Effects of low quality soil, drought and systemic pesticides on plant flowering traits in Brassica Napus L.

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Abstract

One of the most important ecosystem services is animal pollination, which has been found to increase both crop quantity and quality and hence is important for sustainable food production. To provide food to an increasing population, farming systems are intensifying to maintain high crop yields. However, stressors such as climate change and intensified agricultural production schemes may alter plant flowering traits important for pollinator attraction, with the potential consequence of reducing pollination services. In this study, plants of rapeseed (*Brassica napus* L.) were exposed to drought, low soil quality and the neonicotinoid clothianidin, a systemic pesticide. Responses in plant flowering traits including nectar and pollen production, size and number of flowers, time before flowering and water content in pollen were measured to find individual or combined treatment effects. My results show that low soil quality in combination with drought reduced pollen and flower production, in addition to this, pesticide treatment in combination with low soil quality seem to increase the production of pollen. Flower production were reduced by both low soil quality and drought as individual factors, suggesting that the plant adapt by primarily reducing the number of flowers rather than reducing traits such as nectar and pollen quantity on each flower. The other plant traits investigated did not show a treatment response. Negative effects from reduced resources important for foraging may decrease pollinator attraction, consequently reducing seed yield.

Table of content

Introduction

Pollination by animals is an important ecosystem service for food production (Klein et al. 2007) and human welfare (Smith et al. 2015). The benefits of animal pollination on food crops can be seen both in increased crop quantity (Klein et al 2007, Gallai et al 2009) and quality (Klatt et al 2013). 35 percent of the total food production in the world increases with animal pollination, and the majority of global crops could experience a decrease in production if animal pollination is limited (Klein et al 2007). In 2005, the economic value for insect pollination were estimated to €153 billion (Gallai et al 2009), but accounting for the monetary rewards in quality, this value is most likely underestimated (Klatt et al 2013). In addition to this, crops that benefit from animal pollination are an important contribution to human diet (Smith et al 2015). Without a balanced diet of these crops, humans would face health problems like non-communicable diseases and micronutrient deficiencies, with strongest impact in poor countries were nutrient deficit already is a large problem (Smith et al 2015). However, pollination services are under threat. Various factors have been identified to disturb pollinators and pollination services, including climate change and the use of insecticides (Goulson et al. 2015).

Since the mid-20th century, a rapid global warming has been observed, leading to a change in global climate, likely caused by increased greenhouse gas emissions from human activities (IPCC 2013). Such changes in the global climate system are comprehensively affecting the earth natural systems (IPCC 2013). In turn, this will affect the human population, not only by increased temperature and frequencies of extreme weather events (IPCC 2013), but also by loss of ecosystem services (Jansson et al 2015). In addition to climate change, many places are simultaneously experiencing an intensification in agriculture landscapes (Matson et al 1997). Monocultures are growing larger, decreasing biodiversity and increasing crop sensitiveness to pests and insects. To protect crop yields from losses, many farmers choose to use chemical pesticides (Matson et al 1997). This might have negative

effects on the pollinator community which have been found to benefit from small fields with high crop rotation and organic management (Kennedy et al 2013).

It is hard to assess the impacts of climate change on ecosystem services, and the direct effects will in most cases be different from the long-term effects (Jansson et al. 2015). However, to characterize ecosystem responses to climate change is important for identifying vulnerabilities and adaptation capacity (Grimm et al. 2015). One major effect of climate change on pollinators and their services could be alterations in the availability of plant resources (Burkle&Runyon 2015, Al-Ghzawi et al 2009). Increasing temperatures in the spring could create a temporal mismatch between pollinator and flowering period (Hegland et al 2008), meaning that the plant either starts flowering earlier than the bees appear (Kudo et al 2004) or vice versa (Gordo & Sans 2005). These interactions may vary among species and regions (Hegland et al 2008). In addition to this, there are a lot of climatic factors affecting development and growth of both plants and pollinators that will be affected by climate change, such as changed precipitation and temperature patterns causing drought and poor soil conditions (IPCC 2013). Drought and desiccation have been found to be a limiting factor for plant growth (Aslam et al 2015, Alqudah et al 2011), both directly by reducing photosynthesis rate (Aslam et al 2015), and indirectly by altering flower traits important for pollinator attraction (Burkle & Runyon 2015, Zimmerman&Pyke 1988).

To match food demands for a growing population, the world crop production is intensifying, simultaneously increasing the usage of pesticides. The use of chemical insecticides is preserving about one-fifth of the crop yield in intense farming systems (Oerke & Dehne 2004). Neonicotinoids are among the recently developed insecticides that affect the central nervous system of insects (Blacquiere et al 2012). The neonicotinoid family consist of Imidacloprid, Acetamiprid and Clothianidin among others, with increasing usage since the introduction of the compounds in the 1990s (Blacquiere et al 2012). Besides direct effects from neonicotinoids on the survival and fitness of bees (Godfray et al. 2014, 2015), their application could also have indirect effects by influencing the plant metabolism. It has been shown that neonicotinoids increase the resistance of crop plants against herbivorous insect pests (Elbert et al 2008) and increase plant physiological activity (Gonias et al 2007, 2008, Gnanadhas&Johnsson) however, they can also induce defense responses similar to herbivore

attacks (Ford et al. 2010). Thus, the availability of neonicotinoids will likely also affect the availability of plant resources.

Aims and hypothesis

This study aims to investigate possible alterations in the production of flower resources for *Brassica napus* L., when the plant is exposed to stressors related to intensive agriculture and climate change. I will test individual and combined effects from changes in irrigation and soil quality, but also from the treatment with systemic pesticides on the production of flowering resources. In detail I will address the following hypothesis:

1. Individual treatments will alter the assessed plant traits. I expect negative effects from changes in irrigation and soil quality, whereas effects from the treatment with systemic pesticides will be positive.

