Understanding pollinator foraging behaviour and transition rates between flowers is important to maximize seed set in hybrid crops

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Abstract – Hybrid cauliflower production predominately relies on pollen transfer from hermaphrodite to female lines by honeybees. However, the presence of other pollinators may impact pollination success. Here, we investigate how honeybee visitation frequency and behaviour vary with plant sex and presence of blowflies and affect seed and pod set. We found substantial pollen limitation when honeybees were alone. This was likely due to their higher visitation to hermaphrodite flowers, infrequent transition from hermaphrodite to female flowers and high nectar theft in female flowers. Pollen foragers fed on nectar on hermaphrodite, but not female flowers. Moreover, when blowflies were present, the seed set was lower than that with honeybees alone. Our study highlights the importance of understanding the plant mating system and pollinator foraging behaviour with and without other species present in order to maximize seed set in hybrid crops.

blowfly / honeybee / pollination / nectar theft / pollen theft

1. INTRODUCTION

Pollen transfer in insect-pollinated angiosperms is generally highly inefficient and only a small percent of removed pollen reaches conspecific stigmas (Harder and Johnson 2008). This can limit seed production and have diverse ecological and evolutionary consequences for plants (Ashman et al. 2004). Pollen transfer by different species is commonly

approximated by their flower visitation rate, yet increasing visitation rate does not always equate to more successful pollination. For example, some visitors can be inefficient at transferring pollen; there can be competition between growing pollen tubes or single visits per flower may be sufficient (Mayfield et al. 2001; Garratt et al. 2014; Harder et al. 2016). Additionally, behaviour of the visitor (i.e. nectar and pollen theft) and the frequency at which individuals transfer pollen between male and female lines can have large consequences for plant reproduction (Malloof and Inouye 2000; Irwin et al. 2001; Hargreaves et al. 2009; Marzinzig et al. 2018), but is rarely investigated in combination with yield data in existing crop pollination studies.

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Bees, particularly honeybees (*Apis* sp.), are the most commonly managed pollinators, including for pollination of cauliflower (*Brassica oleracea* var. *botrytis* L., Verma and Partap 1994; Selvakumar et al. 2006; Abrol 2012; Singh et al. 2018). They feed on pollen for proteins and nectar for sugar needs (Thorp 2000). Honeybees collect pollen and pack it into their pollen baskets (corbiculae) moistened with nectar for later use to feed larvae in the colony (Thorp 2000). Exclusive pollen foraging in honeybees can result in pollen theft and reduce available pollen for dispersal to stigmas (Hargreaves et al. 2009, but see Cane and Schiffhauer 2001; Javorek et al. 2002). However, the actual pollination success depends on the fate of other pollen which passively accumulates on insect bodies and is inaccessible to bees by grooming (Hargreaves et al. 2009; Koch et al. 2017).

Several studies in hybrid seed crops have found that honeybees are not efficient pollinators (Abrol 2012; Gaffney et al. 2019; Robinson 2019). Failure to set seed in hybrid crops has often been attributed to the poor attractiveness of female plants to honeybees that are foraging for pollen since they have no pollen reward (Parker 1982). In addition to female flower attractiveness, reproductive success of the hybrid plants depends on the specific nectar foraging behaviour of insects in female flowers. Honeybees can act both as legitimate pollinators and nectar thieves across different wild and cultivated plants (Maloof and Inouye 2000). Nectar theft or “side working” is a type of nectar larceny where similar to the legitimate pollinators, insects enter through floral openings to feed on nectar, but without the benefit of pollen deposition (Inouye 1980; Irwin et al. 2010). Generally, nectar theft is considered negative for pollen-limited and self-incompatible plants (Irwin et al. 2001; Burkle et al. 2007). It can differ between plant sexes and it has been found to occur more often on male/hermaphrodite than on female plants in *Glechoma longituba* (Zhang et al. 2009) and hybrid seed canola (Robinson 2019). If similar behaviour occurs in crops where only female plants are harvested, such as hybrid cauliflower, nectar theft would likely not have large consequences for yield.

