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The effect of pollen amount and its caloric value in hybrid lucerne (*Medicago x varia*) on its attractiveness to bumble bees (*Bombus terrestris*)

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Abstract. Bumble bees forage for pollen and nectar on various plant species, but individuals often restrict their choice to a few flower types. The aim of this work was to study whether there is variation in certain pollen characteristics between differently coloured flowers of hybrid lucerne and whether this could be the reason why bumble bees prefer one flower type to another. The pollen amount, pollen grain size and the caloric values from different flower types were analysed to study the reasons for flower colour preference. On two cultivars of hybrid lucerne (*Medicago x varia* Mart.) the yellow flowers were preferred, but the reasons for the preference differed.

Key words: *Medicago x varia*, colour morphs, bumble bees, pollen amount, caloric value

INTRODUCTION

Bumble bees are generalists and pollinate an enormous number of wild plants. However, individuals exhibit selective behaviour to some morphological or colour flower types (Gumbert et al., 1999). Pohtio & Teräs (1995) considered that bumble bees have no innate flower colour preferences, although they may learn to link a profitable flower and its colour. There can be several reasons for flower colour preferences. Flowers vary in their mechanism for release of pollen and nectar and foraging bees need different techniques to collect, transport and store these different food commodities (Hofstede & Sommeijer, 2006). Beside the interspecific variation in the amount or qualities of flower rewards there can occur also intraspecific variation between different flower (colour) types (Pohtio & Teräs, 1995). Bees are capable of learning profitable flower types and efficient flower handling techniques, and develop constancy to type to minimise foraging costs (Dukas & Real, 1993).

Hybrid lucerne *Medicago x varia* Mart. is a crop which may have flowers with very variable petal colour (Teuber & Brick, 1988). It has been shown that attractiveness of hybrid lucerne to pollen collecting bumble bees varies widely between different clones and is a heritable characteristic (Pedersen & Bohart, 1953). Flower colour is genetically determined in this plant (Washington, 1972) and the variation occurs between clones. The flower colour preference of bumble bees on hybrid lucerne

is not permanent; it may change from year to year (Hiiemets et al., 2000). The preference may be caused by varying flower rewards, but in the case of hybrid lucerne it has not been studied much. The aim of this work was to study whether there is variation in certain pollen characteristics (amount and caloric value) between differently coloured flowers of hybrid lucerne and whether this could be the reason why bumble bees prefer one flower type to another.

MATERIALS AND METHODS

The study was conducted at the Jõgeva Plant Breeding Institute, Jõgeva County, Estonia, in July 2005. There were two fields (each 1.0 ha) of local cultivars of the hybrid lucerne *M. x varia,* 'Karlu' and 'Jõgeva 118', under observation. The fields were located 2 km apart. The flowers of both cultivars may appear in light or dark yellow, light or dark purple, and white colour; in addition there may appear also variegated flower colours that may range from a very dark blue to a green or yellow green (Teuber & Brick, 1988).

Washington's classification (1972) was used to determine flower colour. In 2005 the cultivar 'Karlu' had 82% yellow, 15% purple and 3% variegated flowers. On the cultivar 'Jõgeva 118' the yellow and purple flowers were represented almost evenly (accordingly 46% and 49%) and the proportion of flowers with variegated colour were found in less than 5% of plants. Because of the low numbers of variegated flowers these were left out of the analysis.

To determine pollen caloric values, yellow and purple flowers of both cultivars were gathered and dried in an electrical stove with 30°C. The pollen was separated from the dried flowers using a series of analytical sieves ('Retsch' (Din-iso 3310/1)). The pollen caloric values were determined with a microbomb-calorimeter MBK-2 (Ivask, 1999).

To analyse the pollen amount yellow and purple flowers were gathered, dried and acetylised. The numbers of pollen grains were counted from the Fuchs-Rosenthal chamber and calculated per flower. Pollen grain diameters were measured using light microscopy. On the basis of mean pollen grain diameters and number of pollen grains per flower, the pollen volumes per flower were calculated.

The observations for pollinators' behaviour were carried out by counting the visits of bumble bees to the differently coloured inflorescences. The observations were made twice a week over a three week period from 20 1x10 m observation plots on both cultivars. The inflorescences of different colours were counted on the same days as the bumble bees in three 20x20 cm randomly chosen squares per plot. On the basis of the numbers of differently coloured inflorescences and bumble bee visits to them, the expected numbers of flower visits were calculated.

In the statistical analysis the program STATISTICA 7.0 was used. Data on pollen caloric values and pollen grain numbers, diameters and volumes were analysed by *t*-test. The χ^2-test was used to analyse the differences in the observed and expected numbers of bumble bees on the inflorescences.

