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Variation in highbush blueberry floral volatile profiles as a function of pollination status, cultivar, time of day and flower part: implications for flower visitation by bees

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• *Background and Aims* Studies of the effects of pollination on floral scent and bee visitation remain rare, particularly in agricultural crops. To fill this gap, the hypothesis that bee visitation to flowers decreases after pollination through reduced floral volatile emissions in highbush blueberries, *Vaccinium corymbosum*, was tested. Other sources of variation in floral emissions and the role of floral volatiles in bee attraction were also examined.
• *Methods* Pollinator visitation to blueberry flowers was manipulated by bagging all flowers within a bush (pollinator excluded) or leaving them unbagged (open pollinated), and then the effect on floral volatile emissions and future bee visitation were measured. Floral volatiles were also measured from different blueberry cultivars, times of the day and flower parts, and a study was conducted to test the attraction of bees to floral volatiles.
• *Key Results* Open-pollinated blueberry flowers had 32 % lower volatile emissions than pollinator-excluded flowers. In particular, cinnamyl alcohol, a major component of the floral blend that is emitted exclusively from petals, was emitted in lower quantities from open-pollinated flowers. Although, no differences in cinnamyl alcohol emissions were detected among three blueberry cultivars or at different times of day, some components of the blueberry floral blend were emitted in higher amounts from certain cultivars and at mid-day. Field observations showed that more bees visited bushes with pollinator-excluded flowers. Also, more honey bees were caught in traps baited with a synthetic blueberry floral blend than in unbaited traps.
• *Conclusions* Greater volatile emissions may help guide bees to unpollinated flowers, and thus increase plant fitness and bee energetic return when foraging in blueberries. Furthermore, the variation in volatile emissions from blueberry flowers depending on pollination status, plant cultivar and time of day suggests an adaptive role of floral signals in increasing pollination of flowers.

Key words: *Vaccinium corymbosum*, honey bees, bumble bees, volatile organic compounds, diurnal rhythm, nectar production, site of emission.

INTRODUCTION

Most flowers emit highly complex, species-specific bouquets of volatile organic chemicals (Knudsen *et al.*, 2006; Knudsen and Gersherzon, 2006; Raguso, 2008; Schiestl, 2010). The composition of these floral scents may vary according to plant genotype (Chang *et al.*, 1988; Pham-Delegue *et al.*, 1989), pollination status (Tollsten and Bergström, 1989; Moya and Ackerman, 1993; Schiestl *et al.*, 1997; Schiestl and Ayasse, 2001; Theis and Raguso, 2005), flower age (Pichersky *et al.*, 1994; Dudareva *et al.*, 2000) or circadian rhythms (Loughrin *et al.*, 1990; Effmert *et al.*, 2005). High floral emissions often coincide with maximal pollinator activity (Hoballah *et al.*, 2005), suggesting that floral signals play an adaptive role in guiding pollinators to flowers (Pichersky and Gershenzon, 2002; Dobson, 2006; Raguso, 2008). For instance, many bees utilize floral volatiles as cues to locate and recognize food sources (Smith and Getz, 1994; Blight *et al.*, 1997; Burger *et al.*, 2010). These cues are extremely important for bees since most species rely completely on floral rewards (i.e. pollen and nectar) to provision offspring and feed themselves (Michener, 2000). Moreover, foraging bees are capable of discriminating between rewarding and non-rewarding flowers within a patch (Weatherwax, 1986; Goulson *et al.*, 2001; Dötterl and Vereecken, 2010), thereby minimizing the time invested to locate resources. While the mechanisms by which this is accomplished are not entirely clear, studies suggest that some bees rely primarily on olfactory cues (e.g. Giurfa and Núñez, 1992; Howell and Alarcón, 2007; Dötterl and Vereecken, 2010).

If floral volatile emissions incur a physiological or ecological cost to plants, it is predicted that plants should reduce their odour emission after pollination to reduce these costs. Ecological costs may occur if floral volatile emissions attract herbivores as well as pollinators (Andrews *et al.*, 2007; Kessler and Halitschke, 2009). Thus, a decrease in floral scent emissions after pollination might be adaptive both to conserve the costs of odour production and to reduce the

likelihood of attracting consumers (Gori, 1983). This has been shown for the orchid *Ophrys sphegodes* where total amounts of floral scent emissions were lower 2–4 d after pollination compared with unpollinated flowers (Schiestl *et al.*, 1997). Similarly, Theis and Raguso (2005) reported an 89 % post-pollination decline in scent emission rates in Canada thistle, *Cirsium arvense*, and sandhill thistle, *C. repandum*.

Furthermore, post-pollination changes in floral scent may benefit both plants and pollinators by influencing flower visitation by pollinators. It is argued (e.g. Ayasse, 2006) that reduced floral emission after pollination may reduce competition among flowers for pollinators within plants by directing pollinators to unpollinated flowers, thus increasing the plant's reproductive success. In fact, Schiestl *et al.* (1997) found that reduced floral scent in *O. sphegodes* after pollination results in lower pollinator attraction. Nectar is a reward commonly offered by flowering plants to flower visitors in return for providing a pollination service (Simpson and Neff, 1983). Nectar volume often diminishes after pollination (e.g. Luyt and Johnson, 2002); thus, bees can increase their net energetic return if they avoid previously pollinated flowers that may provide low reward (Williams, 1998; Stout and Goulson, 2002).

Except for a few studies (e.g. Tollsten and Bergström, 1989; Schiestl *et al.*, 1997; Theis and Raguso, 2005), the effect of pollination on odour production and flower visitation by bees has remained largely unexplored. In the present study, the hypothesis that bee visitation to flowers decreases after pollination through reduced floral volatile emissions in an agricultural ecosystem was tested. Highbush blueberry, *Vaccinium corymbosum*, is a native North American species with entomophilous flowers, i.e. adapted for insect pollination (West and McCutcheon, 2009), that emit a complex blend of 35 volatiles rich in esters and terpenoid compounds (Szendrei *et al.*, 2009). Insect visitation is essential for pollination of highbush blueberries because insects pick up, transport and deposit pollen leading to subsequent germination of pollen, fertilization of ovules and hormonal changes that result in increased fruit set, size and ripening of berries (MacKenzie, 2009). Bumble bees (*Bombus* spp.), honey bees (*Apis mellifera*) and solitary bees (e.g. *Andrena* spp.) comprise the primary pollinators of highbush blueberry fields across the United States (Rao *et al.*, 2009; West and McCutcheon, 2009; Isaacs and Kirk, 2010). These bees differ, however, in the manner in which they pollinate flowers of this species. For example, bumble bees employ buzz pollination to release pollen from the anthers, whereas non-native honey bees do not (Buchmann, 1983; MacKenzie, 2009).

Despite the high dependency of many *Vaccinium* species on bees for pollination, little is known about the role of flower volatiles in blueberry–bee interactions. Accordingly, the objectives in this study were to examine how pollinator visitation influences floral volatile emissions and subsequent bee visitation, and to investigate sources of variation in floral emissions and the role of floral volatiles in bee attraction. Specifically, the following questions were asked. Does pollination influence floral volatile emissions? Is there variation among cultivars in floral emissions? Does time of day affect floral volatile emissions and amount of nectar reward? What is the site of volatile emissions from flowers? To test the effects of pollination on bees, the following questions were asked. Is bee visitation to flowers influenced by pollination and, if so, does flower pollination affect honey bees and bumble bees differently? Finally, to investigate further the importance of floral volatiles in bee attraction in the system, the question was asked: are bees attracted to single blueberry floral compounds or a floral blend?

MATERIALS AND METHODS

Pollination effects on volatile emissions

Experiments were conducted to compare changes in volatile emissions between open-pollinated and pollinator-excluded blueberry flowers. Forty bushes were randomly selected from a field of highbush blueberry, *Vaccinium corymbosum* 'Bluecrop', located at the P.E. Marucci Research and Extension Center, Rutgers University (Chatsworth, NJ, USA). Blueberry bushes are propagated vegetatively, so bushes of the same cultivar within a field were genetically identical. From each bush, two stems containing insect- and disease-free flower clusters were selected. One of the stems from each bush ($n = 40$) was bagged with a white organdy mesh bag prior to flower opening. Bags prevented bee access to flowers but allowed exchange of air between ambient air and air inside. A week after (when flowers were fully open), five flower clusters from different plants and from each of the treatments were cut at the stem, placed in a glass vial containing water, and then placed inside chambers for volatile collections (see methods below). Volatiles were collected for 3 h (1100–1400 h), and each treatment was replicated 4 times on two consecutive days. Each replicate consisted of five flower clusters. The same procedure was followed 2 weeks after bagging the flowers (i.e. $n = 20$ clusters of each treatment at day 7 and $n = 20$ of each treatment at day 14).