2. In combination, I expect interactive effects from the irrigation, soil quality and pesticide treatments, however these may vary between combinations and plant traits.

Material and method

Brassica napus L were chosen for the study due to the crops importance in agricultural landscape, and for its increasing cultivation both in Sweden (Jordbruksverket 2016) and globally (FAO 2016). 48 plants of *Brassica napus* L. (Rapeseed) were planted in a controlled environment in a greenhouse. They received light 24 h per day until the growth of the hypocotyl stopped and the plants stood upright, after which 14 h of light per day were received. The temperature was 18°C in daytime and 10°C at night. An automatic irrigation system adjusted the watering rate according to the plant needs. When the plants reached leave stage 3-5, 24 of the 48 plants received 50% less water to imitate drought conditions while the other 24 plants received water according to the plant's needs. Half of the plants in drought condition and half of the plants in normal irrigation conditions were grown in soil with 50 volume percent of sand to simulate low quality soil conditions, and the other half of plants were grown in soil with 50 volume percent of vermiculite to simulate high quality soil conditions. In addition, the seeds of 12 of the plants in drought conditions (6 of them in sandy soil and 6 in vermiculite) and 12 of the plants in normal water conditions (6 of them in low soil quality and 6 in high soil quality) were treated with Elado® (Bayer Crop Science) and the fungicide Rovral before planting. Elado® (Bayer Crop Science) is a co-formulation of two active substances, the neonicotinoid insecticide clothianidin and fungicide beta-cyflutrin. Beta-cyflutrin is non-systemic and is found in very small concentrations in the above ground plant parts (Rundlöf et al, 2015). A schematic drawing of the set up in the greenhouse is shown in figure 1. To make sure no personal selection were possible during sampling, the thesis supervisor planted the seeds and the sampler was not knowing of the treatments until end of the statistical analysis.
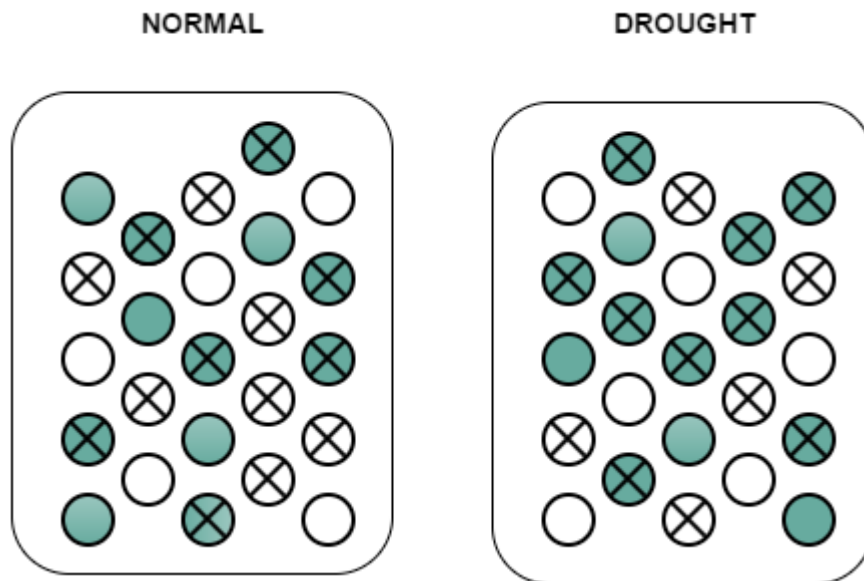
Figure 1, Experiment set up. The figure shows how the samples were distributed. The circles symbolize the plants. The filled circles symbolize the plants treated with neonicotinoid before planting, and the unfilled circles symbolize the plants without neonicotinoid. The X-marked circles illustrates the plants with low soil conditions, while the ones without X illustrates the plants with high quality soil.

Measurements

The measurements were carried out between 2016-02-03 and 2016-03-04. The flowers selected for measurements were primarily in their mature state where the crown pedals are flat (See figure 2, "Plan") to ensure that the nectar production were at its peak, and the pollen were still attached to the stamens. For some flowers, additional samples were taken in the state before the flat state (See figure 2, Tratt) and the one after the flat state (See figure 2, "Nerviken") to get a sufficient amount of data. The sampling was random apart from selection of flowers in the suitable growth stages, and all plants had samples from at least two different days of collection to minimize the impact of daily nectar/pollen production variation. No samples were taken from flowers which pistils were longer than the stamen. Three plants were extracted from the results (plant 15,32 and 20) due to unusual growth and

lack of pollen production. Since no obvious pattern between treatment and these conditions could be found, it was decided not to include these in the results to avoid bias.
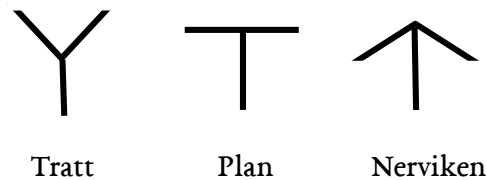

Tratt   Plan   Nerviken

Figure 2, The three different flower stages included in the study. The figure shows a schematic drawing of how the flower pedals were displayed on those flowers subject for measuring. The stage "Plan" were used primarily. The picture is redrawn from a description from statens växtsskyddsanstalt (1953), see reference list for closer description.

Pollen collection

Quantity of pollen was measured by cutting off the flower stamen and collecting it in 5 ml tubes (VWR, USA). Before collection of the pollen, the tubes were weighted (Ac100-A85995, Mettler AC 100, Switzerland). The flower stamens were then cut off, collected in the tube, and weighted. The weight of the empty tube was subtracted to get the weight of the stamen with pollen alone. The tube was then placed without a lid close to a heater during one week to dry the pollen. The container with dry pollen was weighted again and by subtracting the mass of the container, the mass of dry pollen alone were received. 4-7 flowers on each plant were sampled during the growth period.

