EFFECTS OF POLLINATION ON POD DISTRIBUTION IN FABA BEAN *(VICIA FABA* L.)

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Tiivistelmä — Referat — Abstract

Kiinnostus härkäpavun (*Vicia faba* L.) viljelyä kohtaan on kasvanut Suomessa muun muassa pavun korkean proteiinipitoisuuden ansiosta. Härkäpapu korvaa osan kotieläinten rehujen proteiinilisänä käytetystä tuontisoijasta. Kiinnostus härkäpapua kohtaan on myös kasvanut elintarvikemarkkinoilla kasviperäisenä lihan korvikkeena.

Härkäpapu on osittain itsepölytteinen, mutta hyönteispölytyksellä on havaittu olevan positiivinen vaikutus satoon ja sadon luotettavuuteen. Tarhamehiläinen (*Apis mellifera* L.) on yksi härkäpavun tärkeimmistä pölyttäjistä Suomessa.

Tämän maisterintutkielman kokeellisena tavoitteena oli tutkia tarhamehiläisen pölytysvaikutusta ja vuorovaikutusta härkäpavun kukissa. Pääasiallisena toteutuksena toimi häkkikoe, jonka avulla tutkittiin vaikutuksia palkojen lukumäärään ja sijoittumiseen pavun varressa ilman mehiläisiä ja mehiläisten ollessa läsnä kasvustossa. Toisena ja kolmantena tavoitteena oli havainnoida mehiläisten kukkakäyttäytymistä härkäpavun kukissa ja saada lisää tietoa pölyttäjien määrästä ja hyönteislajien monimuotoisuudesta härkäpapupelloilla. Kukkakäyttäytymistä ja pölyttäjien määrää tutkittiin linjalaskelmin kahdeksalta eri pellolta Suomen eteläosissa.

Pölytyksestä johtuva palkojen määrän kasvu oli 54 % verrattuna tyhjiin häkkeihin. Vastaavasti palkojen määrä lehtihankaa kohden kasvoi 19 %. Palot sijoittuivat kasvissa enemmän kasvin keskiosiin mehiläisten pölyttämissä kasveissa. Tarhamehiläisten määrä pelloilla korreloi vahvasti palkojen määrän kanssa lehtihankaa kohden. Tarhamehiläiset vierailivat keskimäärin 1,5 kukassa kasviyksilöä kohden ja kävivät kukissa kukan etuosan kautta 55 % kaikista kukkavierailukerroista. Pölyttäjien määrä ja lajimonimuotoisuus vaihteli huomattavasti eri havaintopeltojen kesken.

Tutkimuksen tulokset puoltavat tarhamehiläisen satoa lisäävää pölytysvaikutusta härkäpavulla ja osoittavat lähtökohtia aiheen jatkotutkimusta ajatellen. Taustatavoitteena tutkimukselle on lähtökohtien pohjustaminen mehiläistarhaajien ja härkäpavun viljelijöiden väliselle yhteistyölle, erityisesti kaupallisten pölytyspalveluiden näkökulmasta. Tulosten perusteella kaupallisten pölytyspalveluiden käyttöä härkäpapuviljelmillä voi suositella. Yhteistyökumppanina tutkimuksessa toimi Suomen mehiläishoitajainliitto (SML ry).

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Tiivistelmä — Referat — Abstract

The faba bean (*Vicia faba* L.) has increased its cultivation area in Finland because of its high protein content. It replaces some of the imported plant proteins, such as soybean (*Glycine max*) meal, as feed for livestock and has increasing interest as an ingredient in plant-based meat-substitute food products.

The faba bean is partly self-pollinating, but insect pollination has been shown to increase yield and reliability. Honeybees (*Apis mellifera*) are one of the most important pollinators for the bean in Finland.

The aims of the thesis were to study the effects of honeybee interactions with faba bean flowers and pollination. This was done mainly via a cage experiment about the pod count and distribution along the plant stem, with and without pollination from honeybees. The second and third aims were to find out more about the honeybee behaviour on the faba bean flowers and the abundance of pollinators and species richness on faba bean fields. For these parts a field survey was conducted on eight different faba bean fields in Southern Finland.

The increase in pod count following bee activity was found to be as high as 65% and the increase in pods per node 19%. The distribution of the pods was increased towards the middle of the plant in the plants that were insect pollinated, compared to the plants that were not. There was a strong correlation between the number of honeybees (*Apis mellifera*) on the fields and the pod counts per plant node. Honeybees visited 1.5 flowers per plant on average and frontal visits constituted 55% of all visits recorded. Pollinator numbers and species diversity varied greatly from field to field.

The results show that pollination by of honeybees benefits faba bean yield. They give a good understanding into the potential yield effects and provide pointers for future research on the topic. The study is aimed to prepare the background for introducing a better basis for cooperation between beekeepers and faba bean farmers. The main goal is to increase knowledge about honeybee pollination effects on the faba bean yield and give a solid start into building prospects for commercial pollination services in Finnish agriculture in general. The study was done in collaboration with The Finnish Beekeeping Association.

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faba bean, pollination, pod distribution, honeybee, floral behaviour

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LIST OF ABBREVIATIONS

EFN	extrafloral nectaries
s. str.	sensu stricto
sp.	species (unknown species of the taxon)
spp.	species (unknown species of the taxon, plural)

1 INTRODUCTION

The faba bean (*Vicia faba* L.) (also known as field bean, broad bean, fava bean, horse bean, bell bean or tic bean) is a plant in the pea and bean family *Fabaceae.* The faba bean is a high protein pulse used as food and feed for livestock. It has a long cultivation history in Finland and during recent years the total cultivation area in Finland has increased. The main reasons for this are the increasing demand of high quality plant based proteins and the properties of the bean in nitrogen fixation and soil health. Faba bean can replace some of the imported soybean (*Glycine max*) meal as feed for livestock and is used in novel food products as a plant-based meat substitute.

A number of factors affect the yield components of the faba bean. Environmental conditions, length of growing season, cultivar, sowing date and rate are all important factors and pollination effects of insects can add great value for the farmer in terms of yield amount and reliability in the next generation. The faba bean is partly self-pollinating, but cross-pollination via insect visits is greatly beneficial for pod development.

Studies by several authors have been made on the pollination mechanisms of the faba bean flower, the degree of cross pollination and about the effects on yield, but the northern location of Finland adds new possibilities for local research in pollination. The honeybee (*Apis mellifera*) is one of the most important pollinators for the bean in Finland. Other important pollinators include wild bumblebees (*Bombus* spp.) and solitary bees (e.g. *Xylocopa* spp.). However, the effects of pollination are very dependent on the local environment, climate and weather conditions. There has not been much research about the effects of pollination on the yield components in Finland.

The faba bean offers bees both pollen and nectar. Honeybees forage the protein-rich pollen for the brood and nectar for producing honey. The faba bean produces a great number of flowers, the flowering period is long and starts early. Thus, the plant supplies these commodities for the bees throughout the summer season, making it potentially a very important crop in terms of hive development and honey production.

This thesis is done in collaboration with the Finnish Beekeeping Association (SML ry).

2 FABA BEAN CULTIVATION AND CROP FORMATION

2.1 Morphology of the faba bean

The faba bean is a leafy, annual herb with thick, square stems. Most cultivars have indeterminate growth and can grow up to two meters tall, depending on the cultivar and growing conditions. The main stem can branch out from the base. The leaves are compound with ovate leaflets. Leaflet shape, size and number varies. Towards the end of the growing cycle, the stems may break and lodge.

There are two recognised subspecies: *paucijuga* and *faba*. The latter has three varieties that have developed under domestication of faba bean, small, medium and large in seed size: *Vicia faba* var. *minor*, *major* and *equina* (Smartt 1990, Adsule and Akpapunam 1996). A wild type has been recently discovered in Israel (Caracuta *et al.* 2016). Out of the three variants var. *minor* is likely to be closest to the wild progenitor.

Flowers form on axillary racemes and produce up to 15 flowers per node (fig. 1) (Knott 1990). The flowers are usually white, with dark markings on the wing petals, but purple or pink pigments can occur. The pods are usually straight and dark green, but turn dark brown or black when ripe.

Seeds per pod vary from two to eight. The seeds in var. *minor* are small, quite round or elliptical, with a thousand seed weight of <560 g. Small seeds are mostly used for feed and small size makes grain drying more efficient. Thousand seed weight of var. *equina* ranges from 560-100 and var. *major* is <1000 mg. (Knott 1990). The shape, size and colour of the seed varies depending on the cultivar. The seeds are high in protein approximately 30% (270- 320 g/kg in commercial varieties) from dry seed weight (Sjödin 1982, Crépon *et al.* 2010), with high protein digestibility (Multari *et al.* 2015).

Figure 1. Morphological features of *Vicia faba*, cultivar ‘Kontu’. (Illustration by the author)

Domestication of the faba bean started in the Neolithic era (Zohary and Hopf, 1973). The oldest seeds have been dated to late 10th millennium B.P. in Syria (Tanno and Willcox 2006). Cultivation in Europe started in the Mediterranean region but is now very widespread with many cultivars bred for cooler climates. The temperature optimum is between 10-30 °C. Faba bean can tolerate acidic soils but prefers neutral to slightly alkaline soils rich in loam or clay (Adsule and Akpapunam 1996).

China is the biggest producer of faba bean, responsible for almost half of the crop production worldwide. Other significant producers are Ethiopia, Egypt, Australia and France, along with some other European countries (FAO 2015). As faba bean is a good source of protein, it is commonly used as both food for humans and feed for livestock. Recently, possibilities in using faba bean and other pulses as meat substitutes in food products has sparked increasing interest (Multari *et al.* 2015, Kumar *et al.* 2017). Over the past decade, annual faba bean world production has stayed over 4 million tons (FAO 2015).

Additionally the faba bean benefits the soil with Rhizobium symbiosis that has nitrogen fixation properties. It is possible to get benefit from the whole plant, not just the beans. Top leaves of the plant can be used as food and the stems can be ploughed into the ground as soil improvement or crop waste can be used as fodder.

2.2 Cultivation of faba bean in Finland

The faba bean is one of the oldest cultivated plants in the world. It also has a long history in Finnish agriculture, and was first cultivated for human consumption (Stoddard *et al.*, 2009). The oldest findings of cultivation in the Nordic region are from Sweden and date to the Stone Age. In Finland, a sacrificial mound was found in Laitila under a burial ground, containing many grains, including faba beans. It was dated to the Vendel Period (600-800 AD) (Huurre 2003). The first known cultivars were grown in the 1700 century Southern Finland and in the 19th century in the Karelia region. The landraces were almost lost, but were collected for breeding in the 1960s (Stoddard *et al.* 2009). All cultivars bred in Finland belong in var. *minor*.

The faba bean has never been very extensively cultivated in Finland, partly because of its need for a relatively long growing season. Two events in Finnish agricultural history aided in the reintroduction of faba bean as a viable option amongst the grain legumes. The first event moving the faba bean cultivation forward was when Artturi Ilmari Virtanen patented the AIV fodder system in 1932. This system emphasised the importance of crop rotation, nitrogen fixation crops (e.g. clover) and silage preservation with mineral acid. This system made N fixation crops more interesting for the farmer and increased research on the subject. (Stoddard *et al.* 2009)

The second breakthrough was when the remaining landraces were collected from Karelia in the 1960s. In 1979 The Academy of Finland launched a biological nitrogen (N) fixation research programme, which lead to Hankkija's Plant Breeding Institute crossbreeding foreign cultivars with the landraces and research in nitrogen fixation symbionts as inoculants. Within 1984-1997 this breeding programme yielded three cultivars, 'Mikko', 'Ukko' and 'Kontu'. Finland joining the EU in 1995 made new cultivars more available for Finnish markets, but they have not became popular because of their lateness of maturity. (Stoddard *et al.* 2009)

The faba bean started to gain popularity in Finland after the 1990s as livestock feed. Lately interest has grown further because of the high protein content of the bean and useful properties in nitrogen fixation, soil-borne cereal disease control, improvement of farm viability, as well as positive effects on ecosystem diversification (Ghaouti and Link 2009, Köpke and Nemecek 2010). Furthermore, it has the potential to improve self-sufficiency in protein feed production in Finland by replacing imported soybean meal. Dependency on soybean for feed in the EU is substantial. Soybean derived products used in feeds constitute 64% of all protein feeds, but the self-sufficiency of soybean meal is merely 3% (de Visser *et al.* 2014). Total Finnish protein self-sufficiency is high (90% including grass fodder). However, supplemental protein imports are high and self-sufficiency in this category is only 15% (Kaukovirta-Norja *et al.* 2015).

Production in Finland was 34 million kg in 2017 and has quite steadily almost doubled during 2010-2016 (fig 2). In 2017 the production was 15 % lower than in 2016, because of the poor summer weather, but the total area was 35 % greater (fig. 3) (OSF 2018a). The most common cultivar in 2017 was overwhelmingly 'Kontu', accounting for over

89% of all faba bean seeds. Second most common cultivars were Ukko (3.3%) and Sampo (2.9%) (Table 1) (Mavi 2017). ‘Sampo’ is the newest Finnish cultivar and is slightly earlier than ‘Kontu’.