Non-bee pollinators have been observed visiting cruciferous plants, but their role in pollination of these crops is less clear (Sihag 1986). Blowflies (*Calliphoridae*) visit flowers for nectar and can inadvertently cause cross-pollination (Heath 1982). They are the second most common non-bee visitor of crop flowers worldwide (Rader et al. 2019). In some crops, such as onions, carrots and strawberries, blowflies have been shown to be efficient pollinators (Heath 1982). For example, seed yield of onions can be similar to or higher when pollinated by flies than honeybees (Currah and Ockendon 1983). In *Brassica* seed crops, blowflies can be preferred pollinators because they discriminate less between lines in comparison with honeybees (Faulkner 1978). Although the importance of blowflies versus honeybees has been investigated in several studies (Faulkner 1978; Currah and Ockendon 1983; Abrol 2012), the two pollinators have rarely been tested together.

Positive effects of other species on honeybee pollination have been reported in almonds (Brittain et al. 2013) and hybrid sunflowers (Greenleaf and Kremen 2006) where the pollination behaviour was altered and efficiency of honeybees increased in the presence of non-*Apis* species. For example, non-*Apis* bees caused honeybees to move more often between rows of hybrid sunflower (*Helianthus annuus* L.), increasing the number of seeds produced per visit (Greenleaf and Kremen 2006). Brosi and Briggs (2013) demonstrated that the removal of one pollinator species reduced floral fidelity of the remaining pollinators, resulting in fewer *Delphinium barbeyi* seeds on average. However, it remains unknown whether a similar positive effect exists between blowflies and honeybees, and whether the presence of both insects reduces pollen limitation of the crop seed production.

In this study, we use cauliflower as a model hybrid crop system. Cauliflower is an important crop accounting for 1,258,000 ha of production worldwide and is grown for use as both a fresh and pickled vegetable (Selvakumar 2003; Singh et al. 2018). Hybrids are found to have higher yield, larger curd size and better quality, greater disease resistance and uniform maturity (Selvakumar et al. 2006). Plants with

cytoplasmatic male sterility or self-incompatibility which rely on cross-pollination for reproduction are used for commercial seed production (Selvakumar 2003; Singh et al. 2018). Large-scale hybrid seed production is achieved using female parental lines that require pollination by insects that carry pollen from hermaphrodite flowers (Selvakumar 2003). Nectar foraging bees can carry equal or more cauliflower pollen grains on their bodies compared with pollen foragers, but this is not always the case and the opposite have also been shown in cauliflower and onion (see review by Partap 2011). Moreover, it remains unclear how efficiently this pollen is transferred to female cauliflower plants.

Here, we observed honeybee and blowfly visitation rates and honeybee behaviour to test their contribution to cauliflower pollination and seed/pod set. We asked the following research questions: (i) Is hybrid cauliflower seed production pollen limited when pollinated by managed honeybees? (ii) Does the addition of blowflies together with honeybees increase overall pollination success? (iii) Do honeybees and blowflies differ in visitation frequency to and in transitions between hermaphrodite and female flowers? (iv) To what extent does honeybee nectar foraging behaviour vary with plant sex and the presence of pollen in the pollen baskets? Additionally, we investigate if increased visitation number per flower increases seed set or probability of pod presence.

2. MATERIAL AND METHODS

2.1. Study design

The study was conducted in 2017, in six closed tunnels (6 × 60 m, Supplementary Figure 1). Within each tunnel, there were 8 rows of cauliflower plants grown for high-purity hybrid seed. Rows 1, 3 and 8 were plants with male fertile (hermaphrodite) flowers of one line and other rows were male sterile (female) plants of another line. Sampling was conducted in rows 4 and 5 as these bordered the walkway. There were three combinations of hermaphrodite and female lines across six tunnels (Supplementary Table 1). Plants were watered and given nutrients via a drip irrigation system. We used plants with cytoplasmatic

male sterility. In our study plants, hermaphrodite flowers produced pollen from fully developed anthers, whereas female flowers typically had underdeveloped anthers that did not produce pollen (Supplementary Figure 2). The bloom cycle of each line-sex combination was asynchronous, causing the size of the plants and the total flower units for hermaphrodite and female plants to be unequal across the tunnels. This is because a portion of the flowering stems of hermaphrodite plants were trimmed to manipulate flowering times and create a succession of flowering stages so that pollen was available at all times for the female plants. However, plants of similar sizes and flowering stages were used in observational and experimental testing.

