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# **Reproduction in the asphalt jungle: Diverging impacts of urbanisation and beekeeping on seed production in native and invasive plants**

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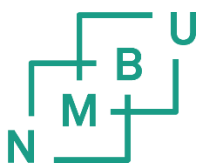
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universitet

Ås, 12.05.2023

A handwritten signature in black ink that reads "Rebekka Sundøy Haldorsen". The signature is written in a cursive style with a horizontal line underneath the name.

Rebekka Sundøy Haldorsen

## Abstract

1. Urbanisation is increasing worldwide, posing a potential threat to pollinator communities and subsequently the delivery of pollination services to wild plants. However, studies are diverging on whether urbanisation positively, neutrally, or negatively correlates with wild pollinators – often due to variations in the surrounding landscape or different definitions of ‘urban’. Furthermore, the apiculture of honey bees (*Apis mellifera*) shows tendencies of a negative influence on wild pollinators and increased spread of invasive plant species. Therefore, it is interesting to investigate whether urbanisation and apiculture influence the dichotomy of conserving native plants while extirpating invasive plants.
2. I have conducted a study on pollen limitation in the two plant species *Bunias orientalis* (invasive) and *Lotus corniculatus* (native) along a landscape diversity and urbanisation gradient in 35 sites in Porsgrunn and Skien municipalities, Norway. I measured pollen limitation by comparing seed numbers and weight for hand pollinated flowers versus controls (naturally pollinated flowers) along the landscape gradients. Further, visits of insect pollinators were recorded to investigate how the pollinator community correlated with the landscape gradients, how their presence correlated with pollen limitation in *B. orientalis* and *L. corniculatus*, and how honey bees correlated with wild insects.
3. I found that seed numbers in the invasive *B. orientalis* increased with landscape diversity, indicating lower pollen limitation and a good ability to spread in heterogeneous landscapes. Seed numbers in the native *L. corniculatus* correlated negatively with urbanisation, indicating high pollen limitation and a lower ability to spread in urban areas. Considering insect visits, I want to highlight the finding of a positive correlation between seed numbers and honey bee visits in *B. orientalis*. As a consequence, honey bees might reduce pollen limitation in the invasive *B. orientalis*, potentially leading to increased spread.
4. *Synthesis*: The invasive *B. orientalis* and native *L. corniculatus* are interesting from a management perspective since they represent the contradictive management goals of extirpating invasive plants and conserving native plants. This study shows that knowing how landscape variables affect pollen limitation in a plant is important before applying management initiatives to reassure that the desired effect applies. The indication of increased seed set in the invasive *B. orientalis* from honey bee visits highlights the need for precaution when placing beehives in urban areas.

## Sammendrag

1. Urbanisering øker globalt og utgjør en potensiell trussel mot pollinatorsamfunn og pollinerings tjenester til ville planter. Likevel er det sprikende resultater fra studier om hvorvidt urbanisering har en positiv, nøytral eller negativ påvirkning på ville pollinatorer – ofte grunnet variasjoner i det omkringliggende landskapet eller ulike definisjoner av 'urbant'. I tillegg er det tendenser til at birøkt av honningbier (*Apis mellifera*) har en negativ innvirkning på ville pollinatorer og bidrar til økt spredning av invasive plantearter. Det er derfor interessant å undersøke om urbanisering og birøkt har en innvirkning på dikotomien av å bevare stedhørende planter samtidig som man bekjemper invasive fremmede planter.
2. Jeg har gjennomført en studie på pollenbegrensning i to plantearter, *Bunias orientalis* (invasiv) og *Lotus corniculatus* (stedhørende), langs en landskapsdiversitets- og urbaniseringsgradient på 35 lokaliteter i Porsgrunn og Skien kommuner, Norge. Jeg målte pollenbegrensning ved å sammenligne antall og vekt av frø for håndpollinerte blomster versus kontroller (naturlig pollinerte blomster) langs landskapsgradientene. Videre ble besøk av insektpollinatorer registrert for å undersøke hvordan pollinatorsamfunnet korrelerte med landskapsgradientene, hvordan deres tilstedeværelse korrelerte med pollenbegrensning i *B. orientalis* og *L. corniculatus*, og hvordan honningbier korrelerte med ville insekter.
3. Jeg fant at antall frø hos *B. orientalis* økte med landskapsdiversitet, noe som indikerer lavere pollenbegrensning og god evne til å spre seg i heterogene landskap. Antall frø hos *L. corniculatus* korrelerte negativt med urbanisering, noe som indikerer høy pollenbegrensning og en lavere evne til å spre seg i urbane områder. Angående insektbesøk, vil jeg fremheve funnet av at honningbier ser ut til å øke frøsettingen til den invasive plantearten *B. orientalis*, hvilket kan redusere pollen begrensningen til planten, og dermed potensielt styrke dens spredning.
4. *Syntese*: Den invasive *B. orientalis* og den stedhørende *L. corniculatus* er interessante fra et forvaltningsperspektiv siden de representerer motstridende forvaltningsmål om å utrydde invasive planter og bevare stedhørende planter. Denne studien viser at det er viktig å vite hvordan landskapsvariabler påvirker pollenbegrensning hos en plante før man implementerer forvaltningsinitiativer for å sikre at ønsket effekt oppnås. På grunn av indikasjonen på at honningbier øker frøsettingen i den invasive planten, fremhever dessuten studien behovet for forsiktighet når man plasserer bikuber i urbane områder.

# Table of Contents

1	Introduction .....	1
2	Materials and methods.....	4
2.1	Study species.....	4
2.2	Study area .....	5
2.3	Study design.....	6
2.3.1	Flower-visiting insects .....	7
2.3.2	Hand pollination and seed sampling .....	8
2.4	Data analyses.....	9
2.4.1	Pollen limitation and landscape variables .....	9
2.4.2	Pollen limitation and insect groups.....	10
2.4.3	Insect groups and landscape variables .....	11
2.4.4	Insect groups and honey bees.....	11
2.4.5	General.....	11
3	Results.....	12
3.1	Average seed number, seed weight, and insect visits.....	12
3.1.1	<i>Bunias orientalis</i> .....	12
3.1.2	<i>Lotus corniculatus</i> .....	12
3.2	Pollen limitation and landscape variables.....	13
3.3	Pollen limitation and insect groups .....	16
3.4	Insect groups and landscape variables .....	16
3.5	Wild insects and honey bees .....	19
4	Discussion.....	20
4.1	Effects of urbanisation.....	20
4.2	Effects of landscape diversity .....	22
4.3	Effects of honey bees.....	23
5	Conclusion.....	24
6	References .....	26
7	Attachments.....	31
7.1	Attachment 1 .....	31
7.2	Attachment 2 .....	32

# 1 INTRODUCTION

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Insects are in decline worldwide (Dicks et al., 2021; Potts et al., 2010). Several reviews on the causes of the insect decline have been conducted, finding land-use change to be the principal driver (Dicks et al., 2021; Kearns et al., 1998; Potts et al., 2010; Vanbergen et al., 2013; Wagner, 2020). A part of the global land-use change is urbanisation, which is the transformation of rural areas into urban areas (United Nations, 2019). The initial phase of urbanisation is urban sprawl along the city margins, and the second phase is urban densification making the city more compact (Tratalos et al., 2007). The United Nations (2019) expect the proportion of global urban dwellers to increase from 55 % in 2018 to 68 % in 2050, further expecting the number of megacities (> 10 million inhabitants) to increase from 33 in 2018 to 43 in 2030. Consequently, more knowledge about the effect of urbanisation on insects is needed.

Insects make up a great share of animal pollinators and perform pollination services on both wild plants and crops. Considering the world's species richness of angiosperms, 87,5% are pollinated by animal pollinators (Ollerton et al., 2011). Thus, reductions in insect pollinators might weaken pollination of wild flora, possibly leading to lower plant species richness (Ollerton et al., 2011). Regarding crop production, around 75 % of the leading global crop species are benefitting from animal pollination (Klein et al., 2007). These crop species constitute around 35 % of the volume consumed, as the remaining crop volume and species are wind- or self-pollinated crops like rice, wheat, and corn (Klein et al., 2007). Animal pollination is found to constitute 0.5 % of global gross domestic product (GDP) (Lautenbach et al., 2012), whereas approximately 10 % of the total value of food production is attributed to insect pollinators (Gallai et al., 2009; Lautenbach et al., 2012). In one study, approximately 79 % of the crop production attributed to insects was from honey bees (*Apis mellifera*) (Calderone, 2012). Evidently, wild insect pollinators have great value for both nature itself and humans.

Many studies worldwide have investigated how urbanisation affects insect pollinators, but the results diverge. How urbanisation affects insects range from positive, to neutral, and negative impacts (Prendergast et al., 2022; Wenzel et al., 2020). This inconsistency is partly explained by what the urban areas are compared with, where intensively managed agricultural areas tend to be more negative and natural areas tend to be more positive for insects, compared to urban areas (Prendergast et al., 2022; Wenzel et al., 2020). Despite a lack of an overall general trend of urbanisation on insects (Wenzel et al., 2020), distinguishing between different taxa shows

different patterns. Butterflies, beetles, and flies are often negatively impacted by urbanisation, whereas bees more often show a positive association with urbanisation (Baldock et al., 2015; Banaszak-Cibicka et al., 2018; Bergerot et al., 2010; Deguines et al., 2012; Geslin et al., 2013; Hall et al., 2017).

Despite diverging overall trends in the impact of urbanisation on insects, we see an agreement on certain effects. Firstly, there is a strong agreement on urbanisation leading to lower species richness with fewer specialist species, and more generalist species (Ayers & Rehan, 2021; Biesmeijer et al., 2006; Prendergast et al., 2022; Wenzel et al., 2020). Negative trends concern pollinators who forage on very few plant species, have long tongues, brood once a year (Biesmeijer et al., 2006), or are ground-nesting species and early spring species (Wenzel et al., 2020). Further, there is a trend of urban sprawl benefitting wild insect pollinators, whereas urban densification is harmful to them (Wenzel et al., 2020). Subsequently, more research is needed on how urban areas can facilitate those insect pollinators that are negatively impacted by urbanisation.

The diverging trends of how urbanisation affects insect pollinators are also pointing to a second important factor: landscape diversity. Landscape diversity is a term which embraces the different landscape types and their respective share within a certain area (Gamez-Virues et al., 2015). Expansion of agriculture or transforming diverse landscapes for human settlement are typical land use changes of concern. Insect specialists benefit from diverse landscapes and at the same time suffer from habitat reduction or increased management intensity (Gamez-Virues et al., 2015), while bee diversity decreases when landscapes are increasingly dominated by monoculture farming (Martins et al., 2017). However, a meta-analysis from 2021 found that the effect of expanding areas of arable land on bee visits is highly context-dependent, having different impacts depending on the type of management and what it replaces (Herbertsson et al., 2021).