Nectar collection

Nectar was collected with a microcapillary (CAMAG, Switzerland; Dungan et al 2004). The microcapillary had the volume of 1 µl, and a length of 32.0 mm. The nectar was sucked up in the capillary, and the height at which the nectar reached by capillary force was measured with a digital calliper (Pro Tools, Germany). The height was converted to volume (µl) by division with 32.00. 4-7 flowers on each plant were sampled during the growth period.

Other plant trait measurements

At every sampling date, size of the flowers where the nectar and pollen were collected were measured. The total number of flowers developed during the entire growth period was counted after all plants had finished flowering. This was made by counting the number of seed pods which were left after the flower withered. Time between planting and first flowering were counted, except for one day when the greenhouse was not visited (2016-02-04). Statistical analysis was therefore conducted including and excluding the flowers that opened during this time from the dataset. These abbreviations were used to describe the different combinations of treatments (Table 1):

Table 1, Abbreviations used to describe the treatment combinations. The table shows the different combination of treatments and their abbreviations used in the report.

Abbreviation	Treatment
NVE	**N**ormal water condition **V**ermiculite soil **E**xposed to clothianidin
NVU	**N**ormal water condition **V**ermiculite soil **U**ntreated (no clothianidin)
NSE	**N**ormal water condition **S**andy soil **E**xposed to clothianidin
NSU	**N**ormal water condition **S**andy soil **U**ntreated (no clothianidin)
DVU	**D**rought condition **V**ermiculite soil **U**ntreated (no clothianidin)
DVE	**D**rought condition **V**ermiculite soil **E**xposed to clothianidin
DSE	**D**rought condition **S**andy soil **E**xposed to clothianidin
DSU	**D**rought condition **S**andy soil **U**ntreated (no clothianidin)

Statistics

The statistical analysis were made in the program R 3.2.4 (R Developmental Core Team 2016) using linear mixed effects models (LME) ('lme'-function; package nlme) (Everitt&Hothorn 2006). In individual models, the observed data from pollen, nectar, water content of the pollen and flower size were respectively used as fixed effects. The plants were used as a random effects, since the collected data were repeated measurements on the same plant. Residuals were inspected for model assumptions of homogeneity and normality ('plotresid'-function; package RVAideMemoire). When such assumptions were violated, in a first step, data were transformed using square root and logarithmic transformation and, in a second step,

aggregated across plants if model assumptions were still violated after transformation. Aggregated data were analyzed using generalized linear models (GLM) (Everitt&Hothorn 2006) and if model assumptions were still violated, analysis were conducted using Kruskal-Wallis tests (Everitt&Hothorn 2006).

Generalized linear models ('glm'-function; package base) were used to analyze the time between sowing and first flowering as well as the total number of developed flowers. The same approach as with the linear mixed effect model was used, were the residuals were inspected to validate model assumptions. Since this data were counted, the most likely data distribution is the Poisson distribution, which was added to the model (family=poisson). The model was also checked for overdispersion, which could not be detected.

It is possible that 5 of the plants could have started flowering during the day in which the greenhouse was not visited, therefore, the generalized linear model was fitted to the time before flowering dataset excluding these plants. To see if this might affect the result of the following Chi-square test, a mean value of these 5 plants was calculated to 50,5. This data was then put in the model, but without the statement that the data is Poisson distributed since this assumption were no longer fulfilled. The following Chi-square test did not show a qualitative difference from when the plants were extracted, why the results presented will be excluding these plants.

A summary of the data transformations and statistical tests used for the analyses of the measured and counted variables can be found in the appendix, table 3.

Result

Pollen and water content of pollen

The Kruskal-Wallis rank sum test showed that treatment had a significant effect on pollen weight (Chi-square = 32.918 ; df = 7 ; P = < 0.001; Appendix table 4 figure 3). The result from the Wilcox rank sum test is presented in table 2.

Drought conditions in combination with low soil quality was found to be the main driver for reduced pollen weight. Plants subject for these conditions without clothianidin treatment (DSU) produced 27,7 % less pollen in average compared to plants exposed to same water condition and insecticide treatment, but under good soil quality (DVU). No individual treatment effects were detected.

However, plants grown under normal irrigation conditions combined with low soil quality (NSU) produces pollen with significantly lower weight compared to plants grown under drought conditions in high quality soil (DVU), with no clothianidin treatment. What is interesting to add, is that when clothianidin treatment is added to the previously described conditions (NSE compared to DVU), the difference in pollen weight loose its significance. Another example of this is when comparing plants grown in low quality soil conditions, normal irrigation and clothianidin (NSE) with plants in drought condition and low soil quality (DSU). The NSE treated plants produces significantly higher pollen quantity compared to DSU treated ones, but when the clothianidin treatment is removed, the pollen production decreases and no significance can be found (NSU treatment compared to DSU).

The results from the analysis on water content in pollen show that treatment had an overall effect (Kruskal-Wallis test chi-squared = 14.144, df = 7, p-value = 0.04868, appendix table 4), but individual treatments did not differ significantly (Table 2; Appendix Figure 4).

Number of developed flowers

The analysis show that treatment had a significant effect on the total number of developed flowers (GLM df = 7, p= < 0.001, appendix table 4 ; figure 5). The result from the multiple comparison is presented in table 2. Plants exposed to the combination of low soil quality and drought (DSU, DSE) did not differ between one another, but produced significantly lower amounts of flowers compared to all other treatments, except when DSE plants were compared to plants exposed to the same treatment combination but with high quality soil (DVE). DSU and DSE plants produced on average about half as many flowers during their growth period compared to plants grown in vermiculite soil and normal water condition (NVU, NVE), and one third less flowers then plants exposed to drought and vermiculite soil (DVU, DVE) and plants with normal water conditions and sandy soil (NSU, NSE). No treatment effects from clothianidin could be detected.

