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RESEARCH PAPER

Pollen dispersal and fruit production in *Vaccinium oxycoccos* and comparison with its sympatric congener *V. uliginosum*

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Keywords

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ABSTRACT

Investigating plant–pollinator interactions and pollen dispersal are particularly relevant for understanding processes ensuring long-term viability of fragmented plant populations. Pollen dispersal patterns may vary strongly, even between similar congeneric species, depending on the mating system, pollinator assemblages and floral traits. We investigated pollen dispersal and fruit production in a population of *Vaccinium oxycoccos*, an insect-pollinated shrub, and compared the pollen dispersal pattern with a co-flowering, sympatric congener, *V. uliginosum*. We examined whether they share pollinators (through interspecific fluorescent dye transfers) and may differently attract pollinators, by comparing their floral colour as perceived by insects. Fluorescent dyes were mainly dispersed over short distances (80% within 40.4 m (max. 94.5 m) for *V. oxycoccos* and 3.0 m (max. 141.3 m) for *V. uliginosum*). Dye dispersal in *V. oxycoccos* was not significantly affected by plant area, floral display or the proximity to *V. uliginosum* plants. Interspecific dye transfers were observed, indicating pollinator sharing. The significantly lower dye deposition on *V. oxycoccos* stigmas suggests lower visitation rates by pollinators, despite higher flower density and local abundance. The spectral reflectance analysis indicates that bees are unlikely to be able to discriminate between the two species based on floral colour alone. Fruit production increased with increasing floral display, but was not affected by proximity to *V. uliginosum* plants. Our study highlights that fragmented populations of *V. oxycoccos*, when sympatric with co-flowering *V. uliginosum*, might incur increased competition for the shared pollinators in the case of pollination disruption, which might then reduce outcrossed seed set.

INTRODUCTION

Most angiosperm species depend on animal pollinators for their sexual reproduction (Ollerton *et al.* 2011). Modern conservation plans targeting fragmented or endangered plant populations have highlighted the need to incorporate the ecological requirements of each interacting partner to ensure the long-term stability and persistence of plant–pollinator networks (Potts *et al.* 2010; Vereecken *et al.* 2010; Burkle & Alarcon 2011). Pollination disruption has regularly been reported in fragmented habitats (Kwak *et al.* 1998; Wilcock & Neiland 2002; Ghazoul 2005; Kolb 2008; Kiers *et al.* 2010), and thus the quality of plant–pollinator relationships often represents a determining factor in the implementation of conservation measures aimed at pollen flow restoration (Van Geert *et al.* 2010; Van Rossum & Triest 2010; Vereecken *et al.* 2010). Investigating plant–pollinator interactions and the resulting pollen dispersal patterns are therefore particularly relevant for understanding processes ensuring long-term viability of fragmented plant populations (Burkle & Alarcon 2011; Mayer *et al.* 2011).

Pollen dispersal patterns may considerably differ between plant species, depending on plant mating system, pollinator assemblages and floral structure (such as anther and stigma accessibility) (Real 1983; Vekemans & Hardy 2004; Ghazoul 2005). Visitation patterns of pollinators are known to be influenced by particular floral traits, such as flower colour and symmetry, the presence of a floral reward (*e.g.* nectar), floral scent and not only pollen quantity, but also quality, *e.g.* the balance of essential amino acids (Roulston *et al.* 2000; Goulson 2003; Raine *et al.* 2006; Leonard *et al.* 2011). Even between similar congeneric species, there may be strong differences in some floral traits, associated with the attraction of different pollen vectors (*e.g.* Jacquemart 2003; Armbruster & Muchhala 2009), which may therefore differently affect pollen dispersal patterns.

Within a population, the size of an individual plant (that can be measured by its area) and its floral display (estimated by the total number of flowers and flower density) may influence the plant's attraction signal to pollinators, increasing visitation when higher and also changing pollinator behaviour, *e.g.* the time spent on the plants (Kwak *et al.* 1998; Rademaker & de Jong 1998; Makino *et al.* 2007; Ishii *et al.* 2008). The presence of co-occurring and co-flowering insect-pollinated plant species, when sharing pollinators, may increase (facilitation) or reduce (competition) pollinator

visitation, and lead to pollen wastage through interspecific pollen transfers (Ghazoul 2006; Mitchell *et al.* 2009a; Flanagan *et al.* 2011). If the sympatric co-flowering species are phylogenetically closely related and present similar floral morphologies and rewards, a stronger competition for pollinators may be expected, as well as interspecific pollen transfers that may lead to hybridisation (Kwak 1978; Esfeld *et al.* 2009; Field *et al.* 2011). These features may in turn affect pollen dispersal, fruit and seed production and outcrossing rates (Kwak *et al.* 1998; Bell *et al.* 2005; Morales & Traveset 2008; Mitchell *et al.* 2009a,b; Van Rossum & Triest 2010; Flanagan *et al.* 2011). To our knowledge, pollen dispersal patterns have rarely been investigated for congeneric species occurring in similar environmental conditions, *i.e.* under the same pollinator service, thus potentially shared by both species (Arnold *et al.* 1992; Irwin 2001; Esfeld *et al.* 2009; Field *et al.* 2011).

