (interviewed on film) for a massive open online course (MOOC) on food security.

2017

- Cucurbit growers group (Cambridgeshire, UK) - Invited presentation
- Royal Entomological Society's Pollinator Special Interest Group (Edinburgh, UK) - Contributed presentation
- Agriculture and Horticulture Development Boards' annual Studentship Conference (Birmingham, UK) - Invited presentation; presentation awarded 2nd Place

- Co-produced two lesson packs on ecology and land management which included lesson plans, PowerPoint presentations, extension worksheets, a bespoke board game, short film, and a structured town hall style debate with props.
- Royal Cornwall Show - how to identify bees.

2016

- Memmott's Lab (Prof J. Memmott, Bristol University, UK) - Invited presentation
- Cucurbit growers group (Cambridgeshire, UK) - Invited presentation
- Exploring Research in Cornwall (Penryn, UK) – Poster, awarded 1st place

2015

- Cucurbit growers group (Cambridgeshire, UK) - Invited presentation
- Royal Entomological Society's Annual Conference (Trinity College, Dublin) – Poster
- Royal Cornwall Show - how to identify bees and flowers
- Open Farm Sunday – how to identify bees and flowers

PRODUCING PERFECT PRODUCE BY PARTHENO-CARPY

Can the traditional methods of pollination in cucurbits be bypassed to create perfect produce via parthenocarpy? *Jessica Knapp, University of Exeter, investigates*

It is no secret that, like most plants that produce a fruit, Cucurbit crops generally require pollination from insects for their fruit to be large and well formed.

However, the process requires co-operation from the insects and sometimes considerable work from the grower to ensure that each plant achieves the pollination required.

So, if there was an 'easier' way to ensure cucurbits achieved marketable size then it would be a significant boost to growers everywhere in terms of savings on pollinators and labour.

This is where 'parthenocarpy' – the term used for fruit set in the absence of fertilisation – becomes of interest because of its potential to help cucurbits to reach maturity via the use of selective breeding.

AHDB project 'CP 118 Cucurbit pollination: mechanisms and management to optimise field crop quality and quantity' set out to investigate if pollination is limiting to fruit quality and quantity, and if so, under what environmental conditions, in courgettes (variant: Tosca).

Initial findings showed that while pollination increases the size, weight, and growth rate of courgettes, natural parthenocarpy means that many fruits were able to reach marketable size and shape without any pollination at all.

Results of a literature review showed that all techniques to induce parthenocarpy (selective breeding, hormone application, or genetic modification) were able to increase fruit quantity without adversely affecting quality in 18 pollinator-dependent crop species (not including seed and nut crops as parthenocarpy causes seedlessness) which traditionally require pollination for fruit set.

Since a crop's need for pollination could be greatly reduced, parthenocarpy could allow producers to extend their growing seasons to exploit environmental conditions usually adverse for pollinators, furthering their economic advantage, increasing agricultural resilience, and improving food security.

Despite the natural parthenocarpic tendency of courgette, no selectively-bred parthenocarpic varieties are currently grown at a commercial scale in the United Kingdom. Pollination experiments show that 41% of fruit set is still dependent on natural pollination, with percentage fruit set, the size and weight, but not sugar content, of courgettes being significantly increased with pollination.

Fortunately, at most of the study sites the pollination rates were so high that there was no statistical difference in the yield of hand (artificial) and open-pollinated (insect pollinated) fruits. This may explain why, when commercial bumblebee colonies were introduced, there was no difference in yield (percentage fruit set, weight, or length) between fields with or without colonies.

Of course, pollination is entirely dependent on the availability of pollinators and suitable habitat to sustain their populations. Therefore, the high level of pollination observed in this study is likely to vary between courgette-growing regions. For example, our study across different sites in the UK showed that more wild flowers in field margins resulted in more pollinators in courgette fields. Therefore, allowing uncultivated areas around the crop to be colonised by wild flowers could be an effective way of boosting pollinator visitation, which in turn may increase yield.

Since the total economic value of insect pollination to courgette is estimated to be worth £2.7 million in the UK (£3,400 per hectare), growers may wish to preserve their wild flowers on the edge of fields as a way of mitigating potential fluctuations in pollinator populations and yield. In doing so, they may also enhance other ecosystem services vital for agricultural production, such as natural pest control.

“ Many fruits were able to reach marketable size and shape without any pollination ”

AHDB project code: CP 118
Project lead: Jessica Knapp,
University of Exeter
AHDB contact: Grace Choto

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Supplementary Information: Additional Work



Figure 27 *Eristalis arbustorum* L. collecting nectar from hogweed on a courgette field margin. Photograph by Daphne Wong.

Re-evaluating strategies for pollinator-dependent crops: how useful is parthenocarpy?

Introduction

Globally, agricultural land is continuing to expand and agricultural practices continue to intensify to meet rising food demands (Bommarco et al., 2013). It is argued that sustainably maximising agricultural yield requires ecosystem services to be optimised through improved soil quality, water efficiency and management of beneficial insects for pest control and pollination (Bommarco et al., 2013; Tilman et al., 2002). Insect-mediated pollination (the transfer of pollen within or between flowers via an insect) is a key regulating service for many crops and wild plants (Klein et al., 2007; Wilcock and Neiland, 2002). Thus any detrimental impact on pollination services, for example from habitat loss, introduced pests and diseases, and practices associated with intensive agriculture, could have a negative effect on crop yields and farmers' profits (Goulson et al., 2015; Potts et al., 2010; Steffan-Dewenter et al., 2005). Observed losses of pollinator species combined with our dependence on their contribution to food security, has led to a widespread concern that we are facing a 'pollinator crisis' (Steffan-Dewenter *et al.* 2002; Potts *et al.* 2010; although see Ghazoul 2005). However, whilst the plethora of recent reviews and studies on this subject come to similar conclusions that improving habitat and environmental conditions for pollinators will have a positive impact on crop production by increasing the 'supply' of pollinators (wild or managed); none of these studies consider the alternative option of reducing 'demand' for crop pollinators via technological innovation or management of crops. This can lead to a narrow (and potentially out-dated) perspective given that, in the meantime, plant breeders and farmers are finding ways of short-circuiting the need for pollination by developing and using new varieties which can set fruit without pollen vectors (Pandolfini et al., 2009).

The need for insect pollination in crops is usually measured in two ways: 1) Pollinator dependence is quantified by comparing the yield of open-or hand-pollinated crops with the yield of crops from which pollinators have been excluded. However, this is often only done for single cultivars in particular environmental conditions (funding objective 2); 2) Pollination deficit estimates the additional pollination needed to achieve maximum yields in a particular context by comparing open-pollinated with hand-pollinated crops (Vaissière, 2010). This technique has identified pollination deficits in a range of pollinator-dependent crop species and is a vital step to evidence the need to implement management interventions to promote pollinator populations. Realistic estimates of the 'value' of insect pollination to global agriculture need to account for not only the variability

in pollination deficit that might result from variable pollinator densities and environmental conditions, but also the variability in pollinator dependence between varieties of single crop species, for which there is currently little good evidence (Melathopoulos et al., 2015). In the wider context, discussion and strategies for improving horticultural crop production (in particular) need to incorporate evidence on the variety of options available for increasing fruit and seed set by manipulating pollination systems, and not just assume that the only way to do this is by maximising pollination. To improve estimates of pollinator dependence in crops, and to widen the debate about how to guide farmers in improving seed and fruit production, we present a meta-analysis of studies inducing parthenocarpy in horticultural crops.

Parthenocarpy (fruit set in the absence of fertilisation) is a trait which has the potential to make many 'pollinator-dependent' species produce fruit without pollination (Vardi et al., 2008). Parthenocarpy is thought to increase fruit quantity as plants are able to set fruit in conditions adverse for fertilisation, for example due to poor pollen maturation or few pollinating species, typically seen in greenhouses or during periods of poor light and cold temperatures (Pandolfini, 2009). Without parthenocarpy, and under these conditions, growers would ordinarily experience high rates of fruit abortion due to an insufficient number of pollen grains delivered to stigmas (Pandolfini, 2009).

Parthenocarpy also has the potential to improve fruit quality as seedlessness (caused by no pollination and therefore fertilisation) can be a desirable trait. This is different to stenospermocarpy, where seedlessness is achieved by seeds being aborted after fertilisation (and therefore pollination) such as with triploid watermelons (Varoquaux et al., 2000). For example, it is thought to extend shelf-life in some species, such as reduced browning in aubergine (Acciarri et al., 2002), is advantageous in fruit processing, such as tinned tomatoes (Pandolfini et al., 2002), and is generally favoured by consumers for convenience in preparation and consumption (Vardi et al., 2008). However, evidence suggests that some parthenocarpic plants may still produce a greater quantity and quality (including higher sugar content (Hayata et al., 2000; Shin et al., 2007)) of fruits when pollinated by insects (Martínez et al., 2013; Nicodemo et al., 2013; Robinson and Reiners, 1999).

Fertilisation of the ovules and seed/ fruit development is co-ordinated by various phytohormones, including auxins, gibberellins, and cytokinins which originate from the developing embryos (Gillaspy et al., 1993). Phytohormones, present in developing seeds are vital for regulating fruit growth and development (Gillaspy et al., 1993). However, in parthenocarpic (and therefore seedless) fruit set, endogenous phytohormones are elevated, suggesting that phytohormones from sources other than developing seeds can

regulate fruit growth (Gustafson, 1936). Consequently, parthenocarpy may be initiated through exogenous application of phytohormones. Auxins, gibberellins, and cytokinins or mixtures of these have all been proven to be effective in inducing fruit development in the absence of fertilization and have been shown to increase productivity in various horticultural crops (Reviewed in Pandolfini 2009). However, little is known about the effect of these hormones on the environment and implementation is expensive and labour-intensive (Saito et al., 2009). Consequently, scientists are increasingly finding ways to exploit genetic parthenocarpy.

Traditionally, approaches to genetic parthenocarpy have largely focused on selective breeding programs for seedlessness (reviewed in Vardi *et al.* 2008 and Varoquaux *et al.* 2000). For example, selective breeding of parthenocarpic sweet pepper (Honda et al., 2012; Tiwari et al., 2007), papaya (Rimberia et al., 2007), and summer squash (Kurtar, 2003; Robinson and Reiners, 1999) varieties have all been shown to increase productivity. More recently, scientists have focused on genetic engineering approaches for parthenocarpic fruit set, through modification of auxin synthesis (*iaaM*), auxin sensitivity (*rolB*), auxin content (*Aucsia*), auxin signal transduction (*IAA9* or *ARF8*), and gibberellin signal transduction (*DELLA*) (reviewed in Pandolfini 2009). For example, the chimeric auxin synthesising *DefH9-iaaM* gene has been shown to increase productivity in aubergine (Acciarri et al., 2002; Donzella et al., 2000; Rotino et al., 1997), tomato (Molesini et al., 2009; Pandolfini et al., 2002), cucumber (Yin et al., 2006), strawberry (Mezzetti et al., 2004), and raspberry (Mezzetti et al., 2004). Auxin-synthesis parthenocarpy is facultative, meaning that it is seedless in conditions adverse for pollination/ fertilisation and seeded (although much reduced in number (Rotino et al., 2005)) in conditions where pollination occurs (Pandolfini et al., 2009). Breeding for genetic parthenocarpy also has the potential to combine multiple desirable traits. For example, parthenocarpy, pistillate-flowering time, improved fruit quality and disease resistance have been combined in cucumbers (Kushnereva, 2008).

Using parthenocarpy to promote fruit set under unfavourable environmental conditions could improve the quality and quantity of pollinator-dependent crops by reducing the number of poorly formed fruits caused by insufficient pollination (Pandolfini, 2009). This could extend current geographic and climatic agricultural ranges of production, simultaneously improving food security and the economic prospects of commercial growers. Methods to induce parthenocarpy should therefore be considered when calculating the contribution of pollinators to fruit set, to avoid over-estimating our dependence on them. Klein *et al.* (2007) provide the most comprehensive review of global crop pollinator dependence, and they acknowledge that their results are often

based on studies from single cultivars and/or single regions because of the difficulty of finding comprehensive evidence. However, their data have been used to subsequently estimate the global value of pollination (Breeze et al., 2011; Gallai et al., 2009) and consequently justify the prediction of a 'pollination crisis' (Potts et al., 2010; Steffan-Dewenter et al., 2005) without substantiated information at the individual crop level, as highlighted by Melathopolous *et al.* (2015).

In this paper we aim to go beyond previous reviews of parthenocarpy (Gorguet et al., 2005; Pandolfini, 2009; Pandolfini et al., 2009; Vardi et al., 2008; Varoquaux et al., 2000) by using meta-analysis techniques to review and synthesise the literature on the extent of parthenocarpy promoting techniques currently being used commercially or experimentally on pollinator-dependent crops across the world. Systematically reviewing plant science literature and applying it to pollination biology provides a broader perspective on the pollinator debate.

Research Questions

1. Does artificial or genetic parthenocarpy increase the quantity and quality of fruits in (normally) pollinator-dependent crop species?
2. Which method for conferring parthenocarpy: selective breeding, genetic modification or growth hormones, is most effective for parthenocarpic fruit set?

Methods

Data Collection

We searched the ISI Web of Science, SCOPUS, Science Direct, Directory of Open Access Journals, AGRICOLA databases, and, Google Scholar, for studies that investigated the effect of genetic and artificial parthenocarpy on the quantity or quality of yield in pollinator-dependent crops as defined by Klein *et al.* (2007), where pollinator dependence is classified as 'essential', 'great', 'modest' or 'little' (Table 10). Searches were conducted from 1945 to March 2016 using the search terms: (Parthenocarp*) AND (genetic mod* OR GM OR genetic* engineer* OR chimeric gene* OR selective breed* OR artificial selection OR hormone) AND (yield OR weight OR Brix). To avoid possible publication bias, patents were included and authors were emailed for relevant reports and unpublished studies (Koricheva et al., 2013).

Studies were included that met all the following criteria: (1) They were a pollinator-dependent horticultural crop species; (2) presented an effect of induced parthenocarpy on yield; (3) reported the sample size; (4) reported the mean, and if possible, the standard deviation for each treatment (for independent categorical variables). Methods

to induce parthenocarpy were selective breeding or genetic modification (genetic parthenocarpy), or application of growth hormones (artificial parthenocarpy). Each intervention was compared to its own (negative) control. So, selective breeding compared parthenocarpic varieties with non-parthenocarpic varieties (SB), growth hormones compared application with no application (HA), and genetic modification compared modified with non-modified plants (GM). Effectiveness was measured in terms of crop quantity (e.g. weight per plant, or yield) and quality in terms of sugar content (e.g. ° Brix where one-degree Brix is 1 gram of sucrose in 100 grams of nectar).

Authors of the original studies quantified the effect of parthenocarpy (i.e. compared parthenocarpic treatment with non-parthenocarpic control) within different 'test environments' which can be broadly classified into hand pollination (this includes one example of experimental flowers being 'selfed', i.e. fertilised by pollen from the same plant (Molesini et al., 2009)) (hereafter, HP), no pollination, (hereafter, NP), or open pollination (hereafter, OP). In both OP and HP conditions only, pollen from plants of the same genetic material were used. Conditions for which the plants were open pollinated vary between studies, from glasshouses supplemented with *Bombus terrestris* L. colonies to 'open field' conditions. The ecological complexity, i.e. availability of pollinators at these 'open fields' was not provided. These test environments thus have differing background levels of potential pollination and were therefore included as a fixed effect in the analysis. The reasons for this were twofold: (1) to see if NP conditions resulted in larger effect sizes (due to non-parthenocarpic controls setting no fruit) and likewise smaller effect sizes in OP and HP conditions for the opposite reason (due to non-parthenocarpic controls setting fruit), and (2) to ensure that test environment did not influence treatment effectiveness. For OP and HP conditions to be included in the meta-analysis, authors had to evidence parthenocarpic fruit set through either a much reduced number of seeds or that fruit set occurred in conditions adverse for pollinators (Pandolfini, 2009).

Calculation of Effect Sizes

Within individual studies different plant species, varieties and pure-bred lines may be tested to determine which one has the best parthenocarpic potential for industrial development. Therefore, each genetic line was independent and thus included as separate cases in the dataset. As a result, many studies contributed more than one entry to the dataset. If a study examined multiple years or more than one treatment level of hormone concentration, then the largest sample size, or in cases with equal sample sizes the treatment level with the greatest effect, was selected.

Hedges' d was used as a measure of effect size in our meta-analysis. This measure is not affected by unequal sample sizes and includes a correction factor for small sample sizes (Koricheva et al., 2013). Hedges' d was calculated for each treatment-control pair in the dataset (Table 11), based on the mean, standard deviation and sample size using the 'metafor' R package (Viechtbauer, 2010).

We used bootstrapped analyses to fill in missing standard deviations (22 quantity samples and 4 quality samples), using 1000 resampled data sets following 'hot deck' imputation, outlined in Koricheva *et al.* (2013). We also include Forest plots showing effect sizes using only complete data (without bootstrapping) in Figures A2.1-2.3.

Meta-analyses

Within a single attempt at inducing parthenocarpy, for example with multiple concentrations of hormones, the concentration which resulted in the greatest effect size (measured by Hedges' d) was selected. This was done to be representative of how these experimental studies would inform industry, i.e. only the best lines and methods would be put forward for development.

All effect sizes were normalised for their positive skew using a real-solution cube-root transform (following Tukey's ladder of powers). To assess the importance of parthenocarpy-inducing methods on crop quality and quantity, one sample two-tailed t -tests were used. The relative effectiveness of parthenocarpy-inducing methods and the effect of different test environments were investigated with analysis of variance (ANOVA). Possible interactions between these two effects were investigated with generalised linear models, using backward stepwise model selection (Crawley, 2012).

Results

Following a key word search of the literature, 161 studies investigated the effect of parthenocarpy in 33 pollinator-dependent crop species. Of these, 35 did not supply full-text, 8 were not in English, and 78 used a study design unsuitable for inclusion (Table 10). The remaining database included 184 effect sizes from 40 studies. Following our selection of the most effective treatments from each experiment (to reflect those which would be taken forward for development) our final sample size was 69 effect sizes (29 for genetic modification, 31 for hormone application, and 9 for selective breeding) (Table 11). These techniques had been used experimentally and/ or commercially on 18 pollinator-dependent crop species, of which 3 have an 'essential' need, 6 have a 'great' need, 3 have a 'modest' need, and 3 have a 'little' need for insect-mediated pollination (3 pollinator-dependent species were unclassified) (Klein et al., 2007). Tomato was the

most commonly studied species (16 studies), followed by aubergine (4 studies) and sweet pepper (3 studies). There was a notable absence of seed and nut crops; this was to be expected given that parthenocarpy causes seedlessness, an undesirable trait in these species. Likewise, an additional 14 pollinator-dependent species showed no evidence of experimental or commercial parthenocarpy in the literature (Table 10).

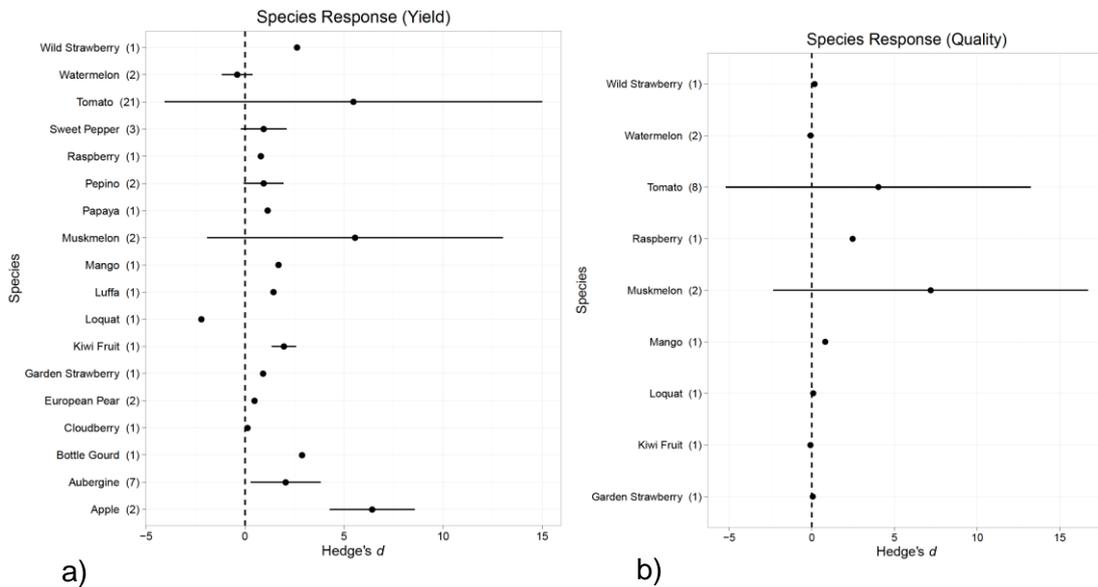


Figure 28 Mean effect sizes for all methods combined to induce parthenocarpy (genetic modification, hormone application, and selective breeding) split by crop species (y axis) for (a) fruit quantity (b) fruit quality. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

Across all species methods to induce parthenocarpy significantly increased fruit quantity ($T_{50} = 8.41$, $P < 0.001$) (Figure 28 a) and quality ($T_{17} = 3.57$, $P = 0.002$) (Figure 28 b). However, there were no significant differences in the effectiveness of genetic modification, selective breeding, or hormone application for increasing fruit quantity ($F_{48} = 0.41$, $P = 0.666$) (Figure 29 **Error! Reference source not found.a**) or quality ($F_{16} = 0.86$, $P = 0.367$) (Figure 29 b).

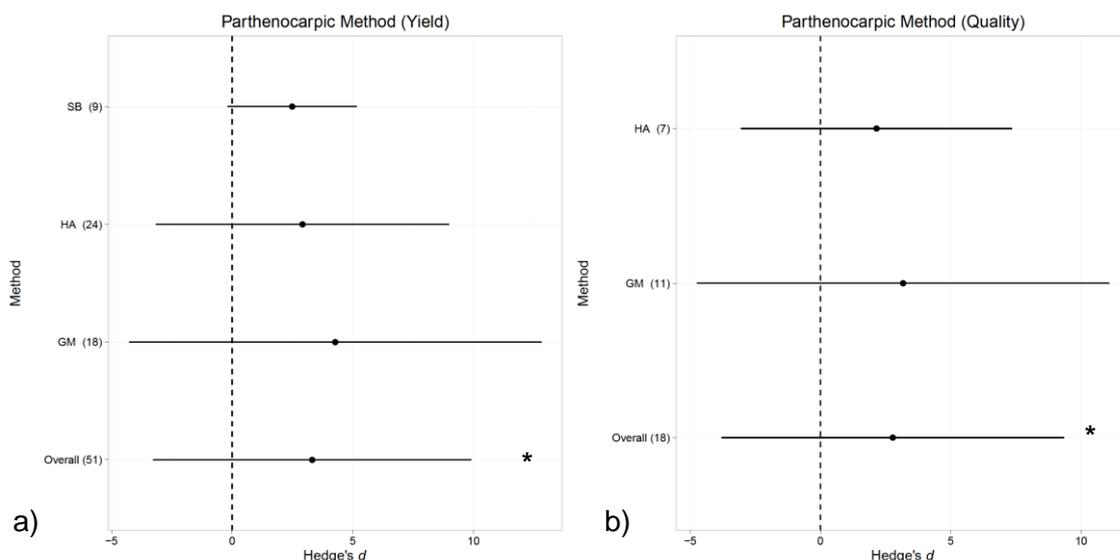


Figure 29 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) (y axis) for (a) fruit quantity and (b) quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses. Significant one sample two-tailed *t*-tests are indicated with an asterisk (*) ($P < 0.05$).

