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Trees for bees: could woody plant pollen be used as a consistent resource in bee-focused agri-environment schemes?

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With 4 figures and 5 tables

Abstract: Bee populations have declined in many parts of the world, raising concerns over their conservation and the pollination services they provide. As a result of declines in agricultural areas, agri-environment schemes have been designed and implemented in order to reverse these trends. Until now, these schemes have largely focused on providing an abundance of herbaceous flowering plants which predominantly provide pollen and nectar during the summer, but flowering trees and shrubs may have been overlooked as a source of earlier-flowering resources. Using *Bombus terrestris* (L.) microcolonies, we investigated differences in pollen quality from eight woody and six herbaceous plant species using compositional analyses and efficacy assays. Pollen from herbaceous plants had a higher average crude protein and lipid content, but there were no differences in the protein:lipid ratio when compared to woody plants. However, when measuring amino acids directly, woody plants had a slightly higher total and essential amino acid content. Despite these minor differences, microcolonies fed on woody plant pollen produced a greater mass of larval offspring and had a lower rate of larval ejection. There was substantial variation between individual studied plant species, and whilst no individual woody plant pollen outperformed the best herbaceous pollen, they all exceeded the performance of the worst herbaceous pollen. This consistent performance suggests that woody plants may be good candidates for inclusion in bee-focused agri-environment schemes in order to provide suitable pollen resources in the early part of the season.

Keywords: bee conservation; farmland; Apoidea; habitat quality; amino acids; protein:lipid ratio; larval mortality; protein content

1 Introduction

Through facilitating the sexual reproduction of a majority of flowering plant species, including a majority of important crop plants, bees are crucial for both the functioning of terrestrial ecosystems and the maintenance of human health (Klein et al. 2007; Ollerton et al. 2011; Calderone 2012). However, the provision of these ecosystem services have been threatened by declines in bee populations that have been most pronounced in more intensively managed agricultural areas (Kremen et al. 2002; Le Féon et al. 2010; Senapathi et al. 2015). There is therefore a desire for management interventions to halt and reverse these declines and to maintain stable bee populations in both agricultural regions and the wider landscape.

Bees require access to floral resources, specifically pollen and nectar, in order to support their adult metabolism and larval development (Michener 2007), and a reduction in the quantity and quality of floral resources in agricultural habitats has therefore been proposed as a major cause of their decline (Kleijn & Raemakers 2008; Roulston & Goodell 2011; Scheper et al. 2014). Though bees can feed on the pollen and nectar provided by crop plants, they also require additional sources from wild and uncultivated plants that bloom outside the narrow flowering window provided by mass-flowering crops (Westphal et al. 2009; Wood et al.

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2018a). Ensuring that appropriate complementary resources are available is therefore critical for supporting and maintaining wild bee populations on farmland (M'Gonigle et al. 2015; Sutter et al. 2017).

In order to provide additional floral resources, the current dominant strategy in northern hemisphere temperate environments has focused on planting strips of herbaceous wildflowers alongside fields (e.g. Pywell et al. 2015; Scheper et al. 2015; Wood et al. 2018a, though see M'Gonigle et al. 2015). The identity of these wildflowers is usually chosen on the basis of expert opinion (e.g. Scheper et al. 2015), with a strong focus towards plants that flower in June, July, and August, the natural flowering period for most herbaceous plants found in these regions. However, there has also recently been an increase in attention paid to the role that flowering trees play in providing pollen for bees in the early part of the season, predominantly in April and May (Kämper et al. 2016; Somme et al. 2016; Persson et al. 2018; Wood et al. 2018a; Bertrand et al. 2019). Genera such as *Acer, Prunus, Quercus*, and *Salix* comprise a major part of the diet of solitary bee species that fly only in the spring, but also of the diet of social bee species that establish their colonies at this time (Kämper et al. 2016; Wood et al. 2018b; Bertrand et al. 2019). Of the 20 most important wild bee crop pollinators identified by Kleijn et al. (2015), only two species are solitary bees that are active solely during the summer. The other species are either solitary and fly only in the spring (eight species), solitary and bivoltine, active in the spring and summer (one species), or are social or subsocial and fly throughout the year (nine species). This phenological pattern would suggest that providing resources during the spring would be a good strategy to support important populations of wild crop pollinators, but until now flowering trees have received little attention in this regard.

When assessing appropriate floral resources to support bee populations, resource quality is an important consideration (Ruedenauer et al. 2019). Whilst nectar is used as the main source of carbohydrates, pollen is the source of all other required nutrients (Roulston & Cane 2000). The chemical composition of pollen is complex and variable, and not all plant species produce pollen of a suitable quality for optimal bee development (Ribeiro et al. 1996; Trunz et al. 2020). Variation in pollen quality can even affect a highly generalised bumblebee species that naturally collects from a wide range of botanical families (Vanderplanck et al. 2018). The chemical composition of pollen is consequently a major factor determining bee growth (Roulston & Cane 2000; Hanley et al. 2008; Vanderplanck et al. 2014a). Plant pollens with a high protein content can support bumblebee colony development (Moerman et al. 2016), and plants with pollen-rich pollen such as members of the Fabaceae have been favoured in herbaceous, pollinator-focused schemes (e.g. Pywell et al. 2015). However, additional factors that structure pollen-foraging choices and developmental success are increasingly being documented, most notably the importance of protein:lipid ratios (Vaudo et al. 2016; 2020; Kraus et al. 2019; Ruedenauer et al. 2020). This suggests that using protein content analyses alone to identify the most important pollen sources for bees (e.g. Somerville and Nicol 2006; Somme et al. 2016; Pamminger et al. 2019) may not capture their true utility in isolation. Relatively few studies have experimentally tested whether these differences directly translate into fitness benefits for wild bee species; this should be an important consideration when considering which resources to include when designing bee-focused schemes.

With these considerations in mind, we aimed to test whether the pollen from trees and shrubs is of high quality for developing bees relative to pollen from herbaceous plants, first by measuring their protein, lipid, and amino acid composition, and secondly by using bioassays with the model bee species *Bombus terrestris* (L.). We hypothesise that the quality of pollen from herbaceous plants will be, on average, of higher variability compared to that from woody plants. This is because increased variability in resource quality between different plant types can drive increased specialisation in bee foraging behaviour (Waser et al. 1996), and the majority of bee species in temperate areas that show pollen specialisation are associated with herbaceous and not woody plants (Westrich 1989; Wood et al. 2018b). This suggests that the quality of herbaceous plant pollen may be more variable than that from woody plants, and therefore potentially of lower average quality, contributing to this observed pattern of specialisation.

