

Nectar foragers contribute to the pollination of buzz-pollinated plant species¹

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PREMISE OF THE STUDY: Pollination performance may depend on the type of floral resource (pollen or nectar) foraged by visitors. In buzz-pollinated plant species, the poricidal anthers release pollen during active pollen collection that induces flower vibrations. These buzz-pollinated species generally do not produce nectar. Nevertheless, several Ericaceae are buzz-pollinated and produce nectar. We estimated the relative effectiveness of visitors according to the type of resource collected, nectar or pollen (buzzing).

METHODS: We compared the relative performance of pollen removal, transport, and deposition (effectiveness) of the main insect visitors on four ericaceous species: three buzz-pollinated species with different pore sizes, *Erica tetralix*, *Vaccinium myrtillus*, and *V. vitis-idaea*; and one non-buzz-pollinated species, *Calluna vulgaris*.

KEY RESULTS: Bumblebees were the main pollinators for the three buzz-pollinated species, whereas hoverflies were the main pollinators for the non-buzz-pollinated generalist *C. vulgaris*. For the studied plant species, we observed no difference in pollination effectiveness among bumblebee species. Buzzing bumblebees were the most effective visitors for pollination per flower visit for the two *Vaccinium* species, whereas nectar foragers were the most effective visitors for pollination of *E. tetralix*. In the case of *Vaccinium myrtillus*, nectar foragers contributed the most to pollination success because they were more abundant than pollen foragers.

CONCLUSIONS: We showed that consideration of the resource collected by visitors and their behavior is necessary to compare their relative performance. The combination of visitation rate and effectiveness per visit reveals that nectar foragers make a substantial contribution to pollination of the buzz-pollinated ericaceous species.

KEY WORDS *Bombus*; Ericaceae; pollen deposition; pollen removal; pollinator behavior; pollinator effectiveness; poricidal anthers

Measuring visitor pollination performance has become increasingly important in conservation, for understanding the effects of pollinator declines on wild plant population survival; and in agriculture, for maintaining crop production (King et al., 2013; Ballantyne et al., 2015). The term *pollination* refers to the transfer of pollen from the anthers to the stigmas, which occurs in three stages: (1) pollen removal from the anthers, (2) pollen transport on an insect body, and (3) pollen deposition onto the stigmas. Thus, a visitor is considered a pollinator only when it contacts the reproductive organs and deposits conspecific, viable pollen on

receptive stigmas (Inouye et al., 1994; Wilcock and Neiland, 2002; Ne'eman et al., 2010). Visitors that extract floral resources during illegitimate visits without contacting the reproductive organs are considered robbers (Maloof and Inouye, 2000). Among pollinators, species often differ in their contributions to pollination, either in their relative frequencies of visitation or in their performance in pollen transfer and deposition (Javorek et al., 2002; Sahli and Conner, 2007; King et al., 2013). Several studies have compared visitor performances among insect taxa (Conner et al., 1995; Javorek et al., 2002; Larsson, 2005; Rader et al., 2009), but comparisons of performance among insect behaviors or types of resources collected remain rare (Thomson and Goodell, 2001; Ballantyne et al., 2015).

Two types of resources are collected by flower visitors: nectar and pollen. These resources are located in different parts of the flower. Therefore, insects have different postures, behaviors, and body movements for gathering the different resource types.

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These differences may also lead to differences in pollen removal, deposition, or the likelihood of contact with the reproductive organs (Shivanna et al., 2005). The first type of resource, nectar, consists of a blend of sugars and serves mainly to attract visitors (De la Barrera and Nobel, 2004; Heil, 2011; Escalante-Pérez and Heil, 2012). Nectar represents a major energy source for many flower visitors, which often preferentially visit plants that produce large quantities of nectar per flower (Klinkhamer and de Jong, 1993; Mitchell, 1993; Cnaani et al., 2006). The presence of nectar clearly enhances benefits to plants by increasing pollination rates (Neiland and Wilcock, 1998). In exchange for the nectar reward, “legitimate” visitors passively accumulate pollen on their body and participate in pollination (Brian, 1951). Nectar may be unconcealed or hidden in a deep corolla tube. In the latter case, visitors have to enter deeper into the flower and thus attain a more precise position that favors contact with anthers or stigmas (Thomson and Plowright, 1980; Westerkamp, 1996). Moreover, some visitors actively collect the pollen reward. Pollen consists mostly of proteins and lipids (Roulston and Cane, 2000) and represents the major nutrient source for egg production by females and for larval development in bees (Haslett, 1989; Woodcock et al., 2014). As bees collect pollen for larvae, they regularly groom during pollen transfer to agglomerate pollen on specific transport structures (i.e., scopa or corbicula; Thomson, 1986; Buchmann and Cane, 1989; Parker et al., 2015). These packed pollen grains are no longer available for pollination and thus constitute a net loss in pollination effectiveness.

To increase pollination performance and limit pollen loss, some plant species have developed mechanisms for “pollen dispensing” (Harder and Thomson, 1989; Harder, 1990; Castellanos et al., 2006), distributing pollen among visitors by limiting the quantity of pollen removed per visit. For example, poricidal anthers release pollen in response to vibrations at a particular frequency (Buchmann and Cane, 1989; Harder and Barclay, 1994). The capacity to generate vibrations for buzz pollination is principally found in bee species from seven families and >50 genera, like *Bombus* and *Andrena*, whereas, for example, *Apis mellifera* cannot buzz (De Luca and Vallejo-Marín, 2013). Poricidal anthers may also favor pollen deposition onto specific areas of the visitor’s body, parts that the bee is less likely to groom or, more likely, parts that contact the stigmas (Harder and Barclay, 1994; Vallejo-Marín et al., 2010; De Luca and Vallejo-Marín, 2013). In some buzz-pollinated taxa, only buzzing visitors can achieve effective pollination, which suggests that buzzing is not only an adaptation for pollen release, but also for pollen collection and deposition onto stigmas (Arceo-Gómez et al., 2011).

Buzz-pollinated flowers have evolved independently several times, occurring in species from 65 families (Buchmann, 1983; De Luca and Vallejo-Marín, 2013). Most buzz-pollinated plant taxa lack nectar, including *Solanum* spp. (Solanaceae) and species belonging to the Myrtaceae (Dukas and Dafni, 1990; Proença, 1992; De Luca and Vallejo-Marín, 2013). Several studies have demonstrated a loss of nectar secretion concomitant with the evolution of buzz-pollination in plant families such as Boraginaceae and Violaceae (Vogel, 1978; Dukas and Dafni, 1990; Knudsen and Olesen, 1993; Freitas and Sazima, 2003). Nectar secretion represents a cost for the plant. Plants are expected to allocate energy to nectar secretion only if it yields sufficient benefits, in terms of reproductive success, to offset the production costs (Pyke, 1991; Mitchell, 1993). When only pollen foragers are effective for pollination

(Dukas and Dafni, 1990; Javorek et al., 2002), nectar secretion has been considered to provide no benefits to the plant. Nevertheless, nectar secretion persists in some plant families. Vallejo-Marín et al. (2010) reported nectar secretion in all species belonging to three poricidal plant families (out of 14 studied families). For example, Ericaceae have poricidal anthers and produce large amounts of nectar (Jacquemart, 1992; Moquet et al., 2017). Other studies of the pollination effectiveness for buzz-pollinated flowers have only examined pollen collection in nectarless flowers (Proença, 1992; Larson and Barrett, 1999; Kawai and Kudo, 2009; De Luca and Vallejo-Marín, 2013). The role of nectar secretion in buzz-pollination systems was never tested previously, and no studies have evaluated pollination effectiveness with respect to the floral resource chosen by individual visitors for buzz-pollinated plant species.

We compared pollination effectiveness and the importance of different types of insect visitors on four ericaceous plant species. As proposed by several authors (Mayfield et al., 2001; Kawai and Kudo, 2009; Neëman et al., 2010), we estimated the relative “effectiveness” as the number of conspecific pollen grains deposited on a virgin stigma in a single visit. This measure avoids the effects of postpollination factors, like resource allocation, which can interfere with seed- or fruit-set measurements (Neëman et al., 2010). To summarize all these contributions to the pollination success of a particular plant species, the term *pollinator importance* refers to the combination of pollination effectiveness and the relative visitation rates of the different visitor species (Lindsey, 1984; Olsen, 1996; Ballantyne et al., 2015).

The four ericaceous species we studied are *Calluna vulgaris*, *Erica tetralix*, *Vaccinium myrtillus*, and *V. vitis-idaea*. These species differ in anther morphology and present a gradient of pollen accessibility: the *Vaccinium* species have small circular and poricidal pores on the end of prominent tubes, *E. tetralix* has large oval pores and no anther tubes, and *C. vulgaris* has anthers with longitudinal split (Fig. 1). These ericaceous species are visited by several insect species. Bumblebees (Apidae, Hymenoptera) are the most abundant visitors of the two *Vaccinium* species and *E. tetralix* (Bannister, 1966; Jacquemart, 1993), including *Bombus jonellus*, a species particularly dependent on ericaceous species (Moquet et al., 2017). Oligolectic species—like *Andrena lapponica*, which collect pollen mainly from *Vaccinium* species—have also been observed (Chambers, 1968). The deep corollas limit nectar harvesting to long-tongued species, and the poricidal anthers limit pollen collection (Jacquemart, 1993; Mayer et al., 2012; Moquet et al., 2017). By contrast, the floral resources of *C. vulgaris* are more accessible than those of the other ericaceous species studied here. In consequence, a diverse guild of insect species, including hoverflies, visit this generalist plant species (Mahy et al., 1998; Dupont et al., 2011; Descamps et al., 2015).