To test the efficacy of the manipulations, two additional experiments were conducted: one experiment tested the effect of using bags on floral volatile emissions. Potted 2-year-old Bluecrop blueberry plants were placed in a walk-in cold room to mimic winter temperatures. In the spring, plants were moved to a pollinator-free greenhouse ($23 \pm 2\ ^{\circ}C$, 70 % RH, 15 : 9 h light : dark). Treatments consisted of flower clusters enclosed in bags and flower clusters without bags (controls). Each treatment was replicated 4 times ($n = 4$ plants per treatment). The experiment was repeated to collect flower volatiles 7 and 14 d after bagging them. Another experiment was conducted to determine the effect of cutting the stem on the emission of volatiles from flowers. A separate set of blueberry plants ($n = 8$) were grown as described above to promote flowering in the greenhouse. In half of these plants, one stem containing flower clusters was cut at the base, placed in a glass vial, and the flower clusters placed inside a chamber for volatile collections. In the other half of the plants, an intact stem bearing flower clusters was placed inside the volatile collection chamber. Volatiles were identified and quantified using methods described below. It should be noted that bee-visited flowers would be expected to vary in pollination status, pollen amounts and nectar amounts, and may have physical signals associated with bee visitation.

Variation in floral emissions among cultivars

Floral emissions were compared among three highbush blueberry cultivars differing in bloom times: 'Duke' (early cultivar), 'Bluecrop' (mid-season), and 'Elliott' (late). Four bushes were randomly selected from each cultivar located at the P.E. Marucci Center. From each bush, one insect-free flower cluster was bagged prior to flower opening. One week later (when flowers were fully open), flower clusters were cut at the stem, placed in a glass vial containing water, and then placed inside chambers for volatile collections ($n = 4$ per cultivar). Volatiles were collected for 3 h (1100–1400 h), and identified and quantified via gas chromatograph (GC) and mass spectrometry (MS) (see methods below). Volatile collections were conducted at different dates during the blueberry-growing season because of the differences in flowering times among cultivars.

Diurnal pattern of floral volatile emissions and nectar reward

Volatile emissions from highbush blueberry 'Bluecrop', flowers in the day versus night were compared by conducting two consecutive 12-h volatile collections, starting at 0600 h. In addition, to determine the diurnal pattern of volatile emissions, headspace collections were performed at four different times of the day (0600–0900, 0900–1200, 1200–1500 and 1500–1800 h). For both experiments, stems containing two to seven flower clusters were collected from greenhouse-grown, unpollinated blueberries. Two stems (each containing 14–37 open flowers) from four different bushes were placed in a 4-L volatile collection chamber ($n = 4$ per time interval), and volatiles were identified and quantified using the methods described below.

The amount of nectar per flower was quantified from 20 open flowers collected at random from plants growing in the greenhouse. Nectar was extracted with a micropipette, spotted onto a pre-weighed strip of Whatman No. 1 filter paper, and then re-weighed. Nectar amounts were measured at 0700, 0900, 1100, 1300, 1500 and 1700 h. Different flowers of similar age (1–2 d after opening) were used in this study.

Site of volatile emissions in flowers

To determine the floral parts responsible for scent emission, headspace volatiles were collected from intact flowers and from same-age flowers in which certain organs were removed to leave only petals, only the ovary, style and anthers, or only the ovary and style. Flowers were harvested soon after full opening from greenhouse-grown, unpollinated highbush blueberries 'Bluecrop'. Each floral part, from a total of 20 flowers, was placed in a Wheaton 20-mL glass scintillation vial (Fisher Scientific, Pittsburgh, PA, USA). For comparison, volatiles from 20 intact flowers were also collected. Flowers came from four or five different blueberry plants. Headspace volatiles from vials were simultaneously collected from the group of 20 intact flowers or flower parts for three consecutive hours (1100–1400 h) and analysed as described below. Volatiles were collected simultaneously from all treatments, and each treatment was replicated 8 times. Each replicate used a different set of 20 intact flowers or flower parts.

Volatile collections and analysis

A push–pull volatile collection system (Tholl and Röse, 2006; Rodriguez-Saona *et al.*, 2009; Szendrei *et al.*, 2009) was used to measure emissions from blueberry flower clusters. The volatile collection apparatus was located in a greenhouse (under the conditions described above), and consisted of four 4-L glass chambers (Analytical Research Systems, Inc., Gainesville, FL, USA). The chambers had a guillotine-like split plate with a hole in the centre at the base that closed loosely around the stem of the plant. Clean air passed through each chamber at 2 L min^{-1} and was pulled at 1 L min^{-1} through a filter trap containing 30 mg of a Super-Q adsorbent (Analytical Research Systems, Inc.). The remainder of the air vented out through the hole in the bottom of the system to maintain a positive pressure and ensure clean samples. After volatile collection, all open flowers within a cluster were counted to calculate the amounts (ng) of volatiles emitted per flower per hour. A pull volatile collection system (Tholl and Röse, 2006) located in the laboratory at 25 °C, 60 % RH, and 16 : 8 h light : dark, was used to measure emissions from flower parts. Air was pulled at approx. 1·5 L min^{-1} from 22-mL glass vials (Agilent Technologies, Palo Alto, CA, USA), containing each part, into a Super-Q trap that was connected to the vials through an opening in the cap.

To quantify and identify volatiles, filter traps were eluted with 150 μL of dichloromethane. An internal standard of 400 ng of *n*-octane was added to the extract. A 1- μL aliquot of each extract was injected in splitless mode onto a Hewlett Packard 6890 GC equipped with a 10 m × 0·53 mm × 2·65 μm HP-1 column (Agilent Technologies) and a flame ionization detector. Helium was the carrier gas at a 5 mL min^{-1} flow rate. The temperature programme began at 40 °C for 1 min and ramped at 14 °C min^{-1} up to 180 °C, where it was held for 2 min, then ramped again at 40 °C min^{-1} up to 200 °C, where it was held for 2 min. The identity of compounds detected was determined by GC–MS, using a combination of retention index and mass spectral data from NIST library as described in Szendrei *et al.* (2009). The approximate total amount of each compound in each sample was calculated by relating its peak area to that of the internal standard. Volatile emissions were calculated as ng h^{-1} $flower^{-1}$, and only numbers of open flowers were used in this calculation.

Pollination effects on bee visitation and behaviour

A study was conducted to compare bee visitation and behaviours between open-pollinated and pollinator-excluded blueberry flowers. This study was carried out in an unsprayed highbush blueberry 'Jersey', field located at the Horticulture Teaching and Research Center, Michigan State University (East Lansing, Michigan). All plants chosen were free of damage by insects or phytopathogens. The abundance of bees on blueberry flowers was observed over 10 d at the end of May 2009. Ten bushes each containing >60 flower clusters were randomly selected. All flower clusters from five of these bushes were bagged prior to flower

opening (pollinator-excluded bushes), and the remaining five bushes were left unbagged (open-pollinated bushes), with treatments assigned randomly.

One week after initial bagging (when flowers were fully open and when the weather conditions were appropriate for the activity of bees), bags were removed to allow observations of bees on pollinator-excluded and open-pollinated bushes. All observations began at 1100 h, when conditions were suitable for bee activity. The number of bees visiting each of the ten blueberry bushes was determined during 10-min observations per bush. Bees were identified as honey bees, bumble bees or other native bees. A second set of observations was conducted starting at about 1430 h (with the exception of day 5, where the count was stopped because of rain). After each sampling period, the pollinator-excluded bushes were re-covered with the bags, and the procedure described above was repeated every 2 d for 5 total observation days.

In addition, the number of blueberry flowers visited by each bee and time spent per flower were quantified. Because of the difficulty in accurately counting visitation by different types of bees at the same time as quantifying visit duration, these observations began at 1300 h, after the first of the bee observations described above, and using the same bushes. Each bush was observed for 10 min and bees visiting the bushes were monitored to record the number of legitimate visits (flowers visited and touched), and the total time of visiting flowers. From this, the time spent per flower in each visit was calculated. During each observation day, each of the ten bushes was observed a second time from 1600–1800 h.

Bee response to single flower volatiles and floral blend

This study tested the attraction of bees to single blueberry floral compounds or a floral blend and was conducted in 2008 in eight different fields of commercial highbush blueberry 'Bluecrop', four in New Jersey and four in Michigan. In each field, 29 different lures were tested by hanging them inside white plastic Delta traps with sticky liners (ISCA Technologies, Inc., Riverside, CA, USA). All lures were manufactured by ISCA Technologies, Inc. with commercially available compounds, and placed in small plastic vials (2-mL polypropylene screw-cap microcentrifuge tube; Fisher Scientific). Vials were made to release one of 26 single-component flower odour components, a flower blend or a leaf blend, or they were control vials with no blueberry volatiles. All chemical and control vials contained mineral oil. Single component lures contained *n*-tridecane (TCI America, Portland, OR, USA; >99 % purity), 2-undecanone (Bedoukian Research Inc., Danbury, CT, USA; 98 %), α-pinene (Penta Manufacturing Co., Fairfield, NJ, USA; ≥97 %), β-pinene (TCI America; >94 %), caryophyllene (Sigma-Aldrich Corp., St Louis, MO, USA; ≥98·5 %), eucalyptol (Sigma-Aldrich; 99 %), humulene (Sigma-Aldrich; ≥90 %), limonene (Cargill Inc., Minneapolis, MN, USA; ≥95 %), myrcene (TCI America; >70 %), farnesene (Bedoukian Research Inc.; unknown purity), β-farnesene (Bedoukian Research Inc.; 90 %), linalool (Alfa Aesar, Ward Hill, MA, USA; 97 %), ocimene (Sigma-Aldrich; ≥90 %), *cis*-3-hexenyl acetate (Bedoukian Research Inc.; 98 %), *cis*-3-hexenyl butyrate (Bedoukian Research Inc.; 98 %), *cis*-3-hexenyl hexanoate (Bedoukian Research Inc.; 98 %), *cis*-3-hexenyl propionate (Bedoukian Research Inc.; 98 %), *cis*-3-hexenyl methylbutyrate (Bedoukian Research Inc.; 98 %), hexyl acetate (Alfa Aesar; 99 %), hexyl butyrate (TCI America; >98 %), hexyl methylbutyrate (Bedoukian Research Inc.; 95 %), methyl salicylate (Alfa Aesar; 98 %), nonanal (Sigma-Aldrich; 95 %), cinnamyl alcohol (Alfa Aesar; 98 %), linalool oxide (Sigma Aldrich; ≥97 %) or caryophyllene oxide (Sigma-Aldrich; ≥95 %).

