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Qualitative and quantitative analyses of flower scent in *Silene latifolia*

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Abstract

The quantitative and qualitative variability in floral scent of 98 specimens of the dioecious species *Silene latifolia* belonging to 15 European and 19 North American populations was determined. Floral scent was collected from single flowers using dynamic headspace methods, and analysed by Micro-SPE and GC-MS methods. The flowers showed a nocturnal rhythm, and scent was emitted only at night. The amount of emitted volatiles varied greatly during the season, from 400 ng/flower/2 min in June to 50 ng/flower/2 min in August and September. The qualitative variability in the floral scent was high and different chemotypes, characterised by specific scent compounds, were found. Female and male flowers emitted the same type and amount of volatiles. The differences in floral scent composition between European and North American populations were small. Typical compounds were isoprenoids like lilac aldehyde isomers, or *trans*-β-ocimene, and benzenoids like benzaldehyde, phenyl acetaldehyde, or veratrole. Some of these compounds are known to attract nocturnal Lepidoptera species. The high qualitative variability is discussed in relation to the pollination biology of *S. latifolia*, and the results are compared with other studies investigating intraspecific variability of flower scent.

Keywords: *Silene latifolia*; Caryophyllaceae; White campion; Flower scent; Intraspecific variation; Micro-SPE, GC-MS, CNESS; Nocturnal rhythmic emission

1. Introduction

Floral odours are important signals for chemical communication between flowering plants and animal pollinators (Pellmyr and Thien, 1986), and may be of importance for reproductive isolation among sympatric, closely related species (e.g. Knudsen, 1999; Levin et al., 2001). Scent is particularly important in night-blooming species when visual cues become inefficient due to darkness (Jürgens et al., 2002; Knudsen and Tollsten, 1993; Miyake et al., 1998; Raguso et al., 2003; Raguso and Pichersky, 1995). *Silene latifolia* Poiret (Caryophyllaceae) is a dioecious European native perennial and produces flowers that open at dusk and normally close soon after dawn. Jürgens et al. (2002) studied the floral scent of *S. latifolia* and found high amounts of lilac aldehyde isomers, veratrole, and benzyl acetate. At least the lilac compounds and benzylacetate are known to attract, alone or together with other compounds, noctuid moths, and they are known as effective antennal stimulants in electroantennographic detections (Meagher, 2002; Plepys et al., 2002a; Raguso and Light, 1998; Raguso et al., 1996). However, Jürgens et al. (2002) conducted their study at night and it is unknown the degree to which floral scent production is related to flower opening or how it varies through the entire anthesis period. Furthermore, last called authors did not distinguish between the scent of male and female flowers of dioecious *S. latifolia*, and as they collected scent from several flowers of an individual, it was also not possible to analyse the scent emitted by single flowers over the entire period of anthesis.

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Several attributes of this plant species make it an interesting subject for the study of evolution and function of floral scent. The dominant flower visitors in Europe are nocturnal Lepidoptera (Altizer et al., 1998; Ellis and Ellis-Adam, 1993; Jürgens et al., 1996), and *S. latifolia* has a specialised relationship with one noctuid species, *Hadena bicruris* Hufnagel (Bopp, 2003; Brantjes, 1976a,b). The female moths pollinate the flowers and then lay their eggs in the female flowers, and the larvae subsequently feed on the developing seeds (Bopp, 2003; Brantjes, 1976a,b). This interaction is one of the 13 known nursery pollination systems (Dufay and Anstett, 2003). *H. bicruris* has dramatic effects on the fitness of its host plant in Europe and is responsible for the destruction of about 25% of all *S. latifolia* fruits produced (Wolfe, 2002). Adult moths are attracted to the *S. latifolia* flowers by the floral scent (Brantjes, 1976a,b).