We addressed the following three questions: (1) Which visitor species and behavior were the most effective for pollen collection? We hypothesized that oligolectic visitors like *A. lapponica* are able to collect pollen more efficiently on their host plant than polylectic species because of their behavioral adaptations to efficiently handle host plant flowers (Strickler, 1979; Laverty and Plowright, 1988; Larsson, 2005). (2) Which visitor species and behavior were the most effective for pollen deposition? We predicted that nectar foragers would be less effective than buzzing individuals, even if they might contribute to pollination when

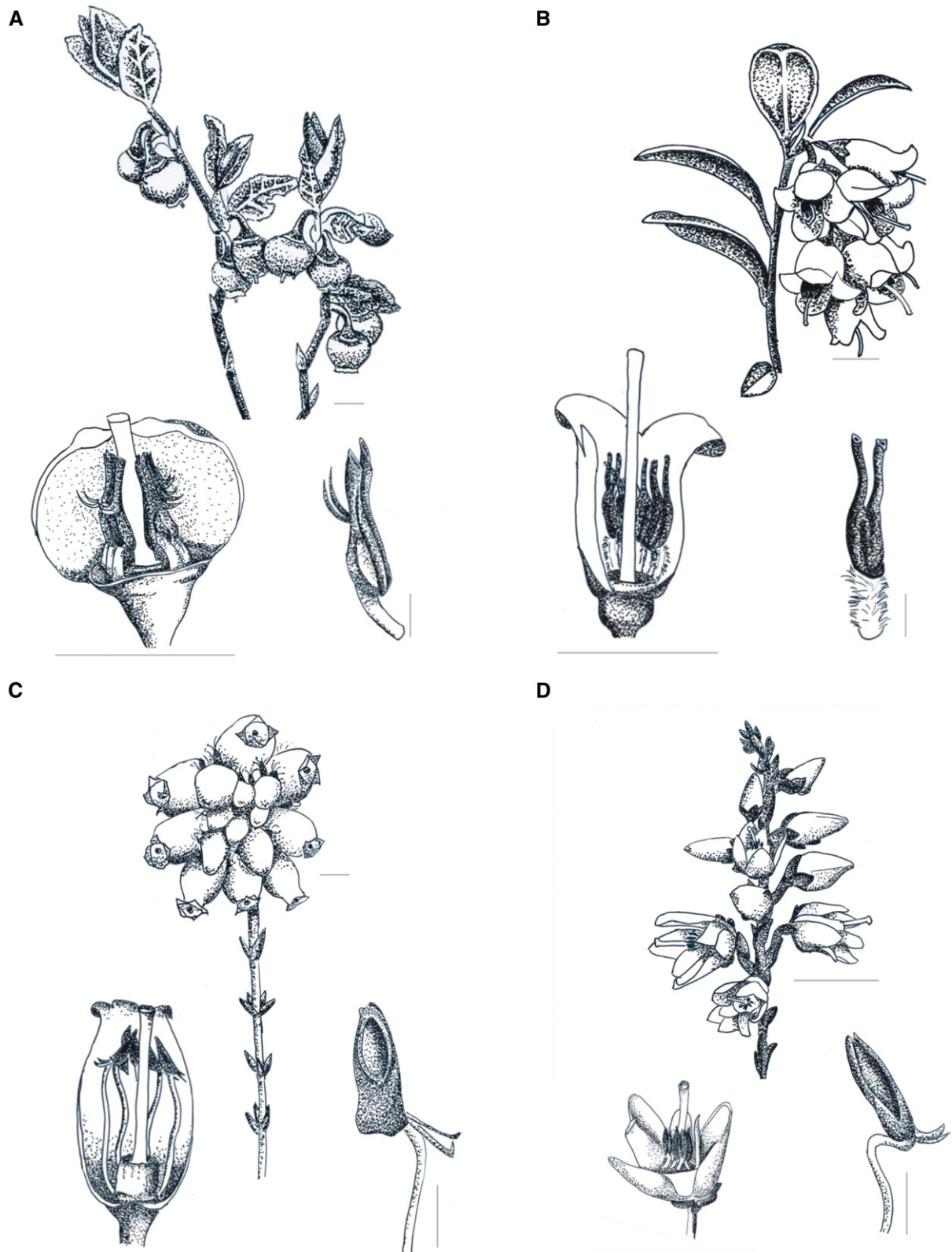


FIGURE 1 Inflorescences, flowers in longitudinal section, and anthers of (A) *Vaccinium myrtillus*, (B) *V. vitis-idaea*, (C) *Erica tetralix*, and (D) *Calluna vulgaris*. Horizontal scale bars = 5 mm. Vertical scale bars for anthers = 0.5 mm. (Drawing by L. Moquet.)

their visitation rates are high. (3) Did floral resource accessibility influence pollination effectiveness? We hypothesized that the small pores of *Vaccinium* limit pollen collection and pollination

to buzzing individuals. On the contrary, large apertures in anthers of *E. tetralix* and *C. vulgaris* allow pollination by nectar foragers.

MATERIALS AND METHODS

Study sites—Experiments were carried out in 10 heathland sites located at Plateau des Tailles in the Upper Ardenne, Belgium (50°10'N, 5°43'E). Eight sites were wet and dry heathlands and the other two, Fange aux Mochettes and Grand Passage, were ombrotrophic mires (i.e., raised bogs receiving all water supplies from precipitation). Heathland site size ranged from 0.3 to 40.7 ha.

Studied plant species—We studied visitor performance in the four most abundant ericaceous species in these heathlands—namely, in order of flowering, *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix*, and *Calluna vulgaris* (hereafter “target plant species”). These shrubby, entomophilous, ericaceous species share several floral traits. Their hermaphroditic flowers produce both nectar and pollen. Pollen grains are grouped in tetrads. Hereafter, pollen unit will be expressed in pollen grain number (tetrads multiplied by 4). *Erica tetralix* and the *Vaccinium* species have poricidal anthers and *C. vulgaris* has anthers with a longitudinal split (Table 1). All four species are self-compatible (Jacquemart and Thompson, 1996; Hermann and Palser, 2000; Jacquemart, 2003).

Vaccinium vitis-idaea (Fig. 1B) flowers in May and June. It is an evergreen shrub, 10–30 cm tall. Flowers are grouped in pendulous racemes of 2 to 12 flowers. The campanulate white corolla is 5–7 mm long (Ritchie, 1955). The 8 to 10 stamens have circular pores (0.2 mm in diameter) on the end of prominent tubes and contain about 46,800 ± 7280 pollen grains per flower. There are about 64 ± 5 ovules per ovary (Jacquemart, 2003).

Vaccinium myrtillus (Fig. 1A) flowers from mid-April to early June. It is a deciduous dwarf shrub, 10–70 cm tall. Flowers are single or in pairs. The globose pink corolla is 4–6 mm long (Ritchie, 1956). The 10 stamens have oval pores (0.4 mm long × 0.2 mm wide) on the end of prominent tubes and contain about 51,680 ± 11,600 pollen grains per flower. There are about 91 ± 14 ovules per ovary (Jacquemart, 2003).

Erica tetralix (Fig. 1C) flowers in July and August. It is an evergreen shrub, 15–80 cm tall. Flowers are grouped in terminal racemes of 9 to 12 flowers. The urceolate pink corolla is 6–7 mm long (Bannister, 1966). The eight stamens have oval pores (0.6 mm long × 0.2 mm wide) and contain approximately 12,320 ± 2680 pollen grains per flower. There are about 97 ± 11 ovules per ovary (Jacquemart, 2003).

Calluna vulgaris (Fig. 1D) flowers in August. It is an evergreen shrub, 15–80 cm tall. The flowers are grouped in racemes. The open pink flowers are 3–4 mm long (Gimingham, 1960). The eight stamens have longitudinal splits and contain about 15,120 ± 7280 pollen

grains per flower. There are about 31 ± 3 ovules per ovary (Jacquemart, 2003).

Visitation rates—In 2014 and 2015, insect visitors on each target plant species were observed during one to four entire days per site per year. Observations were performed on each target plant species in at least six of the 10 study sites, depending on the availability of flowering plant individuals. Observations were done using a standardized method, on quadrats of 10 m² (for *C. vulgaris*, quadrats were limited to 1 m² because of its high floral density) for 20 min and were repeated every hour between 0900 and 1820 hours (Mayer et al., 2011; Descamps et al., 2015; Moquet et al., 2015). Observation sessions were separated by 40 min. Flowers were observed for a total time of 280 h in 2 yr. When insects arrived in the quadrat, the number of flowers and inflorescences visited and visitor behavior were recorded. Three types of behavior were considered: (1) “legitimate non-buzzing,” when the insect inserted its head, or at least its proboscis, inside the corolla (bumblebees collected nectar during this behavior, but it was difficult to distinguish between pollen and nectar collection for other taxa, such as Syrphidae, other Diptera, solitary bees, etc.); (2) “legitimate buzzing,” when insect visitors made vibrations by contracting their thoracic muscles and only collected pollen; and (3) “robbing,” when insect visitors (bumblebees) pierced the corolla in the vicinity of the nectaries (corolla base) and extracted the nectar directly without any contact with the reproductive organs. Just before the end of the foraging bout, when insects left the quadrat, visitors were collected with an insect net. Insects were identified in the field to morphotypes and were released on the quadrat immediately after the 20 min session. We differentiated 10 morphotypes (Table 2): *Andrena* spp., *Apis mellifera*, *Bombus hortorum/jonellus*, *B. hypnorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, *B. terrestris* group (grouping together *B. cryptarum*, *B. lucorum*, *B. magnus*, *B. terrestris*), Syrphidae, and other Diptera (Bibionidae, Calliphoridae, Conopidae, Empididae, Muscidae, Sarcophagidae, Sepsidae, and Tachinidae). Morphotypes were defined according to (1) taxonomic grouping (bees vs. hoverflies vs. other Diptera) and (2) the preference for heathland habitat (*B. jonellus*). Some bumblebee species were grouped according to the color pattern (Terzo and Rasmont, 2007) because species discrimination in the field is not possible. Other visitors—such as Cetoniidae, Halictidae (*Lasioglossum* spp. and *Halictus* spp.), Ichneumonidae, Lepidoptera, *Nomada* spp., *Psithyrus*, and Vespidae—were rarely observed, and their visitation rates were too low to study their pollination effectiveness. On each day of observation, the floral density of the target plant species was estimated in the studied quadrat. This was done by counting the numbers of open flowers on four plots of 1 m² (except for *C. vulgaris*: because of its

TABLE 1. Floral traits of *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix*, and *Calluna vulgaris*.^a

Floral trait	<i>V. myrtillus</i>	<i>V. vitis-idaea</i>	<i>E. tetralix</i>	<i>C. vulgaris</i>
Flower diameter (mm)	5.6 ± 0.2	4.9 ± 0.3	3.5 ± 0.4	3.8 ± 0.5
Flower length (mm)	4.7 ± 0.2	5.0 ± 0.2	6.3 ± 0.3	3.3 ± 0.2
Ovules per flower	90.8 ± 13.5	64.1 ± 5.0	97.1 ± 10.6	31.2 ± 3.3
Pollen grains per flower	51,668 ± 11,608	46,792 ± 7272	12,332 ± 2680	15,128 ± 7288
Style exertion ^b (mm)	0.9 ± 0.2	1.9 ± 0.4	0.4 ± 0.06	0.4 ± 0.04
Anther dehiscence	Poricidal	Poricidal	Poricidal	Longitudinal
Pore morphology	Oval	Circular	Oval	NA
Pore length × width (mm)	0.4 × 0.2	0.2 × 0.2	0.6 × 0.2	NA

^a After Hermann and Palser (2000), Jacquemart (2003), and Stephens (2013).