2 Materials and methods

2.1 Selected pollen diets

We selected monofloral diets of six herbaceous plant taxa (*Cirsium* spp., *Helianthus annuus*, *Papaver rhoeas*, *Taraxacum* agg., *Trifolium repens*, and *Zea mays*) and eight woody plant taxa (*Cistus, Crataegus monogyna, Castanea sativa, Frangula alnus, Prunus cerasus, Quercus pyrenaica, Salix caprea*, and *Salix fragilis*). Pollen from these different plant species were collected from honey bee (Apis mellifera L.) colonies in Belgium and France fitted with pollen traps by two companies (Pollenergie France, Ruchers de Lorraine) and two private beekeepers (see Table 1). These selected plant species are commonly found in central and northern Europe and are often collected in large quantities by honey bees (Requier et al. 2015). For each plant species, pollen was hand-sorted by colour to obtain experimental pollen that was as pure as possible (around 300 g per plant species). The purity of each pollen type after sorting was then was assessed by CARI asbl (Louvain-La-Neuve, Belgium) using light microscopy (median purity = 94%, Table 1).

Table 1. Summary of the pollen types used in this study along with their collection details.

Plant type	Dominant taxa	Dominance (%)	Company	Country of origin	Year of collection
Herbaceous	*Cirsium* spp.	92	Private beekeeper	Belgium, Mons	2014
Herbaceous	*Helianthus annuus*	80	Private beekeeper	France, Arribedieu	2016
Herbaceous	*Papaver rhoeas*	99	Private beekeeper	France, Noaillan	2016
Herbaceous	*Taraxacum* agg.	94	Ruchers de Lorraine	France, Nancy	2016
Herbaceous	*Trifolium repens*	94	Private beekeeper	Belgium, Mons	2014
Herbaceous	*Zea mays*	71	Private beekeeper	France, Arribedieu	2016
Woody	*Castanea sativa*	95	Pollenergie	France, Saint-Hilaire-de-Lusignan	2015
Woody	*Cistus* spp.	94	Pollenergie	France, Saint-Hilaire-de-Lusignan	2015
Woody	*Crataegus monogyna*	91	Pollenergie	France, Saint-Hilaire-de-Lusignan	2015
Woody	*Frangula alnus*	78	Private beekeeper	France, Arribedieu	2015
Woody	*Prunus cerasus*	94	Pollenergie	France, Saint-Hilaire-de-Lusignan	2015
Woody	*Quercus pyrenaica.*	94	Private beekeeper	France, Léogeats	2016
Woody	*Salix caprea*	72	Ruchers de Lorraine	France, Nancy	2014
Woody	*Salix fragilis*	98	Private beekeeper	Belgium, Mons	2015

2.2 Chemical analyses

Pollen protein concentration was measured using the Bradford assay. To prepare the samples for analysis, pollen samples were divided into three ~1 mg replications for each individual diet in 1.7 mL microcentrifuge tubes. Each tube was filled with 1.5 mL of 0.1 M NaOH and vortexed for 10 min. All samples were allowed to sit for 24 hours. We conducted the Bradford assay with the Bio-Rad Protein Assay Kit microassay 300 µL microplate protocol using bovine γ-globulin as the protein standard (Bio-Rad Laboratories, Inc., Hercules, CA). Due to the high protein concentration of the pollen, we diluted 50 µL of each replicate into 100 µL 0.1M NaOH in each well of a sterile non-tissue culture treated 96 well plate. We used three technical replications for each biological replication and measured absorbance at 595 nm using a SpectraMax 190 spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA). Protein concentrations calculated using linear regression analysis from the protein standards. Back calculations to µg protein/mg pollen were made by multiplying concentrations obtained from the spectrophotometer by three for the dilution factor and 1.5 for the initial 1.5 mL extraction, divided by initial mg sample mass.

Pollen lipid concentrations were determined using a modified protocol from Van Handel & Day (1988). To prepare the samples for analysis, we divided the pollen into three ~1mg replications for each individual diet in 2.0 mL microcentrifuge tubes. We added 200 µL 2% sodium sulfate vortexed for 30s. We then added 1.6 mL chloroform/methanol and centrifuged the samples for 5 min. We transferred the supernatant to a clean glass tube, added 600 µL DI water, and centrifuged for 5 min. We separated the top carbohydrate/water/methanol fraction and the remaining chloroform fraction was used for lipid analysis. The lipid/chloroform fraction was left overnight in a fume hood to completely evaporate the solvent. We added 200 µL sulfuric acid to the sample and heated at 100°C for 10 min and then added 5 mL vanillin/phosphoric acid reagent, vortexed for 5 s, and allowed to cool. We used three 300 µL technical replications for each biological replication and measured absorbance at 525 nm. Lipid concentrations were calculated using linear regression analysis from vegetable oil standards, then divided by the initial mg sample weight. Pollen concentrations of protein and lipids are reported as µg nutrient/mg pollen, and subsequent protein:lipid (P:L) ratios were determined for each diet.

For the analysis of total amino acids, 1 mL of hydrolysis solution (6N HCl, 0.1% phenol and 500 µM norleucine) was added to 3–5 mg (dry weight) of pollen (Vanderplanck et al. 2014b). The tube was placed for 1 min under nitrogen to avoid methionine degradation, and then incubated for 24 hours at 110°C. The hydrolysate was evaporated until dryness under vacuum in a boiling bath at 100°C. Afterwards, 1 mL of the sodium citrate buffer pH 2.2 was added into the tube. The sample solution was mixed and poured in an HPLC vial after filtration (0.2 µm filter). Each amino acid was measured separately with an ion-exchange chromatograph (Biochrom 20plus amino acid analyzer). A post-column ninhydrin reaction produced coloured derivatives, which was monitored via a UV detector. For amino acid quantification, norleucine was used as internal standard. This analysis includes essential amino acids that bee cannot synthesize, as well as the non-essential amino acids. The essential amino acids were established by DeGroot (1953) for honeybees; namely

arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Only tryptophan was omitted because its isolation requires a separate alkaline hydrolysis from additional amounts of sample, and it is almost never a limiting essential amino acid (Standifer et al. 1980).

2.3 Model species for bioassays

Bombus terrestris L. was chosen as the bee model for these experiments. *Bombus terrestris* is a common and widely polylectic West Palearctic species and its use as a model organism for investigating the impact of nutrition on development is well established (Ribeiro et al. 1996; Vanderplanck et al. 2014a; Moerman et al. 2016; Roger et al. 2017). Commercial colonies were obtained from Biobest (Westerlo, Belgium) and were fed *ad libitum* with sugar syrup (Biogluc®, Biobest sprl) containing methyl and propyl hydroxybenzoate at a concentration of 0.6% in order to prevent microbial infections and to allow for standardised conditions between treatments. Pollen candies of mixed origin (in the ratio 15 g of syrup for 50 g of pollen) were provided to allow colony growth and maintenance. All colonies were maintained in a dark room at 26–28°C and 65% relative humidity.