We investigated pollen dispersal and fruit production in a large population of *Vaccinium oxycoccos*, a shrub usually considered as allogamous, but rarely visited by insects in Belgium (Jacquemart 1997, 2003), and compared the pollen dispersal pattern with that of a co-flowering, sympatric congeneric species, *V. uliginosum*. This species has been reported to have regular insect visitors, in particular bumblebees (Jacquemart 1996; Brédat 2010). We used fluorescent powdered dye as a pollen analogue. Dye dispersal may be considered a reliable estimator of pollen dispersal for insect-pollinated species (*e.g.* Waser 1988; Rademaker *et al.* 1997; Van Rossum *et al.* 2011; but see Thomson *et al.* 1986; Campbell 1991; Adler & Irwin 2006). To examine whether pollinators might be differently attracted by the flowers of the two species, we compared the floral colours as perceived by the pollinators. Specifically, we addressed the following questions: (i) do pollen dispersal patterns differ between the two *Vaccinium* species; (ii) does floral colour as perceived by insects (spectral reflectance) differ between *V. oxycoccos* and *V. uliginosum*; (iii) is there evidence for interspecific dye transfers, indicating pollinator sharing between the two *Vaccinium* species; and (iv) do individual plant area, floral display (total number of flowers, flower density) and the proximity of *V. uliginosum* influence pollen dispersal and fruit production in *V. oxycoccos*? We discuss the results in the light of differences in species-specific floral traits (rewards, morphology, spectral reflectance) and in terms of biological consequences.

MATERIAL AND METHODS

Study species

Vaccinium oxycoccos and *V. uliginosum* (Ericaceae) are shrubs (Figure S1) that occur in waterlogged, oligotrophic *Sphagnum* bogs and mires, and in acid upland heath and bogs, respectively. They both have a circumboreal distribution, extending to southern mountain areas in Europe (Jacquemart 1996, 1997). In Belgium, both species are considered rare and declining, occurring in highly fragmented habitats (Saintenoy-Simon *et al.* 2006; Walloon Flora Atlas Group, unpublished data). *Vaccinium oxycoccos* typically forms patches of slender, usually prostrate (<10-cm high) stems on *Sphagnum* hummocks that can reach up to 0.5-m high. It flowers in June, producing pink-reddish flowers, with eight stamens forming a tube around the style (Knuth 1909; Jacquemart 1997, 2003). The flowers are protandrous, and are visited by insects, but self-pollination occurs frequently (Jacquemart 1997). Bushes of *V. uliginosum* flower in May–June and can reach up to 80-cm high. Its flowers are ovoid, whitish to pale pink, and weakly protandrous. Both species produce nectar and are pollinated when the main visitors, several species of bumblebee (*Bombus* spp., Apidae) and solitary bee (Hymenoptera), vibrate the anthers (buzz pollination). Other occasional pollinators include syrphid flies and butterflies (Knuth 1909; Jacquemart 1996, 1997, 2003; Brédat 2010).

The blooming period of the two *Vaccinium* species overlaps in the study site, although in 2010 *V. oxycoccos* started to flower 3 weeks later than *V. uliginosum*. Very few visitors were observed on *V. oxycoccos*: a few workers of *Bombus pratorum*, ants and a few flies (F. Van Rossum, personal observations). In contrast, flowers of *V. uliginosum* were regularly visited by queens and workers of several *Bombus* species, mainly *B. lapidarius*, *B. pascuorum*, *B. pratorum*, *B. terrestris s.l.* and *B. vestalis*, and by other solitary bee species, including the *Vaccinium* specialist *Andrena lapponica*. Syrphid flies (*Rhingia campestris*, *Sericomyia lappona* and *Eristalis tenax*) were also observed (Brédat 2010; Mayer *et al.*, 2012). Both species not only reproduce sexually through seeds contained in edible berries, but also propagate clonally, through rooting of the creeping shoots for *V. oxycoccos* and through horizontal rhizomes for *V. uliginosum* (Jacquemart 1996, 1997).

Studied populations

We investigated pollen dispersal in a sympatric population of about 250 flowering patches located on hummocks (referred to hereafter as 'individuals') of *V. oxycoccos* and of about 60 flowering individual bushes of *V. uliginosum* in the study site of the Grande Fange (Régné, 50°14′40″N, 05°46′45″E), a bog located in the Plateau des Tailles region, SE Belgium.

Estimating pollen dispersal using fluorescent dye

We used three colours (orange, pink and blue) of fluorescent dye (Radiant Color Corp., Belgium; Series Radglo© R) as pollen analogues to estimate pollen dispersal. Previous studies showed no difference in dispersal patterns among these dye colours (*e.g.* Van Rossum 2009, 2010; Van Rossum & Triest 2010). We marked flowers with dye at peak flowering season in early June 2010, during a window of 2 days of dry, relatively sunny weather. Because of the unforeseen delay in flowering of *V. oxycoccos* (however with the opening of numerous flowers simultaneously), the experiment had to be carried out for *V. oxycoccos* 5 days later than for *V. uliginosum*. On day 1, we applied dyes once, early in the morning, with wooden toothpicks to dehiscing anthers of 200 flowers over about 0.45 m^2 of two individuals of *V. oxycoccos* (orange and pink dye) and of 60 flowers over about 0.25 m^2 of a *V. uliginosum* bush (blue dye). On day 2, stigmas that had been receptive during the experimental period were harvested from 15 to 31 and five to seven flowers on recipient individuals of *V. oxycoccos* and *V. uliginosum*, respectively (Table 1). Sampling of recipient individuals covered the entire area occupied by the population. The stigmas were embedded in a semi-permanent mount of glycerine