The domesticated honey bee is of great use to humans due to its pollination service and honey production and has therefore been distributed by humans outside its natural range for apiculture. Honey bees have been introduced to almost every country in the world, and typically visit over hundred different plant species within a region (Goulson, 2003). While foraging, honey bee individuals typically stick to one plant species at a time (Wright et al., 2018), potentially depleting abundant nectar resources and displacing native insect pollinators (Goulson, 2003; Valido et al., 2019). Apiculture is, in some experimental studies, found to drive a reduction in wild insect pollinator diversity (Valido et al., 2019) and density (Lindström et al., 2016), as

well as having a preference for non-native plant species (Goulson, 2003). Whether honey bees negatively influence wild insect pollinators and if they contribute to the spread of invasive plant species are not yet conclusive. Nevertheless, pollinators, including honey bees, provide pollination services on plants, thereby affecting both plant population growth and viability.

The quality and efficiency of pollination services from insects have impacts on the plant community, and one way to measure the level of pollination success is through pollen limitation. Pollen limitation may arise in plants, where reduced reproductive success leads to lower seed quality or quantity (Ashman et al., 2004). Regarding animal-pollinated plants, pollen limitation may arise if the frequency of pollinator visits decreases, or if the pollen delivered is in low numbers or are incompatible with the recipient species (Ashman et al., 2004). A diverse insect pollinator community support high plant species richness and abundance (Fontaine et al., 2005), and increasing numbers of wild insect pollinators reduce pollen limitation in crop areas (Garibaldi et al., 2011). Despite using honey bees for pollination services (Junqueira et al., 2021), plant seed set has been shown to decrease when an increase in honey bees is parallel to a decrease in wild insects (Valido et al., 2019). This suggests that a diverse pollinator community supports lower pollen limitation and that honey bees do not necessarily secure low pollen limitation. We must gain deeper insights into the impact of apiculture on flora and wild insect pollinators so that efforts made to promote native plant species and prevent the spread of invasive plant species are not in vain.

I have studied pollen limitation in the native plant species *Lotus corniculatus* and the invasive species *Bunias orientalis* along two landscape gradients, urbanisation and landscape diversity, in a total of 35 sites in Porsgrunn and Skien cities, Norway. The study aims to assess how urbanisation and landscape diversity correlates with the pollinator community and pollination services (through pollen limitation), and how honey bees correlate with the wild flower-visiting insects.

I predicted that (I) increased urbanisation positively correlates with pollen limitation in both study species, (II) increased visits of flower-visiting insects negatively correlate with pollen limitation in both study species, (III) increased urbanisation negatively correlates with visits from flower-visiting insects, and that (IV) high abundance of honey bees negatively correlates with the number of flower-visiting insects.



## 2 MATERIALS AND METHODS

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### 2.1 STUDY SPECIES

Study species are *Bunias orientalis* and *Lotus corniculatus*, chosen since they are common, as well as being native and invasive respectively (Figure 1). The latter is interesting from the perspective of management since it is of global interest to keep the spread of invasive species to a minimum. Therefore, investigating potential drivers for increased seed set and thus spread is important. On the contrary, *L. corniculatus* is a species that is very common in flower meadows and other grassy habitats, hence representing flower meadow plant communities (Clapham et al., 1962, p. 348). Semi-natural grasslands, where *L. corniculatus* frequently occur, is assessed as *vulnerable* in the Norwegian Red List for Ecosystems and Habitat Types (Hovstad et al., 2018), and is therefore of interest to protect. When it comes to pollinators visiting the two plant species, higher bee diversity is found to visit the genus *Lotus* than *Bunias* (Rasmussen et al., 2021). The majority of pollinators on *L. corniculatus* are bumblebees (The Database of Pollinator Interactions, n.d.), while *B. orientalis* have a more generalised pollination system and are visited by various small bees and flies (Clapham et al., 1962, p. 154).



Figure 1: The pictures show *Bunias orientalis* to the left, and *Lotus corniculatus* to the right, both with bright yellow flowers. The invasive *B. orientalis* has open generalist flowers, while the native *L. corniculatus* has specialised tubular pea flowers. Photos: Rebekka Sundøy Haldorsen.

*Bunias orientalis* is biennial, sometimes perennial, and belongs to the cabbage family, *Brassicaceae*. The plant grows in nutrient-rich clay as well as on sandy soils, reaching heights of 50-120 cm (Mossberg & Stenberg, 2018, p. 555). Typical habitat is waste ground, road verges, and along railways (Lid & Lid, 2005, p. 331; Mossberg & Stenberg, 2018, p. 555). The style is short (Clapham et al., 1962, p. 154), and flowers are open and yellow with flowering time between June and July (Mossberg & Stenberg, 2018, p. 555). Flowers are homogamous and thus self-pollinate, and fruits can contain up to 2 seeds (Clapham et al., 1962, p. 154). The

species originated in Southeast Europe and West Asia and likely arrived in Norway through the import of grain or ballast around the year 1800 (Norwegian Biodiversity Information Centre, 2018). Distribution is within the nemoral, southern boreal and middle boreal zone (Lid & Lid, 2005, p. 331). *B. orientalis* is categorized as *severe impact* on the Norwegian Alien Species List (Norwegian Biodiversity Information Centre, 2018).

*Lotus corniculatus* is perennial and belongs to the pea family, *Fabaceae*. Flowers are yellow and partly red, typically with 4-7 flowers in the inflorescence (Mossberg & Stenberg, 2018, p. 384). Flowering season stretch between June and August (Mossberg & Stenberg, 2018, p. 384). The plant is highly self-incompatible and thus dependent on pollinators, and pods contain 1-30+ seeds (Ollerton, 1993; Ollerton & Lack, 1998). It is widely distributed in the nemoral to middle boreal/northern boreal zone in Norway (Lid & Lid, 2005, p. 497), growing in sandy and poor soils, ground covering to a height of 10-30 cm (Mossberg & Stenberg, 2018, p. 384). Typical habitat is road verges, dry land, waste ground, and grasslands (Lid & Lid, 2005, p. 497; Mossberg & Stenberg, 2018, p. 384).

## **2.2 STUDY AREA**

I conducted the fieldwork in Porsgrunn and Skien municipalities, Vestfold and Telemark County, on the southeast coast of Norway. Porsgrunn and Skien are neighbouring municipalities, with the area of Porsgrunn being approximately 22 km<sup>2</sup>, and accommodating roughly 35 000 inhabitants, whereas Skien is approximately 27 km<sup>2</sup> and accommodates roughly 50 000 inhabitants, per 2022 (Statistics Norway, n.d.). The cities are located in the boreonemoral zone and slightly oceanic section (Moen, 1999, p. 142). Important characteristics of the boreonemoral zone are agricultural land with the coexistence of deciduous and coniferous woodlands (Moen, 1999, p. 100). The area is characterised by precipitation between 700-1000 mm/y and medium snow cover at 100-124 days (Moen, 1999, pp. 24, 151). The growing season, days with temperature  $\geq 5$  °C, is 190-200 days/year with a mean yearly temperature of 6-4 °C, and an average July temperature of  $> 16$  °C (Moen, 1999, pp. 21-23). The bedrock in the study area consists of shale, sandstone, marl, limestone and granite (The Geological Survey of Norway, 2022). The most urban areas in the municipalities are the two connected city centres north in Porsgrunn and south in Skien, with agricultural land lining the urban, thereafter surrounded by forest.

### 2.3 STUDY DESIGN

The study comprised hand pollination and recording of insect visits and was carried out between the 09<sup>th</sup> of June and the 05<sup>th</sup> of July 2022. Fieldwork was conducted in two rounds, the first on both study species, and the second on only *L. corniculatus*. The selection of study sites was done ahead of fieldwork by searching digitally and live by car. Digitally, I first checked registered observations in Norway's Species Map Service (Norwegian Biodiversity Information Centre, n.d.), and second I "walked" in Google Maps by the function 'street view', looking for bright yellow flowers (Google Maps, n.d.). Coordinates of potential locations were recorded to be visited in person to ensure a good fit with the other criteria (see below). Searching by car was done by driving around in expected suitable areas where I had not already found sites digitally. I drove until finding a potential site, stopped, and walked alongside roads to see if I could find either of the two study species.

Criteria for site selection were planned beforehand, but with some practical modifications in the field. The maximum site size was approximately 20x20 meters without a lower limit, adjusted depending on infrastructure and access to the respective species, with a minimum of 10 individuals of the study species per site. Plant individuals were attempted to be distributed over the entire site area. The between-site distance was set to a minimum of 500 meters both because solitary bees usually forage within 500 metres from the nest (Gathmann & Tscharrnke, 2002; Goulson, 2003), and because Porsgrunn and Skien are small cities with limited amounts of urban areas. I strived for sites to cover the range of urbanisation in the study area, while simultaneously attempting to keep landscape diversity as constant as possible to isolate the effect of urbanisation on pollen limitation. Nevertheless, this was proven difficult due to other selection criteria. Initially, I planned for 25 sites per plant species, however in round one 16 sites with the invasive *B. orientalis* and 19 sites with the native *L. corniculatus* fit the criteria, one site being the same for both species (Figure 2). In round two, all sites of *B. orientalis* and three sites of *L. corniculatus* were eliminated due to withering plants, leaving 16 sites of *L. corniculatus* for data collection. All sites were approved by the municipalities, with an agreement to not perform maintenance during the research period. Additionally, each site was marked with signs stating, "research area", as a second means to avoid municipal maintenance.