Test environment was shown to influence how effective treatments were on fruit quantity ($F_{48} = 8.35$, $P < 0.001$), with 'no pollination' environments having the largest effect size (Figure 30). However, test environment did not influence the effectiveness of parthenocarpy-inducing methods on fruit quality ($F_{15} = 0.391$, $P = 0.683$) (Figure 30 b). Notably, there was no interactions between treatments and test environment ($F_{43} = 1.63$, $P = 0.197$), showing that the influence of test environments on treatment effectiveness was not biased against any parthenocarpy-inducing method.

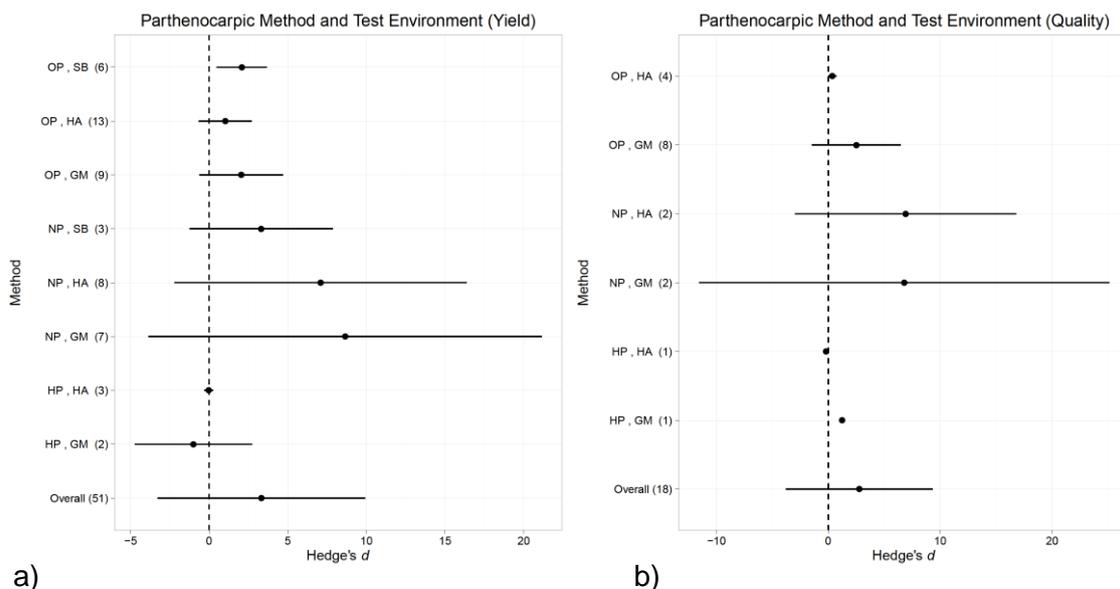


Figure 30 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) and test environment (NP, OP, and HP) (y axis) for (a) fruit quantity (b) fruit quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

Discussion

Artificial and genetic parthenocarpy have proven to be successful at increasing fruit quantity (Figure 28 **Error! Reference source not found.a**), without negatively affecting quality in all crop species studied (Figure 28 **Error! Reference source not found.b**). This is vitally important for commercial acceptance of parthenocarpy as it is only valuable to growers if there are no adverse effects on fruit quality. For example, damaging normal vegetative growth (other than a reduced number of seeds), or a reduction in sugar and nutritional content (Pandolfini, 2009). In this study °Brix was used as a measure of quality as this was the only metric consistently recorded in studies.

The most studied method for inducing parthenocarpy is hormone application, which was shown to positively increase crop quantity and quality (Figure 29). This method is currently the most widely used by commercial growers, and although usually used prophylactically could be a very good temporary practice for periods of unfavourable environmental conditions.

Selective breeding (creating F1 hybrids) could provide a longer-term solution for inducing parthenocarpy, which despite being investigated in fewer studies, still proved very successful at increasing yield (Figure 29 **Error! Reference source not found.a**). This complements yield trials not included in this meta-analysis (see funding objective 2**Error! Reference source not found.**) which have found evidence of genetic parthenocarpy in pollinator-dependent species. For example, 66 per cent of sweet pepper varieties (Honda *et al.* 2012) and 33 per cent of squash varieties examined (Robinson and Reiners 1999) were found to set parthenocarpic fruit. Although an effective method, selective breeding has its limitations. Principally, that crop species can only be crossed with ones that they can sexually reproduce with, and undesirable traits may be inherited alongside desirable ones during crossing. Likewise, selective breeding of varieties is expensive and time consuming, with varieties taking 5-10 years to be released (De Vries *et al.*, 1997). This is because pure lines need to be maintained over many years to ensure their quality, and hybridisation of pure lines often needs to be done by hand. Likewise, seeds grown from F1 hybrids often produce inferior yields to parental crops and consequently growers will need to purchase new F1 seeds each year (Tripp, 1994).

Genetic modification for parthenocarpy could speed up this process by removing the need for back crossing and has been shown to be the most effective method in this meta-analysis (Figure 29). This is supported by Donzella *et al.* (2000) who showed genetic modification to be more effective than hormone spraying at increasing yield. The authors concluded that genetic modification enabled a 10 per cent reduction in production costs

(less labour needed for the hormonal sprays) and increased profit from improved quality following the genetic modification. Interestingly genetic modification in strawberry and raspberry (Mezzetti et al., 2004), and tomato (García-Hurtado et al., 2012; Medina et al., 2013) has been shown to increase the number of flowers per plant, demonstrating the role that phytohormones also play in fecundity. Therefore, yield per plant may be greater than yield per fruit. Genetic methods could also use alternative methods of genetic engineering such as cisgenesis. This could increase the likelihood of regulatory and consumer acceptance by transferring genes between organisms that could otherwise be conventionally bred (Telem et al., 2013; Tester and Langridge, 2010).

The range of effect sizes observed in this study (Figure 29) demonstrates the negative effects that unsuccessful parthenocarpy attempts can have on yield, alongside the highly positive effects that successful parthenocarpic treatments can have, for example those shown in tomato and muskmelon (Figure 28). The variation in the strength of these responses is primarily due to species-specific responses to growth hormones (both applied and genetically modified). For example, if the expression of auxin coding transgenes (in genetically modified) or auxin concentration (from hormone application) is too high, then fruit may appear malformed, particularly in auxin sensitive species (Gorguet *et al.* 2005; Gemici *et al.* 2006). Likewise, relationships between different phytohormones are complex and vary greatly depending on species. This demonstrates the need for continued, multi-treatment experiments to test the most effective strengths and types of hormones, tailored to individual crop species.

Investigating fruit quality and quantity in different test environments can allow us to assess how useful parthenocarpy could be in the total absence of pollination and fertilisation. In the example of genetically modified aubergine, Acciarri *et al.* (2002) found a 30 to 35 per cent increase in productivity, without any effect on quality under both greenhouse and open field conditions. In both test environments the fruit was always seedless therefore, positively influencing fruit quality and the economic value of production. Larger effect sizes in no pollination conditions (Figure 30), demonstrate the greater effect that parthenocarpy will have in conditions where fruit set would ordinarily be very low. Consequently in conditions where hand pollination is required for improved fruit set, artificial and genetic parthenocarpy could be a cost effective alternative (Allsopp et al., 2008; Niu et al., 2015). Conversely effect sizes tend to be smaller in open and hand pollinated environments where pollen is available (Figure 30). This is likely to be because in these conditions the non-parthenocarpic controls are successfully pollinated to some extent. However, in all test environments plants may have experienced some pollination deficit (i.e. if plants were selfed, pollinated from just one donor plant, or if

experiments were conducted in areas with low pollinator abundance). It is not possible to ascertain the degree of pollination deficit in the HP and OP test environments, and to what extent these limitations represent real world growing conditions. So, these results may over-estimate the effect of parthenocarpy compared to yield resulting from open pollination in an environment where pollinators are not limiting, and natural pollination is thus optimal.

Nonetheless, parthenocarpy could still be useful in open pollination environments, where it can minimise the potential for pollination deficits whilst improving fruit uniformity caused by stochastic poor pollination (Pandolfini, 2009). Therefore, parthenocarpy could be advantageous to all crops, whether they are experiencing a pollination deficit or not. In return, these parthenocarpic crops can continue to provide valuable nectar and pollen resources for our wild and managed bees, and other flower-visiting insects. However, there is no information available as to how the quality and quantity of nectar and pollen varies between parthenocarpic and non-parthenocarpic plants, or how selective breeding for parthenocarpy will affect a plant's nectar and pollen production over time. It is also worth remembering that parthenocarpic fruit set and therefore seedlessness is not always desirable, such as crop species where seeds are the edible part and for creating of seed stock.

Incomplete routes of communication between the plant breeding industry, ecologists, and apiculturists have resulted in a mixed and potentially inaccurate message about the extent of our dependence on pollinators for food production (Ghazoul, 2005; Kleijn et al., 2015; Melathopoulos et al., 2015). Studies which value the contribution of insects to pollination are based on pollinator dependence, i.e. the extent that a plant depends on pollinators for fruit set. However, this metric assumes that dependence is constant within a single crop (Gallai et al., 2009; Klein et al., 2007). In reality pollinator dependence is strongly dependent on variety, the spatial and temporal context of the surrounding landscape, and the responses of farmers, consumers and technological innovation to pollinator decline. Therefore, we highlight that there may be over-estimation of pollinator dependence if studies overlook research and development currently underway to reduce the need for pollination. We found evidence for studies inducing parthenocarpy in four out of 13 of the global crops for which pollination is considered essential (according to Klein *et al.* 2007); and 13 out of 30 of the crops for which the need for pollination is considered great. This indicates that research into reducing *demand* for pollination has occurred in 40% of the crops for which ecologists are currently primarily only advocating an increase in *supply* of pollinators as the solution to improving crop yields and quality (Carvalho et al., 2013; Garibaldi et al., 2011). Indeed, there are three crop species in

the top twenty crops for global production (Mt/yr in Klein *et al.* 2007) which benefit from insect pollination and appear in this meta-analysis of parthenocarpy studies (tomato #12; watermelon #15; apple #19). It is not surprising that, if a crop is showing a yield deficit, then different routes are explored to solve the problem; but it is surprising that evidence of the effectiveness of different approaches is not brought together more comprehensively to build an accurate picture for a crop. Single successes presented in this meta-analysis could lead to profound changes in production of certain crops, for example nearly all bananas on the global market are of the Cavendish variety, selectively bred to be parthenocarpic.

Data are not currently available to assess variety choice by farmers, or the level of parthenocarpy in the varieties that they choose. The results of this meta-analysis support the conclusions of Klein *et al.* (2007) and Melathopoulos *et al.* (2015) that to get a more complete picture, varietal information is required – both in terms of pollinator dependence, but also in terms of choices that farmers are making.

Parthenocarpy may be able to reduce the need for pollinators in many horticultural crops but should not be used as a panacea for agricultural success. Biodiversity decline in agricultural landscapes is often an indicator of poor ecosystem health, which can also cause poor fruit set. Thus, agricultural growers should carefully consider causes of poor fruit set and ideally use parthenocarpic species (which can still provide an important nectar and pollen source for pollinator species) in addition to other environmentally considerate practices. Likewise, parthenocarpy could further the pollinator crisis by removing the imperative for conserving pollinators as our 'dependence' on them is reduced (Brown *et al.* 2016). This could affect pollination of non-parthenocarpic pollinator-dependent crops as well as wild plants. Ultimately, widespread implementation of these practices will be limited to countries that have access to and can afford skilled personnel and equipment. Thus, free communication of resources and capabilities from developers to users is essential for the benefits of parthenocarpy to reach the areas of the world that are most in need of its benefits.

This study shows that genetic and artificial parthenocarpy has a great potential to improve fruit quantity, without affecting quality in a range of horticultural crops. Potentially the most promising method for inducing parthenocarpy is genetic modification; the most effective for increasing fruit quality and quantity, whilst being the quickest to implement. However, whilst acceptance for genetic modification, particularly in Europe, remains equivocal, selective breeding may be a more attainable way for achieving genetic

parthenocarpy. This method is also relatively cost-effective for many horticultural growers already growing hybrid varieties. Although currently a popular choice, hormone application remains an expensive and un-sustainable option for many horticultural growers. Nonetheless, any additional costs for agricultural growers associated with implementing genetic and artificial parthenocarpy could be offset by increasing the quality and quantity of crops. Unfortunately, no studies have directly compared the cost of parthenocarpy to traditional methods of supplemented pollination, such as introduced honeybee hives and hand pollination. Climate change could also increase pressure to develop parthenocarpic crop species as changes in pollinator distributions or declines in their populations are likely to be detrimental to food production (Kerr et al., 2015). Thus, parthenocarpic crop plants could allow producers to extend their growing seasons in otherwise adverse climatic and environmental conditions, furthering their economic advantage, increasing agricultural resilience, and improving food security.

Re-evaluating parthenocarpy

Crop species with no references demonstrate current areas where parthenocarpy has not been used commercially or experimentally. References in bold are included in the meta-analysis, references in black were identified in the systematic review but not included in the meta-analysis (i.e. they did not fulfil all the search requirements), and references in blue only had an abstract available.

Species were pollinator-dependent if animal pollination was shown to increase production (fruit set, fruit weight and/or quality, seed number and/or seed quality, and/or increased pollen deposition (an indirect measure)) for at least one variety per crop (Klein *et al.*, 2007). Only crop species with essential, great, modest, and/or little dependence on pollinators were included in analyses. Essential = pollinators essential for most varieties (production reduction by $\geq 90\%$ comparing experiments with and without animal pollinators). Great = great production increase/ animal pollinators are strongly needed (40 - $<90\%$ reduction). Modest = modest production increase/ animal pollinators are clearly beneficial (10 - $<40\%$ reduction) (Klein *et al.*, 2007).

Seed and nut crops are absent from this table as parthenocarpy (therefore, seedlessness) would not be a desired trait. Entries are alphabetically ordered by genus.

Table 10 Pollinator-dependent crops, as defined by Klein *et al.* (2007) and studies (identified by the key word search) which investigate methods to induce parthenocarpy.

		From Klein et al 2007		Studies which promote the effect of parthenocarpy on yield identified found from the systematic review of the literature. Studies in bold were included in the meta-analysis, studies in black were included in the systematic review and studies in blue only had abstracts available.		
Crop species	Crop name	Requirement for animal pollination	Rank# in list of top global crops Mt/yr	Selective breeding	Genetic engineering	Growth hormones
<i>Abelmoschus esculentus</i>	Okra	modest	#56			
<i>Actinidia deliciosa</i>	Kiwifruit	essential				Iwahori <i>et al.</i> 1988 <i>Ohara et al. 1997</i>
<i>Annona squamosa</i>	Atemoya, Cherimoya, Custard apple	essential			Lora <i>et al.</i> 2011	
<i>Asimina triloba</i>	Pawpaw, Indiana banana	essential				
<i>Averrhoa carambola</i>	Carambola, Starfruit	great				

<i>Capsicum annum, C. frutescens</i>	Chilli pepper, Red pepper, Bell pepper, Green pepper, Allspice, Pimento	little	#33	Carrizo 2011 Honda <i>et al.</i> 2012 Tiwari <i>et al.</i> 2007		Balakbir <i>et al.</i> 1998 Gustafson 1936 Heuvelink & Korner 2001 Thanopoulos <i>et al.</i> 2013 Tiwari <i>et al.</i> 2012 Wien & Zhang 1991
<i>Carica papaya</i>	Papaya	little	#52	Rimberia <i>et al.</i> 2007		
<i>Citrullus lanatus</i>	Watermelon	essential	#15			Hayata <i>et al.</i> 1995 Huitrón <i>et al.</i> 2007 Kwon <i>et al.</i> 2006 Maroto <i>et al.</i> 2005 Sedgley <i>et al.</i> 1977 Hayata <i>et al.</i> 1994 Hikosaka <i>et al.</i> 2015 Newbury <i>et al.</i> 1977 Pak 1993 Miguel <i>et al.</i> 2000
<i>Citrus</i> spp.	Bergamont, Chinotto, Citron, Clementine, Grapefruit, Kumquat, Lemmon, Lime,	little parthenocarp y listed as a breeding mechanism	#13	Mesejo <i>et al.</i> 2013		García-Martínez & García-Papí 1979 Guardiola <i>et al.</i> 1993 Talon <i>et al.</i> 1992

	Mandarine, Orange, Pomelo, Tangerine					
<i>Cucumis melo</i>	Cantaloupe, Melon	essential	#29			Hayata et al. 2000 Risser 1976 Masuda 1990
<i>Cucumis sativus</i>	Cucumber, Gherkin	great	#24	Kushnereva 2008 Li et al. 2014 Shaw et al. 2007 Sun et al. 2006 Yan et al. 2012 Dean et al. 1983 Dennijs et al. 1991	Yin et al. 2006	Fu et al. 2008 Hikosaka & Sugiyama 2015 Kim et al. 1992 Shin et al. 2007
<i>Cucurbita maxima, C. mixta, C. moschata, C. pepo</i>	Pumpkin, Squash, Gourd, Marrow, Courgette	essential	#36	Kurtar 2003 Martínez et al. 2014 Nogueira et al. 2011 Robinson & Reiners 1999		Martínez et al. 2013 Yu 1999
<i>Durio zibethinus</i>	Durian	great				
<i>Eriobotrya japonica</i>	Loquat, Japanese plum, Japanese medlar	great			Tao et al. 2015	Mesejo et al. 2010 Ding 1988

<i>Fagopyrum esculentum</i>	Buckwheat	great				
<i>Feijoa sellowiana</i>	Feijoa	great				
<i>Ficus carica</i>	Fig	modest				Blondeau and Crane 1949 Crane 1964 Crane <i>et al.</i> 1948
<i>Fragaria</i> spp.	Strawberry	modest			Mezzetti <i>et al.</i> 2004	Mudge <i>et al.</i> 1981
<i>Lagenaria siceraria</i>	Bottle gourd	Not in Klein <i>et al.</i> 2007				Yu 1999
<i>Luffa acutangula</i>	Luffa	Not in Klein <i>et al.</i> 2007				Bisaria 1977
<i>Malus domestica</i>	Apple	great parthenocarp y listed as a breeding mechanism	#19	Watanabe <i>et al.</i> 2008	Yao <i>et al.</i> 2001	Watanabe <i>et al.</i> 2008 Bangerth 1994 Bangerth <i>et al.</i> 1994 Bukovac 1963 Goldwin <i>et al.</i> Goldwin <i>et al.</i> 1989 Greene 1980 Williams 1980 Luckwill 1960

<i>Mangifera indica</i>	Mango	great	#30		Ogata et al. 2010	Ogata 2009 Perez-Barraza et al. 2015
<i>Manilkara zapota</i>	Sapodilla	essential				
<i>Passiflora edulis</i>	Passionfruit	essential				
<i>Persea americana</i>	Avocado	great				
<i>Prunus armeniaca</i>	Apricot	great				Crane et al. 1960
<i>Prunus avium</i>	Sweet cherry	great				Crane et al. 1960
<i>Prunus cerasus</i>	Sour cherry	great				Crane et al. 1960
<i>Prunus domestica, P. spinosa</i>	Plum, Greengage, Mirabelle, Sloe	great	#48			Crane et al. 1960 Hartmann 1984 Jackson 1968
<i>Prunus persica</i>	Peach, Nectarine	great	#42			Crane et al. 1960
<i>Psidium guajava</i>	Guava, Guayaba	modest				
<i>Punica granatum</i>	Pomegranate	modest				

<i>Pyrus communis</i>	Pear	great	#37	Nishitani <i>et al.</i> 2012		Lafer 2008 Niu <i>et al.</i> 2015 Yarushnykov & Blanke 2005 Zhang <i>et al.</i> 2008 Luckwill 1960 Yamada <i>et al.</i> 1991
<i>Ribes nigrum</i> , <i>R. rubrum</i> ,	Black currant, Red currant	modest				
<i>Rosa spp.</i>	Rose hips, Dogroses	great				
<i>Rubus ideaus</i> , <i>R. fruticosus</i> , <i>R. chamaemorus</i> , <i>R. flagellaris</i> , <i>R. trivalis</i>	Raspberry, Blackberry, Cloudberry, Northern Drewberry, Southern Drewberry	great			Mezzetti <i>et al.</i> 2004	Junttila <i>et al.</i> 2002

<p><i>Solanum lycopersicum</i></p> <p>*parthenocarp y listed as one of breeding mechanisms</p>	<p>Tomato</p>	<p>little</p>	<p>#12</p>	<p>Baggett et al. 1997</p> <p>Charbeonboonsit et al. 1985</p> <p>Costa et al. 1992</p> <p>Dutta et al. 2013</p> <p>Fos et al. 2003</p> <p>Gorguet et al. 2005</p> <p>Habashy et al. 2004</p> <p>Mazzucato et al. 1998</p> <p>Mohamed 1998</p> <p>Philouze et al. 1986</p> <p>Sugahara et al. 2002</p> <p>Tang et al. 2015</p>	<p>Barg & Salts 2000</p> <p>Carmi et al. 2003</p> <p>Carrera et al. 2012</p> <p>Ficcadenti et al. 1999</p> <p>García-Hurtado et al. 2012</p> <p>Goetz et al. 2007</p> <p>Ingrosso et al. 2011</p> <p>Marti et al. 2007</p> <p>Medina et al. 2013</p> <p>Molesini et al. 2009</p> <p>Pandolfini et al. 2002</p> <p>Rotino et al. 2005</p> <p>Schijlen et al. 2007</p> <p>Shabtai et al. 2007</p>	<p>Fos et al. 2003</p> <p>Gemici et al. 2006</p> <p>Goetz et al. 2007</p> <p>Gustafson 1936</p> <p>Karapanos et al. 2013</p> <p>Nandwani et al. 2014</p> <p>Ramin 2003</p> <p>Rounis et al. 2015</p> <p>Serrani et al. 2008</p> <p>Aguero et al. 2007</p> <p>Alabadi et al. 1996</p> <p>Alabadi et al. 1998</p> <p>Ampomah-Dwamena et al. 2002</p> <p>Bunger-Kibler et al. 1982</p> <p>Costa et al 1985</p> <p>El-Habbasha et al.1999</p> <p>Gorecka et al. 1987</p> <p>Gustafson 1960</p> <p>Mapelli et al. 1987</p> <p>Mariotti et al. 2011</p>
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						Matsuo et al, 2012 Mazzucato et al. 1999 Mignolli et al. 2012 Shinozaki et al. 2015 Sjut 1982 Sjut 1984 varga et al 1986 Gelmesa et al. 2013
<i>Solanum melongena</i>	Aubergine	modest	#27	Kikuchi <i>et al.</i> 2008 Mori <i>et al.</i> 2013 Saito <i>et al.</i> 2009 Boyaci 2009 Takeshi et al. 2010	Acciarri <i>et al.</i> 2002 Donzella <i>et al.</i> 2000 Rotino <i>et al.</i> 1997	Boyaci et al. 2011 Gustafson 1936 Sarma et al. 1997 Sidhu 2007
<i>Solanum muricatum</i>	Pepino dulce, Sweet cucumber	Not in Klein et al. 2007		Nuez et al. 1998 Prohens et al. 2002 Prohens and Nuez 2000		Ercan & Akilli 1996 Maroto <i>et al.</i> 1997

				Rodriguez-Burruezo et al 2011		
<i>Solanum quitoense</i>	Naranjillo	great				
<i>Sorbus aucuparia</i>	Rowanberry	essential				
<i>Vaccinium corymbosum</i> , <i>V. angustifolium</i> , <i>V. ashei</i> , <i>V. myrtillus</i>	Highbrush blueberry, Lowbrush blueberry, Rabbiteye blueberry, Bilberry	great				Junttila et al. 2002
<i>Vaccinium macrocarpon</i> , <i>V. oxycoccus</i>	American cranberry, European cranberry	great				Devlin & Demoranville 1967

Table 11 Final dataset used in the meta-analysis, alphabetically ordered by genus.