2.4 Rearing experiment

Differences in pollen quality were assessed using microcolonies following the methodology of Roger et al. (2017). Two-day old *B. terrestris* workers (based on manufacturer certification) were collected from five different colonies provided by Biobest (Westerloo, Belgium). Workers were placed into plastic boxes (10 × 16 × 16 cm) to form microcolonies, with a total of five workers per microcolony, with each microcolony coming only from a single parent colony. Workers were weighed to allow for correction of fitness metrics (see below). A total of 10 microcolonies were created for each plant taxon (n = 14 pollen diets) with the exception of *Salix fragilis*, for which only eight microcolonies could be created. Microcolonies from different parent colonies were distributed between different diets (i.e. two microcolonies from each founding colony per treatment) in order to avoid any potential confounding effect of colony origin. These colonies continued to be fed with syrup *ad libitum* but mixed pollen candies used to feed the mother colonies were replaced with pollen candies of the tested diet, made at the same 15 g syrup to 50 g pollen ratio. Pollen candies were replaced every two days to ensure that they did not become unpalatable. Microcolonies were reared for 19 days at room at 26–28°C and 65% relative humidity. Experiments were run between 2014 and 2016 using the same protocol, with all experiments conducted in the same year as their respective pollen samples were collected (Table 1).

At the end of the 19-day period, the total weight of all offspring (non-isolated larvae, pre- and post-defecating larvae, and pupae) and the number of ejected larvae were measured. Throughout the experiment, the quantity of syrup and pollen collected by microcolonies was also measured, giving one final value for total pollen collection and total syrup collection per colony. Measurements of pollen collection were not corrected for a potential effect of evaporation. However, since pollen collection is not a direct measure of microcolonies performance, pollen candies were provided *ad libitum*, and pollen candies were changed regularly, a potential bias caused by evaporation will not impact the main results. Offspring production (brood weight) as well as resource collection were adjusted relative to the original total fresh weight of the workers for each microcolony, as individual variations of size and weight are known to impact bumblebee fitness and resource collection (Couvillon & Dornhaus 2010; Shpigler et al. 2013).

2.5 Data analysis

Pollen chemical composition was compared between herbaceous and woody plants (henceforth, plant growth type) using a two-way nested ANOVA with plant species as factor nested within plant type. If a significant effect of plant species was detected, one-way ANOVAs as well as post-hoc Tukey tests were performed separately on herbaceous and woody plants. Percentage data were arcsin transformed prior to statistical analyses. When assumptions of normality (Shapiro test) or homoscedasticity (Levene test) of residues were not met ($p < 0.01$), data were log-transformed (i.e. log-, Box-Cox or rank-transformation). The effect of plant growth type on overall amino acid composition (effectively community composition for the seventeen quantified amino acids, averaged across replicates) was assessed using multivariate permutational analysis of variation (PERMANOVA) tests with Bray-Curtis dissimilarity and the function *adonis* from the *vegan* package (Oksanen et al. 2015). Both absolute (mg/g) and relative (percentage) composition were tested.

Statistical analyses using generalized linear models (GLM, 'glm' command in R-package stats) were conducted to compare diet suitability between plant growth types (pollen and syrup collection, offspring production, brood weight produced, and larval ejection) with plant species as a nested factor within plant type. As bioassays were conducted under controlled conditions using the same experimental design, we did not include year of experiment as a random factor. When a significant effect of plant species was detected (Fisher test using the function 'anova'), GLMs with post-hoc Tukey tests ('glht' function from R-package multcomp) were separately performed on herbaceous and woody plants. This post-hoc test includes a single step procedure to adjust the p-values because of multiple comparisons of means (Bonferroni adjustment). When data were not normally distributed ($p < 0.01$), they were log-transformed or analysed assuming a gamma error distribution. Data on larval ejection only occurred rarely in the woody plant species dataset and were therefore zero inflated, so they were computed as a binary variable (i.e. as zero when no ejection occurred, as

one when any level of ejection occurred). A GLM was then run using a binomial distribution to compare the probability of larval ejection (chi-square test using function 'anova'). To check for the possible confounding effects of phylogenetic relatedness, relationships were additionally tested using phylogenetic least squares regression analyses (PGLS). Because these analyses necessitate node matching, only one averaged value per diet treatment can be used. Therefore, these PGLS analyses were run alongside traditional GLMs (using the same averaged value) and the two results were compared. A plant phylogeny for the 14 species used here was created using the 'S.PhyloMaker' approach of Qian & Jin (2016). This tree was used to apply a phylogenetic correction using the PLSR analyses using the package 'ape' (Paradis & Schliep 2019). All analyses were conducted in R version 3.6.0 (R Core Team 2020). Graphical plots were produced using a jitter effect to allow for visual separation of points laterally, with no statistical implications.

3 Results

3.1 Chemical analyses

Pollen from herbaceous plants had a higher crude protein content than pollen from woody plants (ANOVA based on replicates, $F_{1,28} = 81.1$, $p < 0.001$, GLM based on mean values, $t_{1,13} = 2.926$, $p = 0.013$). This result was largely driven by *Papaver rhoeas* and *Trifolium repens*, and adjustment suggests that this result may have been affected by phylogenetic relatedness (PGLS, $t_{1,13} = 0.839$, $p = 0.418$). For both plant types, protein content varied significantly between species (herbaceous, $F_{5,12} = 19.3$, $p < 0.001$; woody, $F_{7,16} = 7.0$, $p < 0.001$; Table 2). Lipid content was also higher for herbaceous pollen compared to woody pollen (ANOVA based on replicates, $F_{1,28} = 56.2$, $p < 0.001$, GLM based on mean values, $t_{1,13} = 3.417$, $p = 0.005$), though this may also have been affected by phylogenetic relatedness (PGLS, $t_{1,13} = 1.325$, $p = 0.210$). There was interspecific variation within both plant types (herbaceous, $F_{5,12} = 5.15$, $p = 0.009$; woody, $F_{7,16} = 5.62$, $p = 0.002$; Table 2). Consequently, there was no significant difference in the protein:lipid ratio between the two pollen types (ANOVA based on replicates, $F_{1,28} = 0.10$, $p = 0.757$, GLM based on mean values, $t_{1,13} = 0.034$, $p = 0.973$, PGLS, $t_{1,13} = 0.050$, $p = 0.960$, Fig. 1A), but interspecific variation within both plant types remained significant (herbaceous, $F_{5,12} = 9.71$, $p < 0.001$; woody, $F_{7,16} = 10.26$, $p < 0.001$; Figs 1B, C).

Regarding total amino acid content, there were significant differences in total (ANOVA based on replicates, $F_{1,28} = 36.9$, $p < 0.001$; Fig. 2A) and essential amino acid content (ANOVA based on replicates, $F_{1,28} = 40.1$, $p < 0.001$; Table 3), though these were not found for the simple analyses (total amino acid, GLM based on mean values, $t_{1,13} = 0.451$, $p = 0.660$, PGLS, $t_{1,13} = 0.090$, $p = 0.930$; essential amino acid, GLM based on mean values, $t_{1,13} = 0.591$, $p = 0.566$, PGLS, $t_{1,13} = 0.127$, $p = 0.900$). For both plant types, there was significant interspecific variation in total (herbaceous, $F_{5,12} = 67.2$, $p < 0.001$; woody, $F_{7,16} = 402.0$, $p < 0.001$;

Table 2. Protein content, lipid content, and protein:lipid ratio across different monofloral diets (mean ± se). Values followed by different letters are significantly different (ANOVA).

