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– Comparative study on pollinating insects and their efficiency in white clover fields

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– Jämförelsestudie om pollinerande insekter och deras effektivitet i vitklöverfält

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Abstract

Agricultural intensification has altered our environment, and many natural habitats for living organisms have been altered or completely disappeared. To restore and conserve biological systems while keeping the food supplies on an acceptable level, there is a demand for organic practice in the agriculture sector. 75 % of the plants for human consumption and animal forage are dependent on animal pollination therefor it is equally important to increase the variety of the cultivated plants and find sustainable solution that is relatively cheap to grow. Due to its special characteristics (nitrogen fixing ability, highly adaptable to abiotic factors) white clover seem to be a good solution. It provides excellent forage for grazing animals and moreover for pollinating insects. There have been many studies conducted to find out what the most important factors are which could give a satisfactory yield by the least effort and least financial investment. There is an ongoing high-tempered discussion whether it is possible to maintain the harvested seed level without chemical pest control. To find more insight, this study was carried out on both organically (7 fields) and conventionally (6 fields) managed fields in the southern part of Sweden. All the fields were designated for seed production. This study includes 3 surveys on pollinator abundance and diversity regarding white clover pollinating insects. Measurements were taken and recorded on abiotic (sun dominance, wind strength, temperature) and biotic factors (number of blooming white clover flowers, number of florets/flower head, number of seeds 2 weeks after the surveys) and landscape characteristics (field borders, other landscape element edges, flowering crops, land use diversity). The study aim was to investigate how pollination activity and pollination effectiveness differs between the two farming systems and to find factors that explain the results. The results showed that there was a significant difference between the amounts of visiting pollinators, benefiting the conventionally managed fields. Among the abiotic factors, temperature had the strongest effect on the pollination activity. There was a positive correlation between the amount of honey bees and the amount of bumblebees and wild pollinators. With regards to landscape characteristics, the investigation showed that honey bees (*A. mellifera)* abundance negatively correlated with the area of field borders and landscape element edges within the 2 km and 3 km buffer zone. Wild bee abundance negatively correlated with the Simpson land use diversity index within the 2 and 3 km buffers zones. However, the seed production and the pollinator abundance showed no correlation. Conclusively, there is an interesting connection to follow up with regards to landscape features and the amount of pollinators and other effecting factors.

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1. Introduction

Many wild flowers and cultivated crops are pollinated by insects, and in particularly by bees (Potts et al. 2010). The majority of crops worldwide, an estimated 75 % (IPBES, 2016), that constitute human food sources are dependent on insect pollination. This connotes the fact that a decline of pollinator diversity (Biesmeijer et al. 2006; IPBES 2016) would lead to decreased food supply worldwide (Gallai et al. 2009; IPBES 2016; Potts et al. 2010). In Europe around 12 % of all croplands are dependent on pollination by wild and domesticated bees for a satisfactory yield (Schulp et al., 2014). Therefore the conservation measures of pollinators and their status assessments is a major issue (Potts et al., 2016, 2010; Vanbergen et al., 2013). The most commonly known contributing species in crop pollination is the honey-bee, *Apis mellifera* (Potts et al., 2010). Wild and solitary bees also have an influential role in the pollination (Corbet, Williams, Osborne 1991a.b.; Williams 1996), and in the Nordic climate especially bumblebees can be more effective due to their adaptation to cold and rainy weather conditions (Willmer et al. 1994, Corbet et al. 1993). According to Bommarco et al. (2011) there has been a radical shift in bumblebee community evenness and relative abundances in Sweden due to intensification and changed agricultural practices. In the last 70 years, there has been a significant habitat loss for bumblebees, resulting in the complete absence of some species and a relative dominance of the buff-tailed bumblebee (*Bombus terrestris)* and the red-tailed bumblebee (*Bombus lapidarius)*.

White clover

White clover is a quickly growing and establishing forage plant which can be used as "living mulch" (SARE, 2012). It has become popular among organic farmers due to its tolerance of extreme weather conditions and its wide range of soil tolerance (SARE, 2012). Moreover, it can control erosion by its intensive growth and as it is a dominating plant it can suppress weeds and thereby provide a non-chemical weed management (SARE, 2012). But prominently, it improves soil quality by fertilizing it through its nitrogen fixing ability (SARE, 2012).

White clover is also known as a crop used for animal fodder and due to its special characteristics as persistency, broad adaptability and perennial nitrogen producer, it can be fitted well in the sustainable agriculture crop rotation.


Figure 1. White clover, Trifolium repens (Wikipedia)

White clover is seeded for animal grazed pastures in North America and northern Europe (Frame; Newbould, 1986) because of its high nutritive value and feeding value (Caradus, Woodfield Stewart, 1996). According to Frankow-Linberg et al. 1996, Swedish forage production is relying on a limited number of possible crops due to the harsh winter circumstances, therefore the usage of white clover has expanded and the demand for white clover seeds increased (Frankow-Lindberg et al., 1996). Because of its climate conditions Sweden could be an important white clover seed producer, and several efforts have been made to ensure homogeneous, reliable yields that can meet the market demand. The most important factors (weather conditions, supplementation of pollinators, field size, landscape complexity, usage of chemicals, etc) determining seed yield have been studied, but there are still many questions to be answered.

*Figure 2. White clover plant, flower, pod with seeds (*Wikimedia commons*)*

Since autogamous self-pollination is rare in white clover, it has a high genetic variation making it able to easily adapt to many different habitats and conditions (Australian Government, The Biology of *Trifolium repens* L. 2008).White clover has elliptic to egg-shaped leaves that are composed of three leaflets, and grow along stolons. The flower heads (generally 1.5-2 cm wide) consist of 40 to 100 florets on long stalks originating from the leaf axils. Florets are usually white but can be found with pink hue as well. The flowers bloom from June to September and 12 days after the pollination, seeds start to develop. The seeds ripen after a month and the pods generally contain 3-6 seeds weighting from 0.5 to 0.8

gram (Bond et al., 2007). In crops grown for seed production pods can have 1-7 seeds (Goodwin et al., 2011)

White clover can reproduce both in asexual and sexual ways. The sexual reproduction tends to occur when the environmental conditions are non-optimal for vegetative reproduction, such as drought or low temperature (Australian Government, 2008). To set seed, white clover is dependent on insect pollination and it is most commonly pollinated by honey bees (*Apis mellifera*) and bumble bees (*Bombus spp*.) (Australian Government, 2008, Van Der Kooi, 2015). Pollinators are attracted by the flower`s nectar (Australian Government, 2008). According to Green (1956, 1957) 1.2 and 0.39 bees are necessary per m^2 to guarantee pollination of white clover.

Pollinators

There are around 2, 000 species of pollinators, including bees, butterflies, moths, birds, bats, beetles and other insects that are contributing with pollination in Europe (STEP, 2015) . While they visit flowers to forage, they also transport pollen from plant to plant and thereby fertilize them (USDA, 2009). The European honey bee (*A. mellifera*) is the most commonly known pollinator species; it is mostly managed by humans by rearing in hives. (Nserc-Canpolin, 2012). To be able to sustain the colony, honey bee workers start to collect pollen and nectar already at 15^oC, but the nectar flow is best at 18^oC or above (Herbage, 2005). The working hours are determined by the daylight and are not dependent on the cloud cover, but strongly reduced by heavy rain and wind (Corrigan, 2017a). Honey bees can fly up to 8-13 km but prefer the least possible distance to forage (Hammond, 2009).

Bumble or humble bees are eusocial just as honey bees, also building colonies. They are comparatively large and hairy and make a humming sound as they fly. Bumble bees nest in open natural areas, and the queen stays hibernated during the winter period in wood residues or in burrows (Corrigan, 2017b). Bumble bees consume pollen and nectar but do not make a remarkable amount of honey. Their mandibular organ is relatively long, making them able to collect nectar from deeper, narrower flowers than many other bees. Moreover, bumble bees have a special sonication manner, providing an ability to dislodge pollen. Bumble bees pollinate a number of important plants such as blueberry (*Vaccinum spp*), tomatoes (*Lycopersicon spp.*) and several orchard crops. In Sweden there are 37 different bumble bee species (Jordbruksverket, 2016), the buff-tailed bumble bee (*B. terrestris*) is also domesticated and used in a similar way as *A. mellifera* (Corrigan, 2011). To some extent, bumble bees are known to be better pollinators than honey bees since they can withstand cold temperature (active from 10 ^{o}C)(Jordbruksverket, 2007), wind and rain due to their ability to regulate their body temperature using solar radiation (even with cloud coverage) and their "shivering" capability (Heinrich, 1981). For foraging they fly up to 1-2 km from the nesting site.

2. Aim and Hypothesis

This study aims to investigate the differences between organic and conventional farms with regards to pollinator abundance, composition and biodiversity, and also to investigate the pollinator's effectiveness by measuring the seed setting. This study will investigate how biotic and abiotic factors correlate with pollinator activity. In addition, I will investigate how the surrounding landscape composition can support pollinator populations.

In an ecosystem there is always a continuous conjunction between the living entities and the non-living constituents (Encyclopedia Britannica, 2015). In this study I will try to investigate some of these interactions in regards of landscape elements by using landscape complexity measures and abiotic factors such as sun dominance, wind strength and temperature. A comparison of two farming systems, organic and conventional will be included. Although the mineral content of the soil can have a wide effect on the biodiversity, it is not within the scope of this study.

Based on my literature research, these predictions were posed:

Hypothesis 1

Conventional fields will have lower abundance of pollinating insects and lower biodiversity than organic fields.

Several studies have claimed that there is a positive correlation between species complexity and abundance of bumble bees at organic farms (Holzschuh 2007, Rundlöf et al., 2008; Williams & Kremen, 2007) due to the strict regulations on prohibiting the usage of inorganic fertilizers and pesticides and herbicides (Grandi, 2011.; Gomerio et al., 2011). Therefore my basic concept is that I will find greater abundance and higher biodiversity of white clover pollinating insects on the organic fields. Since wild bees have been shown to be the most affected species of the agricultural modernization (Steffan-Dewenter et al., 2005) my general notion is that this difference will be more clearly evincible on wild bee species.