Species	Method to induce Parthenocarpy	Test environment	Measure	Effect Size	Variance
<i>Actinidia chinensis</i>	HA	NP conditions	Sugars	-0.082	0.021109
<i>Actinidia chinensis</i>	HA	NP conditions	Yield	1.996201	0.245295
<i>Capsicum annuum</i>	SB	OP conditions	Yield	1.836082	0.028712
<i>Capsicum annuum</i>	HA	NP conditions	Yield	1.33715	0.005758
<i>Capsicum annuum</i>	HA	HP conditions	Yield	-0.34255	0.012835
<i>Carica papaya</i>	SB	OP conditions	Yield	1.136643	0.003864
<i>Citrullus lanatus</i>	HA	HP conditions	Sugars	-0.20544	0.006123
<i>Citrullus lanatus</i>	HA	HP conditions	Yield	0.155029	0.006113
<i>Citrullus lanatus</i>	HA	OP conditions	Sugars	0.051341	0.021092
<i>Citrullus lanatus</i>	HA	OP conditions	Yield	-0.92558	0.007337
<i>Cucumis melo</i>	HA	NP conditions	Sugars	13.91252	0.159346
<i>Cucumis melo</i>	HA	NP conditions	Yield	10.83453	0.099127
<i>Cucumis melo</i>	HA	OP conditions	Sugars	0.460481	0.006492
<i>Cucumis melo</i>	HA	OP conditions	Yield	0.275551	0.006385
<i>Eriobotrya japonica</i>	HA	OP conditions	Sugars	0.086106	0.001266
<i>Eriobotrya japonica</i>	HA	OP conditions	Yield	-2.20575	0.002034
<i>Fragaria ananassa</i>	GM	OP conditions	Sugars	0.057971	0.002531
<i>Fragaria ananassa</i>	GM	OP conditions	Yield	0.91286	0.001397
<i>Fragaria vesca</i>	GM	OP conditions	Sugars	0.164231	0.006346
<i>Fragaria vesca</i>	GM	OP conditions	Yield	2.621295	0.003919
<i>Lagenaria siceraria</i>	HA	NP conditions	Yield	2.876362	0.003216
<i>Luffa acutangula</i>	HA	OP conditions	Yield	1.4368	0.007957
<i>Malus pumila</i>	HA	NP conditions	Yield	4.924251	0.012747
<i>Malus pumila</i>	SB	NP conditions	Yield	7.911708	3.269794
<i>Mangifera indica</i>	HA	OP conditions	Sugars	0.81371	0.006848
<i>Mangifera indica</i>	HA	OP conditions	Yield	1.687746	0.008576
<i>Pyrus communis</i>	HA	OP conditions	Yield	0.582146	0.001236
<i>Pyrus communis</i>	HA	OP conditions	Yield	0.393657	0.001201
<i>Rubus chamaemorus</i>	HA	HP conditions	Yield	0.121166	0.004547
<i>Rubus idaeus</i>	GM	OP conditions	Sugars	2.470727	0.005575
<i>Rubus idaeus</i>	GM	OP conditions	Yield	0.798029	0.001707
<i>Solanum lycopersicum</i>	SB	OP conditions	Yield	5.113391	0.003374
<i>Solanum lycopersicum</i>	GM	OP conditions	Sugars	0.440201	0.006922
<i>Solanum lycopersicum</i>	GM	OP conditions	Yield	0.605605	0.007052
<i>Solanum lycopersicum</i>	GM	HP conditions	Sugars	1.283145	0.005336
<i>Solanum lycopersicum</i>	GM	HP conditions	Yield	1.650157	0.006492
<i>Solanum lycopersicum</i>	GM	NP conditions	Sugars	15.43655	131.9856
<i>Solanum lycopersicum</i>	GM	NP conditions	Yield	10.62201	12.64204
<i>Solanum lycopersicum</i>	HA	NP conditions	Yield	3.61653	0.015346

<i>Solanum lycopersicum</i>	SB	NP conditions	Yield	3.204817	0.013882
<i>Solanum lycopersicum</i>	GM	OP conditions	Sugars	1.425102	0.01586
<i>Solanum lycopersicum</i>	GM	OP conditions	Yield	1.991905	0.009461
<i>Solanum lycopersicum</i>	GM	OP conditions	Sugars	3.43146	0.031267
<i>Solanum lycopersicum</i>	GM	OP conditions	Yield	1.070159	0.00723
<i>Solanum lycopersicum</i>	HA	OP conditions	Yield	1.336772	0.007737
<i>Solanum lycopersicum</i>	GM	NP conditions	Yield	2.094612	0.001646
<i>Solanum lycopersicum</i>	SB	OP conditions	Yield	0.484457	0.004744
<i>Solanum lycopersicum</i>	HA	OP conditions	Yield	3.244583	0.185242
<i>Solanum lycopersicum</i>	GM	OP conditions	Sugars	11.85771	0.234966
<i>Solanum lycopersicum</i>	GM	OP conditions	Yield	8.835926	0.136094
<i>Solanum lycopersicum</i>	GM	HP conditions	Yield	-3.62953	0.014606
<i>Solanum lycopersicum</i>	GM	NP conditions	Yield	36.08369	1.093537
<i>Solanum lycopersicum</i>	HA	OP conditions	Yield	1.186435	0.038214
<i>Solanum lycopersicum</i>	GM	NP conditions	Yield	2.020992	0.009014
<i>Solanum lycopersicum</i>	HA	OP conditions	Yield	4.556189	0.631613
<i>Solanum lycopersicum</i>	GM	OP conditions	Sugars	0.210216	0.000111
<i>Solanum lycopersicum</i>	GM	OP conditions	Yield	0.143855	0.00317
<i>Solanum lycopersicum</i>	HA	NP conditions	Yield	28.83752	0.829705
<i>Solanum lycopersicum</i>	GM	NP conditions	Sugars	-1.42147	0.001866
<i>Solanum lycopersicum</i>	GM	NP conditions	Yield	1.739234	0.006145
<i>Solanum melongena</i>	GM	OP conditions	Yield	1.444628	0.070885
<i>Solanum melongena</i>	SB	OP conditions	Yield	1.761513	0.01627
<i>Solanum melongena</i>	GM	NP conditions	Yield	3.679541	1.106564
<i>Solanum melongena</i>	HA	NP conditions	Yield	2.399622	0.121138
<i>Solanum melongena</i>	SB	NP conditions	Yield	-1.19352	0.016926
<i>Solanum melongena</i>	GM	NP conditions	Yield	4.184441	1.306233
<i>Solanum melongena</i>	SB	OP conditions	Yield	2.084884	0.007034
<i>Solanum muricatum</i>	HA	OP conditions	Yield	1.662862	0.037947
<i>Solanum muricatum</i>	HA	OP conditions	Yield	0.237487	0.004253

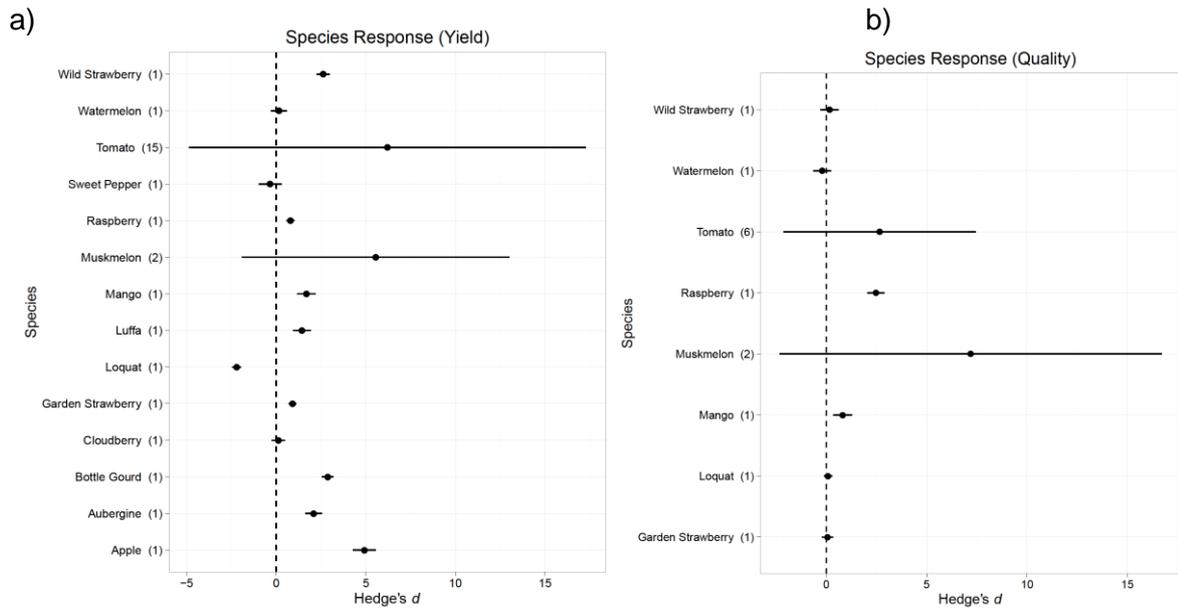


Figure 31 Mean effect sizes for all methods combined to induce parthenocarpy (genetic modification, hormone application, and selective breeding) split by crop species (y axis) for (a) fruit quantity (b) fruit quality. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

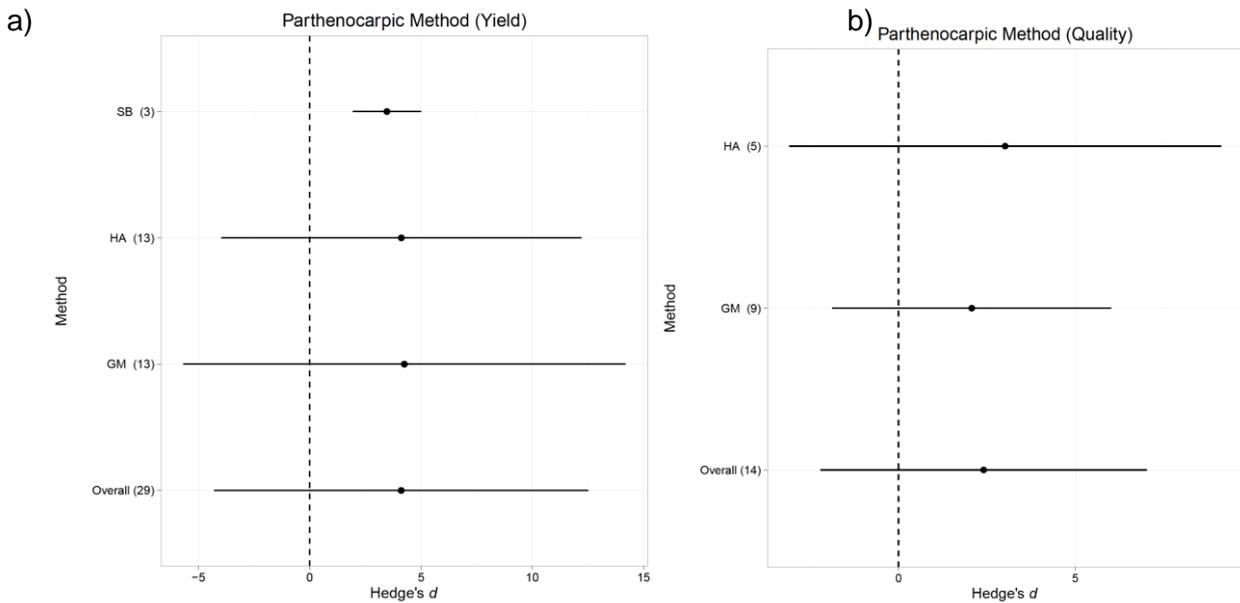


Figure 32 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) (y axis) for (a) fruit quantity and (b) quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

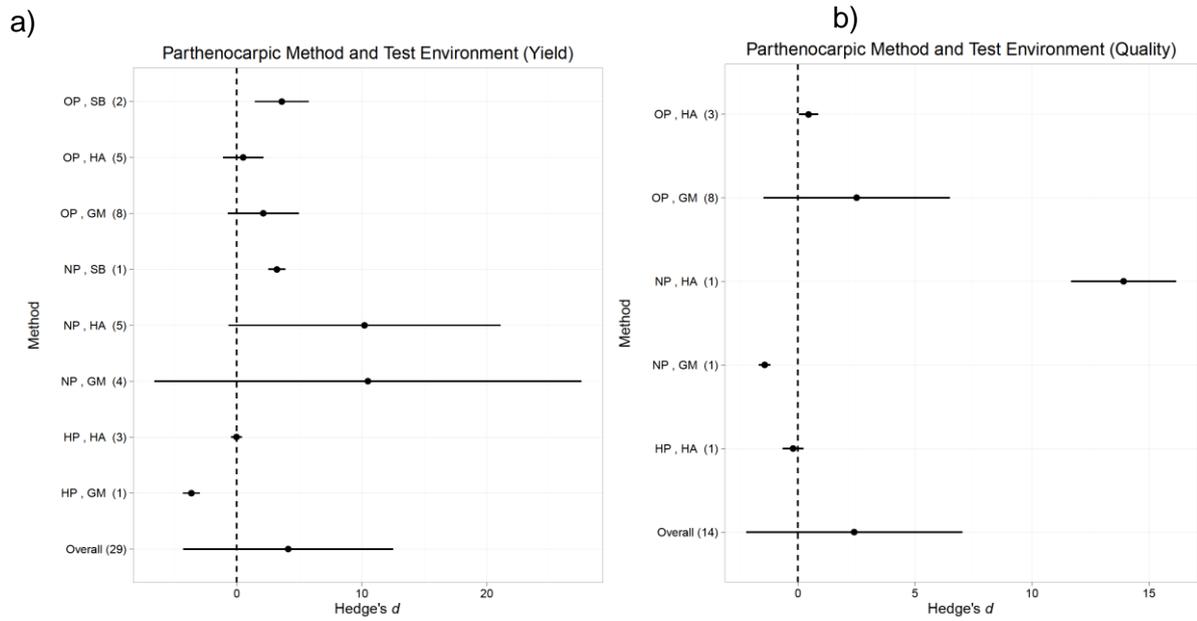


Figure 33 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) and test environment (NP, OP, and HP) (y axis) for (a) fruit quantity (b) fruit quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

Nutritional value of courgette nectar and pollen for bees

Research Questions

- 1) How much pollen and nectar do courgette crops provide?
- 2) How does courgette affect *B. terrestris* colony development at a landscape scale (using *Bumble-BEEHAVE*)?

Materials and methods

Study species

Courgette is monoecious with predominate staminate flowers until pistillate flowers gradually dominate over a season. Within a single day, both types of flower start opening around 05:30 before closing around 12:00 h on the same day, and they do not open again. Although flower anthesis and senescence varies slightly depending on the climate, it is not thought to be directly affected by climatic events such as rainfall (Nepi, Massimo and Pacini, 1993).

In the United Kingdom, courgette is usually grown over two cropping periods (flowering and harvesting lasting around 5 weeks) at two separate sites (often several kilometres apart) to ensure a constant supply of courgette from the beginning of June until the end of August. Hereafter the first cropping period is referred to as 'early courgette' and the second cropping period is referred to as 'late courgette'.

Although all bee species visiting courgette were recorded during pollinator surveys, *Bombus terrestris* was the focus of this study because of their natural abundance at study sites and availability as commercial colonies (Biobest Biological Systems, Belgium) which were required to quantify the proportion of courgette pollen in *B. terrestris*' diet. Colonies were placed in each field (with sugar water but no additional pollen) at a density of three colonies per field.

Study sites

The empirical data for this study were collected in 19 courgette (var. 'Tosca') fields in Cornwall, UK from the beginning of June until the end of August in 2016 (5 fields) and 2017 (14 fields). Each field (average field size of 3.6 ± 0.3 ha *SE*) was situated at least 2 km from any other courgette field so that pollinator communities were unlikely to be shared between fields (Vaissière, 2010). All courgettes were grown conventionally in outdoor (as opposed to protected) conditions in fields surrounded by species-rich hedgerows, where little or no herbicide was used due to the short picking intervals of the crop (P.E. Simmons and Son, personal communication 1st November 2017). This meant that there was a high abundance and species richness of wild flowers within and around the crop.

Quantifying nectar and pollen resources in courgette flowers (2017)

The standing crop of nectar, i.e. the amount of nectar available to visiting insects at a given time, was quantified to show how the volume of courgette nectar changed within a day. On the other hand, the 24 hour secretion rate, i.e. the overall amount of nectar produced by a flower over 24 hours was quantified to show the maximum nectar resource available from all courgette flowers within a landscape per day, which was needed to parameterise *Bumble-BEEHAVE*.

For each flower, full access to the nectary was achieved by removing the stigma or stamen with a scalpel. The standing crop of nectar was calculated from 50 staminate and 50 pistillate flowers every 90 minutes from 05:30 to 12:00 h over five days (10 staminate and 10 pistillate flowers per time point per day). This volume is likely less than when the same flower is repeatedly surveyed due to the flower replenishing resources (Corbet, 2003). In addition, the 24-hour secretion rate of nectar was calculated by securing PVC mesh bags to flowers with wire ties the day before expected anthesis. Bags had a mesh size of 0.2mm, designed to be permeable to wind and rain yet exclude any pollinators (Corbet, 2003). Bags were then removed around 11:00 h and all nectar extracted individually from 40 staminate and 40 pistillate flowers, over four days (10 staminate and 10 pistillate flowers per day).

Nectar volume (μl) was measured using glass microcapillary tubes (sizes 2, 5, 10 and 20 μl microcaps, Drummond Scientific, Broomall PA, USA) and nectar sugar concentration (mg/mg) was measured using a hand-held refractometer modified for small volumes (Eclipse, Bellingham & Stanley, Tunbridge Wells, UK). Nectar sugar concentration as measured by the refractometer, i.e. weight of solute per weight of solution (C ; mg/mg), was converted to nectar sugar concentration in terms of weight of solute per volume of solution (d ; mg/ μl) using $d = (0.0037291C + 0.0000178C^2 + 0.9988603)$ (from Prÿs-Jones & Corbet, 1991). The weight of sugar produced per flower over 24 h (w) (mg) was then calculated using $w = dvc$, where v is volume of nectar (μl) and c is sugar concentration of nectar as a proportion (mg/ μl).

The amount of pollen (mg per flower) available at a given time point was quantified from 20 stamens which were removed from staminate flowers every 90 minutes from 05:30 to 12:00 h over two days (10 stamens per time point per day) to show pollen depletion within a day. In addition, the total amount of pollen produced in 24 hours was quantified from 40 stamens which were removed from staminate flowers (secured with PVC mesh bags the day before expected anthesis) over two days (20 stamens per day). All stamens were placed in centrifuge tubes in the field.

Owing to the vast quantities of pollen on stamens, pollen was weighed rather than counted. This was done by adding 1ml of distilled water, using a pipette, to centrifuge tubes containing stamens, agitating them for 10 seconds at 12,000 rpm, removing stamens with forceps, and centrifuging at 12,000 rpm for 10 minutes. The supernatant was then removed using a pipette and the centrifuge tube placed in a drying cabinet at approximately 40 °C for 24 hours. The samples and centrifuge tubes were weighed on a balance before being washed and returned to the drying cabinet for a further 24 hours and weighed again. The weight of the empty centrifuge tube was then subtracted from the centrifuge tube containing the pollen to provide the weight of pollen per flower (mg per flower).

Effect of courgette on *B. terrestris* colony development (2017)

Pollen loads from B. terrestris

To quantify the proportion of courgette pollen in *B. terrestris*' diet, 'forager trap modules' (Martin et al., 2006) were placed onto all commercial colonies within a field for around 45 minutes (between 07:00 and 09:00 h). Once trapped on returning from a foraging trip, workers were narcotised *in situ* using CO₂ for 30 seconds and the number of bees carrying (and not carrying) pollen loads were recorded. One pollen pellet from one of the corbiculae on each bee, i.e. half of their total pollen load, was placed into a centrifuge tube and taken back to the laboratory. Here all pollen loads were sorted to colour and all yellow pollen loads checked to see if they were from courgette, which has large (180 - 200 µm in diameter) and distinctive pollen grains (Nepi, Massimo and Pacini, 1993). A subset (n=56) of all pollen loads were identified to species (where possible) using Sawyer (1981) and a microscope. All foragers were returned to their colony within an hour of being caught and the pollen loads of bees in each field were surveyed on separate days.

Habitat maps

Habitat maps for each study site were required to estimate the amount of forage and nesting sites (semi-natural habitat and mass-flowering crops) available to bumblebees in the landscape (Figure 34) (Kremen et al., 2004; Westphal et al., 2003). To create these maps, semi-natural habitat (woodlands and heathland), improved grassland, and mass-flowering crops (courgette and maize) were recorded in 750m radii of each field site in 2017 (n = 14). This was done by ground truthing satellite imagery and adapting Land Cover 2007 data (Centre for Ecology and Hydrology 2011) using ArcGIS 10.2.2 (Figure 5.2). Each site had varying quantities of crop and habitat types (Figure 41).