Pollen diets	Protein content (mg/g)	Lipid content (mg/g)	Protein:Lipid ratio
Herbaceous type	101.11 ± 21.37^{a}	60.17 ± 9.59^{a}	2.08 ± 0.63$^{n.s.}$
Cirsium spp.	83.77 ± 15.39^{bc}	52.04 ± 5.86^{ab}	1.61 ± 0.42^{ab}
Helianthus annuus	73.22 ± 8.20^{bc}	82.61 ± 13.10^{a}	0.89 ± 0.13^{b}
Papaver rhoeas	198.12 ± 9.31^{a}	50.24 ± 12.48^{ab}	3.94 ± 1.57^{a}
Taraxacum agg.	65.85 ± 3.37^{c}	93.49 ± 15.10^{a}	0.70 ± 0.15^{b}
Trifolium repens	123.14 ± 20.43^{b}	29.70 ± 3.92^{b}	4.15 ± 1.07^{a}
Zea mays	62.59 ± 5.81^{c}	52.95 ± 5.06^{ab}	1.18 ± 0.22^{b}
Woody type	51.45 ± 8.16^{b}	29.62 ± 3.81^{b}	1.92 ± 0.38$^{n.s.}$
Castanea sativa	36.51 ± 2.36^{c}	26.21 ± 4.60^{abc}	1.39 ± 0.24^{bc}
Cistus spp.	44.95 ± 7.45^{bc}	22.44 ± 1.88^{bc}	2.00 ± 0.17^{b}
Crataegus monogyna	82.92 ± 14.14^{ab}	19.22 ± 0.83^{c}	4.31 ± 0.64^{a}
Frangula alnus	41.06 ± 14.13^{bc}	17.93 ± 0.67^{c}	2.29 ± 0.40^{ab}
Prunus cerasus	92.92 ± 5.21^{a}	47.32 ± 6.36^{a}	1.96 ± 0.37^{b}
Quercus pyrenaica	37.48 ± 6.59^{c}	31.32 ± 4.68^{abc}	1.20 ± 0.26^{bc}
Salix caprea	44.47 ± 7.29^{bc}	29.10 ± 8.55^{abc}	1.53 ± 0.25^{bc}
Salix fragilis	31.30 ± 4.69^{c}	43.48 ± 2.18^{ab}	0.72 ± 0.04^{c}

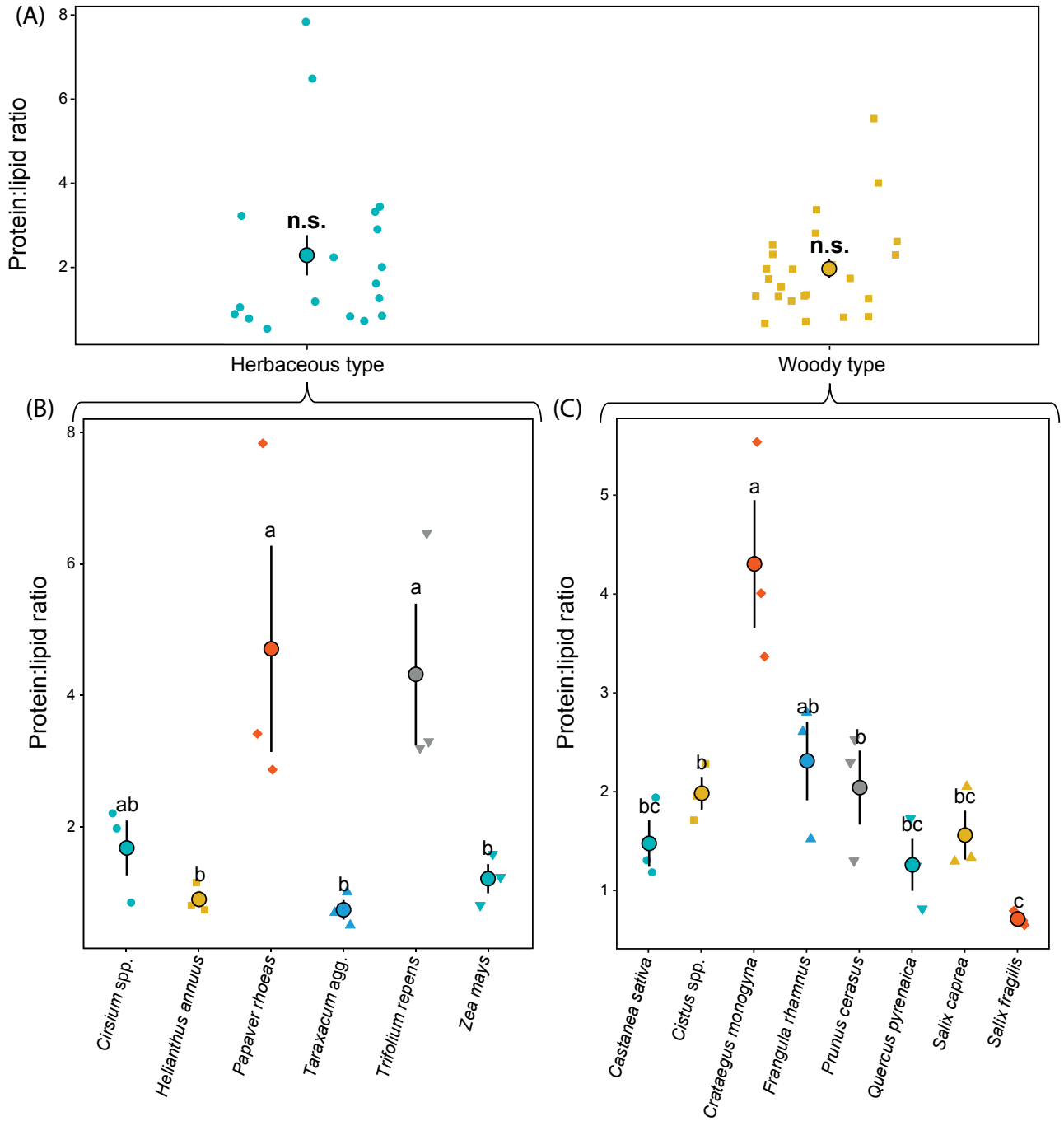

Fig. 1. Differences in protein:lipid ratios for comparisons between selected monofloral diets for **A)** herbaceous and woody plant types, **B)** within herbaceous plant types, and **C)** within woody plant types. Each small data point represents an analytical replicate and large points represent mean values. Error bars indicate standard error of the mean. Letters indicate significant differences ($p < 0.05$).

Figs 2B, C) and essential amino acid content (herbaceous, $F_{5,12} = 46.9$, $p < 0.001$; woody, $F_{7,16} = 291.1$, $p < 0.001$; Table 3). Significant differences between plant types ($F_{1,28} = 14.60$, $p < 0.001$) as well as interspecific variation within each plant type (herbaceous, $F_{5,12} = 8.80$, $p = 0.001$; woody, $F_{7,16} = 28.8$, $p < 0.001$) were also seen for essential amino acids expressed as percentage of total amino acids (Table 3). There was no impact of plant growth type on amino acid composition either in absolute (PERMANOVA, $F_{1,12} = 0.3$, $p = 0.799$) or relative terms ($F_{1,12} = 1.4$, $p = 0.216$).