Honeybees and blowflies were used as managed pollinators. Two commercial honeybee hives were placed in each tunnel at the onset of bloom in female plants. Each double hive contained 6–8 frames of brood and approximately 40–60,000 bees. Plastic trays containing blowfly (*Calliphora stygia*, Calliphoridae) pupae purchased from a bait and tackle company were added to each tunnel near the peak bloom of the female plants. Eight trays of approximately 1500 (12,000 total) pupae were added to tunnels 2 and 3. An estimated 90% of the pupae (10,800) emerged as healthy adults. Eight trays of approximately 750 (6000 total) pupae were added to tunnels 1, 4, 5 and 6 and an estimated 50% of these pupae (3000) emerged as healthy adults. The reduction in emergence was presumably due to heat stress during the transition from cool storage to the tunnels. Unusually warm spring conditions caused early flowering in some tunnels, prior to the availability and pupation of fly pupae, resulting in lower abundances of flies than bees in the tunnels. Additionally, wild, unknown species of flies were caught using raw meat, then reared and released in the tunnels. We recorded four morphospecies of blowflies and one hoverfly (*Eristalis* sp.) across tunnels. The total species richness or abundance of wild flies in each tunnel was unknown. Thus, we consider differences in abundance and species composition of flies across tunnels as underlying, random variability that we do not test for, but account for it in the random structure of the model (see below). As

experiments were conducted on commercially viable farms specifically to compare seed set and quality for growers, it was not possible to construct enclosures in order to simultaneously compare honeybee treatment with and without flies within each tunnel.

2.2. Pollination treatments

Two factors were used in a fully crossed design (all combinations, Supplementary Figure 3), resulting in four treatments per each experimental female plant: (1) hand cross-pollination (2 levels: present and absent) and (2) pollination exclusion (2 levels: open and closed). First, four unopened flower clusters were located on each plant and bagged to exclude pollinators. In the hand pollination treatments, two open male flowers were collected for pollination of each female flower. Male pollen donor flowers were randomly selected from the same tunnel. Using forceps, anthers were removed from the hermaphrodite flowers, placed into an Eppendorf tube and allowed to dehisce before application with a soft paintbrush onto stigmas of open female flowers. The flowers were re-bagged and the same process repeated every 3 days until approximately 10 flowers per cluster were hand pollinated. The treatment without open pollination was bagged until the stigma was no longer receptive (approximately 5 days), while the treatments with open pollination were left open for visits by honeybees or blowflies. Thus, only newly opened flowers were used for the experiment. The sampling was conducted between 16 Oct 2017 and 28 Nov 2017. The open pollination treatment was repeated twice: (1) with honeybees only (before 17 Nov 2017) and (2) with honeybees and blowflies (after 17 Nov 2017). This resulted in four “open” treatments in total (i.e. honeybees alone or both insects present with and without hand pollination). Tunnels 5 and 6 did not have the honeybee-only treatment. In each tunnel, 10–11 plants were sampled, except for tunnel 4 where 37 plants were sampled. The following measurements were recorded: (i) presence/absence of pods and (ii) number of seeds per pod.

2.3. Flower visitation

Visitation by honeybees was recorded on 6 male and 9 female transects (15 plants per transect) of similar flowering stages in the centre of one tunnel. Observations were made three times per day, at 10:30, 12:30 and 14:30 on different plants, for 1 week ending on 30 Oct 2017. Each plant was observed for 20 s and was approximately 30–45 cm in width and height. In November 2017, when female plants were roughly 60–72 cm in height, visitation of flowers by both honeybees and blowflies was observed in all tunnels. The observations were made using a point-transect method where each observation unit consisted of 30 × 30-cm quadrats and lasted for 1 min. The change in observation protocol was due to the plants growing into one another and creating a hedgerow effect. The quadrats were set every four metres from zero to 60 m, totaling 15 quadrats per row. Rows with both males and female plants were observed. Behaviour of the honeybees on the flower (legitimate nectar visits or theft) and presence/absence of pollen in the pollen baskets was recorded. Nectar theft was defined as an observation when a flower visitor foraged for nectar but did not contact the floral reproductive organs and did not cause any damage to flowers (Inouye 1980). If the visitor touched floral reproductive parts, it was considered a legitimate visitor. The observations were again repeated three times per day, at 10:30, 12:30 and 14:30, for four non-consecutive days. This was because nectar and pollen foraging by some bees is asynchronous and pollen foraging may be more frequent early in the morning (Verma and Partap 1994).

