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Abundance of short- and long-tongued bees, and their impact on red clover seed production in four cultivars grown across a large latitude range

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ABSTRACT

Red clover (*Trifolium pratense* L.) is an important insect-pollinated forage crop. Low and variable seed production is a problem in many red clover cultivars, especially tetraploid ones, limiting their marketing potential. The roles of short- and long-tongued bees for red clover seed production, specifically in tetraploid cultivars, are not fully known. Here, we investigate the impact of short- and long-tongued bees, cultivar differences and plant traits for seed production, in one diploid and three tetraploid red clover cultivars managed for seed eating weevils (*Protapion* sp.) over two years. Since we expected the abundance of short- and long-tongued bees to vary across a latitudinal distribution, we included six sites distributed in southern and northern Sweden. We found no clear southern vs. northern pattern for the abundance of short- and long-tongued bees, while seed eating weevils were more abundant in the south. Seed yield (weight per area) was positively related to long-tongued bees, whereas seed set (proportion developed seeds per flower head) was positively related to both short- and long-tongued bees. Cultivars differed in seed production across sites. Moreover, some investigated plant traits - flowering flower head density and florets per flower head - were positively related to seed production. Total flower head density and number of florets per flower head varied among cultivars, sites and years. In conclusion, our results support the significance of both short-, and especially long-tongued bees for red clover seed production, and also suggest the importance of studying underlying genetic and non-genetic influence on cultivar differences.

1. Introduction

Declining trends of bee species richness have been reported frequently in many parts of the world (Biesmeijer et al., 2006; Potts et al., 2010; Zattara and Aizen, 2021). Increased levels of homogeneity in agricultural landscapes accompanied by loss of semi-natural habitats, leading to less suitable nesting habitats and food resources, are assumed main reasons for the decline (Connelly et al., 2015; Kennedy et al., 2013; Öckinger and Smith, 2007; Steckel et al., 2014). Considering bees in the *Bombus* genus, an important taxon in terms of wild pollinators, studies in Scandinavia, for example, have shown that several species are declining (Bartomeus et al., 2013), in particular long-tongued species, while some short-tongued species are unaffected or increasing (Blasi et al., 2023; Bommarco et al., 2012; Dupont et al., 2011). Therefore, the pollinator community available in Scandinavia for crop pollination today is highly skewed towards a few short-tongued bumblebee species and honeybees (Blasi et al., 2023; Bommarco et al., 2012; Dupont et al., 2011). For example, an inventory in red clover fields across Sweden in 2008–2010 found that 89 % of the bumblebees consisted of the two short-tongued *Bombus terrestris* and *B. lapidarius* (Bommarco et al., 2012). A more recent study showed that < 5 % of the pollinators in red clover fields were long-tongued bumblebees (Rundlöf et al., 2018).

Red clover (*Trifolium pratense* L.) is a perennial, insect-pollinated plant of the legume family that is commonly grown in grass-clover leys in many temperate regions of the world (Taylor and Quesenberry, 1996; Boller et al., 2010). With a long cultivation history spanning over hundreds of years, red clover has been and still is today one of the most important forage legumes used for animal fodder (Boller et al., 2010). Red clover has been a target for extensive systematic breeding in recent decades, resulting in over 300 cultivars available on the market today (OECD, 2025). Red clover is naturally diploid, but in the 1950s artificially developed tetraploid cultivars were produced and introduced on

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the market (Boller et al., 2010). This was a breakthrough in red clover breeding, since tetraploids are advantageous in terms of generally larger plants, improved forage yield, disease resistance and persistence (Taylor and Quesenberry, 1996; Boller et al., 2010). However, tetraploid cultivars generate 20–50 % less seed yield than their diploid ancestors, which limits their marketing potential (Boller et al., 2010; Rundlöf et al., 2018). This, together with dramatic between-year variation in seed yield for both diploid and tetraploid cultivars (Bommarco et al., 2012; Jing and Boelt, 2021) constitutes persistent challenges in red clover seed production.

Seed production in red clover is a complex trait influenced by both abiotic (Jing and Boelt, 2021; Petkovic et al., 2017) and biotic factors, such as pollinators (Boller et al., 2010; Free, 1965) and pests (e.g. seed eating *Protapion* sp. weevils, Lundin et al., 2012; Rundlöf et al., 2018). Pollinators - bumblebees and to some extent honeybees (Free, 1993) - are a key component since the plant is almost exclusively self-incompatible and dependent on cross-pollination for reproduction (Free, 1993; Taylor and Quesenberry, 1996). It has been suggested that long-tongued bumblebee species are especially important for seed production due to the long corolla tubes of red clover flowers, and that this is particularly true for tetraploid cultivars because of their longer corolla tubes (reviewed in Free, 1993; Boller et al., 2010). According to this idea, short-tongued bumblebees and honeybees, hereafter collectively referred to as short-tongued bees, might have problems reaching the nectar at the bottom of the corolla tube and therefore reject the flowers or, alternatively, perform nectar robbing where they pierce the flower and obtain nectar without transferring any pollen. Nectar robbing in red clover has been observed in several short-tongued species (Hawkins, 1961; Hänninen, 1962; reviewed in Free, 1993; Hederström et al., 2021). However, the dependence on long-tongued bees for red clover pollination is unclear. In support of the significance of long-tongued bees for seed set, Hawkins (1956) found a positive correlation with abundance of long-tongued bumblebees, but not with short-tongued bees. Moreover, Hederström et al. (2021) showed that short- and medium-tongued bee species visited diploid red clover more frequently than tetraploid red clover when given a choice, whereas long-tongued species visited diploid and tetraploid clover to the same extent. Similar observations that flowers with long corollas are visited more frequently by long-tongued bees than by short-tongued bees have been found in for example lavender (*Lavandula* spp., Balfour et al., 2013). There is also evidence that long-tongued species are more efficient pollinators of red clover than short-tongued species in terms of amount of pollen transfer (Hederström et al., 2021), seeds produced after single visits (Hederström et al., 2021), working speed at nectar collection (Bender, 1999) and number of flowers visited per minute (Nørgaard Holm, 1966). In contrast, several studies have found that short-tongued bees are capable of pollinating red clover to the same extent as long-tongued bees (Palmer-Jones et al., 1966; reviewed in Free, 1993) and that corolla tube length is uncorrelated to seed yield (Vleugels et al., 2015, 2016). It is also possible that pollination services are mostly reliant on common and abundant species, and that the most important pollinators are the most abundant ones (Kleijn et al., 2015). Since there are still uncertainties of how important short- and long-tongued bees are for red clover seed production, more studies are needed (Jing et al., 2021a).

Plant traits are also known to affect red clover seed production, including number of flower heads per plant (Amdahl et al., 2017; Vleugels et al., 2016, 2015), seed weight per flower head (Amdahl et al., 2017) and pollen viability (Jing et al., 2021b). Number of florets per flower head could in theory affect the maximum potential seed production per flower head, but has not been identified as significant for seed yield when tested (Amdahl et al., 2017; Vleugels et al., 2016, 2015). Moreover, fewer studies (but see Hederström et al., 2021; Jing et al., 2021b) have investigated the role of pollinators for cultivar differences in seed production in red clover - for example if pollinators exhibit variation in preference towards different cultivars - which has been observed in some other systems, e.g. sunflower (*Helianthus annuus*, Stejskalová et al., 2018) and apple (*Malus domestica*, Burns and Stanley, 2022). Knowledge about a broader range of plant traits of importance for seed production could also be beneficial for plant breeding.

Due to the recent decline in long-tongued bumblebee species reported in particular in high intensity agricultural landscapes (e.g. Dupont et al., 2011), which appear to favour a dominance (> 95 %) of short-tongued bees in red clover fields in southern Sweden (Rundlöf et al., 2018), it could be valuable to test the impact of pollinators on seed production in red clover across a large geographical range differing in landscape types and climate zones. Moreover, red clover seeds are today only commercially produced in southern Sweden (Öhlund, unpublished data). It would be of interest to evaluate the potential for seed production in northern Sweden where the pollinator community might be more favourable. Older studies in Sweden and Finland have demonstrated geographical differences in the pollinator fauna in red clover, with more honeybees in the south and more bumblebees further north (reviewed in Free, 1993). Similarly, it has been suggested that the relative importance of long-tongued bees gradually increases from south to north in Europe (reviewed in Free, 1993). Latitude and landscape complexity have also been shown to influence the flight period of queens in spring differently for short- and long-tongued bumblebee species: short-tongued and often common species have a better ability to adapt to climate and landscape changes compared to long-tongued and often declining species (Blasi et al., 2023). In summary, to study the impact of different bees for red clover pollination it should be preferable to include several sites distributed in a wide range.