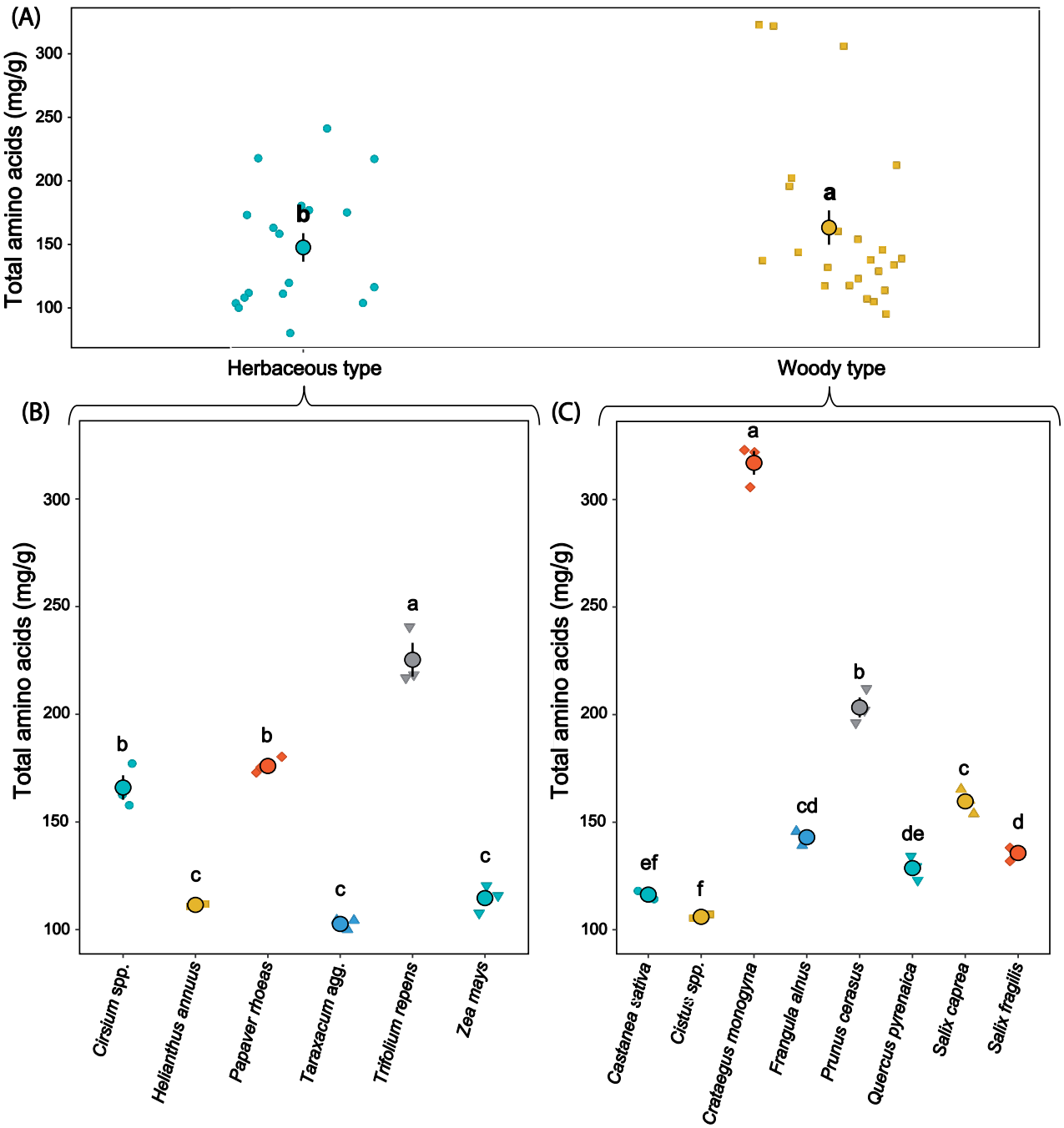

Fig. 2. Total amino acid content for comparisons between selected monofloral diets for **A)** herbaceous and woody plant types, **B)** within herbaceous plant types, and **C)** within woody plant types. Each small data point represents an analytical replicate and large points represent mean values. Error bars indicate standard error of the mean. Letters indicate significant differences ($p < 0.05$).

3.2 Rearing experiment

Microcolonies collected a consistent quantity of pollen across different plant growth types (GLM based on replicates, $F_{1,136} = 0.4$, $p = 0.508$, GLM based on mean values, $t_{1,13} = 0.127$, $p = 0.900$, PGLS, $t_{1,13} = 0.063$, $p = 0.951$), but significant interspecific variation was detected within both plant types (herbaceous, $F_{5,54} = 11.0$, $p < 0.001$; woody, $F_{7,70} = 15.4$, $p < 0.001$, Table 4). Amongst herbaceous plants, microcolonies fed on *Trifolium repens* collected more pollen than those fed on all other herbaceous diets. For woody plants, microcolonies fed on *Salix caprea* collected the greatest quantity of pollen and those fed on *Cistus* spp. the lowest (Table 4). Differences in syrup collection were more pronounced, with microcolonies fed on woody diets collecting

Table 3. Total and essential amino acid content across different monofloral diets (mean ± se). Essential amino acid content expressed as percentage of total amino acids is also presented. Values followed by different letters are significantly different.

Pollen diets	Total amino acids (mg/g)	Essential amino acids (mg/g)	Essential amino acids (% TAA)
Herbaceous type	147.67 ± 20.32^{b}	66.33 ± 8.94^{b}	42.80 ± 0.88^{b}
Cirsium spp.	166.06 ± 5.64^{b}	66.41 ± 3.13^{b}	39.96 ± 0.65^{c}
Helianthus annuus	101.11 ± 10.51^{c}	44.52 ± 5.86^{c}	43.74 ± 1.40^{ab}
Papaver rhoeas	175.97 ± 2.31^{b}	81.07 ± 1.55^{b}	46.06 ± 0.30^{a}
Taraxacum agg.	102.81 ± 1.29^{c}	44.47 ± 0.27^{c}	43.27 ± 0.38^{abc}
Trifolium repens	225.11 ± 7.70^{a}	96.49 ± 3.49^{a}	42.86 ± 0.47^{abc}
Zea mays	114.94 ± 3.40^{c}	47.01 ± 1.01^{c}	40.92 ± 0.58^{bc}
Woody type	163.24 ± 24.43^{a}	71.61 ± 10.22^{a}	44.00 ± 0.86^{a}
Castanea sativa	116.50 ± 1.23^{ef}	52.42 ± 1.04^{f}	44.99 ± 0.60^{ab}
Cistus spp.	102.53 ± 3.85^{f}	40.46 ± 0.97^{g}	39.50 ± 0.64^{d}
Crataegus monogyna	316.78 ± 5.60^{a}	134.25 ± 2.74^{a}	42.37 ± 0.13^{c}
Frangula alnus	142.72 ± 2.13^{cd}	65.51 ± 1.23^{d}	45.89 ± 0.18^{a}
Prunus cerasus	203.19 ± 4.95^{b}	86.80 ± 2.07^{b}	42.72 ± 0.05^{c}
Quercus pyrenaica	128.42 ± 3.33^{de}	55.93 ± 1.85^{ef}	43.55 ± 0.88^{bc}
Salix caprea	159.85 ± 3.18^{c}	74.13 ± 1.62^{c}	46.37 ± 0.21^{a}
Salix fragilis	135.90 ± 1.78^{d}	63.34 ± 0.70^{de}	46.61 ± 0.12^{a}

Table 4. Micro-colony resource collection across different monofloral pollen diets (mean ± se). Values followed by different letters are significantly different.