2.4. Transitions between plants and sexes

To investigate how often honeybees and blowflies transitioned between plants within the same row (same sex) and between rows (same or different sexes), we observed legitimate visits of individual insects for a maximum of 10 min. We recorded the insect species, presence/absence of pollen in the pollen baskets for honeybees, duration of the foraging trip, the number of transitions between plants within rows (same sex), the number of transitions from hermaphrodite to female

flowers and total number of transitions. Observations were conducted during 2 weeks from 13 Nov 2017 to 28 Nov 2017.

2.5. Controlled number of visits: impact on fruit and seed set

We tested the relationship between the number of honeybee visits (one vs. more visits) and seed/pod set of a female line (pod presence/absence and seed number) in one tunnel (tunnel 4). Unopened flower clusters throughout the tunnel were bagged to exclude pollinators. After at least two flowers had opened within a cluster, the bag was removed and the cluster of flowers was observed until one flower was visited by a honeybee. Following visitation, the flower was tagged and the cluster bagged again. For multiple visitations, the same steps were repeated, but the flowers were watched until two, three or four visitations. A visit was recorded only when a visitor touched the floral reproductive parts. The presence of pods and the number of seeds were recorded. The experiment lasted for 1 month starting on 23 Oct 2017 and the seeds were collected 8 to 11 Jan 2018.

2.6. Statistical analyses

We used Generalized Linear Mixed-Effects Models to analyse yield parameters (pod presence/absence and seed number), flower visitation and transitions between plants and rows. For the analysis of seed number and pollinator transitions, we used the Poisson distribution, for the pod presence/absence data, a Bernoulli distribution and for flower visitation rate, a zero-inflated negative binomial distribution (“*nbinom1*” function). For the analyses of seed/pod set from pollination trials, the random structure included flower cluster ID (1:6) nested in plant ID (1:11) and nested in a tunnel ID (1:6). The explanatory variable in these models was a factor with 6 levels: (i) hand pollination closed (HC), (ii) hand pollination open to honeybees (HH), (iii) hand pollination open to honeybees and blowflies (HHB), (iv) no hand pollination closed (C), (v) no hand pollination open to honeybees (H) and (vi) no hand pollination open to honeybees and blowflies (HB). We used general

linear hypotheses and Tukey’s all-pairs comparisons for post hoc pairwise comparisons between treatments. To link the number of visits per flower (one vs. more) to the seed and pod set from the experimental treatments where the number of visits per flower was controlled, we used a model with plant ID in the random structure.

The first model for the visitation rate when only honeybees were present included temporal period (1:3) in the random structure and plant sex as an explanatory variable. Next, we constructed two models to test visitation rates when both insects were present in the tunnels later in the season. The first model included both insects and the explanatory variables were the visitor species (i.e. blowflies or honeybees) in the interaction with plant sex (hermaphrodite or female). The second model included only honeybees and the explanatory variables were plant sex, the presence of pollen in the pollen basket (present or absent), behaviour (legitimate nectar foraging or theft) and their 2-way interactions. The random structure included a temporal period (1:3) crossed with a sampling quadrat ID (1:15) nested in the tunnel ID (1:6). Finally, models estimating the transition of insects between plants within rows and from hermaphrodite to female flowers included insect species as an explanatory variable, tunnel ID in the random structure and log-transformed duration of observation as an offset. Additionally, we tested how transitions of honeybees varied due to the presence or absence of pollen in the pollen baskets using the same model structure.

All models were inspected for over-/underdispersion, zero inflation and distribution of the residuals. Scaled residuals were simulated from the fitted model. Visual inspection of the residuals was then conducted by detecting deviation from uniformity (QQ plot) and by plotting the residuals against predicted values. For testing over-/underdispersion, a non-parametric dispersion test was performed. Finally, to test for zero inflation observed, the number of zeros was compared with the zeros expected from simulations. All analyses were conducted in R (version 3.5.1 2018), using packages “DHARMa” (Hartig 2018), “effects” (Fox 2003), “emmeans” (Lenth 2018), “lme4” (Bates et al. 2015) and “glmmTMB” (Brooks et al. 2017).