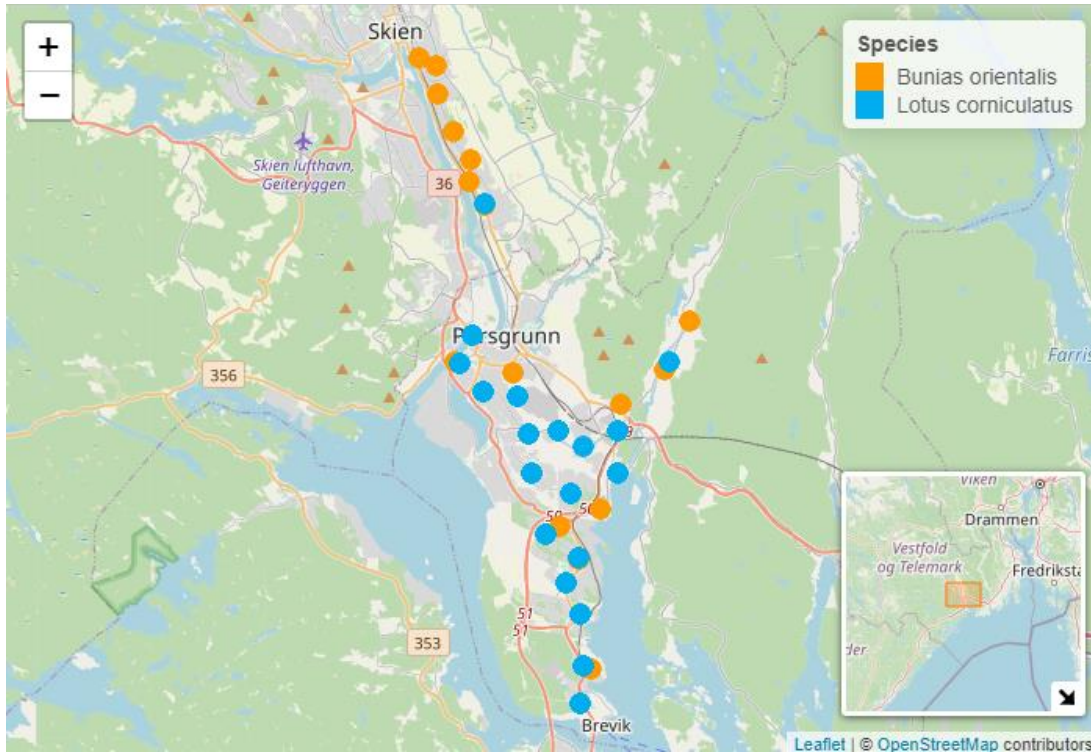


Figure 2: The 35 study sites in Porsgrunn and Skien were located in the central and more urban areas of the municipalities. Orange dots represent sites of *Bunias orientalis* and blue dots represent sites of *Lotus corniculatus*.

The two landscape variables, urbanisation and landscape diversity, were calculated based on the land cover map ELC10 (Venter & Sydenham, 2021). The degree of urbanisation was calculated as the percentage of impervious surfaces within a radius of 250 m around each site. The maximum degree of urbanisation in my chosen sites reached 37 %, meaning that 37 % of the surfaces surrounding the site are classified as impervious. The diversity of land use types was calculated by a Shannon diversity index (hereafter: landscape diversity) (Shannon, 1948). The index is calculated through a combination of how many of the eight different land use types, and the proportion of them, that exists within 250 m radius around the site. The maximum score will be 2.1 (see equation underneath (Shannon, 1948)), and this decreases with fewer land use types and/or increasingly uneven proportions. The values of landscape diversity within my sites ranged between 1.0 and 1.7. Numbers were multiplied by 10 and rounded to integers to reduce the memory required when working with raster maps.

$$H' = -\sum_{i=1}^R p_i \ln p_i$$

### 2.3.1 Flower-visiting insects

Video sampling was performed on each site to record flower-visiting insects. Insects were recorded within three plots per site, with one five-minute video recording per plot. Criteria for plot selection were the presence of flowering plant individuals, as far as possible evenly spread

within the site area without overlapping. Each plot comprises the area covered by a hula hoop with a diameter of 77 cm. All recordings were executed between 09:00 and 18:00 in dry weather and with observations of flying butterflies as an indicator of acceptable wind speed.

I started by placing the hula hoop, waiting for the first insect visitor to arrive on a flower, and then started recording. Each video clip lasted 5:00 minutes, and I filmed all flower-visiting insect individuals on the study species by moving the camera or zooming in on them. After filming a plot, each inflorescence with fresh flowers of the study species was counted.

Post fieldwork each clip was reviewed and insects were recorded to the following groups: wasps, solitary bees, honey bees (*Apis mellifera*), bumblebees, flies, butterflies and beetles. From the recordings, I cannot know which insects actually pollinate the flowers they visit. Therefore, I refer to them as flower-visiting insects.

### **2.3.2 Hand pollination and seed sampling**

To measure pollen limitation, I performed a hand pollination experiment on the study species. I selected 10 plant individuals per site, of which five were controls (naturally pollinated) and five treated (naturally pollinated + hand pollinated), with one meter between plant individuals. Whenever such distance was impossible, the two most closely located individuals were one control and one hand pollinated individual. In round one, one flower per individual was chosen for treatment, and in round two six flowers per individual were chosen for treatment. Flowers were, as far as possible, fresh and bright yellow, and were marked with a cotton string to be re-found for seed collection. Criteria for weather and time were equal to the recording of flower-visiting insects. Flowering was monitored to secure that treatment was performed before flowers started withering.

The hand pollination treatment was performed by directly applying pollen from pollen donor flowers onto the recipient stigma. The number of donor flowers used in the treatment varied in rounds one and two due to time constraints. In round one pollen donor flowers were collected on-site, picking plentiful of flowers from three separate individuals growing outside the study site. Each flower exposed to hand pollination received pollen from one flower of each donor individual, resulting in receiving pollen from three flowers. In round two, I started each day by collecting plentiful of pollen donor flowers from one common area that I used throughout that entire day (Attachment 1). Recipient flowers were treated with one pollen donor flower each. When performing hand pollination, I removed all petals on the donor flowers to expose the anthers with pollen grains before executing the treatment. For *B. orientalis*, whose flowers are

open, the anthers were applied directly on the easily accessible stigma of recipient flowers to deposit pollen, whereas on *L. corniculatus* I had to carefully pull down the keel with a tweezer to expose the stigma before depositing pollen. Donor flowers were used until pollen was no longer visually disposed of on the stigma.

Seed collection was performed approximately three weeks after the hand pollination treatment, between the 03<sup>rd</sup> and 28<sup>th</sup> of July. This interval was chosen since pods were fully formed, as well as the need to secure collection before pods started opening. Fruits were left to air dry on a sunny windowsill in enclosed coffee filters until autumn when seeds were counted manually and weighed on a scale sensitive to four decimals on the gram.

## **2.4 DATA ANALYSES**

All statistical analyses were done separately for the two plant study species, *L. corniculatus* and *B. orientalis*. The models were built under the principle of starting with the most complex model and performing stepwise variable selection. Insignificant variables were thus dropped one at a time until left with only one variable or given (near) significant results.

### **2.4.1 Pollen limitation and landscape variables**

Generalized mixed-effects models were used to test if urbanisation and landscape diversity had an impact on seed numbers. Variables used in model building from the start (many of which were excluded in the final model) were: urbanisation, landscape diversity, hand pollination treatment, flower number (per site), and in the case of *L. corniculatus*: pollen donor site. The model for *B. orientalis* used a Poisson distribution, with fixed effects being treatment (levels: hand pollinated and control) in interaction with landscape diversity (scaled), and with site as a random effect. The model for *L. corniculatus* was zero-inflated with a negative binomial distribution, with fixed effects being treatment (levels: hand pollinated and control) in interaction with the degree of urbanisation (log-transformed), whereas random effects were plant individual nested in site, and round (round of hand pollination).

Running models on average weight per seed against the landscape variables proved difficult. Therefore, I ran a simple linear mixed-effects model on the controls (naturally pollinated seeds) with seed number as the fixed effect, with weight per seed as the response. Due to the issues of running models for seed weight, models for hand pollinated flowers were not built since I was more interested in the correlations between the landscape variables and controls (naturally pollinated flowers) as these represent the current state and impact of urbanisation. The *B.*

*orientalis* model had seed number as fixed effect and site as a random effect. When running the model for *L. corniculatus* it did not meet the model assumptions, and I experienced problems with errors. Limitations in the scale when weighing seeds led to 14 observations recording 0.0000 grams, thus these 14 observations were excluded from the model. Further, I excluded observations from round one, due to few replicates. Ultimately, the model for *L. corniculatus* ended up with weight per seed (log-transformed) as response, with seed numbers (log1p-transformed) as the fixed effect, and random effects being plant individual nested in site, and round. Both models were built with a Gaussian distribution.

I made a map to visualise the spatial distribution of pollen limitation for *B. orientalis* and *L. corniculatus* in Porsgrunn and Skien (Figure 5). The basis for the effect maps is the land cover map ELC10 (Venter & Sydenham, 2021), and colours represent predictions of seed number to the landscape variables. The effect map of *B. orientalis* is based on predictions from the model of seed numbers and its response to landscape diversity. The effect map of *L. corniculatus* is based on predictions from the model of seed numbers and its response to urbanisation. Predictions to values of landscape diversity and urbanisation that were not present in my study sites are cropped away. Therefore, predictions of seed number to landscape diversity under 10 and over 16 and to urbanisation under 1 % and over 35 % are cropped away.

#### **2.4.2 Pollen limitation and insect groups**

Zero-inflated generalized mixed-effects models were used to test if the presence of different insect groups correlated with seed numbers in controls (naturally pollinated flowers). Insect recordings were summed up per site to match the scale at which seeds were measured (as the most detailed location of seeds was “site”, and not “plots”), resulting in the number of insect visits per 15 minutes. I tried using the visitation rate in models considering insect visitation, but that failed to fit the model assumptions in several models. Therefore, insect counts were used throughout the analyses for continuity. Identifying hoverflies from other flies in the video recordings was difficult, and these were therefore combined throughout all the analyses of the study. The model building started with all the different insect groups as fixed effects, but most were eliminated from the final model through stepwise variable selection.

Poisson distribution was used for the *B. orientalis* model, with fixed effects being scaled count (per site) of honey bees and flies, and site as a random effect. Negative binomial distribution was used for *L. corniculatus*, with fixed effects being squared and scaled count (per site) of bumblebees. Since I collected data twice on *L. corniculatus* where the second round included

six flower replicates on each plant individual, the random effects in the model were plant individual nested in site, and round.

### **2.4.3 Insect groups and landscape variables**

Generalized mixed-effects models were run to test how the different insect groups responded to the landscape variables. As a starting point, scaled degree of urbanisation and landscape diversity, as well as their interaction, were used as fixed variables. Assuming that more flowers attract more insects, flower number (per site) was included as an offset variable, but only if a pattern was observed between insect observations and flower numbers. Detecting potential patterns was done by plotting the observations of insects against the number of flowers, and if they were randomly spread the offset was not included in the model. All the models were built with a Poisson distribution.