^b Distance from the stigma to the end of the corolla (mean ± SE).

TABLE 2. Characteristics of insect morphotypes.

Morphotype	Pollen load	Proboscis length ^a (mm)	Insect size ^b (mm)	Buzz	Robbing	Ericaceous specialists
<i>Andrena</i> spp.	Scopa	2–7	8–11	Yes	No	Yes
<i>Apis mellifera</i>	Corbicula	5–7	12–15	No	Yes	No
<i>Bombus hortorum/jonellus</i>	Corbicula	14–16 (<i>B. hortorum</i>), 8–11 (<i>B. jonellus</i>)	9–14	Yes	No	Yes (<i>B. jonellus</i>)
<i>B. hypnorum</i>	Corbicula	8–10	8–18	Yes	No	No
<i>B. lapidarius</i>	Corbicula	10–12	12–16	Yes	No	No
<i>B. pascuorum</i>	Corbicula	12–13	9–15	Yes	No	No
<i>B. pratorum</i>	Corbicula	8–12	9–14	Yes	No	No
<i>B. terrestris</i> group	Corbicula	7–9	9–17	Yes	Yes	No
Syrphidae	None	2–10	5–18	No	No	No
Other Diptera	None	NA	NA	No	No	No

^a According to Knuth (1906), Gilbert (1981), Ranta and Lundberg (1981), and Szymank (1991).

^b According to Gilbert (1985) and Macek et al. (2010).

high flower density, flower numbers were assessed on plots of 0.25 m²). The visitation rate was calculated for each morphotype and each behavior by dividing the total number of visited flowers during the 10 sessions of 20 min by the number of open flowers and multiplying the result by 3 to approximate daily visitation rates (0900 to 1820 hours), assuming that visitations were similar between the 20 min of observations and the 40 min of nonobservation in each hour. Only female bees, which were more abundant on flowers, were studied to estimate visitation rates and pollinator performance (pollen removal, carrying, and deposition capacities) because male individuals do not collect pollen.

Pollen carrying capacity—On each day of observation, several individuals per morphotype (a total of 131 individuals) were killed with ethyl acetate, frozen, and individually stocked before analyses. Pollen was removed from the different insect body parts (head, thoracic tergum and sternum, abdominal tergum and sternum, and legs) by scrubbing them with small cubes of gelatin-glycerine (50 g gelatin to 15 mL glycerine to 175 mL water; Beattie, 1972; Kearns and Inouye, 1993; Mahy et al., 1998). Pollen grains concentrated in the corbicula or scopa were removed and not included in analyses. Gelatin-glycerine cubes were melted on microscope slides, and all the pollen grains were counted by light microscopy. Pollen grains of the target plant species were counted separately from other pollen grains, and the purity of pollen carried was calculated as the number of pollen grains of ericaceous target plant species on which visitors were collected divided by the total number of pollen grains carried. Because of their similar morphology, pollen grains of *V. myrtillus* and *V. vitis-idaea* cannot be distinguished. Pollen grains of ericaceous species carried on the body parts of different insect visitors were represented with a heatmap (“heatmap.2” command, R-package gplot) where visitor morphotypes showing different behavior were clustered by Bray-Curtis dissimilarity index based on the number of ericaceous pollen grains on each body part.

Pollen removal and deposition capacities—In 2015 and 2016, the numbers of pollen grains removed from the anthers and deposited onto the stigmas after a single visit on virgin flowers were compared. Stems of the four target plant species were bagged with fine-mesh nylon bags before flower opening to exclude flower visitors. When the flowers opened, the stems were individually unbagged, cut, and presented to an insect visitor as it foraged on unmanipulated target plant species. In total, 652 flowers from 229 inflorescences were individually visited. Each inflorescence was visited by a single and different individual. When insects moved among flowers on the same inflorescence, the behavior (legitimate non-buzzing, legitimate buzzing, or nectar

robbing), duration of the visit, and identity of the visitor were recorded. After an insect visit, stems were individually rebagged and kept in glasses with tap water for ≥3 h to allow the pollen-tube development and adhesion of tetrads onto the stigma. Each day, some flowers bagged but not visited (72 control flowers) were collected to estimate self-pollen deposition due to flower manipulations (bag opening, stem cutting, flower marking) and wind. Flowers were individually collected and placed in FAA (ethanol 70%: formaldehyde 35%: acetic acid; 8:1:1) before analyses of pollen removal and deposition.

Because it is not possible to directly measure pollen grain removal by insects, the number of pollen grains remaining in flowers after a single visit was quantified from a subsample of the collected flowers (260 flowers). To extract the pollen remaining in the flower, the flowers were sonicated and vortexed in storage tubes to dislodge pollen grains from the anthers and other flower parts. Flowers were rinsed twice in 70% ethanol. Tubes of pollen were centrifuged for 10 min at 6400 rpm to remove the supernatant and allowed to dry in the laboratory. A known volume of Alexander’s stain solution was added (25–100 µL, according to the quantity of pollen grains). Before counting, tubes were sonicated for 10 min and vortexed; 4 µL of the solution was then deposited on a microscope slide and all pollen grains were counted by light microscopy. Three replicates per sample were done to check the homogenization of the solution. An estimation of pollen removed was calculated by the subtraction of the remaining pollen after one visit from the pollen quantity present in control flowers.

To analyze the quantity of pollen deposited onto the stigmas, styles were sequentially rinsed with demineralized water (1 h), sodium hydroxide (3 h), and water (1 h). Styles were then put onto a microscope slide with a drop of aniline blue solution (0.87 g KH₂PO₄, 0.1 g aniline blue, 50 mL water). Pollen grains were counted under a fluorescence microscope (excitation filter 420–440 nm, emission filter 480 nm, Nikon Eclipse). Because flower emasculation was not possible without damaging flower structure, pollen deposited on stigmas can be both cross- and self-pollen.

Statistical analyses—For each target plant species, we analyzed three independent generalized linear mixed models (GLMMs) to test the influences of (1) visitor taxa and behavior (fixed factors) on the number of pollen grains remaining in flowers (response variable); (2) visitor taxa, behavior, and body parts (fixed factors) on the number of ericaceous pollen grains carried by insect individuals (response variable); and (3) visitor taxa and behavior (fixed factors) on the number of pollen grains deposited onto the stigmas (response variable). In the latter case, we divided the number of pollen grains deposited per stigma by the mean number of ovules per ovary. Our 12 models

(three different tests for the four plant species) included interactions between visitor morphotypes and behavior, and sites and insect individuals were included as random factors. Given the overdispersion of the data, a negative binomial distribution was used for all models ("glmer.nb" command, R-package lme4). GLMMs are excellent tools for analyzing nonnormal data that involve random effects. GLMMs make it possible to analyze hierarchically structured and unbalanced datasets such as ours and effectively eliminate the statistical problem of pseudoreplication due to statistically nonindependent data points (e.g., visiting of several flowers in the same inflorescence by a visitor; Bolker et al., 2009; Zuur et al., 2009). When one factor had a significant effect ($P < 0.05$), pairwise comparisons of values of least square means across groups ("lsmeans" command, R-package lsmeans; Lenth, 2016) were computed as a post hoc test with the Tukey HSD method for adjusting P values.

"Visitor importance" combined data on effectiveness (number of pollen grains deposited per stigma divided by mean number of ovules per ovary) and visitation rate. For each visitor morphotype and behavior, importance was calculated as the product of the mean relative effectiveness and the mean relative visitation rate.

Statistical analyses were performed with R version 3.1.2 (R Development Core Team, 2013). Unless indicated otherwise, data are presented as means $\pm$ SE.

RESULTS

Visitation rate—In 2014 and 2015, during 241 h of monitoring, we observed a total of 3544 visitors on the four target plant species. The majority of visitors belonged to the Hymenoptera, mainly

represented by Apidae (*Apis*, 3.6%; *Bombus*, 49.6%) and solitary bees (Andrenidae or Halictidae, 4.3%). Diptera, represented by Syrphidae (21.6%) and other Diptera (17.6%), were also observed. Lepidoptera were not abundant (2.6%). Among bumblebees (Apidae, *Bombus*), seven different morphotypes were observed: *B. pratorum*, *B. pascuorum*, *B. hypnorum*, *B. terrestris* group, *B. lapidarius*, *B. hortorum/jonellus*, and *Bombus* (*Psithyrus*) subsp. (*B. campestris* and *B. vestalis*). Four *Andrena* species were identified: *Andrena apicata*, *A. haemorrhoa*, *A. lapponica*, and *A. prae-cox*. During 1 d (10 h from 0900 to 1820 hours), visitation rates were extrapolated to about 0.99 ± 0.25 visit per flower for *V. myrtillus*, 0.91 ± 0.13 visit per flower for *V. vitis-idaea*, 0.83 ± 0.178 visit per flower for *E. tetralix*, and 0.11 ± 0.16 visit per flower for *C. vulgaris* (Table 3). Bumblebee visits represented 91.4% of visits for *V. myrtillus*, 87.3% for *V. vitis-idaea*, 94.4% for *E. tetralix*, but only 33.4% for *C. vulgaris*. The latter species was mainly visited by diverse Diptera species (63%).

Pollen removal—The number of pollen grains remaining in flowers was significantly different among visitor taxa only for *V. myrtillus* ($\chi^2 = 11.16$, $P = 0.048$; Fig. 2A; Appendix S1, see Supplemental Data with this article). Buzzing individuals of *Andrena* spp. removed significantly more pollen grains ($15,000 \pm 1414$) than individuals of *B. pascuorum* ($Z = -3.65$, $P = 0.005$) and Syrphidae ($Z = -2.30$, $P = 0.027$) regardless of their behavior. For the latter two species, the number of pollen grains remaining in flowers after a single visit was not significantly different from that in unvisited control flowers. On *V. vitis-idaea*, visitor behavior influenced the number of pollen grains removed ($\chi^2 = 7.16$, $P = 0.007$; Fig. 2B), with buzzing individuals removing significantly more pollen grains per flower than legitimate individuals. In contrast to *Andrena* spp. and *B. horto-*

TABLE 3. Visitation rates (number of visits per flower per day, during 0900 to 1820 hours) of different visitor morphotypes and behaviors observed on *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix*, and *Calluna vulgaris*.