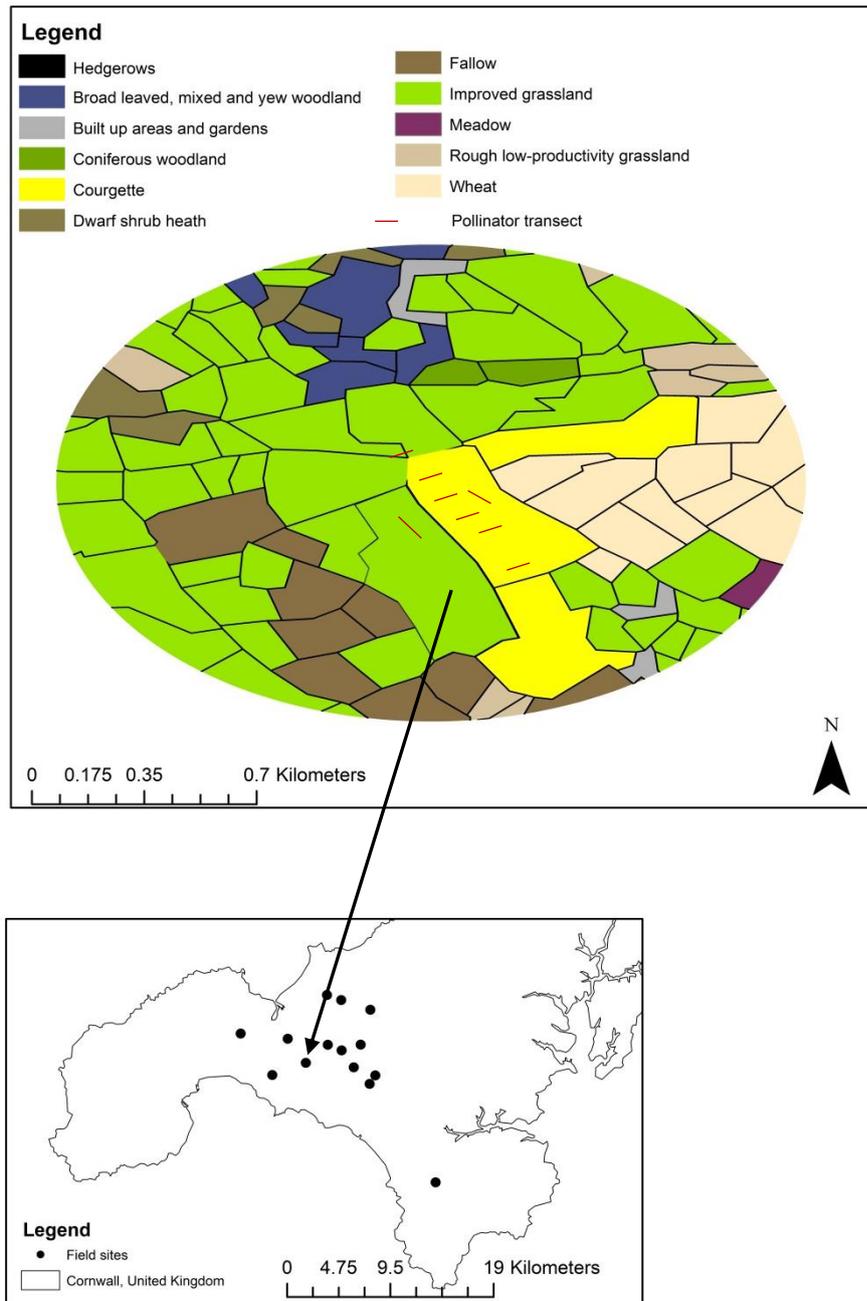


Figure 34 Example landscape map of a study site (including pollinator transects within a courgette field) in the context of all study sites surveyed in 2017 in Cornwall, UK.

Bumble-BEEHAVE simulations using BEE-STEWARD

The default settings for *Bumble-BEEHAVE* provide on the 1st of January simulations with 500 queens who randomly emerge from hibernation on 1st of April (± 28 days *SD*), following a normal distribution. In the model, queens can nest in all types of semi-natural habitat implemented in the model: heathland, species-rich grassland, hedgerow, scrub and woodland (Becher, Twiston-Davies *et al.* 2018).

Habitat types are defined by the presence and abundance of 44 forage plants which provide nectar and or pollen during specified flowering periods. Once a queen has found suitable nesting habitat she must collect sufficient pollen and nectar resources before laying her first batch of eggs. She will then continue to split her time between foraging and brood care until the first adult workers emerge. The queen will then focus on egg-laying whilst workers divide their time between brood-care and foraging. Foraging choices are based on maximising foraging rate (pollen) or energetic efficiency (nectar), which depends on distance, handling time, and the degree of patch depletion. The probability of a bee detecting a new patch is based on the distance of the food source from a colony. Towards the end of colony development female larvae may develop into queens, and the original queen switches from laying diploid eggs to haploid, male eggs. Once new queens are developed they leave their colony, mate and hibernate prior to emergence the following year. For a detailed model description see supplementary material S03 ('ODD protocol') of Becher & Twiston-Davies *et al.* (2018).

BEE-STEWARD's flexible input settings meant that habitat types recorded on surveys, which were not already in the model (i.e. courgette, heathland, and improved grassland) could be easily parametrised in the input files for analysis (Table 12). Courgette fields were specified as either 'early courgette' (flowering from the beginning of June until the middle of July) or 'late courgette' (flowering from the middle of July until the end of August) to reflect the cropping practices of courgette production in the UK. A map of each study site was separately input into the model and manually edited (if needed) using the functions available within the program (Becher *et al.*, 2016).

In order to reduce computational time and to ensure that simulations were based solely on populations in equilibrium (Hui, 2006), a set of preliminary simulations were run in landscapes with no courgette (where courgette fields had been temporarily removed) as a baseline. To determine a suitable number of initial queens for all landscapes, simulations were started with 500 hibernating queens and run over 15 years in each landscape 20 times. The number of queens was then plotted over time to see at what number of queens the population appeared to reach equilibrium (Figure 42). This resulted in 500 hibernating queens as a conservative estimate for all landscapes and simulations. To determine the length of simulations (i.e. time taken to reach equilibrium), simulations were run starting with a population size that was either close to the estimated number of hibernating queens (500) or above it (1000) across all landscapes (with no courgette) 20 times, over 20 years. The population was assumed to be in equilibrium, once both growth curves had converged (Figure 43). Year 11 was taken as the year where all landscapes were in equilibrium.

The effect of courgette on *B. terrestris* population dynamics was explored by re-classifying courgette fields in landscape maps of actual study sites to either 'early season courgette', 'late season courgette' or 'no courgette' in BEE-STEWARD. This created three different cropping scenarios for simulations in *Bumble-BEEHAVE*: 1) no mass-flowering crop (baseline), 2) early season courgette, and 3) late season courgette. All simulations were run 10 times per landscape and cropping scenario, totalling 420 simulations.

The average number of over-wintering queens, colonies, and adult workers, as well as the nectar and pollen stores per colony, were calculated daily for each landscape over 11 years.

Statistical analysis

All analyses were carried out using R (R Core Team, 2017). For empirical data, independent sample *t*-tests were used to compare the differences in mean sugar production (g) between staminate and pistillate flowers (over 24 hours and every 90 minutes), pollen depletion (mg/flower) between 05:30 and 10:00 h, pollen accumulation on stigmas (grains/stigma) between 05:30 and 11:30 h., and *B. terrestris* abundance in the margin and cropped area per hour.

For simulated data the effect of cropping scenario (fixed effect) on the numbers of hibernating queens (day 365), colonies (day 149), adult workers (day 149), and adult workers per colony (day 235) in year 11 was tested using linear models. *Post hoc* Tukey tests were calculated using the multcomp package (Hothorn et al., 2008). All means are presented with their associated standard error unless otherwise stated.

Results

Nectar and pollen measurements from courgette

The secretion rate of nectar, i.e. the weight of sugar produced over 24 hours (from bagged flowers) was greater (although not statistically, $T_{78} = -1.94$, $P = 0.06$) for pistillate flowers (34.41 ± 2.67 mg per flower, $n = 40$) than staminate flowers (26.59 ± 1.56 mg per flower, $n = 40$). These estimates were much higher than the nectar standing crop, i.e. weight of sugar available at a given time point per flower, which at 05:30 h was just 0.52 ± 0.09 mg for pistillate flowers and 1.24 ± 0.16 mg for staminate flowers (Figure 35 a). By 11:30 h nearly all sugar was depleted from both staminate (0.05 ± 0.01 mg) and pistillate (0.07 ± 0.01 mg) flowers (Figure 35 a).

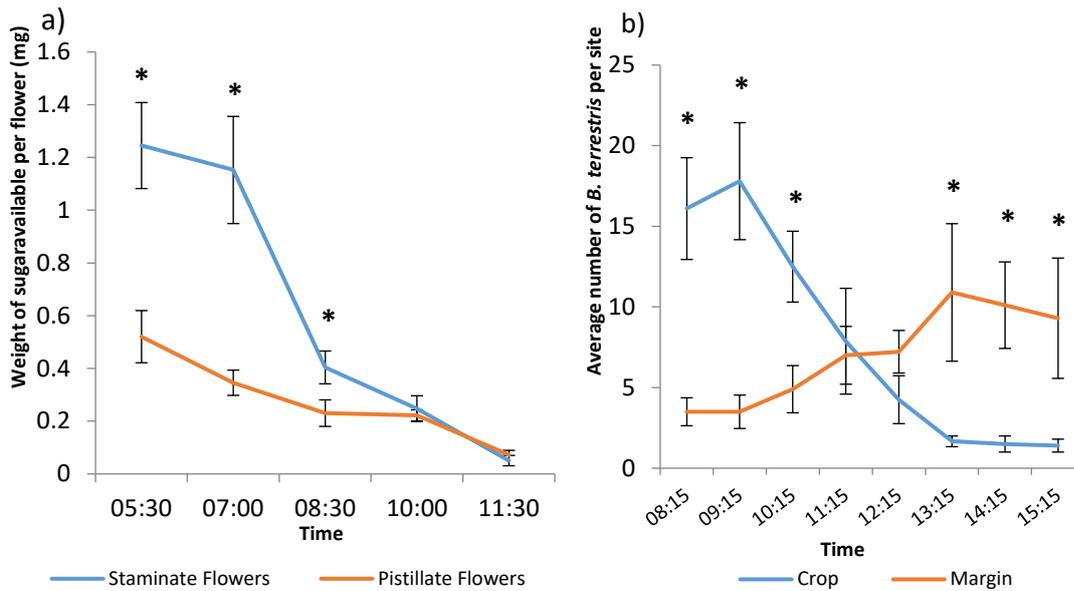


Figure 35 a) Weight of sugar available (\pm SE) every 90 minutes for 40 staminate and 40 pistillate flowers (400 flowers in total) and 2b) average number of *B. terrestris* in the crop and on the margin over time, data were summed per transect in either the crop or on the margin and averaged by site ($n = 10$), all sites contained commercial colonies of *B. terrestris*. Significant independent *t*-tests are indicated with an asterisk (*) for each time point ($P < 0.05$).

The weight of pollen produced over 24 hours (from bagged flowers) was 18.04 ± 0.84 mg per staminate flower ($n = 40$). Again, this was much greater than the weight of pollen available from un-bagged flowers, which was estimated to be 10.96 ± 1.39 mg per flower at 05:30 h. From 05:30 to 10:00 h there was no significant loss ($T_{37} = -1.22$, $P = 0.23$) of pollen (10:00 h = 8.37 ± 1.64 mg per flower) suggesting that much of the pollen is removed around anthesis when the very first pollinator visits occur.

Pollen loads

None of the 394 pollen loads collected from *B. terrestris* contained courgette pollen (Table 13 **Error! Reference source not found.**). Brassica spp. (15), bramble (11), and common poppy (7) were the most common pollen species identified out of a subsample ($n = 56$) of pollen loads (Table 13). Consequently, all courgette flowers were specified as having a pollen resource value of zero in BEE-STEWARD (Table 12).

Bumble-BEEHAVE simulations using BEE-STEWARD

Early courgette landscapes had a higher ‘carrying capacity’ for queen bumblebees, determined by the number of over-wintering queens on the last day of the year compared to no courgette (contrast estimate -419.0 ± 157.9 , $T = 2.65$, $P = 0.03$) and late courgette (contrast estimate -435.7 ± 157.9 , $T = 2.76$, $P = 0.02$) on day 365 (Figure 36). Likewise early courgette resulted in the establishment of more colonies in the landscape compared to no courgette

(contrast estimate -30.53 ± 11.43 , $T = -2.67$, $P = 0.03$), and late season courgette (contrast estimate -31.34 ± 11.43 , $T = -2.74$, $P = 0.03$) on day 149 (day with the largest differences between cropping scenarios) (Figure 37). This resulted in more adult workers on day 149 (peak forager activity) across early courgette landscapes compared to no courgette (contrast estimate -474.86 ± 189.6 , $T = -2.51$, $P = 0.04$) and late courgette (contrast estimate -534.29 ± 189.6 , $T = -2.82$, $P = 0.02$) landscapes (Figure 38). Indeed, the year on year effect of early courgette also increased the abundance of foragers early in the season, before courgette flowering (Figure 5.7). Whilst early courgette landscapes lead to the establishment of more colonies, the average size of each colony did not increase compared to no courgette (contrast estimate -7.13 ± 6.44 , $T = 1.08$, $P = 0.5$) and late courgette (contrast estimate -0.16 ± 6.44 , $T = -0.02$, $P = 1.00$) on day 149 (peak forager activity) (Figure 39). The availability of nectar or pollen per bee was also similar between cropping scenarios (Figure 40). Since pollen and nectar stores per bee were only calculated on days where colonies had more than 100 adult workers (to avoid too many peaks in stores when populations were low), early courgette landscapes appear to have more pollen and nectar available per bee at the beginning and end of the season (Figure 40), however, this is more likely due to more adult workers in these landscapes (Figure 38). The phenology of early season courgette (flowering from beginning of June to the middle of July) is more closely related to forager activity (indicated with the baseline, no courgette) and longer in duration than late season courgette (flowering from middle of July until the end of August) (Figure 38).

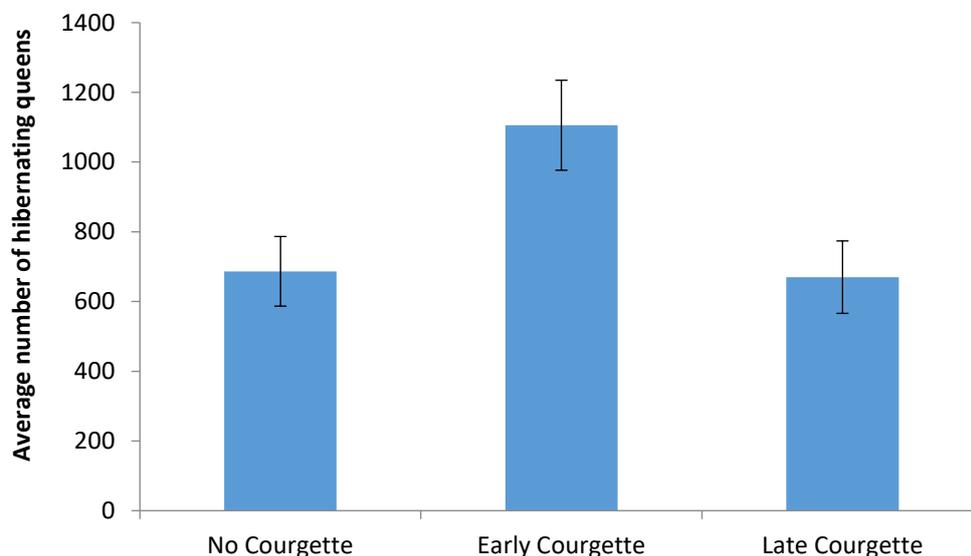


Figure 36 Average number of hibernating queens \pm SE on the last day of year (year 11) for each cropping scenario. Data were averaged across the 10 repeated runs and 14 study sites.

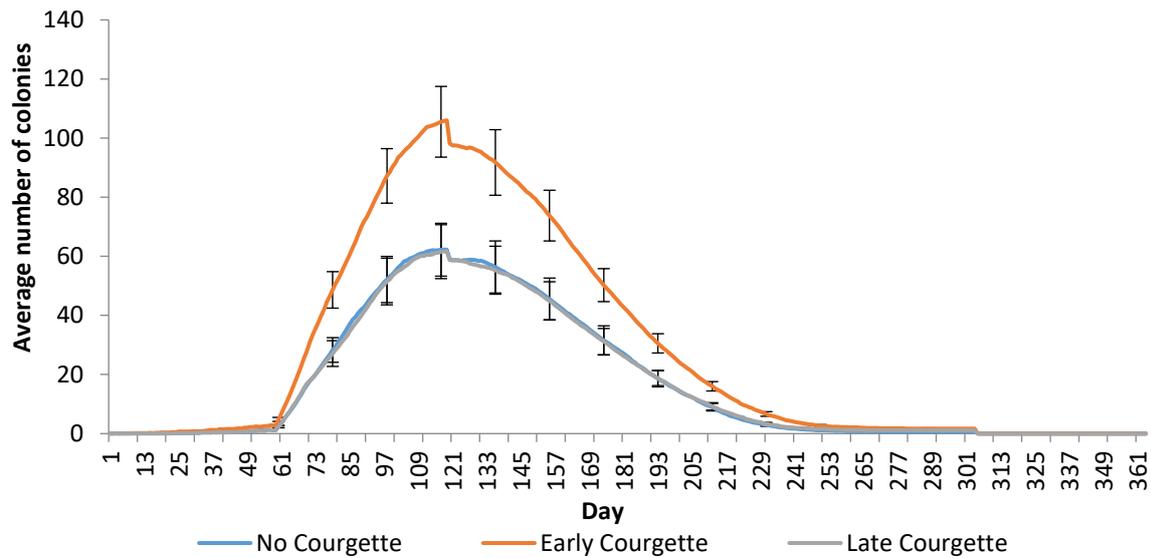


Figure 37 Average number of colonies (\pm SE every 20 days) over the course of a year (year 11) for each cropping scenario. Data were averaged across the 10 repeated runs and 14 study sites.

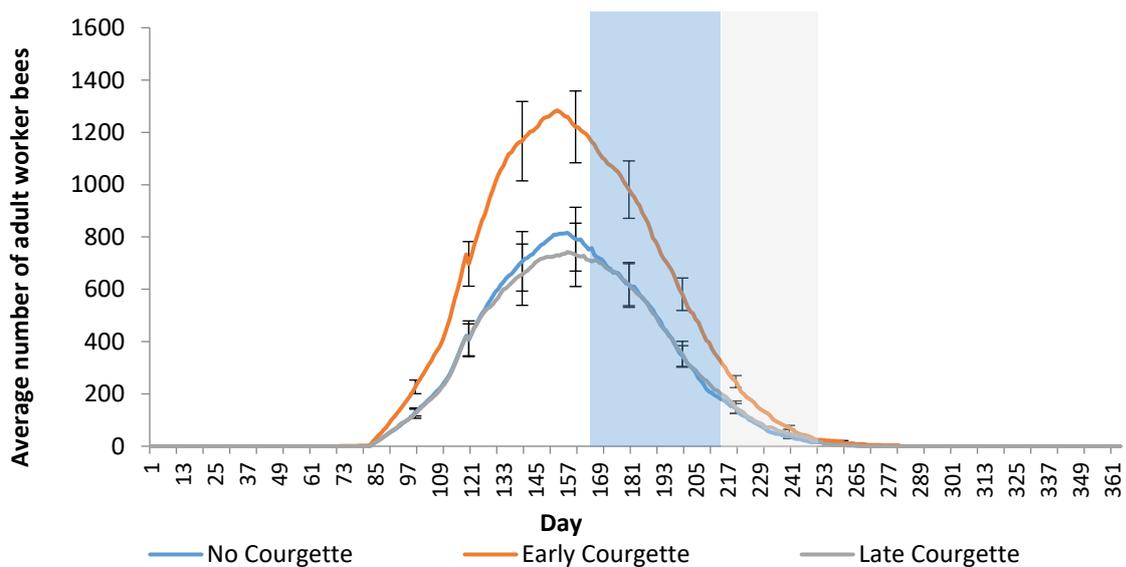


Figure 38 Average number of adult worker bees (\pm SE every 20 days) over the course of a year (year 11) for each cropping scenario. Shaded areas show the flowering times of courgette, early courgette is shown in dark grey, late courgette shown in light grey. Data were averaged across the 10 repeated runs and 14 study sites.

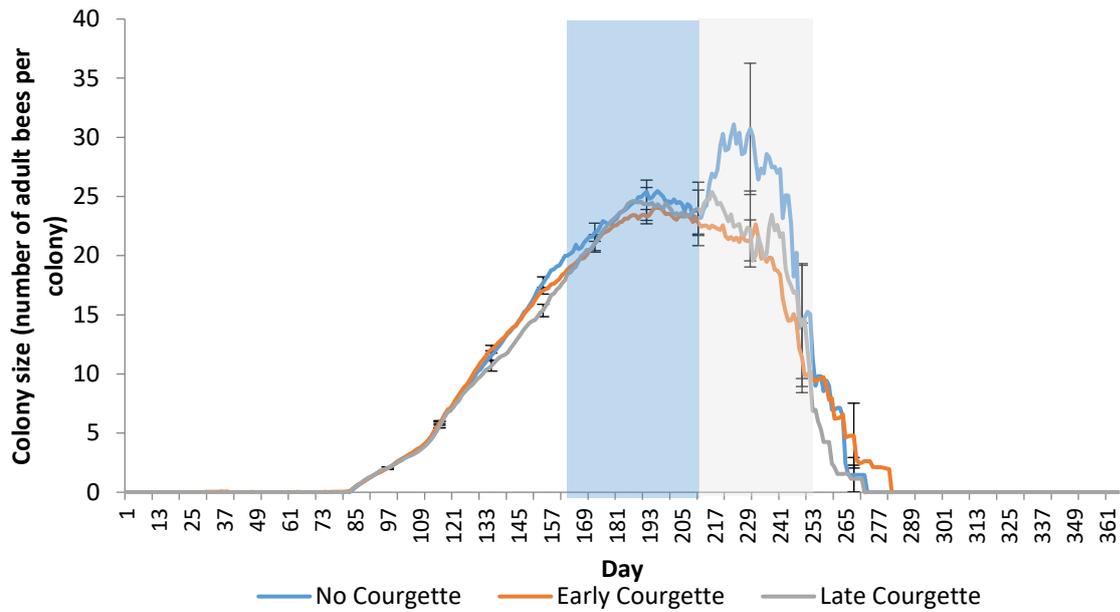


Figure 39 Average size of colonies (number of adult bees per colony) (\pm SE every 20 days) over the course of a year (year 11) for each cropping scenario. Shaded areas show the flowering times of courgette, early courgette is shown in dark grey, late courgette shown in light grey. Data were averaged across the 10 repeated runs and 14 study sites

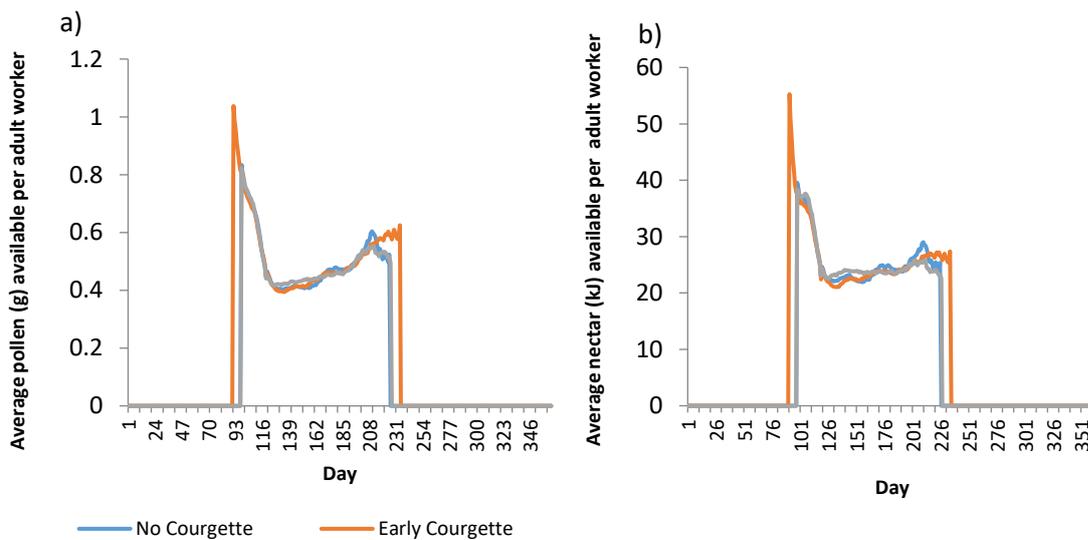


Figure 40 a) pollen (g), and b) nectar (kJ) available per adult worker for each cropping scenario in year 11, n.b. early courgette flowers for longer than late courgette. The total average pollen and nectar stores were divided by the total average number of adult workers, although only when colonies had more than 100 adult workers to avoid too many peaks in stores when populations were low. Data were averaged across the 10 repeated runs and 14 study sites.