Since these models were run with insects as response variables, many insect groups had insufficient numbers of observations and were therefore not built. On the invasive *B. orientalis*, there were too few observations of wasps, beetles, and butterflies. Thus, I made one model each for honey bees, solitary bees, bumblebees, flies and wild insects (in total). Random effects in all five models were plot nested in site. On the native *L. corniculatus*, there were too few observations of wasps, beetles, butterflies, honey bees, and flies. Thus, I made one model each for bumblebees and solitary bees. The model for solitary bees had convergence problems. Therefore, I ran this model with observations of bumblebees and solitary bees merged into wild bees, and a separate model for bumblebees alone. In both models, random effects were plot nested in site, and round.

### **2.4.4 Insect groups and honey bees**

To test if honey bees had an impact on wild insect groups, I ran generalized mixed-effects models with Poisson distribution. Due to few observations of wasps, beetles, and butterflies, they were excluded from these analyses. In total, four models were run, with bumblebees, solitary bees, flies, and wild insects (in total) as response variables. The number of honey bees was used as the only fixed variable and with plot nested in site as random effect. Testing if honey bees correlated with wild insects visiting *L. corniculatus*, was not possible since no honey bees were observed visiting *L. corniculatus*.

### **2.4.5 General**

All analyses were conducted in R version 4.2.2 (R Core Team, 2022) using the ‘glmmTMB’ (Brooks et al., 2017) and ‘lme4’ (Bates et al., 2015) packages for model building, where



'glmmTMB' were used for the zero-inflated models. The 'DHARMA' (Hartig, 2022) and 'blmeco' (Korner-Nievergelt et al., 2015) packages were used for model diagnostics. Variable selection was performed by the function "drop1" in 'lmerTest' (Kuznetsova et al., 2017), with a Chi-square test. Bar plots were created with 'ggplot2' (Wickham, 2016), with graphical additions by 'ggpattern' (FC et al., 2022) and 'ggthemes' (Arnold, 2021). Other plots were built with the package 'effects' (Fox, 2003; Fox & Weisberg, 2019), whereas maps were made with the 'leaflet' package (Cheng et al., 2022) and 'sf' (Pebesma, 2018), or by raster files processed in 'raster' (Hijmans, 2022) and with 'TMB' (Kristensen et al., 2016).

## 3 RESULTS

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### 3.1 AVERAGE SEED NUMBER, SEED WEIGHT, AND INSECT VISITS

#### 3.1.1 *Bunias orientalis*

The mean seed number in the invasive *B. orientalis* was 0.76 seeds for hand pollinated flowers and 0.59 seeds for controls (range: 0-2), but this difference was not significant (Table 1). In hand pollinated flowers, the average weight per seed was 2.1 mg, whereas controls were non-significantly lower at 1.9 mg (Table 1). The share of seed pods from the control and hand pollinated flowers that were re-found was 125 out of 160 possible (Attachment 2). The mean number of flowers per site (sum of flowers within the three observation plots) was 293 (range: 73-707). The mean number of insect visits per site per 15 minutes was 15.1 (range: 4-34), where wild insects contributed 10.9 individuals and honey bees contributed 4.2 individuals. This resulted in a visitation rate of 0.07 insects per flower per 15 minutes. On *B. orientalis* I recorded 242 insects over 240 minutes, where the most abundant insect groups were honey bees (67), flies (65), solitary bees (56) and bumblebees (29) (Figure 3).

#### 3.1.2 *Lotus corniculatus*

Mean seed numbers in the native *L. corniculatus* were 7,9 seeds for hand pollinated flowers and 7,4 seeds in controls (range: 0-31), the difference being non-significant (Table 1). The average weight per seed for hand pollinated flowers was 0,84 mg, whereas controls were non-significantly lower at 0,81 mg (Table 1). The share of seed pods from the control and hand pollinated flowers that were re-found was 822 out of 1150 possible (Attachment 2). The mean number of flowers per site (sum of flowers within the three observation plots) was 409 (range: 93-1357). The mean number of insect visits per site per 15 minutes was 1.24 (range: 0-5), all

of which were wild insects since no honey bees were recorded on *L. corniculatus*. This resulted in a visitation rate of 0.003 insects per flower per 15 minutes. *L. corniculatus* was visited by a low diversity and abundance of species, recording only 48 insects over 530 minutes, most of which were bumblebees (32) (Figure 3).

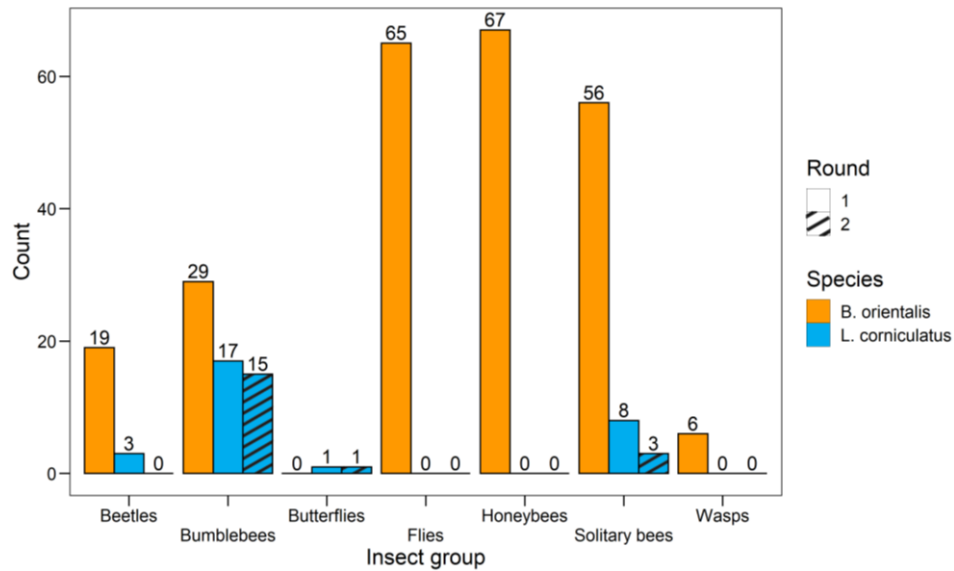


Figure 3: Overview of the number of insect visits on the invasive *Bunias orientalis* (orange) and the native *Lotus corniculatus* (blue). Recordings were performed once on *B. orientalis* and twice on *L. corniculatus*, where no pattern is from round one and stripes are from round two. *B. orientalis* were visited by a high diversity of insects along with a high abundance of especially honey bees, flies and solitary bees, whereas visits on *L. corniculatus* had low diversity, the majority being bumblebees. Insect recordings were performed in the summer of 2022 in Porsgrunn and Skien, Norway.

### 3.2 POLLEN LIMITATION AND LANDSCAPE VARIABLES

Landscape diversity showed a near-significant positive correlation with seed number for control plants in *B. orientalis* (Table 1, Figure 4 A). There was no correlation between seed number and the weight per seed (g) for *B. orientalis* (Table 1, Figure 4 C).

The degree of urbanisation had a near significant negative correlation with seed number in *L. corniculatus* for control plants (Table 1, Figure 4 B). The same results were seen when testing for only round two of data collection (not shown). There was a near significant positive correlation between seed number and weight per seed (g) (Table 1, Figure 4 D).

Pollen limitation in the two plant species is visually represented in maps (Figure 5). The maps show that the spatial distribution of high pollen limitation for both species is centred in the city centre of Porsgrunn and Skien municipalities.

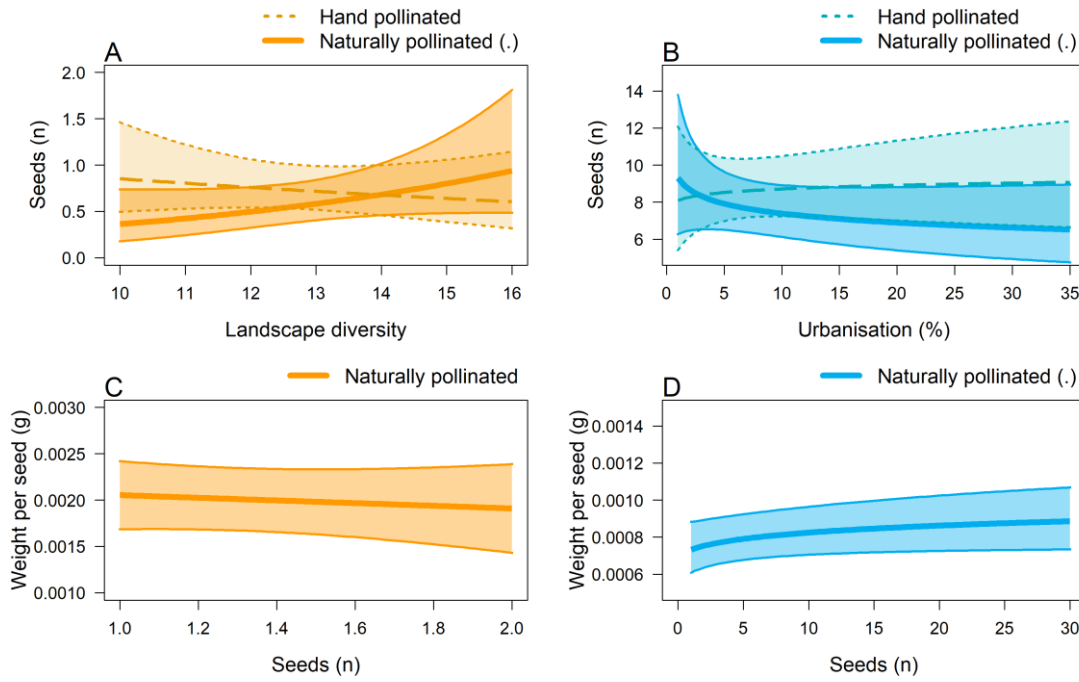


Figure 4: The correlation between landscape variables and seed number and seed weight in two plant species. Orange colours represent *Bunias orientalis*, with a correlation between landscape diversity and seed numbers (A) and a correlation between seed numbers and average weight per seed (g) (C). Blue colours represent *Lotus corniculatus*, with a correlation between urbanisation and seed numbers (B) and a correlation between seed numbers and average weight per seed (g) (D). There was a near-significant correlation between seed number in *L. corniculatus* with urbanisation and with landscape diversity for *B. orientalis* in controls (naturally pollinated flowers). Notice different scales on the axis. Solid lines represent controls (naturally pollinated flowers) and dotted lines are hand-pollinated flowers. Confidence interval at 95%. Data were collected once for *B. orientalis* and twice for *L. corniculatus* in the summer of 2022 in Porsgrunn and Skien, Norway.