Abundance and species composition of pollinating insects will be examined through pollinator surveys. Three of the most influencing abiotic factors (temperature, sun dominance, wind strength) will be recorded and examined in relation to the pollinator activity. In addition, information on some of the affecting biotic factors, the complexity of the landscape around the surveyed fields (within a 1,2 and 3 km radius) in regards of abundance of flowering crops and proportion of field borders and landscape element edges, will be collected. A comparative analysis will be performed between the used farming systems to be able to tell whether the presence of the pollinating insects were a result of the used farming system. The factors will be compared with the overall pollinator abundance to be able to see their relation.

Hypothesis 2

The more complex landscapes will show a higher abundance of pollinating insects.

Several studies have shown close relationship between the pollinator densities and species richness and the landscape elements (Kallioniemi et al., 2017; Holzschuh et al., 2007) such as flower density at both local and landscape level (Kallioniemi et al., 2016), amount of forested area (Garibaldi et al., 2011; Kremen et al., 2004; Zulian et al., 2013), as well as the neighboring agricultural crops (Garibaldi et al. 2016). The main focus will be given to the land use diversity and flower resources in the ambient environment by measuring the diversity of landscape elements including land use properties, amount and diversity (Simpson diversity index) of flowering crops, the proportion of semi natural areas and field borders and landscape element edges. Semi natural areas and field borders usually supports flowering plants and therefore tends to influence the pollinator occurrence (Kallionemi et al., 2016). A more complex landscape, in this study, refers to a landscape with a higher diversity in land use, more diversity in flowering crops and bigger proportion of field borders and borders and edges of other landscape elements (i. e smaller fields and more landscape elements: more heterogeneity).

Hypothesis 3.

The number of seeds will positively correlate with the number of visiting pollinators

Due to the fact that white clover is self-incompatible and the flower morphology does not support wind pollination, insect pollination is required for seed setting. Thus I assume that there will be a positive correlation between the number of seeds and the number of visiting insects (Australian Government, 2008, 2005; Thomas, 2017).

3. Materials and Methods

The data collection for this study was carried out in 13 fields in Scania (southern Sweden) where white clover was grown for seed production. A list of fields categorized by management and ranged by size (ha) including the white clover cultivar is shown in *table 1*. The grown white clover cultivar was unknown in some cases. All the organically managed farms had KRAV certification which is in accordance with EU regulation for organic production (EC) No 834/2007 (KRAV, 2017).

Table 1. The surveyed white clover fields categorized by their management and listed by their size and cultivar (n.a.= information was not available about the cultivated white clover type at the time of this study).

	Field	Size (ha)	Cultivar
Conventional	BOE	5.5	Lena
	SAK	7	n.a.
	OLI	8	SW Hebe
	SVD	9	SW Hebe
	HAS	10	Bombus C
	STW	18	SW Hebe
Conventional Total		**57.5**	
Organic	ACG	5	n.a.
	LAJ	12	SW Hebe
	BEA	13	Undrom
	KRA	22	Jura C krav
	HOO	28	Bombus C krav
	OTE	31	n.a.
	GAN	52	n.a.
Organic Total		**163**	

Pollinator surveys

Picture by Szilvia Johansson

The surveys for pollinator activity were conducted during the summer of 2016. At each field there were three survey rounds conducted between the 6th of June and 12 of July, during the blooming season of white clover. The surveys were preferably performed during certain weather conditions; no more than moderate wind, at least 17^{o}C and at least 30% sun dominance. However, as it was important to collect enough data, some of the surveys did not have the most optimal weather conditions (in some cases the temperature was only 14 C^{o}).

The survey method was previously set up and followed the same procedure as in the Clover project at SLU (SLU, 2017), to enable comparisons of datasets among years. Surveys were conducted along 2 transects in each clover field. Each transect was 50 m long and situated 8-12 meters into the field (*Figure 3*). Six flags (F1-F6) were set out along the transect, with a distance of 12.5 meter from each other as well as from start and stop of the transect. The flags marked the spots for registering inflorescent data and marking of flowers in bloom (explained later). In the case of organic farms, only 3 physical flags (F1-F3) were set out, due to practical reasons related to the rest of the clover project, but the registration of inflorescent was carried out where F4-F6 would have stood also in the organic fields.

The time of execution for the pollinator surveys was 10 minutes (plus additional handling time).

In each field there were two 50 m long and 1 m wide transects (T1 and T2). In organic fields T1 and T2 received the same treatment as the entire field (no insecticide treatment) but in conventional fields T1 was situated in a spray-free zone (24 x 50 m) and did not receive any insecticide treatment (it served as a control plot in other parts of the Clover project (SLU 2017)), whereas T2 were treated as the rest of the field.

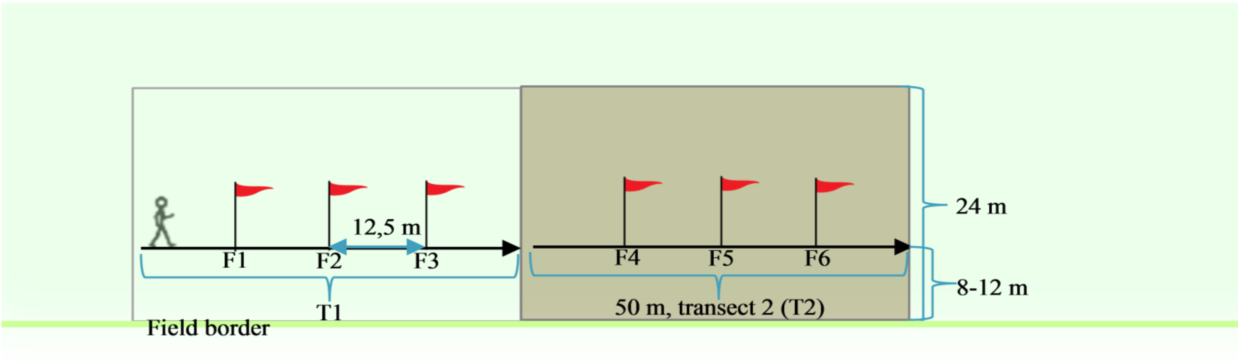


Figure 3. Schematic drawing of the surveyed area, two 50 meters transects. In the case of conventional fields T2 transects were treated as the rest of the field, while T1 did not received any chemical treatments.

During the survey time, the observer walked slowly along the transect and noted all observed pollinators. Bumble bees were collected while all the other pollinators and flying insects were only counted. Bumblebees were identified later in the lab using identification key (Mossberg, Cederberg, 2015; Falk, Lewington, 2015). Solitary bees were categorized as a single group, no further identification was conducted.

Abiotic factors

Four different abiotic factors such as time of the survey, temperature, wind strength, and sun dominance were recorded and incorporated into the statistical analyses, in order to be able to draw conclusion whether the lower pollination activity was due to actual weather conditions or if it was a result of the biotic factors of the surveyed field. Temperature was recorded at the beginning of each survey and the wind strength was determined according to the Beaufort wind scale (SPC, 2017) during the time of survey. Sun dominance was determined by counting the percentage of minutes with sun during the survey time.

Flower frequency assessment

According to Ebeling et al. (2008) flowers in blossom could be an important factor for the pollinator visiting frequency, thus flower frequency assessment was included in the survey.

As the visited fields were in different phenological stages at each survey occasion, flower frequency assessments were carried out in every 12.5 meter, equaling 6 spots at each field (at F1-

F6 in *Figure 3*.). At each spot a frame with an area of 0.5m X 0.5m was placed and within the framed area each flower, bud and over-bloomed flower was counted (according to *Table 2*). If the flower head had more than 5 blooming florets, it was denoted as "in bloom", otherwise as "bud" or "over bloomed", depending on if florets where in bud stage or over bloomed.

Table 2. Flower frequency categories for phenological assessment: At 6 spots in each clover field, a frame of 0,5 x 0,5 m were placed and all flower heads within were categorized according to descriptions in the table below.

	Phenological status		
	Bud	In bloom	Over bloomed
Description of the flowers in the flower head	Mostly buds and less than 5 blooming flowers	More than 5 of the flowers on the head are blooming	Mostly over bloomed but less than 5 blooming flowers

Seed counting

At each pollinator survey, 5 fully blooming flower heads standing adjacent to a flag, were chosen and marked. Meaning that in total 5x6 flower heads were marked at each conventional field and 5x3 flower heads at each organic field during each survey round. These flower heads were then collected two weeks after marking and provided seed setting data enabling us to evaluate the pollination efficiency. The collected flower heads were stored in the freezer to prevent potential pests present in the flowers to hatch and eat the seeds. From each survey round three flower heads were examined randomly (one from each flag, F1, F2, F3 from the organic, and F4, F5, F6 from conventional fields, see *Figure 3*.) by counting the florets and their seeds. Which flower head from the five heads, from each site, that was to be examined was picked randomly.

Florets were removed from the flower head, counted and mixed within each batch; thereafter 25 florets of each batch were randomly chosen for seed counting.

The method was standardized on a principle that the seed counting was continued up until it was done on 25 healthy florets per head.

During the visual examination florets that were damaged (small holes) by insect pests were also noted and the numbers of seeds in them were recorded.

Seed set potential

Seed potential was calculated in order to estimate the number of seeds one flower head could potentially carry if all florets where sufficiently pollinated. The calculation was based on the assumed average seed production of one pod. According to Thomas (1987) in white clover there are at least 5 ovules per floret.

Pollination efficiency

After the seed counting results, assessment of the pollination efficiency was carried out using statistical analysis. Only healthy florets were used to calculate the proportion of florets containing seeds.

Statistical analysis

The statistical analyses were made in SPSS software (ver 22.).

To test whether the number of pollinators was influenced by time of survey or the farming system I used a nested ANOVA including the factors survey round, farming system and their interaction. I also included field as a random factor nested under the faming system type. In regards to pollinators I created three different groups and used them as dependent variables, *Apis mellifera*, *B. terrestris*, and wild bees. The pollinators were divided in to these groups because the presence of *A. mellifera and B. terrestris* to full extent, respectively to some extent, were manipulated by humans. The third group, named wild bee group, consisted of all the other *Bombus spp.* and the solitary bees that were observed during the surveys.

In regards to abiotic (weather data on sun dominance, temperature and wind strength) and biotic factors (number of blooming flowers and interaction between the species) I used the Nested Anova test, including the survey round (survey 1, 2, 3), farming system and interaction between these factors. In compliance with test on the pollinator data (see above) I included the field as a random factor nested under the cultivation method.

To test whether the number of healthy seeds was influenced by time of survey or the farming system I used a nested ANOVA including the factors survey round, farming system and their interaction. I also included field as a random factor nested under the faming system type.