3. RESULTS

3.1. Pollination treatments

Here, we present estimates $\pm$ standard errors and p values for the multiple comparisons of means using Tukey contrasts (see also Supplementary Table 2). Cross-pollination treatments by hand always had more seeds than treatments without hand pollination (Figure 1). Seed number in the closed treatment without hand pollination (C) was zero in all but two pods with one seed each. Without hand cross-pollination, both open treatments (H and HB) had a higher seed number in comparison with the closed treatment (C) (H 6.026 ± 0.719 , $p < 0.001$; HB 2.629 ± 0.782 , $p = 0.008$) and the treatment with both insects present (HB) had lower seed number than the treatment with honeybees only (H) (-3.398 ± 0.337 , $p < 0.001$). Among treatments with hand cross-pollination, the treatment open to honeybees only (HH) had higher seed number than the closed treatment (HC) (0.572 ± 0.172 , $p = 0.008$), but there was no difference between the closed

treatment (HC) and the treatment open to both honeybees and blowflies (HHB).

The treatments without hand cross-pollination, either closed (C -0.925 ± 0.245 , $p = 0.001$) or open to honeybees and blowflies (HB -0.959 ± 0.349 , $p = 0.042$), had lower probabilities of pod presence in comparison with the hand-pollinated closed treatment (HC). There was a tendency for higher probability of pod presence in the treatment without hand cross-pollination open to honeybees (H) in comparison with the closed treatment without hand cross-pollination (C) (0.692 ± 0.270 , $p = 0.069$). There was no difference in the probability of pod presence among other treatments (Supplementary Figure 4).

3.2. Flower visitation rates and honeybee foraging behaviour

Visitation rate per transect (5 min) when only honeybees were present was 40.33 ± 6.87 (mean $\pm$ standard error). With both honeybees and flies present, visitation rate per quadrat (1 min) was 6.64 ± 0.15 (mean $\pm$ standard error).

Figure 1. Seed number per pod in relationship to treatments: (1) hand pollination (present—open circles, and absent—closed circles) and (2) pollination exclusion treatment (closed flowers, open to honeybees and open to both, honeybees and blowflies).

Hermaphrodite flowers were visited more than female flowers when honeybees were the only visitors (0.5852 ± 0.2050 , $p = 0.004$, Supplementary Figure 5) as well as when both insects were present (0.289 ± 0.035 , $p < 0.001$, Figure 2). Overall, the flower visitation rate was significantly lower for blowflies compared with honeybees (-1.646 ± 0.088 , $p < 0.001$). Legitimate visits to female flowers (touching stigma) were lower for honeybees with than without pollen on their corbicula (-3.156 ± 0.183 , $p < 0.001$; Figure 3a). In contrast, legitimate visits to hermaphrodite flowers were higher for honeybees with than without visible pollen in their baskets (2-way interaction 3.770 ± 0.187 , $p < 0.001$; Figure 3b). When honeybees were nectar foragers (pollen baskets had no visible pollen), hermaphrodite flowers had a slightly higher number of legitimate visits (0.175 ± 0.074 , $p = 0.018$) and a lower nectar theft (2-way interaction -1.207 ± 0.095 , $p < 0.001$) than female flowers. In female plants, nectar theft was higher than legitimate visits by nectar foragers (1.224 ± 0.065 , $p < 0.001$). Visitation of female flowers by pollen foragers (visible pollen on their corbicula) was very low and there was no difference between legitimate visits and nectar theft (2-way interaction -1.853 ± 0.110 ,

$p < 0.001$; Figure 3a). In the parentheses are presented model estimates at the link scale $\pm$ standard errors and p values.