In this study, we investigate pollinator abundance and preference, seed production and plant traits in one diploid and three tetraploid cultivars of red clover grown in field trials at six sites in Sweden over two years. Since we expected long-tongued bees to be less abundant in southern compared to northern Sweden, due to a greater extent of homogenous agricultural fields and different climatic zones, we chose to include sites distributed in both the south and the north. We aimed to eliminate the effect of damage by seed eating weevils (*Protapion* sp.) on seeds by treating the plants with an insecticide; however, since there were some weevils found in our field trials, this gave us the opportunity to examine their abundance across the distribution of field sites and potential impact on seed yield. Finally, we tested the ability of the short-tongued bumblebee species *Bombus terrestris* to pollinate tetraploid red clover in the absence of long-tongued species, using cage trials with different pollinator densities. This bumblebee species is the most common in the Swedish bumblebee fauna (Blasi et al., 2023; Bommarco et al., 2012) and is also easily accessible as it is the main bumblebee species used commercially for pollination in agricultural fields and greenhouses. Our main questions were (i) How do abundances of pollinators of different tongue length and *Protapion* weevils differ across the investigated geographic range? (ii) Do pollinators of different tongue length differ in cultivar preference? (iii) How is seed production affected by cultivation site, cultivar, pollinator and weevil abundances? (iv) How are plant traits affected by site and cultivar, and what are their relation to seed production? (v) Is the short-tongued *B. terrestris* able to pollinate tetraploid red clover at high and low densities?

2. Materials and methods

2.1. Field sites and cultivars in field trials

The field trials were conducted in 2020 and 2021 at six trial sites, distributed in southern and northern Sweden with approximately 962 km between the most distant sites (Fig. 1, Supplementary Table S1). One diploid cultivar (SW Yngve, hereafter referred to as 'Yngve') and three tetraploid cultivars (Betty, Peggy and Vicky) were included in the study (Supplementary Table S2). The reason for this unbalanced design was that we were mostly interested in studying differences among tetraploid cultivars, where seed yield is particularly challenging, but

Fig. 1. Map showing the six sites in Sweden for red clover field trials included in the study. The map is based on open GIS data at Lantmäteriet (The Swedish National Land Survey).

wanted to include a diploid as a reference cultivar. The cultivars differ in earliness regarding start of flowering, but their peak flowering periods overlap considerably, and the study was therefore performed within the same time span for all cultivars. The plants were sown one year before data collection, i.e. data collection was related to first year harvest in both years. Per year and site, each cultivar was grown in two 10 × 10 m plots, representing two replicates. The different cultivar plots were kept in the same field and with their placement randomized for each year and site. At each site, different fields were used between the two years. When deemed necessary, plants were treated with an insecticide (or occasionally several insecticides, see products in Supplementary Table S1) against seed eating weevils at the bud stage before flowering following conventional methods. Two sites reported managed honeybee hives 800–1000 m from the field trial (see Supplementary Table S1). No sites reported managed bumblebee hives in the vicinity of the field trials. All data was collected between early July and late August, i.e. during the peak flowering season of the four red clover cultivars. In 2020, all cultivars at Röbäcksdalen and Vicky at Lännäs were excluded due to melting of snow, and ice encasement in spring that damaged the plants.

2.2. Pollinator surveys

To estimate abundance of pollinators in the plots and relative distribution among cultivars (i.e. potential pollinator preferences for cultivars), pollinator surveys were conducted in three survey rounds over the season, approximately one week apart. The surveys were performed between 9 am and 6 pm during days with a minimum temperature of 17°C, at least 30 % sunlight, and no strong winds or heavy rain at least one hour before the survey. Since the activity of pollinators might vary during the day, efforts were made to alternate the survey time of the day (morning, mid-day, and afternoon) between the three different rounds for each site. Pollinators were surveyed in transect walks (modified from Pollard, 1975, 1977) by dividing each plot into five parallel 10-m long and 1-m wide transects, resulting in a total area of 50 m^2 per plot. Total survey time per plot was set to five minutes (excluding time for handling insects). The observer walked slowly along the transects and noted all individuals of bumblebees (*Bombus* sp.) and honeybees (*Apis mellifera*). Only flower-visiting individuals were recorded, and it was noted if an individual was observed nectar robbing. The bees were identified to species level when possible. However, some short-tongued bumblebee species belonging to the subgenus *B. sensu stricto* (*B. terrestris*, *B. lucuorum*, *B. cryptarum* and *B. magnus*) are hard to distinguish (Murray et al., 2008) and were therefore all classified as *B. terrestris* agg. If needed, insects were captured with a net and brought to the lab for species identification. Bumblebees and honeybees were grouped into short-tongued and long-tongued bees based on Goulson et al. (2005) and Balfour et al. (2013) (Supplementary Table S3). In the following text, we refer to short-tongued bumblebees and honeybees collectively as 'short-tongued bees' and long-tongued bumblebees as 'long-tongued bees', respectively.

2.3. Weevil surveys

Even though we wanted to eliminate *Protapion* sp. weevil damage on seeds by treating the plants with an insecticide before the flowering season, we still wanted to evaluate their abundance, and thus potential for impact on seed yield, in the plots at the end of the season. Abundance of weevils was evaluated according to Lundin et al. (2012). At the end of the season, from each plot 60 evenly distributed flower heads were collected, mixed and separated into two different hatching tubes containing approximately 30 flower heads per tube. The flower heads were collected in the appropriate stage for the *Protapion* sp. larvae to have hatched and started to pupate, i.e. when the petals had started to wilt and turn brown, but the stems were still green. The hatching tubes were then kept for at least six weeks inside in a dry and light condition before evaluation. The number of emerging adult weevils were counted, and weevil abundance per flower head was calculated as the number of weevils divided by the number of flower heads in the hatching tube.

2.4. Seed production

Two measurements of seed production were evaluated: seed yield and seed set (Hederström et al., 2021, 2024). The term 'seed production' will in the following text relate to both measurements. Seed yield was estimated as seed weight (g) per area and measured for each plot by harvesting mature flower heads from three 0.25 m^2 sample quadrats evenly distributed in the plot at the end of the flowering season. After harvesting, the material was dried, threshed and cleaned at The Rural Economy and Agricultural Society, Skåne, Sweden. The seed yield for each plot was calculated by weighing the seeds and calculating a mean value from the quadrats. For three of the sites, Kölbäck, Lännäs and Svalöv, measurements of total seed yield harvested from whole plots were also collected to confirm that seed yield measured from the 0.25 m^2 quadrats were satisfactory representatives of the seed yield. Defoliation of the red clover prior to harvest using Reglone was conducted at some of the sites (Lännäs 2021, Svalöv 2020, 2021). Moreover, to get an indication of seed quality for the total yield harvested, thousand seed weight was evaluated in both years and germination percentage was evaluated in 2020 by germinating 2 × 100 seeds per plot following the guidelines of the International Seed Testing Association (ISTA) (2018).

Seed set was estimated as the proportion of developed seeds per flower head and calculated by dividing the number of developed seeds (including seeds with weevil damage) by total number of florets per flower head. This made it possible to get an indication of the contribution of pollination rate to seed set and fertility rate. The flower heads for seed set counting were marked at the same occasion as pollinator surveys were performed and collected three weeks later. For each pollinator survey round, three mature flower heads (at least 80 % of the florets opened) were marked at the start and at the end, respectively, of each transect walk, resulting in six flower heads per plot and survey round.

2.5. Total and flowering flower head densities and florets per flower head

We estimated three plant traits – total flower head density, flowering flower head density and number of florets per flower head. Flower head densities were measured for each plot and survey round. The densities were estimated by placing three 0.25 m^2 quadrats evenly distributed (avoiding edges) in each plot and counting the number of buds ($<$ 5 open florets and the rest immature), flowering flower heads ($>$ 5 open florets) and wilted flower heads ($<$ 5 open florets and the rest wilted) within each quadrat. Total flower head density included buds, flowering and wilted flower heads, and flowering flower head density only included flowering flower heads. The former measure is an indication of the flower head component of the maximum seed yield, while the latter measure is an indication of pollinator attractiveness. Number of florets per flower head was counted from the flower heads collected for evaluation of seed set (see above).