Correlation tests were made between the number of pollinators divided into three groups and abiotic (average sun dominance, average of temperature, average of wind strength) and biotic factors (number of blooming flowers at the time of visits, number of florets/healthy seeds counted from the heads collected 2 weeks after the surveys).

Biodiversity calculation

Since diversity indices are calculated differently and therefore represent partially different aspects about the specific habitat/ecosystem, I have here calculated the most commonly used ones, the species richness (S), the evenness (J), Shannon index (H) and Simpson index (D).

A Species richness value exhibits the number of species in the sample or the sampled area. The weakness of this index is that it doesn't take into account the proportion of each species in the ecological system. Shannon index (also termed Shannon-Wiener index) takes into account species richness and proportion of each species in any biological community. It also accounts for both abundance and evenness of the presented species (Benedek, 2012). Meanwhile Simpson index expresses more the dominating type/species (Nagendra, 2002). The Simpson diversity was calculated on the pollinator abundance. The following equations were used for calculating (Magurran, 2004).

Shannon index: $\mathbf{H'} = -\sum p_i \ln p_i$

Simpson index: $\mathbf{D} = \sum p_i^2$

Landscape variables

The landscape variables were calculated with the help of GIS software for three different buffer zones, with a radius of 1, 2 and 3 km around each field where the surveys were carried out. These distances were chosen according to previous studies of landscape effects on pollinators, saying that bumblebees can possibly fly up to several kilometers to collect nectar (Osborne et al., 2008), but the most frequent distance for foraging is less than 1 km (Hagen et al., 2011) From a data file, received from Läntmäteriet (Swedish National Land Survey) which included information on land use (GEODATA) such as cultivated land, forest and urban area, I could calculate a landscape land use diversity index i.e **Simpson's land use diversity index**. This was done following Persson et al. (2010) and Magurran (2004) by calculating the proportion of land, within each buffer zone, devoted to either of the 6 categories (land use types): arable land, semi natural areas, wetland and water, forest, urban areas, and urban green areas. The Simpson's land use diversity index was calculated as -ln(D), where D is the sum of squared proportions of each land use type within the buffer zone. As in the equation below:

Simpson index: $\mathbf{D} = \sum p_i^{\,2}$

According to Rundlöf et al. (2014) mass flowering crops (MFC) are naturally important for pollinators. Therefore the **proportion of mass flowering crops** (% area of the buffer zone area) close to the focal fields (within the same buffer zones as mentioned above) was calculated with ArcGIS software and included in the analysis. This data was obtained from the Integrated Administration and Control System (IACS), which is managed by The Swedish Board of Agriculture. As linear elements connected to the surveyed areas also can be used as a describing factor to derive the "capacity of agro-ecosystem that enhance the pollinator services" (Zulian et al., 2013), **area of field borders** and landscape element edges in the buffer zones was obtained from the same data and included.

The landscape variables (Simpson's land use diversity index (=-ln(D)), area of field borders and other element edges and percentage of flowering crops) that were all calculated for the 1, 2 and 3 km buffer zones were included and used for statistical analyses separately at each field.

The landscape variables were tested on pollinators by each survey round to be able to include the temporal changes under the entire period of the season; and on all survey rounds together

Implementation and limitations

As this study is aimed to focus on the differences between the organic and the conventional fields, data from the conventional fields were only taken from the T2 transect at each survey round (the spray-free zone and T1 is used as a control and is to be analyzed in another project).

In a case of organic fields, I have corrected the pollinator data and made an average number of the two transect (T1 and T2).

Both conventional and organic fields had supplemented hives of *A. mellifera* and *B. terrestris* placed in the fields during the season, but the exact time of the hive set out is not known, neither the number of colonies used. Since the farmers could not provide reliable data during the time of this thesis work, this study disregards the supplemented stocking data. Instead, I have tested the statistical analyses both with and without these domesticated groups of bees. Since the cultivar was unknown to us in some cases, the difference between cultivars was also not included in the focus of this study.

4. Results

Hypothesis 1. Species abundance and diversity of pollinators

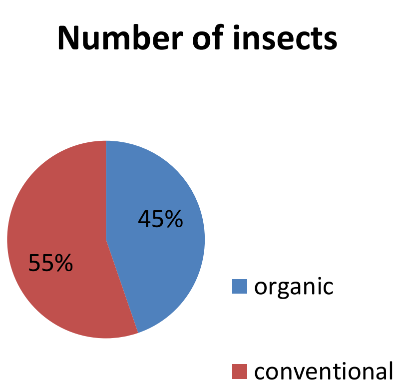


Figure 4. Number of insects in percentage observed during the surveys sorted by the farming system (blue = organic, red = conventional).

There were a total of 3947 individual insects recorded during the surveys, including hoverflies, butterflies, and other flying insects, of which 2182 were observed in conventional fields and 1765 in organic fields (*Figure 4)*. As the table shows (*Appendix 1, Table 1*.) the most dominating species was the honey bee (*A. mellifera*) with 46 %, of all the observed insects, whereas the other main group of clover pollinators, bumble bees (*Bombus sp*.) only constituted 14 % of the total. Hoverfly (*Sphaerophoria sp*.) abundance was relatively high (28 %). The highest abundance of **insects** was observed at OLI (conventional, field size 8 ha) (404 recorded individuals) meanwhile the lowest was observed at BEA (organic, 13 ha) (189 insects recorded) (*Table 3*.).

Table 3. Number of recorded insects and the number of pollinators (the sum of the three survey rounds) sorted by field and farming system.

Farming type	Field	Number of insects	Number of pollinators

conventional	OLI	404	299
	BOE	390	290
	STW	289	246
	SAK	390	216
	HAS	369	210
	SVD	340	169
conventional Total		**2182**	**1430**
organic	HOO	248	189
	OTE	240	182
	LAJ	320	148
	GAN	218	144
	KRA	330	87
	BEA	189	85
	ACG	220	74
organic Total		**1765**	**909**
Grand Total		**3947**	**2339**

White clover pollinators

The number of white clover **pollinators** (only honey bees, bumble bees and solitary bees) recorded was 2339; 1430 were found at the conventional fields and 909 at the organic fields. The highest abundance of pollinators belonged to OLI (conventional, field size 8 ha) (299 recorded individuals) meanwhile the lowest number of pollinators was found at ACG (organic, 5 ha) (74 recorded individuals) when summing up the three survey rounds (*Table 3*). *Figure 5* shows the mean number of pollinators at each field.

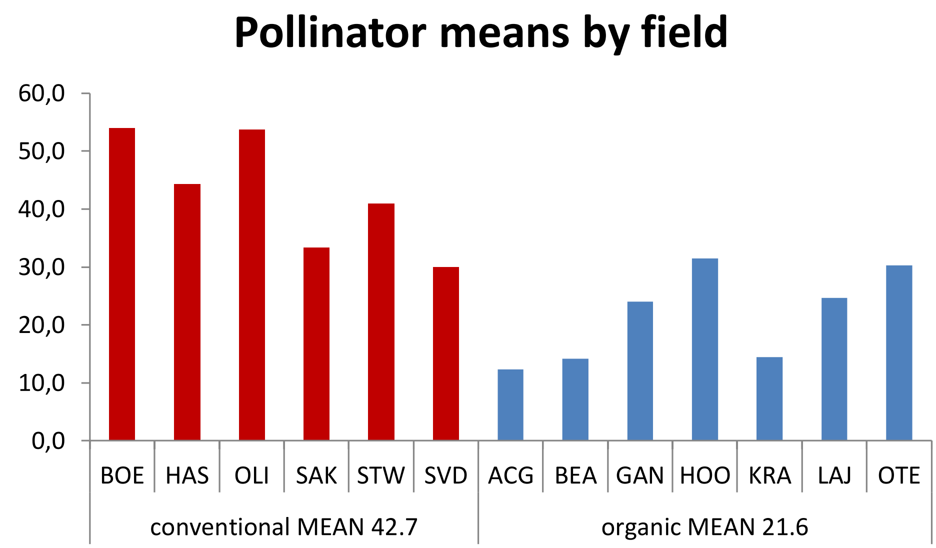


Figure 5. Mean abundance of pollinators sorted by the two types of farming system, including field ID

As the study aimed to compare white clover pollinator presence between the two farming systems, the nested ANOVA test was made both for the total number of pollinator individuals recorded and on the three pollinator groups. There was a significant difference between the fields representing the two types of farming system with regards to the abundance of all pollinators, but there was no significant difference between the three survey rounds (*Table 4)*.

Table 4. Nested ANOVA test on all the pollinators recorded during the season, random factor field and survey rounds as fixed factor. Significant values (p<0.05) shown in bold. Type refers to farming system type. Survey refers to the survey round.

Source of variation	Dependent variable: All pollinators		
	df/Error	F	p
Type	1/11	17.873	**0.001**
Survey	2/22	3.161	0.062
Type*Survey	2/22	2.590	0.098
Type(Field)	11/22	0.516	0.872

The figure below (*Figure 6.*) shows that conventional fields had higher abundance of pollinators (average number of pollinators by survey) than organic fields at each survey round.

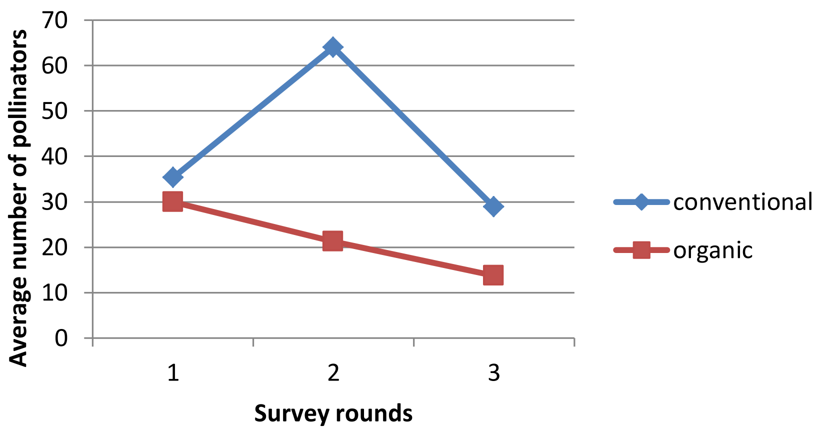


Figure 6. shows the average number of pollinators by farming system at each survey round

The Nested ANOVA test showed (*Table 5.*) a significant difference between the farming system in the abundance of *A. mellifera* (p=0.003) but not for *B. terrestris* (p=0.083), or for the number of wild bees (p=0.987).