Table 1: Parameter estimates, standard error, z-value/t-value and p-value for mixed-effects models for two different plant species, *Bunias orientalis* and *Lotus corniculatus*, testing the correlation between landscape diversity, urbanisation and experimental treatment (hand-pollination and natural-pollination), and seed number and average seed weight (g). For seed numbers, landscape diversity was scaled and urbanisation was log-transformed. For weight in *L. corniculatus*, weight per seed was log-transformed and seed numbers were log<sub>1p</sub>-transformed.

	Estimate	Std. Error	z-value	p-value
<b>Number of seeds, <i>Bunias orientalis</i></b>				
Intercept	-0.33	0.16	-1.98	0.047 *
Landscape diversity	-0.11	0.16	-0.68	0.496
Natural pollination	-0.23	0.22	-1.05	0.295
Landscape diversity : Natural pollination	0.41	0.23	1.81	0.070 .
<b>Number of seeds, <i>Lotus corniculatus</i></b>				
Intercept	2.09	0.20	10.25	<0.001 ***
Urbanisation	0.03	0.09	0.37	0.713
Natural pollination	0.14	0.15	0.91	0.360
Urbanisation : Natural pollination	-0.13	0.07	-1.92	0.055 .
	Estimate	Std. Error	t-value	p-value
<b>Weight per seed, <i>Bunias orientalis</i></b>				
Intercept	0.0022	0.00035	6.32	<0.001 ***
Seed numbers	-0.00014	0.00024	-0.61	0.549
<b>Weight per seed, <i>Lotus corniculatus</i></b>				
Intercept	-7.27	0.11	-65.17	<0.001 ***
Seed numbers	0.069	0.039	1.77	0.078 .

Note: significant p-values are indicated by asterisks (.<0.1, \*<0.05, \*\*<0.01, \*\*\*<0.001)

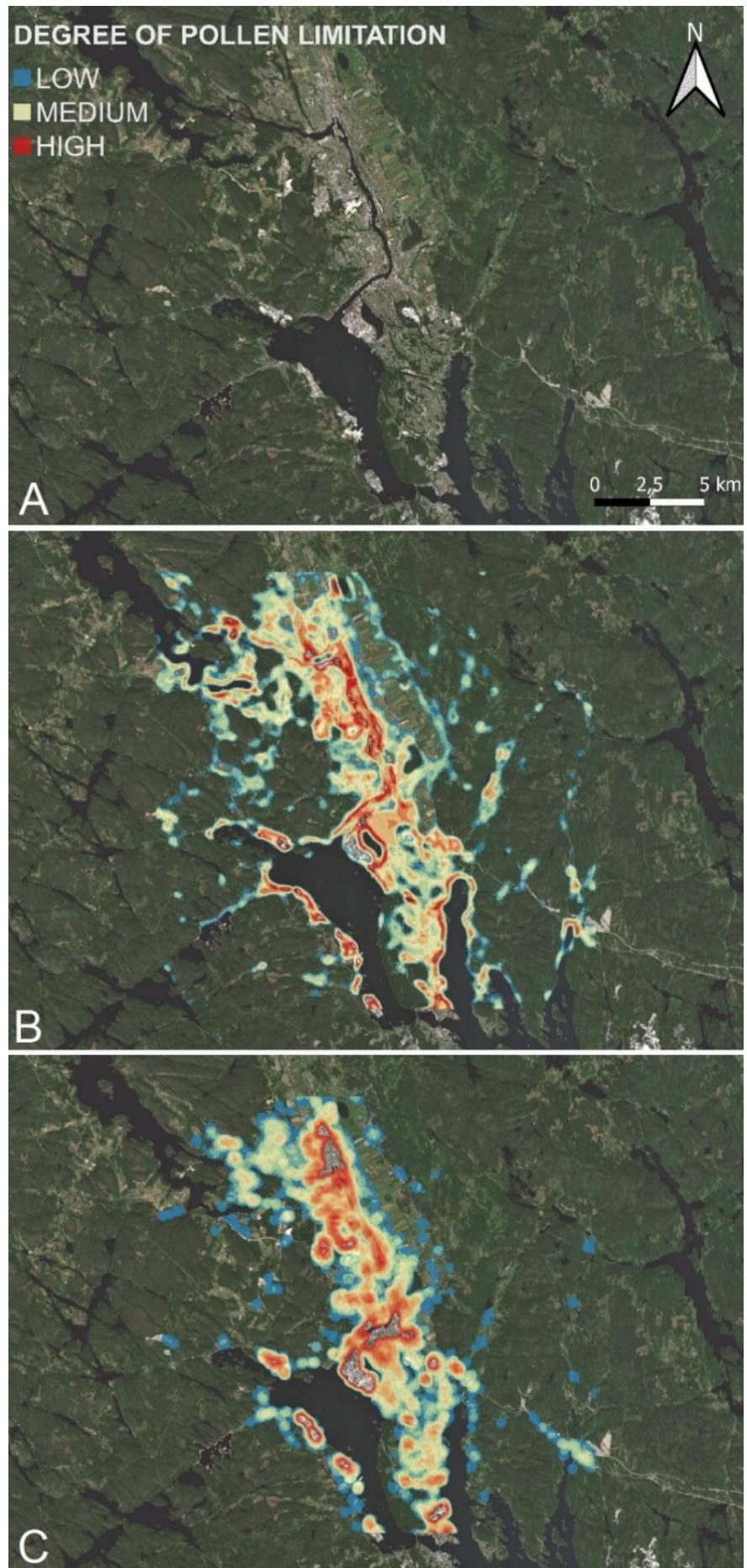


Figure 5: The study area (A), pollen limitation in *Bunias orientalis* (B), and pollen limitation in *Lotus corniculatus* (C). The colours represent the degree of pollen limitation for the two plants, as the number of seeds, with blue colours representing low pollen limitation and red colours representing higher pollen limitation. Predictions are based on the models for how seed number responded to landscape diversity (B. *orientalis*) and urbanisation (L. *corniculatus*). Predictions to values of the landscape variables not present in the study sites, are cropped away from the maps. In terms of nature management, low pollen limitation in native species is positive while it is negative regarding invasive species.

### 3.3 POLLEN LIMITATION AND INSECT GROUPS

The presence of honey bees and flies had a significant positive correlation with seed numbers in *B. orientalis* (Table 2, Figure 6), whereas bumblebees were the only insect group showing a near significant positive correlation with seed numbers in *L. corniculatus*.

Table 2: Parameter estimates, standard error, z-value and p-value for mixed-effects models for two different plant species, *Bunias orientalis* and *Lotus corniculatus*, testing the correlation between different insect groups and seed numbers. All insect groups were scaled, and bumblebees were additionally square root transformed.

	Estimate	Std. Error	z-value	p-value
<b>Number of seeds, <i>Bunias orientalis</i></b>				
Intercept	-0.67	0.20	-3.42	<0.001 ***
Honey bees	0.35	0.18	1.96	0.049 *
Flies	0.45	0.17	2.69	0.007 **
<b>Number of seeds, <i>Lotus corniculatus</i></b>				
Intercept	2.02	0.11	18.37	<0.001 ***
Bumblebees	0.12	0.07	1.80	0.072 .

Note: significant p-values are indicated by asterisks (.<0.1, \*<0.05, \*\*<0.01, \*\*\*<0.001)

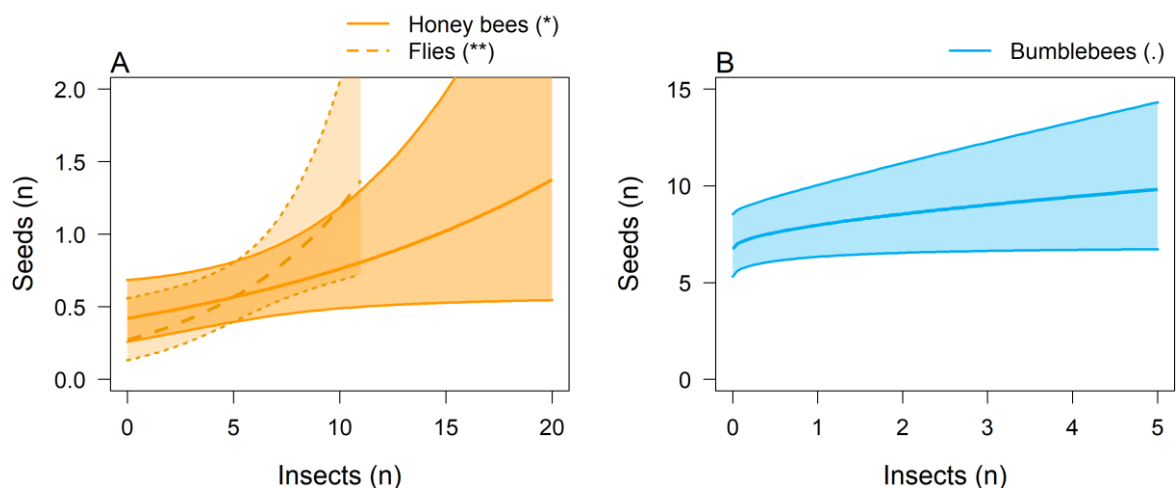


Figure 6: Correlation between different insect groups and the number of seeds in two plant species. Notice the different scales on the axis. Honey bees and flies had significant positive correlations with seed number in *Bunias orientalis* (A), whereas bumblebees had a significant positive correlation with seed number in *Lotus corniculatus* (B). Confidence intervals of 95%. Data on seeds and insect visits were collected once for *B. orientalis* and twice for *L. corniculatus* in the summer of 2022, in Porsgrunn and Skien, Norway.

### 3.4 INSECT GROUPS AND LANDSCAPE VARIABLES

I found no correlation between the two landscape variables and insects visiting *L. corniculatus* (Table 3). For insects visiting *B. orientalis* (Table 3, Figure 7), the number of flies correlated near significantly negative with urbanisation (Figure 7 D), whereas honey bee numbers had a significant positive correlation with landscape diversity (Figure 7 C). The number of both solitary bees and wild insects altogether (Figure 7 A & B), decreased with urbanisation when

landscape diversity was otherwise high, and increased with urbanisation when landscape diversity was low, as shown through the interaction term between urbanisation and landscape diversity.

Table 3: Parameter estimates, standard error, z-value and p-value for mixed-effects models for two different plant species, *Bunias orientalis* and *Lotus corniculatus*, testing the correlation between urbanisation and landscape diversity with different insect groups. All landscape variables were scaled, and in the model for honey bees the landscape diversity was additionally square root transformed.