Taxa/morphotypes		Visits	<i>V. myrtillus</i>	<i>V. vitis-idaea</i>	<i>E. tetralix</i>	<i>C. vulgaris</i>
Andrenidae	<i>Andrena</i> spp.	Legitimate non-buzzing	0.034 $\pm$ 0.015	0.003 $\pm$ 0.002		
	<i>Andrena</i> spp.	Legitimate buzzing	<0.001	0.048 $\pm$ 0.013		
Apidae	<i>Apis mellifera</i>	Legitimate non-buzzing	0.011 $\pm$ 0.010	0.013 $\pm$ 0.013	<0.001	0.011 $\pm$ 0.005
	<i>A. mellifera</i>	Robbing			0.001 $\pm$ 0.001	
	<i>Bombus hortorum/jonellus</i>	Legitimate non-buzzing	0.019 $\pm$ 0.009	0.045 $\pm$ 0.020	0.026 $\pm$ 0.019	0.002 $\pm$ 0.002
	<i>B. hortorum/jonellus</i>	Legitimate buzzing	0.006 $\pm$ 0.004	0.035 $\pm$ 0.014	0.024 $\pm$ 0.012	
	<i>B. hypnorum</i>	Legitimate non-buzzing	0.006 $\pm$ 0.006	0.041 $\pm$ 0.025		
	<i>B. hypnorum</i>	Legitimate buzzing		0.028 $\pm$ 0.013		
	<i>B. lapidarius</i>	Legitimate non-buzzing	0.004 $\pm$ 0.003	0.010 $\pm$ 0.009	0.002 $\pm$ 0.002	
	<i>B. pascuorum</i>	Legitimate non-buzzing	0.269 $\pm$ 0.092	0.049 $\pm$ 0.029	0.172 $\pm$ 0.113	
	<i>B. pascuorum</i>	Legitimate buzzing		0.003 $\pm$ 0.003	0.001 $\pm$ 0.001	
	<i>B. pratorum</i>	Legitimate non-buzzing	0.357 $\pm$ 0.096	0.154 $\pm$ 0.046	0.001 $\pm$ 0.001	0.002 $\pm$ 0.002
	<i>B. pratorum</i>	Legitimate buzzing	0.028 $\pm$ 0.018	0.220 $\pm$ 0.051		
	<i>B. terrestris</i> group	Legitimate non-buzzing	0.206 $\pm$ 0.167	0.037 $\pm$ 0.016	0.104 $\pm$ 0.051	0.109 $\pm$ 0.059
	<i>B. terrestris</i> group	Legitimate buzzing	0.002 $\pm$ 0.002	0.101 $\pm$ 0.055	0.127 $\pm$ 0.042	
	<i>B. terrestris</i> group	Robbing			0.262 $\pm$ 0.137	
	<i>Nomada</i> spp.	Legitimate non-buzzing		0.002 $\pm$ 0.002		
	<i>Bombus</i> (<i>Psithyrus</i>) subsp.	Legitimate non-buzzing		0.046 $\pm$ 0.024	0.001 $\pm$ 0.001	
Other	Halictidae	Legitimate non-buzzing	0.003 $\pm$ 0.003	0.033 $\pm$ 0.012	<0.001	
	Ichneumonidae	Legitimate non-buzzing	0.002 $\pm$ 0.002			
Hymenoptera	Vespidae	Legitimate non-buzzing	0.019 $\pm$ 0.009		<0.001	
	Cetoniidae	Legitimate non-buzzing			<0.001	
Coleoptera	Other Diptera	Legitimate non-buzzing		0.002 $\pm$ 0.001	0.001 $\pm$ 0.001	0.183 $\pm$ 0.114
Diptera	Syrphidae	Legitimate non-buzzing	0.014 $\pm$ 0.008	0.014 $\pm$ 0.004	0.036 $\pm$ 0.009	0.066 $\pm$ 0.030
Lepidoptera	Lepidoptera	Legitimate non-buzzing		0.001 $\pm$ 0.000	0.006 $\pm$ 0.003	

Notes: Visitation rates >0.01 are in bold. "*B. terrestris* group" refers to *B. terrestris*, *B. lucorum*, *B. cryptarum*, and *B. magnus*; "*Psithyrus*" refers to *B. bohemicus*, *B. norvegicus*, *B. sylvestris*, and *B. vestalis*. "Legitimate buzzing" refers to visitors that generate vibrations of the anthers to collect pollen; "legitimate non-buzzing" refers to visitors that insert their proboscis inside the corolla to collect pollen or nectar; "robbing" refers to visitors that use a hole in the corolla to extract nectar. Data are means $\pm$ SE.

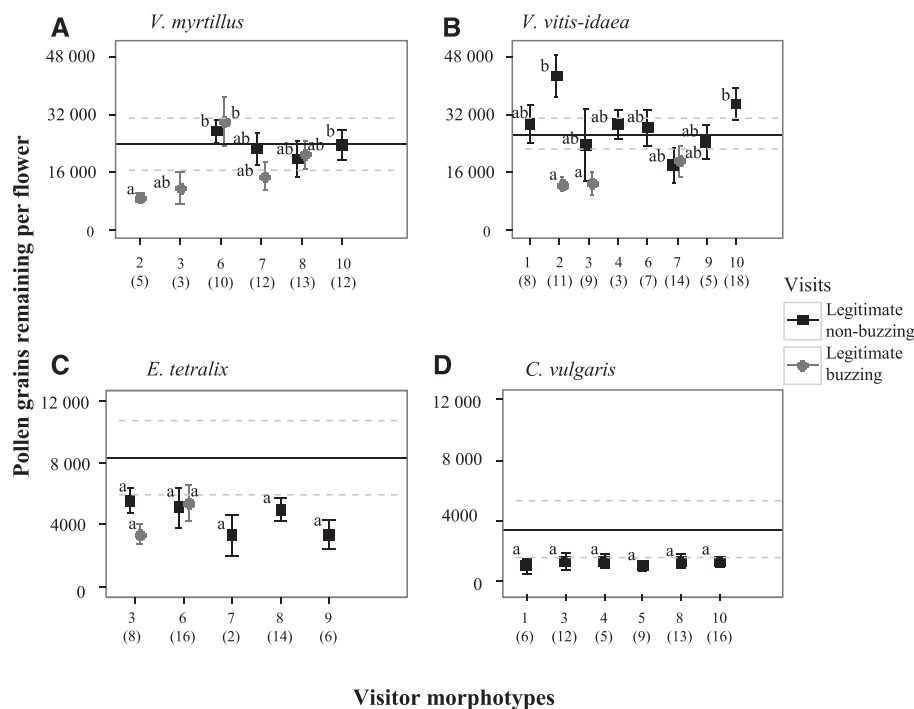


FIGURE 2 Number of pollen grains remaining in the flowers after one visit of insect visitors according to their behavior on (A) *Vaccinium myrtillus*, (B) *V. vitis-idaea*, (C) *Erica tetralix*, and (D) *Calluna vulgaris*. Black lines show the quantity of pollen in unvisited control flowers. Different letters indicate significant differences among pollen grain quantity (tested post hoc). Numbers on x-axes: 1 = *Apis mellifera*, 2 = *Andrena* spp., 3 = *Bombus hortorum/jonellus*, 4 = *B. hypnorum*, 5 = *B. lapidarius*, 6 = *B. pascuorum*, 7 = *B. pratorum*, 8 = *B. terrestris* group, 9 = Diptera (except Syrphidae), and 10 = Syrphidae. All data are means $\pm$ SE; numbers of flowers are in parentheses.

rum/jonellus, buzzing individuals of *B. pratorum* did not remove more pollen grains than legitimate non-buzzing individuals (interactions between behavior and taxa: $\chi^2 = 7.92$, $P = 0.020$). No difference was detected among behaviors or taxa for *E. tetralix* and *C. vulgaris* (Fig. 2D). For *C. vulgaris*, few pollen grains remained in flowers (1180 ± 176 pollen grains).

Pollen carrying capacity and purity—For the four target plant species, the quantity of ericaceous pollen grains carried by insects differed significantly among visitor morphotypes (*V. myrtillus*: $\chi^2_4 = 234.84$, $P < 0.001$; *V. vitis-idaea*: $\chi^2_8 = 447.48$, $P < 0.001$; *E. tetralix*: $\chi^2_2 = 138.17$, $P < 0.001$; *C. vulgaris*: $\chi^2_3 = 47.33$, $P < 0.001$; Fig. 3 and Appendix S1). Individuals of Syrphidae carried significantly fewer pollen grains (107.2 ± 269.6 pollen grains) than *Bombus* spp. (541.2 ± 992.8 pollen grains) or solitary bees (*Andrena* spp., 3434.4 ± 4550.8 pollen grains; *Lasioglossum* spp., 1334.8 ± 1798.0 pollen grains). For all target plant species, the quantity of ericaceous pollen grains carried by insects was significantly different among body parts (*V. myrtillus*: $\chi^2_5 = 23.59$, $P < 0.001$; *V. vitis-idaea*: $\chi^2_5 = 58.57$, $P < 0.001$; *E. tetralix*: $\chi^2_5 = 42.29$, $P < 0.001$; *C. vulgaris*: $\chi^2_3 = 80.91$, $P < 0.001$). The lower parts (legs, lower abdomen, lower thorax) carried more pollen grains than the upper parts. For *E. tetralix* and *V. vitis-idaea*, the number of ericaceous pollen grains carried differed significantly according to the insect behavior ($\chi^2_2 = 21.82$, $P < 0.001$ and $\chi^2_1 = 9.22$, $P = 0.027$). The visitors robbing nectar on *E. tetralix* carried fewer pollen grains than legitimate non-buzzing ($Z = 3.34$, $P = 0.002$) and buzzing visitors ($Z = 5.18$, $P < 0.001$). On *V. vitis-idaea*, buzzing individuals carried significantly more

ericaceous pollen grains than legitimate non-buzzing individuals (gathering nectar, $Z = 2.89$, $P = 0.004$).

For the four target plant species, pollen purity varied with visitor morphotype (*V. myrtillus*: $\chi^2_4 = 7719.70$, $P < 0.001$; *V. vitis-idaea*: $\chi^2_8 = 28,013$, $P < 0.001$; *E. tetralix*: $\chi^2_2 = 214.97$, $P < 0.001$; and *C. vulgaris*: $\chi^2_4 = 246.47$, $P < 0.001$; Table 4). Syrphidae and other Diptera had the lowest purity (from 0.45 ± 0.08 for visitors of *E. tetralix* to 0.93 ± 0.12 for visitors of *C. vulgaris*). The purity of pollen carried by bumblebees varied from 0.73 for *B. pratorum* on *V. myrtillus* to 0.98 for *B. hortorum/jonellus* and the *B. terrestris* group on *C. vulgaris*. On the *Vaccinium* species, the highest purity was found for solitary bee individuals (0.94 for *Lasioglossum* spp. on *V. myrtillus* and 1.00 for *Andrena* spp. on *V. vitis-idaea*). Purity also differed with visitor behavior (*V. myrtillus*: $\chi^2_1 = 68.70$, $P < 0.001$; *V. vitis-idaea*: $\chi^2_1 = 5805.90$, $P < 0.001$; *E. tetralix*: $\chi^2_2 = 1312.39$, $P < 0.001$). Except for *B. hortorum/jonellus* on *V. vitis-idaea*, legitimate buzzing individuals carried a higher proportion of pollen grains of ericaceous target plant species than legitimate non-buzzing individuals.