Table 5. Nested ANOVA test on the three groups of pollinators by adding field and survey rounds as random factors. Significant values (p<0.05) shown in bold. Type refers to farming system type. Survey refers to the survey round.

Source of variation	Dependent variable								
	Apis mellifera			*B. terrestris*			Wild bees		
	df/Error	F	p	df/Error	F	p	df/Error	F	p
Type	1/11	13.788	**0.003**	1/11	3.638	0.083	1/11	0.000	0.987
Survey	2/22	4.229	0.28	2/22	1.692	0.207	2/22	0.318	0.731
Type*Survey	2/22	3.351	0.54	2/22	1.057	0.364	2/22	0.060	0.942
Type(Field)	11/22	0.718	0.71	11/22	1.935	0.090	11/22	1.470	0.212

Correlations between the abundances of different pollinator groups were tested and showed that there was a strong positive correlation between the number of *A. mellifera* and the number of *B. terrestris* (N=39; Pearson correlation; r =0.446, p=0.004), and also a positive correlation between the number of *A. mellifera* and the number of wild bees (N=39; Pearsson correlation; r =0.324, p=0.044).

Abiotic factors

To be able to answer my question on what factor could be the most affecting one that could explain the difference in the pollinator abundance (see *Table 5)*, I analyzed the pollinator abundance with abiotic and biotic factors.

Firstly, I compared the abiotic factors, temperature, sun dominance and wind strength (average per field) between the two farming types, using a Nested ANOVA test (*Table 6*), where the random factor was field and the fixed factor was survey round. The results showed that there was a significant difference in the **sun** dominance.

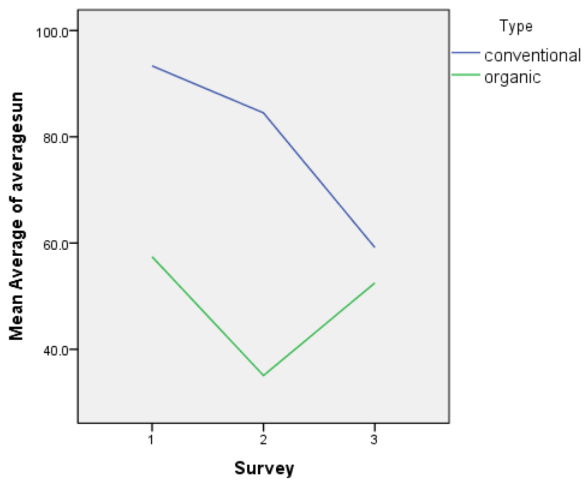


Figure 7. The sun cover throughout the survey rounds, organic farms had lower amount of sunny minutes at each round.

There was always more sun dominance at the surveys of the conventional fields (*Figure 7.*). There was however no difference in the wind strength (*Table 6.*).

There was also a significant difference in the temperature among the survey rounds connected to the farming type category. The average of temperature at the conventional fields was 19.1°C, with the highest at the second survey round (22.2°C). At the organic fields the average was 18.8°C, but

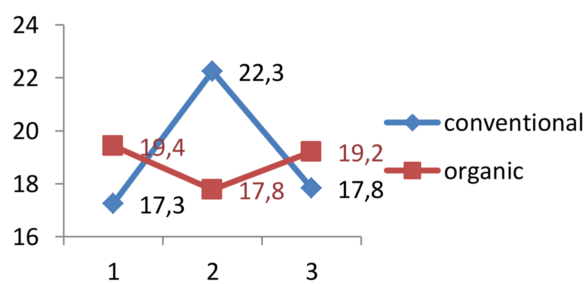


Figure 8. The mean temperature (°C) at the 3 survey rounds by farming type

here none of the survey rounds mean temperature reached 20°C (*Figure 8.*)

Table 6. Nested ANOVA test on the abiotic factors, (random factors: field and survey). Significant values (p<0.05) shown in bold. Type refers to farming system type. Survey refers to the survey round.

Source of variation	Dependent variable								
	Temp			**Sun**			**Wind**		
	df/Error	F	p	df/Error	F	p	df/Error	F	p
Type	1/11	0.186	0.669	1/11	8.801	**0.006**	1/11	1.189	0.283
Survey	2/22	2.307	0.115	2/22	1.333	0.278	2/22	2.587	0.090
Type*Survey	2/22	8.953	**0.001**	2/22	1.490	0.240	2/22	2.827	0.074
Type(Field)	11/22	0.718	0.71	11/22	1.935	0.090	11/22	1.470	0.212

Correlations between the abiotic factors (temperature, sun dominance and wind strength) and the abundance of different pollinator groups showed that the only **abiotic** factor related to pollinator abundance was the **temperature**. Here I found a correlation between the *A. mellifera* abundance (N=39; Pearsson correlation; r=0.612, p=0.000) and the temperature.

B. terrestris was not correlating with temperature, it was only close to the threshold of significance (N=39; Pearsson correlation; r =0.294, p=0.070), whereas wild bee abundance was not correlated at all with the temperature (N=39; Pearsson correlation; r =0.208, p=0.204).

When **abiotic** factors (temperature, sun dominance and wind strength) were **accounted** for in the analysis of abundance only the number of *A. mellifera* and the white clover pollinator abundance showed significant difference between the farming systems (*Table 7.*).

Table 7. Nested ANOVA testing the effects of farming system on pollinator abundance, while accounting for the average sun dominance, average temperature and the average of wind strength (Beaufort scale), with the field assigned as a random factor. Significant values (p<0.05) shown in bold. Type refers to farming system type. Survey refers to the survey round.

Source of variation	Dependent variable											
	Apis mellifera			*B. terrestris*			Wild bee			All pollinators		
	df	F	p	df	F	p	df	F	p	df	F	p
Type	15.549	10.588	**0.005**	13.088	2.965	0.109	17.396	0.027	0.871	18.821	14.509	**0.001**
Survey	18	2.186	0.141	18	0.899	0.424	18	0.141	0.869	18	1.075	0.362
Type*Survey	18	0.040	0.961	18	0.30	0.74	18	0.28	0.75	18	0.020	0.980

					7	0		8	3			
Type(Field)	18	1.037	0.456	18	2.265	0.061	18	0.731	0.697	18	0.590	0.813
Temp	18	13.398	**0.002**	18	3.066	0.097	18	0.091	0.766	18	10.629	**0.004**
Sun	18	0.000	0.988	18	0.284	0.601	18	0.408	0.531	18	0.001	0.976
Wind	18	0.001	0.970	18	1.639	0.217	18	0.022	0.882	18	0.068	0.797

Biotic factors

Correlation tests were made on **biotic** factors recorded each time of the surveys, including the number of blooming flowers/0.25m^2 and the number of florets per flower head (from the result of seed counting). Number of ***B. terrestris*** **had a positive correlation** (N=39; Pearsson correlation; r =0.366, p=0.022) with the **number of florets per flower head**, and the numbers of ***A. mellifera*** **and wild bees** were positively correlated with the **blooming flowers/0.25** m^2 (*A. mellifera*, N=39; Pearsson correlation; r =0.349, p=0.029; wild bees n=39; Pearsson correlation; r =0.485, p=0.002).

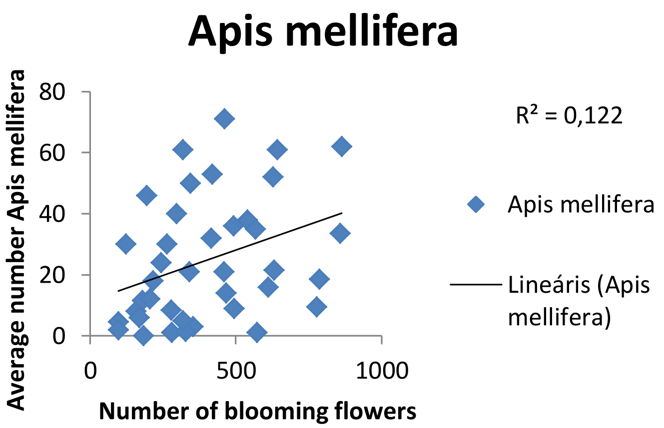
The figure below shows how strong the relation was between the summed number of *A. mellifera* and the blooming flowers/0.25 m^2 (*Figure 9.*), as well as the occurrence of wild bees related to the blooming flowers/0.25 m^2 found on the fields.

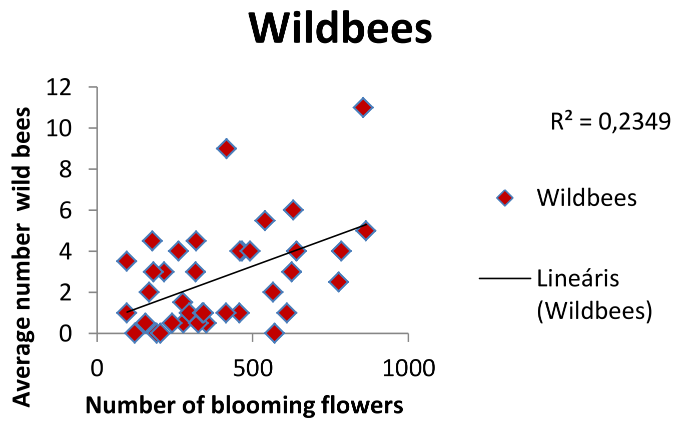
The Nested ANOVA test did not confirm any difference in the number of blooming flowers/0.25 m^2 between the fields belonging to the two different types of farming systems (*Table 8*).

Table 8. Nested ANOVA test on blooming flowers/0.25 m^2 with field as random factor and survey as fixed factor. Type refers to farming system type. Survey refers to the survey round.

Source of variation	Dependent variable Number of blooming flowers/0.25 m^2		
	df/Error	F	p
Type	1/11	0.179	0.681
Survey	2/22	1.315	0.289
Type*Survey	2/22	1.199	0.321
Type(Field)	11/22	1.499	0.201

Figure 9. Relation between (R^2) the number of A. mellifera and the number of blooming flowers/0.25 m^2 (R^2 = 0.122), respectively the number of wild bees and the number of blooming flowers/0.25 m^2. (R^2 = 0.2349)





Biodiversity evaluation

There were 22 different insect species recorded altogether. OTE (organic) had the highest species richness with a number of 14 different species, whereas GAN (organic) had the lowest with 6 different species observed (*Table 9.*). The species richness was higher in the organic (19 different species) than in the conventional (15 different species) fields. (Appendix 2, Table 1.).

