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ORIGINAL PAPER

Early spring floral foraging resources for pollinators in wet heathlands in Belgium

Laura Moquet[1] · **Carolin Mayer**[1] · **Denis Michez**[2] · **Bernard Wathelet**[3] · **Anne-Laure Jacquemart**[1]

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Abstract In temperate regions, foraging resources for pollinating insects are particularly important in early spring, especially for social insects like bumblebees that are initiating colonies. Heathlands, protected open habitats under EU law, provide floral resources (pollen and nectar) for a range of pollinating insects. In early spring, in Belgian heathlands, only two floral resources are available: willows *Salix* spp. (Salicaceae) and bilberry *Vaccinium myrtillus* (Ericaceae). Our objective was to assess whether these two plant species provide quantitative and qualitative resources during early spring. We examined the springtime activity of flower visitors on both *S.* x *multinervis* and *V. myrtillus* flowers in relation to sugar concentration and composition in nectar as well as composition of polypeptides and amino acids in pollen. The chemical composition of pollen differed significantly between the two plant species. *Salix* x *multinervis* offered pollen with higher polypeptide and essential amino acid contents than *V. myrtillus*. However, nectar quantities of *V. myrtillus* flowers were relatively high compared to *S.* x *multinervis*. During the overlapping flowering period of the two plant species, flower visitors seemed to favor high quality and easily accessible pollen of *S.* x *multinervis* species and visited *V. myrtillus* mainly for nectar resources.

Keywords Willow · Bilberry · Bumblebees · Solitary bees · Pollen · Nectar

Introduction

Pollinators play an important role in most terrestrial ecosystems as they contribute to the pollination of about 78 % of plant species in temperate regions (Ollerton et al. 2011). Pollination is considered as a key ecosystem service that is crucial to the reproduction of both wild plant and crop species (Corbet et al. 1991; Davila et al. 2012). Nevertheless, evidence about the decline of insect pollinators, have accumulated worldwide during the past three decades (Kosior et al. 2007; Grixti et al. 2009; Carvalheiro et al. 2013; Nieto et al. 2015). Multiple causes are involved, but one of the primary factors concerns modifications of the landscape matrix (Hendrickx et al. 2007; Goulson et al. 2010; Bennett and Isaacs 2014), especially destruction, fragmentation and degradation of habitats (Rathcke and Jules 1993; Fahrig 2003; Harris and Johnson 2004). These modifications induce shifts or decreases in the quantity and quality of the floral resources available for pollinators in terms of the abundance of flowering plant species and their relative quantity and composition of nectar and pollen rewards (Biesmeijer et al. 2006; Kleijn and Raemakers 2008). Because of their economic importance, much of the research on habitat use by pollinators has been carried out in agricultural ecosystems (Osborne et al. 1999; Persson and Smith 2013); nevertheless, work has also been carried out in semi-natural and natural ecosystems (Lack 1982; Petanidou and Ellis 1993; Carvell

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✉ Laura Moquet
laura.moquet@uclouvain.be

[1] Earth and Life Institute-Research group Genetics, Reproduction, Populations, Université catholique de Louvain, Croix-du-Sud 2, Box L7.05.14, 1348 Louvain-la-Neuve, Belgium

[2] Laboratory of Zoology, Institute of Biosciences, University of Mons, Place du Parc 20, 7000 Mons, Belgium

[3] Industrial Biological Chemistry Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

2002; Potts et al. 2003). Among semi-natural ecosystems, less attention has been paid to heathland ecosystems (but see Descamps et al. 2015; Forup et al. 2007; Kaiser-Bunbury et al. 2009; Mayer et al. 2012).

Heathlands have been recognized for a long time as valuable habitat for honeybees (*Apis mellifera* L.). Hives are set up mainly in late summer, during the flowering period of the heather, *Calluna vulgaris* (L.) Hull (Ericaceae). In contrast, the diversity and abundance of wild pollinators remain poorly explored in this type of habitat. Besides *C. vulgaris*, these open semi-natural ecosystems are dominated by other ericaceous dwarf shrubs (Gimingham 1972). Several specialist pollinators, particularly sensitive to decline, depend on the floral resources of these ericaceous species (Goulson et al. 2005). The solitary bee *Andrena lapponica* Zetterstedt collects pollen mainly from *Vaccinium* species. European heathlands provide the unique habitat for declining bumblebee species, *Bombus monticola* Smith and *Bombus jonellus* Kirby (Rasmont et al. 1993). In Europe, heathlands have largely been destroyed and fragmented during the last two centuries (Aerts and Heil 1993; Webb 1998). They were converted to agricultural or afforested areas (e.g. spruce—*Picea abies*-plantations in Belgium, Clicheroux 1957). As heathlands offer a low floral diversity, especially in spring, the survival of wild pollinator species can be questioned. Early flowering species are important for the diet of pollinators emerging in early spring and for colony establishment of social species. Female bees require pollen in order to complete the maturation of their ovaries and for nest provisioning (Roulston and Cane 2000; Génissel et al. 2002). Pollen represents the main source of polypeptides, amino acids and phytosterols (Roulston and Cane 2000; Génissel et al. 2002). Protein content impacts on reproduction, growth, immunocompetence and longevity of insects (Gilbert 1981; Haslett 1989; Smeets and Duchateau 2003; Alaux et al. 2010). Other studies suggest that the nutritional value of pollen for bees may be defined more accurately by the amino acid composition than by the protein content (Cook et al. 2003; Nicolson 2011; Vanderplanck et al. 2014b). Larvae are larger if fed with pollen containing a high concentration of amino acids. Moreover, ten amino acids are essential for honeybees, which are vital for larval development (De Groot 1953).