2.6. Cage experiments

To confirm the ability of the short-tongued bumblebee species *B. terrestris* to pollinate tetraploid red clover in the absence of long-tongued bees, cage experiments evaluating the pollination efficiency at different abundances of *B. terrestris* were conducted. The experiments were performed in tetraploid cultivars (Supplementary Table S2) in production fields in 2019 and 2020 at five different sites distributed in south (Skåne) and north (Lännäs) (Supplementary Table S1). At each site, four different treatments were set up in the production field: one control square with free access for the local pollinator community, one empty cage with no bumblebees, one cage with low density (~ 5 individuals) and one with high density (~ 50 individuals) of *B. terrestris*. Cages were placed in the field at the start of flowering and left until harvest. To assess the pollinator fauna available for the control squares, pollinator surveys were performed in each production field according to the same method as described above, except that the transects were 50 m long in single lines. The surveys were performed nine times (1 – 4 days in between) in 2019 and three times (8 – 12 days in between) in 2020. In the high-density cages, a commercial *B. terrestris* nest (Natupol normal, www.Lindesro.se) containing a colony of workers, one queen and larvae was placed. In the low-density cages, five bumblebees from the nest in the high-density cage at the same site were released into the cage. The cages were continuously checked at least three times per week and new bumblebees from nests in the high-density cages were added to the low-density cages if needed. The area and height of the cages were 1.4 × 1.8 and 1 m, respectively, in Lännäs, while in Skåne the corresponding measurements were 1.5 × 2 and 1.2 m. The control squares had the same sizes as the cages at each respective site. Seed yield was harvested from the whole cage or square, following the same procedure of drying, threshing and cleaning as described for the seed yield above. Due to the different sizes of the cages, the values for seed yield were standardized to seed yield per 1 m^2 prior to analyses. Prior to harvest, we also estimated total flower head density (including buds, flowering and wilted flower heads), and flowering flower head density (only including flowering flower heads) in one 0.25 m^2 quadrat per cage or open plot, as described above.

2.7. Statistical analyses

To prepare data for analyses, measurements were aggregated as follows: pollinator abundances were summed and flowering flower head density was averaged per year/site/cultivar/plot/survey round for pollinator abundance models (n = 258), averaged per year/site/cultivar for weevil abundance and seed yield models and correlations including seed set or number of florets per flower head (n = 43), averaged per year/site/cultivar/survey round for seed set and florets per flower head models (n = 126), with missing data for survey round 2 for Vicky in Bjertorp 2021 and for survey round 3 for Peggy and Betty in Lännäs 2021), summed per year/site/cultivar/survey round for total flower head density models (n = 129) and averaged per year/site/cultivar/plot for correlations between seed yield and total and flowering flower head densities (n = 86). Statistical analyses were performed using R Studio, version 4.1.2 (R Core team, 2021).

To test how pollinator abundances were influenced by different factors, we used a generalized linear mixed models (GLMMs) with Poisson error distribution and log link using the glmer function in package lme4 (Bates et al., 2015). GLMMs were performed for short-tongued bees, both including and excluding nectar robbing individuals (which were all short-tongued bees), and long-tongued bees separately. In a first step, initial models with flowering flower head density and year, site and cultivar, and their interactions, as fixed factors and survey round nested within site nested within year as a random factor were evaluated with model selection based on AICc-values using the dredge function in package MuMIn (Barton, 2022). For short-tongued bees, a reduced model only retaining flowering flower head density and interactions between year and site and between site and cultivar appeared to be the best one, both when including and excluding nectar robbing individuals. Since the results from abundance models for short-tongued bees both including and excluding nectar robbing individuals gave consistent results, only results from the dataset including nectar robbers are presented. For long-tongued bees, the initial model appeared to be the best one. A significant effect of cultivar on pollinator abundance could be assumed to indicate a cultivar pollinator preference, given that our plots were distributed in the same field. We also considered an alternative analysis with abundance of short- and long-tongued bees in the same GLMM including tongue length as a fixed factor. We do not present the result of this model because it became very complex with significant high-order interactions which made the results hard to interpret. Another possible model that we considered was to exchange the factor site with a north-south location factor that compared the three northern vs. the three southern sites. However, because we could not select the different sites freely (but had to use existing field stations), it was preferable to use a model with site to control for any variation across sites.

To test how weevil abundance was influenced by year, site and cultivar, a generalized linear model (GLM) was used with year, site and their interaction, and cultivar as fixed factors, using the glm function in package stats (R Core team, 2021).

To test how seed yield was influenced by different factors, an initial linear model (LM) including abundance of weevils and two-way interactions between year and site, year and short-tongued bees, year and long-tongued bees, site and short-tongued bees, site and long-tongued bees, cultivar and short-tongued bees, cultivar and long-tongued bees, short- and long-tongued bees, and all main effects, was used with the lm function in package stats (R Core team, 2021). The initial model was evaluated with model selection using the same method as for pollinator abundances named above. This was done for both datasets including and excluding nectar robbing individuals. Abundance of short- and long-tongued bees and weevils were tested for collinearity with Spearman's rank correlation coefficients (Spearman, 1904), showing that abundance of short-tongued bees and weevils were significantly negatively correlated (r = -0.47, p = 0.001). However, neither of these two variables were retained in the best-fitted model. Model selection based on AICc-values as described above resulted in a reduced LM with year in interaction with site, cultivar and abundance of long-tongued bees as fixed factors. This was consistent when performing the model selection on both datasets including and excluding nectar robbing bees. To test what affected seed set, a LM was fitted including year, site and their interaction, cultivar, short-tongued bees, and long-tongued bees as main effects, with interactions between cultivar and each bee group. in interaction with short- and long-tongued bees, respectively, and survey round as fixed factors. The results from the seed set model were also consistent when both including and excluding nectar robbing bees. Since the results for both seed production models were consistent for both datasets, results from datasets including nectar robbing bees are presented in the results.

To confirm that our measure of seed yield (average seed weight of three 0.25 m^2 quadrats) was representative of the seed yield of the whole plot at the three tested sites, we used Pearson correlation for log-transformed values of both measures of seed yield. A relationship between the weight of 1000 seeds from the whole plots and seed yield in the quadrats was tested using Spearman correlation.

For total flower head density, a GLM with negative binomial distribution and log link (due to problems with heteroscedasticity) was specified with year, site and their interaction, cultivar and survey round as fixed factors, using the glm.nb function in package MASS (Venables and Ripley, 2002). For number of florets per flower head, a LM was specified with the same factors as for total flower head density. To test how total flower head density, flowering flower head density and number of florets per flower head were correlated with the two measurements of seed production, Spearman's rank correlation coefficients were calculated for the different plant traits, and seed yield and seed set, respectively. Values for plant traits and seed set were averaged over survey round, and in some cases also averaged over plot (see above).

To test how different densities of *B. terrestris* affected the seed yield in cage trials, a linear mixed model (LMM) was performed with field nested within year as a random factor and treatment as a fixed factor, using the lmer function in package lme4. Similar models were used to test for differences in total and flowering flower head density in the cage trials.

Model assumptions were evaluated by checking the distribution of residuals with Shapiro–Wilks tests, homogeneity of variance between groups with Breusch-Pagan or Levene's tests and if there were any overdispersion using the DHARMa package (Hartig, 2022). For significant variables, post hoc tests were performed by multiple comparisons using estimated marginal means (EMMs) in the emmeans package (Lenth, 2022).

Since data was missing for Vicky in Lännäs and all cultivars in Röbäcksdalen in 2020, it was not possible to obtain EMMs for cultivar effects. Therefore, in figures for cultivar effects showing EMMs from models where cultivar did not have a significant interaction with site or year, we calculated standardized EMMs. This was done by obtaining EMMs for all cultivars in one site and one year where all data were available, and for each cultivar's EMM subtract the mean value of EMMs for all cultivars and add the mean value of the raw data of seed yield for all cultivars.

3. Results

3.1. Abundance and preference of pollinators

A total of 4860 pollinators (1912 in 2020 and 2948 in 2021) were recorded during the transect walks (545 walks in 2020 and 714 walks in 2021) performed during three survey rounds per season across our six field trials in southern and northern Sweden involving four cultivars. In 2020, the number of short-tongued bees was 308 (16 %) and the number of long-tongued bees was 1604 (84 %), whereas in 2021, the number of short-tongued bees was 1916 (65 %) and the number of long-tongued bees was 1032 (35 %) (Supplementary Table S4). Out of the total 4860 pollinators recorded, 85 individuals (1.7 %), which were all *B. terrestris*, were observed nectar robbing.