OTE (organic) had the highest number of white clover pollinator species (10), while GAN (organic) had the lowest following the pattern of the total number of species of all insects (*Table 9.*).

.Table 9. shows the number of species (all insects and the white clover pollinating insects) at each field, found during the surveys and the biodiversity indices of the surveyed fields all together, sorted by their farming system type

		Species richness (S)		Shannon diversity index (H)	Simpson diversity index (D)	Hmax (all insects)	Evenness (all insects)
	Field	**all insects**	**white clover pollinators**				
conventional	BOE	9	5	1.76	0.20	2.20	0.80
	HAS	9	6	1.55	0.57	2.20	0.70
	OLI	10	7	1.60	0.77	2.30	0.69
	SAK	9	6	1.62	0.29	2.20	0.74
	STW	7	4	1.61	0.15	1.95	0.83

	SVD	8	5	1.71	0.16	2.08	0.82
conventional Total		**15**	**11**	**1.34**	**2.85**	**2.71**	**0.49**
organic	ACG	9	5	1.81	0.18	2.20	0.82
	BEA	8	5	1.83	0.16	2.08	0.88
	GAN	6	3	1.37	0.10	1.79	0.76
	HOO	9	7	1.77	0.13	2.20	0.81
	KRA	7	3	1.68	0.16	1.95	0.86
	LAJ	11	8	1.98	0.22	2.40	0.82
	OTE	14	10	2.04	0.17	2.64	0.77
organic Total		**19**	**13**	**1.42**	**3.53**	**2.94**	**0.48**
Grand Total		**22**	**16**				

Some of the species were only found in the conventional fields (*Table 10.*) (*B. subterraneus*), respectively in organic farms *(B. distinguendus, B. jonellus, B. muscorum, B. ruderarius, B. rupestris).* These species were present in very low numbers. *B. lapidarius* (n=127) was the most dominating species from the wild bee group followed by *B. sylvarum* (n=16).

Table 10 List of species and the number of individuals recorded during the surveys sorted by faming system

	Number of individuals		
	conventional	**organic**	**Grand Total**
Apis mellifera	1126	708	1834
B. distinguendus		1	1
B. hortorum	3	4	7
B. jonellus		1	1
B. jonellus/B. hortorum	1		1
B. lapidarius	60	67	127
B. lucorum/B. magnus	1		1
B. muscorum		1	1
B. ruderarius		8	8
B. rupestris		1	1
B. soroëensis	2	2	4

B. subterraneus	1		1
B. sylvarum	3	13	16
B. terrestris	219	86	305
Bombus sp	10	14	24
Melitta/Andrena sp.	4	3	7
Grand Total	**1430**	**909**	**2339**

The three biodiversity indices (D, H, H_{max}) were lower at the conventional fields meaning a lower biodiversity.

There was a positive correlation between the number of pollinator species and the percentage of empty florets (N=39; Pearsson correlation; r =0.509, p=0.001)

Hypothesis 2. Effects of the landscape variables

There was no significant difference between the two types of fields in any of the landscape variables, including Simpson's land use diversity index (=-ln(D)), field borders and other element edges (percentage of total area), or percentage of flowering crops in the three buffer zones (1, 2, 3 km).

The **landscape variables** were not correlated with the number of pollinators regarding any of the three groups when the correlation was made on the average number of each pollinator group for each survey round. The correlation was tested by survey round to be able to see time as a possible factor, meaning that e.g. the number of flowering crops or wild plants in the adjacent environment could change during the season and therefore only affect the number of visitors during certain surveys.

The test showed that in the third survey round there was a negative correlation between *A. mellifera* and the area of field borders and other edges (percentage of the total area) within the 3 km buffer zone (N=13; Pearsson correlation; r =-0.682, p=0.010) and also with the area of field borders and other edges (percentage of the total area) within the 2 km buffer zone (N=13;

Pearsson correlation; r =-0.637, p=0.019). In the third survey round, the wild bees were negatively correlated with the Simpson land use diversity index within the 3 km buffers (N=13; Pearsson correlation; r =-0.686, p=0.010), and with the Simpson land use diversity index within the 2 km buffers (N=13; Pearsson correlation; r =-0.570, p=0.042)

Hypothesis 3. Pollination efficiency

Pollination efficiency was calculated only from the florets that were healthy, by calculating the proportion of those containing seeds. In the case of conventional fields, 84 % of florets contained at least one seed, demonstrating that they had been pollinated, whereas in the organic fields the corresponding value was only 60 %.

The significance test on the percentage of pollinated **florets** showed a significant difference between the two types of farming system, as well within the farming system type (*Table 11*.).

Table 11. Nested ANOVA test on the percentage of pollinated florets, with field as random factor. Significant values (p<0.05) shown in bold. Type refers to farming system type.

	Dependent variable		
	Percentage florets with seeds		
Source of variation	df/Error	F	p
Type	1/11	5.778	**0.035**
Survey	2/22	1.634	0.218
Type*Survey	2/21	2.486	0.106
Type(Field)	11/22	3.896	**0.003**

Figure 10 shows a clear difference between the percentages of pollinated florets and that the conventional farms had a bigger proportion of florets containing seeds. The statistical analysis is shown in Table 11.

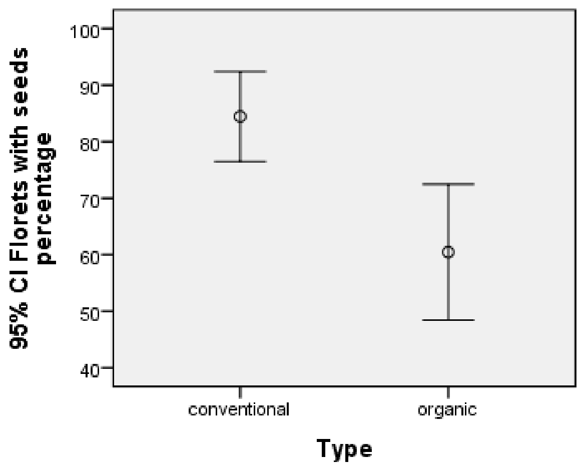


Figure 10. Florets with seeds percentage by farming system type.

There was no correlation found between the percentage of pollinated florets and the number of pollinators of the three groups. (Appendix 3) (*A. mellifera:* N=39, Pearsson correlation; r =0.213, p=0.194; *B. terrestris:* N=39, Pearsson correlation; r =0.266, p=0.101; wild bees: N=39, Pearsson correlation; r =-0.166, p=0.312).

Seed set potential

There were 4354 florets counted with a total of **6603 healthy** and 1494 damaged seeds. At the conventional fields the average number of florets per flower head was 84.2 with an assumed possible seed capacity of 421 seeds per flower head, assuming 5 ovules/pod. At the organic fields it was an average of 79.8 florets per flower head, providing an assumed possible seed capacity of 399 seeds per flower head.

There was a near **significance** in regards to the number of **healthy seeds** between the fields of the two farming types and also within the farming types (DF=11, Error=22, F=2.164, **p=0.059**). The number of damaged seeds did not show any significant difference between the two farming types (DF=1, Error=11, F=2.753, p=0.125).

Figure 11 exhibits the differences between the farming types in the sum of healthy and damaged seeds.

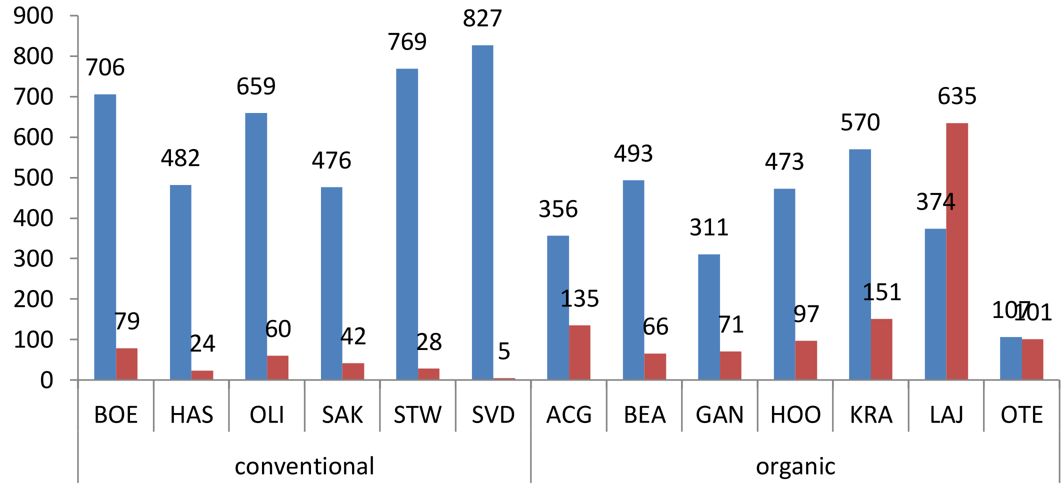


Figure 11. Shows the sums of healthy and damaged seeds of all the three survey rounds sorted by farming system and field (blue columns= healthy seeds, red columns=damaged seeds.

The number of healthy seeds of the three survey rounds was not correlated with any of the pollinator groups, neither with all the pollinators. The number of wild bees showed a trend to be positively correlated with the average number of damaged seeds, but no significant correlation was found (N=39; Pearsson correlation; r =0.302, p=0.061).

5. Discussion

In this study I compared white clover fields with different farming systems to investigate differences between organic and conventional farms in regards to pollinator abundance, composition and biodiversity. Moreover, I examined what factors are the most important to ensure a reliable seed production. I hypothesized that the organic fields would provide an environment where insects would thrive and improve both the biodiversity and the yield. In contrast to that, I believed the conventionally managed fields would have less visiting pollinators, a narrower spectrum of visiting species, and therefore less seeds by the end of the season. The fields altogether were set to be able to not only compare the two types of farming systems but also to be able to explore landscape factors that could possibly determine the yield.

As pollination is a complex ecosystem service (European Commission, 2009), it demands a well-balanced species composition and a fairly good distribution of tasks between each pollinator groups, meaning that more pollinators do not necessarily lead to a better pollination service. This study simply analyzed relationships between well-defined environmental parameters and different measures of pollinator abundance and diversity, but no further in-situ observations on any behavioral characteristics of the pollinators regarding the species interactions with the flowers, which might help to judge or describe pollination success, was performed. All collected data were tested independently on the two different farming systems to be able to detect how all the parameters interact. Contrary to my expectations I have to reject some of my hypotheses.