To maintain their metabolism, especially under suboptimal spring temperatures and unpredictable weather conditions, pollinators also need nectar, the main source of sugars (Waser and Ollerton 2006; Cnaani et al. 2006). The energetic value of nectar to pollinators depends on both the volume and the total concentration of sugars (Cnaani et al. 2006; Nicolson and Thornburg 2007).

In this study, we examined the springtime activity of pollinators in Belgian wet heathlands in relation to amino acid and polypeptide composition in pollen as well as to nectar sugar concentration and composition. In early spring (April–May), the two main floral resources for pollinators in these wet heathlands are *S.* x *multinervis* Döll (Salicaceae) and *V. myrtillus* L. (Ericaceae). The willow *S.* x *multinervis* is a thick, densely branched, bush growing in heathlands, wetlands and woodland margins on acidic peaty soils (Stace 2001; Lambinon and Verloove 2012). In the Ardenne, High Belgium, the flowering season starts as early as March or April (Lambinon and Verloove 2012). *Salix* x *multinervis* thus constitutes the first flowering species in wetlands and wet heathlands (Jacquemart 1992). Willows are dioecious and bear catkins; they are known to attract numerous oligolectic solitary bee species, such as *Andrena* spp. (Dötterl et al. 2005). Visitors of *S.* x *multinervis* have not been studied so far.

The second early flowering species in heathlands is *V. myrtillus*, a deciduous perennial shrub also growing on acidic soils (Coudun and Gégout 2007). The hermaphroditic flowers are single or rarely in pairs and present green to purple pendulous urceolate corollas (Ritchie 1956). *Vaccinium* species have poricidial anthers, therefore pollen is released only by anther vibration which is referred to as buzz pollination (Jacquemart, 2003). Few insects are able to buzz pollinate flowers (Buchmann and Nabhan 1997; De Luca and Vallejo-Marín 2013). The main visitors observed on *V. myrtillus* in Belgian heathlands are bumblebees, which are able to buzz the flowers (Jacquemart 1993).

Our objective was to assess whether these two plant species provide quantitative and qualitative resources during early spring. Our hypothesis posited that ericaceous species, despite their abundance in early spring, do not constitute sufficient resource for bumblebee colony founding. We first investigated whether insect visitor abundance was similar between the two plant species. Then we tested whether the attractiveness could be linked to nectar and/or pollen quantity and composition.

Methods

Study sites and experimental design

Observations were carried out in six wet heathland sites located in the Upper Ardenne, Belgium (Table 1; Fig. 1) covering between 3000 and 407,000 m^2. They are all restricted to an altitude between 550 and 600 m. Population sizes ranged from 250 to 28,000 m^2 for *V. myrtillus* and from 25,000 to 269,000 m^2 for *S.* x *multinervis*. Population size refers to the sum of the area covered by one plant species per site.

We performed the observations during two separate flowering periods. The first period lasted from early April

Table 1 Study sites location and population size of *S.* x *multinervis* and *V. myrtillus*

Sites	Grande fange	Sacrawe	Champfa	Fange aux mochettes	Grand passage	Robiefa
Localities	Vielsalm	Vielsalm	Vielsalm	La Roche-en-Ardenne	Houffalize	Manhay
Coordinates	50°14′40″N 5°46′45″E	50°14′33″N 5°45′46″E	50°13′09″N 5°47′54″E	50°13′21″N 5°40′55″E	50°13′44″N 5°45′46″E	50°15′27″N 5°42′10″E
Total site area (m^2)	2,782,000		1,123,000	721,000	1,267,000	294,000
Estimated population size of *S.* x *multinervis* (m^2)	70,100	209,000	58,000	269,000	33,400	165,000
Estimated population size of *V. myrtillus* (m^2)	250	1500	3300	15,000	16,700	28,000