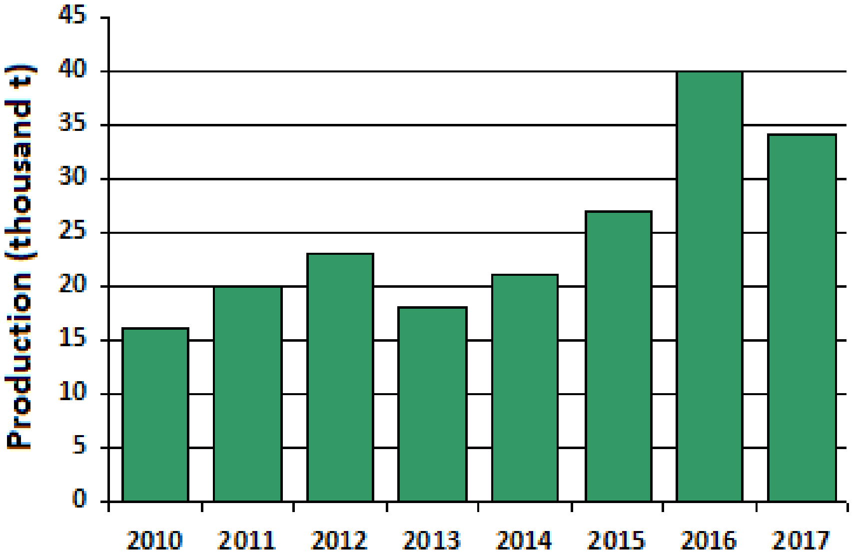

Figure 2. Total faba bean production in Finland from 2010 to 2017. (OSF 2018a)

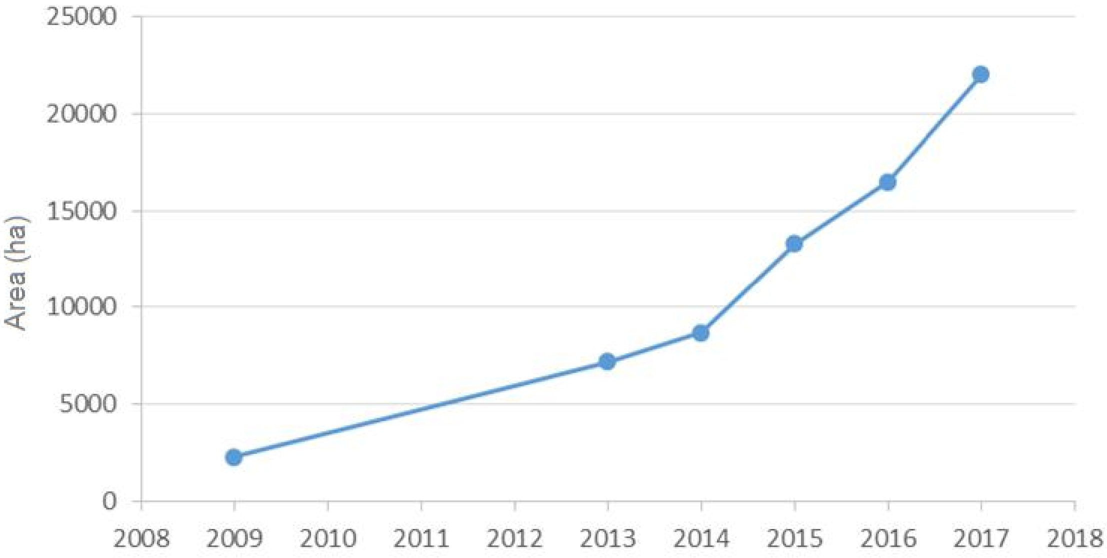

Figure 3. Faba bean cultivation area in Finland from 2009 to 2017. (OSF 2018b)

Table 1. *Most common faba bean cultivars in Finland 2017. (Mavi 2017)*

Cultivar	Area (ha)	Percentage (%)
‘Kontu’(Boreal)	19 687	89,30 %
Other faba bean	832	3,80 %
‘Ukko’(Hankkija)	718	3,30 %
‘Sampo’ (Boreal)	636	2,90 %
‘Bor 61437’ (Boreal)	82	0,40 %
‘Louhi’ (Boreal)	36	0,20 %
‘Vroma’	28	0,10 %
‘Mikko’ (Hankkija)	9	<0,10 %
‘Hangdown’	8	<0,10 %
TOTAL	22 036	100 %

‘Kontu’ is a cultivar bred in 1997 by Hankkija’s Plant Breeding Institute by crossing Hankkija’s ‘Ukko’ and ‘ICARDA-536’. It is beige seeded, high in protein and early, needing 108 days to mature according to the most recent trials. This equals approximately 1124 growing degree days above 5 °C (OSF 2018c).

If national goals towards protein self-sufficiency are to be met and the trend towards plant-based proteins in food products continue, the future of faba bean cultivation looks promising. In research and breeding, themes will likely include earliness, seed quality and facing the challenges that are the result of climate change. Currently the faba bean cultivation area reaches up to Finnish plant hardiness zone IV. The cultivation region will likely increase northwards as the growing season becomes longer. However more area and a warmer climate also means more diseases. Especially the already common Chocolate spot disease (caused by two fungi *Botrytis fabae* and *B. cinerea*) will likely become even more widespread (Ahmed *et al.* 2010).

2.3 Crop formation of faba bean

The growth stages of *V. faba* include germination, vegetative growth, flowering, seed filling, pod senescence (ripening) and stem senescence. Because of the indeterminate nature of flowering, the reproductive and ripening stages can happen concurrently (Knott 1990).

Yield depends on seed size (hundred or thousand seed weight), seed number per pod, pod number per stem, stems per plant and plants per m². Spring beans only have one stem per plant. Other important traits are plant height, days of flowering, and pod distribution on the plant (Thompson and Taylor 1977, Loss and Siddique 1997, Mohsen *et al.* 2013, Li and Yang 2014). The optimal cultivation density is dependent on the environmental conditions (López-Bellido *et al.* 2005). In Finland the cold climate and short growing season limit the yield, and the sowing density is 60-70 seeds/m² (Laine 2017).

2.3.1 Flowering

The flowers of *V. faba* are hermaphroditic. The papilionaceous flower structure consists of three types of petals, flag (or standard), keel and two wing petals (fig. 4).

Flower formation consists of flowering induction and flower retention. Differences in pollination, intra- and inter-plant resource availability and naturally occurring excess flowering affect the flower retention numbers (Patrick and Stoddard 2010). Flower retention is affected by resources and stress factors, such as heat or drought (Bishop *et al.* 2016) and adverse weather conditions within the first four days of the flowering period (Stoddard 1993). The flowers open for the first time around 1 pm, on the next day around 11 am and on the third and following days around 9 am (Stoddard and Bond 1987).

The faba bean flowering time in Finland varies somewhat according to the spring weather conditions. The indeterminate flowering strategy of the faba bean means that the start of flowering usually starts around June and continues until harvest. The peak of flowering lasts approximately 20 days (OSF 2018c). According to Stoddard (1991) in South Australia, the rate of progress of flowering is about three days per node in most cultivars. This suggests that the pollination effects are likely to be most prominent in the first seven nodes. This is supported by increased flower and pod abscission rates, as well as lower outcrossing rates in the higher nodes (Proceddu *et al.* 1980, Bond and Poulsen 1983, Gates *et al.* 1983, Bishop *et al.* 2016).

Figure 4. Morphology of the faba bean flower structure. A) Flag of the flower is located on the top. B) Two wing petals open below the flag. C) The keel petal is located between the wings and protects the stigma and the stamen. The structures protected by the keel are exposed here D) The ovary is long and fully hidden in the petals before pod set. E) The stigma points upwards and is covered in setae. F) Stamens are pointed upwards around the stigma. (Photo by the author)

In terms of appeal to insect pollinators, pollen and nectar production are the key. Both are produced within the flower structure, protected by the surrounding petals (fig. 4). Pollen is released by the stamens into a pocket in the keel petal above the stigma and the anther filaments retract. Nectar is held in a nectary near the base of the ovary (Stoddard and Bond 1987).

A third area of interest for the pollinators is extrafloral nectar produced by the stipules on the base of each leaf node (fig. 5). This nectar is similar, but not identical, in composition to that of the flower. The faba bean extrafloral nectar contains three sugars, glucose, fructose and sucrose, glucose being the most abundant, close to half of total sugar concentration (Engel *et al.* 2001). In addition the EFNs emit volatiles that are close to those of the leaves (Hoffmeister and Junker 2017).

Figure 5. Location of the stipules and the source of extrafloral nectar. A) Red arrows point at black dots on each stipule. The spot produces the nectar. A honeybee can be seen foraging on the stipule. B) *Bombus* s. srt. consuming extrafloral nectar from the stipules close to the apex of the plant stem. The tongue is touching the dark spot on the stipule. (Photos by the author)

2.3.2 Pollination

The pollination mechanism in the faba bean flower happens through tripping. In insect mediated pollination, the pollinator lands on the keel and wing petals and digs its way towards the pollen protected inside the keel petal. The stigma and pollen emerge from the petal and pollen is collected and adheres to the hairs of the insect. In autofertile plants there is no separation, spatially or temporally, between stigma and pollen so the flower is able to self-pollinate more easily without insects burrowing in. Pollination can also sometimes occur with robbing behaviour, but the flower will self-fertilize without cross-pollination.

Cross-pollination is beneficial for the faba bean. Bond and Poulsen (1983) reviewed several papers and found that natural cross-pollination rates in studies range between 4 to 84% and averages around 35%. The reasons for such a wide range can be partly explained by the fact that multiple different methods were used in the different studies. In some cases with high outcrossing rates, the environmental stresses may cause flower or pod abscission for the self-fertilized flowers and skew the results. Therefore the results are not necessarily directly comparable.

More recently, Suso *et al.* (2001) studied the outcrossing rates of five different cultivars in two different locations using isozyme markers. The results indicate that the outcrossing rate increases with greater pollinator abundance and activity. However, when the pollinator numbers are sufficient, adding more does not seem to have an effect on outcrossing. Other factors, such as genetics of the plant come into play when pollinators are abundant.

Factors affecting outcrossing rates are genetic, environmental and ecological. Genetic variation refers to differences between generations rather than differences between cultivars and environmental factors include geographic location and climate (Suso *et al.* 2001). Ecological factors are numerous and point mostly towards pollinator requirements, such as good weather conditions (Link *et al.* 1994). Ecological factors can affect pollinator abundance, species diversity and foraging activity, speed and behaviour, which in turn can have synergistic effects. Additionally access to the flowers within the field (field size, location and plant density) can have an effect on outcrossing (Porceddu *et al.* 1980, Bond and Poulsen 1983, Cunningham and Le Feuvre 2013). According to Cunningham and Le Feuvre (2013), honeybees pollinate most efficiently within 800 meters into the crop. They found that 90 % of the additional 17 % yield increase was within this distance.

The next generation of highly cross-pollinated plants tend to have higher selfing rates, due to hybrid vigour and increased number of pollen grains in the flower. Therefore inbred plants are likelier to need outcrossing, than hybrids. This results in outcrossing working in a cyclical manner, although hybrid rates stay relatively constant within the populations (Stoddard and Bond 1987). In addition, biparental inbreeding is possible, when a flower is pollinated with a close relative. Crossing between relatives can be estimated by comparing single and multilocus genes of outcrossing rates (Suso and Maalouf 2010, Jeffrey *et al.* 2012), but a can also be done by a comparative method comparing inbreeding rates of plants that have had the chance for biparental inbreeding, to those that have not (Kelly and Willis 2002). Overall, inbred plants are likelier to need outcrossing, than hybrids.

Natural intercrossing between cultivars has not been studied extensively, but outcrossing between cultivars in var. *minor* and *major* are common in places where cultivars are grown close to each other (Bond and Poulsen 1983). Artificially crossing var. *minor* and *major* cultivars has been shown to have beneficial effects (Link *et al.* 1996). In Finland natural intercrossing is likely to be very rare due to the popularity of cv. ‘Kontu’ over all other cultivars.

2.3.3 Pod formation

The position of first flower and first pod are not always the same. There are always more flowers formed than develop into pods (Knott 1990, Patrick and Stoddard 2010). Flower retention is higher at a lower position on the stem as is with pod retention. Flower abscission rate is higher with lack of pollinators or stress factors, such as drought, heat or waterlogging, or due to intra-plant competition for assimilates (Knott 1990, López-Bellido *et al.* 2005, Bishop *et al*. 2016). In spring beans vegetative growth happens up to 7-11 nodes before flowering induction.