Pollen diets	Pollen collection (g)	Syrup collection (g)	Pollen dilution (g/g)
Herbaceous type	4.41 ± 0.59$^{n.s.}$	37.34 ± 0.65^{b}	9.63 ± 0.88^{b}
Cirsium spp.	4.34 ± 0.58^{b}	35.65 ± 0.66$^{n.s.}$	9.00 ± 0.72^{a}
Helianthus annuus	3.12 ± 0.12^{b}	38.16 ± 1.49$^{n.s.}$	12.39 ± 0.72^{a}
Papaver rhoeas	4.40 ± 0.51^{b}	38.32 ± 2.08$^{n.s.}$	10.09 ± 1.51^{a}
Taraxacum agg.	3.41 ± 0.12^{b}	35.07 ± 1.06$^{n.s.}$	10.37 ± 0.4^{a}
Trifolium repens	7.19 ± 0.68^{a}	38.92 ± 1.56$^{n.s.}$	5.87 ± 0.61^{b}
Zea mays	3.98 ± 0.27^{b}	37.94 ± 1.59$^{n.s.}$	10.05 ± 0.92^{a}
Woody type	4.61 ± 0.72$^{n.s.}$	47.04 ± 2.19^{a}	12.47 ± 1.48^{a}
Castanea sativa	4.28 ± 0.34^{bc}	50.89 ± 1.69^{a}	12.55 ± 1.07^{ab}
Cistus spp.	3.24 ± 0.20^{a}	49.72 ± 3.52^{a}	15.74 ± 1.39^{a}
Crataegus monogyna	3.91 ± 0.26^{bc}	51.26 ± 1.87^{a}	13.69 ± 1.06^{a}
Frangula alnus	4.03 ± 0.36^{bc}	45.54 ± 3.36^{ab}	12.59 ± 1.81^{ab}
Prunus cerasus	3.58 ± 0.16^{bc}	52.37 ± 3.70^{a}	15.16 ± 1.51^{a}
Quercus pyrenaica	5.05 ± 0.47^{b}	37.31 ± 1.37^{b}	8.33 ± 1.18^{b}
Salix caprea	9.42 ± 0.98^{a}	37.88 ± 1.10^{b}	4.50 ± 0.55^{c}
Salix fragilis	3.34 ± 0.35^{c}	51.38 ± 4.08^{a}	17.18 ± 2.67^{a}

26.0% more syrup than those fed on herbaceous diets (GLM based on replicates, $F_{1,136} = 61.2$, $p < 0.001$, GLM based on mean values, $t_{1,13} = 3.747$, $p = 0.003$, PGLS, $t_{1,13} = 3.154$, $p = 0.008$; Table 4). Whilst no interspecific variation was detected between herbaceous diets ($F_{5,54} = 1.2$, $p = 0.347$), the lowest syrup collection was seen in microcolonies fed on *Quercus pyrenaica* and *Salix caprea* ($F_{7,70} = 5.1$, $p < 0.001$; Table 4). The higher rates of syrup collection by microcolonies fed on woody plant pollen naturally resulted in a higher dilution rate compared to those fed on herbaceous diets (GLM based on replicates, $F_{1,136}=11.43$, $p<0.001$, Table S4), though this was not significant in the

simple analyses (GLM based on mean values, t1,13 = 1.511, p = 0.157, PGLS, t1,13 = 1.130, p = 0.281). Interspecific variation in pollen dilution was detected in both pollen types (herbaceous, F5,54 = 7.4, p < 0.001; woody, F7,70 = 14.6, p < 0.001). For woody plants, although microcolonies fed on *Salix caprea* collected the greatest quantity of pollen, they collected an average quantity of syrup, resulting in the lowest dilution rate (Table 4). Amongst herbaceous plants, microcolonies fed on *Trifolium repens* showed the lowest dilution rate as they collected more pollen but same average amount of syrup (Table 4).

Overall, microcolonies fed on pollen from woody plants produced a 62.0% greater mass of larval offspring (GLM based on replicates, $F_{1,136} = 37.6$, $p < 0.001$, Fig. 3A), though this was not significant in the simple analyses (GLM based on mean values, $t_{1,13} = 1.506$, $p = 0.158$, PGLS, $t_{1,13} = 0.389$, $p = 0.704$). There was interspecific variation within both herbaceous and woody diets (herbaceous, $F_{5,54} = 25.4$, $p < 0.001$; woody, $F_{7,70} = 10.1$, $p < 0.001$; Table 5). Within herbaceous diets, microcolonies fed on *Trifolium repens* produced the greatest mass of offspring whilst those fed on Asteraceae diets (*Cirsium* spp., *Helianthus annuus*, and *Taraxacum* agg.) displayed lower offspring production (Fig. 3B). For woody diets, the highest offspring production was seen in microcolonies fed on *Salix caprea* (Fig. 3C).

This difference in microcolony performance between plant growth types was also reflected in the probability of larval ejection, which was higher in microcolonies fed on herbaceous diets (GLM based on replicates, $F_{1,129} = 159.4$, $p < 0.001$, GLM based on mean values, $t_{1,13} = 4.663$, $p < 0.001$, PGLS, $t_{1,13} = 2.757$, $p = 0.017$; Fig. 4, Table 5). Though interspecific variation was detected within both herbaceous and woody plant diets (herbaceous, $F_{5,48} = 51.7$, $p = 0.033$; woody, $F_{7,69} = 57.1$, $p < 0.001$), post-hoc analyses had a statistical power that was too substantially reduced to determine with confidence the diets with the highest larval ejection rates (no significant differences were detected for the multiple comparisons).

4 Discussion

Though variable, the chemical composition of pollen from woody and herbaceous plants was not strongly differentiated; though herbaceous plant pollen had on average higher lipid and crude protein content, there were no differences in the protein:lipid ratios. For total amino acid content, woody pollen contained a slightly higher concentration than herbaceous plant pollen, but overall composition did not differ, with no lack of essential amino acids. Despite the chemical composition, performance was better on woody plant pollen diets, with all microcolonies fed on woody plant pollen producing more brood than the worst performing herbaceous pollen diets. Microcolonies fed on herbaceous plant diets also had a high and variable rate of larval ejection compared to those fed on woody plant diets, these results being in line with our hypothesis that herbaceous plant pollen is more likely to be of variable quality. However, the universality of this result should be treated with caution, as we only considered a subset of herbaceous plants, including members of the family Asteraceae that are known to have low quality pollen for generalist bee species (Vanderplanck et al. 2018; 2020).