Table 1. Dye dispersal results for *Vaccinium oxycoccus* and *V. uliginosum* populations in the Grande Fange (within species and interspecific, *i.e.* dye from *V. uliginosum* on *V. oxycoccus*: number of sampled recipient individuals (n), potential distance to dye source in m (mean with range), effective distance of dye transfers in m (mean with range and for 80% of dye transfers), proportion of recipient individuals showing dye, mean fraction of dyed stigmas and mean dye abundance (with range).

species	n	distance to dye source (m)	distance of dye transfers (m)	80% dye transfers (m)	ind. with dye	fraction dyed stigmas	dye abundance
V. oxycoccus	95	40.5 (0.2–130.7)	26.6 (0.2–94.5)	40.4	0.36	0.05 (0.00–1.00)	0.07 (0.00–2.21)
V. uliginosum	50	61.1 (1.4–146.7)	23.6 (1.4–141.3)	3.0	0.32	0.17 (0.00–1.00)	0.26 (0.00–1.57)
interspecific	95	87.6 (19.4–146.1)	78.9 (22.9–108.0)	102.5	0.14	0.01 (0.04–0.08)	0.01 (0.04–0.10)

jelly on a microscope slide (Van Rossum 2010). In total, 2048 stigmas collected from 95 flowering individuals of *V. oxycoccos* and 339 stigmas from 50 individual bushes of *V. uliginosum* were examined for dye particles at 10× magnification under a fluorescence microscope. We recorded the presence or absence of fluorescent dye particles of each colour (intra- and interspecific) on each stigma. For each recipient individual, we calculated the proportion of stigmas showing deposited dye (fraction of dyed stigmas = number of stigmas with dye/total number of collected stigmas). We then estimated dye abundance for each stigma and dye colour by assessing the observations into four classes (0: no dye particles observed on stigma; 1: <5 particles; 2: 6–50 particles; 3: >50 particles). Mean dye abundance for each recipient individual and each dye colour was calculated as an average weighted by the number of stigmas within each class (Van Rossum 2009). The geographic coordinates of each recipient and dye source individual were recorded using a GPS (Garmin Oregon 400t, Southampton, UK). The potential distances (range and mean) from dye source to recipient individuals within each species were inferred (Table 1). The minimum distance between recipient individuals of *V. oxycoccos* and individuals of *V. uliginosum* ranged from 4.4 to 39.8 m (mean: 20.2 m).

Floral traits and spectral reflectance analysis of flowers

Many floral traits have been found to differ between *V. oxycoccos* and *V. uliginosum* (Jacquemart 2003; summarised in Table 2; Figure S1). However, how pollinators see the flowers is largely unknown. We determined the similarity of the flower colour as perceived by their pollinators by plotting the reflectance spectra of the two *Vaccinium* species as loci in the bee colour hexagon; a suitable model for colour perception in higher Hymenoptera (Chittka 1992; Chittka & Kevan 2005) that yields largely similar results using either honeybees (*Apis mellifera*) or bumblebees (*Bombus* spp.) (Peitsch *et al.* 1992; Briscoe & Chittka 2001). We used the reflectance curve of *V. uliginosum* leaves as a green standard and a white standard (WS-2, Avantes, Eerbeek, The Netherlands) for calibration of the spectrophotometer (AVASPEC-2048-USB2-UA, Avantes). We used a xenon light source (AVALIGHT-XE, Avantes,) for measurements of the relative (%) reflectance (300–700 nm) of 20 flowers of each species picked randomly in the field.

Measure of reproductive components in *V. oxycoccos*

For each recipient individual of *V. oxycoccos*, the number of open flowers was counted for three 10 × 10 cm plots and total plant (hummock) area measured as an ellipse, and used to calculate open flower density (number of open flowers 100 cm^{-2}) and the total number of flowers per plant over total plant area. In August 2010, the number of fruits was counted for the same previously sampled 95 individuals for three 10 × 10 cm plots, to calculate fruit density (number of fruits 100 cm^{-2}) and the total number of fruits per plant over total plant area.

Table 2. Flowering population size, flower density and floral traits (mean ± SD) of *Vaccinum oxycoccus* and *V. uliginosum*: flower diameter, number of anthers and tetrads per anther, tetrad diameter, volume of pollen produced per anther and position of style in the corolla.

floral trait	*V. oxycoccus*	*V. uliginosum*
flowering population size	250[a]	60
flower density (number of open flowers m^{-2})	1890 ± 1490[a]	142 ± 95
flower diameter (mm)[b]	5.1 ± 0.7[a]	3.8 ± 0.2
number of anthers[b]	8	8–10
number of tetrads per anther[b]	782 ± 397[a]	550 ± 87
tetrad diameter (μm)[b]	31.9 ± 3.3	48.3 ± 2.3[a]
volume of pollen produced by anther (mm^3)[b]	0.0133	0.0327[a]
style in corolla[b]	Exserted	Included

[a]Superior in pollinator choice.
[b]From or based on Jacquemart (2003).