After fertilization, the flower begins the stage of pod set (Knott 1990). Pods start to fill, increasing their mass rapidly via cell division that progressively changes into cell expansion (Patrick and Stoddard 2010). Seeds start to increase in dry matter approximately 28 days after flowering (Adler and Müntz 1983).

3 POLLINATION RELATED INSECT-PLANT INTERACTIONS

3.1 Importance of pollinators in pod development

It is said that 75 % of the 115 most important food crops in the world benefit in various degrees on insect mediated pollination for yield increase, and this equals around one third of global crop production (Klein *et al.* 2007). Faba bean is among these crops. While the faba bean is partly autofertile, it has been extensively shown that yields benefit from insect pollination (Aouar-sadil *et al.* 2008; Bommarco *et al.* 2012; Cunningham and Le Feuvre, 2013; Bishop *et al*. 2016). Without insect pollination, yield results vary greatly both yearly and depending on geographic location and local environment (Garibaldi *et al*. 2011a and 2013). Changes in seasonal growing conditions can affect yield overall and result in slower growth and development. For example, cold temperatures and wet soil affect Rhizobium activity negatively. Cold, cloudy or rainy growing seasons also affect pollinator activity as especially honeybees do not usually fly in temperatures below 10°C (Joshi and Joshi, 2010).

Insect pollinated faba bean pods have been linked to resilience against environmental stresses, better fertilization and a higher yield, as well as greater vigour in the next generation (Stoddard 1986a, Stoddard and Bond 1987, Somerville 1999, Musallam 2004, Bishop *et al.* 2016).

3.2 Insects as pollinators for faba bean

Faba bean insect pollinators in the order Hymenoptera are mostly from the family Apidae, including subfamilies Apinae (e.g. honeybees, bumblebees and stingless bees), Nomadinae (kleptoparasitic cuckoo bees) and Xylocopinae (carpenter bees).

From these taxa, the most common pollinators on *Vicia faba* in Europe have been reported to be restricted to just a few species. In France, the United Kingdom and Germany, the most common bee species recorded are *Apis mellifera*, *Bombus* s. str., *B. hortorum* (subgenus *Megabombus*), *B. lapidarius* (subgenus *Melanobombus)* and *B. pascuorum* (subgenus *Thoracobombus*) (table 2) (Tasei 1976, Pierre *et al.* 1996, Bond and Kirby 1999, Marzinnzig *et al.* 2018). Some sweat bees *Lasioglossum* spp. were observed in the UK and Germany (Nayak *et al.* 2015, Marzinzing *et al.* 2018). In Spain and Algeria bees from the genus *Eucera* can be even more numerous and more efficient in faba bean pollination than other species (Aouar-sadil *et al.* 2008, Suso and del Rio 2015).

Table 2. The most common bee species recorded visiting faba bean flowers and their reported abundance in several studies. In the studies, where abundance numbers were not provided, sightings are marked by "y".

Species	Poulsen 1973	Kendall and Smith 1975	Tasei 1976	Pierre *et al.* 1996	Bond and Kirby 1999	Garratt *et al.* 2014	Marzinnzig *et al.* 2018
	Denmark	UK	France	France	UK	UK	Germany
A. mellifera	32 %	y	80%	69 %	y	y	56 %
Bombus s. str.	5 %	y	8%	28 %	y	y	37 %
B. hortorum	42 %	y	4%	<1 %	28 %	y	4 %
B. pascuorum	14 %	y	-	-	26 %	y	1 %
B. lapidarius	-	-	2 %	-	-	y	2 %
Solitary/sweat bees	-	-	1 %	3 %	46 %	y	<1 %

Bumblebees and honeybees are generalist species that visit a wide variety of plant species. Honeybees (*Apis mellifera*) are among the most important pollinators especially on agricultural crops. Honeybees forage flowers on single species of plant in one trip. This flower-constant behaviour makes the insect a good pollinator of commercial crops (Joshi and Joshi 2010). Faba bean visiting honeybees are reported to have 99.7 % faba bean pollen packed in the corbiculae, and 97.2 % in the body setae (Marzinzig *et al.* 2018).

For insect pollination to happen, the bee must carry enough viable pollen and be in contact with the flower stigma. Pollen can be carried on insect setae, but bees often clean the hairs and deposit the pollen into the corbiculae (pollen baskets) on their hind legs. This packing can affect the functional traits of the pollen (Parker *et al.* 2015).

Insect-mediated pollination efficiency of the faba bean can be categorised into four major factors; pollinator abundance, diversity, activity and behaviour (Barret and Eckert 1990, Suso et al. 2001). Effectiveness of these factors are determined by deposition of pollen grains per visit and the visitation rates (Marzinzig *et al.* 2018). Pollen load carried by honeybees on apple orchards was over 11 mg per bee and foraging time over 3 flowers per minute. (Joshi and Joshi 2010). Negi and Joshi (2006) had similar findings with Indian mustard (*Brassica juncea* L.). Studies in Denmark and Germany found

Bombus hortorum to be the most efficient pollinator for the faba bean, with a faster visitation rate and higher seed set than with honeybees (Poulsen 1973, Marzinzig *et al.* 2018).

3.2.1 Honeybees

Honeybees gather pollen in different quantities during the day and during the growing season. As stated previously, good environmental conditions for flight play an essential role in pollination activity. Flight depends on temperatures over 10 °C, the optimum being close to 20 °C. Honeybees do not fly in rainy weather. The biggest factor after good flight conditions is hive development and the changing need in protein provided by pollen.

The bee colony consists of three kinds of adult bees: female workers, male drones, and a single reproducing female, the queen. Honeybees in flight are mostly workers and consists of younger bees making orientation flights to learn their surroundings and more experienced bees that have already started foraging. Bees collect water, nectar, pollen and resin and occasionally sugary secretions from aphids or wax from scale insects (Seeley 1995). The annual cycle of the colony starts in spring, after the colony has hibernated in a tight ball structure in the middle of the hive. The colony activates and brood productions starts slowly, increasing exponentially after the first flowers start to bloom. A full size colony is regarded as having 30 000 individuals, at which point it starts to reproduce, by rearing males and new queens, as well as by preparing for swarming (Seeley 1995).

The tasks the honey bee workers perform during their lifetime changes according to the age of the bee and its respective genotype. For example some bees have a stronger tendency towards patrolling the hive, while others are more likely to tend the brood or the queen (Seeley 1995). However, the worker bees vary their tasks depending on their age, slowly changing into new sets of tasks. Right after hatching they are likeliest to clean the hive cells, then nurse the brood and build the comb, store nectar and pollen and finally forage (Seeley 1982).

Foraging tasks done by the workers usually start from an age over 21 days (Abou-Shaara 2014). Without any previous knowledge of a new nesting or foraging site, scout bees go on reconnaissance flights to search for one (Janson *et al.* 2007, Liang *et al.*

2012). Scouts for food constitute 5-25 % of total foragers. Nest site foragers in turn make up only <5 % of these foragers and can exhibit more novelty seeking behavior than other workers (Liang *et al*. 2012). Once a prolific foraging area is found, the scouts return to the hive to inform other foragers of the site by an elaborate set of movements, known as dancing. The length of the dance and number of times the bee repeats the dance (number of returning flights) depends on the quality of the resource. This way the better the source is, the more it gets advertised (Janson *et al.* 2007).

Foragers can be further divided into two categories. Persistent bees go outside the hive to check the already known resources and the reticent bees stay in the hive to wait for the information (Van Nest and Moore 2012). The persistent bees return to the hive to confirm the availability with a dance. A reticent bee may start scouting for new sites if they it does not receive a dance.

According to Van Nest and Moore (2012), 40-90 % or the foragers are persistent foragers. A bee can remember the location and time it last visited a good foraging site and can learn to arrive at the site at a favourable time of day (Moore *et al.* 2011). A revisiting reconnaissance flight the next day increases the likelihood of getting to the source at an earlier time than when it was previously found. As different plants have differing times for anthesis, the earliest optimal arrival time varies. The faster the bees can optimize the arrival, the more they can ultimately forage from the specific resource. The revision of the source is also more energy efficient than if all foraging bees get to a depleted or unfavourable foraging site out of habit alone (Van Nest and Moore 2012).

If the death rate of the foragers within a colony is high, the colony will try to adjust this imbalance by sending out bees to forage at a younger age than normal. Foraging is a high-risk activity, lowering the survival of the flyer. Younger foragers are likelier to have a higher death rate than older ones and colony failure may ensue from these dynamics (Woyciechowski and Moron 2009, Khoury *et al.* 2011). According to Danka et al. (1986) the percentage of foragers in a colony differs with colony size and availability of suitable flowering plants. Growing, larger colonies have more brood to nurse, so nurse bee populations are also greater. Nectar and pollen abundance increases the forager numbers regardless of the colony size. The number of foragers in a colony was estimated to be 6.5 % with approximately 26 100 honeybees. Pelototalo (2010) estimated the number of foragers to be 27 %, when water foragers were deducted. In a 30 000 colony this equals about 8 000 individual bees.

The foraging decisions are affected by brood production. Larvae release pheromones that induce pollen foraging behavior and pollen reserves have an inhibiting effect (Traynor *et al.* 2015). The larvae do not consume the pollen directly, instead the nurse bees eat the pollen and secrete a protein rich liquid into the larvae cells (Traynor *et al.* 2015).

3.2.2 Bumblebees

In Finland 37 bumblebee species (*Bombus* spp.) have been recorded up to the year 2016 belonging in 10 different subgenera (FEGH 2016). *Bombus lucorum* is the most common species throughout the country (Finnish Biodiversity Information Facility 2018a).

The short-tongued bumblebees from the subgenus *Bombus* (sensu stricto) *B. lucorum* and *B. terrestris* look very similar and are hard to distinguish on morphological characters, so they are commonly misclassified (Wolf *et al.* 2010). However, *Bombus lucorum* is a native species in Finland while *Bombus terrestris* is invasive (MAFF 2012). In the southern parts of Finland, where the status of the invasive species is unknown (but assumed more common), identification strictly from flight or photos is very unreliable.

In addition, there are two more species that very closely resemble *B. lucorum* morphologically: *B. cryptarum* and *B. magnus.* Both have been recorded as present in Finland. The identification is hard to the extent that this group has been called the *Bombus lucorum* Complex. In Central Europe *B. terrestris* was also considered part of the *B. lucorum* Complex but is currently more commonly excluded. DNA evidence supports this interpretation (Bossert *et al.* 2016). Waters *et al.* (2011) identified species form the *B. lucorum* Complex in Scotland and suggest a combination of molecular and ecological methods for accurate identification. In this study, bumblebees in the *Bombus* s. str. are not identified to species level, but referred as either belonging to the subgenus *Bombus* s. str. or as part of the *B. lucorum* Complex.

Bombus hortorum is a long-tongued bumblebee in the subgenus *Megabombus*. They are specialised in foraging flowers with deep blooms and have been recorded to be very efficient pollinators of faba bean, visiting flowers up to twice as fast as honeybees (Poulsen 1973). According to the sightings information by Finnish Biodiversity Information Facility (2018b), *B. hortorum* is well established in the southern parts of Finland.

Mated bumblebee queens overwinter and start searching for a nesting site in the spring. *Bombus lucorum, B. terrestris, B. lapidarius* and *B. pascuorum* queens start emerging a few weeks earlier than *B. hortorum*. The search season length varies between species, but lasts for approximately 5-8 weeks after first emergence (Kells and Goulson 2003). The number of nest searching queens correlates with the number of nests found in the area (O'Connor *et al.* 2017). The nest are typically subterraneous, old nests of small mammals, such as rodents. Preferred nesting sites vary somewhat depending on the species. *B. terrestris, B. lapidarius,* and *B. lucorum* tend to prefer semi-natural boundaries along banks, while *B. hortorum* nests more often in tussocks (Svensson *et al.* 2000, Kells and Goulson 2003). The nesting site does not necessarily correlate with the location of good foraging plants (O'Connor *et al.* 2017), instead they prefer to forage more than 100 m away from their nest (Dramstad *et al.* 2003).

The colony starts small, with the queen alone laying eggs, nursing and foraging for 8-16 worker bees. These workers then take up the nursing and foraging tasks. The colony can grow up to a few hundred workers (Goulson 2010, O'Connor *et al.* 2017). Foragers are mostly either nurses of foragers, but about a third carry out both tasks. The colony will adjust this behavior according to the needs of the brood. The type of foraging (pollen or nectar) depends on the food reserves in the nest and the presence of larva (Free 1955). Bumblebees are likely to select the most optimal protein/lipid ratio of the resources available depending on the needs of the colony (Vaudo *et al.* 2016).

3.3 Importance of the faba bean pollen and nectar to honeybees

Pollen provides bees with a source of protein. Protein is needed especially in the larval stages of honeybee development. The sugars in the nectar supply the energy resources required to sustain the hive and the foraging needs and grow the colony. The rate of which these resources are foraged depends on the needs of the colony, but is also guided by genotypic traits. Some honeybee worker strains can be genetically more dispositioned for hoarding pollen than nectar (Fewell *et al.* 2000). The nectar sources chosen by this strain were lower in sugar concentration than with bees focusing more on nectar overall (Pankiw *et al.* 2002). The need for pollen is the primary determining factor of resource selection and nectar is only secondary (Fewell and Winston 1996, Aronne *et al.* 2012).