We did not observe any clear pattern of short- or long-tongued bees being more abundant in either south or north (Fig. 2). Instead, the abundance of both short- and long-tongued bees varied significantly among sites in interaction with year (Table 1). For long-tongued bees, the abundance was also significantly affected by an interaction between year, site and cultivar, suggesting a more variable effect of cultivar over years and sites. Relatively high abundances of bees in total were observed in some of the northern sites, represented by Lännäs in 2020 and Ås and Röbäcksdalen in 2021 (Fig. 2).

There was no consistent pattern of cultivar preference of neither short- or long-tongued bees among the years and sites (Supplementary Fig. S1). Instead, the abundances varied significantly among cultivars in interaction with site for short-tongued bees (Supplementary Fig. S2a), and in interaction with site and year for long-tongued bees (Supplementary Fig. S2b). We observed that the abundance of short-tongued bees was higher in the diploid cultivar Yngve than in the tetraploid cultivars, as we expected, at only two of the sites, Svalöv and Lännäs. In contrast, at Ås there were most short-tongued bees observed in the tetraploid Peggy, whereas there was no difference among the cultivars in Bjertorp, Kölbäck or Röbäcksdalen (Supplementary Fig. S2a). For both short- and long-tongued bees, the abundance was significantly affected by a positive effect of flowering flower head density (Table 1, Supplementary Fig. S3).

3.2. Abundance of weevils

We found three species of seed eating *Protapion* weevils in our hatching tubes: *P. apricans*, *P. assimile* and *P. trifolii* (Supplementary Table S5). The total abundance of *Protapion* sp. weevils per flower head investigated at our trial sites, which were all treated with an insecticide, differed significantly between sites in interaction with year (Table 1, Fig. 3a). In both years, the highest total abundance of *Protapion* sp. weevils per flower head was observed in southern sites, except for in 2021 where Svalöv and Bjertorp had a similar value as the northern sites. In the northern sites, the abundances were low in both years. There was an overall effect of cultivar in the model (Table 1), but the effect was not strong enough to significantly separate the cultivars when performing Tukey's post hoc tests with adjustment for multiple comparisons. However, we saw a trend that Yngve had more weevil infestation than the other cultivars, followed by Vicky (Supplementary Fig. S4).

3.3. Seed production

Seed yield, estimated as average seed weight (g) from 0.25 m^2 quadrats, was significantly affected by an interaction between year and site (Fig. 3b), cultivar (Fig. 4a), and by a positive effect of the abundance of long-tongued bees (Fig. 5a) according to the best-fitted model (Table 2). Abundance of short-tongued bees (Fig. 5b) and weevils (Supplementary Fig. S5) were included in the initial analysis but not retained in the best-fitted model, indicating that they were of less importance. The northern sites Lännäs and Ås had relatively high seed yields both years, as did the southern site Svalöv in 2020. In both years, the southern site Bjertorp had a relatively low seed yield, and this was also the case for the southern site Kölbäck in 2021. Among the cultivars, the diploid Yngve and the tetraploid Vicky had a comparable higher seed yield than the tetraploid Peggy (Fig. 4a).

Seed yield from the 0.25 m^2 quadrats was strongly correlated with the seed yield harvested from the whole plots in Svalöv (mean $\pm$ SD = 317.9 $\pm$ 26.5 kg/ha (2020), 285.0 $\pm$ 22.5 kg/ha (2021)), Kölbäck (mean $\pm$ SD = 153.6 $\pm$ 30.2 kg/ha (2020), 96.9 $\pm$ 10.1 kg/ha (2021)) and Lännäs (mean $\pm$ SD = 151.8 $\pm$ 20.2 kg/ha (2020), 244.4 $\pm$ 62.8 kg/ha (2021)) (Pearson r = 0.78, N = 23, $p < 0.001$), indicating that values from the 0.25 m^2 quadrats were satisfactory representatives for seed yield. The weight of 1000 seeds from these whole plots (mean $\pm$ SD = 2.35 $\pm$ 0.39 g) showed a non-significant trend to be negatively

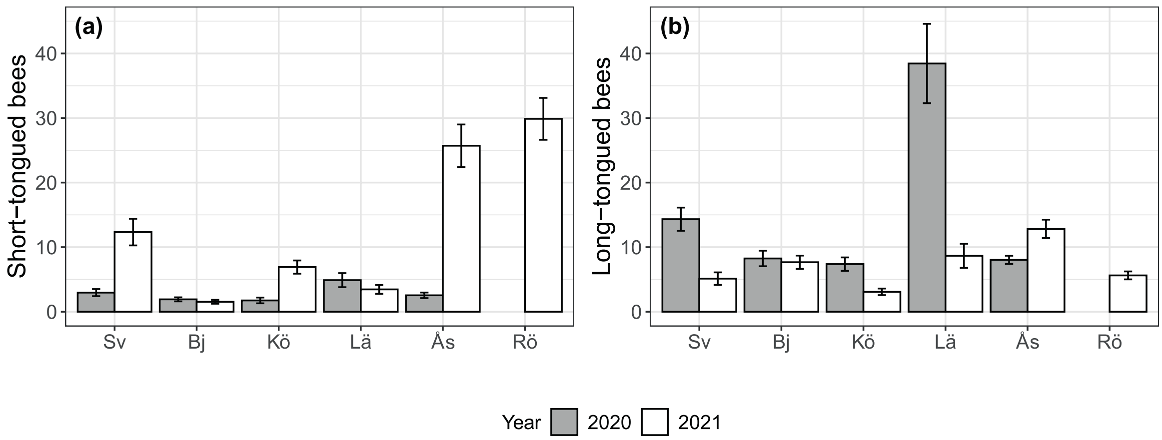

Fig. 2. Mean values of raw data and 95 % confidence intervals of abundance of (a) short- and (b) long-tongued bees in one diploid and three tetraploid cultivars grown at six sites in Sweden over two years. In 2020, data is missing from all cultivars in Röbäcksdalen and from the cultivar Vicky in Lännäs due to melting of snow, and ice encasement in spring. The sites on the x-axes are ordered according to their latitudinal distribution, starting with the southernmost site to the left. Sv = Svalöv, Bj = Bjertorp, Kö = Kölbäck, Lä = Lännäs, Ås = Ås, Rö = Röbäcksdalen.

Table 1
Analysis of Deviance Tables (Type II Wald chi-square tests) of GLMMs for the abundance of short- and long-tongued bees and of a GLM for number of *Protapion* sp. weevils per flower head, measured in four cultivars of red clover grown at six sites from southern to northern Sweden over two years. Probabilities less than 0.05 are shown in bold.

		Short-tongued bees		Long-tongued bees		Weevils	
Source of variation	Df	chisq	p	chisq	p	chisq	p
Year	1	28.84	**< 0.001**	5.17	**0.023**	114.96	**< 0.001**
Site	5	22.36	**< 0.001**	12.06	**0.034**	246.79	**< 0.001**
Cultivar	3	5.63	0.131	6.47	0.091	8.62	**0.035**
Flowering flower head density	1	95.61	**< 0.001**	31.52	**< 0.001**		
Year x Site	4	16.34	**0.003**	11.37	**0.023**	268.24	**< 0.001**
Year x Cultivar	3			4.46	0.216		
Site x Cultivar	15	50.04	**< 0.001**	71.76	**< 0.001**		
Year x Site x Cultivar	11			47.43	**< 0.001**		

correlated to seed yield in the quadrats (Spearman r = -0.35, N = 23, p = 0.11). However, germination percentage evaluated in 2020 was high (> 90 %) across all samples harvested from the whole plots (mean ± SD = 93.9 ± 2.0 %).

Seed set, estimated as the proportion of developed seeds per flower head, was significantly affected by an interaction between year and site (Fig. 3c), a positive effect of the abundance of short-tongued-bees (Fig. 5d) and long-tongued bees in an interaction with cultivar (Fig. 5c, Table 2). In 2020, Ås and Svalöv had the highest values of seed set, whereas Bjertorp had the lowest value. In 2021, less variation was observed but Svalöv stood out as having a relatively low seed set. The interaction between cultivar and abundance of long-tongued bees revealed that the diploid cultivar Yngve had a stronger positive relationship with the abundance of bees than the tetraploid cultivars. The significant main effect of cultivar showed that Yngve had an overall higher seed set (standardized EMM ± SE = 0.51 ± 0.038) than the tetraploid cultivars (standardized EMMs; ± SE 0.41 ± 0.038 for Peggy, 0.40 ± 0.04 for Vicky and 0.40 ± 0.038 for Betty, Fig. 4b).