This is illustrated in the compositional analyses. Though protein and lipid content was higher in herbaceous plants, this is likely to have been driven by phylogenetic structuring rather than a trait inherent to herbaceous plants. Importantly, the protein:lipid ratio amongst selected species showed no difference, suggesting that performance differences observed in microcolonies were not driven by these factors. Plant pollen can also contain a wide range of secondary metabolites that can discourage pollen collection as it can increase rates of larval mortality when consumed by developing bees (Praz et al. 2008; Wang et al. 2019; Brochu et al. 2020). To date, the majority of harmful secondary metabolites reported from plant pollen have been found in botanical families predominantly comprising herbs (e.g. Asteraceae; Boraginaceae, Cucurbitaceae, Dipsacaceae, Praz et al. 2008; Wang et al. 2019; Brochu et al. 2020). In Boraginaceae, the greatest levels of secondary metabolites are found in species that offer only pollen as a reward for pollinators, with lower concentrations found in those species offering both pollen and nectar (Trunz et al. 2020), suggesting that the high levels are present in order to discourage overharvesting of pollen. The absence to date of harmful secondary metabolites reported from the pollen of woody plants in temperate regions may be as a result of their flowering strategy which is to produce a great abundance of easily accessibly pollen (simple, non-complex flowers) in a short time period (mass flowering), thus preventing overharvesting through sheer quantity. Although a detailed investigation into the precise dynamics between pollination strategy and pollen chemical composition is just beginning (Trunz et al. 2020), our results are consistent with the principle that herbaceous and woody plants pursue different strategies. Given the limited number of taxa tested here compared to the huge diversity of flowering plants, representatives of many more botanical families need to be studied before this claim can be supported with a high degree of confidence; for example, there is complexity in the results, as despite producing the greatest brood weight of all the woody pollen diets, *Salix caprea* also had the highest rate of larval ejection in this group, nearly 10 times higher than in its congener *Salix fragilis*. This deserves further investigation.

Separately, the fact that protein content was higher but total amino acid content was lower in herbaceous plants compared to woody plants is not an aberrant result, as total amino acid analyses take into account all amino acids present in the pollen grain whereas crude protein measurements underestimate proteinaceous nitrogen represented by short-chain length oligopeptides (< 10,000 Da) that are less effi-

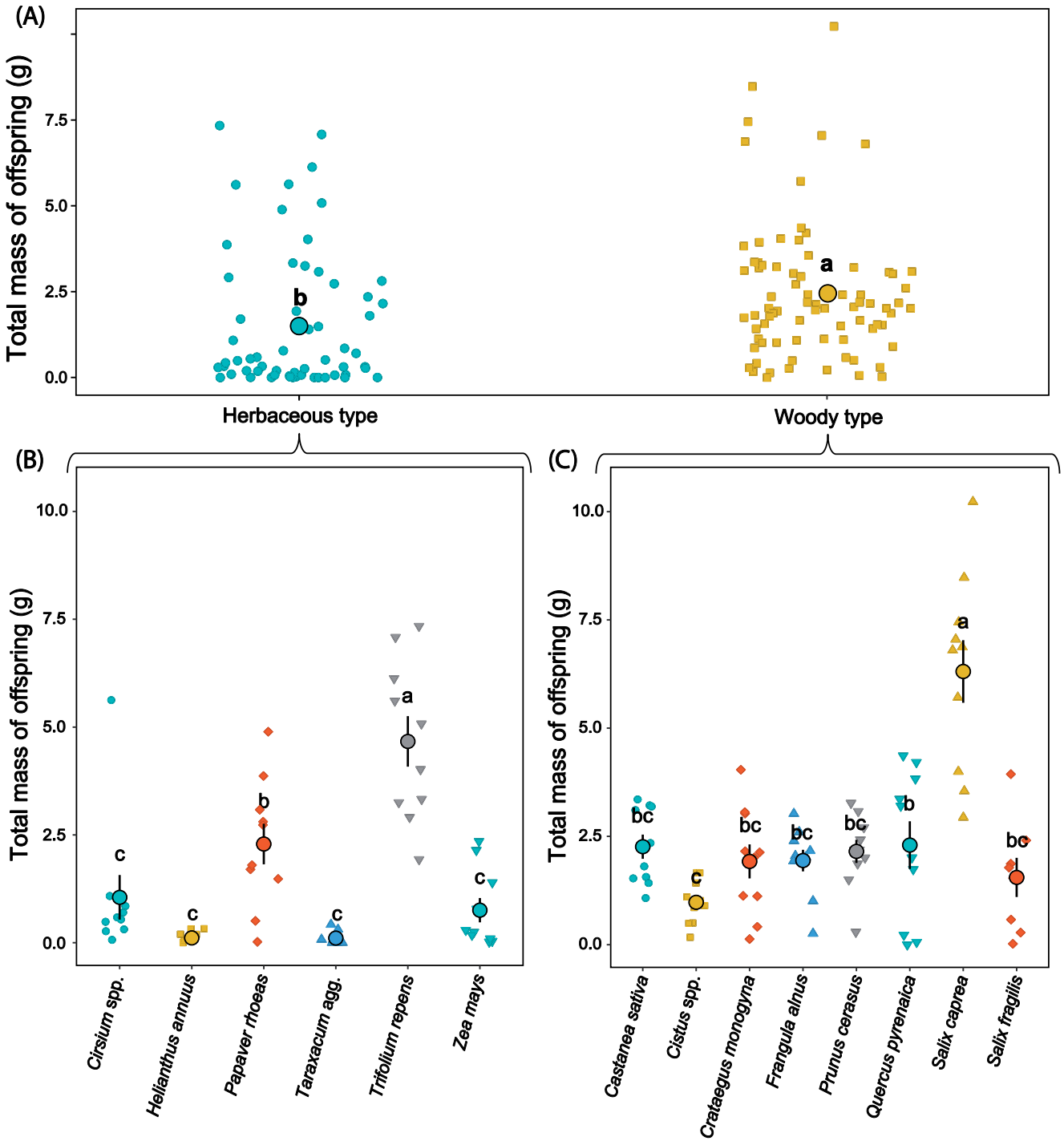

Fig. 3. Offspring production (total fresh weight of brood) for *Bombus terrestris* microcolonies for comparisons between selected monofloral diets for **A)** herbaceous and woody plant types, **B)** within herbaceous plant types, and **C)** within woody plant types. Each small data point represents an analytical replicate and large points represent mean values. Error bars indicate standard error of the mean. Letters indicate significant differences ($p < 0.05$).

ciently extracted (Vanderplanck et al. 2014). The observed difference between the Bradford assay and the total compositional analysis would suggest that a greater proportion of woody plant protein is comprised of oligopeptides, but this has not been investigated in detail elsewhere as crude protein content is the traditional method for assessing 'bee-relevant' protein content in pollen (Pamminger et al. 2019).