Data analysis

Within-population dye dispersal patterns

Since the mean dye abundance (*i.e.* average weighted by number of stigmas within each class) and the fraction of dyed stigmas were highly correlated for both species (Gamma correlation coefficient $\Gamma = 0.991$ and 0.978 for *V. oxycoccos* and *V. uliginosum*, $P < 0.001$), we restricted the analyses to comparisons of the mean dye abundance. Because no difference ($P > 0.05$) was found in dispersal patterns between the two dye colours used for *V. oxycoccos* (GLM test of homogeneity of slopes: $\chi^2 = 2.22$ and 2.97 for dye colour and interaction dye colour × distance to dye source, respectively), the data sets obtained for the two dye sources were pooled for the analyses.

Dye dispersal patterns, overall as well as considering effective – successful (*i.e.* only the recipient plants where at least one stigma had received dye) – dye transfers only, were investigated for each species using multiple (*V. oxycoccos*) or univariate (*V. uliginosum*) regression analysis, to test the relationship between mean dye abundance for each recipient

individual and the distance to the dye source. For *V. oxycoccos*, plant area, floral display (total number of flowers, flower density) and the minimum distance to *V. uliginosum* were also added as predictor variables, and a forward step-wise selection procedure was applied to the regression analysis. Depending on the variable distribution type, the analyses were based on a generalised linear model (GLM, power link function and significance determined using a likelihood ratio chi-square test) or on a linear model (identity link function, significance determined using the F-test).

To test whether dye dispersal patterns (overall and effective transfers) may differ between *V. oxycoccos* and *V. uliginosum*, a test of homogeneity of slopes based on a GLM or a linear model (see here above) was carried out on mean dye abundance, with the distance to dye source as independent variable and species as grouping variable. To this end, the analysis was performed for the same range of distance to dye source, *i.e.* from 1.4 to 131.0 m. The variables were transformed (logarithm, Box Cox) when necessary to achieve normality.

Interspecific dye dispersal by shared pollinators

To test whether the interspecific dye dispersal pattern (overall and effective transfers) may differ from the intraspecific transfers in *V. oxycoccos*, a test of homogeneity of slopes based on a GLM or a linear model (see above) was carried out for the same range of distance to dye source, *i.e.* from 19.4 to 130.7 m, on mean dye abundance, with the distance to dye source as independent variable and category (within-species/interspecific) as grouping variable. The variables were transformed (logarithm, Box Cox) when necessary to achieve normality.

Spectral reflectance analysis of flowers

We assessed both the intra- and interspecific discrimination capability of pollinators towards the colours of *V. oxcycoccus* and *V. uliginosum* by calculating (i) pair-wise Euclidean distances between loci, and (ii) the mean Euclidean distance between the species centroids in the bee colour hexagon. The Euclidean distance between any two loci indicates the perceived colour difference or contrast between the stimuli, and threshold values of hexagon units for colour discrimination usually range between 0.062 (Dyer and Chittka 2004a,b; Dyer *et al.* 2008) and 0.100 (Chittka *et al.* 1997) for bees. We used the pair-wise Euclidean distance matrix between loci as input file in MEGA 5 (Molecular Evolutionary Genetics Analysis 5; Tamura *et al.* 2011) to construct a neighbour-joining (NJ) tree depicting the floral colour differences among samples from the pollinators' perspective.

Effect of plant area, floral display and minimum distance to V. uliginosum on fruit production in V. oxycoccos

We used a multiple regression analysis with a forward step-wise selection procedure to examine whether fruit production (fruit density, total number of fruits) can be related to plant area, floral display (total number of flowers, flower density) and the minimum distance to *V. uliginosum* plants. Floral display and fruit production variables were log-transformed to achieve normality. All analyses were performed using STATISTICA (StatSoft, Tulsa, OK, USA).

RESULTS

Within-population dye dispersal patterns

The proportion of recipient individuals showing dye deposition was 0.36 and 0.32 in *V. oxycoccos* and in *V. uliginosum*, respectively (Table 1). For these dye-receiver individuals, the fraction of dyed stigmas ranged from 0.14 to 1.00 for both species (mean: 0.04 and 0.52, respectively) and mean dye abundance from 0.04 to 2.21 and 0.14 to 1.57 (mean: 0.19 and 0.81, respectively). The effective distance of dye transfers for *V. oxycoccos* varied from 0.2 m to 94.5 m, which was shorter than the maximum potential distance to dye source (130.7 m), with 80% of the dye transfers occurring at <40.4 m (Table 1). In *V. uliginosum*, the effective distance of transfers ranged from 1.4 m to 141.3 m, covering the potential distances to dye source, and with 80% of the dye transfers at <3.0 m.

For both species there was a significantly ($P \leq 0.002$) negative relationship between mean dye abundance and the distance to dye source for overall dye dispersal (GLM model; *V. oxycoccos*: $\Gamma = -0.430$, $\chi^2 = 19.03$; *V. uliginosum*: $\Gamma = -0.674$, $\chi^2 = 31.89$; Fig. 1), and when only considering effective dye transfers (linear model; *V. oxycoccos*: $R^2 = 0.178$, $F_{1,67} = 14.50$, Pearson's correlation coefficient $r = -0.422$, $t = -3.81$; *V. uliginosum*: $R^2 = 0.501$, $F_{1,14} = 14.06$, $r = -0.708$, $t = -3.75$). No significant ($P > 0.05$) relationship between mean dye abundance and the other variables (plant area, floral display and minimum distance to *V. uliginosum*) was found.