RESULTS AND DISCUSSION

The results of the study show that pollen caloric values differed between yellow and purple flowers only in the cultivar 'Karlu' (Fig. 1). There the pollen from yellow flowers had significantly higher mean caloric value than the pollen from purple flowers (t= 2.7; df = 18; P = 0.02). On the cultivar 'Jõgeva 118' no significant difference was found (t = 0.07; df = 18; P = 0.9).

No significant difference in the mean numbers of pollen grains per flower between the two flower colour types was found on either cultivar (Table 1). However pollen grain diameters differed significantly between the colour types on both cultivars. The purple flowers on the cultivar 'Karlu', and the yellow flowers on the cultivar 'Jõgeva 118' , had larger diameter pollen grains. On the cultivar 'Karlu' due to the larger pollen grains the pollen volume per flower was significantly higher in purple flowers. Although yellow flowers had larger pollen grains on the cultivar 'Jõgeva 118', pollen volumes per flower did not differ significantly between yellow and purple flowers.

The observed numbers of bumble bee visits to inflorescences of different colour were compared to the expected number of bumble bee visits to find out flower colour preferences. Indeed, the observed numbers of bumble bees were significantly higher on yellow flowers compared with expected ones on both cultivars ('Karlu': χ^2 = 3.98 P = 0.046; 'Jõgeva 118': χ^2 = 6.64 P = 0.01) (Fig. 2). On purple flowers the actual numbers of bumble bees was significantly lower than expected on the basis of the proportions of flower types.

Seed production in hybrid lucerne usually encounters many problems. Northern areas with cold winters affect the survival rate of the lucerne plants (Free, 1993). Weather conditions during blooming period are not always propitious for ensuring an economically effective seed yield (Bolaños-Aguilar et al., 2002). Excessive humidity encourages vegetative growth of the plants and deters pollinators from foraging. In many agricultural regions the decline of wild pollinator species has also become a problem in lucerne seed production (Strickler & Vinson, 2000).

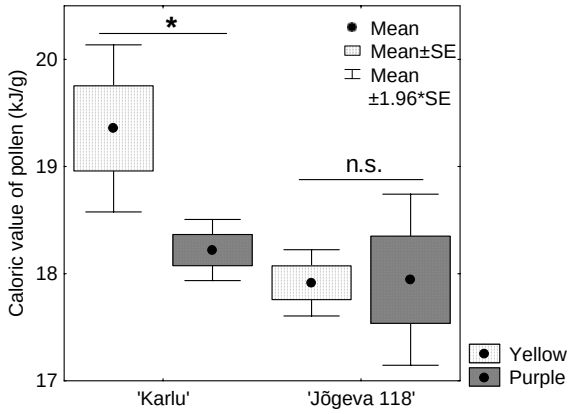

Fig. 1. Caloric values of pollen from yellow and purple flowers on the cultivars 'Karlu' and 'Jõgeva 118'. * P < 0.05; n.s.- not significant.

Table 1. The mean (±Std. error) pollen grain numbers, grain diameters and pollen volumes per flower in yellow and purple flowers of hybrid lucerne. The statistically significant differences between the means were tested with *t*-test.

	Cultivar 'Karlu'		Cultivar 'Jõgeva 118'	
	Yellow	Purple	Yellow	Purple
No. of pollen grains	1395 ±69	1566 ±119	2099 ±146	2196 ±121
	$t = 1.2$; $df = 58$; $P = 0.22$		$t = 0.5$; $df = 58$; $P = 0.61$	
Pollen grain diameter (µm)	33.4 ±0.6	37.9 ±0.7	37.5 ±0.5	35.5 ±0.6
	$t = 4.9$; $df = 98$; $P < 0.001$		$t = 2.5$; $df = 98$; $P = 0.01$	
Pollen volume (mm^3)	0.027 ±0.001	0.045 ±0.003	0.058 ±0.004	0.051 ±0.003
	$t = 4.8$; $df = 58$; $P < 0.001$		$t = 1.3$; $df = 58$; $P = 0.21$	