My **first hypothesis** was that at conventional fields I would find a lower abundance of pollinating insects than at organic fields. According to my findings there was a significant difference in the abundance of pollinating insects between the two types of farming systems. And despite what other researches (Risberg, 2004; Holzschuch et al., 2007) have observed, I found a higher abundance of pollinating insects in the conventional fields. The abundance of honey bees seemed to be responsible for this difference, as this group showed a significant difference when testing the pollinator groups separately. This observed difference could therefore likely be explained by the fact that the field owners set out honey bee hives to improve their yields, although according to Petersen, Reiners & Nault (2013) the previously added *A. mellifera* or *B. impatiens* was not increasing the visitation frequency, neither the pumpkin production in their study. Unfortunately, I currently don’t have the data on the number of managed honey bee hives in the landscape, to further test this. Although it contradicts with the suppositions that insecticide spraying negatively effects pollinators, there could be several other factors that promote the higher visitation, such as landscape characteristics or undamaged flowers (protected from herbivory by insecticide spraying) being more attractive to pollinators.

One of the most influencing **abiotic** factors I observed was the temperature; it was clearly shown in the results that during the second survey at organic fields the temperature was relatively low, in turn neither wind nor sun dominance were shown to significantly affect the pollinators. The group most affected by the temperature was *A. mellifera*, and this correlation has been stated in other studies as well (Wratt, 1968; Boyle-Makowski, Philogene, 1985).

One **biotic** factor recorded at each survey round was the flower frequency, as the percentage and the number of flowers in bloom could definitely affect the number of visiting pollinators. The second biotic factor was measured after the surveys, in the lab, by counting the number of florets and seeds in the flower heads. In this study the flower heads were harvested approximately 2 weeks after the pollination, when app. 90% of seeds were viable (Harris, 1987). Nine flower heads were examined with 225 florets representing each field. The more florets present, the more nectar available to attract pollinators to visit. Both of the measured biotic factors showed the expected effect on the pollinators. *Apis mellifera* and wild bees were positively correlated with the number of flowers in bloom, while *B. terrestris* had a positive correlation with the average number of florets per head.

Moving to the other part of **hypothesis 1**, where I stated that conventional farms would have a lower biodiversity, indeed I found more species in the organic fields. The Shannon - and Simpson diversity indices also showed that organic fields had a higher biodiversity, further supporting my hypothesis.

Although species richness (S) was higher in the case of organic farms, this index only takes into account the number of species found, not the evenness of species proportions. However the evenness (J) value means to describe the maximal diversity with a certain number of species. The higher the index value is the more similar abundance of each species in the biomass. This means that the closer the value is to 0, the more pronounced the dominance of one or few species (*Table 9.*)

According to the statistical analysis on the species composition, there was a positive correlation between *A. mellifera* and *B. terrestris* as well as between *A. mellifera* and wild bees. This positive correlation could be explained by that those farmers who set out *A. mellifera* probably were using managed *B. terrestris* as well to increase their yields, or that when the conditions (i.e. warm and sunny weather, many flowering flowers etc.) are good for one species, it is probably in favor for the others as well.

Despite the commonly known fact on competition between pollinator groups for food resources and nesting areas (Abrol, 2012), in this study the statistical analyses did not show negative correlation between the three pollinator groups, and this is in line with Abrol (2012), saying that this interspecific relation can be mitigated if the surrounding area has relatively good resources both quality and quantity wise, i.e. a white clover field can be viewed as such a resource during its blooming period.

Hypothesis 2 was rejected, since the results showed that the fields surrounded by more complex landscapes (higher diversity in land use, flowering crops, and bigger proportion of field borders and landscape element edges) did not have a higher abundance of pollinating insects. The statistical analyses actually showed a negative correlation between the landscape variables and the amount of *A. mellifera* and wild bees.

In more detail, the Simpson land use diversity index in the 2 and 3 km buffer zones was negatively correlated with the number of wild bee species found on the surveyed field. This is a bit surprising but could possibly have something to do with the conclusion drawn by Carvalheiro et al. (2011), that the diversity of visiting insects was positively correlated to the flower diversity (a white clover field can in many places be consistent of only one plant species, white clover, and not have any diversity at all).

The wild bee group in this study consisted of all the bumble bee species and solitary bees, excluding *B. terrestris*. Most bumble bees are known to be generalist, foraging on many different plants that are available at the time, explaining the positive correlation between the Simpson index (on number of species) and percentage of flowering crops since generalists can utilize several mass flowering crops.

Area of field borders and landscape element edges within the 2 and 3 km buffer zones was negatively correlating with the number of *A. mellifera*. A higher number of field borders and landscape element edges in this study refer to a larger field border and (other) edge area, which equals to smaller field sizes and more landscape elements within the particular zone. Conclusively, *Apis mellifera* was favoring those fields where there were less field border and edge area and larger, cohesive cultivated areas providing larger amounts of one homogeneous flowering crop to forage on. Due to the honey bees foraging habits by visiting one type of plant at a time and choosing the possible best supplied resource in regards of input output energy ratio. It could also be owing to the fact that farmers with bigger sized fields put out more honey bee hives, trying to increase the number of pollinators visiting their crop.

Hypothesis 3 on finding a correlation between the number of visiting pollinators and the seed set is rejected. There was no correlation between the number of visiting pollinators and the number of healthy seeds, meaning that the possible white clover seed yield cannot be predicted by measuring pollinators only. There are other factors that can reduce plant vigor which can lead to insufficient seeding ability. From the biological aspect these are slugs, stem nematodes and weevils (Honwei Cai, 2016). Although there was a significant difference between the numbers of healthy seeds between the two farming systems, with the conventional fields having a higher amount at each survey round, it still did not correlate with the number of recorded pollinating insects. The non-correlation in this study could be elucidated by the fact that the surveys were only 10-minutes long and the pollination activity of course continues after the visits. The weather conditions at the time of some surveys were worse at organic fields but its impact could not bear out my overall results since the entire summer period was nearly the same. As organic field does not receive any chemical treatments, many of the fields were overgrown by other plants, weeds, possibly more tempting to pollinators (especially the "wild" group) and making them forage less on the white clover. In fact, organic fields are tending to increase the insect pollinated weeds, meanwhile it is the opposite at conventional fields where the non-insects pollinated weeds are in majority (Gabriel and Tscharntke, 2007).

The number of produced clover seeds is closely related with the number of present weevils, which are the major pests on white clover in the Nordic countries according to a study in Denmark done on organic white clover fields (Langer et al., 2005). The seed set also seems to be correlated with the plants self-covering (plant different parts overlapping the flowers) during the time of early stages of inflorescence, and not only on the pollination success (Pasumarty, Satyanarayana Venkata, 1990). The foliage canopy of the white clover plant is suggested to be low under this period and even the sun dominance is related to this seed set digression (Pasumarty, Satyanarayana Venkata, 1990) meaning that less sun leads to lower seed set.

6. Conclusion

This study includes a wide set of data on pollinator activity, abundance, biodiversity and factors that are affecting it. However, to draw any sound conclusion, a repetition of the study over several years would be desirable.

My suggestion is that since temperature was an influencing factor, it would be wise to investigate fewer fields but with nearly analogous landscape characteristics and carry out surveys in parallel on the same day, to exclude the effect of temperature, to be able to draw better conclusions on pollinating activity and biodiversity. Since I have not been taking into account the number of pests present at the fields, I would suggest that future experiments should include an assessment of the amount of herbivores and their impact on pollination activity and seed set. Moreover, in regards to weather conditions, humidity can also have a negative effect on the pollination activity, especially when the temperature is low. In the Swedish climate it can be a constraining factor on honey bees (even the pollen lodging slows down); therefore it would be interesting to record data on humidity in further investigations.

As some of the landscape complexity parameters were showing a strong effect on wild pollinators, it would be interesting to carry out surveys on the flora in the buffer zones, focusing only on the organic fields. It could be interesting to see how many organic fields were neighboring the surveyed fields, also how long time ago they were established (positive effect of converting field to organic on the strawberry plant started after 2-4 years, Andersson et al, 2012). To include the flowering crop type would also be important to consider, as different crops bloom at different time during the season. The flowering crop can either keep the pollinators near the surveyed field or attract them away. In addition, it would be interesting to look for other landscape characteristics, such that could provide nesting sites for pollinators. This could give us a better understanding on the abundance and biodiversity of pollinating species in the landscape.

The soil nutrient levels, especially at organic farms, would also be an important factor to include in future studies. It could provide information related to the crop growth and development. In fact, the nectar production can rely on particular soil nutrients, providing more attractive foraging mass for pollinators under certain circumstances (Clifford, White, 1986) an important fact to keep in mind when planning the crop rotation, and when choosing cultivars among flowering crops dependent on insect pollination.

Furthermore, on those organic farms where I observed a lower amount of damaged seeds, it would be interesting to carry out surveys on the wider species biodiversity and investigate how these interact with each other during the season, i.e the interaction between pests, their natural enemies, pollinators, the crop and the surrounding vegetation.

7. References

Abrol, P. D., 2012. *Pollination Biology: Biodiversity Conservation and Agricultural Production*, Springer Dordrecht Heidelberg London New York, ISBN 978-94-007-1941-5, p. 635.

Andersson, K. G., S Rundlöf, M. Smith, G. H., 2012. *Organic Farming Improves Pollination Success in Strawberries*, PLoS ONE 7(2): e31599. doi:10.1371/ journal.pone.0031599, Available at: http://dx.doi.org/10.1371/journal.pone.0031599 (accessed: 2017.02.26)

Australian Government, Office of the Gene Technology Regulator, 2008. *The Biology of Trifolium repens L. (white clover)*, version 2; October Available at: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/clover-3/$FILE/biologywclover2008.pdf, (accessed: 2017.02.26)

Bartomeus, I., Ascher, J.S., Gibbs, J., Danforth, B.N., Wagner, D.L., Hedtke, S.M., Winfree, R. 2013. *Historical changes in northeastern US bee pollinators related to shared ecological traits.* Proceedings of the National Academy of Sciences of the United States of America, 110(12), pp. 4656-4660.

Benedek, Zs., 2012. *Biodiverzitás-indikátorok a döntéshozatalban: a jelenleg népszerű indexek kritikai áttekintése és megoldási javaslatok* Available at: http://korny.uni-corvinus.hu/fem/01-1/benedek.pdf, (accessed: 2017.02.26)

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemueller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. 2006. *Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands*. Science, 313(5785), pp. 351-354.