The floral blend contained a mixture of α-pinene, β-pinene, *cis*-3-hexenyl acetate, hexyl acetate, limonene, linalool, hexenyl propionate, *cis*-3-hexenyl butyrate, hexyl butyrate, *cis*-3-hexenyl methylbutyrate, hexyl methylbutyrate, 2-undecanone, *n*-tridecane, cinnamyl alcohol, *cis*-3-hexenyl hexanoate, and caryophyllene. The leaf blend contained α-pinene, β-pinene, myrcene, ocimene, eucalyptol, linalool oxide, linalool, methyl salicylate, caryophyllene, humulene, β-farnesene and caryophyllene oxide. Although the floral blend contained a few volatiles found also in leaves, it mainly contained compounds emitted from blueberry flowers. Likewise, the leaf blend contained compounds that are emitted almost exclusively from blueberry leaves. Blends were prepared based on amounts of blueberry leaf volatiles reported by Rodriguez-Saona *et al.* (2009) for the leaf blend, and flower volatiles reported by Szendrei *et al.* (2009) for the floral blend. These blends contained ratios of compounds detected in leaves and flowers.

Two concentrations of each lure were tested: low (30 mg) and high (100 mg). To avoid interactions between the low and high concentration volatile lures, all the low concentration lures with a set of control lures were placed in one section of a field within each farm, while the high concentration lures and a separate set of control lures were placed in a nearby section of the same field. Baited traps were randomly placed at peak bloom, in four rows spaced 3·3 m apart and in the parts of fields that were adjacent to wooded areas. Traps were hung in the top third of bushes, and at least 6 m apart within rows. A completely randomized block design was used, with each treatment replicated 4 times (each farm considered a replicate) and blocked by concentration within farms. The trap positions were rotated each week, and the numbers of honey bees and native bees caught in traps were determined after 4 weeks.

Statistical analysis

Because individual compounds could not be treated as independent variables (Hare, 2011), a two-way multivariate analysis of variance (MANOVA) was used to analyse the effects of pollination (open-pollinated versus pollinator-excluded), time (1 or 2 weeks after exclusion) and their interaction, on total volatile emissions from flowering blueberries. Similarly, one-way MANOVAs were done to test for the effects of cultivar, time of day and flower part on blueberry floral emissions. Volatile compounds were grouped based on their biosynthetic origin into C_6 alcohols and alcohol derivatives, monoterpenes/oxygenated monoterpenes, sesquiterpenes, hydrocarbons, oxygenated hydrocarbons or phenyl propanoid derivatives. Only groups containing more than two compounds were considered for MANOVA. A significant MANOVA was followed by ANOVA to determine which compounds within a group were

affected by treatment (Scheiner, 2001). In addition, the effects of pollination, cultivar, time of day and flower part on patterns of volatile emissions were analysed using principal component analyses (PCA) (Hare, 2011). A one-way ANOVA was used to determine the effects of excising and bagging the blueberry stem on total volatile emissions from flowers. ANOVA was also used to test for the effect of time of day on nectar production, and Pearson correlation was conducted to determine if the total amounts of volatile emissions per flower collected at 0600–0900, 0900–1200, 1200–1500 and 1500–1800 h correlate with the average nectar production per flower at 0700, 1100, 1300 and 1700 ($n = 4$ data points). Prior to analyses, and if needed, amounts were either $\log_{10}(x)$- or $\log_{10}(x + 0{\cdot}05)$-transformed depending on whether the data included zero values. All volatile data were analysed using Minitab Release 13·32 (Minitab Inc., State College, PA, USA).

To compare the number of bees visiting open-pollinated and pollinator-excluded bushes, χ^2 analysis, with $\alpha = 0{\cdot}05$, was used. To compare the number of flowers visited and the frequency of flower visitation, unpaired *t*-tests ($P < 0{\cdot}05$) were used to determine whether significant differences existed between the values in open pollinated or pollinator-excluded treatments. The effect of lure type on number of honey bees and native bees captured in traps was analyzed using ANOVA, with treatment (lure versus blank), concentration (block), and their interaction as independent variables. Because numbers of individual species of native bees in traps was low, all native bee species were summed prior to analysis.

RESULTS

Pollination effects on volatile emissions

Pollinator-excluded flowers emitted 46 % higher amount of volatiles than open-pollinated flowers [total floral volatile emissions (ng h^{-1} $flower^{-1}$): pollinator-excluded flowers = 90·2 ± 8·9 (mean ± s.e.); open-pollinated flowers = 61·6 ± 7·5 (ANOVA: $F = 6{\cdot}19$; d.f. = 1,12; $P = 0{\cdot}029$)], indicating that pollination had a significant effect on volatile emissions from blueberry flowers. Neither time of collection (ANOVA: $F = 1{\cdot}96$; d.f. = 1,12; $P = 0{\cdot}187$) nor pollination × time interaction (ANOVA: $F = 0{\cdot}45$; d.f. = 1,12; $P = 0{\cdot}517$) had an effect on total volatile emissions.

PCA for the effects of pollination on floral volatile emissions resulted in a model with the first two components explaining a total of 70 % of the variation. The score plot of PC1 versus PC2 shows that volatiles from open-pollinated and pollinator-excluded flowers slightly overlap 1 week after treatment, but these two treatments clearly separate after 2 weeks (Fig. 1A). The first PC explained 44 % of the variation of the data and separated floral volatiles in week 1 from those of week 2 (temporal variation) (circles vs. triangles; Fig. 1A), while the second PC explained 26 % of the variation and separated volatiles from open-pollinated and pollinator-excluded flowers (pollination effects) (white vs. black symbols; Fig. 1A). When analysed by chemical group, pollination and time but not their interaction affected emissions of ketones and sesquiterpenes (Table 1). Within chemical groups, pollination and time affected the emission of individual volatiles differently: pollinator-excluded flowers emitted greater amounts of the alcohol cinnamyl alcohol (ANOVA: $F = 22{\cdot}9$; d.f. = 1,12; $P < 0{\cdot}001$), the ketone 2-heptanone (ANOVA: $F = 18{\cdot}04$; d.f. = 1,12; $P = 0{\cdot}001$), and the sesquiterpene β-bourbonene (ANOVA: $F = 13{\cdot}51$; d.f. = 1,12; $P = 0{\cdot}003$) compared with open-pollinated flowers (Fig. 2A–C). Time of volatile collection, but not pollination × time interaction ($P > 0{\cdot}05$), had a significant effect on 2-heptanone (ANOVA: $F = 129{\cdot}16$; d.f. = 1,12; $P < 0{\cdot}001$), such that emissions decreased by the 2nd week of collections (Fig. 2B). On the other hand, open-pollinated flowers emitted greater amounts of the sesquiterpenes β-caryophyllene (ANOVA: $F = 5{\cdot}86$; d.f. = 1,12; $P = 0{\cdot}032$) and γ-cadinene (ANOVA: $F = 7{\cdot}17$; d.f. = 1,12; $P = 0{\cdot}02$) compared with pollinator-excluded flowers (Fig. 2D, E). Emissions of β-caryophyllene increased by the second week of collection (ANOVA: $F = 11{\cdot}65$; d.f. = 1,12; $P = 0{\cdot}005$), but there was no pollination × time interaction (ANOVA: $F = 1{\cdot}7$; d.f. = 1,12; $P = 0{\cdot}217$; Fig. 2D).

Cutting stems at their base prior to volatile collections had no effect on floral volatile emissions [total floral volatile emissions (ng h^{-1} $flower^{-1}$): values from cut stems were 42·1 ± 5·6 (mean ± s.e.) compared with 32·5 ± 2·6 from intact stems (ANOVA: $F = 2{\cdot}55$; d.f. = 1,6; $P = 0{\cdot}161$)]. Also, there was no effect of bagging the stems on volatile emissions from flowers either after 1 week of bagging. Average values from bagged stems were 61·4 ± 25·4 ng h^{-1} $flower^{-1}$, compared with 60·1 ± 16·8 from non-bagged stems (ANOVA: $F = 0{\cdot}15$; d.f. = 1,6; $P = 0{\cdot}713$). A similar pattern was seen after 2 weeks of bagging: bagged stems = 65·5 ± 14·9 vs. 74·6 ± 24·0 from non-bagged stems (ANOVA: $F = 0{\cdot}01$; d.f. = 1,6; $P = 0{\cdot}916$). Thus, these results indicate that neither cutting nor bagging the stems affected the total amount of volatiles emitted from blueberry flowers.