Nectar, flower size and time before flowering

The volume of nectar did not differ between the treatments (LME $F_{7,37}$ = 0.3693; p= 0.9143, see table 4; Appendix Fig. 6), neither did the size of flowers (LME $F_{7,37}$ = 0.573; p= 0.773, see table 4; Appendix Fig. 7) nor the start of flowering (GLM df = 7, p= 0.2115, see table 4; Appendix Fig.8).

Table 2 Result from the post hoc statistical analyses. *The table present the results (p-values) from the post hoc tests used on the variables with significant overall treatment effects. Significant p-values are bold and filled.*

Treatment	Pollen weight	Number of flowers	Water content in pollen
DSU – DVU	**0.0018**	**< 0.001**	1.0000
DSU – NSE	**0.0088**	**< 0.001**	1.0000
DSU – NSU	1.0000	**< 0.001**	1.0000
DSU – NVE	0.4594	**< 0.001**	0.79
DSU – NVU	**0.0335**	**< 0.001**	1.0000
DSU – DVE	0.0763	**0.00309**	1.0000
DSU – DSE	1.0000	0.93189	1.0000
DSE – DVU	**0.0431**	**< 0.001**	1.0000
DSE – NSE	0.5693	**< 0.001**	1.0000
DSE – NSU	1.0000	**< 0.001**	1.0000
DSE – NVE	1.0000	**< 0.001**	1.0000
DSE – NVU	0.4142	**< 0.001**	1.0000
DSE – DVE	1.0000	**0.01751**	1.0000
DVE – NVU	1.0000	**< 0.001**	1.0000
DVE – NVE	1.0000	**0.02145**	1.0000
DVE – NSU	0.4142	0.98134	1.0000
DVE – NSE	1.0000	0.99730	1.0000
DVE – DVU	1.0000	0.99977	1.0000
DVU –NVU	1.0000	**< 0.001**	1.0000
DVU – NVE	1.0000	**0.02983**	1.0000
DVU – NSU	**0.0043**	0.99955	1.0000
DVU – NSE	0.1831	1.00000	1.0000
NSE – NVU	1.0000	**0.00109**	1.0000
NSE – NVE	1.0000	**0.04013**	1.0000
NSE – NSU	0.1281	0.99999	1.0000
NSU – NVU	0.1281	**0.00332**	1.0000
NSU – NVE	1.0000	0.08545	0.50
NVU – NVE	1.0000	0.99685	1.0000

Discussion

Drought conditions in combination with low soil quality was found to have greatest influence on pollen weight and the total number of flowers produced. Pollen production was significantly reduced for plants exposed to the combination of drought and low soil quality, while the number of flowers were reduced in plants both by individual effect and combined effects from these two treatments. Water content in pollen was not found to significantly differ between treatments, indicating that the weight reduction caused by the treatments was due to reduced pollen quantity rather than quality (Alqudah et al 2011). Clothianidin treatment did not affect the plant traits individually, but increased pollen production were found as a response to the combination of low soil quality and clothianidin treatment.

These results are consistent with hypothesis 1, regarding combination effects from the different treatments. However, responses in pollen production could only be found for plants grown in low soil quality with clothianidin treatment and for plants exposed to drought and low soil quality. Drought condition and low soil quality additionally reduced the number of produced flowers, but also as an effect from the treatments individually, which is stated in hypothesis 2.

It is likely that pollen production was reduced by a combined effect from low soil quality and drought, since no relationship between soil quality and pollen production could be found when irrigation conditions were normal. Additionally, there was no significant relationship between any of the treatments when keeping clothianidin treatment and soil quality constant and changing irrigation. This suggests that neither soil quality nor water condition affects pollen weight individually. However, there is some evidence that low soil quality is a relatively more important factor for pollen production. Plants grown in low quality soil with normal irrigation (NSU), produces pollen with significantly lower weight compared to plants exposed to drought and high quality soil (DVU). Even though the plant receives sufficient amount of water, low soil quality seems to be sufficient to force the plant

to reduce pollen production. This is probably due sandy soils low water holding capacity, which is a limiting factor for plant productivity, and contribute to reduce the efficiency of water and fertilizer use by plants (Sivapalan 2006). The DVU treatment did not reduce pollen production even though drought condition apply, suggesting that high soil quality can mitigate drought effects (Sivapalan 2006). The level of drought imposed by reduction of water were singlehandedly not enough to give a response in pollen production. This suggests that when the plant is forced to redistribute the resources to adapt to this level of drought, other traits, such as number of developed flowers, is primarily targeted rather than pollen production. This would be a reasonable explanation since the tissue in flowers contains high water amounts (Aslam et al 2015) compared to pollen. However, when soil quality is low in addition to drought, the plant is forced to reduce pollen production as well, probably because the low soil quality creates more intense drought condition (Mazen 2015). This is consistent with several articles (Aslam et al 2015, Majidi 2015) reporting that the level of drought is determining for different responses in plant physiological functions, yield components and growth.