Yet, *S. latifolia* and its specialised pollinator and seed predator have very different global geographic distributions. The plant was accidentally introduced to North America (McNeill, 1977) about 200 years ago and has subsequently spread throughout most of the continent. *H. bicruris*, however, did not accompany its host plant and is still only found in Europe (Hacker, 1996; Wolfe, 2002). In the introduced range of North America, its most important pollinators are also lepidopterans like different noctuids, geometrids, and sphingids (Altizer et al., 1998; Young, 2002) but the taxa differ from Europe. Given that floral scent profiles may be influenced by pollinators, the question remains to be addressed whether there has been an evolutionary shift in odour compounds in North America in *S. latifolia* in the introduced range. It is of interest to point out that there has been genetically based change in other characters in this species since its introduction. North American individuals of *S. latifolia* germinate faster, display greater growth rates, have higher fecundities, and are more susceptible to enemies than are individuals from Europe (Blair and Wolfe, 2004; Wolfe et al., 2004).

The main goals of our study were to determine qualitative and quantitative variability in floral scent, and to determine if *S. latifolia* odour profiles have differentiated between Europe and North America. In reality, very little is known abut intraspecific variability in flower odor. Most studies in the literature are typically based on small sample sizes (Jürgens et al., 2002). Here we present results of head-space volatile collections from single flowers of 98 *S. latifolia* specimens from 15 European and 19 North American populations. We predicted that North American specimens differ in their scent from European specimens. Blair and Wolfe (2004) and Wolfe et al. (2004) found genetically based changes in morphological traits when comparing European and North American populations, and they found an increased susceptibility to enemies in North American plants. These genetically differences between European and North American populations should also correlate with differences in floral scent. It is furthermore hypothesised that local adaptions to differing pollinator spectra between the two continents result in different floral scent mixtures. As only female flowers are attacked by *H. bicruris*, different selective pressures may affect the two sexes of the plants.

2. Results and discussion

The chemical composition of the flower scent of *S. latifolia* from 15 European and 19 North American populations (see Fig. 1) is summarised in Table 1. The compounds listed in Table 1 are ordered in classes, which to some degree reflect their biosynthetic origin (see Knudsen et al., 1993). We detected 58 compounds and identified 51 volatiles. Most of these substances were also found in the specimens of *S. latifolia* studied by Jürgens et al. (2002), and the results of the two studies are generally consistent. Dominant compound classes were isoprenoids and benzenoids. Additionally, we found

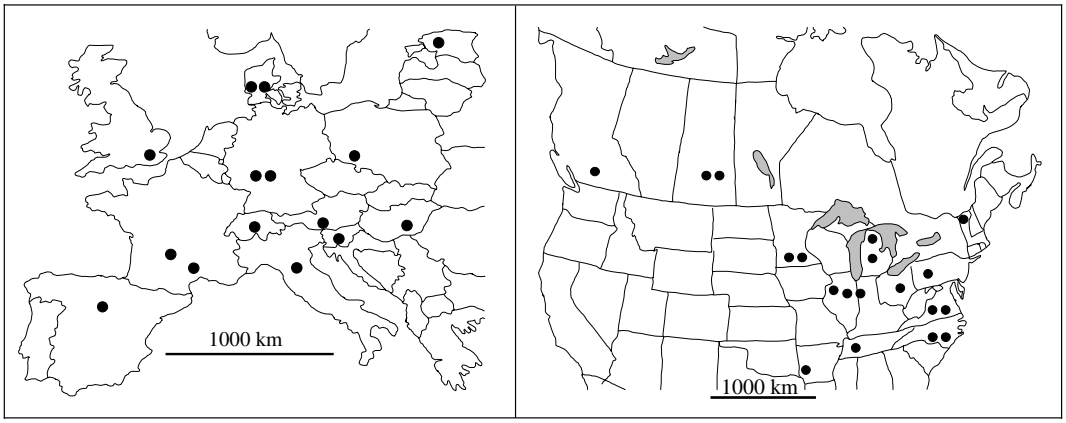

Fig. 1. Geographic origin of the 15 European and 19 North American populations analysed.