Variation in floral emissions among cultivars

Total amounts of volatile emissions were similar among blueberry flowers of *V. corymbosum* 'Duke', 'Bluecrop' and 'Elliott' (Table 2). However, the score plot shows a clear separation of the volatile profiles among blueberry cultivars (Fig. 1B), with the first two PCs explaining a total of 56·4 % of the variation. Several classes of floral volatiles were influenced by cultivar including esters (MANOVA: Wilks' $\lambda < 0{\cdot}01$; $F = 18{\cdot}49$; $P < 0{\cdot}01$), ketones (MANOVA: Wilks' $\lambda = 0{\cdot}29$; $F = 3{\cdot}38$; $P = 0{\cdot}04$), monoterpenes (MANOVA: Wilks' $\lambda < 0{\cdot}01$; $F = 9{\cdot}64$; $P < 0{\cdot}01$) and sesquiterpenes (MANOVA: Wilks' $\lambda = 0{\cdot}03$; $F = 7{\cdot}39$; $P < 0{\cdot}01$). In particular, the emissions of ten individual odour components were influenced by cultivar (Table 2): 'Duke' emitted the highest amounts of ethyl pentanoate, ethyl-3-hexenoate, limonene, *cis*-3-hexenyl butyrate and β-bourbenene of all cultivars; 'Elliott' emitted higher quantities of *cis*-3-hexenyl acetate, hexyl acetate, *cis*-3-hexenyl butyrate, 2-undecanone and hexyl propionate compared with the other cultivars. On the other hand, 'Bluecrop' emitted the lowest amounts of *cis*-3-hexenyl butyrate. Because volatile emissions from different blueberry cultivars were collected at different times of the spring, the differences reported here could be due to cultivar or seasonality.

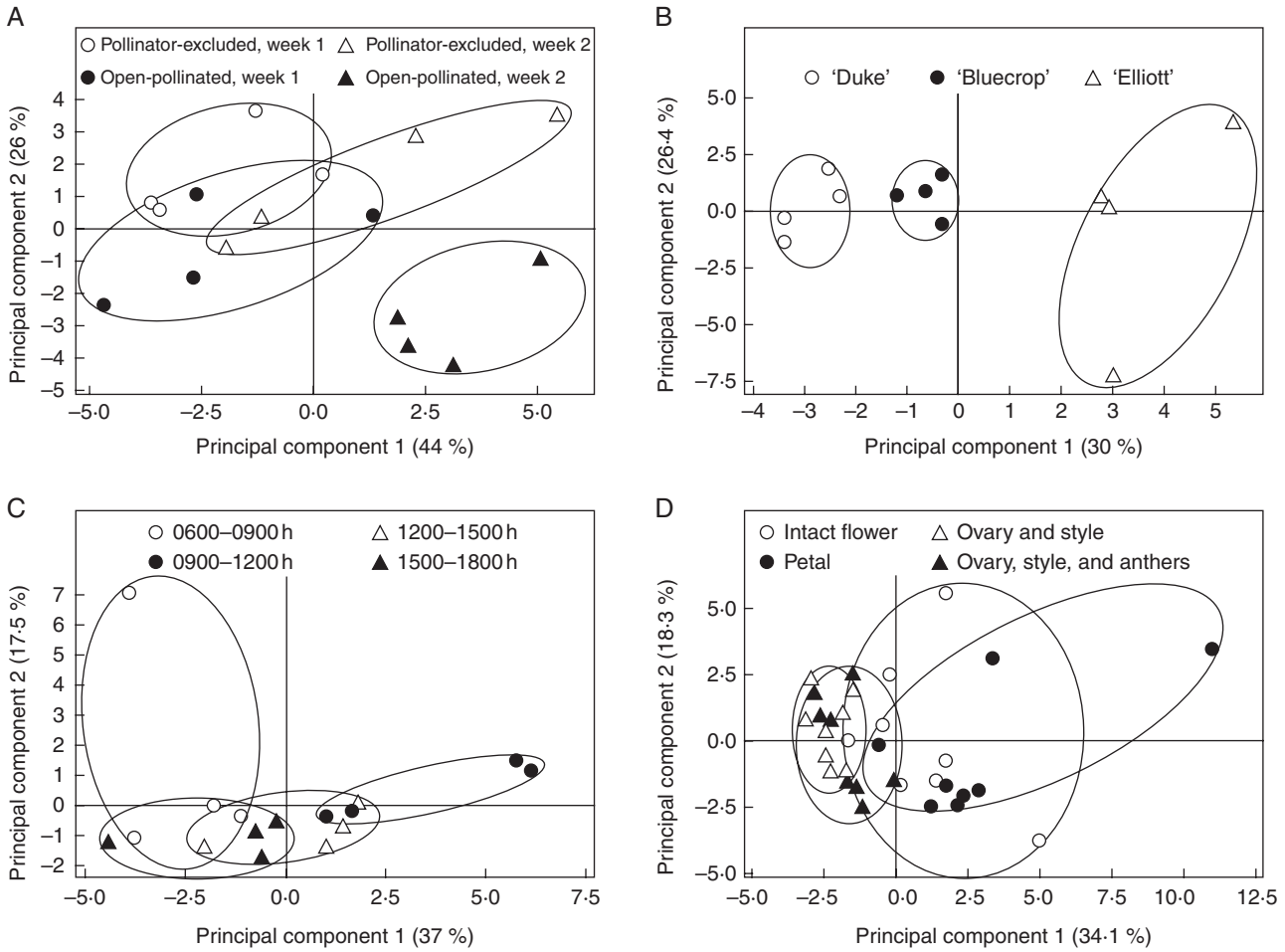

FIG. 1. Score plot of principal component analysis (PCA) for the effects of pollination status (A), cultivar (B), time of day (C) and flower part (D) on the volatile pattern of highbush blueberry (*Vaccinium corymbosum*) flowers. Percentage variation explained by each principal component is indicated in parenthesis.

TABLE 1. *Two-way MANOVA for effects of pollination (open pollinated versus pollinator excluded), time (7 or 14 d after exclusion), and their interaction, on total volatile emissions from blueberry flowers*

	Pollination			Time			Pollination × time		
Volatile class*	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*
Alcohols[†]	0·89	0·67	0·53	0·42	7·73	**< 0·01**	0·59	3·79	0·06
Esters[‡]	0·02	3·58	0·39	<0·01	13·74	0·21	0·04	1·99	0·51
Ketones[§]	0·35	9·86	**< 0·01**	0·06	84·13	**< 0·01**	0·87	0·82	0·47
Monoterpenes[¶]	0·26	3·38	0·07	0·10	10·31	**< 0·01**	0·34	2·27	0·15
Sesquiterpenes[#]	0·20	8·97	**< 0·01**	0·28	5·74	**0·01**	0·55	1·85	0·20

* Significant treatment effects are indicated in bold ($P \leq 0{\cdot}05$).
[†] Alcohols include *cis*-3-hexen-1-ol and hexanol.
[‡] Esters include ethyl pentanoate, ethyl-3-hexenoate, *cis*-3-hexenyl acetate, hexyl acetate, hexenyl acetate isomer, *cis*-3-hexenyl propionate, hexyl propionate, *cis*-3-hexenyl butyrate, hexyl butyrate, *cis*-3-hexenyl methylbutyrate, hexyl methylbutyrate and *cis*-3-hexenyl hexanoate.
[§] Ketones include 2-heptanone and 2-undecanone.
[¶] Monoterpenes include α-pinene, β-pinene, limonene, eucalyptol, ipsdienone and linalool.
[#] Sesquiterpenes include β-bourbonene, β-caryophyllene, γ-cadinene and α-farnesene.

Diurnal pattern of floral volatile emissions and nectar reward

Blueberry flowers emitted approx. 5 times higher amounts of volatiles during the day than at night [mean volatile production (ng h^{-1} $flower^{-1}$ ± s.e.) = 48·6 ± 10·8 in the day, 10·2 ± 2·2 at night (ANOVA: $F = 24{\cdot}04$; d.f. = 1,6; $P = 0{\cdot}003$)]. Emissions during the day were 1·8–2·0 times greater between 0900 and 1200 h compared with between 0600 and 0900 h and 1500 and 1800 h (Table 3). Similarly, the score plot of PC1 versus PC2 shows an overlap between the volatile floral blends emitted at 0600–0900 h and

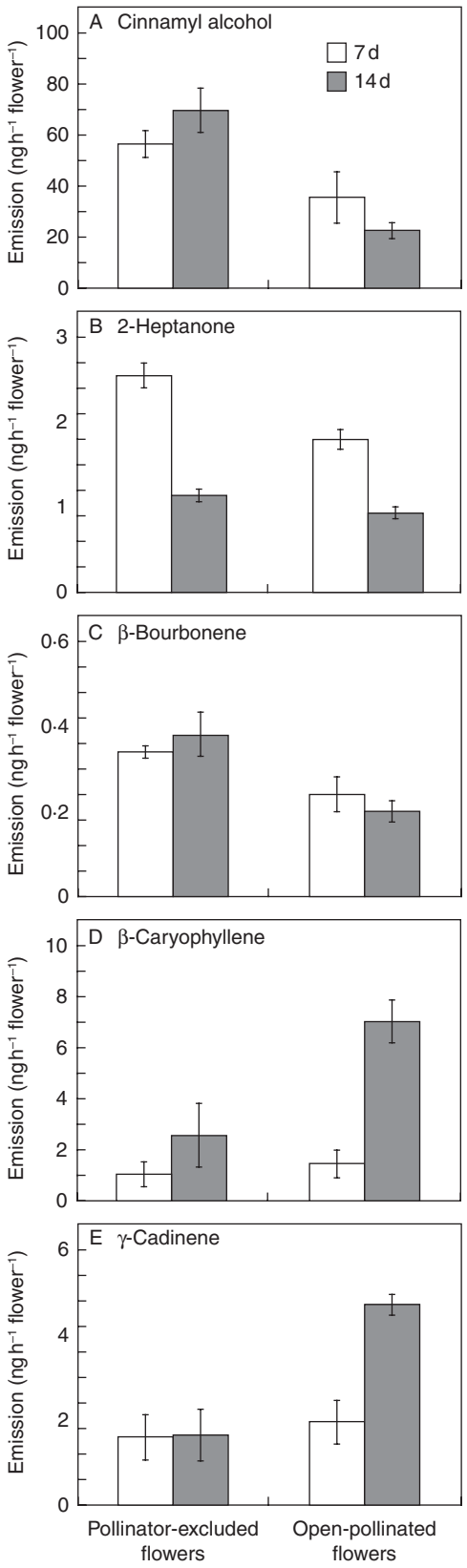

FIG. 2. Emission of selected volatiles (mean ± s.e.) from open-pollinated and pollinator-excluded highbush blueberry (*Vaccinium corymbosum*) flowers 7 and 14 d after exclusion.