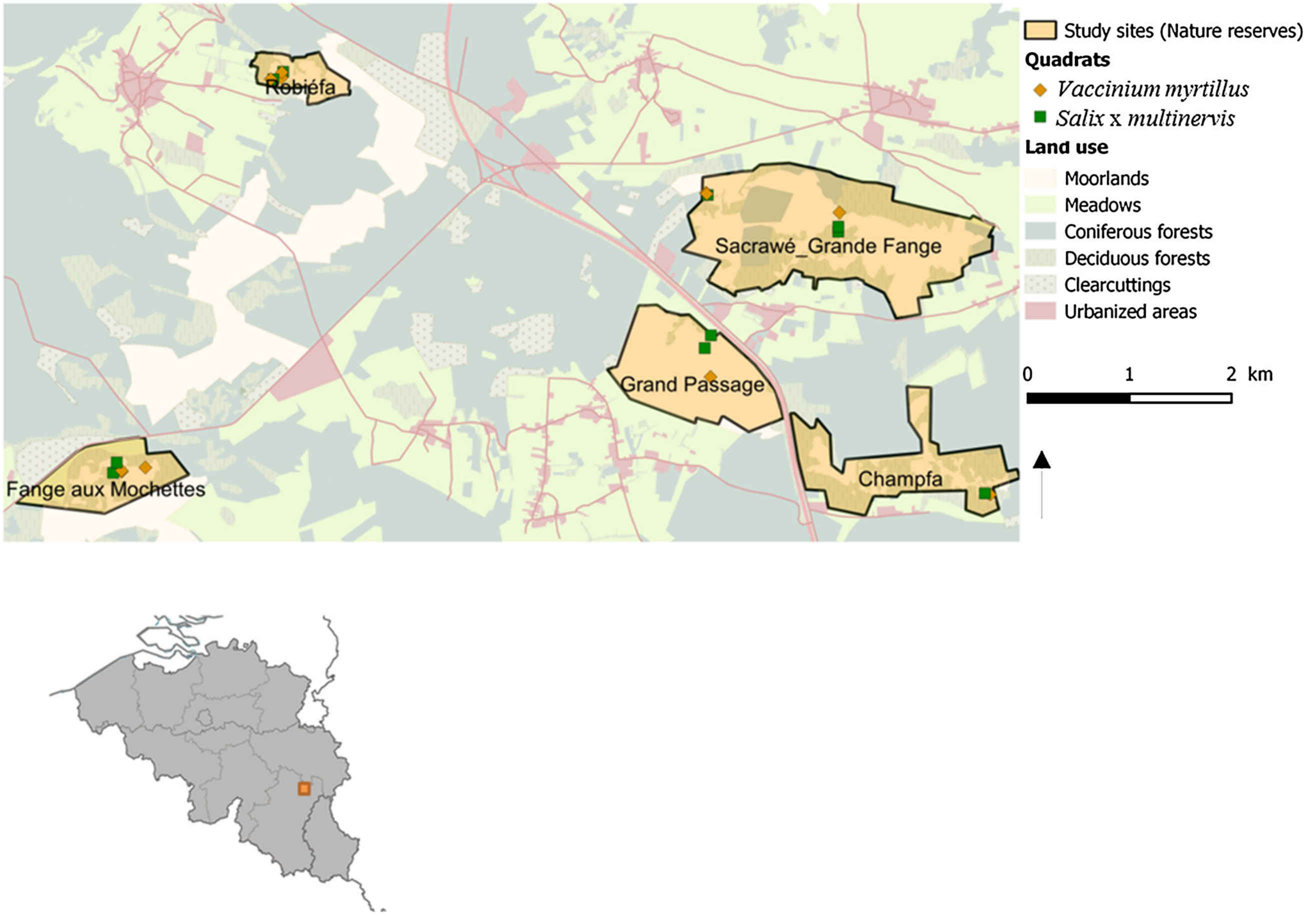

Fig. 1 Location of study sites in the Upper Ardenne, Belgium

to early May when both plant species were flowering. At this time, visitor abundances were studied alternately on the same study site and the same day on *S.* x *multinervis* and *V. myrtillus*. The second period was when *S.* x *multinervis* flowering period was over and only *V. myrtillus* visitors were recorded.

Flower visitors were recorded during one or two entire days (9.00 a.m.–6.00 p.m.) for both sampling periods and in each of the six sites. The number of flowers visited by insect foraging on *V. myrtillus* were recorded on a plot of 10 m^2 continuous shrub cover (>80 %) for 20 min every hour. Visitors were collected with an insect net, identified, and then released. The same protocol was used for insect visitors of *S.* x *multinervis* but on entire catkins. Male catkins offer about 170 flowers whereas female catkins bear about 60 flowers. Observations were performed on a section of a grove corresponding to 500 catkins on average. For bumblebees, determination in the field was made to operational taxonomic units (OTU; Terzo and Rasmont 2007, Table 5) that cluster similar species that are impossible to distinguish under field conditions. To evaluate species diversity within each OTU, we collected specimens at the end of the observations. Specimens were identified using determination keys (Verlinden 1994; Patiny and Terzo 2010; Rasmont and Terzo 2010) and a reference collection from the Université catholique de Louvain. Identifications were confirmed by several specialists (Pauly A., Patiny S., Rasmont P.).

Pollen loads

Distinction between pollen and nectar foraging on individual flowers is quite difficult in the field (Nagamitsu et al. 2000). We thus estimated pollen-host use and fidelity from pollen loads. We sampled pollen loads in the six study sites from bumblebee individuals visiting *V. myrtillus* (56 in 2013 and 12 in 2014). Only nine pollen loads were collected from bumblebees caught on *S.* x *multinervis* in 2014 as the shrubs were dense and bumblebees difficult to access. After the bees had been immobilized in a bee marking cage, one of the two pollen loads was carefully removed using a toothpick. In the lab, pollen loads were acetolyzed (Erdtman 1960, modified) and pollen grains were identified under light microscopy (Leitz Wetzlar) at a magnification of 450x. Pollen identification was based on a reference collection from the Université catholique de Louvain, an identification key (Reille 1992) and a comprehensive list of blooming plants in the study sites. At least 400 randomly chosen pollen grains of each of the 75 pollen loads were identified and counted.

Pollen composition

Pollen collection

Flowers of *V. myrtillus* and *S.* x *multinervis* were collected during the flowering period. For *V. myrtillus,* pollen needs to be extracted by vibrating the flowers due to poricidial anthers. We built small vibrators out of a 5 mm long rod, slightly inclined and attached to a small handle. Rod rotations could be modulated to produce vibrations between 200 and 800 Hz. Pollen from several individuals and study sites was pooled and stored at −20 °C before chemical analysis.

Polypeptide analyses

The polypeptide content (molecular weight >10,000 Da) was quantified from 5 mg dry pollen for each species in triplicate, following the method described by Vanderplanck et al. (2014a). The quantification of total polypeptide content was performed using the standard curve of the BCA Protein Assay Kit (Pierce, Thermo Scientific) at the University of Mons (Mons, Belgium).