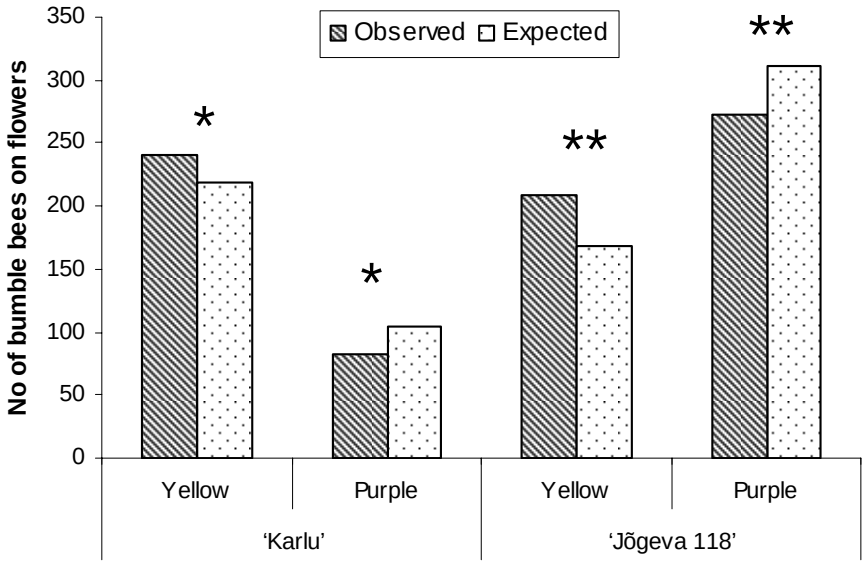

Fig. 2. Bumble bees preferred yellow flowers on both cultivars of hybrid lucerne and discriminated against the purple flowers. * $P < 0.05$; ** $P < 0.01$.

The main pollinators of lucerne crops are wild bees: solitary bees (McGregor, 1976) and in northern regions bumble bees (Mänd et al., 1996). Honey bees do visit the flowers of hybrid lucerne, but they forage for nectar (Free, 1993). Bumble bees visit lucerne flowers mostly for pollen, but they simultaneously also take nectar to supply themselves with energy (McGregor, 1976).

Pollen grains consist of three layers: outer exine, intine and inner protoplasm which contain mostly proteins (Dobson, 1991). Chemically the pollen consists of proteins (caloric value 23.0 kJ/g), starch (17.6 kJ/g), carbohydrates (16.7 kJ/g), lipids (38.9 kJ/g) and pectines (38.9 kJ/g) (Runge, 1973). The proportions of these constituents probably depend on pollen grain size: larger grains contain more protein, smaller grains more pectines and lipids. The small differences in caloric values may reflect large differences in protein or lipid content (Pitelka, 1978). According to this, the higher caloric value should be a cue about the smaller pollen grains that have relatively high content of lipids and pectines.

Our results support partly this logic that pollen grains with larger diameters have lower caloric values. In the cultivar 'Karlu' the purple flowers had pollen grains with significantly larger diameters but low caloric value. However, on the cultivar 'Jõgeva 118' there was no difference in caloric values between flower types, although the pollen grain diameters were larger in the case of yellow flowers. Although the difference in pollen grain diameters in the latter case was statistically significant, it is possible that it was not big enough to cause the difference in caloric values.

Bees are not able to digest the outer layers of pollen grains; therefore the most important for them is the protoplasm that consists of proteins and starch (Rasheed & Harder, 1997). The larger pollen grains with higher volume of protoplasm should be more valuable for bumble bees. Indeed, on the cultivar 'Jõgeva 118' the preference of bumble bees for yellow flowers with larger pollen grains was found, although no difference in the caloric values was detected. On that cultivar also no differences in the numbers of pollen grains and the pollen volumes per flowers were found. On the cultivar 'Karlu' however, yellow flowers were also preferred, but the reasons for this must be different. Here the pollen grains were smaller and with higher caloric value than pollen from purple flowers, the pollen volume per flower was significantly lower also. The reasons for this unexpected result could be in the other valuable contents of pollen for bumble bees. Cook et al. (2003) showed that honey bees forage preferentially for pollen with the greatest available nutritional quality. The quality does not mean the highest content of protein itself, but the highest content of some certain most valuable amino acids that vary between different plant species in large scale. The pollen composition may vary between the hybrids as well. Also other essential nutrients in pollen may influence foraging behaviour, as may phago-stimulants in pollen and defensive metabolites, including pollen toxins, and phago-deterrents (Cook et al., 2003). The exact constituents of the pollen that could take part in determining the flower colour preference of the bumble bees needs further work. It is obvious that the basis of flower colour preference is not simply the energetics of foraging, the protein content nor the quantity of the reward.

CONCLUSIONS

The two cultivars of hybrid lucerne differed in the characteristics of pollen from yellow and purple flowers. The pollen grains from the preferred yellow flowers had a larger diameter on the cultivar 'Jõgeva 118', but a smaller diameter on the cultivar 'Karlu'. Although bumble bees preferred yellow flowers on both cultivars, the reasons for this were different.

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