1500–1800 h, but both of these blends were clearly different from the blend emitted at 0900–1200 h (Fig. 1C). Emissions of esters (MANOVA: Wilks' $\lambda < 0{\cdot}01$; $F = 13{\cdot}12$; $P < 0{\cdot}01$) and sesquiterpenes (MANOVA: Wilks' $\lambda = 0{\cdot}09$; $F = 4{\cdot}72$; $P < 0{\cdot}01$) were influenced by time of day. Emissions of the esters ethyl-3-hexenoate, *cis*-3-hexenyl propionate and hexyl propionate, and the sesquiterpenes caryophyllene and γ-cadinene peaked between 0900 and 1200 h. Only *cis*-3-hexenyl acetate was emitted in greater quantities between 0600 and 0900 h (Table 3).

Nectar production also peaked at 0900 and 1100 h [mean nectar production (mg ± s.e.) = 0·36 ± 0·13 at 0700, 1·45 ± 0·39 at 0900, 1·14 ± 0·45 at 1100, 0·32 ± 0·13 at 1300, 0·65 ± 0·36 at 1500, and 0·05 ± 0·04 at 1700; $F = 6{\cdot}29$; d.f. = 5,114; $P < 0{\cdot}001$)]. There was a positive correlation between the diurnal pattern of volatile emissions and nectar production (Pearson, $r = 0{\cdot}94$; $P = 0{\cdot}05$).

Site of volatile emissions in flowers

Intact flowers and petals emitted comparable and greater amounts of volatiles than all other floral parts (Fig. 1D and Table 4). Except for alcohols (MANOVA: Wilks' $\lambda = 0{\cdot}81$; $F = 0{\cdot}98$; $P = 0{\cdot}45$), emissions of esters (MANOVA: Wilks' $\lambda = 0{\cdot}04$; $F = 3{\cdot}31$; $P < 0{\cdot}01$), ketones (MANOVA: Wilks' $\lambda = 0{\cdot}26$; $F = 8{\cdot}64$; $P < 0{\cdot}01$), monoterpenes (MANOVA: Wilks' $\lambda = 0{\cdot}12$; $F = 3{\cdot}95$; $P < 0{\cdot}01$) and sesquiterpenes (MANOVA: Wilks' $\lambda = 0{\cdot}51$; $F = 2{\cdot}27$; $P = 0{\cdot}03$) varied depending on floral part. 2-Heptanone, α-pinene, hexenyl acetate isomer, *cis*-3-hexenyl propionate, *cis*-3-hexenyl methylbutyrate, hexyl methylbutyrate, cinnamyl alcohol, β-bourbonene and γ-cadinene were emitted in higher quantities from petals than from ovaries, styles or anthers (Table 4). Cinnamyl alcohol was emitted only from petals, whereas the esters *cis*-3-hexenyl acetate and *cis*-3-hexenyl butyrate were emitted in greater quantities from ovaries and styles (Table 4). The monoterpene linalool was detected only in the samples of ovaries and styles.

Pollination effects on bee visitation and behaviour

A total of 446 insects was recorded during the observations. Honey bees (*A. mellifera*) were the dominant group observed on blueberry flowers, comprising 37·9 % of all insects observed. The next most abundant group was other insects (Coleoptera, Lepidoptera, Diptera and non-bee Hymenoptera) with 32·5 %, followed by bumble bees that were mostly *Bombus impatiens* (19·7 %). The remaining species were other bees, mainly Andrenidae (9·9 %).

A significant difference ($P < 0{\cdot}05$) was found in the number of bees visiting exposed bushes that had previously been bagged or not (Fig. 3). There were no significant differences among treatments on days 1 and 3, when the weather was less suitable for bee flight. However, on day 7, honey bees visited approximately twice as many flowers on pollinator-excluded bushes (mean ± s.e. = 6·8 ± 1·59) and this was also seen on day 9 (5·0 ± 0·95), compared with open-pollinated bushes (3·2 ± 0·66 and 2·0 ± 0·68 on days 7 and 9, respectively). These values were significantly different

TABLE 2. *Variation among blueberry (*Vaccinium corymbosum*) cultivars in floral scent emissions**

Volatiles by chemical class†	Emission (mean ± s.e.; ng h^{-1} $flower^{-1}$)‡			F§
	'Duke'	'Bluecrop'	'Elliott'	
Alcohols				
cis-3-Hexen-1-ol	0·11 ± 0·06	0·10 ± 0·10	0·68 ± 0·56	
Hexanol	0·00 ± 0·00	0·03 ± 0·03	1·90 ± 1·90	
Esters				
Ethyl pentanoate	**0·23 ± 0·03^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**686·01****
Ethyl-3-hexenoate	**0·37 ± 0·07^{a}**	**0·17 ± 0·07^{a}**	**0·00 ± 0·00^{b}**	**14·28****
***cis*-3-Hexenyl acetate**	**0·51 ± 0·13^{b}**	**0·38 ± 0·07^{b}**	**7·14 ± 2·91^{a}**	**29·23****
Hexyl acetate	**0·12 ± 0·07^{ab}**	**0·05 ± 0·05^{b}**	**0·60 ± 0·17^{a}**	**5·82***
Hexenyl acetate isomer	0·58 ± 0·40^{a}	0·27 ± 0·07^{a}	0·61 ± 0·08^{a}	0·88
cis-3-Hexenyl propionate	0·35 ± 0·08^{a}	0·04 ± 0·04^{a}	0·20 ± 0·20^{a}	3·92
Hexyl propionate	**0·71 ± 0·14^{b}**	**1·16 ± 0·09^{ab}**	**3·83 ± 2·21^{a}**	**4·53***
***cis*-3-Hexenyl butyrate**	**0·77 ± 0·16^{a}**	**0·06 ± 0·06^{b}**	**0·80 ± 0·28^{a}**	**15·35****
Hexyl butyrate	0·31 ± 0·09^{a}	0·44 ± 0·08^{a}	0·70 ± 0·13^{a}	3·77
***cis*-3-Hexenyl methylbutyrate**	**0·26 ± 0·07^{a}**	**0·08 ± 0·05^{ab}**	**0·00 ± 0·00^{b}**	**11·07****
Hexyl methylbutyrate	1·84 ± 0·58^{a}	2·38 ± 0·36^{a}	1·40 ± 0·25^{a}	1·82
cis-3-Hexenyl hexanoate	0·03 ± 0·03^{a}	0·04 ± 0·04^{a}	0·40 ± 0·15^{a}	2·71
Ketones				
2-Heptanone	1·02 ± 0·20^{a}	0·29 ± 0·03^{a}	0·45 ± 0·23^{a}	0·21
2-Undecanone	**0·03 ± 0·03^{b}**	**0·09 ± 0·05^{ab}**	**0·46 ± 0·04^{a}**	**6·86***
Monoterpenes				
α-Pinene	0·36 ± 0·05^{a}	0·51 ± 0·06^{a}	0·55 ± 0·19^{a}	0·65
β-Pinene	0·19 ± 0·07^{a}	0·12 ± 0·04^{a}	0·13 ± 0·08^{a}	0·24
Limonene	**0·44 ± 0·06^{a}**	**0·32 ± 0·08^{a}**	**0·00 ± 0·00^{b}**	**176·69****
Eucalyptol	0·30 ± 0·06^{a}	0·20 ± 0·08^{a}	0·69 ± 0·23^{a}	0·40
Myrcenone	0·46 ± 0·11^{a}	0·49 ± 0·17^{a}	1·45 ± 0·90^{a}	0·91
Linalool	0·07 ± 0·04^{a}	0·10 ± 0·06^{a}	0·70 ± 0·36^{a}	1·33
Sesquiterpenes				
β-Bourbenene	**0·77 ± 0·15^{a}**	**0·00 ± 0·00^{b}**	**0·09 ± 0·09^{b}**	**18·43****
Caryophyllene	0·74 ± 0·15^{a}	0·60 ± 0·11^{a}	1·30 ± 0·71^{a}	0·56
γ-Cadinene	0·37 ± 0·08^{a}	0·64 ± 0·14^{a}	0·76 ± 0·28^{a}	1·18
Farnesene	0·05 ± 0·05^{a}	0·14 ± 0·11^{a}	0·10 ± 0·10^{a}	0·26
Phenyl propanoid derivatives				
Cinnamyl alcohol	13·83 ± 4·02	19·47 ± 4·65	20·04 ± 1·07	
Hydrocarbons				
n-Tridecane	0·00 ± 0·00	0·00 ± 0·00	0·06 ± 0·06	
Totals	24·83 ± 5·78^{a}	28·16 ± 5·60^{a}	45·06 ± 6·79^{a}	3·07

* Three-hour collections beginning at 1100 h ($n = 4$).
† Emission of compounds in bold were significantly influenced by genotype.
‡ Means within rows with different letters are significantly different ($P \leq 0·05$).
§ Individual ANOVAs were conducted only after a significant MANOVA for the chemical class (d.f. = 2,9; *, $0·05 \geq P \geq 0·01$; *, $P < 0·01$).

between treatments (χ^2 test: $P < 0·05$ for day 7 and $P < 0·025$ for day 9) (Fig. 3A).