Amino acid analyses

The amino acid content was quantified from 3 mg dry pollen in triplicate for each species following the method described in Vanderplanck et al. (2014a). Total amino acids were measured separately with an ion exchange chromatograph and post-column ninhydrin derivatisation (Biochrom 20 plus amino acid analyser) at the University of Liège (Gembloux, Belgium).

Essential amino acids for solitary bees and bumblebees were considered to be the same as those identified for honeybees (i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine, De Groot 1953).

Nectar sampling and chemical analyses

We sampled nectar from male and female catkins of *S.* x *multinervis* as well as *V. myrtillus* flowers at their peak of flowering between early April and late May 2014. On every visitor observation day with good weather conditions (no rain for 24 h), nectar was collected on several bushes from freshly opened flowers with glass capillary tubes of 0.5 or 1 µL (Hirschmann® Laborgeräte, Eberstadt, Germany). The nectar volume was estimated by measuring the length of the nectar column in the capillary tube. In the laboratory, nectar contents were pooled and stored at −80 °C until analyses of their sugar concentration and composition. We needed at least 0.5 mg of nectar for analyses. Sugar composition was determined by gas chromatography, with a Perkin-Elmer Autosystem XL equipped with a split injector (1/20) and helium as the carrier gas (flow of 1 mL/min). The injector and detector temperatures were maintained at 250 and 350 °C, respectively. Sugar (i.e. sucrose, glucose and fructose) analyses for nectar composition were performed in the Centre Apicole de Recherche et d'Information (CARI asbl, Louvain-la-Neuve, Belgium).

Statistical analyses

All analyses were conducted in R (R Development Core Team 2013) and if not indicated otherwise, data are presented as mean ± standard deviation. With only one individual observed, *B. hypnorum* L. was removed from the analyses. Data for pollen collection were also removed for the *B. lapidarius* OTU due to an insufficient sample size.

To determine whether visitor and bumblebee species proportions differed between plant species and between the first and the second flowering periods, we performed Chi-square tests. When conditions for Chi-square were not fulfilled, we used Fisher exact tests. Since the proportions of bumblebee OTUs on the female and the male catkins of *S.* x *multinervis* were similar (Fisher exact; $p = 0.6$), observations of both types of catkins were pooled.

We performed binomial GLMs to determine whether proportions of bumblebees collecting pollen were significantly different among plant and bumblebee species.

Poisson GLMs were performed to test whether the proportion of *Vaccinium* sp. and *Salix* sp. pollen grains and pollen diversity in pollen loads differed among *Bombus* OTUs. Only pollen loads from bumblebees caught on *V. myrtillus* flowers could be analyzed due to the low sample numbers from *S.* x *multinervis*.

We further used Gaussian GLM to compare chemical contents of pollen and nectar between the two plant species.

Results

Visitor abundance and diversity

We recorded 637 insect visitors during *S.* x *multinervis* observations and 136 and 116 visitors during the two periods on *V. myrtillus*. On average, 5.8 ± 5.4 visitors per 20 min session were observed on *S.* x *multinervis* whatever the catkin sex, corresponding to 0.011 visit/hour/catkin in total. On *V. myrtillus,* 1.6 ± 1.3 visitors were observed per 20 min session per flower, resulting in 0.007 visit/hour/flower during the first period, overlapping with *S.* x *multinervis* flowering. After *S.* x *multinervis* flowering had ceased, more visitors were observed on *V. myrtillus* with 3.1 ± 2.0 visitors per flower and per session or 0.017 visit/hour/flower.

We observed 29 different visitor species on *S.* x *multinervis*. Main visitors belonged to the families of Syrphidae (Diptera, 69 %) and Apidae (Hymenoptera, 22 %, Fig. 2a). Syrphidae were mainly represented by the genus *Eristalis* (93 %), especially *E. pertinax* Scopoli. Among Apidae, the genus *Bombus* was predominant (86 %), followed by *Apis* individuals (12 %). Other flower visitors were solitary bees (7 %, especially *Andrena haemorrhoa* Fabricius, *A. clarkella* Kirby and *A. praecox* Scopoli, Hymenoptera, Andrenidae), and other Diptera (1 %, Empididae and Calliphoridae).

We observed 16 different visitor species on *V. myrtillus*. The proportions of the different families of visitors did not differ between the first and the second periods of observation (Fisher exact; $p = 0.481$). Apidae dominated the visitor guild with 88 % of the floral visits (Fig. 2b, c) with mainly *Bombus* individuals (96 %) followed by *Apis* individuals (4 %). Other flower visitors included solitary bees (4 %) belonging to the families of Andrenidae (*Andrena apicata* Smith and *A. haemorrhoa* Fabricius) and *Lasioglossum* spp. (Halictidae), Vespidae (4 %) and Syrphidae (3 %).

Within bumblebee visitors, six different OTUs were observed visiting both *S.* x *multinervis* and *V. myrtillus* flowers. Identification of *B. hortorum* OTU individuals revealed that exclusively *B. jonellus* individuals were present (all 18 specimens caught). The majority (74 %) of the observed bumblebees were queens. Workers were only observed for *B. jonellus* and *B. pratorum* with 39 and 55 % of the visitors, respectively.

The proportion of the five bumblebee OTUs was significantly different between *S.* x *multinervis* and *V. myrtillus* visitors for the same days of observation (Fisher exact; $p < 0.005$; Fig. 3). The main bumblebee visitors on *S.* x *multinervis* belonged to the *B. terrestris* OTU (81 %) while the main visitors on *V. myrtillus* during the co-flowering period were *B. pascuorum* Scopoli (37 %) and *B. pratorum* L. (38 %). At the end of the *S.* x *multinervis* flowering period, the proportion of the *B. terrestris* OTU visiting *V. myrtillus* decreased from 20 to 3 %, the proportion of *B. pascuorum* also decreased whereas the proportion of *B. pratorum* increased (80 %, Fisher exact, $p < 0.005$). The proportion of *B. jonellus* remained quite stable.