Discussion

This study clearly demonstrates a mutualistic interaction between courgette flowers and *B. terrestris* that is beneficial to both, improving pollination success and colony dynamics (Bailes *et al.* 2015; Holzschuh *et al.* 2016). Courgette, like many other mass-flowering crops offers vast quantities of nectar and pollen to attract pollinators to its flowers for pollination (Vidal *et*

al., 2006). Indeed per m², courgette offers more nectar (0.35 ml) than oilseed rape (0.30 ml), field bean (0.092 ml) and sunflower (0.003 ml) (Becher et al., 2016), and is therefore a high value mass-flowering crop in terms of nectar production.

Despite courgette pollen being relatively high in protein (Petersen, Reiners & Nault 2013), its large sticky grains may make it difficult for *B. terrestris* to collect (Vaissière & Vinson 1994). Therefore, *B. terrestris* may avoid collecting *Cucurbita* pollen, since as a generalist species it can visit alternative, more easily obtainable pollen, unlike *Peponapis* and *Xenoglossa* spp. which as *Cucurbita* specialists are thought to rear their offspring exclusively on *Cucurbita* pollen (Tepedino 1981). This may be why no pollen loads from returning *B. terrestris* foragers contained courgette pollen.

Given courgette's bountiful, yet transient supply of nectar, bumblebee population dynamics were shown (using *Bumble-BEEHAVE*) to improve in landscapes with early flowering courgette compared to a no courgette baseline. As bumblebee foragers are generally most active mid-summer, early courgette was the best cropping scenario for concurrently achieving more forager visits (pollination potential) and more food (nectar only) to be brought back to the colony. Whilst courgette nectar provides additional energy, helping to reduce foraging efforts, bees can only benefit if protein providing pollen is also available to raise their brood. Empirical data showed that, within a day, bees were able to utilise courgette for nectar and wild flowers for pollen (Figure 35 b), thus supporting model results which showed (at a coarser temporal scale) that with more nectar, colonies were able to grow and subsequently forage on more, additional resources for pollen. Subsequently early courgette supports more adult workers (foragers), colonies, and hibernating queens for subsequent years compared to late, and no courgette landscapes. Nonetheless, planting early courgette and late courgette in fields adjacent to each other could improve forager numbers in late courgette and further improve bumblebee populations for subsequent years (Riedinger et al., 2014).

The phenological matching of crops with key periods of pollinator activity is thought to be why the presence of oilseed rape in the landscape (early in the season) can improve the reproductive potential of *Osmia bicornis* L. (Holzschuh et al., 2013; Jauker et al., 2012), but not *Bombus pascuorum* S. (Herrmann et al., 2007) and *B. terrestris* (Westphal et al., 2009). This is because whilst oilseed rape can improve colony establishment and growth of bumblebees, the lack of resources later in the season mean there is no increase in the number of males or queens produced (Herrmann et al., 2007; Westphal et al., 2009).

This lack of phenological matching is also true of late courgette which despite offering resources later in the season (unlike oilseed rape) still misses the key period of bumblebee foraging. However, Rundlöf et al. (2014) observed more queen and male bumblebees on

transects around fields of late-flowering red clover, suggesting results could be specific to flower and pollinator species. Since nectar and pollen supplies in the landscape are directly influenced by colony density, it is perhaps unsurprising that colonies in early courgette landscapes had similar amounts of pollen and nectar available per bee compared to late and no courgette landscapes. Interestingly, the average pollen and nectar store per bee and average number of colonies per landscape decline around day 116, which may be a result of willow species (common to hedgerows and scrub in *Bumble-BEEHAVE*'s input files) no longer flowering.

Combining empirical data on pollinator visitation, nectar and pollen availability, and pollination efficiency, with model simulations has provided a unique insight into the mutualistic relationship between *B. terrestris* and the mass-flowering crop, courgette. Flower-scale data (within a day) showed how effective a pollinator *B. terrestris* is in courgette and the extent to which they utilise courgette flowers for pollen and nectar. Based on this information *Bumble-BEEHAVE* was parameterised to show the effect of courgette management at the crop-scale (within a year) which, whilst theoretical, is consistent with empirical knowledge.

Broadly, these findings show that matching crop phenology with key periods of forager activity can be an effective way of improving bumblebee population dynamics and pollination efficiency. Increased understanding of a plant-pollinator mutualism at different temporal and spatial scales means that management recommendations can be made. For growers this may mean planting mass-flowering crops with complementary phenologies (such as early and late courgette) in fields adjacent to each other. For conservationists it may mean recognising the importance of courgette, alongside other mass-flowering crops, as valuable forage resources for bumblebees, whilst continuing to promote additional sources of forage to fulfil bees' nutritional requirements over space and time. In doing so, it could be possible to simultaneously improve pollination services *and* bumblebee populations in intensive farmland.

Appendix – Nutritional value of courgette for bees

Table 12 Input parameters for additional crop and habitat types for BEE-STEWARD.

Crop type	Flower species	Flower density (flower s/m ²)	Pollen (g/flower)	Nectar (ml/flower)	Proportion of protein in pollen	Sugar concentration of nectar (mol/l)	Flowering start day	Flowering stop day	Corolla depth (mm)	Nectar volume (flower)	flower (myl/)	Internal flower handling time (seconds)	Notes
Courgette	Early courgette	3	0*	0.0765	0*	1.4660	163	212	0	76.47		2.5	1)
	Late courgette	3	0*	0.0765	0*	1.4660	213	251	0	76.47		2.5	
Improved grassland	Dandelion	0.05	0.0004	0.0005	0.0917	1.2947	1	364	1.2	0.4702		0.6	2)
	White clover	2.34	0.0004	0.0007	0.2307	0.9803	151	272	2	0.6666		0.6	
Heath	Ling	465	0**	0.0001	0**	1.17	182	273	4	0.0787		0.6	3)
	Bell Heather	7.17	0**	0.0002	0**	1.17	121	334	5.5	0.1997		0.6	
	Cross-leaved Heather	2.14	0**	0.0002	0**	1.17	152	273	6.5	0.1742		0.6	
	Bilberry	9.23	0**	0.0026	0**	1.17	91	181	5	2.6215		0.6	

Notes (Table 12):

- 1) Empirical observations. * Pollen values set to 0 as no bees were observed bringing back courgette pollen to their colonies.
- 2) Nectar and pollen were already in the model; however, flower species and flower density were based on empirical observations.
- 3) Data from Baude et al. (2016). ** No pollen data available for these species.

Table 13 Plant species identified from a subsample of pollen loads n=56, from the total 394 loads collected. None of the yellow pollen was courgette pollen.

Species name	Common name	Number of pollen loads
<i>Brassica spp.</i>	Brassica spp.	15
<i>Rubus fruticosus</i>	Bramble	11
<i>Papaver rhoeas</i>	Common poppy	7
<i>Veronica filiformis</i>	Speedwell	4
<i>Helianthemum chamaecistus</i>	Common rockrose	3
<i>Linaria vulgaris</i>	Common toadflax	3
<i>Verbascum thapsus</i>	Great mullein	3
<i>Echium vulgare</i>	Viper's bugloss	2
<i>Hedera helix</i>	Common ivy	2
<i>Ribes sanguineum</i>	Flowering currant	2
<i>Calystegia sepium</i>	Hedge bindweed	1
<i>Centaurea cyanus</i>	Cornflower	1
<i>Centranthus ruber</i>	Red valerian	1
<i>Heracleum sphondylium</i>	Hogweed	1

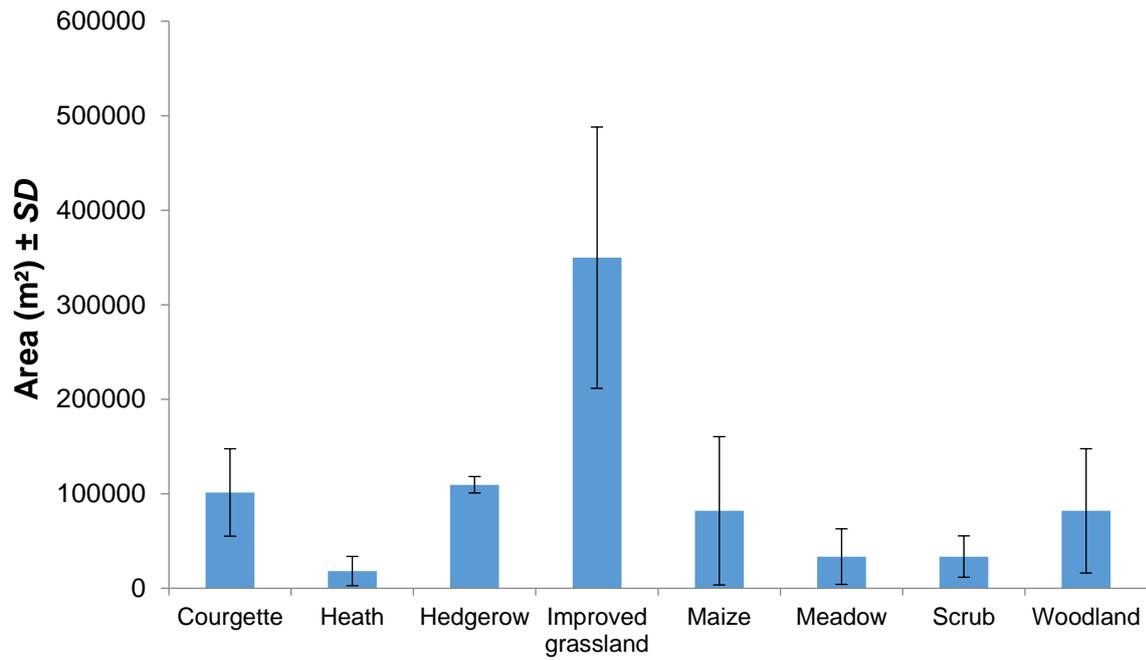


Figure 41 Area (m²) ± SD of the different habitat types providing pollen and/or nectar across the 14 landscapes. In model simulations the area of courgette was specified as either 'early courgette' or 'late courgette' (see Table A 4.1), or 'no courgette' (no habitat specified in the model). *Simulated B. terrestris* were able to nest in heath, hedgerow, meadow, scrub, and woodland.

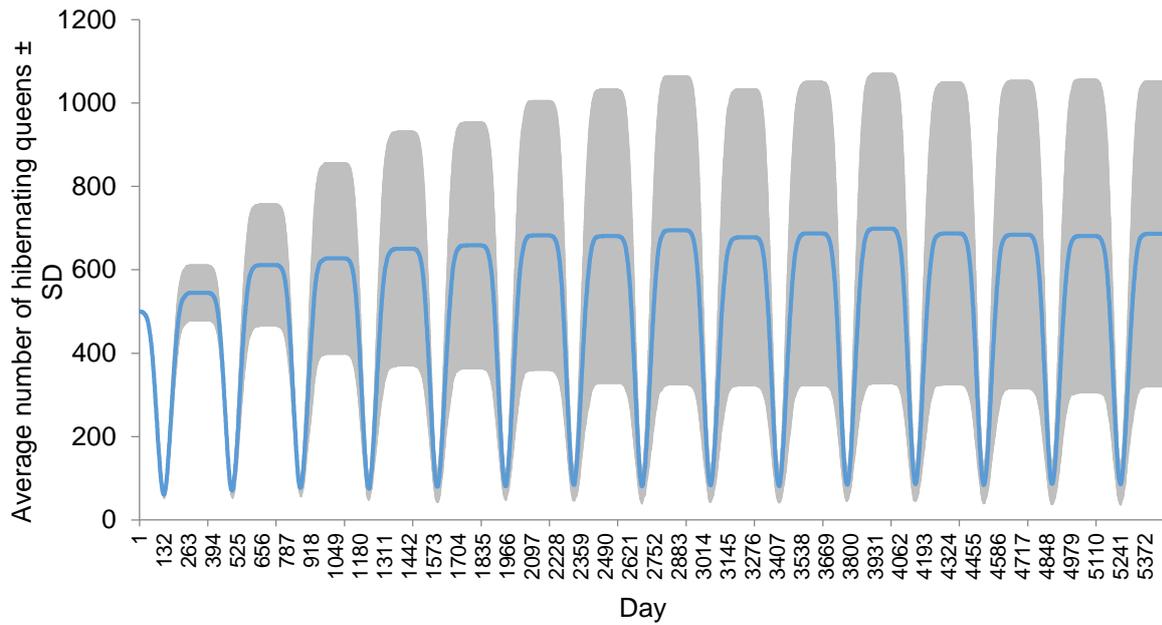


Figure 42 Average number of hibernating queens \pm SD (shaded) across 14 landscapes with no courgette present (baseline) over 15 years. Data were simulated 20 times for each landscape.

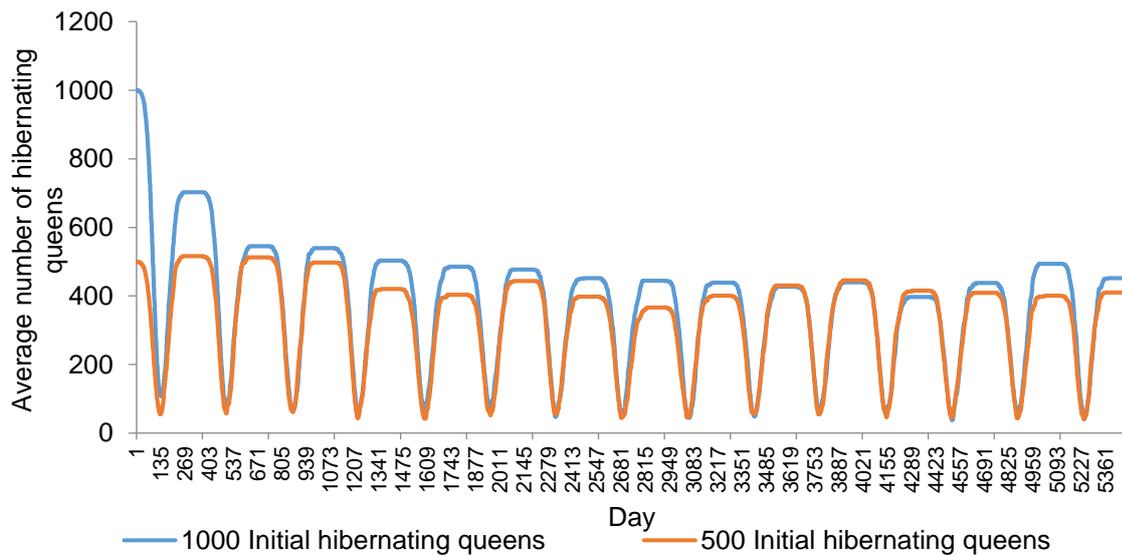


Figure 43 Average numbers of hibernating queens simulated for the study site 10, the last of the 14 different landscapes to reach equilibrium, i.e. when both growth curves have converged; taken as year 11 (a conservative estimate). Data were simulated 20 times for each landscape.

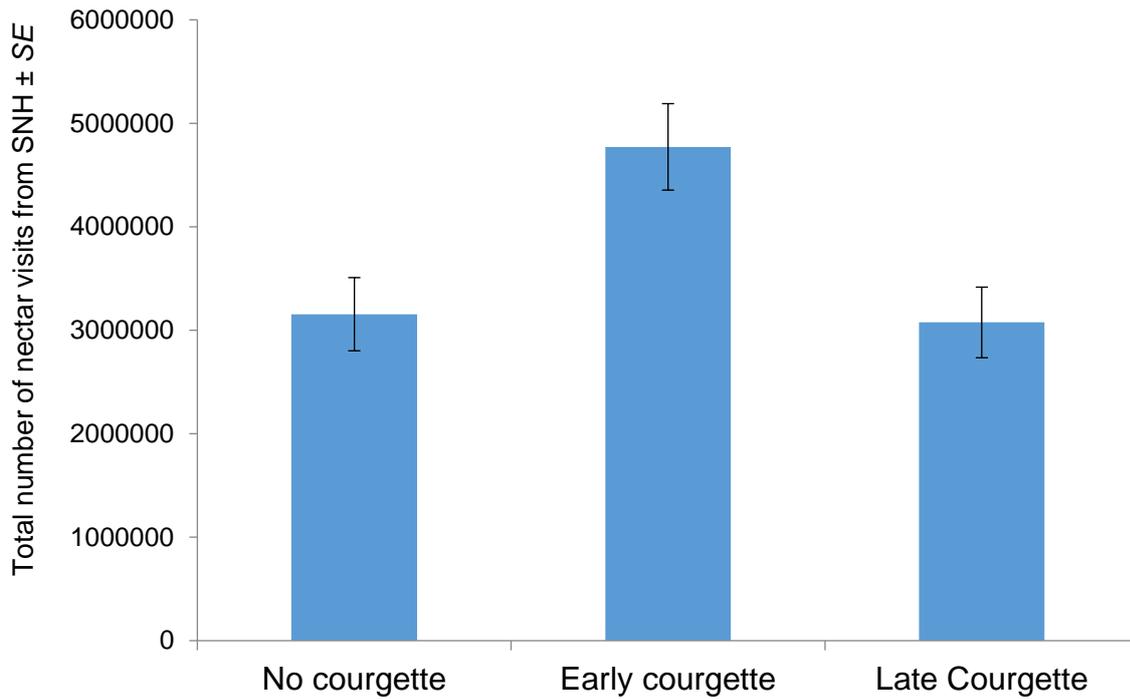


Figure 44 Total simulated number of forager visits \pm SE at the end of the year (year 11) to patches of SNH, semi-natural habitat: heathland, species-rich grassland, hedgerow, scrub and woodland for nectar in each of the three cropping scenarios. Data were averaged across the 10 repeated runs and 14 study sites.



Figure 45 *B. terrestris* removing excess courgette pollen from its body.

Species list of bees and hoverflies recorded in courgette fields

Table 14 Bees and hoverflies collected from pan traps in the cropped area and margins of courgette fields in 2016. No species are of current conservation concern.

Species	Abundance
Honeybee	
<i>Apis mellifera</i>	10
Bumblebees	
<i>Bombus hortorum</i>	7
<i>Bombus hypnorum</i>	1
<i>Bombus lapidarius</i>	4
<i>Bombus lucorum</i>	2
<i>Bombus pascuorum</i>	2
<i>Bombus pratorum</i>	1
<i>Bombus rupestris</i>	2
<i>Bombus terrestris</i>	9
<i>Bombus terrestris</i>	1
Solitary bees	
<i>Andrena angustior</i>	6
<i>Andrena bicolor</i>	10
<i>Andrena dorsata</i>	1
<i>Andrena flavipes</i>	1
<i>Andrena haemorrhoa</i>	2
<i>Andrena humilis</i>	1
<i>Andrena minutula</i>	4
<i>Andrena nigroaenea</i>	1
<i>Andrena subopaca</i>	1
<i>Halictus rubicundus</i>	3
<i>Hyleaus cornutus</i>	1
<i>Lasioglossum calceatum</i>	13
<i>Lasioglossum leucopus</i>	4
<i>Lasioglossum malachurum</i>	20
<i>Lasioglossum morio</i>	2
<i>Lasioglossum pauxillum</i>	1

<i>Lasioglossum punctatissimum</i>	1
<i>Lasioglossum smeathmanellum</i>	13
<i>Lasioglossum viollosulum</i>	5
<i>Megachile versicolor</i>	1
<i>Panurgus banksianus</i>	4
<i>Sphecodes pellucidus</i>	2
<i>Sphecodes puncticeps</i>	1
Species	Abundance

Hoverflies

<i>Anasimyia contracta</i>	1
<i>Baccha obscuripennis</i>	1
<i>Cheilosia pagana</i>	1
<i>Episyrphus balteatus</i>	8
<i>Eristalis arbustorum</i>	18
<i>Eristalis interruptus</i>	1
<i>Eristalis pertinax</i>	1
<i>Eristalis tenax</i>	7
<i>Eupodes corollae</i>	41
<i>Helophilus pendulus</i>	3
<i>Melanstoma mellinum</i>	4
<i>Melanstoma scalare</i>	1
<i>Merodon equestris</i>	8
<i>Neoascia podagrica</i>	3
<i>Platycheirus albimanus</i>	1
<i>Platycheirus clypeatus</i>	4
<i>Platycheirus granditarsus</i>	1
<i>Sphaerophoria scripta</i>	3
<i>Syritta pipiens</i>	4
<i>Syrphus ribesii</i>	2
<i>Volucella bombylans</i>	1

Table 15 Bees recorded on transects in the cropped area and margins of courgette fields in 2016. No species are of current conservation concern.