Pesticide treatment had no individual effect on any of the measured plant traits. However, plants subject for low soil quality seem to increase pollen production when exposed to clothianidin. Plants grown in drought and low quality soil (DSU) produced significantly lower pollen quantity compared to plants in high soil quality and normal irrigation (NVU), but when adding clothianidin treatment (DSE compared to NVU), the pollen production seems to increase slightly so the significant difference from NVU treatment disappears. The same were found in normal water condition, suggesting an interactive effect from clothianidin and low quality soil. It has previously been found that neonicotinoids increase plant metabolism in non-stressed environments (Gnanadhas&Johnsson), however, the positive effect have been found largest during stressed conditions (Gonias 2007). My result indicate that the plants grown in low soil quality is not as severely affected from the stressed conditions when the seed has been treated with clothianidin, which is consistent with both Gonias 2007 and Thielert 2009. No relationship between number of flowers and clothianidin could be found, which might be because the irrigation and drought both had individual effect on the assessed traits, with possibly larger response than from clothianidin.

The average number of flowers on each plant was almost halved when grown in low quality soil in combination with drought, compared to plants grown in high quality soil and normal water condition. This is consistent with the finding regarding pollen production. In contrast to the pollen production, however, both sandy soil and water condition also seem to have an individual effect on the total number of flowers. Significant results can be found when keeping the soil quality and pesticide constant and changing water condition (example NVU compared to DVU), and also when keeping water condition and pesticide constant and changing soil quality (example DSE compared to DVE). This is consistent with Al-Ghzawi et al 2009, who found a decrease in number of produced flowers as a result of drought. My results indicate that a stressed plant saves resources by firstly decrease the number of flowers on the plant without reducing the reproductive quality on that individual (in terms of flower size, pollen or nectar).

Consequences for pollination

The adaptation strategy to reduce the amount of pollen could have effects on pollination services both from the plant fitness but also the pollinating insect's point of view. Since the amount of available pollen is low, the chances of successful fertilization and reproduction of the plant is reduced (Alonso et al 2011). In addition, Al-Ghzawi et al 2009 found that plants grown under drought stress had a lower number of pollinators visiting the flowers compared to normal water condition, suggesting decreased pollinator attraction. These results were mainly due to changes in morphological characteristics including reduced number of flowers, decreased pollen amount and viability. Overall, this can lead to a decrease in crop yield (Alqudah et al 2011). However, Burkle & Runyon 2015 found that in some species, the pollination visitation rate actually increased when the plant were subject of drought, possibly due to increased production of volatile organic compunds, or that less nectar and pollen were collected on each flower (Jones et al 1998). It is therefore hard to predict how the pollination attraction might differ for the plants exposed to the treatments in my experiment, especially since responses differ between plant species and level of stress. Even though the pollinator attraction for the plants might be unaffected or even increased, the rate of germination and

fertilization probably would decrease both due to reduced pollen availability and number of flowers available for pollination (Ejsmond et al 2015, Bishop et al 2016). For pollinating insects, a reduction in pollen quantity could contribute to negative health effects since pollen is an important natural protein source (Brodschneider and Crailsheim 2010). However, if the plant was to reduce the water content of the pollen as a drought adaptation strategy instead of actual quantity, other negative effects could be expected. Low water content in pollen has been correlated to reduced pollen viability in some species (Nepi 2009, Nepi 2001), but the threshold to which pollen can dehydrate without influencing the viability is species specific (Nepi 2001). In turn, a reduced pollen quality would have negative effects on bee physiology and survival (Di Pasquale 2013, Frias et al 2015).

A decrease in number of flowers could have negative influences on plant reproduction due to possible reduction in pollinator attraction (Buide 2005), but also by reducing the number of pods produced. Previous studies have shown that drought stress reduces the number of pods per plant (Ghobadi et al 2006) and the seed weight in Brassica napus (Majidi 2015), with reduced seed yield as a consequence (Weymann 2015, Alqudah et al 2011). In addition to this, pollen production has been found to limit pollination success by reducing the amount of available pollen (Truman & Wallace 1999), which in turn can reduce seed production (Gan et al 2004, Buide 2005). This suggest an overall negative influence on seed yield from the treatments. However, there are other plant traits which might be of greater importance for pollinator attraction and success. Nectar production has been found to influence the pollination visitation rate (Irwin et al 2004) and to be negatively affected by drought stress (Zimmerman & Pyke 1988). My results showed no significant effect on nectar production by the different treatments, which could be an indication that the plant reduces other resources (number of flowers and pollen) before quantity of nectar. Alqudah et al 2011 states that the quantity and quality of nectar is determining for flower attractiveness to pollinators, indicating that even though the amount of flowers in my experiment were reduced, the individual flowers attractiveness might not be severely affected. However, it is possible that the nectar quality changed as a response to stressed environment without changing the volume. One such factor affecting the quality could be the sucrose content, which have been found to change with water stress (Wyatt et al 1992), but this was not

included in my study. Another possible explanation to why no treatment effect on nectar production were found could be due to measuring difficulties. The microcapillaries had variating success in retrieving the nectar, which partly might be explained by different densities or water content in the nectar. Dungan et al -2004 writes that when sugar solutions such as honey dew or nectar are very concentrated, drops are too viscous to be drawn into capillary tubes. McKenna&Thomson -1988 also report that microcapillaries are unsuitable for nectar volumes less than 1 μl. Since problems retrieving nectar into the capillaries occurred during my measuring, it is possible that the composition or volume of the nectar actually were affected by treatments, but another measuring method would have been more suitable. One suggestion would be to use small stripes of filter paper to absorb the nectar, as presented in Mckenna&Thomson -1988.

My result indicate a trend of increasing pollen production for plants grown in low soil quality, if the seed has been treated with clothianidin before planting. Even though increased pollen production might be considered a positive influence on pollination success (Truman & Wallace 1998), neonicotinoids have also been shown to influence the pollinator preference (Kessler et al 2015, Blacquere et al 2012, Rundlöf et al 2015). Rundlöf et al 2015 showed that in fields with plants treated with neonicotinoid coating, the wild bee density, solitary bee nesting and bumblebee colony growth and reproduction were significantly reduced, which would reduce pollination success by limiting the number of pollinators. In contrast to this, other reports state that plants treated with neonicotinoids actually attract pollinators, even though the rewards in energy were constant (Kessler et al 2015). Increasing usage of pesticides has also been linked to the recent bee deaths, with obvious negative effects for pollination service (Goulson et al 2015).