The faba bean pollen is available to bees when the flowers are open. Pollen production varies, but is estimated to be on average 27 000 pollen grains per flower (Suso *et al.* 2008, Bailes *et al.* 2018). Several studies suggest that honeybees prefer pollen with higher essential amino acid concentrations, but the results are mixed. Corby-Harris *et al.* (2018) showed that since foraging bees do not consume pollen, the younger nurse bees might do the sampling. However, the results showed that pollen quality was not assessed or communicated by the nurse bees to the foragers.

Faba bean pollen crude protein value form dry matter is 24 %, which is close to the mean of 62 floral species foraged by the honeybee (Somerville and Nicol 2006). The pollen contains some of the essential amino acids for honeybees, including histidine, threonine, arginine and small amounts of leucine and valine can be found, which are especially important for the bees (De Groot 1953, Cook *et al.* 2003). Fatty acid contents of the pollen are favourable for the honeybees. Especially in the larval stages, bees require palmitic and oleic acids, which are abundant in faba bean pollen (26 % and 15 %, respectively of total fatty acids present) (Manning 2001).

The average amount of nectar in faba bean flowers is relatively low when compared to the average of some plant species foraged by honeybees (Adgaba *et al.* 2017). The nectar amount depends on the flowering stage and time of day. Maximal nectar secretion occurs in the morning, but remains high throughout the day (Pierre *et al.* 1996). Estimations of nectar secretion per flower range from 0.1-3.9 µL (Stoddard and Bond 1987, Pierre *et al.* 1996, Osborne *et al.* 1997, Bailes *et al.* 2018). Nectar in open flowers is estimated to be around 1.15 µL. In closed flowers the amount is about half of the opened ones (Pierre *et al.* 1996).

Sucrose, fructose and glucose are the main components of nectar. In faba bean, the overall glucidic concentrations are reported to be on average 30 %. Sucrose is the dominant sugar, but exact concentrations may vary (Pierre *et al.* 1996, Osborne *et al.* 1997, Bailes *et al.* 2018). Sucrose is the most attractive sugar for the honeybee. It acts as a reward for the insect and strongly influences the foraging decisions of the individual bee (Scheiner *et al.* 2004).

3.4 Apidae behaviour on faba bean

The most important pollinators of faba bean in Europe are the honeybee, several bumblebees and some solitary bees (Stoddard and Bond 1987). The most common faba bean pollinators make three different kinds of visits to the faba bean plants. The so called positive visits are when the insect enters the flower from the front and digs into it, tripping the flower. Depending on the length of the tongue, the frontal foraging might be for nectar or for pollen. Long-tongued bumblebees for example can reach the base from the front, while short-tongued bumblebees and honeybees can reach only the pollen (fig. 5). *Apis mellifera* and *Bombus* s. str. have the shortest tongue length (6 mm and 8-9 mm respectively), *B. lapidarius* has an intermediate length (10-12 mm), while *B. pascuorum* and *B. hortorum* have the longest tongues of these species (12-13 mm and 14-16 mm, respectively) (von Hagen and Aichhorn 2014, as cited in Marzinzig *et al.* 2018). The faba bean flower corolla length is approximately 20-30 mm (Preston and Isely 2012).

Figure 6. Foraging strategy can depend on the tongue length of the bumblebee. A) Short-tongued *Bombus* s. str. robbing the faba bean flower. B) Long tongued *Bombus hortorum* foraging for nectar from the front. (Photos by the author)

The negative visits to the flowers are the result of nectar robbing behaviour (fig. 6 and 7B). Bumblebees such as *Bombus* s.str. bite a hole through the basal calyx of the flower to get to the nectar without entering the flower from the front (fig. 7A and 8). This does not seem to harm the flower (Newton and Hill 1983) and may have a self-pollination effect (Kendall and Smith 1975, Navarro 2000). The floral scent attracts pollinators to the plants. The main volatile components are mono- and sesquiterpenes, such as linalool (Hoffmeister and Junker 2017).

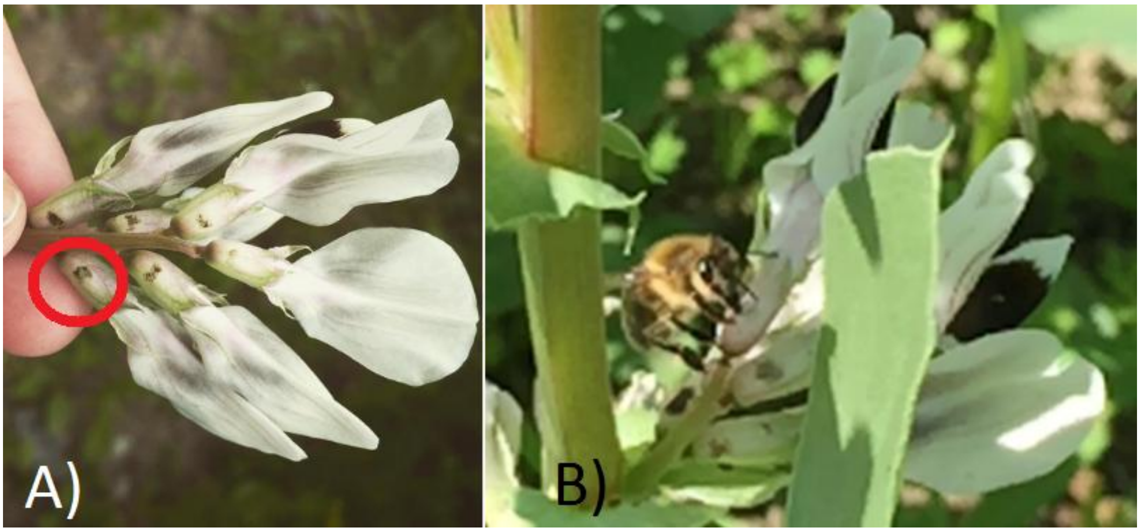

Figure 7. A) Holes in the faba bean flower basal calyx are usually clearly visible. B) Other insects foraging for nectar can utilise the holes made by *Bombus* spp. (Photos by the author)

Figure 8. The holes made by *Bombus* spp. are situated in the basal part of the floral calyx. (Photo by the author)

A third type of visiting the plant is not primarily related to the flower, but to the stipules on the base of the nodes (fig. 9C). These are called the extrafloral nectaries (EFNs). A larger concentration of stipules can be found on plant apex where the new leaf node structures are forming. These stipules produce a nectar-like substance (Davis *et al.* 1988), which attracts insects (Nuessly *et al*. 2004, Katayama and Suzuki 2004).

Visits to these sources does not contribute to the pollination of the plant, but may increase the attractiveness to pollinators. Major scent components are excreted from the dark-coloured spots on the stipules and are made from compounds from the leaves. The scent profile is less complex than in floral nectar, the main component being benzaldehyde (Hoffmeister and Junker 2017). This compound has been shown to attract pollinators (Theis 2006). Floral nectar availability is limited to the flowering period, but extrafloral nectar is available before and after flowering. Nectar from EFNs is also more readily available, because it is not confined within the flower.

EFNs also serve as the natural defence mechanism of the plant. When herbivory occurs on the plant, the exudates and visual traits of the EFNs change to attract natural enemies

(Jones *et al.* 2011). This was also shown to alter the behaviour of *B. terrestris* that became more interested in the EFNs after the experimental treatment due to changes in olfactory and visual traits (Hoffmeister and Junker 2017).

Figure 9. Different floral behaviours of *Apis mellifera* A) *A. mellifera* entering the faba bean flower from the front. Pollen from the *V. faba* flowers are gray. B) *A. mellifera* robbing the flower C) *A. mellifera* on the extrafloral nectaries. (Photos by the author)

A diverse pollinator profile in species and abundance on a field with differing activity and behaviour can have substantial effects on the pollination of a crop (Suso *et al.* 2001). For example especially the short-tongued bumblebees can be fast fliers, but their behaviour can result in lower cross-pollination rate due to high rates of robbing (Aouar-sadil *et al.* 2008). Honeybee behaviour on the other hand can have a bigger impact on cross-pollination because each foraging bee generally goes out for robbing nectar, gathering pollen or visiting external nectaries (stipules) at one time (Page *et al.* 1995, Abou-Shaara 2014) and exhibit high flower constancy (Marzinzig *et al.* 2018). *Bombus hortorum* is similarly focused on just one species at a time, preferring flowers with a long corolla and making almost exclusively positive visits in faba bean flowers (Marzinzig *et al.* 2018). In contrast *Bombus terrestris* and *B. lucorum* are not always flower constant and can visit multiple species during one foraging flight (Free 1970, Marzinzig *et al.* 2018).

3.5 Pollination services in Finland

Apiculture has a long history in Europe and before commercial hives, people managed wild colonies (Chauzat *et al*. 2013). Due to the highly adaptable nature of the European honeybee, it has become the most common managed bee species in the world. The generalist, flower constant nature and ability to forage over long distances makes it a very good commercial pollinator species (van Engelsdorp and Meixner 2010). Bumblebees, especially *B. terrestris* have also been reared commercially and used successfully for pollination in greenhouses and even with field crops (Velthuis and van Doorn 2005).

Commercial pollination services mean that a crop farmer buys pollination services by renting honeybee hives from the beekeeper in order to increase the pollination rates of their crops. Overall these services are closely linked to honey production. The more there is honey production, the more there are possibilities for honeybee pollination services to grow. Honey production in Finland has been growing since 2011 and is currently around 55 000 hives in production (SML 2018). The Finnish Beekeeping Association estimates that the value of honeybee pollination out of the yield value is 13%. Lehtonen (2012) estimates that the value of bee pollination of faba bean in Finland is 0.4 million euros out of total yield.

There are approximately 3 000 beekeepers in Finland, and close to a hundred of them are professional beekeepers. Bees are kept throughout the country, but most of the farms are situated in the southern parts of Finland. Hives per beekeeper vary from one to over a thousand (SML 2018). However, the availability of commercial pollination services is rather low, only about 120 beekeepers offer commercial pollination services (SML 2018). The situation in the UK is similar, with < 10% of beekeepers responding to a survey actively providing pollination services (Breeze *et al.* 2017).

By comparison, the United States is the leading provider of commercial pollination services in the world. Pollination market values in the USA exceed the value of honey production. Farmers pay less for pollinating honey crops and more for crops that do not provide as much nectar for the bees (non-honey crops). It has been estimated that service fees for commercial pollination are in total 296 million euros per year (Rucker *et al*. 2012). Hives are transported hundreds at a time to their service locations.

Reasons for the low number of service providers in Finland may be because farmers already have a working deal free of charge with beekeepers or they have their own hives. Some farmers have found it difficult to find local service providers. Likewise, the beekeepers report getting only very few queries from farmers. This indicates problems with communication within the pollination markets. There may also be difficulties with setting the appropriate price for the service – this is especially relevant, when the profitability of farming is low. In these cases the farmers are not willing to pay a high compensation for pollination, even if they acknowledge its importance. On the other hand, there are many beekeepers and farmers (e.g. apple orchards), who make agreements outside of the system set up by the Finnish Beekeeping Association (SML, Eeva-Liisa Korpela 2018, personal communication). There is currently no way to document the number of these agreements, thus they are not included in the register of 120 pollination service providers.

Commercial services in Finland have so far been mostly been associated with greenhouse crops, by bumblebees (Ruottinen 2005). Field pollination by honeybees has mostly been for oilseed rape (*Brassica* sp.), caraway (*Carum carvi*), berry and fruit crops, such as strawberries (*Fragaria*), raspberries (*Rubus*) and apple orchards (*Malus*), but also clover (*Trifolium* sp.) and faba bean (Peltotalo 2010).

Labour and transport costs are estimated to be the biggest costs for the service provider, while 62 % of overall beekeeping expenses go into disease management (Breeze *et al.* 2017). Moving the bee hives does not have adverse effects to the foraging of the colony (Riddell Pearce *et al.* 2013), but moving them too frequently can disturb the colony development, add stress and increase winter losses (Simone-Finstrom *et al.* 2016). Therefore it is advisable to leave the hives with the crops for the duration of a few more brood cycles, even after the prime flowering time has ended.

The Finnish Beekeeping Association provides farmers and beekeepers with a pollination agreement form and suggests a price range of 80-150 € per hive, depending on the pollinated plant species. Research for a more crop specific pricing is under way by the association. As an example, in the UK 14 % of beekeepers providing services to faba bean fields received a median payment of 36 € per hive, which is relatively low, considering the potential for increased faba bean yield and cost for the beekeeper. Faba bean fields are recommended to have 2-8 hives per pollinated area (SML 2018), but the optimal number for hives per hectare has not been assessed. 2.5 hives/ha have been used by Scriven *et al.* (1961). Garrat *et al.* (2014) suggested that with an observed average pollination efficiency of 0.0004 flowers per minute, only 58 % of the open flowers would be visited once in optimal weather conditions. Therefore, the efficiency can be increased by increasing the number of pollinators on the field, but can be negatively affected by poor weather.