Species	Abundance
Honey bee	
<i>Apis mellifera</i>	369
Bumblebees	
<i>Bombus hortorum</i>	17
<i>Bombus hypnorum</i>	3
<i>Bombus lapidarius</i>	51
<i>Bombus pascuorum</i>	20
<i>Bombus pratorum</i>	2
<i>Bombus terrestris/ lucorum</i>	186

References

- Acciarri, N., Restaino, F., Vitelli, G., Perrone, D., Zottini, M., Pandolfini, T., Spena, A., Rotino, G., 2002. Genetically modified parthenocarpic eggplants: improved fruit productivity under both greenhouse and open field cultivation. *BMC Biotechnol.* 2, 1–7. <https://doi.org/10.1186/1472-6750-2-4>
- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M., 2008. Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency. *Curr. Biol.* 18, 1572–1575. <https://doi.org/10.1016/j.cub.2008.08.066>
- Ali, M., Saeed, S., Sajjad, A., Akbar, A., 2015. Linking Pollination Effectiveness and Interspecific Displacement Success in Bees. *Neotrop. Entomol.* 44, 101–108. <https://doi.org/10.1007/s13744-014-0259-0>
- Ali, M., Saeed, S., Sajjad, A., Bashir, M.A., 2014. Exploring the Best Native Pollinators for Pumpkin (*Cucurbita pepo*) Production in Punjab, Pakistan. *Pak. J. Zool.* 46, 531–539.
- Allsopp, M.H., de Lange, W.J., Veldtman, R., 2008. Valuing Insect Pollination Services with Cost of Replacement. *PLoS One* 3, 1–8. <https://doi.org/10.1371/journal.pone.0003128>
- Artz, D., Nault, B., 2011. Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as pollinators of pumpkin. *J. Econ. Entomol.* 104, 1153–1161. <https://doi.org/10.1603/EC10431>
- Artz, D.R., Hsu, C.L., Nault, B.A., 2011. Influence of honey bee, *Apis mellifera*, hives and field size on foraging activity of native bee species in pumpkin fields. *Environ. Entomol.* 40, 1144–1158. <https://doi.org/10.1603/EN10218>
- Avila-Sakar, G., Krupnick, G., Stephenson, A., 2001. Growth and resource allocation in *Cucurbita pepo* ssp. *texana*: effects of fruit removal. *Int. J. Plant Sci.* 162, 1089–1095.
- Baggett, J., Kean, D., Mansour, N.S., 1997. “Siletz” Parthenocarpic Tomato. *HortScience* 32, 1299–1300.
- Bailes, E.J., Ollerton, J., Patrick, J.G., Glover, B.J., 2015. How can an understanding of plant–pollinator interactions contribute to global food security? *Curr. Opin. Plant Biol.* 26, 72–79. <https://doi.org/10.1016/j.pbi.2015.06.002>
- Balakbir, A., Ruiz, J., Romero, L., 1998. Yield and Fruit Quality of Pepper (*Capsicum annum* L.) in Response to Bioregulators. *HortScience* 33, 85–87.
- Barg, R., Salts, Y., 2000. Method for the induction of genetic parthenocarpy in plants. 6114602.
- Barton, K., 2017. MuMIn: Multi-Model Inference. R package version 1.40.0. <https://CRAN.R-project.org/package=MuMIn>.
- Batáry, P., Báldi, A., Kleijn, D., Tscharntke, T., 2011. Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. *Proc. R. Soc. B* 278, 1894–1902. <https://doi.org/10.1098/rspb.2010.1923>
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models using lme4. *J. Stat. Softw.* 67. <https://doi.org/10.18637/jss.v067.i01>
- Bates, D.M., Robinson, R.W., Jeffrey, C., 1990. *Biology and Utilization of the Cucurbitaceae*. Comstock Publishing Associates, Ithaca and London.
- Baude, M., Kunin, W.E., Boatman, N.D., Conyers, S., Davies, N., Gillespie, M.A., Morton, R.D., Smart, S.M., Memmott, J., 2016. Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature* 530, 85–88. <https://doi.org/10.1038/nature16532>
- Becher, Matthias A. Twiston-Davies, G., Penny, T., Goulson, D., Rotheray, E., Osborne, J., 2018. Bumble-BEEHAVE: a systems model for exploring multifactorial causes of bumblebee decline at individual, colony, population and community level. *J. Appl. Ecol.* <https://doi.org/10.1111/1365-2664.13165>
- Becher, M.A., Grimm, V., Knapp, J., Horn, J., Twiston-Davies, G., Osborne, J.L., 2016. BEE SCOUT: A model of bee scouting behaviour and a software tool for characterizing nectar/pollen landscapes for BEEHAVE. *Ecol. Modell.* 340, 126–133. <https://doi.org/10.1016/j.ecolmodel.2016.09.013>

- Becher, M.A., Grimm, V., Thorbek, P., Horn, J., Kennedy, P.J., Osborne, J.L., 2014. BEEHAVE: A systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. *J. Appl. Ecol.* 51, 470–482. <https://doi.org/10.1111/1365-2664.12222>
- Beduschi, T., Kormann, U.G., Tschardt, T., Scherber, C., 2018. Spatial community turnover of pollinators is relaxed by semi-natural habitats, but not by mass-flowering crops in agricultural landscapes. *Biol. Conserv.* 221, 59–66. <https://doi.org/10.1016/j.biocon.2018.01.016>
- Bisaria, A., 1977. Effect of a morphactin on growth, sex expression, fruit-set, and yield in *Luffa acutangula*. *Can. J. Bot.* 55, 752–756.
- Blaauw, B.R., Isaacs, R., 2014. Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *J. Appl. Ecol.* 51, 890–898. <https://doi.org/10.1111/1365-2664.12257>
- Bommarco, R., Kleijn, D., Potts, S.G., 2013. Ecological intensification: Harnessing ecosystem services for food security. *Trends Ecol. Evol.* 28, 230–238. <https://doi.org/10.1016/j.tree.2012.10.012>
- 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. *Proc. Natl. Acad. Sci. U. S. A.* 110, 8387–92. <https://doi.org/10.1073/pnas.1210590110>
- Bos, M.M., Veddeler, D., Bogdanski, A.K., Klein, A.M., Tschardt, T., Steffan-Dewenter, I., Tylianakis, J.M., 2007. Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination. *Ecol. Appl.* 17, 1841–1849. <https://doi.org/10.1890/06-1763.1>
- Boualem, A., Troadec, C., Camps, C., Lemhemdi, A., Morin, H., Sari, M.A., Fraenkel-Zagouri, R., Kovalski, I., Dogimont, C., Perl-Treves, R., Bendahmane, A., 2015. A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. *Science* (80-). 350, 688–691. <https://doi.org/10.1126/science.aac8370>
- Boyaci, H.F., Oguz, A., Yazici, K.M., Eren, A., 2011. The efficacy of endogenous gibberellic acid for parthenocarp in eggplant (*Solanum melongena* L.). *J. Biotechnol.* 10, 6522–6528. <https://doi.org/10.5897/AJB11.443>
- Breeze, T.D., Bailey, a. P., Balcombe, K.G., Potts, S.G., 2011. Pollination services in the UK: How important are honeybees? *Agric. Ecosyst. Environ.* 142, 137–143. <https://doi.org/10.1016/j.agee.2011.03.020>
- Bretagnolle, V., Gaba, S., 2015. Weeds for bees? A review. *Agron. Sustain. Dev.* 35, 891–909. <https://doi.org/10.1007/s13593-015-0302-5>
- Brown, M.J.F., Dicks, L. V., Paxton, R.J., Baldock, K.C.R., Barron, A.B., Chauzat, M.-P., Freitas, B.M., Goulson, D., Jepsen, S., Kremen, C., Li, J., Neumann, P., Pattermore, D.E., Potts, S.G., Schweiger, O., Seymour, C.L., Stout, J.C., 2016. A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ* 4, e2249. <https://doi.org/10.7717/peerj.2249>
- Brown, M.J.F., Paxton, R.J., 2009. The conservation of bees: a global perspective. *Apidologie* 40, 410–416. <https://doi.org/10.1051/apido/2009019>
- Bullock, J.M., Dhanjal-Adams, K.L., Milne, A., Oliver, T.H., Todman, L.C., Whitmore, A.P., Pywell, R.F., 2017. Resilience and food security: rethinking an ecological concept. *J. Ecol.* 105, 880–884. <https://doi.org/10.1111/1365-2745.12791>
- Canto-aguilar, A., Veterinaria, M., 2000. Importance of conserving alternative pollinators: assessing the pollination efficiency of the squash bee, *Peponapis limitaris* in *Cucurbita moschata* (Cucurbitaceae). *J. Insect Conserv.* 4, 203–210. <https://doi.org/10.1023/A:1009685422587>
- Carmi, N., Salts, Y., Dedicova, B., Shabtai, S., Barg, R., 2003. Induction of parthenocarp in tomato via specific expression of the rolB gene in the ovary. *Planta* 217, 726–735. <https://doi.org/10.1007/s00425-003-1052-1>
- Carrera, E., Ruiz-Rivero, O., Peres, L.E.P., Atares, A., Garcia-Martinez, J.L., 2012. Characterization of the procerca Tomato Mutant Shows Novel Functions of the SIDELLA

- Protein in the Control of Flower Morphology, Cell Division and Expansion, and the Auxin-Signaling Pathway during Fruit-Set and Development. *Plant Physiol.* 160, 1581–1596. <https://doi.org/10.1104/pp.112.204552>
- Carrizo, C., 2011. Fruit characteristics, seed production and pollen tube growth in the wild chilli pepper *Capsicum flexuosum*. *Flora Morphol. Distrib. Funct. Ecol. Plants* 206, 334–340. <https://doi.org/10.1016/j.flora.2010.05.008>
- Carvalho, L.G., Kunin, W.E., Keil, P., Aguirre-Gutiérrez, J., Ellis, W.N., Fox, R., Groom, Q., Hennekens, S., Van Landuyt, W., Maes, D., Van de Meutter, F., Michez, D., Rasmont, P., Ode, B., Potts, S.G., Reemer, M., Roberts, S.P.M., Schaminée, J., Wallisdevries, M.F., Biesmeijer, J.C., 2013. Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. *Ecol. Lett.* 16, 870–878. <https://doi.org/10.1111/ele.12121>
- Carvell, C., Meek, W.R., Pywell, R.F., Goulson, D., Nowakowski, M., 2007. Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. *J. Appl. Ecol.* 44, 29–40. <https://doi.org/10.1111/j.1365-2664.2006.01249.x>
- Carvell, C., Osborne, J.L., Bourke, A.F.G., Freeman, S.N., Pywell, R.F., Heard, M.S., 2015. Bumble bee species' responses to a targeted conservation measure depend on landscape context and habitat quality. *Ecol. Appl.* 21, 1760–1771.
- Centre for Ecology and Hydrology, 2016. CEH Land Cover plus: Crops | Centre for Ecology & Hydrology [WWW Document]. URL <https://www.ceh.ac.uk/crops2015> (accessed 8.14.17).
- Centre for Ecology and Hydrology, 2011. Land Cover Map 2007 | Centre for Ecology and Hydrology [WWW Document]. URL <https://www.ceh.ac.uk/services/land-cover-map-2007> (accessed 8.14.17).
- Charbeonboonsit, S., Splittstoesser, W., George, W., 1985. The effects of pollination methods and auxin applications upon parthenocarpic fruit set and development in tomato. *Sci. Hortic. (Amsterdam)*. 27, 1–8.
- Connelly, H., Poveda, K., Loeb, G., 2015. Landscape simplification decreases wild bee pollination services to strawberry. *Agric. Ecosyst. Environ.* 211, 51–56. <https://doi.org/10.1016/j.agee.2015.05.004>
- Corbet, S., 2003. Nectar sugar content: estimating standing crop and nectar secretion in the field. *Apidologie* 34, 1–10. <https://doi.org/10.1051/apido>
- Costa, J., Catala, S., Botella, F., Nuez, F., Cuartero, J., 1992. Freda - a New Tomato Parthenocarpic Hybrid. *Hortscience* 27, 185–186.
- Crawley, M., 2012. *The R Book*, 2nd Editio. ed. John Wiley and Sons Ltd., Chichester.
- Crone, E.E., Williams, N.M., 2016. Bumble bee colony dynamics: Quantifying the importance of land use and floral resources for colony growth and queen production. *Ecol. Lett.* 19, 460–468. <https://doi.org/10.1111/ele.12581>
- Darvill, B., Knight, M.E., Goulson, D., 2004. Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos* 107, 471–478. <https://doi.org/10.1111/j.0030-1299.2004.13510.x>
- De Vries, F., Rabbinge, R., Groot, J., 1997. Potential and attainable food production and food security in different regions. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* 352, 917–928.
- DEFRA, 2016. Weekly and historical monthly fruit and vegetable wholesale prices. Retrieved 11 October, 2016, from <https://www.gov.uk/government/collections/fruit-and-vegetable-wholesale-prices>.
- Devlin, R.M., Demoranville, I.E., 1967. Influence of Gibberellic Acid and Gibrel on Fruit Set and Yield in *Vaccinium macro-carpon* cv. Early Black. *Physiol. Plant.* 20, 587–592. <https://doi.org/10.1111/j.1399-3054.1967.tb07199.x>
- Dicks, L. V., Baude, M., Roberts, S.P.M., Phillips, J., Green, M., Carvell, C., 2015. How much flower-rich habitat is enough for wild pollinators? Answering a key policy question with incomplete knowledge. *Ecol. Entomol.* 40, 22–35. <https://doi.org/10.1111/een.12226>
- Donzella, G., Spena, A., Rotino, G.L., 2000. Transgenic parthenocarpic eggplants: Superior

- germplasm for increased winter production. *Mol. Breed.* 6, 79–86. <https://doi.org/10.1023/A:1009613529099>
- Dutta, A.K., Akhtar, S., Karak, C., Hazra, P., 2013. Gene actions for fruit yield and quality characters of tomato through generation mean analysis. *Indian J. Hortic.* 70, 230–237.
- Ercan, N., Akilli, M., 1996. Reasons for parthenocarpy and the effects of various hormone treatments on fruit set in pepino (*Solanum muricatum* Ait.). *Sci. Hortic. (Amsterdam)*. 66, 141–147.
- Ficcadenti, N., Sestili, S., Pandolfini, T., Cirillo, C., Leonardo, G., Spena, A., 1999. Genetic engineering of parthenocarpic fruit development in tomato. *Mol. Breed.* 5, 463–470.
- Fos, M., Proaño, K., Alabadí, D., Nuez, F., Carbonell, J., García-Martínez, J.L., 2003. Polyamine metabolism is altered in unpollinated parthenocarpic pat-2 tomato ovaries. *Plant Physiol.* 131, 359–366. <https://doi.org/10.1104/pp.013037>
- Free, J.B., 1993. *Insect pollination of crops*. Academic Press Inc, London.
- Fu, F.Q., Mao, W.H., Shi, K., Zhou, Y.H., Asami, T., Yu, J.Q., 2008. A role of brassinosteroids in early fruit development in cucumber. *J. Exp. Bot.* 59, 2299–2308. <https://doi.org/10.1093/jxb/ern093>
- Gallai, N., Salles, J.M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 68, 810–821. <https://doi.org/10.1016/j.ecolecon.2008.06.014>
- García-Hurtado, N., Carrera, E., Ruiz-Rivero, O., López-Gresa, M.P., Hedden, P., Gong, F., García-Martínez, J.L., 2012. The characterization of transgenic tomato overexpressing gibberellin 20 oxidase reveals induction of parthenocarpic fruit growth, higher yield, and alteration of the gibberellin biosynthetic pathway. *J. Exp. Bot.* 63, 695–709. <https://doi.org/10.1093/jxb/err313>
- García-Martínez, J., García-Papí, M., 1979. The Influence of Gibberellic acid, 2,4-D dichlorophenoxyacetic acid and 6-benzylaminopurine on fruit-set of clementine mandarin. *Sci. Hortic. (Amsterdam)*. 10, 285–293.
- Garibaldi, L.A., Carvalheiro, L.G., Leonhardt, S.D., Aizen, M.A., Blaauw, B.R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A.M., Kremen, C., Morandin, L., Scheper, J., Winfree, R., 2014. From research to action: practices to enhance crop yield through wild pollinators. *Front. Ecol. Environ.* 12, 439–447. <https://doi.org/10.1890/130330>
- Garibaldi, L.A., Carvalheiro, L.G., Vaissière, B.E., Gemmill-herren, B., Hipólito, J., Freitas, B.M., Ngo, H.T., Azzu, N., Sáez, A., Åström, J., An, J., Blochtein, B., 2016. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science* 351, 388–391. <https://doi.org/10.1126/science.aac7287>
- Garibaldi, L.A., Steffan-Dewenter, I., Kremen, C., Morales, J.M., Bommarco, R., Cunningham, S.A., Carvalheiro, L.G., Chacoff, N.P., Dudenhöffer, J.H., Greenleaf, S.S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L.A., Potts, S.G., Ricketts, T.H., Szentgyörgyi, H., Viana, B.F., Westphal, C., Winfree, R., Klein, A.M., 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol. Lett.* 14, 1062–1072. <https://doi.org/10.1111/j.1461-0248.2011.01669.x>
- Garratt, M., Truslove, C.L., Coston, D.J., Evans, R.L., Moss, E.D., Dodson, C., Jenner, N., Biesmeijer, J.C., Potts, S., 2013. Pollination deficits in UK apple orchards. *J. Pollinat. Ecol.* 12, 9–14.
- Garratt, M.P.D., Breeze, T.D., Jenner, N., Polce, C., Biesmeijer, J.C., Potts, S.G., 2013. Avoiding a bad apple: insect pollination enhances fruit quality and economic value. *Agric. Ecosyst. Environ.* 184, 34–40. <https://doi.org/10.1016/j.agee.2013.10.032>
- Garratt, M.P.D., Senapathi, D., Coston, D.J., Mortimer, S.R., Potts, S.G., 2017. The benefits of hedgerows for pollinators and natural enemies depends on hedge quality and landscape context. *Agric. Ecosyst. Environ.* 247, 363–370. <https://doi.org/10.1016/j.agee.2017.06.048>
- Gathmann, A., Tschardt, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71, 757–764.
- Gemici, M., Türkyılmaz, B., Tan, K., 2006. Effects of 2,4-D and 4-CPA on yield and quality of

- the tomato, *Lycopersicon esculentum* Miller. J. Food Sci. 29, 24–32.
- Ghazoul, J., 2005. Buzziness as usual? Questioning the global pollination crisis. Trends Ecol. Evol. 20, 367–373. <https://doi.org/10.1016/j.tree.2005.04.026>
- Gillaspy, G., Ben-David, H., Gruissem, W., 1993. Fruits: A Developmental Perspective. Plant Cell 5, 1439–1451. <https://doi.org/10.1105/tpc.5.10.1439>
- Gingras, D., Gingras, J., DeOliveira, D., 1999. Visits of honeybees (Hymenoptera : Apidae) and their effects on cucumber yields in the field. J. Econ. Entomol. 92, 435–438.
- Goetz, M., Hooper, L.C., Johnson, S.D., Rodrigues, J.C.M., Vivian-Smith, A., Koltunow, a. M., 2007. Expression of Aberrant Forms of AUXIN RESPONSE FACTOR8 Stimulates Parthenocarpy in Arabidopsis and Tomato. Plant Physiol. 145, 351–366. <https://doi.org/10.1104/pp.107.104174>
- Gorguet, B., Van Heusden, A.W., Lindhout, P., 2005. Parthenocarpic fruit development in tomato. Plant Biol. 7, 131–139. <https://doi.org/10.1055/s-2005-837494>
- Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Scienceexpress 347, 1–16. <https://doi.org/10.1126/science.1255957>
- Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia 153, 589–596. <https://doi.org/10.1007/s00442-007-0752-9>
- Guardiola, J.L., Barrés, M.T., Albert, C., García-Luis, A., 1993. Effects of Exogenous Growth Regulators on Fruit Development in Citrus unshiu. Ann. Bot. <https://doi.org/10.1006/anbo.1993.1021>
- Gustafson, F.G., 1936. Inducement of Fruit Development by Growth-Promoting Chemicals. Proc. Natl. Acad. Sci. U. S. A. 22, 628–636. <https://doi.org/10.1073/pnas.22.11.628>
- Habashy, a. a., Testa, G., Mosconi, P., Caccia, R., Mazzucato, A., Santange-Lo, E., Soressi, G.P., 2004. Parthenocarpy restores fruitfulness in sterile triploid (3x) tomatoes artificially obtained by crossing 4x x 2x somaclones. J. Hortic. Sci. Biotechnol. 79, 322–328. <https://doi.org/10.1080/14620316.2004.11511768>
- Hanley, N., Breeze, T.D., Ellis, C., Goulson, D., 2014. Measuring the economic value of pollination services: Principles, evidence and knowledge gaps. Ecosyst. Serv. 1–9. <https://doi.org/10.1016/j.ecoser.2014.09.013>
- Hardman, C.J., Norris, K., Nevard, T.D., Hughes, B., Potts, S.G., 2016. Delivery of floral resources and pollination services on farmland under three different wildlife-friendly schemes. Agric. Ecosyst. Environ. 220, 142–151. <https://doi.org/10.1016/j.agee.2016.01.015>
- Häussler, J., Sahlin, U., Baey, C., Smith, H.G., Clough, Y., 2017. Pollinator population size and pollination ecosystem service responses to enhancing floral and nesting resources. Ecol. Evol. 7, 1898–1908. <https://doi.org/10.1002/ece3.2765>
- Hayata, Y., Niimi, Y., Inoue, K., Kondo, S., 2000. CPPU and BA, with and without pollination, affect set, growth, and quality of muskmelon fruit. HortScience 35, 868–870.
- Hayata, Y., Niimi, Y., Iwasaki, N., 1995. Synthetic Cytokinin-1-(2=chloro=4=pyridyl)-3-phenylurea (CPPU)-Promotes Fruit Set and Induces Parthenocarpy in Watermelon. J. Am. Soc. Hortic. Sci. 120, 997–1000.
- Heard, M.S., Carvell, C., Carreck, N.L., Rothery, P., Osborne, J.L., Bourke, A.F.G., 2007. Landscape context not patch size determines bumble-bee density on flower mixtures sown for agri-environment schemes. Biol. Lett. 3, 638–641. <https://doi.org/10.1098/rsbl.2007.0425>
- Herbertsson, L., Jönsson, A.M., Andersson, G.K.S., Seibel, K., Rundlöf, M., Ekroos, J., Stjernman, M., Olsson, O., Smith, H.G., 2018. The impact of sown flower strips on plant reproductive success in Southern Sweden varies with landscape context. Agric. Ecosyst. Environ. 259, 127–134. <https://doi.org/10.1016/j.agee.2018.03.006>
- Herbertsson, L., Lindstrom, S.A.M., Rundlof, M., Bommarco, R., Smith, H.G., 2016. Competition between managed honeybees and wild bumblebees depends on landscape context. Basic Appl. Ecol. 17, 609–616. <https://doi.org/10.1016/j.baae.2016.05.001>
- Herrmann, F., Westphal, C., Moritz, R.F.A., Steffan-Dewenter, I., 2007. Genetic diversity and