Pollen grain deposition—The number of ericaceous pollen grains deposited per stigma after one visit ranged from zero to 436 on *V. myrtillus*, from zero to 564 on *V. vitis-idaea*, from zero to 716 on *E. tetralix*, and from 12 to 800 on *C. vulgaris*. For all target plant species except *C. vulgaris*, the number of pollen grains deposited per stigma differed according to insect morphotype (*V. myrtillus*: $\chi^2_7 = 24.64$, $P < 0.001$; *V. vitis-idaea*: $\chi^2_8 = 24.24$, $P = 0.001$; *E. tetralix*: $\chi^2_6 = 29.20$, $P < 0.001$; Fig. 4 and Appendix S1). For these three target plant species, the number of pollen grains on the stigmas was not significantly higher after visits by Syrphidae and other Diptera than on unvisited flowers ($P > 0.05$). On *V. myrtillus*, buzzing visitors of *B. hortorum/jonellus* deposited significantly more pollen grains than Syrphidae ($Z = 3.49$, $P = 0.016$ and $Z = 0.25$, $P < 0.01$, respectively) and other Diptera ($Z = 3.14$, $P = 0.050$ and $Z = 3.46$, $P = 0.018$). On *V. vitis-idaea*, individuals of *Andrena* spp. deposited more pollen grains than individuals in Syrphidae ($Z = 3.67$, $P = 0.011$). On *E. tetralix*, *B. hortorum/jonellus* and *B. pascuorum* visitors deposited significantly more pollen grains during legitimate non-buzzing visits than individuals in Syrphidae ($Z = 3.57$, $P = 0.012$ and $Z = 4.30$, $P < 0.001$, respectively).

Behavior influenced the quantity of pollen grains deposited per stigma for *V. vitis-idaea* ($\chi^2_1 = 9.51$, $P = 0.002$) and *E. tetralix* ($\chi^2_2 = 24.82$, $P < 0.001$), but not for *V. myrtillus* ($\chi^2_1 = 0.748$, $P = 0.387$). Individuals buzzing flowers of *V. vitis-idaea* deposited significantly more pollen grains (103.6 ± 123.2 pollen grains per stigma or 1.6 ± 1.9 pollen grains per ovule) than non-buzzing individuals (40.4 ± 62.0 pollen grains per stigma or 0.6 ± 1.0 pollen grains per ovule). By contrast, individuals buzzing flowers of *E. tetralix* deposited fewer pollen grains (81.6 ± 116.8 pollen grains per stigma or 0.8 ± 1.2 pollen grains per ovule) than non-buzzing individuals (179.6 ± 147.6 pollen grains per stigma or 1.8 ± 1.5 pollen grains per ovule). Robbing

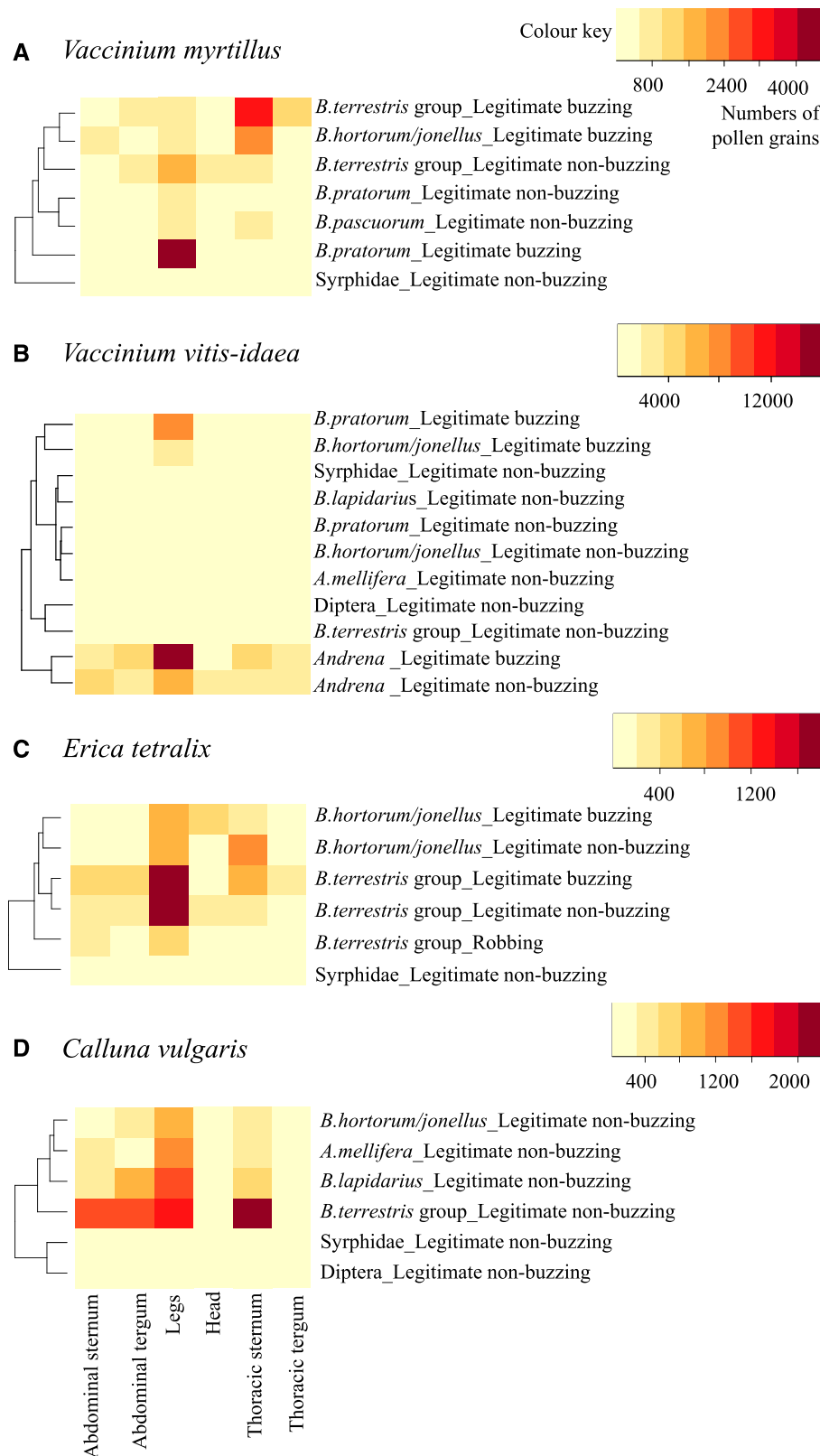


FIGURE 3 Heatmap of the amount of pollen grains of the four target ericaceous plant species on the different insect body parts according to visitor morphotypes and their behavior on (A) *Vaccinium myrtillus*, (B) *V. vitis-idaea*, (C) *Erica tetralix*, and (D) *Calluna vulgaris*. Visitor types were clustered depending on the Bray-Curtis dissimilarity index, based on the number of ericaceous pollen grains on each body part.

individuals did not deposit more pollen grains than control (76.4 ± 25.7 pollen grains per stigma or 0.79 ± 0.26 pollen grains per ovule). On *E. tetralix*, buzzing individuals of *B. terrestris* group deposited significantly fewer pollen grains than other species (interactions between insect morphotype and behavior, $\chi^2 = 9.12$, $P = 0.010$).

Visitor importance—We combined visitation rates and effectiveness (pollen grain deposition) to estimate visitor importance. Bumblebees were the most important visitors of the *Vaccinium* species and *E. tetralix*, whereas Syrphidae, other Diptera, solitary bees (*Andrena* spp.), and honeybees had very limited importance (Fig. 5). On *V. myrtillus* and *E. tetralix*, legitimate non-buzzing bumblebees were more important for pollination than buzzing bumblebees, but it was the opposite for *V. vitis-idaea*. On *C. vulgaris*, *B. terrestris* group and Diptera species were the two most important taxa.

DISCUSSION

Which species and behavior are the most effective for pollen removal?—During buzzing, bees removed 28–60% (*V. myrtillus* and *E. tetralix*) of the pollen grains present in anthers. These proportions were lower than those measured on non-buzz-pollinated species ($\leq 88\%$; Schmid-Hempel and Speiser, 1988; Harder, 1990; Thøstenes and Olesen, 1996; Suzuki et al., 2002; Kawai and Kudo, 2009) and reflected the regulation of pollen delivery in buzz-pollinated species using a “pollen dispensing mechanism” (Harder and Barclay, 1994; Larson and Barrett, 1999; Kawai and Kudo, 2009). We observed differences in pollen removal capacity among visitor morphotypes. *Bombus hortorum* morphotype (including *B. jonellus*) and *A. lapponica*, two taxa with a strong preference for heathland plant species, were the two most effective taxa at pollen removal on the *Vaccinium* species. Twice less pollen remained in *V. myrtillus* anthers after a buzzing visit of these two specialist species (10,000 pollen grains on average) than after a buzzing visit by other polylectic bumblebee species (22,000 pollen grains). Differences in removal performance among visitor species have been observed on other buzz-pollinated species (Kawai and Kudo, 2009). We supposed that oligolectic visitors have behavioral adaptations to efficiently handle their host plant’s flowers (Strickler, 1979; Laverty and Plowright, 1988; Larsson, 2005; Sampson et al., 2016; Tepedino et al., 2016).

TABLE 4. Purity (percentage of pollen grains of the four target ericaceous species on which visitors were collected) of different visitor morphotypes and behaviors observed on *Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix*, and *Calluna vulgaris*.