3.3. Transitions between plants and sexes

We observed 78 foraging trips, lasting an average of 352 (± 24) seconds. In total, we observed 124 transitions between plants in the same row (sex) and 42 transitions between plants in different rows (same or different sex). Only 11.9% of all transitions between plant rows were from hermaphrodite to female plants, while the remaining transitions were between plants of the same sex (85.7%) or from female to hermaphrodite plants (2.4%). Transition rate between plant rows, independent of direction (same or different sex), or from hermaphrodite to female plants, did not differ between honeybees and blowflies. However, the transition rate between plants within the same row (the same sex) occurred more often by honeybees than blowflies (model estimate 0.462 ± 0.191 , $p = 0.016$). Honeybees carrying full pollen baskets had lower transition rate between plants within a row (model estimate -1.389 ± 0.361 , $p < 0.001$) and between rows (model estimate $-$

Figure 2. Visitation rate of hermaphrodite and female flowers by blowflies (open circles) and honeybees (filled circles). Visitation rate is the number of visits per quadrat per minute.

Figure 3. Honeybee visitation rate of **a** female and **b** hermaphrodite flowers in relationship to the bee behaviour (legitimate visit, i.e. touching plant reproductive parts—open circles, and nectar thieving—filled circles) and presence/absence of pollen in pollen baskets. Visitation rate is the number of visits per quadrat per minute.

1.599 ± 0.615 , $p = 0.009$) than those without pollen in their baskets.

0.638 ± 0.306 , $p = 0.037$; Figure 4b) with increased number visits per flower by honeybees.

3.4. Number of visits, fruit and seed set

We found increased seed number (model estimate 0.554 ± 0.279 , $p = 0.047$; Figure 4a) and probability of pod presence (model estimate

4. DISCUSSION

Identifying the main factors that ensure high hybrid crop pollination success requires an examination of different pollinator species and their

Figure 4. Number of legitimate honeybee visits related to **a** number of seeds per pod and **b** probability of pod presence.

foraging behaviour (Maloof and Inouye 2000; Irwin et al. 2001; Hargreaves et al. 2009; Marzinzig et al. 2018). Our results show that hybrid cauliflower seed production is pollen-limited when pollinated by honeybees (at stocking rates we used) and surprisingly, adding blowflies reduced seed set. Furthermore, we show that low seed set when pollinated by honeybees is likely due to their low transition from hermaphrodite to female flowers and high nectar theft in female flowers.

First, we asked if hybrid cauliflower seed production is pollen-limited when pollinated by managed honeybees and if so, does the addition of blowflies increase overall pollination success. We found that visitation by honeybees alone and visitation in the presence of both honeybees and flies both resulted in lower seed set in comparison with the hand pollination treatment. This shows strong pollen limitation with these pollination management strategies. Moreover, unlike previous studies that showed increased honeybee pollination efficiency in the presence of non-*Apis* species (Greenleaf and Kremen 2006; Brittain et al. 2013), adding blowflies together with honeybees demonstrated no benefit for cauliflower seed production in our study and, in fact, the seed set was reduced. As bees are known to react to perceived danger by avoiding flowers containing a predator, a dead bee or the scent of a dead bee (Dukas 2001; Abbott 2006), it is possible that bee decreased foraging in the presence of flies due to a perceived risk of death associated with a scent of dead/decaying flies and meat. However, the specific causes of this reduced seed set are unknown and additional research is needed where treatments with and without blowflies are tested simultaneously (e.g. within enclosure cages). Blowflies visited fewer flowers than honeybees, possibly due to their lower abundances, but how this affected seed set and whether it influenced honeybee behaviour remains to be investigated.

Second, to better understand the mechanisms behind low pollination success, we investigated pollinator visitation rate, transition between plant sexes and honeybee foraging behaviour. The low cauliflower seed production when pollinated by honeybees is likely due to their low transition rates from hermaphrodite to female lines,

particularly during pollen foraging trips. Pollen-collecting honeybees have been shown to act as pollen thieves because they rarely or never visited female flowers (Verma and Partap 1994; do Carmo and Franceschinelli 2004; Hargreaves et al. 2009; Waytes 2017; Robinson 2019). These infrequent transitions between plant sexes may be due to differences in the attractiveness of plant sexes, spatial organisation of plant sexes and/or constancy of honeybees on a particular line (Faulkner 1978). Previous studies that investigated honeybee attractiveness to cauliflower plant sexes only observed visitation frequency and show conflicting results. Selvakumar (2003) found no difference in visitation related to plant sex in self-incompatible cauliflower lines, yet Abrol (2012) showed that honeybees can be 3.5 times more common on female cauliflower lines, presumably because female lines produced larger amounts and more nectar sugar content. In contrast, we found that both insects visited more hermaphrodite than female lines. Similar was found in other hybrid crops, such as carrots (Gaffney et al. 2019), rapeseed mustard, sunflower and faba bean (Abrol 2012).