Bumblebees collecting pollen

During the co-flowering period, a higher proportion of the bumblebee visitors collected pollen on *S.* x *multinervis* than on *V. myrtillus* (35.2 vs. 11.1 % respectively). Only *B. jonellus* individuals collected pollen on *V. myrtillus*. After the flowering period of *S.* x *multinervis*, the proportion of bumblebees collecting pollen on *V. myrtillus* significantly increased (GLM: $X^2_2 = 9.9; p = 0.007$) and reached 24.7 % (Fig. 4).

Pollen loads

Pollen collected by bumblebees caught on *V. myrtillus* largely originated from *Vaccinium* flowers (59.0 % of total pollen, Table 2). The number of plant taxa per pollen load ranged from 1 to 4 species (1.6 ± 0.8 species) and did not differ significantly among bumblebee species (GLM: $X^2_4 = 2.3$; $p = 0.66$). A mean of 72.1 % of pollen loads were monospecific (>95 % conspecific pollen grains), 69.4 % of them contained only pollen of *Vaccinium* and 20.4 % of them only contained pollen of *Salix*. Other resources collected by visitors caught on *V. myrtillus* were *Sambucus racemosa* L. (11 %), *Vicia* sp. (3 %), *Malus sylvestris* L. (3 %), *Cytisus scoparius* L. (1 %) and *Acer* sp. (0.5 %). *Salix* pollen in pollen loads averaged 21.9 %. The average proportion of *Vaccinium* pollen in pollen loads

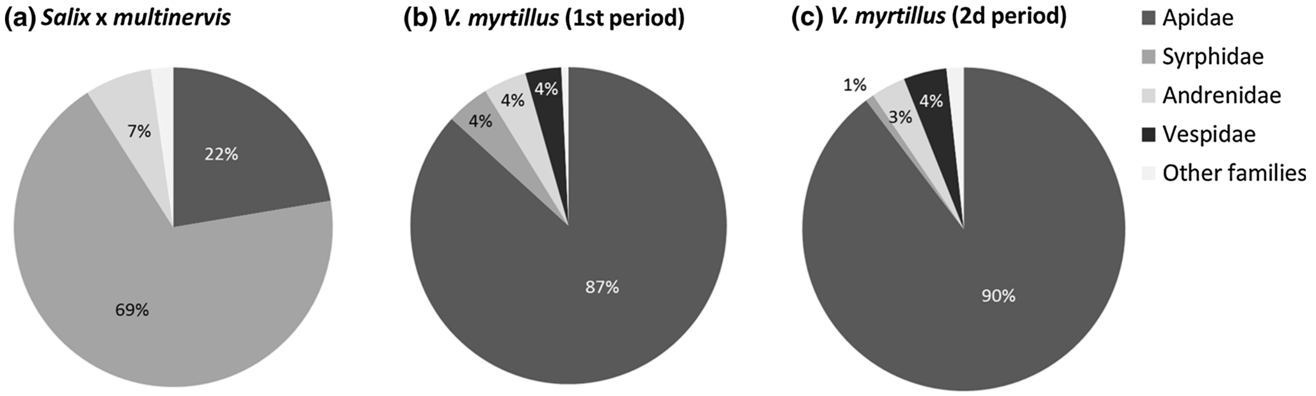

Fig. 2 Visitor proportions of **a** *Salix* x *multinervis* and **b** *V. myrtillus* during the first period (from early April to early May) and **c** *V. myrtillus* during the second period (mid-May) recorded in six sites in the Upper Ardenne, Belgium. "Other families" refers to taxa <2 % of individuals collected

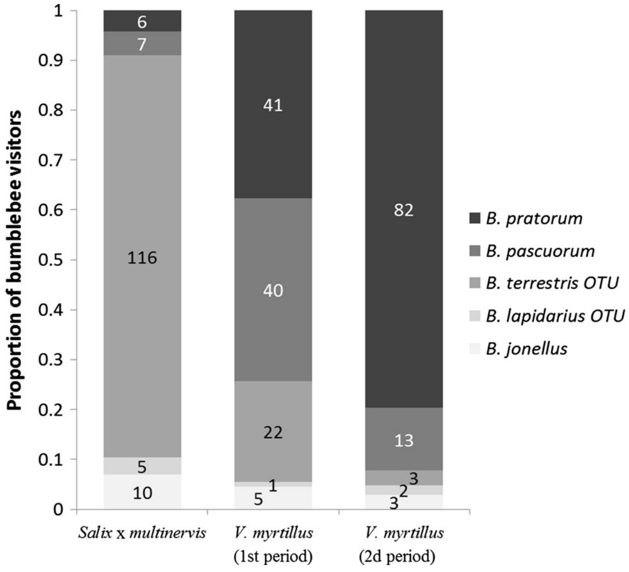

Fig. 3 Bumblebee visitors of *S.* x *multinervis* and of *V. myrtillus* during the first period (from early April to early May) and the second period (mid-May) recorded in six sites in the Upper Ardenne, Belgium. Figures in brackets indicated the numbers of individuals observed. *OTU* operational taxonomic units

varied between 44.9 % for *B. pascuorum* and 78.1 % for *B. jonellus*.