Visitor morphotypes	Visits	<i>V. myrtillus</i>	<i>V. vitis-idaea</i>	<i>E. tetralix</i>	<i>C. vulgaris</i>
<i>A. mellifera</i>	Legitimate non-buzzing		91.7 ± 0.1 (3)		98.2 ± 0.4 (2)
<i>Andrena</i> spp.	Legitimate non-buzzing		87.2 ± 4.1 (12)		
<i>Andrena</i> spp.	Legitimate buzzing		99.7 (3)		
<i>B. hortorum/jonellus</i>	Legitimate non-buzzing		88.5 ± 3.2 (8)	60.7 (1)	98.1 ± 0.3 (3)
<i>B. hortorum/jonellus</i>	Legitimate buzzing		89 ± 8.4 (3)	92.6 ± 4.2 (4)	
<i>B. pascuorum</i>	Legitimate non-buzzing	72.5 ± 4.5 (2)			
<i>B. pratorum</i>	Legitimate non-buzzing	94.1 (1)	63.3 ± 14.2 (6)		
<i>B. pratorum</i>	Legitimate buzzing	93.9 (1)	82.8 ± 15.5 (2)		
<i>B. terrestris</i> group	Legitimate non-buzzing	75.3 (1)		69.7 ± 15 (2)	98.2 ± 0.7 (4)
<i>B. terrestris</i> group	Legitimate buzzing	83.3 (1)		82.5 ± 10.2 (4)	
<i>B. terrestris</i> group	Robbing			62.7 ± 16 (4)	
Diptera	Legitimate non-buzzing		39.8 ± 12.8 (6)		88 ± 3.3 (4)
<i>Lasioglossum</i> spp.	Legitimate non-buzzing	93.9 ± 5.9 (2)	65.8 ± 8.6 (8)		
Lepidoptera	Legitimate non-buzzing		39.9 ± 21.7 (2)		
Syrphidae	Legitimate non-buzzing	52.5 ± 6.4 (9)	47.3 ± 8.4 (15)	45.4 ± 8.3 (6)	93.1 ± 1.2 (10)

Notes: “*B. terrestris* group” refers to *B. terrestris*, *B. lucorum*, *B. cryptarum*, and *B. magnus*. “Legitimate buzzing” refers to visitors that generate vibrations of the anthers to collect pollen; “legitimate non-buzzing” refers to visitors that insert their proboscis inside the corolla to collect pollen or nectar; “robbing” refers to visitors that use a hole in the corolla to extract nectar. Data are means ± SE. Numbers of individuals are in parentheses.

Moreover, in some cases (e.g., *B. terrestris* group and *B. pascuorum* on *V. myrtillus*), we observed that pollen foragers did not remove more pollen than nectar foragers (legitimate non-buzzing bumblebees). In this particular case, buzzing individuals were not highly effective in removing pollen. Each bumblebee species produces vibrations with different duration, frequency, and amplitude (Buchmann and Hurley, 1978; De Luca and Vallejo-Marín, 2013).

These vibrations can be ineffective for releasing pollen when the frequency or amplitude is too weak (Harder and Barclay, 1994). The low performance of some buzzing bumblebee species in collecting pollen may explain their low visitation rates on ericaceous species for pollen resources. In our study, *B. pascuorum* had both low pollen-removal capacities and low visitation rate for pollen collection compared to *B. jonellus*.

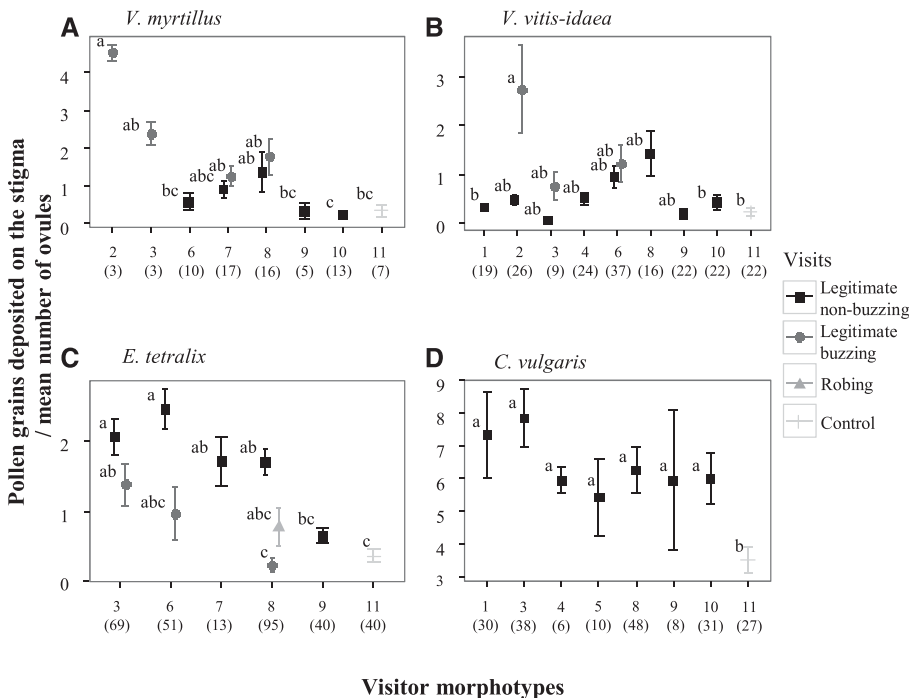


FIGURE 4 Number of pollen grains deposited on the stigma after a single visit of different visitor morphotypes divided by the mean number of ovules according to their behavior on (A) *Vaccinium myrtillus*, (B) *V. vitis-idaea*, (C) *Erica tetralix*, and (D) *Calluna vulgaris*. Controls are unvisited flowers, manipulated only during bagging. Different letters indicate significant differences in pollen grain quantity (tested post hoc). Numbers on x-axes: 1 = *Apis mellifera*, 2 = *Andrena* spp., 3 = *Bombus hortorum/jonellus*, 4 = *B. hypnorum*, 5 = *B. lapidarius*, 6 = *B. pascuorum*, 7 = *B. pratorum*, 8 = *B. terrestris* group, 9 = Diptera (except Syrphidae), 10 = Syrphidae, and 11 = control. All data are means ± SE; numbers of flowers are in parentheses.

Among the four identified *Andrena*, only the oligolectic species, *Andrena lapponica* (Falk, 2015), performed the buzz pollination on the two studied *Vaccinium* species. Buzzing is poorly developed in the Andrenidae (Buchmann, 1983). However, rare studies have reported that other *Andrena* species (*A. carolina* and *A. mexicanorum*) perform buzz pollination on *Vaccinium* spp. and *Solanum elaeagnifolium* (Cane et al., 1985; Cane and Buchmann, 1989; Javorek et al., 2002). There is little information about buzz pollination in non-Apidae species or about why, in closely related species like *A. apicata*, *A. lapponica*, and *A. praecox*, some species use vibrations as a pollen-harvesting technique but others do not.

Which species and behavior are the most effective for pollen deposition?—The oligolectic species *Andrena lapponica* was the most effective species for pollen deposition on the two *Vaccinium* species (Fig. 4). *Andrena* spp. and other solitary bees are often considered effective pollinators and can be even more effective than bumblebees in some cases (Schemske and Horvitz, 1984; Javorek et al., 2002; Moeller, 2005). Their effectiveness probably results from the large amount of pollen they can carry (>20,000 pollen grains) with a high purity (>98%). High purity increases the probability of depositing conspecific pollen grains onto the stigmas and reduces pollen interference like stigma clogging (Waser, 1986; Wilcock and Neiland, 2002).

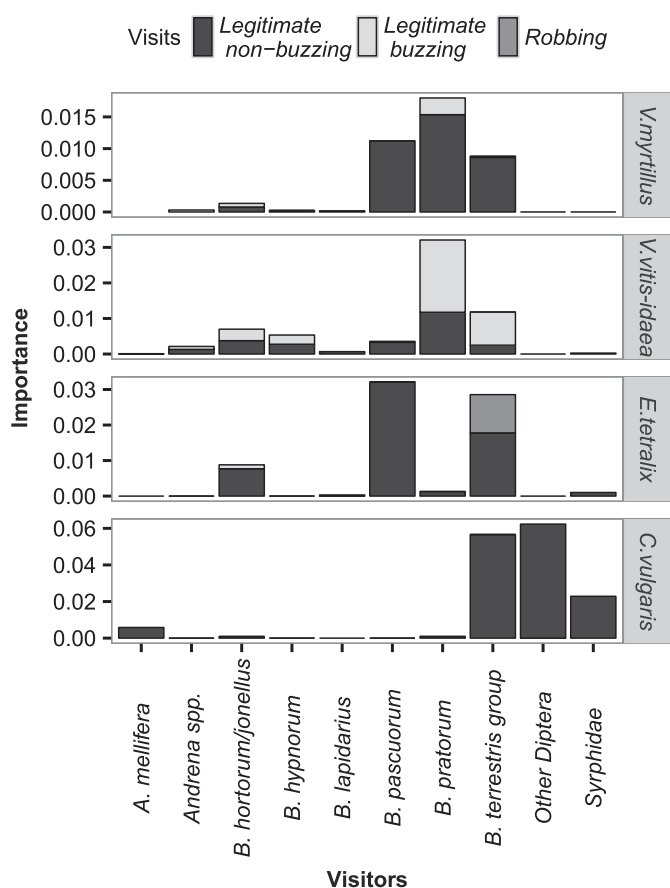


FIGURE 5 Importance (relative visitation rate multiplied by relative effectiveness) of different visitors in relation to the four target ericaceous plant species (*Vaccinium myrtillus*, *V. vitis-idaea*, *Erica tetralix*, and *Calluna vulgaris*) and insect behavior.

We did not detect a difference in pollen deposition among bumblebee species for any of the target plant species. Nevertheless, we observed differences according to visitor behavior and the resource selected by the visitors. Buzzing individuals were the most effective bumblebees for the two *Vaccinium* species, whereas nectar foragers were the most effective bumblebee pollinators for *E. tetralix*. To our knowledge, no studies have been able to analyze pollinator performance according to individual behavior on buzz-pollinated species. On non-buzz-pollinated species, the few previous studies showed contrasting results according to both plant and pollinator species (Thomson and Goodell, 2001; Monzón et al., 2004). These contrasting results indicate interspecific differences in the strategy developed for pollen and nectar dispensing by the plants, as well as in pollen and nectar collection by the visitors.

When we combined pollinator effectiveness with visitation rates, the most important visitor morphotype for pollination differed according to the studied ericaceous plant species. Moreover, the large variation in visitation rates among species seemed to affect visitor importance more than pollen deposition effectiveness. Even when less effective, the most abundant visitor species (e.g., *B. pascuorum* and *B. pratorum* visiting *V. myrtillus*) were more important than species that deposited high quantities of pollen onto the stigmas but that were less frequent visitors (e.g., *A. lapponica* visiting *V. myrtillus*). Therefore, bumblebee robbers, even if they deposited a very small quantity of pollen onto stigmas,

have nonnegligible importance for pollination of *E. tetralix* due to their abundance and their high visitation rates. Positive effects of nectar robbing have also been reported in other studies (Maloof and Inouye, 2000; Sampson et al., 2004; Rojas-Nossa et al., 2015). Maloof and Inouye (2000) called these visitors “robber-like pollinators” because, despite the illegitimate visit, they contribute directly to pollination. On *E. tetralix*, a self-compatible species, the pollen deposited can be self-pollen that falls on stigmas during manipulations of the flower by insects. We observed that slight movements of flowers contributed negligibly to stigmatic deposition on unvisited flowers (controls). Large visitors like bumblebees, however, probably generated sufficient flower movements to allow deposition of self-pollen.