Honey production from faba bean has not been well researched. A complex set of environmental and ecological considerations regulate honeybee foraging decisions (Musallam *et al.* 2004, Nyak *et al.* 2015) and due to the short tongue of the bee (Hawkins 1969) nectar robbing is mostly reliant on the holes made in the corolla by short-tongued bumblebees. The EFNs can provide nectar even after the flowering period, but the effects to the honey yield have not been studied. The honey production adjacent to faba bean fields can be lower during the start of the season, than in non-crop areas. However, the added value of a single crop honey reduces the loss in early honey production (Breeze *et al.* 2017). Some added value may be derived from the qualities of faba bean honey. The nectar from faba bean has a fructose/glucose ratio of 2.2 (Pierre *et al.* 1996), thus making the sugar in the honey likely to be slow to crystallise, depending on the glucose/water ratio (Salonen 2011).

4 OBJECTIVES

As the cultivated area of the broad bean continues to grow, there has been interest towards the effects of pollination to the yield components. Especially the honeybee and commercial bumblebee hives have gained interest as a way to increase yield. In the point of view of the farmer, it is most useful to know more about insect pollination as a way of increasing yield. In the perspective of the beekeeper, the focus is more on the wellbeing of the hive (feed in the form of pollen) and the overall yield of honey and its properties. The main question to ask is whether one can affect the pollination success (and thus yield) by increasing the number of pollinators on the faba bean fields.

The first aim of the thesis is to examine the effects of pollination on the pod count and distribution along the plant stem, with and without pollination from honeybees. This was done via a cage experiment and a field survey on eight different faba bean fields. Pod and pod-bearing node counts give pointers towards the effects of pollination on the yield components and make it possible to predict potential yield increase. The hypotheses were that honeybee pollination will increase the pod counts and average pods per node on the plant stem and that there is a positive correlation between honeybee numbers on the fields and pod counts per node.

The second aim is to get a better understanding on honeybee and bumblebee floral behaviour on faba bean flowers and the rate at which they make frontal visits to the flowers. This was conducted as an observation survey on eight faba bean fields. Knowledge on how many flowers bees visit per plant and which part of the flower they forage on are crucial to assess the cross pollination potential. The hypotheses were that honeybees will collect pollen from the front of the flowers and visit multiple individual plants during one flight.

The third aim was to have gain a preliminary evidence on how the pollinator abundance and species diversity differs between faba bean fields. This was done as a field survey with the floral behaviour observations. The hypothesis was that bee pollinators will be among the most numerous species observed on these fields.

With the results, the goal is to look for possibilities to increase knowledge of yield benefits and further the co-operation between faba bean farmers and beekeepers for potential commercial pollination services.

5 MATERIALS AND METHODS

5.1 Viikki cage experiments

The first part of the study was carried out as a cage experiment on the fields of the Viikki experimental farm in Helsinki from May to September 2015.

Nine net cages were placed on a field with faba bean (cultivar ‘Kontu’) in its early flowering stages in June (fig 10), before the buds were open. Plots were assigned into four different treatments with three replicates (fig.11):

O: open cages, where pollinators were free to enter the cage from one side
E: empty cages, where pollinators had no access
B: cages with honeybees (*Apis mellifera*) had a small nuclear hive within the cage that was otherwise closed from other pollinators
C: control area with no cage.

Dimensions of the cages used were 7.50 x 1.20 x 1.50 m.

Figure 10. Cages were placed on the experimental fields in Viikki.

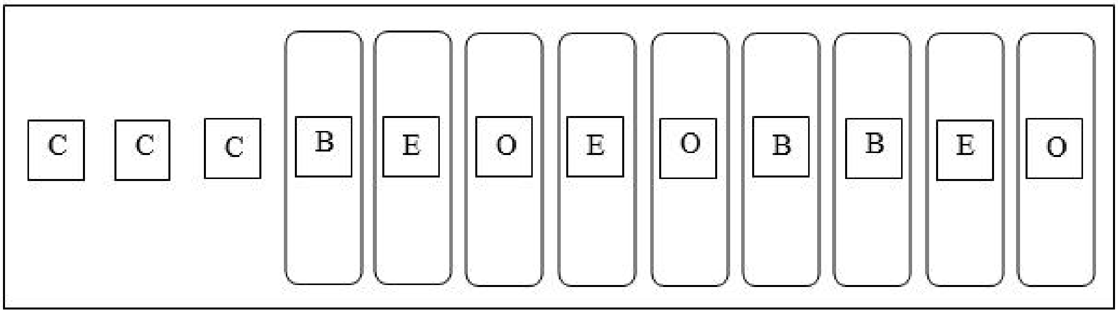

Figure 11. Experimental layout of the cage experiment. C = Control treatment, B = cage with honeybees, O = open cages, E = empty cages.

The cages were placed on the field in a randomized complete block design. Nucleus hives (fig. 12) each containing a few hundred bees were placed inside the cages for twenty days during the main flowering season. One hive was also placed outside of all cages to increase likelihood of pollination outside the cages.

After flowering ended, cages were removed 21 August and the pods were left to ripen until the end of August. Pods per node were then counted from the base of the plant, treating the firsr podded node as number one, from 100 plants per treatment. After the last node with pods, nodes that did not bear pods were not taken into account.

Figure 12. Small nucleus hives were placed inside three cages.

5.2 Field research

The field survey was conducted on eight different faba bean fields in Southern Finland, southern most field was located near Salo and the northern most field was situated in Sahalahti (table 3).

Table 3. Observation fields and their approximate locations and altitudes.

Field code	Township	Latitude	Longitude	Altitude (m)
A	Myrskylä	60.64 N	25.85 E	48
B	Kantele	60.67 N	25.66 E	47
C	Sahalahti	61.47 N	24.43 E	102
D	Salo	60.35 N	22.01 E	30
E	Loppi	60.72 N	24.44 E	129
F	Otalampi	60.38 N	24.56 E	60
G1	Askola	60.53 N	25.71 E	21
G2	Askola	60.50 N	25.70 E	21

Insect observations were conducted after the start of flowering, within the 20 days of best flowering period. Observations were done 8 – 21 July 2015, when the temperature was above 15 °C. The temperatures on the fields ranged from 17 – 22 °C and the wind was below 6 m/s (± 1-6 m/s). Surveys on each location were done between 12 pm and 4 pm during the optimal flight time of honeybees (Poulsen 1973), which coincides with the time when the faba bean flowers tend to be open for the first time (Stoddard and Bond 1987). The vicinity of the closest beehives was not confirmed, but approximations ranged from 200 m to 6 km.

5.2.1 Honeybee floral behaviour

Honeybee floral behaviour was observed on each field. Target minimum observation count was 30 bees per field. Unfortunately this was not possible on all fields, as there were very few honeybees in flight in some of them. Positive, negative and extra-floral visits were recorded as well as visits per one plant stem before going to the next. The colour of the pollen in the corbicula was recorded.

5.2.2 Field surveys on pollinator abundance and species diversity

Three 60 meter transect lines were chosen in each field and insects recorded based on taxa from genus level (e.g. *Syrphus*, *Bombus*) and in a few cases up to species level (*Apis mellifera*, *Bombus hortorum*). The main focus was on recording honeybees and different *Bombus* species to determine the numbers of potential pollinators. The different *Bombus* species from the *Bombus* s. str. subgenus were not identified to species level, because of the difficulty of identification. A number of other insect taxa were recorded as well to serve as an indicator of species abundance and diversity on the field.

5.2.3 Pod distribution and count

After pod set, the fields were visited again in August and the pods per node on approximately 100 plants per field were counted.

5.3 Interpreting results

Measurements of pod counts and distribution along the plant were then analysed using one-way analysis of variance. Post hoc comparison was done with Tukey HSD. Pearson correlation coefficient and regression analysis were used to determine the effects of honeybee numbers on the average pods per node. All statistical analysis was made using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA)

6 RESULTS

6.1 Cage experiment

The presence of open cages reduced pod number to half of the control (P<0.001) (fig. 13). Closing the cage did not cause a further significant reduction in pod numbers (P=0.158). Including bees in the cage increased pod numbers per plant by 54% above that in the closed cages (P<0.001), but the number was still less than the open field (P<0.001). The same pattern was seen in terms of pods per node (fig. 14). There was a significant increase of 19% the number of pods per node when comparing the cage with bees to the empty cage. The cage decreased the mean number of pods per node by 32 %.

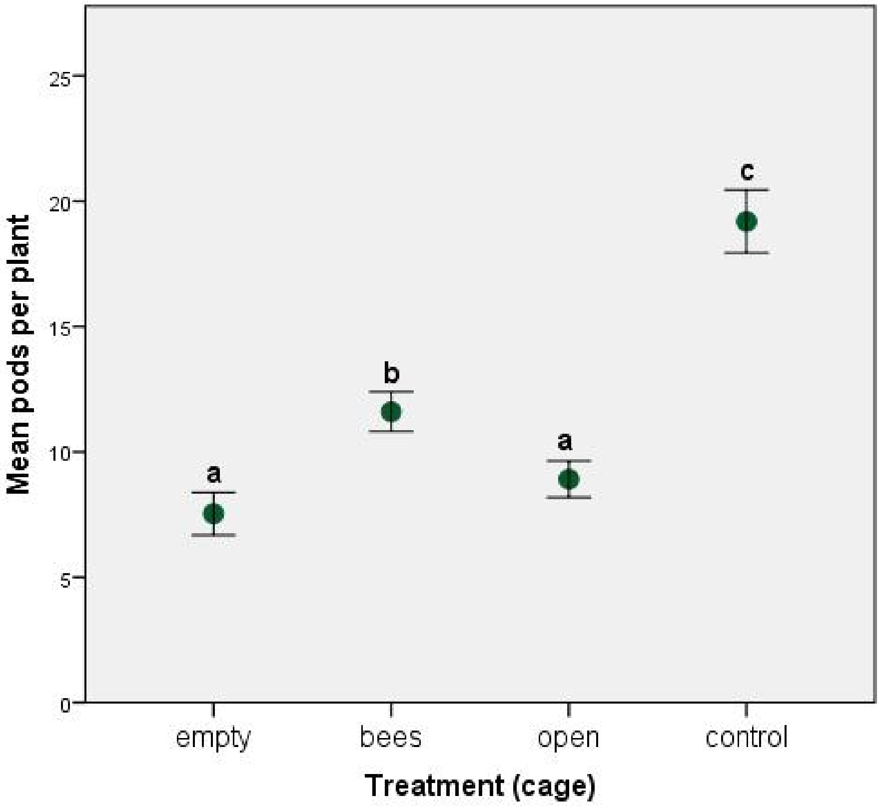

Figure 13. Mean of pods per plant in each treatment (cage). Error bars: 95% CI

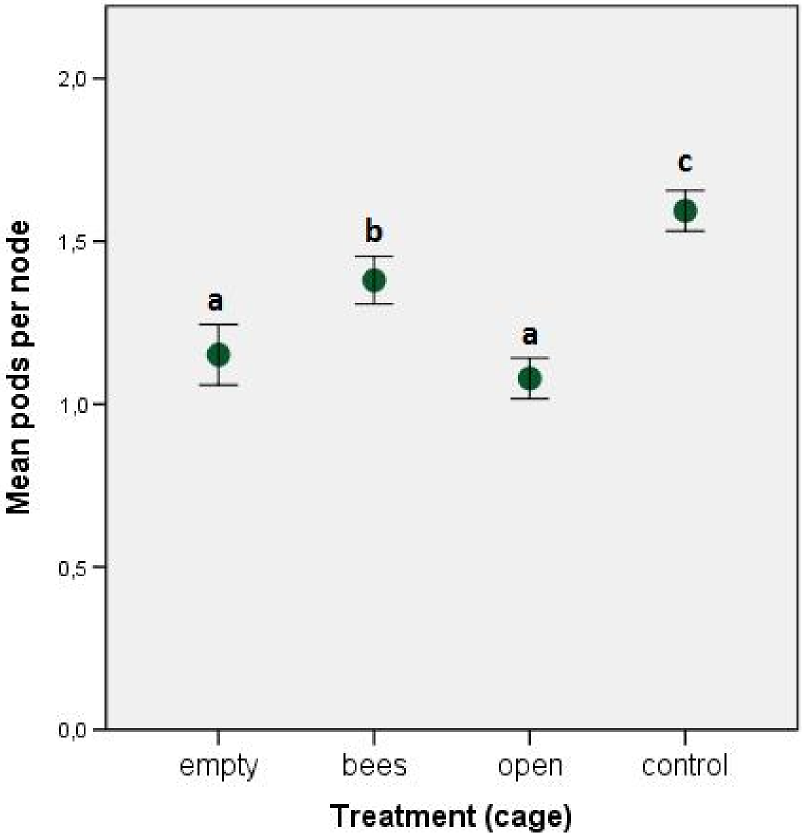

Figure 14. Mean of pods per node in each treatment (cage). Error bars: 95% CI.