For the nine pollen loads collected by bumblebee visitors caught on *S.* x *multinervis*, 8 of 9 loads contained pure pollen of *Salix*.

Nectar volume and composition

Vaccinium myrtillus produced more nectar per flower (0.77 ± 1.38 µl) than female catkins of *S.* x *multinervis* (0.10 ± 0.13 µl; GLM: $t_2 = 4.0$; $p < 0.005$). Sugar concentration of the nectar of *V. myrtillus* flowers was significantly lower than both female and male catkins of *S.* x *multinervis* (GLM: $t_2 = 5.4$; $p < 0.005$ and $t_2 = 2.7$; $p = 0.04$, respectively). Nectar from male catkins of *S.* x *multinervis* and *V. myrtillus* flowers were sucrose dominant whereas nectar from female catkins of *S.* x *multinervis* was hexose rich. Nectar of female catkins offered the highest concentrations in both glucose and fructose (GLM: $F_2 = 23.4$, $p < 0.005$ and $F_2 = 16.0$, $p < 0.005$ respectively; Table 3).

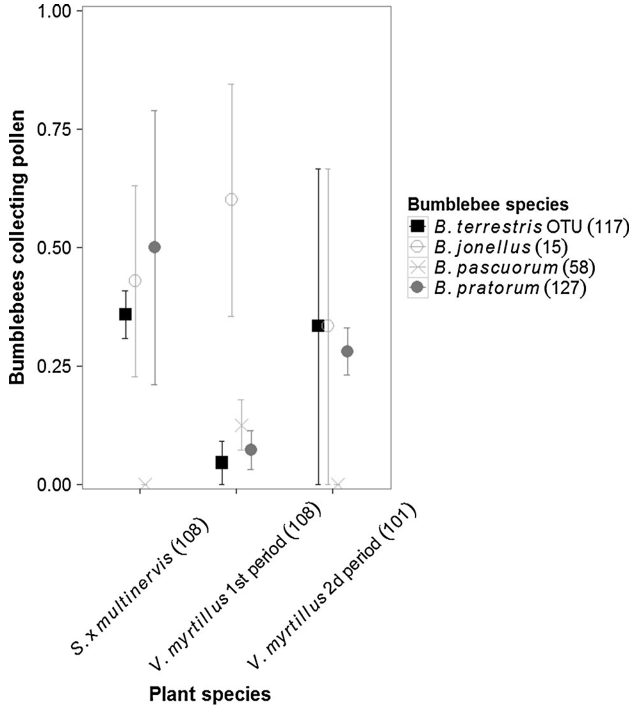

Fig. 4 Proportion of bumblebees collecting pollen on *S.* x *multinervis* and *V. myrtillus* during the first period (from early April to early May) and the second period (mid-May) caught in six sites in the Upper Ardenne, Belgium. *OTU* operational taxonomic units (see "Appendix" for species information). Data show mean ± standard error. Numbers of individuals are indicated in *brackets*

Table 2 Percentage (mean ± SD) of pollen grains of the different plant species present in pollen loads of bumblebee individuals caught on *S.* x *multinervis* and *V. myrtillus*

Plant species	Bumblebee species	*Salix* sp.	*Vaccinium* sp.	*Sambucus racemosa*	*Vicia* sp.	*Malus sylvestris*	*Cytisus scoparius*	*Acer* sp.	Other species
S. x *multinervis*	*B. lapidarius* OTU (2)	97.1 ± 4.2							2.9 ± 4.2
	B. terrestris OTU (7)	99.8 ± 0.1	0.1 ± 0.2						
V. myrtillus	*B. jonellus* (16)	8.6 ± 21.1	78.1 ± 36.8	12.9 ± 33.6		0.1 ± 0.2			0.3 ± 0.6
	B. lapidarius OTU (7)	14.3 ± 37.8	56.0 ± 52.0	0.2 ± 0.3		28.9 ± 48.0			0.6 ± 1.3
	B. terrestris OTU (11)	28.2 ± 45.4	43.9 ± 49.4	25.7 ± 43.7				1.7 ± 4.9	0.4 ± 0.6
	B. pascuorum (19)	28.7 ± 42.8	44.9 ± 43.7	14.6 ± 34.4	10.1 ± 25.1	0.2 ± 0.6	0.1 ± 0.2	0.8 ± 2.9	0.7 ± 1.5
	B. pratorum (15)	26.7 ± 42.4	68.1 ± 43.4	0.1 ± 0.2			4.6 ± 18.0		0.5 ± 1.1

Numbers of pollen loads are indicated in brackets. Other species correspond to species represented by less than 0.1 % of total pollen grains

OTU operational taxonomic units (see Table 5)

Polypeptide and total amino acid contents

Salix x *multinervis* pollen contained significantly higher amounts of polypeptides than *V. myrtillus* pollen (60.0 ± 5.9 vs. 18.9 ± 7.0 µg/mg; GLM: $F_1 = 71.7$, $p < 0.005$, Table 4). However, *V. myrtillus* pollen contained more amino acids than *S.* x *multinervis* (324.1 ± 4.4 vs. 284.2 ± 12.5 µg/g; GLM: $F_1 = 27.3$, $p = 0.006$) while the relative proportion of essential amino acids was significantly lower (48.9 ± 0.16 vs. 52.2 ± 0.6 %; GLM: $F_1 = 116.0$; $p < 0.005$).

Discussion

Which species are the floral visitors in wet heathlands in early spring?