Limited transition from hermaphrodite to female flowers is unlikely to be related to exclusive pollen foraging in a single trip in our study. Although Verma and Partap (1994) found that honeybees forage exclusively on cauliflower pollen or nectar in one foraging trip, our results indicate that mixed pollen and nectar foraging is relatively common in a single trip, but only in hermaphrodite flowers (as opposed to foraging on pollen in hermaphrodite and transition to nectar in female flowers). These mixed foraging visits to hermaphrodite flowers are likely a more efficient way to obtain resources (Irwin et al. 2001), as foraging for nectar on hermaphrodite plants does not require additional search time for pollen foragers. Additionally, even though cauliflower sugar concentration can be lower in male flowers (Abrol 2012), pollen foragers can respond more strongly to lower sugar concentrations than nectar foragers (Page and Erber 2002). Thus, although beneficial for honeybees in terms of resource acquisition, switching from pollen to nectar foraging had no benefit for hybrid cauliflower seed production since it only occurred within hermaphrodite flowers.

Female flowers have experienced not only low visitation by pollen foragers in our study, but also high nectar theft, which may have further affected pollen limitation of seed production. Although the potential of nectar theft to strongly affect the reproductive success of male and female plants has been widely discussed (Maloof and Inouye 2000; Irwin et al. 2001, 2010), the differences in frequency of nectar theft between plant sexes have rarely been investigated. It is thus unclear which floral traits might be associated with higher rates of nectar theft in female than hermaphrodite flowers in our study, but it could indicate gender-biased nectar production and flower morphology (Carlson and Harms 2006; Abrol 2012). For example, some *Brassica* lines with cytoplasmic male fertility have petals of the corolla tubes that are not fused at the base only in female flowers, allowing side entry to the nectaries without contact to the stigmas (Supplementary Figure 2). These high rates of nectar theft in female flowers can have large consequences for cauliflower seed production because honeybees do not touch plant reproductive organs and therefore do not contribute to pollination even if they carry pollen.

In conclusion, we show that the success of cauliflower pollination by honeybees is likely to be related to honeybee behaviour. Furthermore, we show that adding another species does not necessarily increase pollination success and may in fact be detrimental due to changes in honeybee behaviour. Overall, increasing the efficiency of honeybees and flies as cauliflower pollinators requires a better understanding of the causes of high pollen and nectar theft, low transition rates between sexes and changes in behaviour and efficiency in the presence of other species. Although our study demonstrates relative differences in seed/pod set related to pollination treatments, applying these treatments at the whole plant level and additionally measuring seed weight would provide better links to the potential yield benefits (Adamidis et al. 2019; Robinson 2019). Finally, we show that seed set and probability of pod presence can increase with increased number of honeybee visits per flower, but to better understand this relationship, a greater sample sizes and gradients in visit number may be required.

Nevertheless, this indicates the potential benefit of increasing honeybee stocking rates if they are positively related to cauliflower visit number. Increased quantities of managed pollinators are also shown to reduce floral constancy in hybrid canola (Waytes 2017), but this remains to be investigated in cauliflowers. Future research of benefit to industry could explore appropriate ratios and spatial organisation of hermaphrodite to female lines and pollinator number/stocking rates and composition in order to optimize cauliflower pollination.

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AUTHOR CONTRIBUTIONS

RR, LKK and LK designed the study. LKK, JJ, LK and JK conducted the fieldwork. CS conducted the seed testing. VG analysed data and wrote the manuscript. All authors contributed to the final manuscript.

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Il est important de comprendre le comportement alimentaire des pollinisateurs et les taux de transition entre les fleurs pour maximiser la production de semences dans les cultures hybrides.

mouche à viande / abeille mellifère / pollinisation / vol de nectar / vol de pollen.

Das Verständnis des Sammelverhaltens von Bestäubern und die Übertragungsraten zwischen den Blüten ist wichtig, um den Samenansatz von Hybridkulturen zu maximieren.

Schmeißfliegen / Honigbienen / Bestäubung / Nektardiebstahl / Pollendiebstahl.

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