3.4. Plant traits

Both total flower head density, estimated as total number of buds, flowering and wilted flower heads per 0.75 m^2, and number of florets per flower head varied between sites in interaction with year (Fig. 3d and e, Table 3). In 2020, the northern site Lännäs had the highest value of total flower head density, whereas Bjertorp in the south had the lowest value. In 2021, all northern sites had lower values of total flower head density than the southern sites, and Svalöv in the south had the highest value (Fig. 3d). For number of florets per flower head, Ås in the north had the highest value in 2020, and Bjertorp the lowest number. In 2021, the same pattern was observed, but Ås was accompanied by Röbäcksdalen in north (Fig. 3e). Among the cultivars, Yngve had significantly higher values of both traits than Peggy, whereas Betty and Vicky did not significantly differ from the other two cultivars (Fig. 4c and d). Furthermore, total flower head density differed significantly between the survey rounds, represented by the highest value in the third round and lowest in the first round. Number of florets per flower head was significantly higher in the first round than the second and third round.

Correlation analyses showed that there were no significant correlations between total flower head density and seed yield (Spearman r = -0.017, N = 86, p = 0.9) or seed set (Spearman r = -0.27, N = 43, p = 0.08). However, for flowering flower head density, there were positive correlations with both seed yield (Spearman r = 0.70, N = 86, p < 0.001) and seed set (Spearman r = 0.45, N = 43, p = 0.002). Additionally for number of florets per flower head, there were positive correlations between seed yield (Spearman r = 0.74, N = 43, p < 0.001) and seed set (Spearman r = 0.60, N = 43, p < 0.001).

3.5. Cage trials

The seed yield obtained from cages with high densities of the short-tongued *B. terrestris* and from open plots were significantly higher than the yield from low density and empty cages (F_3 = 12.59, p < 0.001, Fig. 6). This result indicates that high densities of *B. terrestris* gave the same level of seed yield as the wild pollinators did in the open plots. Surveys of the wild pollinators in the open plots revealed that in four of the five sites, short-tongued bees dominated the pollinator community

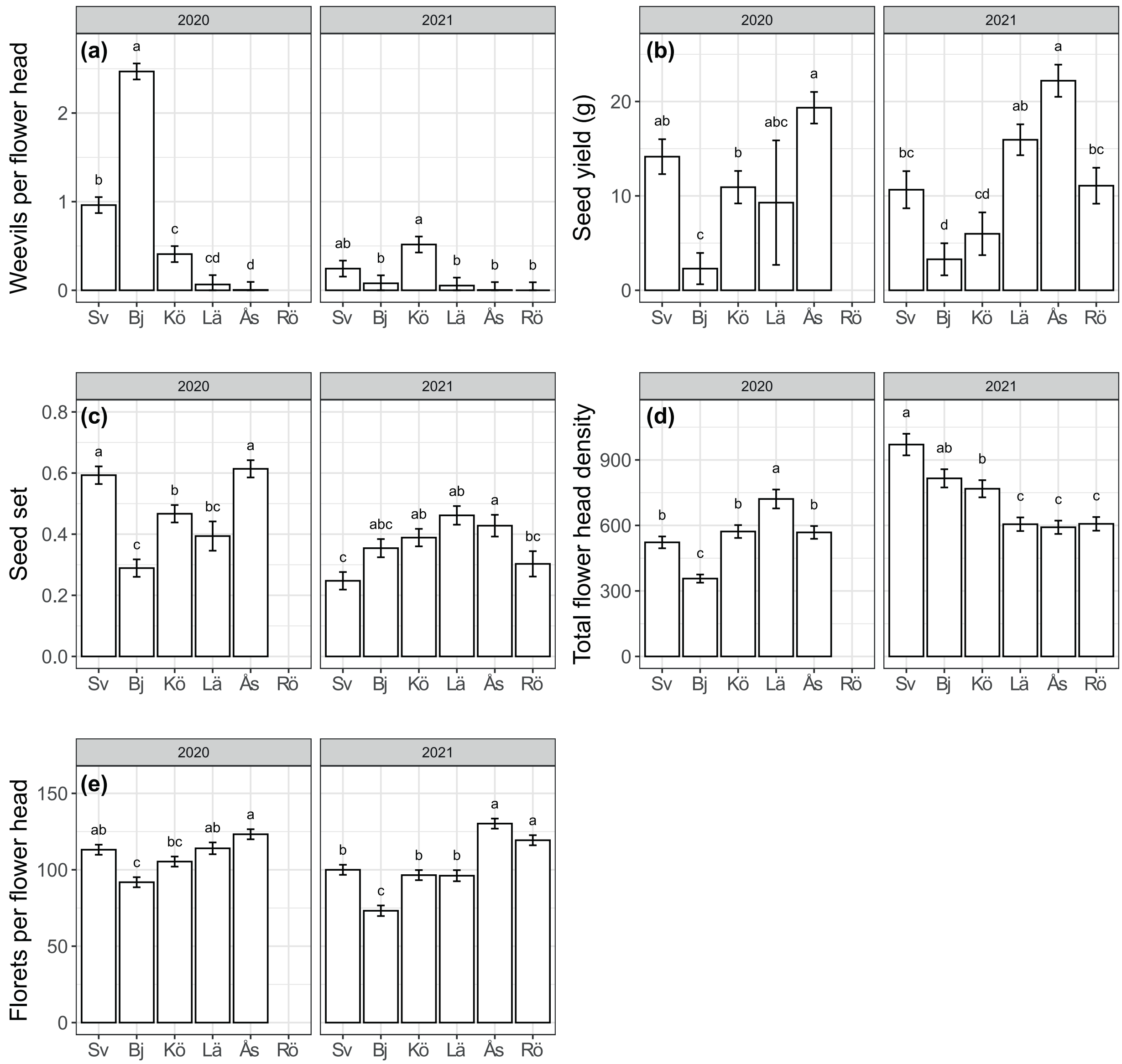

Fig. 3. Estimated marginal means (EMMs) and SE of (a) number of *Protapion* sp. weevils per flower head, (b) seed yield (estimated as average seed weight from two plots per cultivar with three 0.25 m^2 quadrats in each), (c) seed set (calculated as the proportion of developed seeds per flower head), (d) total flower head density (estimated as the sum from three 0.25 m^2 quadrats per plot) and (e) number of florets per flower head in one diploid and three tetraploid cultivars grown at six sites in Sweden over two years. In 2020, data is missing from all cultivars in Röbäcksdalen and from the cultivar Vicky in Lännäs due to melting of snow, and ice encasement in spring. The sites on the x-axes are ordered according to their latitudinal distribution, starting with the southernmost site to the left. Sv = Svalöv, Bj = Bjertorp, Kö = Kölbäck, Lä = Lännäs, Ås = Ås, Rö = Röbäcksdalen. Cultivars sharing a letter are not significantly different at 0.05 significance level.

(90–99 %), and this was especially true for the two southern sites (98–99 %) (Supplementary Table S6). The northern site North 1 stood out from the other sites, being dominated by long-tongued bees (83.5 %).

There was no difference in total flower head density among the four treatments at the end of season ($F_{3,15.2} = 0.776$, $p = 0.52$). However, flowering flower head density differed among treatments ($F_{3,15.3} = 6.88$, $p = 0.004$), with a significantly lower mean in the high-density cage (EMM = 2.96 flower heads per 0.25 m^2) compared to in the other treatments (EMMs; open plot = 18.6, low-density cage = 21.1, empty cage = 23.5). Thus, the high-density cage, with increased pollination, showed shortened flowering time.

4. Discussion

We investigated the abundance of short- and long-tongued bees and seed eating weevils, as well as their role for seed production in four cultivars of red clover grown at six sites distributed in southern and northern Sweden over two years. We also investigated how seed production and plant traits known for or potentially related to seed production varied among the cultivars, sites and years. The abundance of short- and long-tongued bees varied greatly between sites and years, with no pattern of a consistent southern or northern distribution for any of the tongue length groups. However, some northern sites had relatively higher abundances of bees in total. Seed eating weevils were more abundant in the south. Long-tongued bees were more important than short-tongued bees and weevils when considering seed yield, but for seed set, both short- and long-tongued bees had a positive impact. We found cultivar differences in seed production and plant traits, as well as variation between sites and years, indicating that both environmental and genetic factors are likely involved.