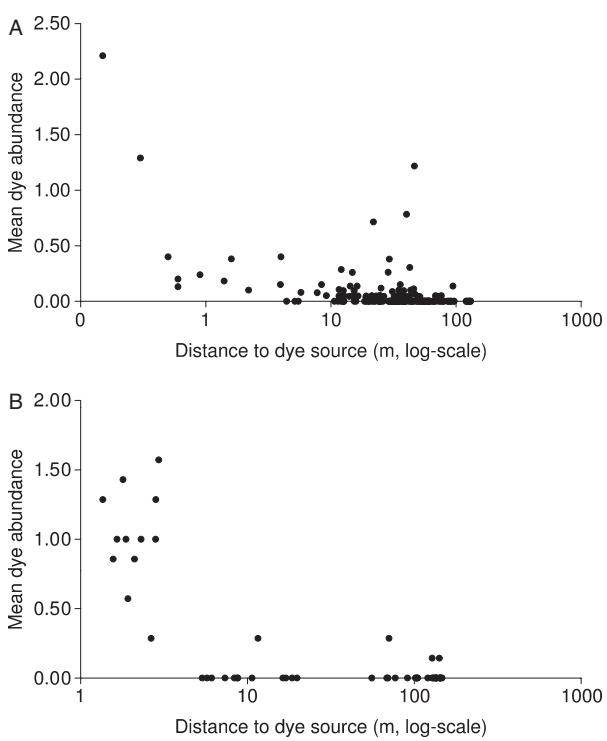

Fig. 1. Distribution of dye deposition (mean dye abundance) as a function of the distance to dye source (log-scale), A: for *Vaccinium oxycoccus* (gamma correlation coefficient $\Gamma = -0.430$, $P < 0.001$); B: for *V. uliginosum* ($\Gamma = -0.674$, $P < 0.001$).

Differences in dye deposition between the two species, for the same distance range (1.4–131.0 m), were tested using a test of homogeneity of slopes (GLM or linear model). This test revealed that mean dye abundance was significantly ($P < 0.001$) lower for *V. oxycoccos* than for *V. uliginosum* (overall dispersal: means = 0.05 and 0.32, respectively, $\chi^2 = 63.45$; effective dye transfers: means = 0.28 and 0.85, respectively; $F_{1,73} = 11.88$). The interaction between species and distance to dye source was significant for the overall dispersal ($\chi^2 = 34.68$, $P < 0.001$), but not for the effective dye transfers ($F_{1,73} = 0.10$, $P > 0.10$). The test also showed a significantly negative relationship between mean dye abundance and the distance to dye source (overall: $\chi^2 = 61.00$, $P < 0.001$; effective dye transfers: $F_{1,73} = 8.38$, $P = 0.005$).

Interspecific dye dispersal by shared pollinators

We observed dye deposition from the *V. uliginosum* dye source on 13.7% (13 out of 95) of the *V. oxycoccos* recipient plants, with the fraction of dyed stigmas ranging from 0.04 to 0.08 and a dye abundance of 0.04 to 0.10, for a distance to dye source varying from 22.9 to 108.0 m (Table 1). The test of homogeneity of slopes (GLM or linear model) for the same distance range (19.4–130.7 m) revealed no significant ($P > 0.10$) differences in dye deposition on *V. oxyccocus* stigmas between *V. oxycoccos* (within species) and *V. uliginosum* (interspecific) dye sources (overall dispersal: $\chi^2 = 1.24$ and effective dye transfers: $F_{1,48} = 0.04$), and no significant ($P > 0.10$) interaction between category (within-species/interspecific patterns) and distance to dye source (overall dispersal: $\chi^2 = 0.97$ and effective dye transfers: $F_{1,48} = 0.10$).

Spectral reflectance analysis of flowers

The flowers of *V. oxycoccos* and *V. uliginosum* appear whitish-pinkish to the human eye and their spectral curves indicate that they have non-overlapping patterns of reflectance between 300 and 640 nm (Fig. 2). The two species slightly overlap in the region of the red end of the spectrum between 640 and 700 nm (Fig. 2). Our measurements indicate that neither species strongly reflects ultraviolet (UV) light: flowers of *V. oxycoccos* have virtually no UV reflection, while *V. uliginosum* flowers only exhibit a weak reflectance in the UV range (300–400 nm, relative reflectance <10%). Overall, the relative reflectance of *V. uliginosum* flowers is higher than that of *V. oxycoccos* across the best part of the visible spectrum for bees, *i.e.* 300–640 nm range (Fig. 2).

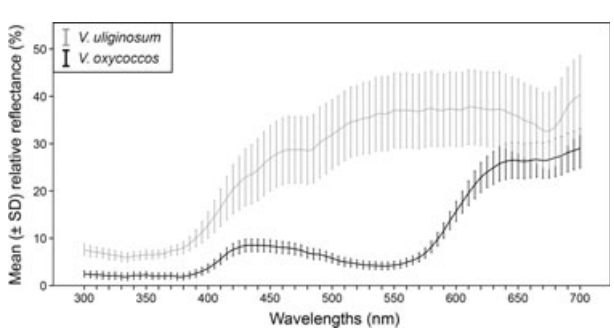

Fig. 2. Reflectance functions (mean relative reflectance ±SD) measured on 20 flowers of *Vaccinium uliginosum* (grey line) and of *V. oxyccocus* (black line) as a function of wavelength, between 300 and 700 nm.