	Estimate	Std. Error	z-value	p-value
<b><i>Bunias orientalis</i></b>				
Numbers of wild insects				
Intercept	1.35	0.17	8.11	<0.001 ***
Urbanisation	-0.16	0.15	-0.99	0.324
Landscape diversity	0.16	0.15	1.10	0.270
Urbanisation : Landscape diversity	-0.55	0.22	-2.48	0.013 *
Numbers of honey bees				
Intercept	-5.75	0.66	-8.68	<0.001 ***
Landscape diversity	1.35	0.57	2.39	0.017 *
Number of solitary bees				
Intercept	0.35	0.23	1.55	0.122
Urbanisation	-0.05	0.19	-0.26	0.797
Landscape diversity	0.17	0.22	0.80	0.423
Urbanisation : Landscape diversity	-0.97	0.31	-3.13	0.002 **
Number of bumblebees				
Intercept	-5.66	0.49	-11.59	<0.001 ***
Urbanisation	0.17	0.38	0.44	0.659
Number of flies				
Intercept	-0.03	0.22	-0.16	0.874
Urbanisation	-0.38	0.20	-1.92	0.055 .
<b><i>Lotus corniculatus</i></b>				
Number of wild bees				
Intercept	-5.81	0.18	-31.82	<0.001 ***
Urbanisation	-0.09	0.18	-0.50	0.619
Number of bumblebees				
Intercept	-1.42	0.31	-4.59	<0.001 ***
Urbanisation	-0.23	0.25	-0.92	0.357

Note: significant p-values are indicated by asterisks (.<0.1, \*<0.05, \*\*<0.01, \*\*\*<0.001)

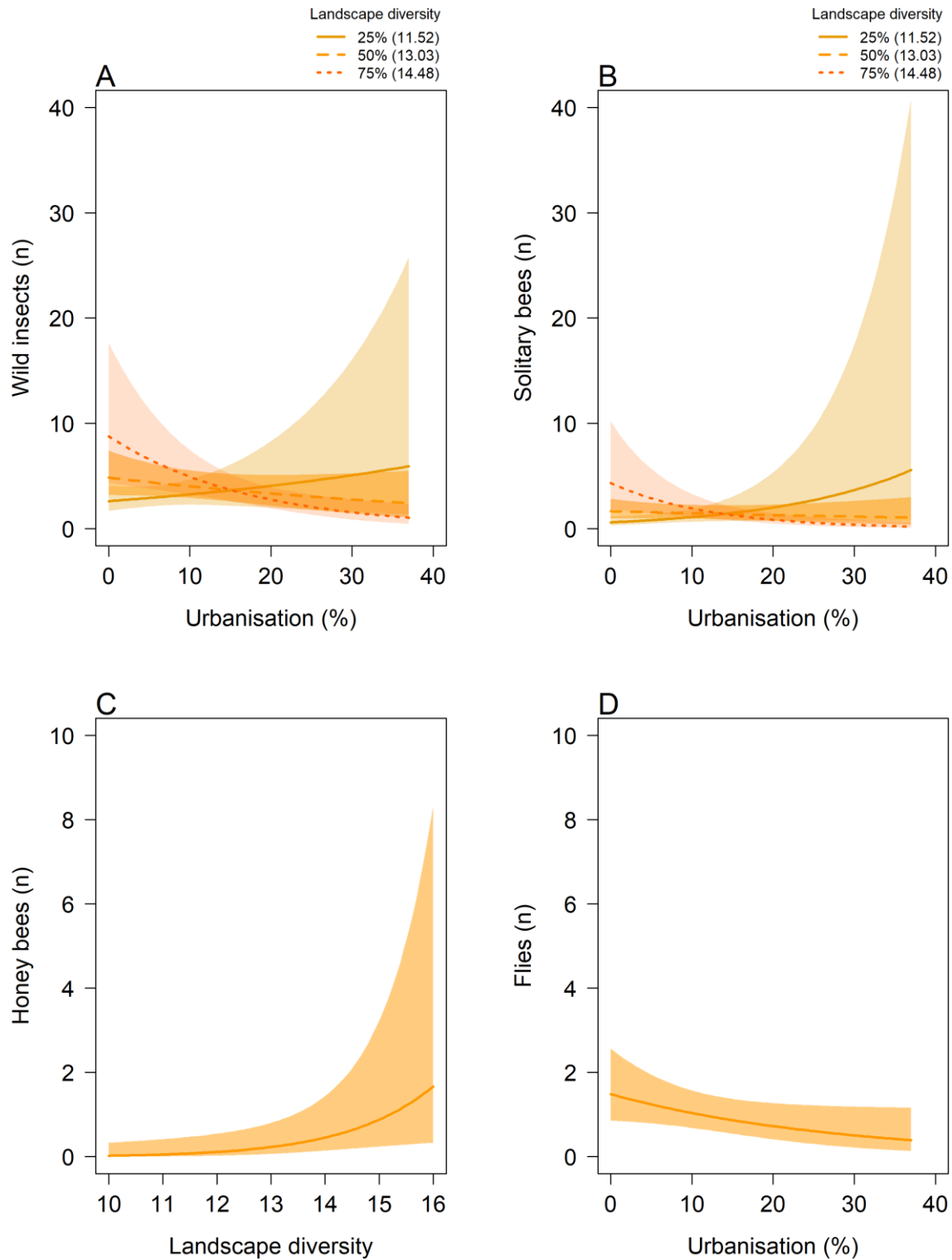


Figure 7: Correlation between landscape variables and the number of insect visits on the plant species *Bunias orientalis*. Notice the different scales on the axis. Numbers of insect visits from wild insects (A) and solitary bees (B) both correlated negatively with the interaction between urbanisation and landscape diversity, thus with higher landscape diversity increasing proportion of urbanisation negatively correlated with the numbers of visits (see the red, dotted line representing the upper quantile of landscape diversity in my study sites). The number of honey bee visits (C) positively correlated with increasing landscape diversity. The number of fly visits (D) correlated near-significantly negative with increasing urbanisation. Confidence intervals of 95%. Data on insect visits were collected once for *B. orientalis* in the summer of 2022, in Porsgrunn and Skien, Norway.

### 3.5 WILD INSECTS AND HONEY BEES

The presence of honey bees had a near significant positive correlation with bumblebee numbers but no correlation with wild insects, solitary bees, or flies (Table 4, Figure 8).

Table 4: Parameter estimates, standard error, z-value and p-value for mixed-effects models for the plant species, *Bunias orientalis*, testing the correlation between honey bees and different insect groups.

	Estimate	Std. Error	z-value	p-value
<b><i>Bunias orientalis</i></b>				
<b>Number of wild insects</b>				
Intercept	0.96	0.17	5.53	<0.001 ***
Honey bees	0.06	0.05	1.06	0.292
<b>Number of bumblebees</b>				
Intercept	-1.59	0.56	-2.84	0.004 ***
Honey bees	0.18	0.10	1.73	0.083 .
<b>Number of solitary bees</b>				
Intercept	-0.34	0.29	-1.20	0.232
Honey bees	0.08	0.07	1.04	0.297
<b>Number of flies</b>				
Intercept	-0.01	0.25	-0.05	0.961
Honey bees	-0.02	0.08	-0.21	0.836

Note: significant p-values are indicated by asterisks (.<0.1, \*<0.05, \*\*<0.01, \*\*\*<0.001)

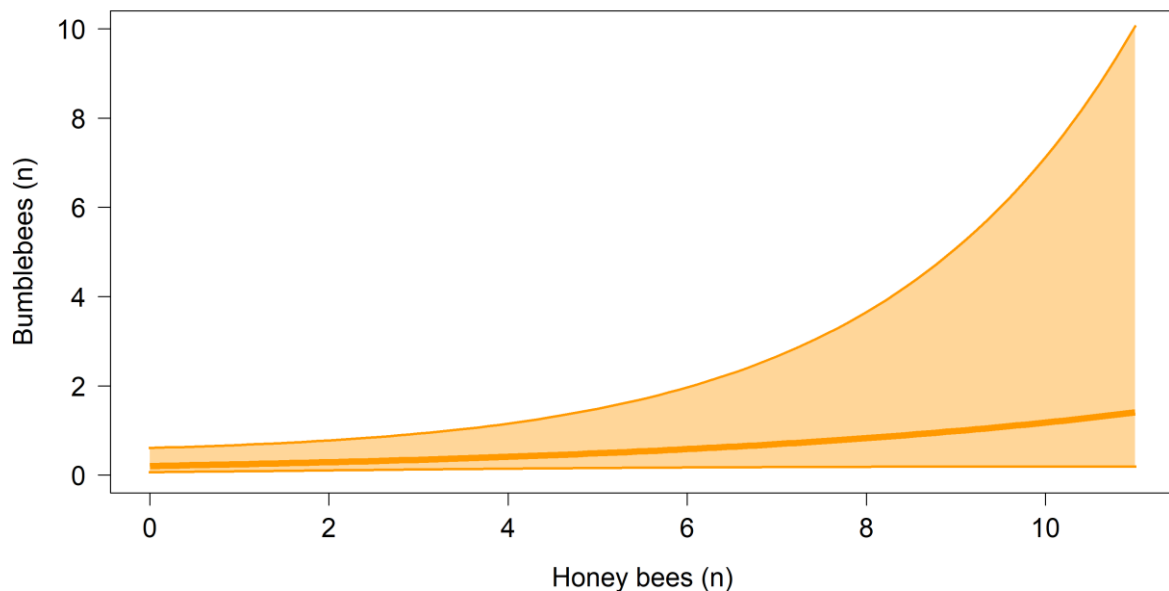


Figure 8: Near significantly positive correlation between honey bee visits and bumblebee visits, on the plant species *Bunias orientalis*. Confidence intervals of 95%. Data on insect visits were collected once for *B. orientalis* in the summer of 2022, in Porsgrunn and Skien, Norway.



## 4 DISCUSSION

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The number of seeds in *Bunias orientalis* and *Lotus corniculatus*, and thereby their degree of pollen limitation, correlate differently with urbanisation and landscape diversity. I found that seed numbers in the invasive *B. orientalis* correlated near-significantly positive with increasing landscape diversity, while simultaneously being positively correlated with the presence of honey bees and flies. Seed numbers in the native *L. corniculatus* were near-significantly negatively correlated with increasing urbanisation but near-significantly positively correlated with the presence of bumblebees. Studying how insects correlated with the landscape variables, I found no correlation for the insect groups visiting *L. corniculatus*. On the contrary, wild insects visiting *B. orientalis* correlated negatively with increasing urbanisation in areas with high landscape diversity. Honey bees, on the other hand, did not respond to urbanisation and correlated positively with increasing landscape diversity while simultaneously having a near-significant positive correlation with bumblebee numbers. Thereby, the predictions in this study were only partially supported by the results.