Bommarco R., Lundin O., Smith G.H., Rundlöf M., 2011. *Drastic historic shifts in bumble-bee community composition in Sweden* Proc. R. Soc. B 2012 279 309-315 first published online 15 June 2011doi: 10.1098/rspb.2011.0647

Bond, W. Davies, G. Turner, R. 2007. *The biology and non-chemical control of White Clover*, HDRA, Ryton Organic Gardens, Coventry, CV8, 3LG, UK, Available at: https://www.gardenorganic.org.uk/sites/www.gardenorganic.org.uk/files/organic-weeds/trifolium-repens.pdf, (accessed: 2017.02.01)

Boyle-Makowski, R.M.D., Philogène, B.J.R. 1985. *Pollinator activity and abiotic factors in an apple orchard, Canadian Entomologist*, Vol. 117, Issue 12, , pp. 1509-1521

Caradus; J. R. Woodfield, D. R. Stewart, A. V. 1996. *Overview and vision for white clover*, Agronomy Society of New-Zealand Special Publication No. 11/Grassland Research and Practice series No. 6, Available at: https://www.researchgate.net/profile/John_Caradus/publication/309075194_Overview_and_vision_for_white_clover_white_clover_New_Zealand's_competitive_edge/links/582b33d908aef19cb806af6a.pdf, (accessed: 2017.02.01)

Carvalheiro, LG, Veldtman R, Shenkute AG, Tesfay G, Werner CW, Donaldson JS, Nicolson SW (2011) *Natural and within-farmland biodiversity enhances crop productivity*. Ecology Letters 14, 251– 259

Clifford, P.T.P.; White, S.D., 1986. *A sulphur response: white clover seed production*. New Zealand Journal of Experimental Agriculture 14: 97–99.

Corbet SA, Williams IH, Osborne JL. 1991a. *Bees and the pollination of crops and wild flowers: changes in the European Community.* Review commissioned by Scientific and Technical Options Assessment, European Parliament.

Corbet SA, Williams IH, Osborne JL. 1991b. *Bees and the pollination of crops and wild flowers in the European Community*. Bee World 1991; 72: 47

Corbet, S. A., M. Fussell, et al. 1993. *"Temperature and the pollinating activity of social bees."* Ecological Entomology 18(1): 17-30.

Corrigan, B. EOL, 2017a. Available at: http://eol.org/data_objects/13447547, (accessed: 2017.02.25)

Corrigan, Bob, Encyclopedia of Life website 2017b. Available at: http://eol.org/data_objects/134475456, (accessed: 2017.02.26.)

Ebeling, A., Klein, A-M, Schumacher, J, Weisser, W. W. and Tscharntke, T., 2008. *How does plant richness affect pollinator richness and temporal stability of flower visits?*, Oikos 117: 1808-1815, Available at: http://www.toek.wzw.tum.de/fileadmin/1_Datein/PDF_Publikationen/2008-03.pdf, (accessed: 2017.02.25.)

Encyclopedia of Life website, Available at: http://eol.org/data_objects/31378217, (accessed: 2017.02.25.)

European Commission, 2009, Ecosystem Goods and Services, factsheet, Sept 2009. Available at: http://ec.europa.eu/environment/pubs/pdf/factsheets/Eco-systems%20goods%20and%20Services/Ecosystem_EN.pdf (accessed: 2017.04.29)

Falk, S., Lewington, R. 2015. *Field guide to the Bees of Great Britain and Ireland*. London. British Wildlife publishing, Bloomsberry Publishing Plc.

Frame, P. Newbould, 1986. *Agronomy of white clover, Advances in agronomy*, Elsevier Available at: http://www.sciencedirect.com/science/article/pii/S0065211308602801, (accessed: 2017.02.25.)

Frankow-Lindberg, B.E., Salomonsson, L. & Torstensson, A. 1996. *The relationship between stolon characteristics, total non-structural carbohydrates, winter survival and spring yield of white clover in Sweden.* Recent Research and Development on White Clover in Europe, REU Technical Series, 42, 51-54.

Gabriel, D. and Tscharntke, T. 2007. *Insect pollinated plants benefit from organic farming*, Agr. Ecosyst. Environ.118:43–48.

Gaile, Z. 2005. *Organic seed propagation: current status and problems in Europe*, report, May 31-June 3, Available at: http://orgprints.org/5707/1/EnvirfoodReportOrgSeedProd.pdf , (accessed 2017.02.23.)

Gallai N, Salles J-M, Settele J, Vaissi`ere B. E. 2009. *Economic valuation of the vulnerability of world agriculture confronted with pollinator decline.* Ecological Economics, Elsevier, 68 (3), pp.810-821.

Goodwin, RM. Cox, HM. Taylor, MA. Evans, LJ. McBrydie, HM. 2011. *Number of honey bee visits required to fully pollinate white clover (Trifolium repens) seed crops in Canterbury, New Zealand*, New Zealand Journal of Crop and Horticultural Science, Available at: http://www.tandfonline.com/doi/pdf/10.1080/01140671.2010.520164, (accessed: 2017.02.25.)

Hagen M, Wikelski M, Kissling WD. 2011. *Space Use of Bumblebees (Bombus spp.) Revealed by Radio-Tracking.* PLoS ONE 6(5): e19997. doi:10.1371/ journal.pone.0019997

Hammond,G., 2009. *Apis mellifera, honey bee*, Madison Blankenship, Animal diversity Web, Available at: http://animaldiversity.org/site/accounts/information/Apis_mellifera.html, (accessed: 2017.02.25.)

Harris, W. 1987. *Population dynamics and competition. In "White Clover"*, MJ Baker, WM Williams, eds. CAB International, Wallingford. pp 203-278.

Heinrich, B. 1981. *Insect Thermoregulation*. Krieger Publishing Company. ISBN 0-471-05144-6.

Herbage, 2005. *White clover- Understanding growth & development*, September. No. Available at: http://maxa.maf.govt.nz/sff/about-projects/search/05-034/05-034clover-development.pdf, (accessed: 2017.03.10.)

Holzschuh, A, Steffan-Dewenter I., Kleijn, D. Tscharntke, T. 2007. *Diversity of flower-visiting bees in cereal fields; effects of farming system, landscape composition and regional context*, Journal of applied Ecology., 44, 41-49

Hongwei, C., Toshihiko Y., Chittaranjan, K. (eds) 2016 (first edition) *Genetics, Genomics and Breeding of Forage Crops* (CRC Press) ISBN 978-1-4822-0811-5 (eBook PDF) USA

Idaho Plant Materials Program, Plant Guide, *White clover*, USDA, NRCS, 2009. Available at:https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_046139.pdf, (accessed 2017.02.21.)

Intergovernmental Science/Policy Platform on Biodiversity and Ecosystem Services (IPBES) 2016. *The assessment report on pollinators, pollination and food production*. Germany. Available at:http://www.ipbes.net/sites/default/files/downloads/pdf/spm_deliverable_3a_pollination_20170222.pdf , (accessed: 2017.02.21.)

Jordruksverket, *Öka skörden med honungsbin och jordhumlor*, 2007, Jönköping, Sweden Available at: http://www.svenskraps.se/vallfrotill10000/pdf/sjv-oka-skorden-bihumlor-2007.pdf, (accessed: 2017.04.26).

Kallioniemia E., Åströma J., Ruscha M. G., Dahlea S., Åströma S., Gjershauga J. O. 2017. *Local resources, linear elements and mass-flowering crops determine bumblebee occurrences in moderately intensified farmlands* Agriculture, Ecosystems & Environment, Volume 239, 15 February 2017, Pages 90–100

Kremen C, Williams NM, Bugg RL, Fay JP, Thorp RW 2004. *The area requirements of an ecosystem service: crop pollination by native bee communities in California*. Ecol Lett 7:1109–1119

KRAV website, Available at: http://www.krav.se/krav-standards, (accessed: 2017.05.22)

Langer, V., Rohde, B. 2005. *Factors reducing yield in organic white clover seed production in Denmark.* Grass and Forage Science, 60 (2), pp. 168-174.

Magurran, A.E. 2004. *Measuring Biological Diversity*. Wiley&Blackwell Science. Ltd/Publishing UK. Available at: https://books.google.se/books?hl=sv&lr=&id=fIjsaxmL_S8C&oi=fnd&pg=PT7&dq=Null+Models+Chapter+2+Studies+of+species+diversity+are+a+major+component+of+community+ecology+and&ots=ay-jH-15Ae&sig=NdMiS8ES82qIh-A9vfhedrgofQ0&redir_esc=y#v=onepage&q&f=false, (accessed:2017.04.26.)

Mossberg, B. and Cederberg, B. 2015. *Humlor, 40 arter att älska och förundras över, i Sverige.* Värnamo. Bonnier Fakta.

Nagendra, H., 2002. *Opposite trends in response for the Shannon and Simpson indices of landscape diversity*, Applied Geography, Vol. 22, Issue 2, p 175-186.

Osborne JL, Martin AP, Carreck NL, Swain JL, Knight ME, Goulson D, Hale RJ, Sanderson RA. 2008. *Bumblebee flight distances in relation to the forage landscape.* J Anim Ecol. 2008 Mar; 77(2):406-15.

Pasumarty, Satyanarayana Venkata, 1990. *Influence of light on inflorescence development and seed yield in white clover (Trifolium repens L.)* a thesis presented in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Plant Science at Massey University, Palmerston North, New Zealand, , Massey University, NZ, Available at: http://mro.massey.ac.nz/bitstream/handle/10179/3495/02_whole.pdf?sequence=1&isAllowed=y, (accessed: 2017.02.29.)

Persson A. S., Olsson, O., Rundlöf, M., and Smith, H. G. 2010. *Land use intensity and landscape complexity – Analysis of landscape characteristics in an agricultural region in Southern Sweden.* Agriculture Ecosystems & Environment, 136, 169-176.

Petersen, D. J., Reiners, S., Nault, A. B. 2013. *Pollination Services Provided by Bees in Pumpkin Fields Supplemented with Either Apis mellifera or Bombus impatiens or Not Supplemented*, Available at: http://dx.doi.org/10.1371/journal.pone.0069819, (accessed: 2017.02.25)

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE., 2010. *Global pollinator declines: trends, impacts and drivers.* 2010 Jun;25(6):345-53. doi: 10.1016/j.tree.2010.01.007. Epub 2010 Feb 24.