The floral resources of S. x multinervis attracted a diverse range of insect species (29 different species). Unlike other *Salix* species (Füssel 2007; O'Rourke et al. 2014), Syrphidae (Diptera, 69 %) were the dominant visitors during our observations. Bumblebees represented 22 % of visitors caught on *S.* x *multinervis* and consisted mainly of individuals of the B. terrestris OTU. Visitors also included solitary bees, especially *Andrena* species (7 %). Among them, *A. praecox* and *A. clarkella* are oligolectic, and collect pollen exclusively on *Salix* (Moroń et al. 2008).

Similar to other studies on *Vaccinium* species (Jacquemart 1993; MacKenzie and Eickwort 1996; Javorek et al. 2002; Mayer et al. 2012) bumblebees were the main visitors of *V. myrtillus*. *Andrena* species were also caught on *V. myrtillus* flowers. *Bombus* and *Andrena* are able to buzz-pollinate (Buchmann 1983; Javorek et al. 2002), which explains their high abundance on *Vaccinium* flowers.

Different bumblebee species visited the two plant species: *V. myrtillus* was mainly visited by *B. pascuorum* and *B. pratorum* whilst the *B. terrestris* OTU was particularly associated with *S.* x *multinervis* catkins. This could be the result of resource partitioning, a mechanism facilitating species coexistence through resource exploitation at different times, or resource use in different locations (Morse 1977; Inouye 1978; Westphal et al. 2006). Body size may determine the ability of a bee individual to manipulate flowers (Harder 1985) and may explain the suspected resource partitioning. Queens of the *B. terrestris* OTU are quite large with an individual length >20 mm whereas queens of *B. pascuorum* and *B. pratorum* are considered smaller being 17 and 16 mm in length, respectively (Benton 2006). A smaller body size seems to be an advantage while manipulating and buzzing the flowers of *V. myrtillus* (4–6 mm long and 5–7 mm broad, Ritchie 1956).

Table 3 Fructose, glucose and sucrose content (g/100 g; mean ± SD) and quantity of nectar per flower or catkin (µl; mean ± SD)

	Glucose (g/100 g)	Fructose (g/100 g)	Sucrose (g/100 g)	Quantity (µl)
S. x *multinervis* ♂	7.2 ± 3.7	8.8 ± 4.8	22.3 ± 9.1	0.14 ± 0.13
S. x *multinervis* ♀	26.1 ± 7.2	27.5 ± 8.9	9.2 ± 9.4	0.10 ± 0.18
V. myrtillus	0.7 ± 0.6	1.1 ± 0.5	13.2 ± 0.8	0.77 ± 1.38

♂ correspond to male catkins and ♀ to female catkins. N = 3 replicates for nectar ratio and composition. N > 40 floral units for nectar quantities (all sites pooled)

Table 4 Polypeptide and total amino acid contents of pollen expressed as a percentage of dry weight (mean ± SD) and essential amino acid proportion expressed as a percentage of total amino acid content (mean ± SD)

	Polypeptide content (µg/mg)	Total amino acid content (µg/mg)	Essential amino acids (%)
S. x multinervis	60.0 ± 5.9	284.2 ± 12.5	52.5 ± 0.56
V. myrtillus	18.9 ± 7.0	324.1 ± 4.4	48.9 ± 0.16

N = 3 replicates

The majority of observed individuals were queens. Since they collected pollen, we can suppose that colony foundation was underway. Workers were only observed for *B. pratorum* and *B. jonellus*, their colonies could have reached the ergonomic phase. These two bumblebees species are known to be early emerging species (Prŷs-Jones and Corbet 1987; Goulson et al. 2005).

Did the plant species present valuable resources of nectar?

On *S.* x *multinervis*, the quantity of nectar was quite low per male or female catkin (0.14 and 0.10 µl per catkin respectively, i.e. ~0.0010 and ~0.0016 µl per flower). At the same time, nectar was highly concentrated and total sugar concentration averaged 38.3 % for male and 62.8 % for female catkins. These results are congruent with other studies (Elmqvist et al. 1988; Füssel 2007), which supposed that female flowers compensate for the lack of pollen with a higher concentration of sugars in nectar than males. Sugar ratios also differed between flower sexes, with nectar of male flowers being sucrose-dominant, whereas the nectar of female flowers was hexose-dominant. Sexual differences in nectar composition have been observed in other species such as *Salix myrsinifolia* x *phylicifolia* or *Salix caprea* (Katoh et al. 1985; Elmqvist et al. 1988; Dötterl et al. 2014). The consequences of such differences in sugar proportions for pollinator foraging remain to be elucidated.

In contrast, nectar quantities per *V. myrtillus* flower were relatively high (0.77 µl on average) and could contribute to the attractiveness of the flowers. The amounts observed here were low compared to other studies on *V. myrtillus* (Jacquemart 1992), but can be explained by dry weather conditions during early spring 2014. Nectar of *V. myrtillus* is sucrose dominant. Some studies assume that sucrose dominant nectar is associated with long tongued bees, like bumblebees (Percival 1961; Baker and Baker 1983, 1990; Petanidou 2005).

Our results showed that nectar composition and quantity differed between the two plant species. Flowers of *V. myrtillus* offered higher nectar quantities than those of *S.* x *multinervis* catkins, irrespective of their sex. To collect the same nectar quantity, insects need to forage on several hundreds of flowers on *S.* x *multinervis* for only one *V. myrtillus* flower. However, sugar concentration of *V. myrtillus* nectar was lower than that of *S.* x *multinervis* nectar. Our results corresponded with others demonstrating the importance of nectar quantity for pollinator choice (Waddington and Gottlieb 1990). Other studies showed that concentration and composition are the main parameters influencing pollinator choices (Baker and Baker 1983; Cnaani et al. 2006). These trade-offs between handling cost, nectar volume and nectar concentration still need to be studied.