- mass resources promote colony size and forager densities of a social bee (*Bombus pascuorum*) in agricultural landscapes. *Mol. Ecol.* 16, 1167–1178. <https://doi.org/10.1111/j.1365-294X.2007.03226.x>
- Heuvelink, E., Korner, O., 2001. Parthenocarpic fruit growth reduces yield fluctuation and blossom-end rot in sweet pepper. *Ann. Bot.* 88, 69–74. <https://doi.org/10.1006/anbo.2001.1427>
- Hikosaka, S., Sugiyama, N., 2015. Effects of Exogenous Plant Growth Regulators on Yield, Fruit Growth, and Concentration of Endogenous Hormones in Gynoecious Parthenocarpic Cucumber (*Cucumis sativus* L.). *Hortic. J.* 84, 342–349. <https://doi.org/10.2503/hortj.MI-051>
- Hoehn, P., Tscharrntke, T., Tylianakis, J.M., Steffan-Dewenter, I., 2008. Functional group diversity of bee pollinators increases crop yield. *Proc. Biol. Sci.* 275, 2283–2291. <https://doi.org/10.1098/rspb.2008.0405>
- Holzschuh, A., Dainese, M., Gonzalez-Varo, J., Mudri-Stojnic, S., Riedinger, V., Rundlof, M., Scheper, J., Wickens, J., Wickens, V., Bommarco, R., Kleijn, D., Potts, S., Roberts, S., Smith, H.G., Montserrat, V., Vujic, A., Steffan-Dewenter, I., 2016. Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecol. Lett.* <https://doi.org/10.1111/ele.12657>
- Holzschuh, A., Dormann, C.F., Tscharrntke, T., Steffan-Dewenter, I., 2013. Mass-flowering crops enhance wild bee abundance. *Oecologia* 172, 477–484. <https://doi.org/10.1007/s00442-012-2515-5>
- Honda, I., Matsunaga, H., Kikuchi, K., Matsuo, S., Fukuda, M., 2012. Identification of pepper (*Capsicum annuum* L.) accessions with large or small fruit that have a high degree of parthenocarpy. *Sci. Hortic. (Amsterdam)*. 135, 68–70. <https://doi.org/10.1016/j.scienta.2011.12.014>
- Hooper, D.U., Chapin III, F.S., Ewel, J.J., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3–35. <https://doi.org/10.1890/04-0922>
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical J.* 50, 346–363. <https://doi.org/10.1002/bimj.200810425>
- Hui, C., 2006. Carrying capacity, population equilibrium, and environment's maximal load. *Ecol. Modell.* 192, 317–320. <https://doi.org/10.1016/j.ecolmodel.2005.07.001>
- Huitrón, M.V., Diaz, M., Diáñez, F., Camacho, F., Valverde, A., 2007. Effect of 2,4-D and CPPU on triploid watermelon production and quality. *HortScience* 42, 559–564.
- Hurd, P.D., Linsley, E.G., Michelbacher, A.D., 1974. Ecology of the squash and gourd bee, *Peponapis pruinosa*, on cultivated cucurbits in California (Hymenoptera: Apoidea). *Smithson. Contrib. to Zool.* 1–17. <https://doi.org/10.5479/si.00810282.168>
- Ingrosso, I., Bonsegna, S., De Domenico, S., Laddomada, B., Blando, F., Santino, A., Giovanazzo, G., 2011. Over-expression of a grape stilbene synthase gene in tomato induces parthenocarpy and causes abnormal pollen development. *Plant Physiol. Biochem.* 49, 1092–9. <https://doi.org/10.1016/j.plaphy.2011.07.012>
- Iwahori, S., Tominaga, S., Yamasaki, T., 1988. Stimulation of Fruit Growth of Kiwifruit, *Actinidia chinensis* Planch., by N-(2-chloro-4-pyridyl)-N'-phenylurea, a Diphenylurea-Derivative Cytokinin 35, 109–115. [https://doi.org/10.1016/0304-4238\(88\)90042-8](https://doi.org/10.1016/0304-4238(88)90042-8)
- Jauker, F., Peter, F., Wolters, V., Diekötter, T., 2012. Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outbalance post-flowering disadvantages. *Basic Appl. Ecol.* 13, 268–276. <https://doi.org/10.1016/j.baae.2012.03.010>
- Julier, H.E., Roulston, T.H., 2009. Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects. *J. Econ. Entomol.* 102, 563–573. <https://doi.org/10.1603/029.102.0214>
- Junttila, O., Martinussen, I., Ernstsén, A., Nilsen, G., Bhuvaneshwari, T. V., 2002. Parthenocarpic fruit development in cloudberry (*Rubus chamaemorus* L.) is induced by 3B-hydroxylated gibberellins. *J. Hortic. Sci. Biotechnol.* 77, 9–12. <https://doi.org/10.1080/14620316.2002.11511448>
- Karapanos, I.C., Alexopoulos, A.A., Akoumianakis, K.A., Grigoriou, F., Miliordos, D.,

- Rigakis, K., Skandalou, I., Passam, H.C., To, 2013. Application of β -naphthoxyacetic acid (β -NOA) improves fruit yield and marketable quality in out- of-season cherry tomatoes (*Solanum lycopersicum* L. var. *cerasiforme* (Dunal) D. M. Spooner, G. J. Anderson and R. K. Jansen) cultivated in unheated gr. J. Hortic. Sci. Biotechnol. ISSN 88, 165–172. <https://doi.org/10.1080/14620316.2013.11512952>
- Kennedy, C.M., Lonsdorf, E., Neel, M.C., Williams, N.M., Ricketts, T.H., Winfree, R., Bommarco, R., Brittain, C., Burley, A.L., Cariveau, D., Carvalho, L.G., Chacoff, N.P., Cunningham, S.A., Danforth, B.N., Dudenhöffer, J.H., Elle, E., Gaines, H.R., Garibaldi, L.A., Gratton, C., Holzschuh, A., Isaacs, R., Javorek, S.K., Jha, S., Klein, A.M., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L., Neame, L.A., Otieno, M., Park, M., Potts, S.G., Rundlöf, M., Saez, A., Steffan-Dewenter, I., Taki, H., Viana, B.F., Westphal, C., Wilson, J.K., Greenleaf, S.S., Kremen, C., 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecol. Lett. 16, 584–599. <https://doi.org/10.1111/ele.12082>
- Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., Gall, L.F., 2015. Climate change impacts on bumblebees converge across continents. Science (80-). 349, 177–180.
- Kikuchi, K., Honda, I., Matsuo, S., Fukuda, M., Saito, T., 2008. Stability of fruit set of newly selected parthenocarpic eggplant lines. Sci. Hortic. (Amsterdam). 115, 111–116. <https://doi.org/10.1016/j.scienta.2007.08.001>
- Kim, I.S., Okubo, H., Fujieda, K., 1992. Endogenous levels of IAA in relation to parthenocarpy in cucumber (*Cucumis sativus* L.). Sci. Hortic. (Amsterdam). 52, 1–8. [https://doi.org/10.1016/0304-4238\(92\)90002-T](https://doi.org/10.1016/0304-4238(92)90002-T)
- Kleijn, D., Winfree, R., Bartomeus, I., Carvalho, L.G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'Gonigle, L.K., Rader, R., Ricketts, T.H., Williams, N.M., Adamson, N.L., Ascher, J.S., Báldi, A., Batáry, P., Benjamin, F., Biesmeijer, J.C., Blitzer, E.J., Bommarco, R., Brand, M.R., Bretagnolle, V., Button, L., Cariveau, D.P., Chifflet, R., Colville, J.F., Danforth, B.N., Elle, E., Garratt, M.P.D., Herzog, F., Holzschuh, A., Howlett, B.G., Jauker, F., Jha, S., Knop, E., Krewenka, K.M., Le Féon, V., Mandelik, Y., May, E.A., Park, M.G., Pisanty, G., Reemer, M., Riedinger, V., Rollin, O., Rundlöf, M., Sardiñas, H.S., Scheper, J., Sciligo, A.R., Smith, H.G., Steffan-Dewenter, I., Thorp, R., Tscharnkte, T., Verhulst, J., Viana, B.F., Vaissière, B.E., Veldtman, R., Westphal, C., Potts, S.G., 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 7414. <https://doi.org/10.1038/ncomms8414>
- Klein, A.-M., Hendrix, S.D., Clough, Y., Scofield, A., Kremen, C., 2014. Interacting effects of pollination, water and nutrients on fruit tree performance. Plant Biol. 17, 1–8. <https://doi.org/10.1111/plb.12180>
- Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharnkte, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B 274, 303–313. <https://doi.org/10.1098/rspb.2006.3721>
- Kleinhenz, M.D., Bumgarner, N.R., 2013. Using °Brix as an Indicator of Vegetable Quality [WWW Document]. Ohio State Univ. Ext. URL <http://ohioline.osu.edu/factsheet/HYG-1651>
- Knapp, J.L., Bartlett, L.J., Osborne, J.L., 2017. Re-evaluating strategies for pollinator-dependent crops: How useful is parthenocarpy? J. Appl. Ecol. 54. <https://doi.org/10.1111/1365-2664.12813>
- Knapp, J.L., Osborne, J.L., 2017. Courgette Production: Pollination Demand, Supply, and Value. J. Econ. Entomol. 110, 1973–1979. <https://doi.org/10.1093/jee/tox184>
- Knapp, J.L., Shaw, R., Osborne, J.L., 2018. Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation. Basic Appl. Ecol. - Under Rev.
- Knight, M.E., Martin, A.P., Bishop, S., Osborne, J.L., Hale, R.J., Sanderson, R.A., Goulson, D., 2005. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol. Ecol. 14, 1811–1820. <https://doi.org/10.1111/j.1365->

294X.2005.02540.x

- Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of Meta-analysis in Ecology and Evolution. Princeton University Press.
- Kouonon, L.C., Jacquemart, A.L., Zoro Bi, A.I., Bertin, P., Baudoin, J.P., Dje, Y., 2009. Reproductive biology of the andromonoecious *Cucumis melo* subsp. *agrestis* (*Cucurbitaceae*). *Ann. Bot.* 104, 1129–1139. <https://doi.org/10.1093/aob/mcp196>
- Kremen, C., Miles, A., 2012. Ecosystem services in biologically diversified versus conventional farming systems: Benefits, externalities, and trade-offs. *Ecol. Soc.* 17. <https://doi.org/10.5751/ES-05035-170440>
- Kremen, C., Williams, N.M., Bugg, R.L., Fay, J.P., Thorp, R.W., 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecol. Lett.* 7, 1109–1119. <https://doi.org/10.1111/j.1461-0248.2004.00662.x>
- Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl. Acad. Sci. U. S. A.* 99, 16812–16816. <https://doi.org/10.1073/pnas.262413599>
- Kumar, R., 2016. Cucurbits: History, Nomenclature, Taxonomy, and Reproductive Growth, in: Pessaraki, M. (Ed.), Handbook of Cucurbits: Growth, Cultural Practices, and Physiology. pp. 3–22.
- Kurtar, E., 2003. An investigation of parthenocarpy in some summer squash (*Cucurbita pepo* L.) cultivars. *J. Agron.* 2, 209–213. <https://doi.org/10.3923/ja.2003.209.213>
- Kushnereva, V., 2008. Breeding of cucumber (*Cucumis sativus*) for resistance to multiple diseases and other traits, in: Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. Avignon (France), pp. 429–432.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016. Package ‘ImerTest.’
- Kwon, S.W., Jaskani, M.J., Ko, B.R., 2006. Evaluation of Soft X-Ray Irradiated Pollen and CPPU for Diploid Seedless Watermelon Production. *Acta Hort.* 289–294.
- Lafer, G., 2008. Effects of different bioregulator applications on fruit set, yield and fruit quality of “Williams” pears. *Acta Hort.* 183–187.
- Larsen, T.H., Williams, N.M., Kremen, C., 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecol. Lett.* 8, 538–547. <https://doi.org/10.1111/j.1461-0248.2005.00749.x>
- Lau Tak-Cheung, Stephenson, A., 1993. Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (*Cucurbitaceae*). *Am. J. Bot.* 80, 763–768. <https://doi.org/10.2307/2445596>
- Li, J., Wu, Z., Cui, L., Zhang, T., Guo, Q., Xu, J., Jia, L., Lou, Q., Huang, S., Li, Z., Chen, J., 2014. Transcriptome Comparison of Global Distinctive Features Between Pollination and Parthenocarpic Fruit Set Reveals Transcriptional Phytohormone Cross-Talk in Cucumber (*Cucumis sativus* L.). *Plant Cell Physiol.* 55, 1325–1342. <https://doi.org/10.1093/pcp/pcu051>
- Lora, J., Hormaza, J.I., Herrero, M., Gasser, C.S., 2011. Seedless fruits and the disruption of a conserved genetic pathway in angiosperm ovule development. *Proc. Natl. Acad. Sci. U. S. A.* 108, 5461–5465. <https://doi.org/10.1073/pnas.1014514108>
- Lundin, O., Ward, K.L., Artz, D.R., Boyle, N.K., Pitts-Singer, T.L., Williams, N.M., 2017. Wildflower plantings do not compete with neighboring almond orchards for pollinator visits. *Environ. Entomol.* 46, 559–564. <https://doi.org/10.1093/ee/nvx052>
- Mader, E., Spivak, M., Evans, E., 2010. Managing Alternative Pollinators; A Handbook for Beekeepers, Growers and Conservationists. SARE and NRAES.
- Maroto, J.V., Lopez-Galarza, S., Pascual, B., Bono, M.S., San Bautista, A., Alagarda, J., 1997. Procarpil Enhances Earliness and Parthenocarpy of Pepino (*Solanum muricatum* Ait.). *HortScience* 32, 133.
- Maroto, J. V., Miguel, A., Lopez-Galarza, S., San Bautista, A., Pascual, B., Alagarda, J., Guardiola, J.L., 2005. Parthenocarpic fruit set in triploid watermelon induced by CPPU and 2,4-D applications. *Plant Growth Regul.* 45, 209–213. <https://doi.org/10.1007/s10725-005-3992-x>
- Marti, C., Orzaez, D., Ellul, P., Moreno, V., Carbonell, J., Granell, A., 2007. Silencing of DELLA

- induces facultative parthenocarpy in tomato fruits. *Plant J.* 52, 865–876. <https://doi.org/10.1111/j.1365-313X.2007.03282.x>
- Martin, A.P., Carreck, N.L., Swain, J.L., Goulson, D., Knight, M.E., Hale, R.J., Sanderson, R.A., Osborne, J.L., 2006. A modular system for trapping and mass-marking bumblebees: applications for studying food choice and foraging range. *Apidologie* 37, 341–350. <https://doi.org/10.1051/apido>
- Martínez, C., Manzano, S., Megías, Z., Garrido, D., Picó, B., Jamilena, M., 2014. Sources of parthenocarpy for zucchini breeding: relationship with ethylene production and sensitivity. *Euphytica* 200, 349–362. <https://doi.org/10.1007/s10681-014-1155-8>
- Martínez, C., Manzano, S., Megías, Z., Garrido, D., Picó, B., Jamilena, M., 2013. Involvement of ethylene biosynthesis and signalling in fruit set and early fruit development in zucchini squash (*Cucurbita pepo* L.). *BMC Plant Biol.* 13, 1–14. <https://doi.org/10.1186/1471-2229-13-139>
- Mazzucato, a, Taddei, a R., Soressi, G.P., 1998. The parthenocarpic fruit (pat) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seedless fruits and has aberrant anther and ovule development. *Development* 125, 107–114.
- McCreight, J., Staub, J., Wehner, T., Dhillon, N., 2013. Gone Global: Familiar and Exotic Cucurbits Have Asian Origins. *HortScience* 48, 1078–1089.
- Medina, M., Roque, E., Pineda, B., Cañas, L., Rodríguez-Concepción, M., Beltrán, J.P., Gómez-Mena, C., 2013. Early anther ablation triggers parthenocarpic fruit development in tomato. *Plant Biotechnol. J.* 11, 770–9. <https://doi.org/10.1111/pbi.12069>
- Melathopoulos, A.P., Cutler, G.C., Tyedmers, P., 2015. Where is the value in valuing pollination ecosystem services to agriculture? *Ecol. Econ.* 109, 59–70. <https://doi.org/10.1016/j.ecolecon.2014.11.007>
- Mesejo, C., Reig, C., Martínez-Fuentes, A., Agustí, M., 2010. Parthenocarpic fruit production in loquat (*Eriobotrya japonica* Lindl.) by using gibberellic acid. *Sci. Hortic. (Amsterdam)*. 126, 37–41. <https://doi.org/10.1016/j.scienta.2010.06.009>
- Mesejo, C., Yuste, R., Martínez-Fuentes, A., Reig, C., Iglesias, D.J., Primo-Millo, E., Agustí, M., 2013. Self-pollination and parthenocarpic ability in developing ovaries of self-incompatible Clementine mandarins (*Citrus clementina*). *Physiol. Plant.* 148, 87–96. <https://doi.org/10.1111/j.1399-3054.2012.01697.x>
- Metcalf, R.L., Rhodes, A.M., Metcalf, R.A., Ferguson, J., Metcalf, E.R., Lu, P., 1982. Cucurbitacin Contents and Diabroticite (*Coleoptera Chrysomelidae*) feeding upon *Cucurbita* spp. *Entomol. Soc. Am.* 11, 931–937. <https://doi.org/10.1093/ee/11.4.931>
- Mezzetti, B., Landi, L., Pandolfini, T., Spena, A., 2004. The defH9-iaaM auxin-synthesizing gene increases plant fecundity and fruit production in strawberry and raspberry. *BMC Biotechnol.* 4, 4. <https://doi.org/10.1186/1472-6750-4-4>
- Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M., Karron, J.D., 2009. New frontiers in competition for pollination. *Ann. Bot.* 103, 1403–1413. <https://doi.org/10.1093/aob/mcp062>
- Mohamed, M.F., 1998. Characteristics and inheritance of natural facultative-parthenocarpic fruit-set in “Nadja” tomato under low temperature conditions. *Euphytica* 103, 211–217.
- Molesini, B., Pandolfini, T., Rotino, G.L., Dani, V., Spena, A., 2009. Aucsia Gene Silencing Causes Parthenocarpic Fruit Development in Tomato. *Plant Physiol.* 149, 534–548. <https://doi.org/10.1104/pp.108.131367>
- Montero-Castaño, A., Ortiz-Sánchez, F.J., Vilà, M., 2016. Mass flowering crops in a patchy agricultural landscape can reduce bee abundance in adjacent shrublands. *Agric. Ecosyst. Environ.* 223, 22–30. <https://doi.org/10.1016/j.agee.2016.02.019>
- Morandin, L.A., Winston, M.L., 2006. Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agric. Ecosyst. Environ.* 116, 289–292. <https://doi.org/10.1016/j.agee.2006.02.012>
- Morandin, L.A., Winston, M.L., 2005. Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecol. Appl.* 15, 871–881. <https://doi.org/10.1890/03-5271>
- Mori, T., Umeda, T., Honda, T., Zushi, K., Wajima, T., Matsuzoe, N., 2013. Varietal differences

- in the chlorogenic acid, anthocyanin, soluble sugar, organic acid, and amino acid concentrations of eggplant fruit. *J. Hortic. Sci. Biotechnol.* 88, 657–663. <https://doi.org/10.1080/14620316.2013.11513021>
- Motzke, I., Tschardtke, T., T., W., Klein, A., 2015. Pollination mitigates cucumber yield gaps more than pesticide and fertilizer use in tropical smallholder gardens. *J. Appl. Ecol.* 52, 261–269. <https://doi.org/10.1111/1365-2664.12357>
- Murray, T.E., Fitzpatrick, Ú., Brown, M.J.F., Paxton, R.J., 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. *Conserv. Genet.* 9, 653–666. <https://doi.org/10.1007/s10592-007-9394-z>
- Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Nandwani, D., Dennerly, S., Balkaran, S., 2014. Effect of 4-CPA on Fruit Set and Yield of Beefsteak Tomato (*Solanum lycopersicum* L.) on the Island of St. Croix. *Acta Hortic.* 255–260.
- Ne'eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S.G., Dafni, A., 2010. A framework for comparing pollinator performance: Effectiveness and efficiency. *Biol. Rev.* 85, 435–451. <https://doi.org/10.1111/j.1469-185X.2009.00108.x>
- Nepi, Massimo and Pacini, E., 1993. Pollination, Pollen Viability and Pistil Receptivity in *Cucurbita pepo*. *Ann. Bot.* 72, 527–536.
- Nerson, H., 2007. Seed production and germinability of cucurbit crops. *Seed Sci. Biotechnol.* 1, 1–10.
- Nicholls, C.I., Altieri, M.A., 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. *Agron. Sustain. Dev.* 33, 257–274. <https://doi.org/10.1007/s13593-012-0092-y>
- Nicodemo, D., Malheiros, E.B., Jong, D. De, Couto, R.H.N., 2013. Enhanced production of parthenocarpic cucumbers pollinated with stingless bees and Africanized honey bees in greenhouses. *Semin. Agrar.* 34, 3625–3634. <https://doi.org/10.5433/1679-0359.2013v34n6Supl1p3625>
- Nishitani, C., Yamaguchi-Nakamura, A., Hosaka, F., Terakami, S., Shimizu, T., Yano, K., Itai, A., Saito, T., Yamamoto, T., 2012. Parthenocarpic genetic resources and gene expression related to parthenocarpy among four species in pear (*Pyrus* spp.). *Sci. Hortic. (Amsterdam)*. 136, 101–109. <https://doi.org/10.1016/j.scienta.2011.12.029>
- Niu, Q., Wang, T., Li, J., 2015. Effects of exogenous application of GA4+7 and N-(2-chloro-4-pyridyl)-N'-phenylurea on induced parthenocarpy and fruit quality in *Pyrus pyrifolia* 'Cuiguan.' *Plant Growth Regul.* 251–258. <https://doi.org/10.1007/s10725-014-9995-8>
- Nogueira, D.W., Maluf, W.R., Dos Reis Figueira, A., Maciel, G.M., Gomes, L.A.A., Benavente, C.A.T., 2011. Combining ability of summer-squash lines with different degrees of parthenocarpy and PRSV-W resistance. *Genet. Mol. Biol.* 34, 616–23. <https://doi.org/10.1590/S1415-47572011005000039>
- Ogata, T., Tamura, H., Hamada, K., Hasegawa, K., 2010. Effect of Gibberelin on Setting and Growth of Non-Pollinated Parthenocarpic Fruit in Mango 597–604.
- Olsson, O., Bolin, A., Smith, H.G., Lonsdorf, E. V., 2015. Modeling pollinating bee visitation rates in heterogeneous landscapes from foraging theory. *Ecol. Modell.* 316, 133–143. <https://doi.org/10.1016/j.ecolmodel.2015.08.009>
- Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, a D., Reynolds, R., Edwards, a S., 1999. A landscape-scale of bumble bee foraging study range and constancy using harmonic radar. *J. Appl. Ecol.* 36, 519–533.
- Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., Sanderson, R. a., 2008a. Bumblebee flight distances in relation to the forage landscape. *J. Anim. Ecol.* 77, 406–415. <https://doi.org/10.1111/j.1365-2656.2007.01333.x>
- Osborne, J.L., Martin, A.P., Shortall, C.R., Todd, A.D., Goulson, D., Knight, M.E., Hale, R.J., Sanderson, R. a., 2008b. Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. *J. Appl. Ecol.* 45, 784–792. <https://doi.org/10.1111/j.1365-2664.2007.01359.x>