By analysing the colour differences between flowers of *V. oxycoccos* and *V. uliginosum* from the pollinators' perspective using the bee colour space, we found that all colour loci fell at the intersection between the 'bee blue' and the 'bee UV-blue' categories of the colour hexagon (Fig. 3). The two species formed discrete clusters in the bee colour hexagon, with only one sample of *V. oxycoccos* (oxyc16) located apart from the rest of its conspecific samples and with a reflectance that was more similar to that of the *V. uliginosum* samples (see arrows on Fig. 3). We found that the mean interspecific Euclidean distance in floral colour was 0.072 hexagon units. Intraspecific colour contrasts varied, ranging from 0.000 to 0.124 for *V. oxycoccos* and from 0.000 to 0.108 for *V. uliginosum*.

Effect of plant area, floral display and minimum distance to *V. uliginosum* on fruit production in *V. oxycoccos*

Mean and range values of the reproductive components in *V. oxycoccos* are presented in Table 3. Fruit production (fruit density, total number of fruits) was significantly ($P = 0.013$ and <0.001, respectively) positively related to the total number of flowers (Table 4). While fruit density was positively ($P < 0.001$) related to flower density, the relationship was negative ($P = 0.001$) for the total number of fruits in the multiple regression analysis (Table 4). Plant area and the minimum distance to *V. uliginosum* were eliminated in the forward step-wise selection procedure ($P > 0.05$).

DISCUSSION

Within-population dye dispersal patterns

For both species the main factor influencing the amount of within-population dye deposition on stigmas appears to be the distance to dye source, with dye dispersal showing a rapidly decaying distribution curve with increasing spatial distance. Such a dispersal pattern, with most dye transfers at short distances (within a few tens of meters), and a few long-distance events, has been reported for several other bee-pollinated plant species (*e.g.* Kwak *et al.* 1998; Van Geert *et al.* 2010; Van Rossum *et al.* 2011; but see Van Rossum 2010; Van Rossum & Triest 2010 reporting more extended dye dispersal). This feature may be the result of the combination of (i) bee foraging behaviour (systematic visits to flowers of a plant and then moving to the next closest plant, grooming, and leaving the patch when the pollen load is sufficiently high), (ii) passive loss of pollen grains during flight, (iii) plant distribution, and (iv) secondary transfers during foraging (*e.g.* Inouye *et al.* 1994; Rademaker *et al.* 1997; Kwak *et al.* 1998).

The amount of dye deposition on stigmas may also be affected by factors other than distance to dye source. For instance, a large floral display (in size or density) may be more rewarding to insects, in particular to bees, and therefore be perceived as more attractive, thus increasing visitation rates (Makino *et al.* 2007; Ishii *et al.* 2008). The presence of other floral resources, when sharing the same pollinator guild, may lead to interspecific competition as well as facilitation (Ghazoul 2006; Flanagan *et al.* 2011). Our results show no evidence of an effect of plant area, total flower number, flower density, and of minimum distance to *V. uliginosum* on

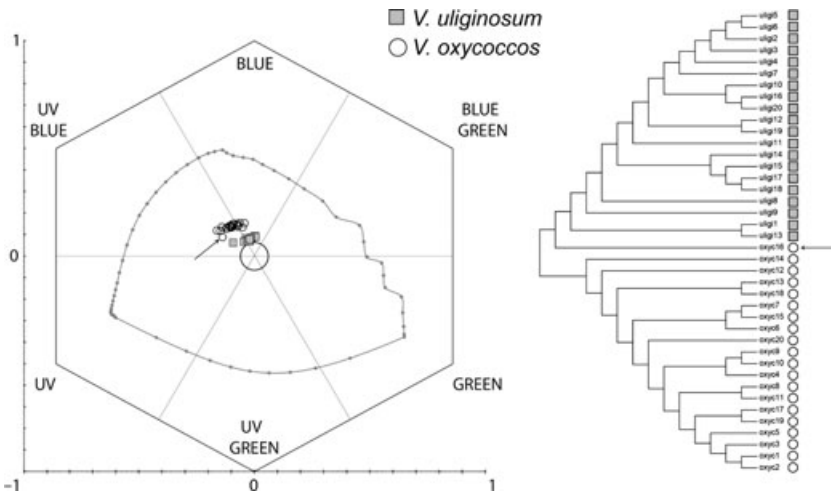

Fig. 3. Floral colours of *Vaccinium oxyccocus* (open circles) and *V. uliginosum* (filled squares) in the bee colour hexagon (left) and NJ tree constructed with the pair-wise Euclidean distances among loci (right). The curved grey line in the hexagon represents the loci of monochromatic light at background intensity (see Chittka 1992 for methodology). Each segment dividing the hexagon corresponds to one bee colour category. All samples except 'oxyc16' (indicated by the arrow on the graphs) clustered species-specifically.