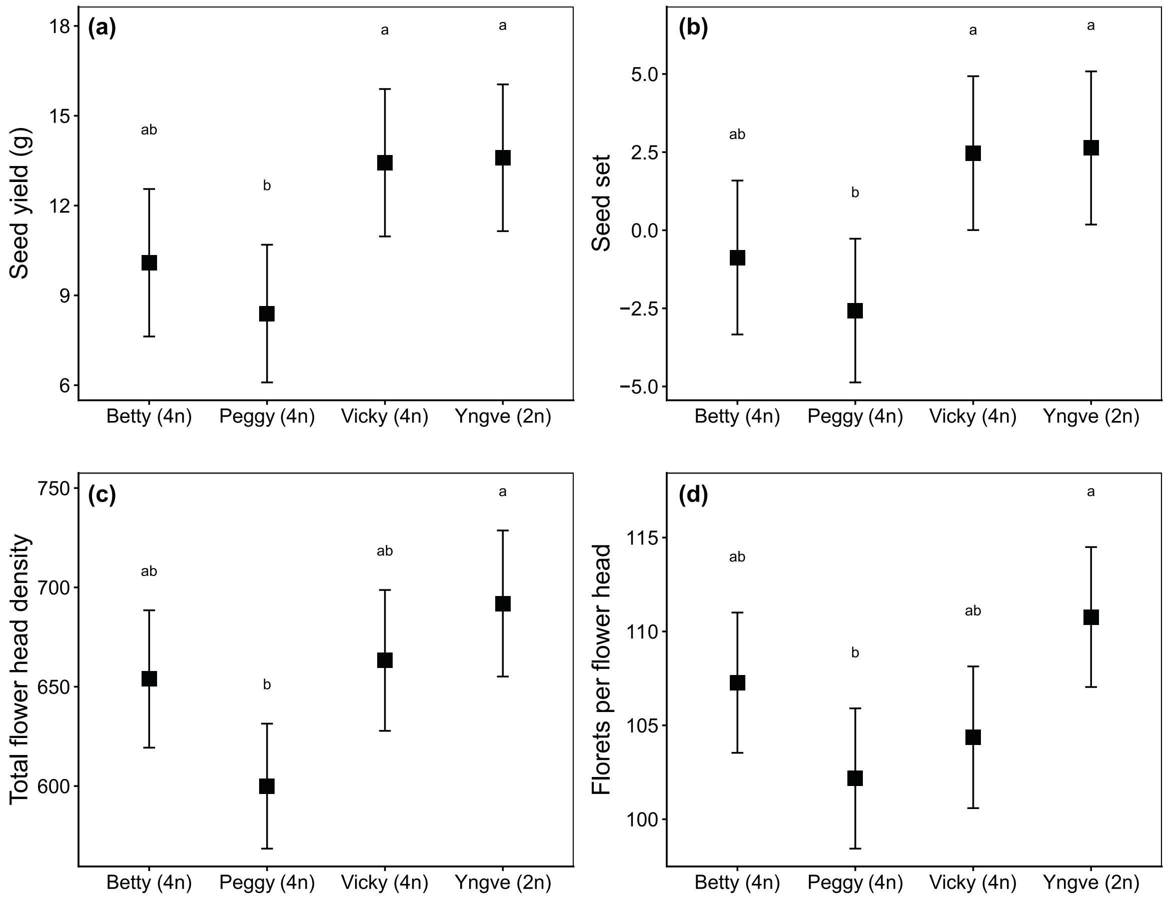

Fig. 4. Standardized estimated marginal means (EMMs) and SE for (a) seed yield (estimated as average seed weight from two plots per cultivar with three 0.25 m^2 quadrats in each), (b) seed set (calculated as the proportion of developed seeds per flower head), (c) total flower head density (estimated as the sum from three 0.25 m^2 quadrats per plot), (d) number of florets per flower head among one diploid and three tetraploid cultivars grown at six sites in Sweden over two years. 2 n = diploid cultivar, 4 n = tetraploid cultivar. Cultivars sharing a letter are not significantly different at 0.05 significance level.

4.1. Pollinator and weevil abundance among sites across Sweden

The worldwide declining trend of species richness in bees, such as the *Bombus* genus (Biesmeijer et al., 2006; Potts et al., 2010; Zattara and Aizen, 2021) has been observed in Scandinavia in particular for long-tongued species, while some short-tongued species are unaffected or have benefitted (Blasi et al., 2023; Bommarco et al., 2012; Dupont et al., 2011). Since some of the underlying reasons for the detected declines is believed to be related to increased levels of homogeneity in agricultural landscapes accompanied by loss of semi-natural habitats, nesting habitats and food resources (Connelly et al., 2015; Kennedy et al., 2013; Öckinger and Smith, 2007; Steckel et al., 2014), we wanted to investigate the abundance of short- and long-tongued species in a wide latitudinal range in Sweden. The southern parts of Sweden are composed of particularly high agricultural production landscapes compared to the north, suggesting a higher landscape complexity in the north, but specific sites may differ. When comparing our three southern and three northern red clover sites, we found that abundance of both short- and long-tongued bees were mostly affected by year in interaction with site, with no clear pattern of more short- or long-tongued bees in either southern or northern Sweden in our separate tests of the short- vs. long-tongued bees. This result was contrary to our expectation and to that seen in some older studies, where honeybees were suggested as the most important pollinators in southern Sweden and Finland, whereas bumblebees were more important in northern and central parts (reviewed in Free, 1993). It is possible that abundance of different pollinators in our study were highly affected by site-specific abiotic and biotic factors fluctuating between years, for example weather conditions (Reeves et al., 2024; Vincze et al., 2024), surrounding flower strips (Rundlöf et al., 2018), crops (Holzschuh et al., 2016) or wild flower resources (Blaauw and Isaacs, 2014), available nesting sites or proximity to commercial bee nests (Ropars et al., 2019; Wermuth and Dupont, 2010), so that we would have needed more data to investigate a difference in geographical distribution. In an expanded study, a landscape context analysis would be particularly valuable (cf. Hederström et al., 2024). Nevertheless, the northern sites Lännäs in 2020, and Ås and Röbäcksdalen in 2021, had the highest abundances of bees in total, possibly indicating that northern sites can favour a high bee abundance or that these sites were specifically favourable for bees. Because we used field stations, which also sometimes had honeybee hives placed in nearby fields (Röbäcksdalen and Ås 2021, Supplementary Table S1), for our study sites, it is uncertain how well our results would translate to that in farmers' fields. We have, for example, seen higher bee diversity in the most southern field station in Svalöv compared to nearby farmers' fields (Lankinen et al., unpublished results) and to the two farmers' fields used for cage experiments in Skåne. Potentially, this could be due to a larger diversity of crops in field stations compared to in agricultural landscapes with regular fields.

To better understand the cultivation conditions for red clover in the north of Sweden, we also investigated the presence of seed eating weevils at our six sites. To the best of our knowledge, there has not been any systematic study investigating the abundance of *Protapion* sp. weevils in red clover fields over as large distribution range as in our study. Even though the plants in our experiment were sprayed with an insecticide to control for weevils when needed, we still found some weevils in our field trials. The weevil abundance varied between sites and years,