Did the species offer valuable resources of pollen?

The chemical composition of pollen differed significantly between the two plant species. *Salix* x *multinervis* offered pollen with higher polypeptide and essential amino acid contents than *V. myrtillus.* The composition of *S.* x *multinervis* pollen might be attractive for insect visitors. The pollen contained 60.0 ± 5.9 µg/mg of polypeptides and 284.2 ± 12.5 µg/g of amino acids (free and bound-protein amino acids). Moreover, 52.5 % of amino acids were essential for insect development. Similar results were found for *Salix caprea* pollen where polypeptide content averaged 71.5 ± 0.84 µg/mg and amino acids content reached 217.2 ± 11.2 µg/mg (Vanderplanck et al. 2014a). In contrast, the pollen of *V. myrtillus* flowers was not particularly rich in polypeptides as it contained only 18.9 ± 7.0 µg/mg of polypeptides, 324.1 ± 4.4 µg/g of amino acids, of which 48.9 % are considered essential. Polypeptide content was even lower than in *Calluna vulgaris* pollen, another Ericaceae species considered as poor pollen resource due to the low polypeptide content that induced a low performance for colony development (Tasei and Aupinel 2008; Vanderplanck et al. 2014a). These results are different to other studies that predict high quality of pollen for flowers with poricidial anthers (Roulston and Cane 2000; De Luca and Vallejo-Marín 2013).

Moreover, pollen quantity and availability were different between *V. myrtillus* and *S.* x *multinervis*. In *S.* x *multinervis* catkins, pollen is easily accessible and is present in high quantity. Indeed, *S.* x *multinervis* presents open and accessible tiny flowers with approximately 170 flowers in male catkins and 60 flowers in female catkins. Furthermore, in *Salix* species, each male flower contains a high amount of pollen (from 1700 to 5000 pollen grains per anther depending on the species, Peeters and Totland 1999). In contrast, flowers of *V. myrtillus* only offer on average 1300 pollen tetrads that require buzzing (Jacquemart 2003).

What governs bumblebee choices for pollen resources?

These differences in pollen quantity and quality were reflected in visitor choice. With the exception of *B. jonellus*, pollinators seemed to favor the high quality and easily accessible pollen of *S.* x *multinervis* and they visited *V. myrtillus* almost exclusively for nectar resources. Only when *S.* x *multinervis* had ceased flowering, bumblebees switched to pollen collection from *V. myrtillus* and their proportion increased to 29.4 %. Our results are consistent with other studies suggesting that bees are able to detect and select pollen according to its chemical composition (Cook et al. 2003; Hanley et al. 2008; O'Rourke et al. 2014).

Fidelity to pollen resources differed among bumblebee species caught on *V. myrtillus*. *Bombus jonellus* collected *Vaccinium* pollen in higher proportions (78.1 %) than the polylectic *B. pascuorum* (44.9 %) for example. This result can be explained by the relative specialization of *B. jonellus* for ericaceous species compared with the highly polylectic species *B. pascuorum* (Goulson et al. 2005). But, contrary to other studies (Carvell et al. 2006; Somme et al. 2014), we did not find any differences among bumblebee species concerning the proportion of pure pollen loads or the number of plant taxa in individual pollen loads. This is probably owed to the restricted resources in early spring, which limits the possibility for bees to enlarge their diet.

Bumblebee individuals sampled on catkins of *S.* x *multinervis* were extremely constant during pollen collection (94 % conspecific pollen). The bumblebee visitors caught on *V. myrtillus* were relatively less constant (59.0 % conspecific pollen). Due to the poor quality of the pollen, bumblebee visitors of *V. myrtillus* could supplement their diet with other species like other *Salix* species (Salicaceae), *Sambucus racemosa* (Adoxaceae), *Vicia* sp. (Fabaceae), *Malus sylvestris* (Rosaceae) as shown in other studies (Eckhardt et al. 2013). The majority of the other plant species identified in pollen loads were not present within our study sites but were encountered in the surroundings. These results reflect the importance of a landscape matrix of floral resources for pollinators and thus, for pollinator conservation.

Conclusion

In Belgian wet heathlands, *S.* x *multinervis* and *V. myrtillus* provided different but important resources for insects during emergence and development in early spring. These two species were complementary in terms of visitors and of floral resources. *Salix* x *multinervis* mainly acted as a pollen resource while *V. myrtillus* offered valuable resources of nectar. Future heathland management needs to consider the maintenance of both plant species for the survival of the pollination ecosystem services throughout the entire flowering season. These flowering species seem crucial for pollinator conservation even at a landscape level.

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Appendix

See Table 5.

Table 5 Bumblebee OTU composition adapted for Belgium (operational taxonomic units, from Terzo and Rasmont 2007)

OTU	Species
B. hortorum OTU	*B. barbutellus* Kirby
	B. hortorum L.
	B. jonellus Kirby
	B. ruderatus Fabricius
B. hypnorum OTU	*B. hypnorum* L.
B. lapidarius OTU	*B. lapidarius* L.
	B. rupestris Fabricius
	B. soroeensis Fabricius
	B. wurfleinii Radoszkowski
B. pascuorum OTU	*B. humilis* Illiger
	B. pascuorum Scopoli
B. pratorum OTU	*B. pratorum* L.
B. terrestris OTU	*B. cryptarum* Fabricius
	B. lucorum L.
	B. magnus Vogt
	B. terrestris L.

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