No treatment effect could be found for flower size or time between sowing and appearance of first flower. Other observations have found that drought reduces the flower size (Zimmerman & Pyke 1988, Burkle & Runyon 2015), however, Buide et al 2005 found that the number of flowers produced is of greater importance for pollinator attraction than size of flower. Delay in flowering has also been found to be an effect of drought condition (Alqudah et al 2011), but Al-Ghzawi et al 2009 observed the opposite, that the time required for flower development during drought stress was less than the time usually required for

normal plants. Overall, it is difficult to interpret how these changes in plant traits will affect the plants pollinator attraction, and responses often differ between plant species (Goulson et al 2015).

Treatment effects and climate change

The changes in global climate will likely increase the areas subjected to desiccation and low soil quality (IPCC 2013). My results indicate that plants grown in areas with low soil quality and drought will have to adapt to the stress by reducing resources vital for foraging processes and pollinator attraction, reducing plant fitness. It is likely that areas with these characteristics will increase in abundance, not only due to increasing temperatures but also due to over grazing from cattle and human activities such as deforestation (Houérou 1997). Plants grown in areas were drought and low soil quality is a consequence from rising temperatures, would not just experience a change in plant resources but could also be greatly affected by decoupling in plant-insect interaction, further decreasing pollination success. It is possible that increasing spring temperature has a greater response either in plant flowering (Kudo et al 2004) or first pollinator appearance (Gordo & Sans 2005), creating a temporal mismatch which reduce the time in which both pollinator and plant reproductive traits are active and fully developed (Hegland et al 2008). My results did however not show a significant change in start of flowering, which indicates that no enhancement of the possible plant-pollinator decoupling will be present in areas exposed to the treatments compared to other areas. For pollen production, Ejsmond et al 2015 found that the trade-off between number of pollen grains produced and size of each grain will favor large grain sizes in higher temperature, regardless of intensity of desiccation. Larger pollen size increase the seed size for a range of species creating a more competitive seed (Ejsmond et al 2015), especially in stressed environments (Blake et al 2004). I found the quantity of pollen to decrease as a response to low quality soil and drought, in a warmer climate, the number of pollen grain might decrease further to increase size of each pollen instead. However, the seed establishment might benefit from more competitive seeds, despite low soil quality and drought. Although it is unclear how the pollinator attraction will change due to treatment

effects on the plant traits, it previously has been found that pollen availability is a limiting factor for crop pollination (Truman &Wallace 1998). In an increasingly warmer world, the importance of functional plant-pollinator interactions are proving to be vital for sustaining high crop yields since insect pollination help plants mitigate to a high temperatures (Bishop et al 2016).

It is important to add that the soil quality and water availability in reality seldom is constant in three dimensions, and precipitation is rarely acting in this predictable consequent manor as in the study, but is variation in both space and time, and with expected larger variations and enhancements of the extreme events.

Conclusion

This study aimed to investigate potential responses from individual or combined treatment effects of climate change and systemic insecticides on foraging resources in *Brassica napus*. Areas with low soil quality and dry climate will probably increase in abundance due to climate change, consequently leading to higher amount of agricultural crops experiencing negative responses in pollen and flower production. However, clothianidin treatment might increase the pollen production in low quality soil. In spite of this positive response, neonicotinoids have been found connected to negative effects for pollinator communities. This suggests that treatment with neonicotinoids would not be a long-term strategy for plant mitigation to stressed environments, since the positive effects in plant metabolism might be canceled out by negative effects in pollination services. There are many factors influencing plant attractiveness to pollinators other than the responses from the treatments. However, the observed decrease in pollen quantity and flower density will probably reduce pollen availability for pollinators, with negative consequences both for pollinator community and plant fitness, leading to decreased seed yield and yield components. In a warmer world, plant-pollinator interactions might be increasingly disturbed, but are an important component to sustain high crop yields.

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Picture source

Figure 1

Persson, Brita (1953), *Observationer rörande blommornas biologi hos raps (Brassica Napus L.) med hänsyn till faran för bidöd vid behandling av rapsfält med insekticider*, Statens växtskyddsanstalt meddelande nr: 66, 1953.

Appendix

This appendix presents the steps used for analyzing the data collected for the different plant traits (Table 3), a summary of the results from the statistical analysis (Table 4) and the distribution plots for the different variables to visualize the data dispersion.

Table 3 summary of the different steps in the statistical analysis. *The table presents the data characteristics, possible transformation and which tests were used for the statistical analysis.*

	Data	**Distribution**	**Model**	**Treatment test**	**Post hoc test**	**p-value adjustment method**
Nectar	Measured	Normal	LME with square root transformation	F-test	-	-
Flower size	Measured	Normal	LME	F-test	-	-
Water content of pollen	Measured and aggregated	Normal	-	Kruskal Wallis	Wilcox rank sum test	Holm
Pollen	Measured and aggregated	Normal	-	Kruskal Wallis	Wilcox rank sum test	Holm
Time before flowering	Counted	Poisson	GLM	Chi-square	-	-
Number of flowers	Counted	Poisson	GLM	Chi-square	Multiple comparison	Tukey

Table 4 Summary of the result from the statistical analysis. The table present the result from Kruskal Wallis rank sum test, F-test and Chi-square test for the different variables.