Did floral-resource accessibility influence pollination effectiveness?

We observed differences in the schedule of pollen dispersal among the generalist *C. vulgaris* and the three other buzz-pollinated ericaceous plant species. On *C. vulgaris*, a large proportion of pollen grains was removed during the first visit. According to Li et al. (2014), anther characteristics, such as the degree of anther opening and anther presentation (exposed vs. hidden), explain the difference in pollen removal per visit. In *C. vulgaris*, anthers split completely and are exposed (open corolla), increasing the probability and surface of contacts between the anthers and the visitor's body. Such high pollen removal could be detrimental for pollination because many pollen grains could be lost during flight or grooming (Wilson and Thomson, 1996). The high quantity of pollen removed per flower visit is offset by a “pollen dosing” strategy such as “packaging mechanism” that divides global pollen production in many flowers that sequentially become available to pollinators. Pollen production of *C. vulgaris* is separated into many flowers with fewer pollen grains per flower (~4000) than in the two buzz-pollinated *Vaccinium* species (~12,000).

Erica tetralix and the *Vaccinium* species have poricidal anthers that limit pollen access and act as a pollen-dispensing mechanism. On the *Vaccinium* species, we showed that buzzing visitors were far more effective for pollen deposition than legitimate nectar visitors. Furthermore, the higher nectar quantity of these plants attracted nectar foragers (Jacquemart, 1992; Stephens, 2013; Moquet et al., 2017). Nectar production is not frequent in buzz-pollinated species (Buchmann, 1983; Knudsen and Olesen, 1993; De Luca and Vallejo-Marín, 2013). To explore the adaptive nature of floral nectar secretion, it is necessary to evaluate costs and benefits for plants (Zimmerman, 1988; Pyke, 1991). The benefits might be substantial when nectar foragers are abundant and/or effective pollinators. For *V. myrtillus*, nectar foragers were less effective than pollen foragers but were considerably more abundant and contributed more significantly to pollination than did pollen foragers. The abundant nectar added to the attractiveness of this plant species, increasing pollination success when pollen visitors were scarce or when the pollen was not very attractive (Moquet et al., 2015).

Unlike in the *Vaccinium* species, nectar foragers were more effective for pollen deposition than buzzing individuals on *E. tetralix*. From a plant perspective, nectar foragers were also more efficient for pollen transfer. “Efficiency” refers to the ratio of the number of pollen grains removed from the anthers and the number deposited onto the stigmas. Because nectar foragers collected less pollen but deposited more pollen grains, they were more efficient for the plant than pollen foragers that removed lots of pollen and stored a large fraction in the corbicula. Floral traits explained these differences

among plant species. For example, low style exsertion (distance from stigma to the end of the corolla, ~0.4 mm for *E. tetralix*; Jacquemart, 2003) promoted contacts of the stigma with the insect's head during nectar foraging but limited contacts during buzzing visits. Moreover, large anther pores (~0.6 mm long; Hermann and Palser, 2000) allowed passive pollen collection by legitimate nectar foragers. Depending on anther characteristics, the dispensing mechanism is more or less restrictive. Large anther apertures release a large proportion of pollen (60% for *E. tetralix*) with visits of buzzing individuals and, to a lesser extent, with visits of nectar foragers that generate movements during flower manipulations. The different anther morphology of the studied ericaceous species reflects different strategies of pollen dispersal (Marazzi et al., 2007). When the anther pores are larger, more pollen is lost during visits by pollen foragers whereas pollination by nectar foragers is more effective. No studies have attempted to analyze the amplitude and frequency of buzz needed for pollen removal with respect to different anther (pore diameter, the presence of anther tubes) and flower morphology (open, campanulate, or urceolate corolla), and future work in this area is needed.

CONCLUSIONS

Because visitors have different postures and behaviors, it is crucial to consider which resource they collect for comparison of visitor performance in pollen collection and deposition. The combination of visitation records and pollen grain deposition (effectiveness) reveals that nectar foragers make a substantial contribution to pollination of the buzz-pollinated ericaceous species.

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LITERATURE CITED

- Arceo-Gómez, G., M. L. Martínez, V. Parra-Tabla, and J. G. García-Franco. 2011. Anther and stigma morphology in mirror-image flowers of *Chamaecrista chamaecristoides* (Fabaceae): Implications for buzz pollination. *Plant Biology* 13: 19–24.
- Ballantyne, G., K. C. Baldock, and P. G. Willmer. 2015. Constructing more informative plant–pollinator networks: Visitation and pollen deposition networks in a heathland plant community. *Proceedings. Biological Sciences* 282: 20151130.
- Bannister, P. 1966. Biological flora of the British Isles: *Erica tetralix* L. *Journal of Ecology* 54: 795–813.
- Beattie, A. J. 1972. A technique for the study of insect-borne pollen. *The Pan-Pacific Entomologist* 47: 82.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24: 127–135.
- Brian, A. D. 1951. The pollen collected by bumble-bees. *Journal of Animal Ecology* 20: 191–194.
- Buchmann, S. L. 1983. Buzz pollination in angiosperms. In C. E. Jones, and R. J. Little [eds.], *Handbook of experimental pollination biology*, 73–113. Van Nostrand Reinhold, New York, New York, USA.
- Buchmann, S. L., and J. H. Cane. 1989. Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* 81: 289–294.
- Buchmann, S. L., and J. P. Hurley. 1978. A biophysical model for buzz pollination in angiosperms. *Journal of Theoretical Biology* 72: 639–657.
- Cane, J. H., and S. L. Buchmann. 1989. Novel pollen-harvesting behavior by the bee *Protandrena mexicanorum* (Hymenoptera: Andrenidae). *Journal of Insect Behavior* 2: 431–436.
- Cane, J. H., G. C. Eickwort, F. R. Wesley, and J. Spielholz. 1985. Pollination ecology of *Vaccinium tamineum* (Ericaceae: Vaccinioideae). *American Journal of Botany* 72: 135–142.
- Castellanos, M. C., P. Wilson, S. J. Keller, A. D. Wolfe, and J. D. Thomson. 2006. Anther evolution: Pollen presentation strategies when pollinators differ. *American Naturalist* 167: 288–296.
- Chambers, V. H. 1968. Pollens collected by species of *Andrena* (Hymenoptera: Apidae). *Proceedings of the Royal Entomological Society of London, Series A* 43: 155–160.
- Cnaani, J., J. D. Thomson, and D. R. Papaj. 2006. Flower choice and learning in foraging bumblebees: Effects of variation in nectar volume and concentration. *Ethology* 112: 278–285.
- Conner, J. K., R. Davis, and S. Rush. 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* 104: 234–245.
- De la Barrera, E., and P. S. Nobel. 2004. Nectar: properties, floral aspects, and speculations on origin. *Trends in Plant Science* 9: 65–69.
- De Luca, P. A., and M. Vallejo-Marín. 2013. What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. *Current Opinion in Plant Biology* 16: 429–435.
- Descamps, C., L. Moquet, M. Migon, and A.-L. Jacquemart. 2015. Diversity of the insect visitors on *Calluna vulgaris* (Ericaceae) in Southern France heathlands. *Journal of Insect Science* 15: 130.
- Dukas, R., and A. Dafni. 1990. Buzz-pollination in three nectariferous Boraginaceae and possible evolution of buzz-pollinated flowers. *Plant Systematics and Evolution* 169: 65–68.
- Dupont, Y. L., C. Damgaard, and V. Simonsen. 2011. Quantitative historical change in bumblebee (*Bombus* spp.) assemblages of red clover fields. *PLoS One* 6: e25172.
- Escalante-Pérez, M., and M. Heil. 2012. Nectar secretion: Its ecological context and physiological regulation. In *Secretions and exudates in biological systems*, 187–219. Springer, Berlin, Germany.
- Falk, S. 2015. Field guide to the bees of Great Britain and Ireland. British Wildlife, London, UK.
- Freitas, L., and M. Sazima. 2003. Floral biology and pollination mechanisms in two *Viola* species—from nectar to pollen flowers? *Annals of Botany* 91: 311–317.
- Gilbert, F. S. 1981. Foraging ecology of hoverflies: Morphology of the mouthparts in relation to feeding on nectar and pollen in some common urban species. *Ecological Entomology* 6: 245–262.
- Gilbert, F. S. 1985. Morphometric patterns in hoverflies (Diptera, Syrphidae). *Proceedings of the Royal Society of London. Series B, Biological Sciences* 224: 79–90.
- Gimingham, C. H. 1960. Biological flora of the British Isles: *Calluna Salisb.* *Journal of Ecology* 48: 455–483.
- Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. *Oecologia* 85: 41–47.
- Harder, L. D., and R. M. R. Barclay. 1994. The functional significance of poricidal anthers and buzz pollination: Controlled pollen removal from *dodecatheon*. *Functional Ecology* 8: 509–517.
- Harder, L. D., and J. D. Thomson. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* 133: 323–344.