Examining the pod distribution shows where the treatments' differences developed. The control had many more pods than the caged treatments, particularly above node 8 (fig. 15). The counted plants were taller whereas the caged plants were constrained in their lower cages. However, no pod numbers were different between cages with bees and the control at nodes one, two, three, four, six and seven and at node five the significance was low (P=0.029). Open and empty cages had significant difference from the control except at the first node (fig. 15).

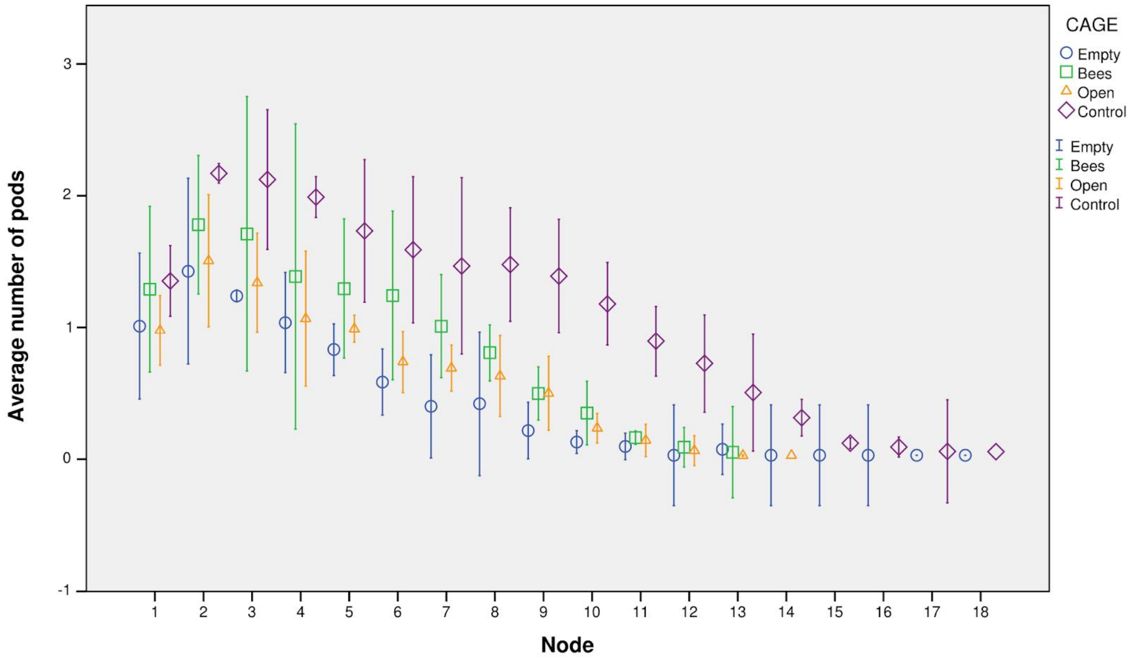

Figure 15. Effects of the treatment (cage) on the average number of pods per node on the plant stem. Error bars: 95% CI.

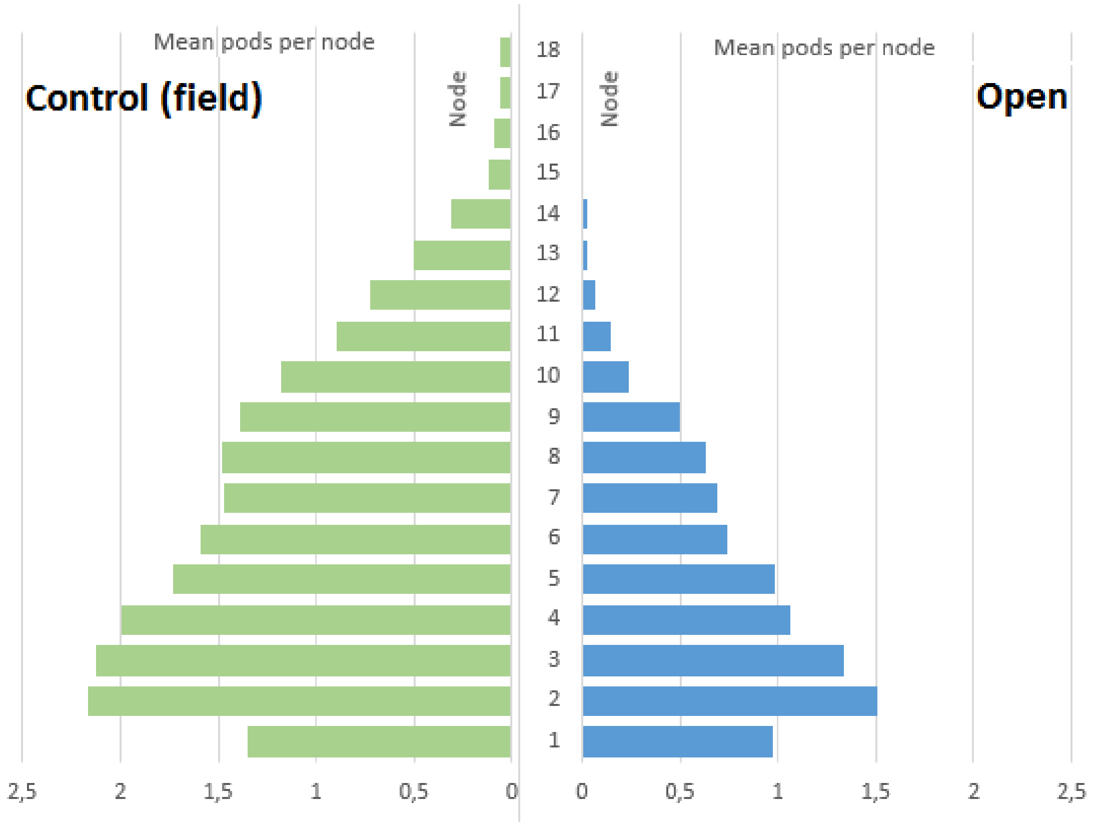

Figure 16. Average number of pods per node in the field and in the open cage.

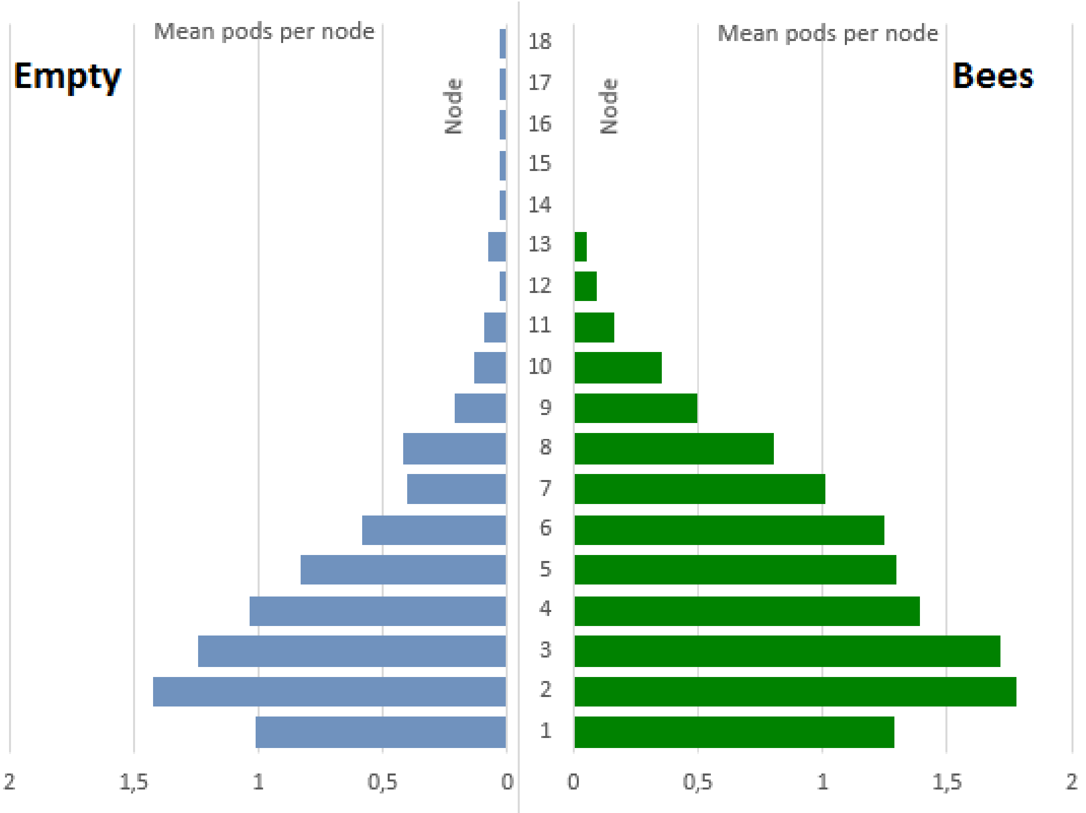

Figure 17. Average number of pods per node in the empty and in the cage with bees.

The cage with bees had more pods at the 5th, 6th and 7th nodes, when compared to the empty cage (fig. 17, table 2). Nodes 2-4 in cages ‘empty’ and ‘open’ show significantly fewer pods per node than the control. Nodes 8-13 had no significant difference between groups ‘empty’, ‘bees’ and ‘open’, but all had significant difference between them and the control treatment in the open field (table 4). Some plants had pods on them as far as the 17th node. Data from nodes 14 to 17 were not used, since there was not sufficient data from all the treatments.

Table 4. Probability values of pairwise differences between treatments in mean numbers of pods at each of the first 8 podded nodes according to Tukey's HSD.

Nodes	Bees	Open	Control
1st node			
Empty	0.319	0.996	0.184
Bees	-	0.241	0.974
Open	-	-	0.136
2nd node			
Empty	0.227	0.962	0.009**
Bees	-	0.412	0.169
Open	-	-	0.017*
3rd node			
Empty	0.171	0.958	0.010**
Bees	-	0.327	0.249
Open	-	-	0.020*
4th node			
Empty	0.430	0.999	0.010**
Bees	-	.500	0.094
Open	-	-	0.013*
5th node			
Empty	0.029*	0.629	0.001***
Bees	-	0.164	0.039*
Open	-	-	0.002**
6th node			
Empty	0.010**	0.744	0.001***
Bees	-	0.040*	0.174
Open	-	-	0.002**
7th node			
Empty	0.013*	0.270	0.000***
Bees	-	0.204	0.055
Open	-	-	0.003**
8th node			
Empty	0.069	0.423	0.000***
Bees	-	0.553	0.004**
Open	-	-	0.001***

6.2 Field surveys

There was a strong correlation between the average number of honeybee sightings in the field and the average pods per node recorded (r = 0.75, n= 8, P= 0,021) (fig 18). Field C was clearly an outlier and removing it increased the correlation (r = 0.97, n= 7, P<0.001). There was no significant correlation found between the number of *Bombus* spp. and the mean number of pods per node (r =0.310, n=8, P= 0.455) (fig. 19).

A strong negative correlation was found between the average number of Coccinellidae and the mean number of pods per nod (r = -0.76, n= 8, P= 0.029).

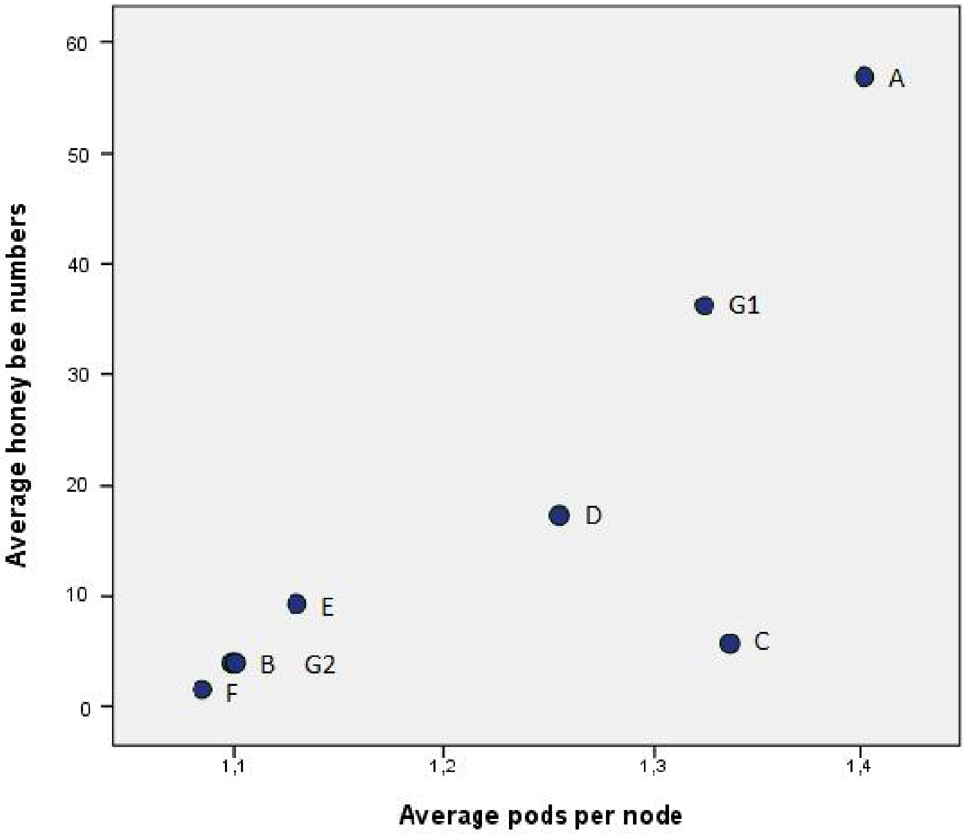

Figure 18. Honeybee numbers during surveys in July and average pods per node in August in eight different fields. Letter codes for the fields are explained in Table 3.