Table 3. Reproductive components (mean and ranges) of *Vaccinum oxycoccus*: plant area, floral display (total number of flowers, flower density) and fruit production (total number of fruits, fruit density).

variable	mean	range
plant area (cm^2)	2611	259–15080
total number of flowers	573	15–4342
flower density (/100 cm^2)	19	1–70
total number of fruits	398	0–3016
fruit density (/100 cm^2)	13	0–36

Table 4. Multiple regression analyses of plant area, floral display and distance to *Vaccinum uliginosum* on fruit production (fruit density, total number of fruits) in *V. oxycoccus*. Only the selected predictor variables, after forward step-wise selection procedure ($P < 0.05$), are shown.

variable	β	t	*P*
fruit density ($R^2 = 0.690$, $F_{2,92} = 102.43$, $P < 0.001$)			
number of flowers	0.268	2.54	0.013
flower density	0.594	5.63	<0.001
total number of fruits ($R^2 = 0.835$, $F_{2,92} = 232.44$, $P < 0.001$)			
number of flowers	0.834	14.52	<0.001
flower density	−0.330	−3.35	0.001

R^2 = multiple determination coefficient; β = standardised partial regression coefficient; t = t-statistic (two-tailed test of whether the partial regression coefficient differs from zero); *P* = significance probability.

dye deposition on *V. oxycoccos* flowers. However, in *V. oxycoccos* as well as in *V. uliginosum*, no trace of dye deposition was found on the majority of individuals, even at short distances. This was also the case for other *V. uliginosum* populations in the Plateau des Tailles (Brédat 2010). Unlike other plant species previously studied for dye dispersal (*e.g.* Van Rossum 2009, 2010; Van Geert *et al.* 2010; Van Rossum & Triest 2010), also in the same study sites (F. Van Rossum *et al.*, unpublished results), plants of these two *Vaccinium* species form patches or bushes that can produce large clumps of flowers, up to a few thousand. It may be difficult to detect dye deposition events in such large clumps after 1 day following dye marking, given the number of collected flowers within a patch. We may therefore have missed visited flowers, suggesting the need to sample a larger number of flowers for such extensively flowering plants.

Interspecific dye dispersal by shared pollinators

There is evidence of dye deposition from the *V. uliginosum* dye source on stigmas of *V. oxycoccos* for 13.7% of the individuals at up to 108 m distance, indicating that pollinators of *V. uliginosum* also visit *V. oxycoccos* flowers, suggesting that both species, when occurring in sympatry, at least partially share the same generalist pollinator guild. When between- and within-species dye deposition patterns are comparable, as found for *V. oxycoccos* (however with *V. uliginosum* source plants marked a few days earlier), we may ask whether heterospecific pollen transfers might lead to stigma clogging for *V. oxycoccos* (but also to pollen wastage for *V. uliginosum*), and how this might affect seed set, taking into account the ability for this species for autogamous self-pollination (Jacquemart 1997, 2003). Moreover, for phylogenetically related taxa, this might lead to hybridisation (*e.g.* Kwak 1978; Morales & Traveset 2008; Esfeld *et al.* 2009). This is unlikely for *V. uliginosum* and *V. oxycoccos*, as they belong to two different sections (*Vaccinium* and *Oxycoccus*, respectively) located in separate clades, indicating polyphyly, and do not form hybrids (Powell & Kron 2002; Vander Kloet & Dickinson 2009).

Differences in dye dispersal patterns related to species-specific characteristics

Dye abundance on stigmas (as well as the proportion of stigmas with dye) was much lower for *V. oxycoccos* than *V. u-*

liginosum, despite the higher number of dye source flowers and of recipient flowers collected. This finding suggests that *V. oxycoccos* flowers are less visited than *V. uliginosum* flowers, which confirms observations of insect visitation patterns for each *Vaccinium* species at the study site (Jacquemart 1996, 1997; Brédat 2010). Such differences in insect visitation rates might result from several factors related to variation in species-specific characteristics (detected as unique or combined, see Leonard *et al.* 2011) and abundance. First, the two *Vaccinium* species significantly differ in height: bushes of *V. uliginosum* are taller and flowers are located higher than in the prostrate plants of *V. oxycoccos* (Jacquemart 2003), which might facilitate access to individual flowers by large insects such as bumblebees.

Second, plant species attractiveness may be positively related to its relative local flowering density (Kwak *et al.* 1998; Ghazoul 2005; Van Rossum & Triest 2010). Moreover, pollen-foraging bees can exhibit floral constancy, *i.e.* a learned fidelity to flowers of particular species that have previously provided a reward, which is often highly linked to local species abundance (Chittka *et al.* 1997; Goulson 2000; Esfeld *et al.* 2009). Flower density and flowering population size appear, however, to be higher in *V. oxycoccos* than in *V. uliginosum* (Table 2). Moreover, flowers are also bigger (Table 2), suggesting that flowering patches of *V. oxycoccos* might constitute a more visible signal for pollinators than *V. uliginosum*. Despite this, *V. oxycoccos* is less visited by pollinators.

Third, species that contrast in their floral colours as perceived by the insects may differently attracts pollinators, especially bee species (Giurfa *et al.* 1995; Arnold *et al.* 2009). In this study, we found that all colour loci of *V. oxycoccos* and *V. uliginosum* fall at the intersection between the 'bee blue' and the 'bee UV-blue' (Fig. 2), two categories of the colour hexagon for which bees are known to have an innate preference (Giurfa *et al.* 1995; Raine *et al.* 2006). 'Bee blue' and 'bee UV-blue' flowers are usually found in May–June, at a time when the two *Vaccinium* species are in bloom, however they are less often recorded in flower-rich habitats (Arnold *et al.* 2009).