Table 1
Occurrence and relative abundance of compounds found in 98 flower scent samples of 15 European populations (=41 samples), and 19 North American populations (=57 samples)

Compound[a]	R_I[b]	Occurence		Relative abundance[e]					
		P[c] (S[d])		Europe			North America		
		Europe	North America	Median	Quartile	Min–Max	Median	Quartile	Min–Max
Fatty acid derivatives									
cis-3-Hexenol	860	14 (32)	19 (48)	0.45	0.04–0.70	0–10.97	0.31	0.10–1.29	0–6.32
cis-3-Hexenyl acetate*[f]	1016	15 (38)	19 (57)	2.66	1.07–5.24	0–26.02	3.70	1.72–6.48	0.19–24.52
2-Hexenol acetate	1025	13 (21)	18 (41)	0	0–0.34	0–1.60	0.12	0–0.36	0–4.44
4-Oxoisophorone	1159	–	1 (2)	–	–	–	0	0–0	0–0.76
Benzenoids									
Benzaldehyde*	982	5 (41)	19 (57)	1.33	0.62–2.45	0.14–74.28	2.04	1.04–4.36	0.17–64.41
Benzyl alcohol*	1050	14 (37)	19 (56)	0.32	0.15–1.00	0–5.45	0.59	0.28–1.17	0–7.56
Phenyl acetaldehyde*	1060	15 (38)	19 (54)	3.36	0.52–8.57	0–47.24	2.16	0.68–23.66	0–83.13
2-Methoxy phenol*	1102	14 (34)	19 (54)	0.75	0.07–1.79	0–10.02	0.91	0.35–2.09	0–9.96
Methyl benzoate	1107	7 (13)	15 (25)	0	0–0.10	0–5.46	0	0–0.50	0–28.69
2-Phenylethanol	1129	14 (30)	18 (46)	0.05	0–0.40	0–9.07	0.12	0.03–0.58	0–3.80
Veratrole*	1153	12 (22)	10 (16)	0.06	0–14.41	0–38.61	0.00	0–0.04	0–23.22
Benzyl acetate*	1174	5 (9)	8 (15)	0	0–0	0–39.94	0	0–0.03	0–23.22
Methyl salicylate	1208	15 (35)	18 (52)	1.16	0.25–2.48	0–14.64	1.04	0.32–2.94	0–14.91
2-Methyl benzaldehyde	1217	1 (3)	–	0	0–0	0–0.40	–	–	–
Benzyl isobutanoate	1306	–	1 (1)	–	–	–	0	0–0	0–0.04
Dimethyl salicylate	1347	3 (3)	3 (3)	0	0–0	0–0.04	0	0–0	0–0.50
Benzyl benzoate*	1789	15 (39)	19 (56)	0.47	0.09–1.27	0–13.03	0.76	0.13–1.56	0–11.82
Phenylpropanoids									
Benzenepropanal	1178	2 (3)	7 (8)	0	0–0	0–5.02	0	0–0	0–1.70
Benzenepropanol	1243	7 (12)	11 (18)	0	0–0.03	0–4.53	0	0–0.30	0–13.87
trans-Cinnamaldehyde	1287	12 (23)	16 (41)	0.03	0–0.25	0–2.05	0.13	0–0.73	0–7.04
trans-Cinnamyl alcohol	1319	10 (17)	15 (34)	0	0–1.07	0–8.41	0.26	0–1.98	0–20.90
Benzenepropyl acetate	1380	1 (1)	4 (5)	0	0–0	0–0.67	0	0–0	0–0.56
Methyleugenol	1408	–	1 (1)	–	–	–	0	0–0	0–0.09
Cinnamyl alcohol acetate	1454	2 (3)	7 (8)	0	0–0	0–0.23	0	0–0	0–0.22
trans-Isoeugenol*	1463	–	1 (1)	–	–	–	0	0–0	0–0.05
N-bearing compounds									
3-Methyl-butyl aldoxime*	858	6 (9)	12 (21)	0	0–0	0–3.84	0	0–0.54	0–5.17
3-Methyl-butyl aldoxime*	870	6 (8)	11 (17)	0	0–0	0–2.30	0	0–0.14	0–3.23
Indole*	1307	3 (5)	7 (15)	0	0–0	0–6.09	0	0–0.01	0–3.36
Monoterpenoids									
α-Pinene*	957	15 (39)	18 (51)	0.22	0.11–1.30	0–6.41	0.35	0.10–0.74	0–5.90
β-Pinene*	995	15 (35)	17 (45)	0.19	0.07–0.69	0–5.94	0.25	0.03–0.57	0–5.69
β-Myrcene*	1001	–	1 (3)	–	–	–	0	0–0	0–6.09
trans-β-Ocimene*	1058	11 (27)	6 (39)	1.91	0–6.94	0–25.84	3.24	0–8.90	0–22.68
Lilac aldehyde A*	1154	15 (41)	19 (57)	18.28	12.41–22.37	2.22–29.71	14.19	10.20–19.04	0.18–31.88
Lilac aldehyde B + C*	1163	15 (41)	19 (57)	28.41	18.61–33.56	3.88–41.22	22.45	15.68–28.75	0.23–40.33
Lilac aldehyde D*	1178	15 (41)	19 (56)	5.19	4.51–6.77	1.12–11.36	5.56	3.19–7.28	0–10.20
Lilac alcohol A*	1211	15 (40)	19 (54)	0.24	0.09–0.57	0–5.01	0.15	0.09–0.26	0–2.87
Lilac alcohol B + C*	1219	14 (37)	19 (52)	0.2	0.12–0.32	0–1.29	0.22	0.12–0.39	0–1.66
Lilac alcohol D*	1232	14 (37)	19 (51)	0.12	0.04–0.23	0–1.04	0.11	0.06–0.20	0–3.59
Lilac degradation	1341	15 (40)	18 (48)	0.21	0.15–0.26	0–0.39	0.18	0.10–0.26	0–0.41
Lilac degradation	1352	15 (41)	19 (57)	6.23	4.31–7.66	0.82–10.21	5.13	3.48–6.54	0.09–9.26
Lilac alcohol formate	1360	4 (5)	2 (2)	0	0–0	0–0.15	0	0–0	0–0.13
Sesquiterpenoids									
α-Longipinene	1377	2 (2)	3 (4)	0	0–0	0–0.30	0	0–0	0–0.16
Longicyclene	1401	2 (2)	3 (4)	0	0–0	0–0.17	0	0–0	0–0.15
α-*cis*-Bergamotene	1430	–	1 (1)	–	–	–	0	0–0	0–0.34
ST	1441	12 (18)	14 (31)	0	0–0.02	0–0.23	0.01	0–0.06	0–0.35
trans-β-Caryophyllene*	1450	5 (5)	4 (7)	0	0–0	0–0.36	0	0–0	0–0.30
α-*trans*-Bergamotene	1452	–	1 (1)	–	–	–	0	0–0	0–0.02
(*E,E*)-α-Farnesene	1512	3 (4)	6 (10)	0	0–0	0–0.64	0	0–0	0–2.30
α-Selinene	1521	–	1 (81)	–	–	–	0	0–0	0–0.04