	Kruskal Wallis rank sum test		
	chi-square	numDF	p-value
Water content	14.144	7	0.04868
Pollen	32.918	7	2.743e-05
	F -test		
	numDF	denDF	F-value	p-value
Nectar	7	37	0.3693	0.9143
Flower size	7	37	0.573	0.773
	Chi-square test		
	numDF	denDF	p-value
Time before flowering	7	37	0.2115
Number of flowers	7	37	< 2.2e-16

Pollen

Average pollen weight by treatment
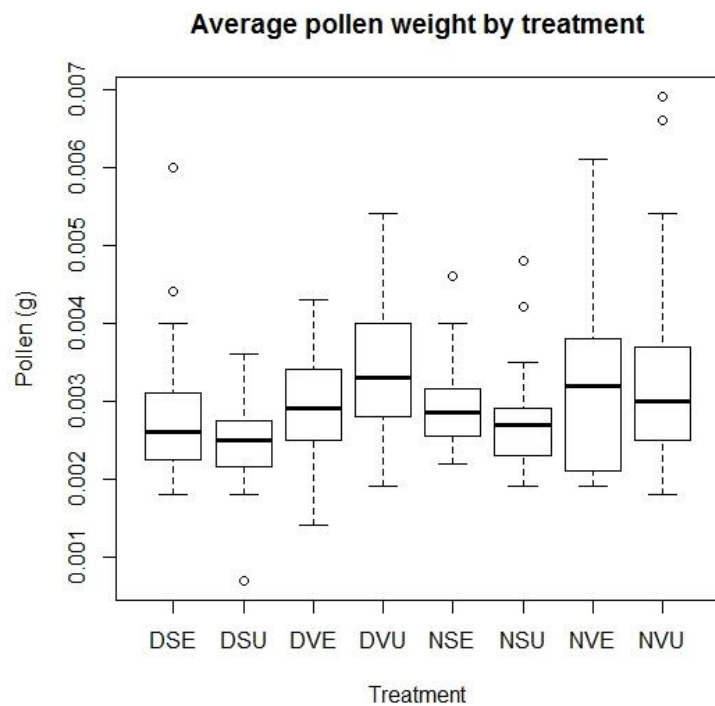
Figure 3 Sample distribution of pollen weight by treatment. The boxplot show the pollen weight for the different treatments. The boxes capture 50 % of the sample variance and the black line is the median. The dotted lines include 95 % of the variance and the small circles represent outliers.
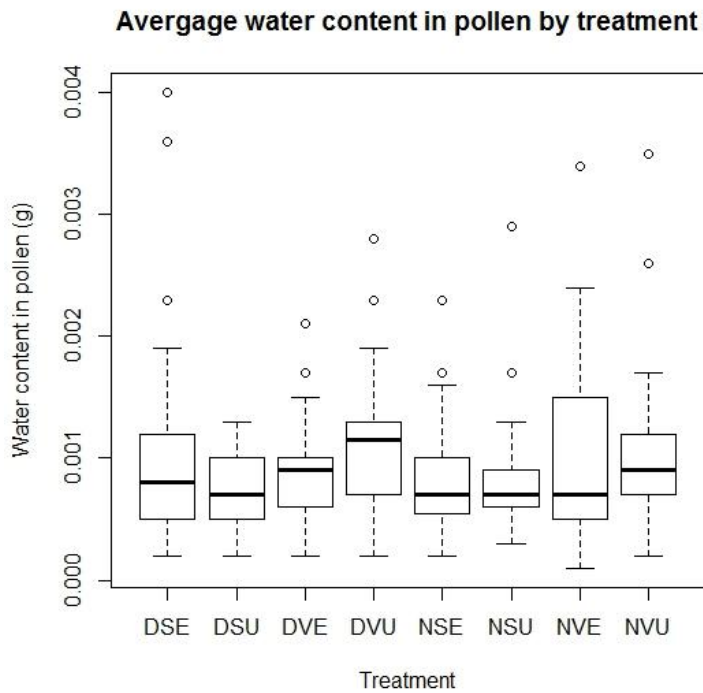
Water content

Figure 4 Sample distribution of water content in pollen for the treatments. The boxplot show the water content in pollen for the different treatments. The boxes capture 50 % of the sample variance and the black line is the median. The dotted lines include 95 % of the variance and the small circles represent outliers.

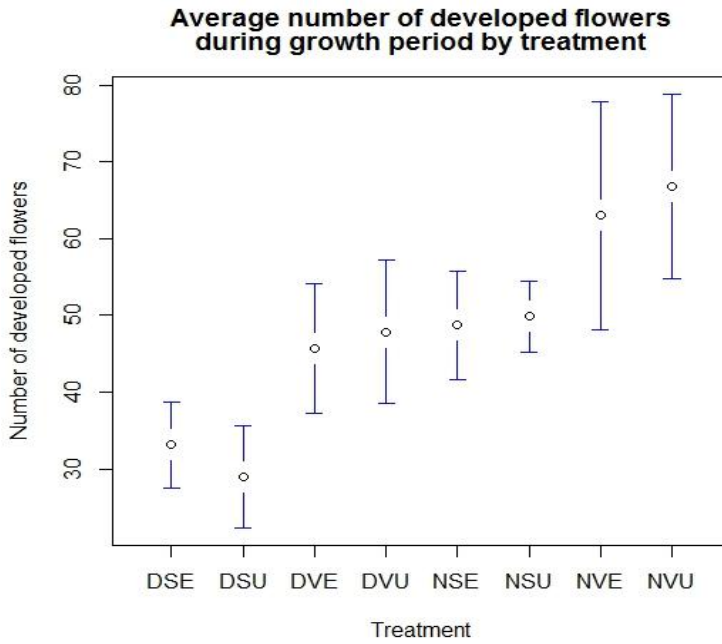Number of developed flowers during growth period