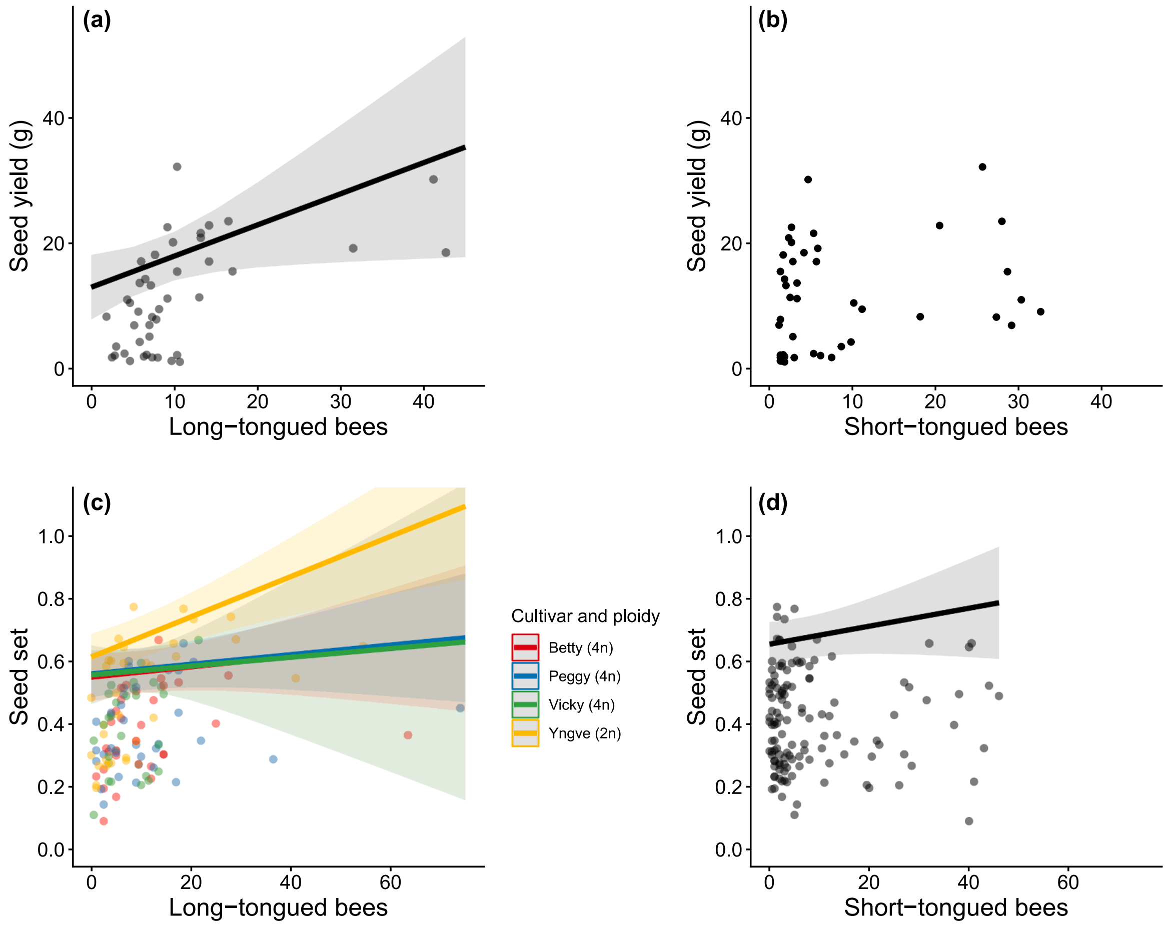

Fig. 5. (a) Seed yield is predicted to increase with the abundance of long-tongued bees ($p = 0.039$), (b) abundance of short-tongued bees did not significantly affect the seed yield, and seed set is predicted to increase with abundance of (c) long-tongued and (d) short-tongued bees. The lines represent the predicted effects based on the seed yield (a) and seed set (c, d) models, with 95 % confidence interval. The data points represent raw data. 2 n = diploid cultivar, 4 n = tetraploid cultivar.

Table 2
Type II ANOVA tables of LMs for seed yield (estimated as seed weight (g) from 0.25 m^2 quadrats) and seed set (proportional number of developed seeds per flower head) in one diploid and three tetraploid cultivars grown at six sites from southern to northern Sweden over two years. Probabilities less than 0.05 are shown in bold.

	Seed yield (g)			Seed set	
Source of variation	Df	F	p	F	p
Year	1	0.34	0.565	12.13	**< 0.001**
Site	5	27.29	**< 0.001**	14.30	**< 0.001**
Cultivar	3	6.72	**0.001**	11.24	**< 0.001**
Survey round	2			2.58	0.080
Short-tongued bees	1			4.33	**0.040**
Long-tongued bees	1	4.89	**0.035**	5.56	**0.020**
Year x Site	4	3.47	**0.020**	16.67	**< 0.001**
Cultivar x Short-tongued bees	3			0.44	0.723
Cultivar x Long-tongued bees	3			2.90	**0.039**

Table 3
Analysis of Deviance Table (Type II tests) of a GLM for total flower head density and a LM for number of florets per flower head, measured in four cultivars of red clover grown at six sites from southern to northern Sweden over two years. Probabilities less than 0.05 are shown in bold.

	Total flower head density			Number of florets per flower head	
Source of variation	df	chisq	p	F	p
Year	1	99.0	**< 0.001**	21.3	**< 0.001**
Site	5	44.9	**< 0.001**	41.4	**< 0.001**
Cultivar	3	13.3	**0.004**	3.42	**0.020**
Survey round	2	86.4	**< 0.001**	21.03	**< 0.001**
Year x Site	4	118.3	**< 0.001**	4.84	**0.001**

but in general, there was a trend of more weevils in the southern sites, suggesting that weevils are less abundant in northern Sweden. Therefore, red clover seed production in northern Sweden should likely be less negatively affected by weevil damage than in southern Sweden. However, because we do find some weevils in the north, it would be important to follow the recommendations for integrated pest and pollinator management (IPPM), such as spraying before flowering or moving fields between years (Lundin et al., 2012; Stephansson and Lundin, 2014) to avoid future build-up of pest populations.

4.2. *Seed production in relation to sites, pollinators and cultivar differences*

Variability in seed yield between sites and years is a well-known phenomenon in red clover (Amdahl et al., 2016; Bommarco et al., 2012; Jing and Boelt, 2021). For example, in a study investigating seed yield in four different sites in Norway and Sweden, the seed yield was significantly affected by location (Amdahl et al., 2016). Similarly, we found that seed yield and seed set varied between sites and years. Considering separate years, some of the highest values of seed production was observed in some of the northern sites, except from seed set in 2020 where this was also accompanied by Svalöv in the south. The variation in seed production between sites and years might be a result of

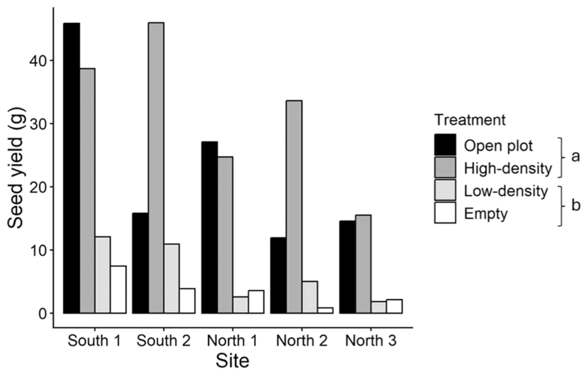

Fig. 6. Standardized seed yield (g per m^2, mean values of raw data) obtained from five fields with unreplicated cage treatments, including the treatments open plots (black), high-density (~ 50 individuals of *B. terrestris*, dark grey), low-density (~ 5 individuals of *B. terrestris*, light grey) and empty (white) cages. The following tetraploid cultivars were used: Vicky in South 1, Kelly in South 2, Peggy in North 1 and 3, and Betty in North 2. In North 2 and 3, second-year plants were used which caused comparably lower seed yield than in the first-year plants used in the other sites. South 1 and 2 = southern Sweden (Skåne), North 1, 2 and 3 = northern Sweden (Västernorrland). Treatments sharing a letter are not significantly different at 0.05 significance level.

fluctuating abiotic and biotic factors not tested here, such as precipitation (Jing and Boelt, 2021), or low species richness of pollinators, which have been suggested as a driver of between-year fluctuation in seed production of crops that are heavily reliant on pollinator services (Yachi and Loreau, 1999). Our results suggest that red clover seed production has the potential to be at least as successful in northern as in southern Sweden, given that this would fit with the cultivation strategy. However, there is an increased risk of ice damage in spring due to a colder climate, which in 2020 damaged all our plants in Röbäcksdalen and plants of the cultivar Vicky in Lännäs.