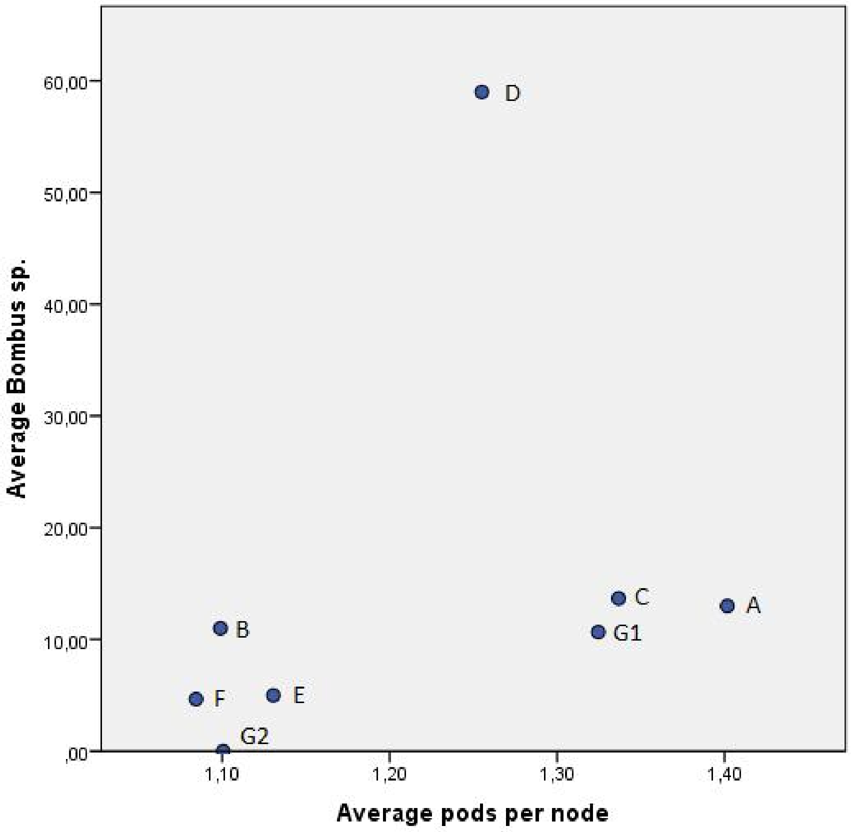

Figure 19. Bumblebee numbers during surveys in July and average pods per node in August in eight different fields. Letter codes for the fields are explained in Table 3.

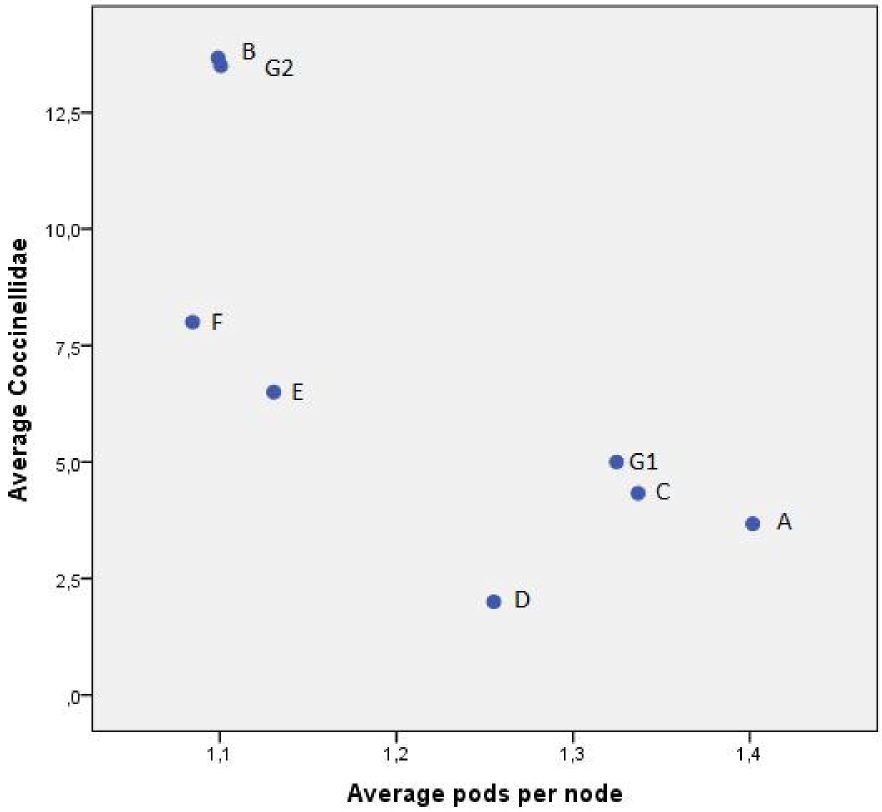

Figure 20. Coccinellid numbers during surveys in July and average pods per node in August in eight different fields. Letter codes for the fields are explained in Table 3.

In the floral behaviour survey it was observed that bees that foraged for the pollen gathered it from flower to flower on the same plant species and did not switch between species during the sample observation period. Pollen in the corbiculae was recorded to be grey on all front-visiting honeybees, showing that the pollen was from faba bean. Honeybees were also observed to visit *Trifolium* flowers in some fields, but they did not switch to faba bean when foraging this species. No bumblebees from the subgenera *Bombus* s.str. were observed to visit flowers from the front. One long-tongued bumblebee (*Bombus hortorum*) (fig. 6B) was observed to visit a flower from the front apparently gathering nectar in field C.

On average from the whole field survey observations (n=126), honeybees visited 1.5 (SD 0.5) flowers per plant before moving to another plant. The average per field did not exceed 1.8 flower visits per plant. Frontal visits constituted 55% of all floral behaviour (mixed visits included) (table 5).

Robbing behaviour by honeybees was documented in five of the eight fields and ranged from no robbing behaviour to 84 % robbing on the observation day (Fig. 9). Some bees were recorded foraging pollen and exhibiting robbing behaviour. Extrafloral visits were recorded in six of the eight fields an accounted for 19 % of all recorded behaviour (table 5). Most bees went only from stipule to stipule, but honeybees were also seen to visit stipules after frontal visits on two fields.

Table 5. Observed floral behaviour of *A. mellifera* on faba bean fields. Mixed visits of robbing and EFN were not recorded.

Visit type	Observations (n=241)	Percentage (%)
Frontal visits only	119	51 %
Robbing only	61	26 %
EFN only	45	19 %
Mixed: frontal and robbing	6	3 %
Mixed: frontal and EFN	4	2 %
TOTAL	241	100 %

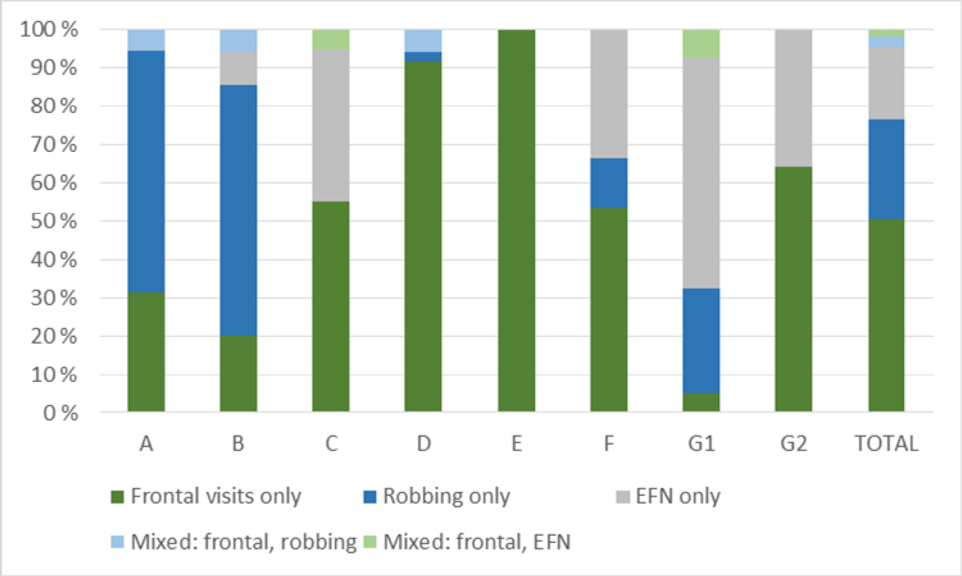

Figure 21. Types of *A. mellifera* floral behaviour and the occurrences (%) on different faba bean fields. Letter codes for the fields are explained in Table 3.

6.3 Pollinator abundance and species diversity

The most abundant species was *Apis mellifera*, representing 41% of all arthropod taxa recorded, with an average of 0.9 bees per square meter (±0.98 SD). *Bombus* spp. was found on average 0.7 bees per square meter (±0.93 SD), and accounted for 35% of all taxa recorded. The third most numerous taxon was Coccinellidae spp., 12% of all taxa, with an average of 0.2 per square meter (±0.12 SD). The remaining taxa accounted for 12% of species and included tachina flies (Tachinidae) (3%), hoverflies (Syrphidae) (2%), other Diptera (1%), soldier beetles (Cantharidae) (3%). Butterflies (Lepidoptera) were also seen (2%) and some wasps (Vespidae), shield bugs (Pentatomoidea), lacewings (Neuroptera), dragonflies and damselflies (Odonata) as well as spiders (Araneae).

As with foraging behaviour, the insect numbers on different fields varied greatly. In field A, honeybees were the most abundant species, accounting for over 76 % of all insects. On the other hand, on field D the most abundant taxon was *Bombus* s.str., 71%. In total, Apidae (*A. mellifera* and *Bombus* spp.) were most numerous on fields A, D and G (fig. 22).

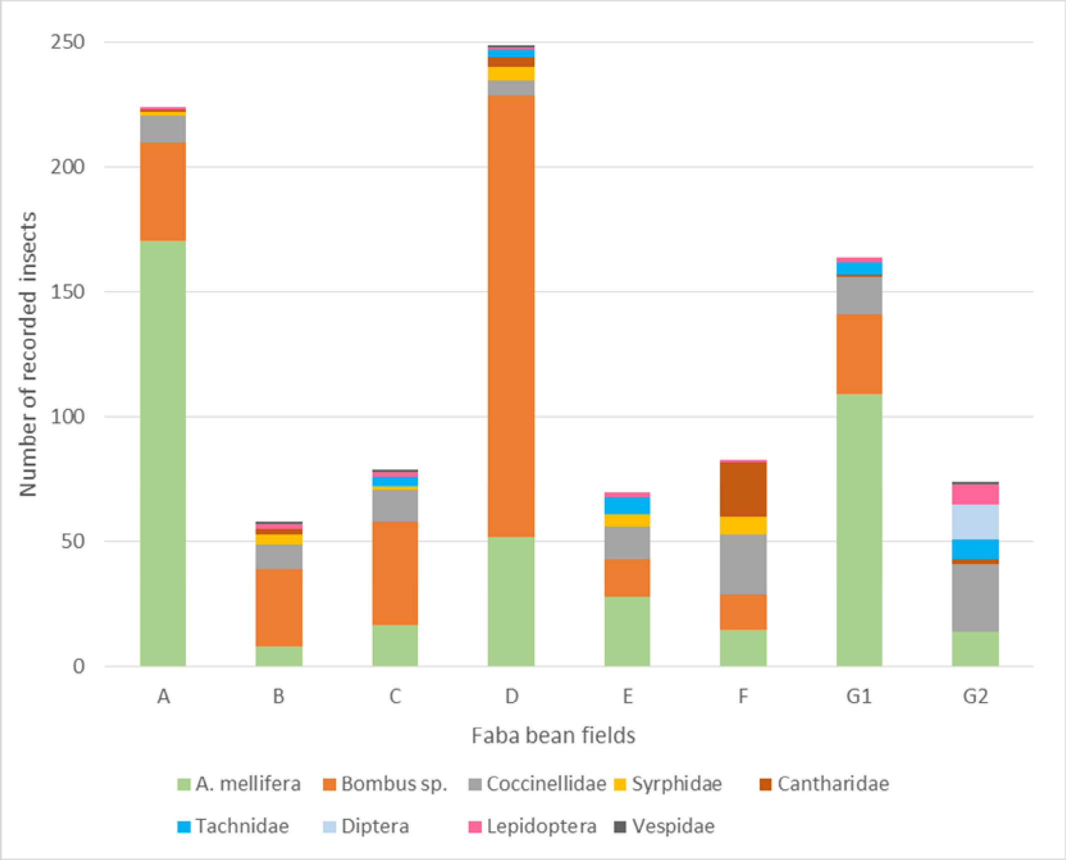

Figure 22. Number of insects of different taxa varied greatly between the eight faba bean fields. Letter codes for the fields are explained in Table 3.