Figure 5 Sample distribution of number of flowers by treatment. The plot shows average number of flowers for the different treatments during the growth period, including the confidence intervals (blue lines) which shows the variance of the samples.
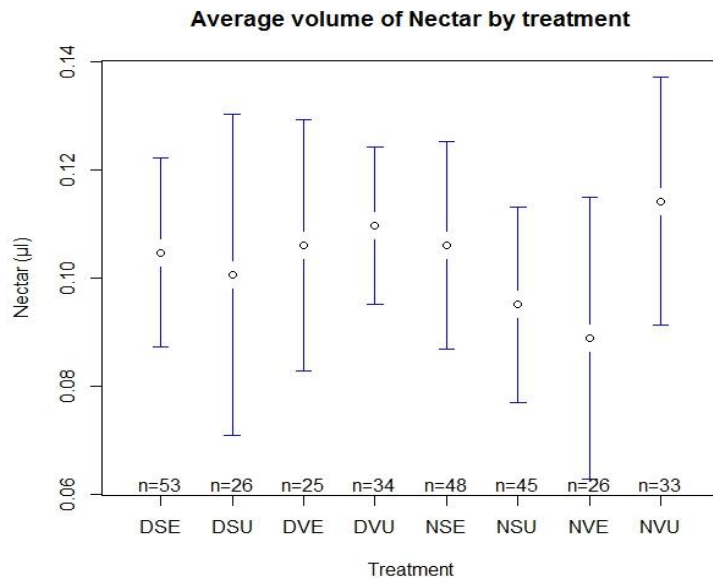
Nectar

Figure 6 Sample distribution of volume of nectar by treatment. The plot shows average nectar volume for the different treatments, including the confidence intervals (blue lines) which shows the variance of the measurements, and the number of samples in each treatment (n).
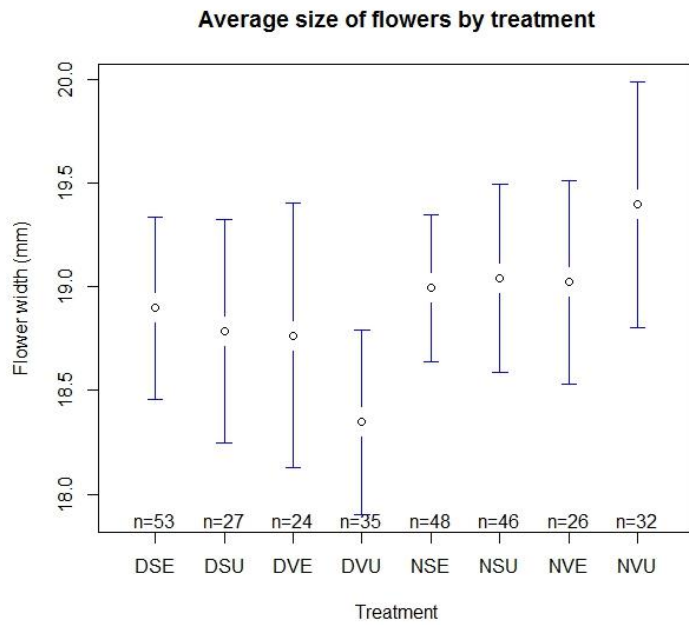
Flower size

Figure 7 Sample distribution of flower size by treatment. The plot shows average flower size for the different treatments, including the confidence intervals (blue lines) which shows the variance of the measurements, and the number of samples in each treatment (n).
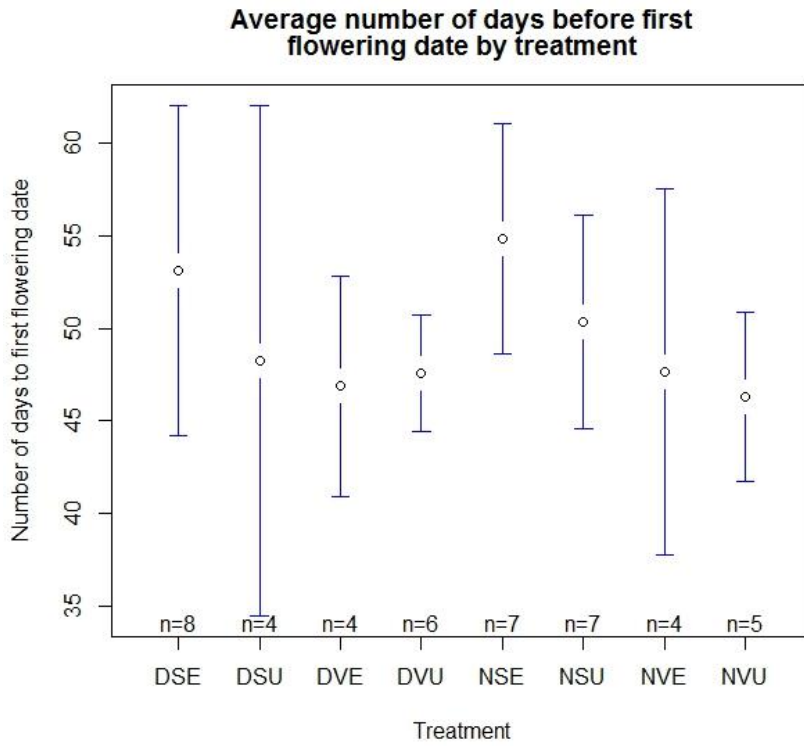
Time before flowering

Figure 8 Sample distribution of days before flowering by treatment. *The plot shows average time between sowing and first flower appearance for the different treatments, including the confidence intervals (blue lines) which shows the variance of the measurements, and the number of samples in each treatment (n).*