- Pandolfini, T., 2009. Seedless fruit production by hormonal regulation of fruit set. *Nutrients* 1, 168–177. <https://doi.org/10.3390/nu1020168>
- Pandolfini, T., Molesini, B., Spena, A., 2009. Parthenocarpy in Crop Plants, in: *Annual Plant Reviews*. pp. 326–345.
- Pandolfini, T., Rotino, G.L., Camerini, S., Defez, R., Spena, A., 2002. Optimisation of transgene action at the post-transcriptional level: high quality parthenocarpic fruits in industrial tomatoes. *BMC Biotechnol.* 2. <https://doi.org/10.1186/1472-6750-2-1>
- Pelletier, L., McNeil, J.N., 2003. The effect of food supplementation on reproductive success in bumblebee field colonies. *Oikos* 103, 688–694. <https://doi.org/10.1034/j.1600-0706.2003.12592.x>
- Petersen, Jessica, D., Nault, B., 2014. Landscape diversity moderates the effects of bee visitation frequency to flowers on crop production. *J. Appl. Ecol.* 1347–1356. <https://doi.org/10.1111/1365-2664.12287>
- Petersen, J.D., Huseth, A.S., Nault, B.A., 2014. Evaluating pollination deficits in pumpkin production in New York. *Plant-Insect Interact.* 1247–1253.
- Petersen, J.D., Reiners, S., Nault, B., 2013. Pollination services provided by bees in pumpkin fields supplemented with either *Apis mellifera* or *Bombus impatiens* or not supplemented. *PLoS One* 8, 1–8. <https://doi.org/10.1371/journal.pone.0069819>
- Phillips, B.W., Gardiner, M.M., 2015. Use of video surveillance to measure the influences of habitat management and landscape composition on pollinator visitation and pollen deposition in pumpkin (*Cucurbita pepo*) agroecosystems. *PeerJ* 3, e1342. <https://doi.org/10.7717/peerj.1342>
- Pisanty, G., Afik, O., Wajnberg, E., Mandelik, Y., 2015. Watermelon pollinators exhibit complementarity in both visitation rate and single-visit pollination efficiency. *J. Appl. Ecol.* 360–370. <https://doi.org/10.1111/1365-2664.12574>
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: Trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353. <https://doi.org/10.1016/j.tree.2010.01.007>
- Power, A.G., 2010. Ecosystem services and agriculture: tradeoffs and synergies. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2959–71. <https://doi.org/10.1098/rstb.2010.0143>
- Pretty, J., Bharucha, Z.P., 2014. Sustainable intensification in agricultural systems. *Ann. Bot.* 114, 1571–1596. <https://doi.org/10.1093/aob/mcu205>
- Pufal, G., Steffan-dewenter, I., Klein, A., 2017. Crop pollination services at the landscape scale. *Curr. Opin. Insect Sci.* 91–97. <https://doi.org/10.1016/j.cois.2017.05.021>
- R Core Team, 2017. R: A language and environment for statistical computing.
- Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., Bommarco, R., Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M., Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog, F., Hipólito, J., Jaggard, S., Jauker, F., Klein, A.-M., Kleijn, D., Krishnan, S., Lemos, C.Q., Lindström, S.A.M., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilsson, L., Pattemore, D.E., de O. Pereira, N., Pisanty, G., Potts, S.G., Reemer, M., Rundlöf, M., Sheffield, C.S., Scheper, J., Schüepp, C., Smith, H.G., Stanley, D.A., Stout, J.C., Szentgyörgyi, H., Taki, H., Vergara, C.H., Viana, B.F., Woyciechowski, M., 2016. Non-bee insects are important contributors to global crop pollination. *Proc. Natl. Acad. Sci.* 113, 146–151. <https://doi.org/10.1073/pnas.1517092112>
- Ramin, a a, 2003. Effects of auxin application on fruit formation in tomato growing under stress temperatures in the field. *J. Hortic. Sci. Biotechnol.* 78, 706–710. <https://doi.org/10.1080/14620316.2003.11511687>
- Riedinger, V., Renner, M., Rundlöf, M., Steffan-Dewenter, I., Holzschuh, A., 2014. Early mass-flowering crops mitigate pollinator dilution in late-flowering crops. *Landsc. Ecol.* 29, 425–435. <https://doi.org/10.1007/s10980-013-9973-y>
- Rimberia, F.K., Adaniya, S., Ishimine, Y., Etoh, T., 2007. Morphology of papaya plants derived via anther culture. *Sci. Hortic. (Amsterdam)*. 111, 213–219. <https://doi.org/10.1016/j.scienta.2006.10.015>

- Risser, G., 1976. Induction of parthenocarpy in *Cucumis melo* by "PH 30.13." *Sci. Hortic.* (Amsterdam). 5, 73–76.
- Robinson, R.W., 2000. Rationale and Methods for Producing Hybrid Cucurbit Seed. *J. New Seeds* 1, 49–67. <https://doi.org/10.1300/J153v01n03>
- Robinson, R.W., Reiners, S., 1999. Parthenocarpy in summer squash. *HortScience* 34, 715–717.
- Rodriguez-Granados, N.Y., Lemhemdi, A., Choucha, F.A., Latrasse, D., Benhamed, M., Adnane, B., Bendahmane, A., 2017. Sex Determination in *Cucumis*, in: Grumet R., Katzir N., G.-M.J. (Ed.), *Genetics and Genomics of Cucurbitaceae*. Springer, Cham.
- Roldán-Serrano, a. S., Guerra-Sanz, J.M., 2005. Reward attractions of zucchini flowers (*Cucurbita pepo* L.) to bumblebees (*Bombus terrestris* L.). *Eur. J. Hortic. Sci.* 70, 23–28.
- Rotheray, E.L., Osborne, J.L., Goulson, D., 2017. Quantifying the food requirements and effects of food stress on bumble bee colony development. *J. Apic. Res.* 56, 288–299. <https://doi.org/10.1080/00218839.2017.1307712>
- Rotino, G.L., Acciarri, N., Sabatini, E., Mennella, G., Lo Scalzo, R., Maestrelli, A., Molesini, B., Pandolfini, T., Scalzo, J., Mezzetti, B., Spena, A., 2005. Open field trial of genetically modified parthenocarpic tomato: seedlessness and fruit quality. *BMC Biotechnol.* 5. <https://doi.org/10.1186/1472-6750-5-32>
- Rotino, G.L., Perri, E., Zottini, M., Sommer, H., Spena, A., 1997. Genetic engineering of parthenocarpic plants. *Nat. Biotechnol.* 15, 1398–1401. <https://doi.org/10.1038/nbt1297-1398>
- Roulston, T.H., Goodell, K., 2011. The role of resources and risks in regulating wild bee populations. *Annu. Rev. Entomol.* 56, 293–312. <https://doi.org/10.1146/annurev-ento-120709-144802>
- Rounis, V., Skarmoutsos, K., Tsaniklidis, G., Nikoloudakis, N., Delis, C., Karapanos, I., Aivalakis, G., 2015. Seeded and Parthenocarpic Cherry Tomato Fruits Exhibit Similar Sucrose, Glucose, and Fructose Levels, Despite Dissimilarities in UGPase and SPS Gene Expression and Enzyme Activity. *J. Plant Growth Regul.* 34, 47–56. <https://doi.org/10.1007/s00344-014-9441-1>
- Rundlöf, M., Persson, A.S., Smith, H.G., Bommarco, R., 2014. Late-season mass-flowering red clover increases bumble bee queen and male densities. *Biol. Conserv.* 172, 138–145. <https://doi.org/10.1016/j.biocon.2014.02.027>
- Saito, T., Yoshida, T., Monma, S., Matsunaga, H., Sato, T., Saito, A., Yamada, T., 2009. Development of the Parthenocarpic Eggplant Cultivar "Anominori." *Japan Agric. Res. Q.* 43, 123–127.
- Saturni, F.T., Jaffé, R., Metzger, J.P., 2016. Landscape structure influences bee community and coffee pollination at different spatial scales. *Agric. Ecosyst. Environ.* 235, 1–12. <https://doi.org/10.1016/j.agee.2016.10.008>
- Sawyer, R., 1981. *Pollen identification for beekeepers*. University of Cardiff Press, Cardiff.
- Scheper, J., Holzschuh, A., Kuussaari, M., Potts, S.G., Rundlöf, M., Smith, H.G., Kleijn, D., Letters, E., 2013. Environmental factors driving the effectiveness of European agri-environmental measures in mitigating pollinator loss - a meta-analysis. *Ecol. Lett.* 16, 912–920. <https://doi.org/10.1111/ele.12128>
- Schijlen, E.G.W.M., de Vos, C.H.R., Martens, S., Jonker, H.H., Rosin, F.M., Molthoff, J.W., Tikunov, Y.M., Angenent, G.C., van Tunen, A.J., Bovy, A.G., 2007. RNA Interference Silencing of Chalcone Synthase, the First Step in the Flavonoid Biosynthesis Pathway, Leads to Parthenocarpic Tomato Fruits. *Plant Physiol.* 144, 1520–1530. <https://doi.org/10.1104/pp.107.100305>
- Schulp, C.J.E., Lautenbach, S., Verburg, P.H., 2014. Quantifying and mapping ecosystem services: demand and supply of pollination in the European Union. *Ecol. Indic.* 36, 131–141. <https://doi.org/10.1016/j.ecolind.2013.07.014>
- Sedgley, M., Newbury, H.J., Possingham, J. V., 1977. Early fruit development in the watermelon: anatomical comparison of pollinated, auxin-induced parthenocarpic and unpollinated fruits. *Ann. Bot.* 41, 1345–1355.
- Serrani, J.C., Ruiz-Rivero, O., Fos, M., García-Martínez, J.L., 2008. Auxin-induced fruit-set in

- tomato is mediated in part by gibberellins. *Plant J.* 56, 922–934. <https://doi.org/10.1111/j.1365-313X.2008.03654.x>
- Shabtai, S., Salts, Y., Kaluzky, G., Barg, R., 2007. Improved yielding and reduced puffiness under extreme temperatures induced by fruit-specific expression of rolB in processing tomatoes. *Theor. Appl. Genet.* 114, 1203–1209. <https://doi.org/10.1007/s00122-007-0511-7>
- Shaw, N., Cantliffe, D., Stofella, P., 2007. A New Crop for North American Greenhouse Growers: Beit Alpha Cucumber - Progress of Production Technology through University Research Trials. *Acta Hortic.* 731, 251–258.
- Shin, Y.S., Park, S.D., Kim, J.H., 2007. Influence of pollination methods on fruit development and sugar contents of oriental melon (*Cucumis melo* L. cv. Sagyejeol-Ggul). *Sci. Hortic. (Amsterdam)*. 112, 388–392. <https://doi.org/10.1016/j.scienta.2007.01.025>
- Shuler, R.E., Roulston, T.H., Farris, G.E., 2005. Farming practices influence wild pollinator populations on squash and pumpkin. *J. Econ. Entomol.* 98, 790–795. <https://doi.org/10.1603/0022-0493-98.3.790>
- Stanghellini, M.S., Ambrose, J.T., Schultheis, J.R., 1998. Seed production in watermelon: A comparison between two commercially available pollinators. *HortScience*.
- Stanghellini, M.S., Schultheis, J.R., Ambrose, J.T., 2002. Pollen Mobilization in Selected *Cucurbitaceae* and the Putative Effects of Pollinator Abundance on Pollen Depletion Rates. *J. Am. Soc. Hortic. Sci.* 127, 729–736.
- Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., Tsch, 2002. Scale-dependant effects of landscape context on three pollinator guilds. *Ecology* 83, 1421–1432. [https://doi.org/10.1890/0012-9658\(2002\)083\[1421:SDEOLC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1421:SDEOLC]2.0.CO;2)
- Steffan-Dewenter, I., Potts, S.G., Packer, L., Ghazoul, J., 2005. Pollinator diversity and crop pollination services are at risk. *Trends Ecol. Evol.* 20, 651–653. <https://doi.org/10.1016/j.tree.2005.09.004>
- Stephenson, A.G., Devlin, B., Horton, J.B., 1988. The effects of seed number and prior fruit dominance on the pattern of fruit production in *Cucurbita pepo* (zucchini squash). *Ann. Bot.* 62, 653–661.
- Sun, Z., Lower, R.L., Staub, J.E., 2006. Variance component analysis of parthenocarpy in elite U.S. processing type cucumber (*Cucumis sativus* L.) lines. *Euphytica* 148, 331–339. <https://doi.org/10.1007/s10681-005-9041-z>
- 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. *Behav. Ecol. Sociobiol.* 65, 13–21. <https://doi.org/10.1007/s00265-010-1037-6>
- Telem, R.S., Wani, S.H., Singh, N.B., Nandini, R., Sadhukhan, R., Bhattacharya, S., Mandal, N., 2013. Cisgenics - a sustainable approach for crop improvement. *Curr. Genomics* 14, 468–76. <https://doi.org/10.2174/13892029113146660013>
- Tepedino, V., 1981. The Pollination Efficiency of the Squash Bee (*Peponapis pruinosa*) and the Honey Bee (*Apis mellifera*) on Summer Squash (*Cucurbita pepo*). *J. Kansas (Central States) Entomol. Soc.* 54, 359–377.
- Tester, M., Langridge, P., 2010. Breeding Technologies to Increase Crop Production in a Changing World. *Science (80-.)*. 327, 818–822. <https://doi.org/10.1126/science.1183700>
- Thanopoulos, C., Bouranis, D., Passam, H.C., 2013. Comparative development, maturation and ripening of seedless and seed-containing bell pepper fruits. *Sci. Hortic. (Amsterdam)*. 164, 573–577. <https://doi.org/10.1016/j.scienta.2013.10.010>
- Tilman, D., Cassman, K.G., Matson, P. a, Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418, 671–677. <https://doi.org/10.1038/nature01014>
- Tiwari, a., Dassen, H., Heuvelink, E., 2007. Selection of sweet pepper (*Capsicum annum* L.) genotypes for parthenocarpic fruit growth. *Acta Hortic.* 2007, 135–140. <https://doi.org/10.17660/ActaHortic.2007.761.16>
- Tiwari, A., Offringa, R., Heuvelink, E., 2012. Auxin-induced Fruit Set in *Capsicum annum* L. Requires Downstream Gibberellin Biosynthesis. *J. Plant Growth Regul.* 31, 570–578. <https://doi.org/10.1007/s00344-012-9267-7>

- Tripp, R., 1994. Biodiversity and Modern Crop Varieties : Sharpening the Debate. *Agric. Human Values* 13, 48–63. <https://doi.org/10.1007/BF01530523>
- Tscharntke, T., Tylianakis, J.M., Rand, T.A., Didham, R.K., Fahrig, L., Batáry, P., Bengtsson, J., Clough, Y., Crist, T.O., Dormann, C.F., Ewers, R.M., Fründ, J., Holt, R.D., Holzschuh, A., Klein, A.M., Kleijn, D., Kremen, C., Landis, D.A., Laurance, W., Lindenmayer, D., Scherber, C., Sodhi, N., Steffan-Dewenter, I., Thies, C., van der Putten, W.H., Westphal, C., 2012. Landscape moderation of biodiversity patterns and processes - eight hypotheses. *Biol. Rev.* 87, 661–685. <https://doi.org/10.1111/j.1469-185X.2011.00216.x>
- Vaissière, B.E., 2010. Protocol to Detect and Assess Pollination Deficits in Crops, FAO / IFAD Project: development of tools and methods for conservation and management of pollinator services for sustainable agriculture.
- Vaissière, B.E., Vinson, S.B., 1994. Pollen morphology and its effect on pollen collection by honey bees, *apis mellifera* L. (hymenoptera: Apidae), with special reference to upland cotton, *gossypium hirsutum* L. (malvaceae). *Grana* 33, 128–138. <https://doi.org/10.1080/00173139409428989>
- Vanbergen, A.J., 2013. Threats to an ecosystem service: Pressures on pollinators. *Front. Ecol. Environ.* 11, 251–259. <https://doi.org/10.1890/120126>
- Vardi, A., Levin, I., Carmi, N., 2008. Induction of Seedlessness in Citrus : From Classical Techniques to Emerging Biotechnological Approaches. *J. Am. Soc. Hortic. Sci.* 133, 117–126.
- Varoquaux, F., Blanvillain, R., Delseny, M., Gallois, P., 2000. Less is better: New approaches for seedless fruit production. *Trends Biotechnol.* 18, 233–242. [https://doi.org/10.1016/S0167-7799\(00\)01448-7](https://doi.org/10.1016/S0167-7799(00)01448-7)
- Vidal, M.D.G., Jong, D. De, Wien, H.C., Morse, R. a., 2010. Pollination and fruit set in pumpkin (*Cucurbita pepo*) by honey bees. *Rev. Bras. Botânica* 33, 106–113. <https://doi.org/10.1590/S0100-84042010000100010>
- Vidal, M.D.G., Jong, D. De, Wien, H.C., Morse, R. a., 2006. Nectar and pollen production in pumpkin (*Cucurbita pepo* L.). *Rev. Bras. Botânica* 29, 267–273. <https://doi.org/10.1590/S0100-84042006000200008>
- Viechtbauer, W., 2010. Conducting Meta-Analyses in R with the metafor Package. *J. Stat. Softw.* 36, 1–48. <https://doi.org/10.1103/PhysRevB.91.121108>
- von Frisch, K., 1967. The dance language and orientation of bees. Harvard University Press, Cambridge.
- Walters, A., Taylor, B., 2006. Effects of honey bee pollination on pumpkin fruit and seed yield. *HortScience* 41, 370–373.
- Walters, S.A., 2005. Honey bee pollination requirements for triploid watermelon. *HortScience* 40, 1268–1270.
- Walther-Hellwig, K., Frankl, R., 2000. Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. *J. Appl. Entomol.* 124, 299–306. <https://doi.org/10.1046/j.1439-0418.2000.00484.x>
- Watanabe, M., Segawa, H., Murakami, M., Sagawa, S., Komori, S., 2008. Effects of Plant Growth Regulators on Fruit Set and Fruit Shape of Parthenocarpic Apple Fruits. *J. Japanese Soc. Hortic. Sci.* 77, 350–357. <https://doi.org/10.2503/jjshs1.77.350>
- Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G., Roberts, S.P.M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B.E., Woyciechowski, M., Biesmeuer, J.C., Kunin, W.E., Settele, J., Steffan-Dewenter, I., 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecol. Monogr.* 78, 653–671. <https://doi.org/10.1890/07-1292.1>
- Westphal, C., Steffan-Dewenter, I., Tscharntke, T., 2009. Mass flowering oilseed rape improves early colony growth but not sexual reproduction of bumblebees. *J. Appl. Ecol.* 46, 187–193. <https://doi.org/10.1111/j.1365-2664.2008.01580.x>
- Westphal, C., Steffan-Dewenter, I., Tscharntke, T., 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecol. Lett.* 6, 961–965. <https://doi.org/10.1046/j.1461-0248.2003.00523.x>
- Wien, H.C., Zhang, Y., 1991. Prevention of Flower Abscission in Bell Pepper. *J. Am. Soc.*

- Hortic. Sci. 16, 516–519.
- Wilcock, C., Neiland, R., 2002. Pollination failure in plants: Why it happens and when it matters. *Trends Plant Sci.* 7, 270–277. [https://doi.org/10.1016/S1360-1385\(02\)02258-6](https://doi.org/10.1016/S1360-1385(02)02258-6)
- Willcox, B.K., Aizen, M.A., Cunningham, S.A., Mayfield, M.M., Rader, R., 2017. Deconstructing pollinator community effectiveness. *Curr. Opin. Insect Sci.* 21, 98–104. <https://doi.org/10.1016/j.cois.2017.05.012>
- Winfree, R., Aguilar, R., Vázquez, D.P., Lebuhn, G., Aizen, M. a., 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90, 2068–2076. <https://doi.org/10.1890/08-1245.1>
- Winfree, R., W. Fox, J., Williams, N.M., Reilly, J.R., Cariveau, D.P., 2015. Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecol. Lett.* 18, 626–635. <https://doi.org/10.1111/ele.12424>
- Winfree, R., Williams, N.M., Gaines, H., Ascher, J.S., Kremen, C., 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *J. Appl. Ecol.* 45, 793–802. <https://doi.org/10.1111/j.1365-2664.2007.01418.x>
- Wood, T.J., Holland, J.M., Goulson, D., 2015a. Pollinator-friendly management does not increase the diversity of farmland bees and wasps. *Biol. Conserv.* 187, 120–126. <https://doi.org/10.1016/j.biocon.2015.04.022>
- Wood, T.J., Holland, J.M., Hughes, W.O.H., Goulson, D., 2015b. Targeted agri-environment schemes significantly improve the population size of common farmland bumblebee species. *Mol. Ecol.* 24, 1668–1680. <https://doi.org/10.1111/mec.13144>
- Yan, L.Y., Lou, L.N., Li, X.L., Feng, Z.H., Lou, Q.F., Chen, J.F., 2012. Inheritance of parthenocarpy in cucumber under the same background. *Acta Hortic.* 935, 55–59.
- Yao, J., Dong, Y., Morris, B. a., 2001. Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1306–1311. <https://doi.org/10.1073/pnas.98.3.1306>
- Yarushnykov, V. V., Blanke, M.M., 2005. Alleviation of frost damage to pear flowers by application of gibberellin. *Plant Growth Regul.* 45, 21–27. <https://doi.org/10.1007/s10725-004-6893-5>
- Yin, Z., Malinowski, R., Ziółkowska, A., Sommer, H., Plcader, W., Malepszy, S., 2006. The DefH9-iaaM-containing construct efficiently induces parthenocarpy in cucumber. *Cell. Mol. Biol. Lett.* 11, 279–290. <https://doi.org/10.2478/s11658-006-0024-4>
- Yu, J., 1999. Parthenocarpy induced by N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) prevents flower abortion in Chinese white-flowered gourd (*Lagenaria leucantha*). *Environ. Exp. Bot.* 42, 121–128. [https://doi.org/10.1016/S0098-8472\(99\)00025-8](https://doi.org/10.1016/S0098-8472(99)00025-8)
- Zhang, C., Lee, U., Tanabe, K., 2008. Hormonal regulation of fruit set, parthenogenesis induction and fruit expansion in Japanese pear. *Plant Growth Regul.* 55, 231–240. <https://doi.org/10.1007/s10725-008-9279-2>
- Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. *Ecol. Econ.* 64, 253–260. <https://doi.org/10.1016/j.ecolecon.2007.02.024>