Collectively, the results of our spectral reflectance analysis indicate that although bees might occasionally discriminate between strongly contrasted flowers within species, they are unlikely to be able to discriminate between the two *Vaccinium* species based on their floral colour alone, although they are separately clustered in the bee colour space (Fig. 3). They only slightly overlap at the red end of the spectrum (640–700 nm), where the bees' discrimination capabilities are more limited (Chittka & Waser 1997). Indeed, the intraspecific contrasts may be high (up to 0.108–0.124), whereas the mean interspecific contrast (0.072) is below the 0.100 practical discriminability threshold value for bees (Chittka *et al.* 1997; but see Dyer and Chittka 2004a,b; Dyer *et al.* 2008 who used 0.062). Our hypothesis that the *Vaccinium* pollinators might experience difficulty in distinguishing between colours of *V. oxycoccos* and *V. uliginosum* fits general observations that bees often generalise to similar colours (*e.g.* Chittka *et al.* 1997), and is therefore in agreement with our results on dye dispersal indicating pollinator sharing between the two plant species.

Fourth, we cannot exclude that pollinators might compensate for their inability to discriminate between similar floral colours by using other floral traits that may help them to identify the plants they visit. These traits potentially include the reward quality (pollen and nectar quantity and quality), floral scent (Goulson 2003; Raine *et al.* 2006; Dötterl & Vereecken 2010; Leonard *et al.* 2011) or scent marks (Giurfa *et al.* 1994). Although the number of stamens is quite similar, *V. oxycoccos* produces more numerous but smaller tetrads per anther than *V. uliginosum*, and as a consequence the mean total volume of pollen produced per anther appears to be 2.46 times higher in *V. uliginosum* than in *V. oxycoccos* (Table 2; Jacquemart 2003). This suggests that flowers of *V. uliginosum* might be more rewarding in pollen quantity, which could contribute to higher visitation. Pollen chemical content, such as crude protein, should be similar between the two species, as they are closely related taxa (Buchmann 1986; Roulston *et al.* 2000). A flower of *V. uliginosum* produces on average 1.6 µl of nectar, dominated by fructose and glucose (Jacquemart 1996), which makes bushes of this species and their high number of flowers a good resource for bees. Nectar production is yet to be investigated for *V. oxycoccos.* It is also unknown whether the two *Vaccinium* species differ in floral scent, which might contribute to the pollinator ability to discriminate between flowers (Goulson 2003; Dötterl & Vereecken 2010). These points certainly deserve further attention.

Besides causing possible differences in visitation rates, flower morphology may also influence the efficiency of dye deposition on the stigmas (*e.g.* Real 1983). Style exsertion from the corolla is higher for *V. oxycoccos* than *V. uliginosum* (Jacquemart 2003), which might facilitate pollen (dye) deposition (Wyatt 1983). However, higher dye abundance was found on *V. uliginosum* stigmas. Whether pollen adherence on the stigma differs between the two *Vaccinium* species is still unknown and merits investigation. Finally, we found a significant difference between the two species in regression slopes of the overall dye dispersal and 80% transfer distance was lower for *V. uliginosum*. This may be related to differences in plant spatial arrangement of the two *Vaccinium* species in the study site.

Effect of plant area, floral display and minimum distance to *V. uliginosum* on fruit production in *V. oxycoccos*

Large floral displays of *V. oxycoccos* produce more fruits than small ones. However, when the total number of flowers is held constant in the model, we found a significant negative effect of flower density on the total number of fruits. As found for dye deposition, the proximity to *V. uliginosum* plants did not affect fruit production. Moreover, *V. oxycoccos* fruits can develop from self-pollinated flowers through autogamy and geitonogamy, although leading to lower seed set (Jacquemart 2003). These findings suggest no pollination limitation for *V. oxycoccos*. As this species occurs in oligotrophic habitats, and produces fleshy – potentially costly – fruits, this negative relationship might indicate a limited resource availability effect, as reported for boreal *V. oxycoccos* plants (Fröborg 1996) and the closely related *V. macrocarpon* (Brown & McNeil 2006).

CONCLUSION

Insect pollination, especially by bumblebee and other bee species is known to promote outcrossing in *Vaccinium* species

(Jacquemart 1996, 2003). In fragmented habitats, where the pollinator guilds may be less diverse and abundant, competition for pollinators may arise between co-flowering plant species sharing the same pollinators, with the effect of reducing their outcrossed seed set (*e.g.* Kwak *et al.* 1998; Wilcock & Neiland 2002; Mitchell *et al.* 2009a,b). Given (i) the lower pollen deposition found for *V. oxycoccos*, (ii) that *V. uliginosum* and *V. oxycoccos* share pollinators when in sympatry, and (iii) that *V. uliginosum* has been reported as an important nectar and pollen resource for bumblebee colonies (Brédat 2010), populations of *V. oxycoccos* co-occurring with co-flowering *V. uliginosum* in highly fragmented habitats might incur increased competition and thus reduced outcrossing rates, possibly leading to erosion of genetic diversity in the long term.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Photos of flowers and plants of *Vaccinium oxycoccos* and *V. uliginosum*.

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