Risberg, O. J. 2004. *Humlor (Bombus) på ekologiska och konventionella gårdar*, Uppsala, Available at: http://www.jordbruksverket.se/download/18.51c5369e120aee363f080002056/1370040757154/humlor1.pdf, (accessed: 2017.02.25)

Rundlöf , M., Nilsson, H., Smith, G. H., 2007. *Interacting effects of farming practice and landscape context on bumble bees,* Available at: http://dx.doi.org/10.1016/j.biocon.2007.10.011, (accessed: 2017.03.14.)

Rundlöf M, Persson S. A., Smith G. H., Bommarco R. 2014. *Late-season mass-flowering red clover increases bumble bee queen and male densities* Original Research Article Biological Conservation, Volume 172, April 2014, Pages 138-145

Rundlöf M., Nilsson H. & Smith H.G. 2008. *Interacting effects of farming practice and landscape context on bumble bees.* Biological Conservation, 141, 417-426

SARE, Sustainable Agriculture Research & Education, 2012, Available at: http://www.sare.org/Learning-Center/Books/Managing-Cover-Crops-Profitably-3rd-Edition/Text-Version/Legume-Cover-Crops/White-Clover, (accessed: 2017.02.23.)

Schmiedel, I., Culmsee, H., 2016.*The influence of landscape* ***fragmentation****, expressed by the 'Effective Mesh Size Index', on regional patterns of vascular plant species richness in Lower Saxony*, Germany, Landscape and Urban Planning, Germany, Available at: http://eds.a.ebscohost.com/eds/detail/detail?vid=7&sid=1cd4a2b2-e821-4d57-8ec4-2ec7fbccd8db%40sessionmgr4006&hid=4208&bdata=JnNpdGU9ZWRzLWxpdmUmc2NvcGU9c2l0ZQ%3d%3d#AN=S0169204616300317&db=edselp,(accessed: 2017.02.23.)

Schulp CJE, Lautenbach S, Verburg PH. 2014. *Quantifying and mapping ecosystem services: demand and supply of pollination in the European Union*. Ecol Indic.; 36:131–141.

SLU website, 2017. *Seed-eating weevils in clover seed production* Available at: http://www.slu.se/en/Collaborative-Centres-and-Projects/ekoforsk-en/projectlist-2011-2013/weevils-odor/?si=65A5C58D8177C86093DC88702FF85438&rid=1168921787&sn=sluEPi6-prodSearchIndex (accessed: 2017.02.23.)

Steffan-Dewenter, I. Potts, G.S., Packer, L. 2005. *Pollinator diversity and crop pollination services are at risk* Available at: http://dx.doi.org/10.1016/j.tree.2005.09.004, (accessed: 2017.03.14.)

STEP project, Status and Trends of European Pollinators 2015. ISBN: 978-954-642-762-5, p. 9. (accessed: 2017.02.23.)

Storm predictor center, Beaufort Wind Scale, 2017. Available at: http://www.spc.noaa.gov/faq/tornado/beaufort.html, (accessed: 2017.02.26.)

The Canadian Pollination Initiative website (NSERC-CANPOLIN), 2012. Available at: http://www.pollinator.ca/bestpractices/honeybee.html, 2012 October, (accessed: 2017.02.25.)

The editors of Encyclopedia Britannica, Ecosystem, 2015. Available at: https://global.britannica.com/science/ecosystem, (accessed: 2017.02.23.)

Thomas, R.G. 1987. *Reproductive development*. pp. 63–123. In: Baker, M.J.; Williams, W.M. (eds). White Clover. CAB International, Wallingford, U.K.

USDA official web page, 2017. Available at: https://www.nrcs.usda.gov/wps/portal/nrcs/main/national/plantsanimals/pollinate/, (accessed: 2017.02.23.)
Van Der Kooi, C. J.; Pen, I.; Staal, M.; Stavenga, D. G.; Elzenga, J. T. M. 2015. *"Competition for pollinators and intra-communal spectral dissimilarity of flowers*". Plant Biology. doi:10.1111/plb.12328.

Vanbergen, A.J, 2013. *Threats to an ecosystem service: pressures on pollinators*, Frontiers in Ecology and the Environment, Volume 11, Issue 5, June 2013,Pages 251–259

Walther-Hellwig, K.; Frankl, R. 2000. "*Foraging distances of Bombus muscorum, Bombus lapidarius, and Bombus terrestris (Hymenoptera, Apidae)*".Journal of Insect Behavior13 (2): 239–246. doi:10.1023/A:1007740315207

Williams IH. 1994. *The dependence of crop production within the European Union on pollination by honey bees*. Agricultural Science Reviews 1994; 6: 229-257

Willmer P.G., Finlayson K. 2014. *Big bees do a better job: intraspecific size variation influences pollination effectiveness.* Journal of Pollination Ecology, 14(23), 2014, pp 244-254 **p 251**

Wratt, E. C., 1968. *The Pollinating Activities of Bumble Bees and Honeybees in Relation to Temperature, Competing Forage Plants, and Competition from Other Foragers*, Journal of Apicultural Research, Vol. 7., , p. 61-66

Zulian G. Maes, J. Paracchini, M.L., 2013. *Linking land cover data and crop yields for mapping and assessment of pollinator services in Europe. Land 2*. 472-492 Available at: file:///C:/Users/mypc/Downloads/land-02-00472.pdf, (accessed: 2017.02.26.)

8. Appendices

Appendix 1.

Table 1. The Species composition during the surveys

Species	Number of individuals (conventional)	Number of individuals (organic)	Total of individuals	Percentage
Apis mellifera	1126	708	1834	46%
Sphaerophoria sp.	574	541	1115	28%
unidentified species	145	275	420	11%
B. terrestris	219	86	305	8%
B. lapidarius	60	67	127	3%
butterfly	29	19	48	1%
Bombus sp	10	14	24	1%
B. sylvarum	3	13	16	< 1%
flies		13	13	< 1%
lady bug	4	7	11	< 1%
B. ruderarius		8	8	< 1%
B. hortorum	3	4	7	< 1%
Melitta/Andrena sp.	4	3	7	< 1%
B. soroëensis	2	2	4	< 1%
B. distinguendus		1	1	< 1%
B. jonellus		1	1	< 1%
B. jonellus/B. hortorum	1		1	< 1%
B. lucorum/B. Magnus	1		1	< 1%
B. muscorum		1	1	< 1%
B. rupestris		1	1	< 1%
B. subterraneus	1		1	< 1%
grasshopper		1	1	< 1%
Grand Total	2182	1765	3947	

** Bombus sp were unidentified due to not been able to catch them*

Appendix 2

Table 1 List of species by field with the recorded individuals (uni= unidentified flying insect)

Field	Number of individuals
conventional	**2024**
BOE	**390**
Apis mellifera	262
B. jonellus/B. hortorum	1
B. lapidarius	16

B. terrestris	10
butterfly	2
lady bug	4
Melitta/Andrena sp.	1
Sphaerophoria sp.	66
uni	28
HAS	**280**
Apis mellifera	110
B. lapidarius	4
B. soroëensis	2
B. sylvarum	1
B. terrestris	41
Bombus sp	6
butterfly	2
Sphaerophoria sp.	66
uni	48
OLI	**404**
Apis mellifera	195
B. hortorum	1
B. lapidarius	19
B. lucorum/B. magnus	1
B. subterraneus	1
B. terrestris	79
butterfly	5
Melitta/Andrena sp.	3
Sphaerophoria sp.	80
uni	20
SAK	**390**
Apis mellifera	174
B. hortorum	2
B. lapidarius	4
B. sylvarum	1
B. terrestris	34
Bombus sp	1
butterfly	2
Sphaerophoria sp.	158
uni	14
STW	**220**
Apis mellifera	159
B. lapidarius	7
B. terrestris	14
Bombus sp	1
Sphaerophoria sp.	24

uni	15
SVD	**340**
Apis mellifera	144
B. lapidarius	6
B. sylvarum	1
B. terrestris	16
Bombus sp	2
butterfly	17
Sphaerophoria sp.	150
uni	4
organic	**1765**
ACG	**220**
Apis mellifera	41
B. lapidarius	10
B. ruderarius	1
B. soroëensis	1
B. terrestris	21
butterfly	2
fly	13
Sphaerophoria sp.	80
uni	51
BEA	**189**
Apis mellifera	49
B. lapidarius	8
B. sylvarum	4
B. terrestris	22
Bombus sp	2
butterfly	4
Sphaerophoria sp.	35
uni	65
GAN	**218**
Apis mellifera	142
B. terrestris	1
butterfly	5
Melitta/Andrena sp.	1
Sphaerophoria sp.	33
uni	36
HOO	**248**
Apis mellifera	159
B. distinguendus	1
B. hortorum	1
B. lapidarius	9
B. ruderarius	2

B. soroëensis	1
B. terrestris	16
Sphaerophoria sp.	45
uni	14
KRA	**330**
Apis mellifera	67
B. lapidarius	10
B. terrestris	10
butterfly	2
lady bug	7
Sphaerophoria sp.	201
uni	33
LAJ	**320**
Apis mellifera	100
B. lapidarius	26
B. ruderarius	3
B. rupestris	1
B. sylvarum	5
B. terrestris	8
Bombus sp	4
butterfly	3
Melitta/Andrena sp.	1
Sphaerophoria sp.	100
uni	69
OTE	**240**
Apis mellifera	150
B. hortorum	3
B. jonellus	1
B. lapidarius	4
B. muscorum	1
B. ruderarius	2
B. sylvarum	4
B. terrestris	8
Bombus sp	8
butterfly	3
grasshopper	1
Melitta/Andrena sp.	1
Sphaerophoria sp.	47
uni	7
Grand Total	**3789**

Appendix 3

Table1. Correlation between the three pollinator groups and the pollinated florets (in the table called "florets with seed percentage"). No correlation was found

Correlations

		Florets with seeds percentage
Apis melifera	Pearson Correlation	.213
	Sig. (2-tailed)	.194
	N	39
B. terrestris	Pearson Correlation	.266
	Sig. (2-tailed)	.101
	N	39
Wildbees	Pearson Correlation	-.166
	Sig. (2-tailed)	.312
	N	39
Florets with seeds percentage	Pearson Correlation	1
	Sig. (2-tailed)	
	N	39

**. Correlation is significant at the 0.01 level (2-tailed).