The importance of short- and long-tongued bees for diploid and tetraploid red clover pollination and seed production has for a long time been uncertain and previous studies are ambiguous (Bender, 1999; Hederström et al., 2021; Rundlöf et al., 2018; Wermuth and Dupont, 2010). We found that the abundance of long-tongued bees had a significant positive effect on seed yield whereas for seed set there was a positive effect of both the abundance of short- and long-tongued bees. Because our measure of seed set allowed us to get a better estimate of pollination success - excluding the effects of weevil damage - compared to seed yield, our results suggest the importance of both short- and long-tonged bees at least for initial seed production. It is not fully clear why the impact of short-tongued bees was less important for seed yield than for seed set. We can hypothesise that the presence of weevils might have decreased the positive impact of pollinators on seed yield, as was found in white clover in Hederström et al. (2024). However, weevil damage was relatively low in our sprayed field trials and did not significantly affect seed yield. Another possibility is nectar robbing of the short-tongued bees, but the results for both measures of seed production were consistent when excluding these from the dataset. The ability of short-tongued bees to pollinate tetraploid red clover, despite their long corolla tubes, was further supported by the results from the cage trials with tetraploid red clover where high densities of *B. terrestris* generated the same seed yield as open pollination outside of the cages (Fig. 6). It should be noted that commercial use of *B. terrestris* in agricultural fields may pose risks to wild bumble bee species (Chandler et al., 2019; Osterman et al., 2021). Long-tongued bees have in several studies been identified as more efficient pollinators than short-tongued bees in red clover (Nørgaard Holm, 1966; Bender, 1999; Hederström et al., 2021), which might explain their significant impact on seed yield. Moreover, seed yield is not only influenced by pollination success but also by other factors, such as number of flower heads per plant (Vleugels et al., 2015, 2016; Amdahl et al., 2017), which may mask the importance of pollinators.

It is well known that diploid red clover generates higher seed yield (Taylor and Quesenberry, 1996; Boller et al., 2010; Vleugels et al., 2016; Jing and Boelt, 2021) and seed set (Vleugels et al., 2015, 2016; Hederström et al., 2021; Jing et al., 2021b) than tetraploid red clover. In line with previous studies, our values of proportional seed set were lower in all three investigated tetraploid cultivars (mean = 0.38 – 0.39) compared to the tested diploid cultivar (mean = 0.5). Seed yield was variable among the tetraploid cultivars, with higher values in Vicky compared to Peggy. Because tetraploid cultivars in general have larger and heavier seeds than diploid cultivars (Vleugels et al., 2016; Hederström et al., 2021), it is possible that the lower seed production seen in our study could have been mitigated if we had taken seed quality into consideration. However, we did see high values (> 90 % germination) of seed quality in all plots at the three sites were this was measured, suggesting that seed yield should be a good estimate of the final yield. Interestingly, we found an interaction between cultivar and number of long-tongued pollinators for seed set, showing that the seed set was predicted to reach higher levels with increasing abundance of the long-tongued bees in the diploid cultivar Yngve than in the tetraploid cultivars (Fig. 5c). Thus, we do not find evidence that seed set of our three tetraploid cultivars are limited by the abundance of long-tongued pollinators. A previous study found some evidence that short-tongued pollinators prefer diploid over tetraploid cultivars when growing close together (Hederström et al., 2021), whereas another study did not support a difference in preference (Vanommeslaeghe et al., 2018). In our study, we did not observe that the tetraploid cultivars were less visited by any pollinators than the diploid cultivar in our 10 m by 10 m plots. It should be noted that because we only included one diploid cultivar, our study had limited power to detect differences between ploidy levels. However, it appears more likely that lower seed production in tetraploid cultivars is influenced by fertility traits linked to ploidy level, as shown in (Büyükkartal, 2003; Jing et al., 2021b; Vleugels et al., 2019). While it may be important to investigate the role of cultivar and ploidy differences in fertility traits in the future, it would also be of interest with continued work on cultivar differences (cf. Stejskalová et al., 2018; Burns and Stanley, 2022) in pollinator attraction traits for seed production in red clover (e.g. floral scents) as well as for other insect pollinated crops.

4.3. Plant trait variation between sites and cultivars

Previous studies have found cultivar differences for flower head density (Amdahl et al., 2017; Hederström et al., 2021; Vleugels et al., 2015) and number of florets per flower head (Jing and Boelt, 2021), implying that these traits are to some extent genetically determined, which is of high relevance for plant breeding. However, few studies (but see Amdahl et al., 2016) have investigated how these traits might vary within cultivar due to abiotic factors, for example between different sites and years. We discovered that both total flower head density and number of florets per flower head not only differed among cultivars, but that they also were influenced by site in interaction with year and time of the season. The site by year interaction suggests that these traits are also affected by abiotic factors, for example if effects of day length vary with environmental factors. Similar results were found in Amdahl et al. (2016), who found a significant effect of location in southern and northern Sweden and Norway on flower head density. Our results showed that number of florets per flower head decreased during the season, as the total flower head density increased, potentially indicating a trade-off between the traits.

Despite the effects of sites and years on the two investigated plant traits, we observed higher values for both traits in the diploid cultivar Yngve compared to one of the tetraploid cultivars, Peggy, but not the other two tetraploid cultivars (Fig. 4c and d). Our results are therefore partly in concordance with previous studies which reported that diploid cultivars produce more flower heads per plant (Hederström et al., 2021; Vleugels et al., 2015, 2016) and a higher number of florets per flower head (Jing and Boelt, 2021), whereas some studies do not find any ploidy difference in number of florets per flower head (Hederström et al., 2021; Vleugels et al., 2015, 2016). However, our study included only one diploid cultivar and to examine a ploidy effect on traits, an experimental setup with several cultivars of each ploidy level should ideally be conducted. Moreover, it would be important to link differences in plant traits not only to seed production, but potentially also to pollinator attraction. Previous studies have found that increased amount of flowering flower heads have a positive impact on pollinator attraction (Hederström et al., 2021; Lundin et al., 2017), as we found in the pollinator abundance models in our study. Similarly, from our correlation analyses we found that both seed yield and seed set were positively correlated with flowering flower head density and number of florets per flower head. However, there were no correlation between the two measurements of seed production and total flower head density. Given that there is no trade-off between flowering flower head density and number of florets per flower head, and seed development, seed yield could be directly affected by the traits. Since there still was a positive correlation between the traits and seed set, this indicates that increased amount of flowering flower heads and number of florets per flower head favours pollinator attraction and thereby pollination rate and seed production.

5. Conclusions

In the present study on red clover seed production across Sweden, we found no clear trends of a higher number of short- or long-tongued bees in red clover field trial sites in southern or northern Sweden, despite the relatively higher proportion of arable land in the landscape in the south. Instead, the bee abundance fluctuated between sites and years. Furthermore, we found that seed eating *Protapion* sp. weevils were mostly abundant in southern sites and almost absent in the northern sites. Despite previous evidence that long-tongued bees are more efficient pollinators of red clover (Nørgaard Holm, 1966; Bender, 1999; Hederström et al., 2021), we conclude that short-tongued bees are able to pollinate both diploid and tetraploid red clover and do not consequently reject tetraploid cultivars. However, our results also showed that long-tongued bees were more important for predicting seed yield, and that, even though both short- and long-tongued bees had a significant impact on seed set, increased abundance of long-tongued bees led to higher predicted seed set in the diploid cultivar. This supports the importance of both short-, and especially long-tongued bees as a potentially limiting factor for red clover seed production. Furthermore, our results suggest that the lower seed production often observed in tetraploid cultivars (Taylor and Quesenberry, 1996; Boller et al., 2010; Vleugels et al., 2015, 2016; Hederström et al., 2021; Jing et al., 2021b; Jing and Boelt, 2021) is not mainly caused by insufficient pollination, but probably also caused by other factors including differences in fertility (Jing et al., 2021b; Vleugels et al., 2019). In addition, our results suggest the importance of a better understanding of cultivar traits (e.g. flower head density and number of florets per flower head) influencing not only seed production but also pollinator attraction (for flowering flower head density). We suggest that future studies on seed production in red clover cultivars as well as other insect pollinated crops combine investigations of how genetic vs. environmental factors impact seed production with studies of cultivar differences in pollinator attraction.

CRediT authorship contribution statement

Kajsa Svensson: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Veronika Hederström:** Writing – review & editing, Validation, Supervision, Conceptualization. **Ida Valentin:** Supervision, Methodology, Investigation, Formal analysis. **Sara Lindholm:** Methodology, Investigation. **Linda Öhlund:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Mattias C. Larsson:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Åsa Lankinen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

Linda Ohlund reports a relationship with Lantmannen Lantbruk that includes: employment. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2026.110272.

Data availability

Data are available from the Swedish National Data Service (SND) Repository: https://doi.org/10.5878/5v4x-rd92 (Svensson et al. 2026).

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