The responses of native bumble bees (*Bombus* spp.) to blueberry bushes were similar to those of honey bees (Fig. 3B). Bumble bees averaged 2·0 ± 0·41 visits to pollinator-excluded bushes compared with no visits to the open-pollinated bushes on day 5 (χ^2 test: $P < 0·05$), and during that day there was heavy rainfall (12·95 mm) in the afternoon, which halted the counting of bees. On day 7, there was an increase in the average number of visits by bumble bees to the pollinator-excluded bushes (mean ± s.e. = 5·6 ± 2·5 vs. 1·2 ± 0·2; $P < 0·025$). In contrast, on day 9 there was no significant difference between the two treatments in the number of bumble bee visits to bushes (mean ± s.e. = 2·8 ± 0·74 vs. 3·6 ± 0·75; $P = 0·5$).

There were no statistical differences in the number of flowers visited by honey bees or bumble bees between pollinator-excluded or open-pollinated bushes, in any of the days of observations (Table 5). As expected, bumble bees generally visited blueberry flowers faster than honey bees. On pollinator-excluded bushes, the range of time spent per blueberry flower was between 4·4 and 10·4 s $flower^{-1}$ for bumble bees, compared with 10·0 to 18·4 s $flower^{-1}$ for honey bees. On open-pollinated bushes, the range was 6·0–15·2 s $flower^{-1}$ for bumble bees and 5·0–24·4 s $flower^{-1}$ for honey bees (Table 5). Although there was no difference between treatments in the number of seconds spent per flower by honey bees; for bumble bees, variation in the time spent per flower was mixed between the treatments. On day 3, bumble bee visiting speed was higher in the open-pollinated bushes than the pollinator-excluded bushes (unpaired *t*-test: $P = 0·025$) (Table 5). In contrast, on day 7, bumble bees spent more time visiting flowers of pollinator-excluded bushes compared with open-pollinated bushes (unpaired *t*-test: $P = 0·008$).

TABLE 3. *Diurnal course of volatile emissions from blueberry (*Vaccinium corymbosum*) flowers**

Volatiles by chemical class[†]	Emission (mean ± s.e. ng h^{-1} $flower^{-1}$)[‡]> 0600–0900 h	0900–1200 h	1200–1500 h	1500–1800 h	F[§]
Alcohols					
cis-3-Hexen-1-ol	0·31 ± 0·21	0·17 ± 0·10	0·06 ± 0·06	0·00 ± 0·00	
Hexanol	0·00 ± 0·00	0·04 ± 0·04	0·00 ± 0·00	0·00 ± 0·00	
Esters					
Ethyl pentanoate	0·05 ± 0·05^{a}	0·25 ± 0·04^{a}	0·10 ± 0·06^{a}	0·12 ± 0·04^{a}	2·31
Ethyl-3-hexenoate	**0·00 ± 0·00^{b}**	**0·14 ± 0·02^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**656·22****
***cis*-3-Hexenyl acetate**	**0·80 ± 0·53^{a}**	**0·17 ± 0·02^{ab}**	**0·07 ± 0·04^{ab}**	**0·05 ± 0·03^{b}**	**3·99***
Hexyl acetate	0·05 ± 0·05^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	1·00
Hexenyl acetate isomer	0·08 ± 0·05^{a}	0·21 ± 0·01^{a}	0·09 ± 0·05^{a}	0·07 ± 0·04^{a}	1·44
***cis*-3-Hexenyl propionate**	**0·00 ± 0·00^{b}**	**0·24 ± 0·05^{a}**	**0·23 ± 0·02^{a}**	**0·16 ± 0·06^{a}**	**13·82****
Hexyl propionate	**0·04 ± 0·04^{b}**	**0·45 ± 0·13^{a}**	**0·27 ± 0·02^{a}**	**0·16 ± 0·05^{ab}**	**5·81****
cis-3-Hexenyl butyrate	0·06 ± 0·06^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	1·00
Hexyl butyrate	0·18 ± 0·11^{a}	0·26 ± 0·04^{a}	0·16 ± 0·06^{a}	0·11 ± 0·04^{a}	0·77
cis-3-Hexenyl methylbutyrate	0·06 ± 0·06^{a}	0·14 ± 0·05^{a}	0·08 ± 0·08^{a}	0·05 ± 0·05^{a}	0·85
Hexyl methylbutyrate	0·40 ± 0·04^{a}	0·54 ± 0·09^{a}	0·39 ± 0·05^{a}	0·27 ± 0·09^{a}	1·44
Ketones					
2-Heptanone	0·41 ± 0·07	0·64 ± 0·08	0·60 ± 0·05	0·44 ± 0·10	
2-Undecanone	0·13 ± 0·05	0·28 ± 0·07	0·16 ± 0·06	0·07 ± 0·04	
Monoterpenes					
α-Pinene	0·00 ± 0·00	0·17 ± 0·08	0·09 ± 0·05	0·04 ± 0·04	
β-Pinene	0·00 ± 0·00	0·06 ± 0·06	0·00 ± 0·00	0·04 ± 0·04	
Limonene	0·07 ± 0·07	0·19 ± 0·03	0·13 ± 0·05	0·02 ± 0·02	
Eucalyptol	0·00 ± 0·00	0·06 ± 0·06	0·19 ± 0·08	0·18 ± 0·10	
Ipsdienone	0·00 ± 0·00	0·21 ± 0·10	0·19 ± 0·08	0·04 ± 0·04	
Linalool	0·05 ± 0·05	0·25 ± 0·07	0·32 ± 0·14	0·17 ± 0·09	
Sesquiterpenes					
β-Bourbenene	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	0·05 ± 0·05^{a}	0·00 ± 0·00^{a}	1·00
Caryophyllene	**0·00 ± 0·00^{b}**	**0·37 ± 0·09^{a}**	**0·11 ± 0·06^{ab}**	**0·02 ± 0·02^{b}**	**7·87****
γ-Cadinene	**0·00 ± 0·00^{b}**	**0·68 ± 0·22^{a}**	**0·66 ± 0·22^{a}**	**0·26 ± 0·26^{b}**	**10·50****
Phenyl propanoid derivatives					
Cinnamyl alcohol	8·27 ± 1·82	14·25 ± 2·47	10·09 ± 1·95	7·60 ± 2·45	
Totals	10·96 ± 1·15^{b}	19·78 ± 1·87^{a}	14·04 ± 1·50^{ab}	9·87 ± 3·01^{b}	4·88*

* Collections were conducted in 3-h intervals over a 12-h sampling period ($n = 4$).
[†] Emission of compounds in bold were significant influenced by time of day.
[‡] Means within rows with different letters are significantly different ($P \leq 0·05$).
[§] Individual ANOVAs were conducted only after a signifcant MANOVA for the chemical class (d.f. = 3,12; *, $0·05 \geq P \geq 0·01$; **, $P < 0·01$).

Bee response to single flower volatiles and floral blend

Traps baited with the floral blend attracted 3·6 and 2·5 times more honey bees in New Jersey and Michigan, respectively, than non-baited control traps (ANOVA, New Jersey: $F = 11·88$, d.f. = 1,12, $P = 0·005$; Michigan: $F = 10·32$, d.f. = 1,12, $P = 0·007$) (Fig. 4, and Table S1 in Supplementary data, available online) (Fig. 4). There was no effect of concentration (ANOVA, New Jersey: $F = 0·19$, d.f. = 1,12, $P = 0·669$; Michigan: $F = 2·48$, d.f. = 1,12, $P = 0·141$) or any evidence of a treatment × concentration interaction (ANOVA, New Jersey: $F = 0·47$, d.f. = 1,12, $P = 0·507$; Michigan: $F = 0·28$, d.f. = 1,12, $P = 0·605$). None of the single-compound lures or the leaf blend had an effect on honey bees or native bees (all P-values $>0·05$). In Michigan, a trap baited with a single volatile compound, a floral blend or a leaf blend caught on average 41·1 ± 2·1 (s.e.), 87·6 ± 16·7, and 47·3 ± 10·6 honey bees, and 0·8 ± 0·1, 1·5 ± 0·6 and 1·0 ± 0·3 native bees, respectively. In New Jersey, a trap baited with a single volatile compound, a floral blend or a leaf blend caught on average 18·0 ± 1·4, 55·5 ± 8·5 and 42·3 ± 15·5 honey bees and 1·5 ± 0·3, 2·5 ± 1·1 and 1·1 ± 0·6 native bees, respectively.