Given these differences, the question remains at to whether or not flowering trees and shrubs have been overlooked as suitable species to be included in bee-focused

Table 5. Micro-colony performance across different monofloral pollen diets (mean ± se). Values followed by different letters are significantly different.

Pollen diets	Brood weight (g)	Pollen efficacy	Larval ejection rate (%)
Herbaceous type	1.50 ± 0.71^{b}	0.28 ± 0.12^{b}	18.75 ± 5.41^{a}
Cirsium spp.	1.05 ± 0.52^{c}	0.19 ± 0.05^{ab}	34.60 ± 5.44
Helianthus annuus	0.11 ± 0.04^{c}	0.04 ± 0.01^{b}	10.91 ± 6.12
Papaver rhoeas	2.29 ± 0.47^{b}	0.49 ± 0.07^{a}	5.22 ± 2.03
Taraxacum agg.	0.11 ± 0.05^{c}	0.03 ± 0.01^{b}	36.20 ± 16.05
Trifolium repens	4.67 ± 0.59^{a}	0.76 ± 0.04^{a}	14.62 ± 3.53
Zea mays	0.76 ± 0.28^{c}	0.16 ± 0.06^{ab}	10.98 ± 4.89
Woody type	2.43 ± 0.58^{a}	0.48 ± 0.05^{a}	2.65 ± 1.26^{b}
Castanea sativa	2.26 ± 0.28^{bc}	0.52 ± 0.04^{ab}	0.71 ± 0.71
Cistus spp.	0.98 ± 0.15^{c}	0.29 ± 0.04^{b}	0.89 ± 0.62
Crataegus monogyna	1.92 ± 0.39^{bc}	0.48 ± 0.08^{ab}	1.53 ± 0.91
Frangula alnus	1.94 ± 0.25^{bc}	0.46 ± 0.05^{ab}	0 ± 0
Prunus cerasus	2.15 ± 0.27^{bc}	0.58 ± 0.06^{ab}	1.75 ± 1.16
Quercus pyrenaica	2.30 ± 0.55^{b}	0.39 ± 0.08^{ab}	4.29 ± 1.71
Salix caprea	6.31 ± 0.72^{a}	0.72 ± 0.04^{a}	10.90 ± 2.77
Salix fragilis	1.55 ± 0.45^{bc}	0.43 ± 0.12^{ab}	1.14 ± 1.14

agri-environment schemes (see also Requier & Leonhardt 2020 for a non-floral resource perspective). Though the results presented here suggest that woody plants provide consistently good pollen and their addition to the landscape is likely to benefit spring-flying bee species, their potential use must be set in context and considered against several caveats. There is a functional argument for including woody plant species, as the addition of flowering trees would provide resources for species with short flight periods that do not extend into the summer, and hence are unable to interact with herbaceous enhancements. This can be seen in the genus *Andrena*, an important pollinator of spring fruit crops (Park et al. 2015). Most spring-flying species are univoltine and are associated with flowering trees and shrubs (Wood et al. 2018a), and so do not respond to the addition of herbaceous wildflower strips (Campbell et al. 2017; Wood et al. 2018a). However, species with long flight periods require a continual supply of resources throughout the season, so the use of trees and shrubs should not be seen as a panacea. Simply providing abundant spring resources may result in faster initial colony growth, but has no impact on overall reproductive output and therefore population size (Westphal et al. 2009). The seasonal shift from trees and shrubs to herbaceous plants as the season progresses is well documented (Wood et al. 2018b; Bertrand et al. 2019), and if the desired pollinator community contains species with long flight periods, the choice of plants included in enhancements should reflect that.

From a perspective focused solely on bee conservation, most temperate spring-flying bees have stable population trends (Scheper et al. 2014), whereas this is not the case

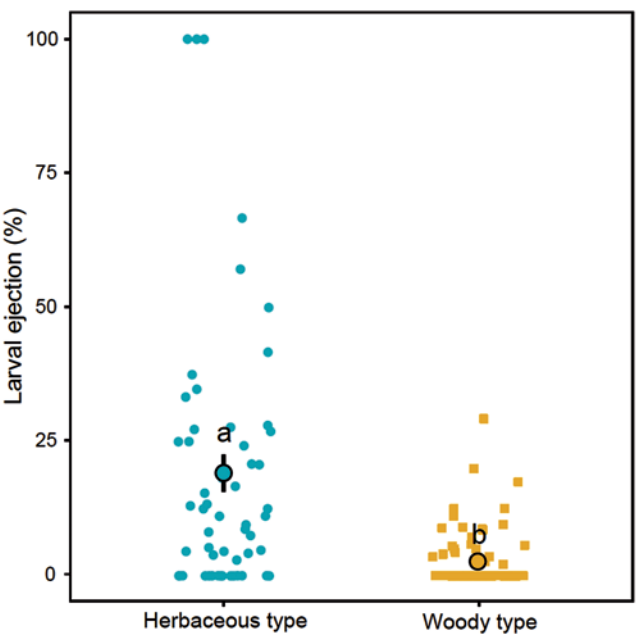

Fig. 4. Larval ejection rate for *Bombus terrestris* microcolonies fed on monofloral diets from herbaceous or woody plants. Each small data point represents an analytical replicate and large points represent mean values. Error bars indicate standard error of the mean. Letters indicate significant differences ($p < 0.05$).

for bees that fly during the summer (Hofmann et al. 2019). Except in the most intensified regions, woody features such as hedgerows or single trees can still persist in agricultural landscapes in a way that flowering grasslands cannot unless

specifically conserved (Scheper et al. 2014). Woody plants are also grow vertically and can provide a greater density of flowering resources than herbaceous plants in the same area of land, so temperate landscapes often retain more spring-flowering than summer-flowering resources (Scheper et al. 2014). Therefore, adding more spring-flowering woody plants to such landscapes will not benefit the most threatened bee species which fly in the summer and preferentially feed on herbaceous plants (Hofmann et al. 2019; Drossart et al. 2019). This tension exemplifies the conflict between identifying an optimal strategy to provide ecosystem services and support bee conservation concurrently (Kleijn et al. 2015). Finally, there is a practical consideration which is that it is simply much faster to add herbaceous plants to a landscape than it is to add woody plants which may take several years between planting and flowering, particularly in the case of trees. Adding woody plants as part of an agri-environment scheme therefore requires support and planning on a decade long timescale, and must therefore be serious considered by land managers before implementation.

In conclusion, our results show that the pollen from woody plants supports bumblebee colony growth more consistently than pollen from herbaceous plants, with very low rates of larval mortality. This suggests that these plants may therefore be suitable candidates for inclusion in agri-environments schemes aimed at increasing the population size of wild bee species. This consistent pollen quality in woody plants may derive from traits inherent to their pollination strategy, but greater taxon sampling is necessary to establish this with confidence. The use of woody plants in agri-environment schemes should not be considered in isolation and as a replacement for more traditional schemes that use herbaceous plants, except in the most extreme cases where the vast majority of a desired wild bee pollinator community is made up of species that fly only in the spring (Wood et al. 2018a).

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