7 DISCUSSION

This work has shown that faba bean cv. ‘Kontu’ benefits from bee-mediated pollination, with honeybees playing an important role. The crop was shown to be important for many other insect groups, particularly bumblebees.

7.1 Honeybee pollination effects

In this study the number of pods per plant was 54% higher and average pods per node was 19% higher in the treatment with bees than without. In previous studies on the yield benefits of pollination have shown differing results. Somerville in Australia (1999) recorded a 25 % increase in yield, Musallam *et al*. in Egypt (2004) in a 49 % increase and Cunningham and Le Feuvre in Australia (2013) recorded a 17 % yield increase. There are many factors that come into play with yield increase due to insect pollination.

Climate conditions, pollinator community (Stoddard 1993), difference in variety in autofertility and other reproductive traits in the plant itself or constrained experimental conditions can make results vary significantly.

Cages can have an effect on pollinator densities, pollinator movement and can limit the growth of the plants by restricting growth in the confined space or with shade (Blanche *et al.* 2006, Cunningham and Le Feuvre 2013). Small-scale studies can have skewed results, because results at flower or stem level do not usually translate well into the field or plot scale. In this experiment, the effect of the cage was significant. The plants were much taller and stronger in the field control. In the treatments, after the cages were removed, the stems of the plants were so fragile that almost all plants toppled within a day. The cage restricted the vertical growth and provided cover from wind.

The distribution of the pods on the plant stem suggests that statistically up to the middle of the plant, counted by nodes, bees have a pollination effect compared to the restricted cages, therefore making the pod distribution more even than without any pollination. It has been shown that pods formed form from bee-visited flowers have more seeds than those set by autofertility (Musallam *et al.* 2004). Kendall and Smith (1975) found that there was more cross-pollination in the lower nodes, and the upper nodes were more often pollinated by autofertile means. Environmental stress, such as heat can also affect pod set so that the first node to set is moved higher on the stem. Non-yield biomass was reduced while yield increased, suggesting allocation of resources elsewhere on the plant (Bishop *et al.* 2016).

This study does not include data about any other yield components than the pod counts. Number of seeds per pod was not measured, nor the overall yield harvested. The great increase in pod numbers by honeybee pollination, implies an overall yield increase since cross-pollination can also affects bean numbers per pod (Kendall and Smith 1975). Single seed weight stays relatively constant and the total seed yield is dependent on the seeds per plant (Thompson and Taylor 1977, Li and Yang 2014).

In terms of the yield components, seed size (mg) is mostly determined by pollination and fertilization of the flower, the pods per plant by genotype x environment, and the plants per m2 by management practices (Stoddard 1986b). Autofertility often gives only 1-2 seeds/pod (i.e. 0.5 seeds/ovule) whereas insect-mediated pollination allows 0.95

seeds /ovule. This means that insect-mediated pollination enables the fulfilment of the genetic potential of seeds per pod. A large ratio of pods with only one or two seeds, instead of the 3-4 achievable by the cultivar, will affect the seed number on the plant, thus adding less than expected to the overall yield (Li and Yang 2014). It is reasonable to assume the seed numbers to be higher on plants pollinated by front-visiting bees in this study, thus also increasing the yield.

Insects, especially heavier bumblebees can disrupt the barrier between stigma and anthers and trip the flower to self-pollinate even when there is no positive visit inside the flower (Kendall and Smith 1975). Hence, yield may benefit even if the main activity is robbing. In this case the cross-pollination rate would be low. In the cage experiment honeybees were used. The mouthparts of these bees are not strong enough to cut a hole in the base of the flower for nectar robbing and the insect itself is relatively small and light compared to the flower. Therefore in the cage experiment it is safe to assume that every visit to a flower was a positive one and tripping by the insect was done via burrowing into the flower. This results in pollen extraction by the bee and facilitates cross-pollination when moving from plant to plant. Furthermore, no scars from robbing (fig. 8) were seen on the caged plants.

The benefit to the beekeeper from the faba bean comes mostly from the added floral abundance when other flowering plants are not in flower, providing the honeybees with pollen resources (Bond and Poulsen 1983). In regard to the interests of the faba bean farmer, potential yield increase can provide additional income. With a recommended sowing rate of 60-70 plants per m^2, faba bean average yield in Finland in 2015 was 2 360 kg per hectare (OSF 2018a). With a 17% yield increase (Cunningham and Le Feuvre 2013), 400 kg/ha would be gained. Considering the average selling price in 2018 was 187 €/tonne, the yield increase would benefit the farmer approximately 75 € per hectare.

7.2 Honeybee floral behaviour

Honeybee behaviour was shown to be very supportive to potential cross-pollination. With average positive floral visitations being 1.5 flowers per plant, the rate is sufficient to enable cross-pollination (Garrat *et al.* 2014). However, honeybee pollination is very dependent on good weather conditions. Sub-optimal flight conditions during the best flowering days can therefore decrease the cross-pollination rate. Overall weather in June had more rainfall than average and on many days the temperatures stayed below 15 °C (the minimum temperature set for field surveys for honeybee floral behaviour).

Other factors to consider are the effect of time of the day and seasonal changes in foraging behaviour. Observations were made only once per field, on slightly differing times of day. The floral abundance close to the fields was not assessed. Different flowering plants nearby at time of observations may be a factor in the honeybee foraging behaviour. For example Cook *et al.* (2003) concluded that the honey bee prefers oilseed rape pollen over faba bean pollen under certain conditions.

Counts of robbed flowers with holes on them were not conducted in this study, but it was observed that open, mature flowers on every field had an aperture in the calyx at the base of the flower. Newton and Hill (1983) estimated in UK conditions, that as the flowering progressed, the number of pierced flowers on the field decreased. This might be due to alternative sources of nectar being available. Poulsen (1973) had a differing result in Denmark, where the portion of robbed flowers increased during the growing period. For honeybee pollination, this should have no effect, as the foraging via the positive visits is for pollen, but for nectar foraging these holes are needed. Thus, for the honeybee to be able to forage these flowers for nectar for honey production, the holes are important.

There were no positive visits seen with *Bombus* s. str. behaviour. This is backed by the low correlation from the regression analysis of the number of bumblebees on the field and pod numbers per node. Although bumblebees may cause tripping of the flowers while robbing, the visit would not result in cross-pollination. Poulsen (1973) observed the short tongued bumblebee to make positive visits to the flowers, but they were only about 20 % of total visits. However, long tongued bumblebees, such as the observed *B. hortorum,* can be very efficient pollinators (Poulsen 1973, Tasei 1976, Marzinzig *et al.* 2018).

7.3 Pollinator abundance and species diversity

Pollinator abundance in this study was observed to be very limited. Mostly honeybees and bumblebees were recorded making floral visits. Out of these, honeybees and only one bumblebee species was seen to make frontal visits. However, bumblebee abundance on the fields can have a clear benefit for honeybees in terms of nectar gathering. As explained in the previous chapter, the fact that observations were made only once per field limits the accuracy of the results. Other pollinator species and other insect species may have visited the fields during the long flowering period and the abundance of these may vary during the season.

Both positive and negative flower visits may have a positive effect on the yield (Kendall and Smith 1975, Navarro 2000). Therefore the most importance factors in pollination efficiency are pollinator activity (pollinating visits per flower) and abundance (pollinators per flower). Just one species visiting the flowers in sufficient quantities can be enough to facilitate the pollination of the whole plant population (Suso *et al.* 2001). In this study, the main focus on the species survey were potential macrofauna pollinators and no single species was generally identified. For a better understanding of the species diversity and wild pollinators, more thorough surveys are needed.

Sufficient wild pollinator abundance can be important to field crops especially when honeybees are not available. Less numerous pollinators can also be beneficial to the pollination due to pollinator abundance effect. Mass-flowering plants like the faba bean, that offer both pollen and nectar sources can be very attractive to bumblebees as well as other beneficial insects, increasing the overall pollinator abundance in the immediate area of the field (Köpke and Nemecek 2010). In fact, there is a synergistic effect of mass-flowering plants close to semi-natural grasslands on the abundance of solitary bees (Holzschuh *et al.* 2013).

More diverse habitats with natural and semi-natural surroundings can increase the flower visitor richness, visitation and fruit set of crops while honeybee visits stay the same (Garibaldi *et al.,* 2011b). For example, solitary bees have higher flower visitation rates in areas with more semi-natural habitats (Woodcock *et al.* 2013). Organic farming management and a more diverse environment can improve overall pollinator success in faba bean pod numbers and beans per pod (Andersson *et al.* 2008). The landscape structure surrounding the survey fields was not assessed, but there is an overall declining trend in semi-natural habitat patch densities in Finland (Hietala-Koivu *et al.* 2004). Managing these landscapes may increase wild pollinator abundance. However, in order to promote the most cost-effective conservation measures, it is essential to know which species are most important for the crop and choose a strategy suited for them specifically (Kleijn *et al.* 2015). For example, the efficient faba bean pollinator *B. hortorum* can benefit from increasing the nesting sites near the crops. This species prefers field-forest, semi-natural boundaries with tussocks (Kells and Goulson 2003).

Climate change can affect faba bean pollination in multiple different ways, including drought, heat and change in pollinator species diversity (Stoddard 2017). Water deficit may result in low pollen deposition and germination (Stoddard 1986b), while heat stress can affect the early flower formation and damage pollen (Bishop, Jones and Potts 2016). The warming climate may also increase the numbers of long-tongued wild pollinators by increasing their suitable habitat northwards (Martinet *et al.* 2015, Rasmont *et al.* 2015). This might mean better pollination efficiency by wild pollinator species in the future.

Interestingly, there was a negative correlation found between the number of Coccinellidae on a field and the average number of pods per node. This might indicate that the bees are less likely to land on the flowers with beetles on them. Kirk et al. (1995) found that bees are less likely to choose flowers with the pollen beetle *Meligethes aeneus*. What is more, a large population of aphids may attract ants that in turn can affect bee flower selection and discourage landing (Stoddard and Bond 1987). In this study, aphids and ants were not counted in the survey, even though they were found to be present on some fields. Coccinellidae are predators of aphids and therefore their numbers can correlate with the numbers of aphids (Freier *et al*. 2007). Ants and coccinellids might discourage bees from visiting the flowers, and thus have a negative effect on the pollination efficiency.

8 CONCLUSION

8.1 Practical implications

The positive effect on pod counts and pods per node by honeybee pollination was confirmed. Honeybees restricted in the cages made frontal visits and provided the plants with better pollination than found in the empty cage without the bees. Honeybees were also confirmed to cross-pollinate by foraging only in flowers from one species at a time and visiting flowers from different plants of the same species.

This research indicates promising results for the faba bean farmer considering commercial pollination services or integrating beekeeping into the farming in other ways. For the beekeeper, the bees do forage both pollen and nectar from the faba bean. For nectar gathering, the holes made by bumblebees are needed. This is likely not an issue, since holes were detected on all fields visited.

8.2 Theoretical implications

Providing good weather conditions, the faba bean yield increase can be considerably by insect-mediated pollination. The long-tongued pollinator *Bombus hortorum* adds significant value to pollination efficiency. Supporting wild pollinators with more diverse semi-natural habitats can be beneficial to pollinator abundance and thus, faba bean yield. The speed, size and behaviour of *B. hortorum* make it a very good cross-pollinator for the faba bean. However, the honeybee can compensate for its slower, less efficient pollination with abundance, thus making it an important species for faba bean pollination, especially when wild species are less numerous.

8.3 Future research

For the faba bean, a closer analysis of the yield components can generate more accurate predictions for the actual seed yield. The wild pollinator abundance research in this thesis was very limited. Therefore more thorough and comprehensive field surveys could reveal more interactions with the faba bean and bumblebees, especially long-tongued species, such as *B. hortorum*. The honey amounts gathered from faba bean fields, properties of the honey from the nectar and the pollen utilisation by the honeybee has not been studied in detail and calls for additional research.

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