DISCUSSION

The interactions between flowers and their pollinators result from the balance of competing interests. Flowering plants provide nutrients and signals sufficient to attract pollinators to visit flowers while also minimizing investment in these signals, whereas pollinators maximize their nutritional uptake from flowers by exhibiting sensory capacity and behaviours that increase the chance of visiting rewarding flowers. The series of studies presented here illuminate the mechanisms of these interactions for a flower–pollinator interaction between highbush blueberry and bees. Bushes of this plant attract a diverse suite of pollinators dominated by honey bees and bumble bees (Rao *et al.*, 2009; Tuell *et al.*, 2009), and pollination by these insects has become increasingly important as this plant has been domesticated around the world in the past 50 years (Strick and Yarborough, 2005; MacKenzie, 2009).

On the plant side of this interaction, over 50 % of the odour components did not vary significantly among treatments, indicating some stability within the basic flower odour complex. However, it was also found that specific components of the complex floral blend vary after pollination, among cultivars,

TABLE 4. *Emissions of volatiles from parts of blueberry (*Vaccinium corymbosum*) flowers**

Volatiles by chemical class†	Emission (mean ± s.e.; ng h^{-1})‡				F§
	Intact flower	Petals	Ovaries + styles + anthers	Ovaries + styles	
Alcohols					
cis-3-Hexen-1-ol	1·55 ± 0·64	0·80 ± 0·3	1·06 ± 0·50	1·37 ± 0·50	
Hexanol	0·67 ± 0·21	0·76 ± 0·16	0·41 ± 0·15	0·36 ± 0·15	
Esters					
Ethyl pentanoate	0·11 ± 0·11^{a}	0·15 ± 0·10^{a}	0·11 ± 0·07^{a}	0·00 ± 0·00^{a}	0·72
cis-3-Hexenyl acetate	**2·29 ± 0·65^{a}**	**0·78 ± 0·23^{b}**	**2·80 ± 0·81^{a}**	**2·53 ± 0·70^{a}**	**2·86***
Hexyl acetate	0·25 ± 0·12^{a}	0·29 ± 0·17^{a}	0·40 ± 0·14^{a}	0·28 ± 0·15^{a}	0·36
Hexenyl acetate isomer	**0·42 ± 0·14^{a}**	**1·01 ± 0·21^{b}**	**0·00 ± 0·00^{c}**	**0·00 ± 0·00^{c}**	**29·04****
cis-3-Hexenyl propionate	**0·93 ± 0·17^{a}**	**1·28 ± 0·23^{a}**	**0·00 ± 0·00^{b}**	**0·06 ± 0·06^{b}**	**78·47****
Hexyl propionate	0·61 ± 0·12^{a}	0·98 ± 0·13^{a}	0·76 ± 0·15^{a}	0·63 ± 0·06^{a}	1·09
cis-3-Hexenyl butyrate	**0·34 ± 0·15^{ab}**	**0·00 ± 0·00^{b}**	**0·64 ± 0·25^{a}**	**0·90 ± 0·30^{a}**	**3·22***
Hexyl butyrate	0·76 ± 0·29^{a}	0·87 ± 0·31^{a}	1·00 ± 0·08^{a}	0·59 ± 0·10^{a}	1·06
cis-3-Hexenyl methylbutyrate	**1·14 ± 0·30^{a}**	**2·31 ± 0·87^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**48·27****
Hexyl methylbutyrate	**2·06 ± 0·37^{ab}**	**4·21 ± 1·88^{a}**	**0·68 ± 0·21^{b}**	**0·15 ± 0·10^{b}**	**18·37****
cis-3-Hexenyl hexanoate	0·11 ± 0·07^{a}	0·13 ± 0·09^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	1·55
Ketones					
2-Heptanone	**2·06 ± 0·40^{a}**	**2·98 ± 0·47^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**67·27****
2-Undecanone	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	0·07 ± 0·07^{a}	0·00 ± 0·00^{a}	1·00
Monoterpenes					
α-Pinene	**16·74 ± 4·71^{a}**	**24·17 ± 5·12^{a}**	**0·62 ± 0·25^{b}**	**0·31 ± 0·13^{b}**	**30·64****
β-Pinene	0·85 ± 0·62^{a}	0·20 ± 0·11^{a}	0·18 ± 0·09^{a}	0·00 ± 0·00^{a}	1·97
Limonene	0·12 ± 0·08^{a}	0·18 ± 0·09^{a}	0·13 ± 0·09^{a}	0·06 ± 0·06^{a}	0·39
Eucalyptol	0·26 ± 0·13^{a}	0·38 ± 0·16^{a}	0·21 ± 0·10^{a}	0·13 ± 0·08^{a}	0·49
Ipsdienone	0·00 ± 0·00^{a}	0·05 ± 0·05^{a}	0·00 ± 0·00^{a}	0·00 ± 0·00^{a}	1·00
Linalool	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**0·67 ± 0·25^{a}**	**10·34****
Sesquiterpenes					
β-Bourbenene	**0·34 ± 0·14^{ab}**	**0·55 ± 0·23^{a}**	**0·08 ± 0·08^{b}**	**0·00 ± 0·00^{b}**	**4·22***
γ-Cadinene	**0·48 ± 0·17^{a}**	**0·42 ± 0·18^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**5·84****
Farnesene	0·12 ± 0·08^{a}	0·00 ± 0·00^{a}	0·07 ± 0·07^{a}	0·05 ± 0·05^{a}	0·71
Phenyl propanoid derivatives					
Cinnamyl alcohol	**32·91 ± 13·98^{a}**	**63·11 ± 31·88^{a}**	**0·00 ± 0·00^{b}**	**0·00 ± 0·00^{b}**	**120·68****
Totals	65·11 ± 14·65^{a}	105·61 ± 34·68^{a}	9·23 ± 1·56^{b}	8·08 ± 1·86^{b}	28·97**

* Three-hour collections beginning at 1100 h ($n = 8$).
† Emission of compounds in bold varied by floral part.
‡ Means within rows with different letters are significantly different ($P \leq 0·05$).
§ Individual ANOVAs were conducted only after a significant MANOVA for the chemical class (d.f. = 3,28; *, $0·05 \geq P \geq 0·01$; **, $P < 0·01$).

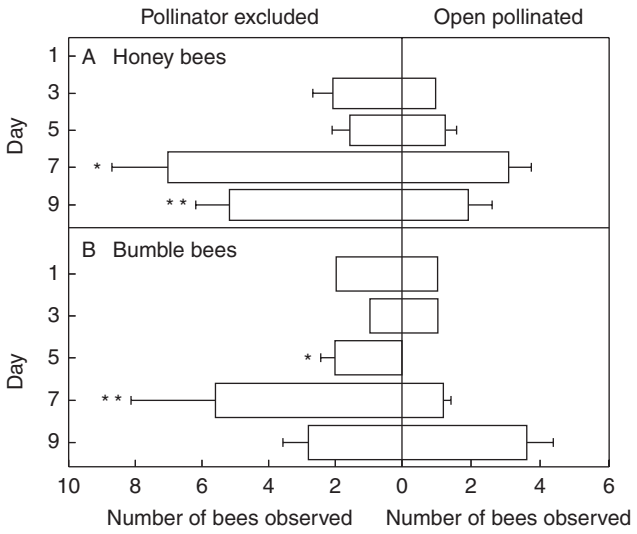

FIG. 3. Number of honey bees (A) and bumble bees (B) (mean ± s.e.) observed in 10-min periods on highbush blueberry (*Vaccinium corymbosum*) plants that were open-pollinated or bagged to exclude pollinators ($n = 10$). *, $P < 0·05$; **, $P < 0·025$.

and over time. Such variability in odour production has been reported previously in other plant systems (Tollsten and Bergström, 1989; Pham-Delegue *et al.*, 1989; Loughrin *et al.*, 1990; Pichersky *et al.*, 1994; Schiestl *et al.*, 1997; Dudareva *et al.*, 2000), and can have important implications for flower discrimination by bees (Pham-Delegue *et al.*, 1989; Schiestl *et al.*, 1997). While there is expected to be less variation within patches of blueberry in the wild than among selected cultivars in this study, the sensitivity of bees to flower odours reported elsewhere (e.g. Wright and Smith, 2004) would suggest that such odours could facilitate bee relocation of patches (or fields) of *V. corymbosum* that are in bloom and also rewarding. Furthermore, emissions from blueberry flowers correlated with nectar production. Honey bees, the main pollinator in the present system, are known to adjust their foraging visits based on nectar production rhythms (Moore *et al.*, 1989), and thus may use floral emissions to time their activity and assess the quality of flowers during foraging.