- Haslett, J. R. 1989. Adult feeding by holometabolous insects: Pollen and nectar as complementary nutrient sources for *Rhingia campestris* (Diptera: Syrphidae). *Oecologia* 81: 361–363.
- Heil, M. 2011. Nectar: Generation, regulation and ecological functions. *Trends in Plant Science* 16: 191–200.
- Hermann, P. M., and B. F. Palser. 2000. Stamen development in the Ericaceae. I. Anther wall, microsporogenesis, inversion, and appendages. *American Journal of Botany* 87: 934–957.
- Inouye, D. W., D. E. Gill, M. R. Dudash, and C. B. Fenster. 1994. A model and lexicon for pollen fate. *American Journal of Botany* 81: 1517–1530.
- Jacquemart, A.-L. 2003. Floral traits of Belgian Ericaceae species: Are they good indicators to assess the breeding systems? *Belgian Journal of Botany* 136: 154–164.
- Jacquemart, A.-L. 1992. Préliminaires sur la production de nectar chez trois espèces de *Vaccinium*. *Apidologie* 23: 453–464.
- Jacquemart, A.-L. 1993. Floral visitors of *Vaccinium* species in the high Ardenne, Belgium. *Flora* 188: 263–273.
- Jacquemart, A.-L., and J. D. Thompson. 1996. Floral and pollination biology of three sympatric *Vaccinium* (Ericaceae) species in the Upper Ardenne, Belgium. *Canadian Journal of Botany* 74: 210–221.
- Javorek, S. K., K. E. Mackenzie, and S. P. Vander Kloet. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). *Annals of the Entomological Society of America* 95: 345–351.
- Kawai, Y., and G. Kudo. 2009. Effectiveness of buzz pollination in *Pedicularis chamissonis*: Significance of multiple visits by bumblebees. *Ecological Research* 24: 215–223.
- Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.
- King, C., G. Ballantyne, and P. G. Willmer. 2013. Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4: 811–818.
- Klinkhamer, P. G. L., and T. J. de Jong. 1993. Attractiveness to pollinators: A plant's dilemma. *Oikos* 66: 180–184.
- Knudsen, J. T., and J. M. Olesen. 1993. Buzz-pollination and patterns in sexual traits in North European Pyrolaceae. *American Journal of Botany* 80: 900–913.
- Knuth, P. 1906. Handbook of flower pollination, vol. 1: Introduction and literature. Clarendon Press, Oxford, UK.
- Larson, B. M. H., and S. C. H. Barrett. 1999. The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae). *Journal of Ecology* 87: 371–381.
- Larsson, M. 2005. Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (Dipsacaceae). *Oecologia* 146: 394–403.
- Laverty, T. M., and R. C. Plowright. 1988. Flower handling by bumblebees: A comparison of specialists and generalists. *Animal Behaviour* 36: 733–740.
- Lenth, R. V. 2016. Least-squares means: The R package lsmeans. *Journal of Statistical Software* 69: 1–33.
- Li, X.-X., H. Wang, R. W. Gituru, Y.-H. Guo, and C.-F. Yang. 2014. Pollen packaging and dispensing: Adaption of patterns of anther dehiscence and flowering traits to pollination in three *Epimedium* species. *Plant Biology* 16: 227–233.
- Lindsey, A. H. 1984. Reproductive biology of Apiaceae. I: Floral visitors to *Thaspium* and *Zizia* and their importance in pollination. *American Journal of Botany* 71: 375–387.
- Macek, J., J. Straka, P. Bogusch, L. Dvořák, P. Bezděčka, and P. Tyrner. 2010. Blanokřídlí České Republiky: Žahadloví. I. Academia, Prague, the Czech Republic.
- Mahy, G., J. R. De Sloover, and A.-L. Jacquemart. 1998. The generalist pollination system and reproductive success of *Calluna vulgaris* in the Upper Ardenne. *Canadian Journal of Botany* 76: 1843–1851.
- Maloo, J. E., and D. W. Inouye. 2000. Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651–2661.
- Marazzi, B., E. Conti, and P. K. Endress. 2007. Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiina). *International Journal of Plant Sciences* 168: 371–391.
- Mayer, C., L. Adler, W. S. Armbruster, A. Dafni, C. Eardley, S.-Q. Huang, P. G. Kevan, et al. 2011. Pollination ecology in the 21st century: Key questions for future research. *Journal of Pollination Ecology* 3: 8–23.
- Mayer, C., D. Michez, A. Chyzy, E. Brédard, and A.-L. Jacquemart. 2012. The abundance and pollen foraging behaviour of bumble bees in relation to population size of whortleberry (*Vaccinium uliginosum*). *PLoS One* 7: e50353.
- Mayfield, M. M., N. M. Waser, and M. V. Price. 2001. Exploring the 'most effective pollinator principle' with complex flowers: Bumblebees and *Ipomopsis aggregata*. *Annals of Botany* 88: 591–596.
- Mitchell, R. J. 1993. Adaptive significance of *Ipomopsis aggregata* nectar production: Observation and experiment in the field. *Evolution* 47: 25–35.
- Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant–pollinator interactions in *Clarkia xantiana* ssp. *xantiana*. *Oecologia* 142: 28–37.
- Monzón, V., J. Bosch, and J. Retana. 2004. Foraging behavior and pollinating effectiveness of *Osmia cornuta* (Hymenoptera: Megachilidae) and *Apis mellifera* (Hymenoptera: Apidae) on “Comice” pear. *Apidologie* 35: 575–585.
- Moquet, L., C. Mayer, D. Michez, B. Wathélet, and A.-L. Jacquemart. 2015. Early spring floral foraging resources for pollinators in wet heathlands in Belgium. *Journal of Insect Conservation* 19: 837–848.
- Moquet, L., M. Vanderplanck, R. Moerman, M. Quinet, N. Roger, D. Michez, and A.-L. Jacquemart. 2017. Bumblebees depend on ericaceous species to survive in temperate heathlands. *Insect Conservation and Diversity* 10: 78–93.
- Ne'eman, G., A. Jürgens, L. Newstrom-Lloyd, S. G. Potts, and A. Dafni. 2010. A framework for comparing pollinator performance: Effectiveness and efficiency. *Biological Reviews of the Cambridge Philosophical Society* 85: 435–451.
- Neiland, M. R. M., and C. C. Wilcock. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* 85: 1657–1671.
- Olsen, K. M. 1996. Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxillaris* (Asteraceae). *Oecologia* 109: 114–121.
- Parker, A. J., J. L. Tran, J. L. Ison, J. D. K. Bai, A. E. Weis, and J. D. Thomson. 2015. Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees. *Arthropod-Plant Interactions* 9: 197–203.
- Proença, C. E. B. 1992. Buzz pollination—older and more widespread than we think? *Journal of Tropical Ecology* 8: 115–120.
- Pyke, G. H. 1991. What does it cost a plant to produce floral nectar? *Nature* 350: 58–59.
- Rader, R., B. G. Howlett, S. A. Cunningham, D. A. Westcott, L. E. Newstrom-Lloyd, M. K. Walker, D. A. J. Teulon, and W. Edwards. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology* 46: 1080–1087.
- Ranta, E., and H. Lundberg. 1981. Food niche analyses of bumblebees: a comparison of three data collecting methods. *Oikos* 36: 12–16.
- R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Ritchie, J. C. 1955. Biological flora of the British Isles: *Vaccinium vitis-idaea* L. *Journal of Ecology* 43: 701–708.
- Ritchie, J. C. 1956. Biological flora of the British Isles: *Vaccinium myrtillus* L. *Journal of Ecology* 44: 291–299.
- Rojas-Nossa, S. V., J. M. Sánchez, and L. Navarro. 2015. Effects of nectar robbing on male and female reproductive success of a pollinator-dependent plant. *Annals of Botany* 117: 291–297.
- Roulston, T. H., and J. H. Cane. 2000. Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution* 222: 187–209.
- Sahlí, H. F., and J. K. Conner. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* 94: 203–209.
- Sampson, B. J., R. G. Danko, and S. J. Stringer. 2004. Nectar robbery by bees *Xylocopa virginica* and *Apis mellifera* contributes to the pollination of rabbiteye blueberry. *Journal of Economic Entomology* 97: 735–740.

- Sampson, B. J., C. T. Pounders, C. T. Werle, T. R. Mallette, D. Larsen, L. Chatelain, and K. C. Lee. 2016. Pollination of *Hibiscus* (section *Trionum*: Malvaceae). *Journal of Pollination Ecology* 18: 7–12.
- Schemske, D. W., and C. C. Horvitz. 1984. Variation among floral visitors in pollination ability: A precondition for mutualism specialization. *Science* 225: 519–521.
- Schmid-Hempel, P., and B. Speiser. 1988. Effects of inflorescence size on pollination in *Epilobium angustifolium*. *Oikos* 53: 98–104.
- Shivanna, K. R., V. K. Sawhney, and R. B. Knox. 2005. Pollen biotechnology for crop production and improvement. Cambridge University Press, Cambridge, UK.
- Ssymank, A. 1991. Rüssel- und Körperlänge von Schwebfliegen (Diptera: Syrphidae) unter Berücksichtigung der Verwendung von Alkoholmaterial. *Mitteilungen der Schweizerische Entomologische Gesellschaft* 64: 67–80.
- Stephens, D. 2013. Pollination ecology and the floral reward of *Vaccinium myrtilloides* and *V. vitis-idaea* (Ericaceae). Ph.D. dissertation, University of Saskatchewan, Saskatoon, Canada.
- Strickler, K. 1979. Specialization and foraging efficiency of solitary bees. *Ecology* 60: 998–1009.
- Suzuki, K., I. Dohzono, K. Hiei, and Y. Fukuda. 2002. Pollination effectiveness of three bumblebee species on flowers of *Hosta sieboldiana* (Liliaceae) and its relation to floral structure and pollinator sizes. *Plant Species Biology* 17: 139–146.
- Tepedino, V., L. A. Horn, and S. Durham. 2016. Pollen removal and deposition by pollen- and nectar-collecting specialist and generalist bee visitors to *Iliamna bakeri* (Malvaceae). *Journal of Pollination Ecology* 19: 50–56.
- Terzo, M., and P. Rasmont. 2007. MALVAS, suivi, étude et vulgarisation sur l'interaction entre les MAE et les abeilles sauvages. Direction Générale de l'Agriculture, Namur Région Wallonne, Belgium.
- Thomson, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. *Journal of Ecology* 74: 329–341.
- Thomson, J. D., and K. Goodell. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38: 1032–1044.
- Thomson, J. D., and R. C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia* 46: 68–74.
- Thøstesen, A. M., and J. M. Olesen. 1996. Pollen removal and deposition by specialist and generalist bumblebees in *Aconitum septentrionale*. *Oikos* 77: 77–84.
- Vallejo-Marín, M., E. M. Da Silva, R. D. Sargent, and S. C. H. Barrett. 2010. Trait correlates and functional significance of heteranthy in flowering plants. *The New Phytologist* 188: 418–425.
- Vogel, S. 1978. Evolutionary shifts from reward to deception in pollen flowers. In A. J. Richards [ed.], *The pollination of flowers by insects*. Linnean Society Symposium Series no. 6, 89–96. Academic Press, London, UK.
- Waser, N. M. 1986. Flower constancy: Definition, cause, and measurement. *American Naturalist* 127: 593–603.
- Westerkamp, C. 1996. Pollen in bee-flower relations some considerations on melittophily. *Botanica Acta* 109: 325–332.
- Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7: 270–277.
- Wilson, P., and J. D. Thomson. 1996. How do flowers diverge? In D. G. Lloyd and S. C. H. Barrett [eds.], *Floral biology: Studies on floral evolution in animal-pollinated plants*, 88–111. Chapman & Hall, New York, New York, USA.
- Woodcock, T. S., B. M. Larson, P. G. Kevan, D. W. Inouye, and K. Lunau. 2014. Flies and flowers II: Floral attractants and rewards. *Journal of Pollination Ecology* 12: 63–94.
- Zimmerman, M. 1988. Nectar production, flowering phenology, and strategies for pollination. In *Plant reproductive ecology: Patterns and strategies*, 157–178. Oxford University Press, New York, New York, USA.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology. Springer, New York, New York, USA.