### 4.1 EFFECTS OF URBANISATION

I found that seed numbers in control flowers of the native *L. corniculatus* correlated negatively with increasing urbanisation, indicating that the plant is more pollen limited in more urban areas. This is presented visually (Figure 5 C), where there is a gradual reduction in pollen limitation from the most urban city centre, and outwards into the municipality. This aligns with previous research, finding that pollen limitation in flowers in urban areas is higher compared to flowers in non-urban areas (Bennett et al., 2020; Carper et al., 2022; Pellissier et al., 2012; but see Verboven et al., 2012). An explanation might be that urban areas offer high flower species richness, resulting in insects visiting a higher number of flower species, and thereby depositing more heterospecific pollen (Baldock et al., 2015; Carper et al., 2022; Martins et al., 2017). In turn, this might weaken the pollination service thus leading to a lower seed set. Another explanation can be the extent of landscape fragmentation, which is found to increase when the degree of urbanisation increases (Pellissier et al., 2013). Fragmentation leads to stronger patch isolation, making it difficult for pollinators to navigate and locate floral resources (Pellissier et al., 2013). Thus, pollen limitation might increase, as is found for *L. corniculatus* (Pellissier et al., 2013). A meta-analysis from 2020 investigating pollen limitation along an urban-rural gradient found that plants exclusively pollinated by functional insect groups other than bees, or plants that were specialists with one or few pollinators, were more pollen limited in urban

habitats than in natural habitats (Bennett et al., 2020). This coincides with studies finding bees to be positively affected by urbanisation (Baldock et al., 2015; Banaszak-Cibicka et al., 2018; Hall et al., 2017; Theodorou et al., 2020), while other insect groups (beetles, flies, and butterflies) show negative impacts (Bergerot et al., 2010; Deguines et al., 2012; Geslin et al., 2013). However, this only partly aligns with my findings: *L. corniculatus* was more pollen limited when urbanisation increased and was visited by only one functional group of pollinators, while the generalist *B. orientalis* was visited by many insect groups and did not show a response to urbanisation. But, the pollinators on *L. corniculatus* were indeed bumblebees and solitary bees, contradicting the research indicating that bees thrive in urban areas and thereby should have reduced pollen limitation in *L. corniculatus* with increasing urbanisation.

For *L. corniculatus*, bumblebees were the only insect group showing a – albeit non-significant – correlation with increased numbers of seeds. The tubular flowers of *L. corniculatus* need heavier insects like bumblebees to pollinate (Pellissier et al., 2012), and since the stigma and stamen are less available to smaller insects it creates less competition for the bumblebees, who might therefore have a strengthened attraction towards this floral resource. Research on whether bumblebees in urban contexts thrive (Meeus et al., 2021; Silva et al., 2023) or suffer (Weissmann et al., 2021; Winfree et al., 2009) is diverging. Perhaps, the study area can support enough resources for the bumblebees to persist in the cities. However, bumblebees constituted the majority of visits on *L. corniculatus*, which in turn showed increased pollen limitation with urbanisation. This might suggest that my chosen study design to record insect visits did not capture differences in bumblebee availability along the urbanisation gradient, and that transects, or other survey schemes, would be more suitable in a similar study system.

Different groups of insects visiting *B. orientalis* showed significant correlations with urbanisation. Firstly, the numbers of solitary bees and wild insects (in total) both correlated significantly negatively with urbanisation in increasingly diverse landscapes. A possible reason for this could be that homogeneous landscapes with low landscape diversity support few insects in the first place and therefore experience modest effects of an increase in urbanisation. This stands in contrast to a heterogeneous landscape that can support more insect biodiversity and thus experience detrimental effects from increasing urbanisation. This indicates a context dependency on whether urbanisation is negative for insects, which is supported by other research (Gamez-Virues et al., 2015; Wenzel et al., 2020). Secondly, flies correlated negatively – although non-significantly – with increasing urbanisation while correlating positively with increasing seed number in *B. orientalis*. This aligns with previous studies showing that flies

respond negatively to urbanisation (Baldock et al., 2015; Ssymank et al., 2008). Flies are highly understudied as pollinators, and despite being diverse and abundant they are not traditionally valued as important pollinators even though they pollinate in harsher weather conditions than bees (Silva et al., 2023; Ssymank et al., 2008). Nevertheless, my results indicate that flies are important pollinators for the invasive *B. orientalis*, and simultaneously suggest that flies are sensitive to urbanisation. This highlights the need for more research on other insect groups than bees on both pollen limitation and the effect of urbanisation.

The study sites reached a level of urbanisation at 37 %, which is an important observation due to the distinction between urban sprawl and urban densification. Urban sprawl is, to a larger extent than urban densification, beneficial for insect biodiversity (Fortel et al., 2014; Lowenstein et al., 2014; Martins et al., 2017). The principal reason why sub-urban areas (urban sprawl) are beneficial for insect biodiversity is the low percentage of impervious surfaces along with parks and residential areas offering diverse habitats and forage (Pellissier et al., 2013; Wenzel et al., 2020). On the other hand, urban densification (causing the degree of impervious surfaces to exceed 50 %) more often shows negative impacts on insect biodiversity (Wenzel et al., 2020). The lack of densely urban sites might hinder the experimental setup to capture the effect of urbanisation on insects (other than flies).

## **4.2 EFFECTS OF LANDSCAPE DIVERSITY**

The seed number of *B. orientalis* correlated positively with increasing honey bee visits and landscape diversity – although the latter was non-significant. The seed number responding to landscape diversity is presented visually (Figure 5 B), where there is the highest pollen limitation in the city centre. Compared to *L. corniculatus*, the degree of pollen limitation is reduced much more abruptly, indicating that despite being urban, the city centre is also heterogeneous regarding the landscape. Flowers of the invasive *B. orientalis* are open, with a short style, are visited by a diverse insect community of flies and bees, and are able to self-pollinate (Clapham et al., 1962, p. 154). Therefore, *B. orientalis* is probably robust towards changes in the pollinator community. Road verges act like corridors for the spread of plants (Lazaro-Lobo & Ervin, 2019), which is a typical habitat for *B. orientalis* as well as wastelands and along railways (Mossberg & Stenberg, 2018, p. 555). Thus, the plant is able to grow in a lot of different areas that are common in urban environments no matter the degree of impervious surfaces. Hence, *B. orientalis* can thrive in urban areas owing to abundant habitats and the partial independence of pollinators.

Honey bees correlated significantly positively with both the seed number in *B. orientalis* and also with increased landscape diversity – similar to *B. orientalis*. This indicates a mutualistic relationship between the two species that are further reinforced by a diverse landscape (Barthell et al., 2001). Domesticated honey bees only need their surroundings to support them with forage, as their hives are provided by humans. Hence, the correlation to landscape diversity might simply originate from the initial placement of hives by beekeepers, who may prefer heterogeneous landscapes rather than forests, fields, or city centres. I did not find a negative correlation between honey bees and urbanisation, contrasting the wild insects who were negatively correlated with urbanisation. This coincides with honey bees being able to fly several kilometres in search of forage (Goulson, 2003), thus possibly making them capable of sustaining in a highly urban environment compared to pollinators more restricted by flight distance.

### **4.3 EFFECTS OF HONEY BEES**

Worldwide, the honey bee is the most frequently recorded insect species in urban areas (Silva et al., 2023). Additionally, it is the insect species most often reported as the most abundant species (Silva et al., 2023). The indication of a mutualistic relationship between honey bees and *B. orientalis* from my data aligns with research finding that honey bees visit managed plant species and contribute to increased seed set in invasive species (Barthell et al., 2001; Iwasaki & Hogendoorn, 2022; Martins et al., 2017; Ropars et al., 2019; but see Harrison & Winfree, 2015). The combination of honey bees searching kilometres for forage (Goulson, 2003) and that invasive plants tend to grow in abundance wherever they manage to establish, possibly makes *B. orientalis* an attractive resource regardless of being located far away from the apiaries. My results indicate that Porsgrunn and Skien have a level of honey bee abundance that may increase the spread of invasive species. From the perspective of nature management, this is concerning, especially since the apiculture of honey bees are advocated for as a means to increase pollination services (Colla, 2022). Furthermore, the possibly strengthened spread of invasive plants makes survival for native plants more difficult, which ultimately may threaten wild insects through losses of important resources.

The discussion of whether honey bees negatively influence wild insect communities has been going on for a long time. The rising concern for wild insect pollinators is enhanced by recent studies, e.g. Herrera (2020), who found that wild bee visits to flowers in the Mediterranean basin was previously four times higher than honey bee visits, but are today at equal proportions. Previous research has shown that the density of honey bees drives a reduction in wild pollinators

(Lindström et al., 2016; Valido et al., 2019), especially bees (Iwasaki & Hogendoorn, 2022; Mallinger et al., 2017), that have similar ecological niches as honey bees (Goulson, 2003; Rasmussen et al., 2021). My results contrast with this, as I found a – albeit non-significant – positive relationship between bumblebee numbers and honey bee densities. This contrasting result might be due to the relatively diverse landscapes of my study sites, as previous research has shown a lower negative impact on wild insects from honey bees whenever the landscape is diverse and thereby supports a multitude of habitat and floral resources (Herbertsson et al., 2016; Lindström et al., 2016; Valido et al., 2019).

Another explanation for the positive correlation between honey bees and bumblebees might be that they are simply attracted to the same resources, or that beekeepers have placed hives near my sites that simultaneously are suitable habitats for bumblebees. Unfortunately, I did not gather information regarding the number or location of apiaries in the study area. In addition, the low quantity of visitation data implies the need for a cautious interpretation of these results. However, my findings are interesting because Porsgrunn and Skien are representative cities in Norway regarding size and number of inhabitants. Competition between insects is complex, and to provide conclusive results, experiments are needed. Therefore, it is important to highlight my results to urge more experiments regarding the relationship between domesticated honey bees and wild insects in urban areas.

## 5 CONCLUSION

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The two main findings of the present study are that urbanisation affects pollen limitation in native and invasive species differently, and that honey bees seem to increase seed set (and thus potentially the spread) of the invasive plant *B. orientalis*.

My study indicates that *L. corniculatus* has a lower seed set with increasing urbanisation. This means that increasing the quantity of green spaces (causing less impervious surfaces) is a potential measure to support native plant species, despite the lack of conclusive experimental studies on the matter (Ayers & Rehan, 2021; Prendergast et al., 2022; Silva et al., 2023). Moreover, the different response to the landscape variables in the invasive *B. orientalis* shows the importance of further investigating how more green spaces affects the spread of both native and invasive plants.