Most studies that have investigated post-pollination changes in odour production have found some components that decline post-pollination and others that increase (e.g. Schiestl *et al.*,

TABLE 5. *Number of flowers visited per foraging visit and flower-visiting speed for honey bees and bumble bees on blueberry (*Vaccinium corymbosum*) plants that were open pollinated or bagged to exclude pollinators (pollinator excluded)*

	No. of flowers visited (mean ± s.e.)			Time per flower (s; mean ± s.e.)[†]		
Day	Pollinator excluded	Open-pollinated	*t*-Test	Pollinator excluded	Open-pollinated	*t*-Test
Honey bees						
1	0·0 ± 0·0	0·0 ± 0·0	–	0·0 ± 0·0	0·0 ± 0·0	–
3	2·0 ± 1·0	4·0 ± 0·0	1·15	10·5 ± 4·5	5·0 ± 0·0	0·72
5	1·0 ± 0·0	3·3 ± 1·5	1·61	10·0 ± 5·0	24·4 ± 7·3	1·42
7	5·4 ± 0·6	5·2 ± 0·5	0·28	18·4 ± 1·4	19·5 ± 1·8	0·48
9	5·7 ± 0·8	4·6 ± 1·6	0·69	17·8 ± 1·2	13·0 ± 3·0	1·55
Bumble bees						
1	7·0 ± 0·0	0·0 ± 0·0	–	4·4 ± 0·0	0·0 ± 0·0	–
3	9·8 ± 2·7	10·5 ± 6·2	0·11	**7·2 ± 0·6**[a]	**15·2 ± 1·0**[b]	**3·77***
5	12·8 ± 5·2	5·8 ± 1·4	1·3	6·3 ± 1·2	6·3 ± 1·7	0·02
7	8·4 ± 2·1	4·6 ± 0·8	1·68	**10·4 ± 1·1**[a]	**6·1 ± 0·3**[b]	**3·87****
9	8·0 ± 2·0	4·6 ± 0·5	1·63	6·4 ± 1·1	6·0 ± 0·5	0·38

[†] Different letters within rows indicate significant differences between means: *, $0{\cdot}05 \geq P \geq 0{\cdot}01$; **, $P < 0{\cdot}01$ (highlighted in bold).

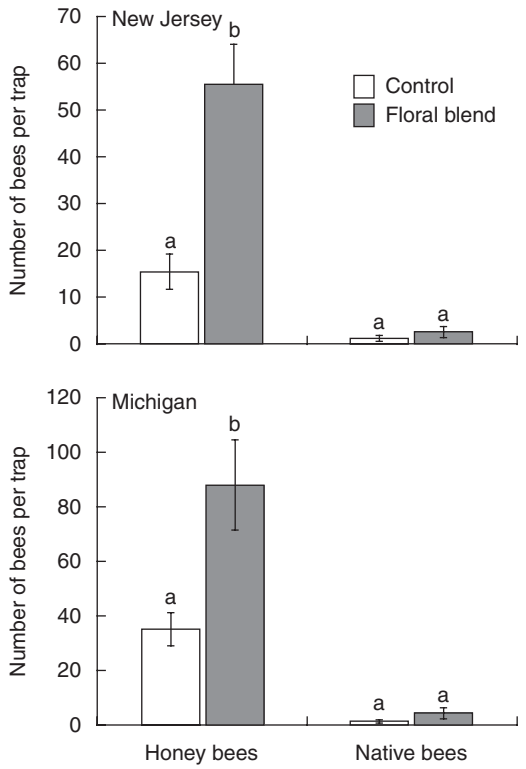

FIG. 4. Numbers of honey bees and native bees (bumble bees and others) captured in control traps and traps baited with a floral blueberry blend. Means with different letters above bars are significantly different ($P \leq 0{\cdot}05$).

1997). Whereas it was found in the present study that release of β-caryophyllene increased after pollination, Theis and Raguso (2005) found that this compound declined within 2–3 d of pollination. This between-system variation in the compounds that change after pollination, and the direction of the change, highlights how flexible generalist pollinators such as honey bees and bumble bees need to be if they are to respond appropriately to odour changes in the plant(s) they are foraging on. The capacity for associative learning of odour cues by honey bees is well documented (e.g. Menzel, 1993; Menzel and Müller, 1996; Komischke *et al.*, 2002). In *V. corymbosum*, flowers with greater β-caryophyllene or γ-cadinene release may provide a sufficient signal for bees to avoid unrewarding flowers, despite the lack of response to specific components in the field trial. Schiestl and Ayasse (2001) reported increased emissions of the bee-repellent farnesyl hexanoate in *O. sphegodes* after pollination. Thus, it is possible that changes in both attractant and repellent floral compounds are involved in bee selection of rewarding flowers. Further manipulative experiments are required to investigate the interplay between rewards from blueberry flowers, associated odours and bee behaviour. It is also possible that transient odour cues left at flowers by visiting bees contribute to the altered odour profile of visited vs. unvisited flowers (e.g. Giurfa and Núñez, 1992), but such changes would be independent of those observed in plant-derived components reported here.

Plants with reduced floral emissions after pollination are expected to increase their reproductive success if pollinators are re-directed to unpollinated flowers (Ayasse, 2006; Dudareva and Pichersky, 2006). In this blueberry system, the greater abundance of honey bees and bumble bees at blueberry bushes that previously had pollinators excluded supports the hypothesis that post-pollination changes in odour reduce bee attraction to flowers. There are two possible explanations for this effect being seen at the level of bee interaction with bushes, but not in their post-arrival behaviour at flowers. First, the changes in flower signals after pollination may operate only over medium distances to attract bees to bushes with some unpollinated flowers, with bees ignoring these cues when foraging within the bush canopy. Alternatively, the bees could use scent differences to locate unpollinated flowers within the high density of flowers on a blueberry bush. The second possibility cannot be ruled out because the open-pollinated bushes had a mix of flowers that were newly opened and those that had been visited previously. If bees can quickly identify these newly opened flowers, similar bee visitation parameters might be expected. It is then possible that these volatile signals may not only benefit a single plant but a group of plants. Use of selective hand-pollination of

flowers on potted bushes in screen cages is one approach that may be worth pursuing for exploring the scale of discrimination of pollination status by bees within and among plants and plant patches. Moreover, it is hypothesized that changes in floral scent after pollination are most important for plants with a low visitation rate and that are pollinator-limited (Dudareva and Pichersky, 2006). This may also apply to blueberries because a single bush produces thousands of flowers and needs to compete with other plants for pollinators.

During bloom of blueberry plants, flowers are visited by both honey bees and bumble bees. Although both species require nectar and pollen for nest survival and growth, honeybee colonies are foraging primarily for nectar whereas bumble bees forage primarily for pollen (Seeley, 1989; Harder, 1990). The results of odour collection from different flower parts suggest that the petals are the primary source of odour in *V. corymbosum.* Two compounds, cinnamyl alcohol (the major component in the floral blend) and 2-heptanone, were emitted exclusively from petals, and both declined after pollination. Although the mechanism for these pollination-induced volatile changes remains unknown, they can be caused by physiological processes associated with flower senescence (Tollsten, 1993; Pichersky *et al.*, 1994). Indeed, post-pollination changes in the chemistry, colour and structure of petals have been observed (Attri *et al.*, 2007; Willmer *et al.*, 2009), and these changes in odour may be related to overall declining health of the flowers as they lose vigour after pollination. In *Plantanthera*, the trigger of the process was suggested to be fertilization of the ovules (Tollsten, 1993).

The fact that honey bees are attracted to a synthetic floral blend indicates that odour cues are important in mediating honey bee interactions with blueberry flowers. Such cues may be more valuable to bees that are visiting flowers to remove nectar since nectar production would be expected to stop as part of the same changes that reduce petal vigour. This is supported by the fact that nectar volume often declines after pollination (Luyt and Johnson, 2002). There may also be some pollen-specific odour cues in *V. corymbosum* that bees foraging for pollen can use to determine the pollen reward status of flowers. A study of three pollen-bearing flowers by Dobson *et al.* (1996) found that pollen-borne odours were chemically distinct from those released by other parts of the flower, similar to the earlier findings of Dobson *et al.* (1990) and Bergström *et al.* (1995). The present data, however, indicate no specific odours in anthers from the comparison of the non-petal parts of the flower and the sample of ovaries and styles.

In conclusion, the present study shows temporal and spatial variation of floral scent emissions in highbush blueberries and documents the role of these scents in blueberry–bee interactions. Consistent with previous studies (Tollsten and Bergström, 1989; Tollsten, 1993; Schiestl *et al.*, 1997; Theis and Raguso, 2005), we showed a clear change in floral scent emission after pollination in highbush blueberries and fewer bees visiting bushes with pollinated flowers. Open-pollinated flowers, however, continued to produce volatiles throughout the duration of the experiment (14 d), albeit at smaller quantities than pollinator-excluded flowers. Schiestl and Ayasse (2001) proposed three possible explanations for this: (1) flowers may need to retain some degree of bee attraction even after pollination; (2) there may be physiological constraints that prevent flowers from ceasing volatile emissions; and (3) a decline in floral volatile emissions might be less detectable to pollinators than an increase in repellent compounds. Although the main blueberry floral volatile cinnamyl alcohol declined after access to pollinators, some minor components increased, suggesting their potential repellent effect. The observed increase in bee attraction together with greater volatile emissions may help guide bees not only to un-visited flowers within plants but also to un-visited flowers from neighbouring plants, thus increasing the fitness of both individual plants and their neighbours. We also showed that emissions of some components from the blueberry floral blend vary among cultivars, time of day and floral part. This high variability in volatile emissions from blueberry flowers as well as the attraction of honey bees to a synthetic floral blend supports an adaptive role of floral signals in guiding bees to flowers.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: complete data set for number of honey bees and native bees captured in the lure-baited traps.

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