Lastly, despite diverging trends in studies that investigate the relationship between honey bees and invasive plants (Iwasaki & Hogendoorn, 2022), my results indicate the need for cautious

placement of beehives since honey bees might enhance the spread of invasive plants. People are increasingly enthusiastic about insects, simultaneously as media and governments advocate for apiculture as a means for sustainable pollination seemingly without scientific support (Colla, 2022; Herrera, 2020). Therefore, results from the present study along with other studies need to be communicated to the public, helping them to make informed and good choices when managing their property regarding apiculture.

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# 7 ATTACHMENTS

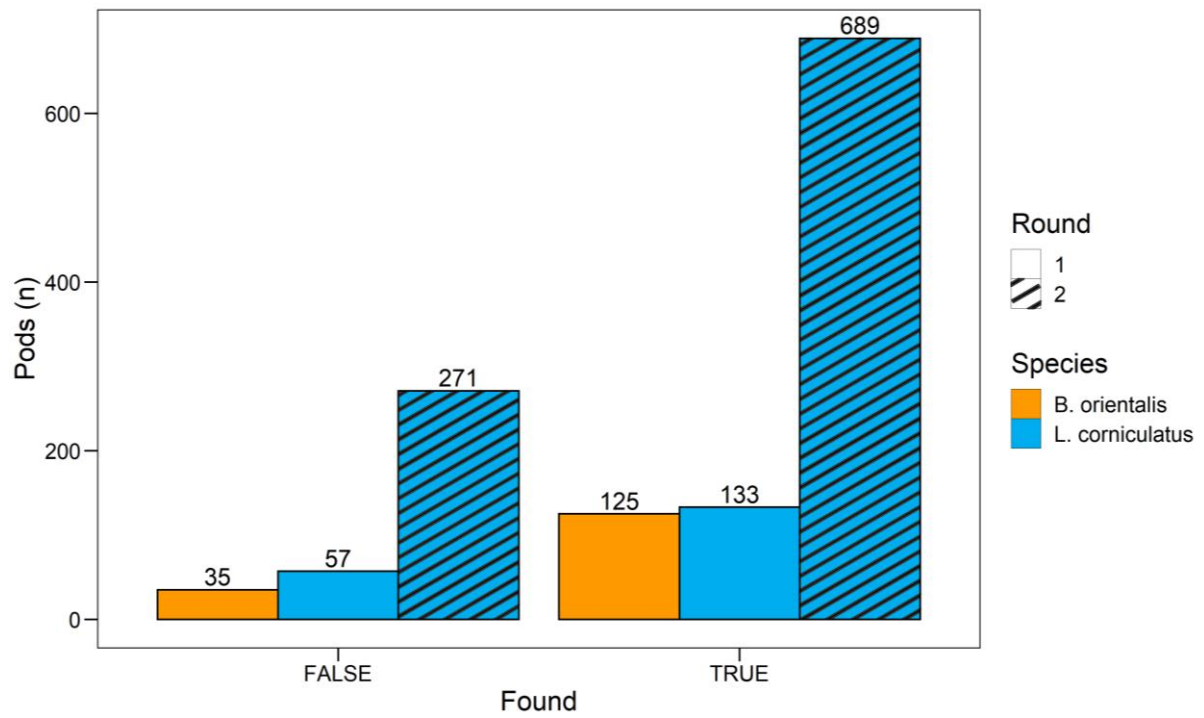
## 7.1 ATTACHMENT 1

*Attachment 1: Overview of Bunias orientalis and Lotus corniculatus over the site locations with the date for marking hand pollinated flowers and control flowers, performing hand pollination, seed collection, degree of landscape diversity and urbanisation, and for L. corniculatus round 2; pollen donor with its location.*

Round	Site	Site nick-name	Species	Date pollinated	Date seed collected	Latitude	Longitude	Urbanisation %	Landscape div.	Pollen donor	Pollen donor latitude	Pollen donor longitude
1	1	A	Bunias	10.06.2022	03.07.2022	59.1348586	9.6338410	21.00	16.00	NA	NA	NA
1	3	C	Bunias	10.06.2022	03.07.2022	59.1250524	9.7067414	18.00	14.00	NA	NA	NA
1	4	D	Bunias	11.06.2022	03.07.2022	59.1017487	9.6974030	7.00	13.00	NA	NA	NA
1	5	E	Bunias	11.06.2022	03.07.2022	59.0976731	9.6799096	2.00	11.00	NA	NA	NA
1	6	F	Bunias	12.06.2022	03.07.2022	59.0904866	9.6881245	6.00	12.00	NA	NA	NA
1	8	H	Bunias	12.06.2022	03.07.2022	59.1329172	9.7253177	1.00	11.00	NA	NA	NA
1	9	I	Bunias	20.06.2022	12.07.2022	59.1437411	9.7364167	0.00	14.00	NA	NA	NA
1	11	K	Bunias	12.06.2022	03.07.2022	59.0656968	9.6931433	37.00	13.00	NA	NA	NA
1	13	M	Bunias	13.06.2022	03.07.2022	59.1323767	9.6592502	8.00	14.00	NA	NA	NA
1	14	N	Bunias	10.06.2022	03.07.2022	59.1700856	9.6469235	22.00	15.00	NA	NA	NA
1	15	O	Bunias	11.06.2022	03.07.2022	59.1750993	9.6396051	19.00	16.00	NA	NA	NA
1	16	P	Bunias	09.06.2022	03.07.2022	59.186592	9.632955	3.00	12.00	NA	NA	NA
1	17	Q	Bunias	09.06.2022	03.07.2022	59.1947903	9.6262882	9.00	11.00	NA	NA	NA
1	18	R	Bunias	09.06.2022	03.07.2022	59.2011641	9.6255858	3.00	10.00	NA	NA	NA
1	19	S	Bunias	09.06.2022	03.07.2022	59.203149	9.617719	28.00	14.00	NA	NA	NA
1	20	T	Bunias	10.06.2022	03.07.2022	59.1799821	9.6403920	8.00	10.00	NA	NA	NA
1	1	NA	Lotus	13.06.2022	04.07.2022	59.1343354	9.6359086	35.00	17.00	NA	NA	NA
1	5	NA	Lotus	16.06.2022	04.07.2022	59.1192723	9.7049665	2.00	13.00	NA	NA	NA
1	7	NA	Lotus	16.06.2022	04.07.2022	59.1098659	9.7049400	3.00	15.00	NA	NA	NA
1	9	NA	Lotus	20.06.2022	12.07.2022	59.1157584	9.6900266	2.00	11.00	NA	NA	NA
1	10	NA	Lotus	21.06.2022	12.07.2022	59.1185127	9.6661056	4.00	13.00	NA	NA	NA
1	11	NA	Lotus	21.06.2022	12.07.2022	59.1193523	9.6792025	17.00	14.00	NA	NA	NA
1	13	NA	Lotus	15.06.2022	04.07.2022	59.1051167	9.6842156	3.00	13.00	NA	NA	NA
1	15	NA	Lotus	16.06.2022	04.07.2022	59.0958617	9.6732892	3.00	13.00	NA	NA	NA
1	16	NA	Lotus	16.06.2022	04.07.2022	59.0905080	9.6881078	6.00	12.00	NA	NA	NA
1	17	NA	Lotus	20.06.2022	12.07.2022	59.0778581	9.6884219	16.00	14.00	NA	NA	NA
1	19	NA	Lotus	13.06.2022	04.07.2022	59.1282164	9.6461714	25.00	14.00	NA	NA	NA
1	21	NA	Lotus	13.06.2022	04.07.2022	59.1408527	9.6415074	27.00	15.00	NA	NA	NA
1	22	NA	Lotus	20.06.2022	12.07.2022	59.1346209	9.7280127	1.00	13.00	NA	NA	NA
1	29	NA	Lotus	15.06.2022	04.07.2022	59.0661856	9.6898640	15.00	13.00	NA	NA	NA
1	30	NA	Lotus	15.06.2022	04.07.2022	59.0579908	9.6885322	14.00	13.00	NA	NA	NA
1	31	NA	Lotus	16.06.2022	04.07.2022	59.0850647	9.6823947	13.00	14.00	NA	NA	NA
1	33	NA	Lotus	16.06.2022	04.07.2022	59.1269667	9.6610544	4.00	12.00	NA	NA	NA
1	34	NA	Lotus	13.06.2022	04.07.2022	59.1702353	9.6469553	22.00	15.00	NA	NA	NA
1	36	NA	Lotus	21.06.2022	12.07.2022	59.1098111	9.6675735	8.00	13.00	NA	NA	NA
2	1	NA	Lotus	02.07.2022	27.07.2022	59.1343354	9.6359086	35.00	17.00	3	59.1638813	9.6622989
2	5	NA	Lotus	01.07.2022	27.07.2022	59.1192723	9.7049665	2.00	13.00	2	59.1977065	9.6313375
2	7	NA	Lotus	05.07.2022	28.07.2022	59.1098659	9.7049400	3.00	15.00	4	59.1634473	9.6339821
2	10	NA	Lotus	02.07.2022	27.07.2022	59.1185127	9.6661056	4.00	13.00	3	59.1638813	9.6622989
2	11	NA	Lotus	01.07.2022	28.07.2022	59.1193523	9.6792025	17.00	14.00	2	59.1977065	9.6313375
2	13	NA	Lotus	30.06.2022	27.07.2022	59.1051167	9.6842156	3.00	13.00	1	59.1332677	9.6324362
2	15	NA	Lotus	30.06.2022	27.07.2022	59.0958617	9.6732892	3.00	13.00	1	59.1332677	9.6324362
2	16	NA	Lotus	05.07.2022	28.07.2022	59.0905080	9.6881078	6.00	12.00	4	59.1634473	9.6339821
2	17	NA	Lotus	02.07.2022	27.07.2022	59.0778581	9.6884219	16.00	14.00	3	59.1638813	9.6622989
2	19	NA	Lotus	30.06.2022	27.07.2022	59.1282164	9.6461714	25.00	14.00	1	59.1332677	9.6324362
2	21	NA	Lotus	30.06.2022	27.07.2022	59.1408527	9.6415074	27.00	15.00	1	59.1332677	9.6324362

## 7.2 ATTACHMENT 2

Attachment 2: Overview of how many control and hand pollinated flowers that were re-found for seed collection. False are the pods not found, and true are the pods found. Lost cotton string or aborted flowers are possible causes of these losses. Orange colours are *Bunias orientalis* and blue colours are *Lotus corniculatus*. Stipes represent round two of the field work.





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