(*continued on next page*)

Table 1 (*continued*)

Compound[a]	R_I[b]	Occurence		Relative abundance[e]					
		P[c] (S[d])		Europe			North America		
		Europe	North America	Median	Quartile	Min–Max	Median	Quartile	Min–Max
Unknown	1535	–	2 (2)	–	–	–	0	0–0	0–0.06
δ-Cadinene	1543	1 (1)	–	0	0–0	0–0.13	–	–	–
7-*epi*-α-Selinene	1548	–	2 (2)	–	–	–	0	0–0	0–0.06
trans-Nerolidol	1569	1 (1)	3 (6)	0	0–0	0–0.06	0	0–0	0–0.80
Dendrolasin	1582	2 (3)	4 (8)	0	0–0	0–0.18	0	0–0	0–0.29
Unknowns									
m/z 93,43,55,111,91,38	1067	11 (24)	15 (34)	0.12	0–0.41	0–1.70	0.09	0–0.46	0–1.71
m/z 93,43,55,111,91,77	1083	10 (12)	11 (17)	0	0–0.06	0–0.79	0	0–0.18	0–1.25
m/z 43,125,85,41,42,56	1145	13 (29)	16 (32)	0.28	0–0.71	0–1.46	0.10	0–0.46	0–1.30

[a] Compounds within classes are listed according to Kovat's index.
[b] Kovat's retention index.
[c] P = number of populations where a compound was detected.
[d] S = number of scent samples where a compound was detected.
[e] Relative proportion (%) of the compounds in the floral scent bouquets of the 98 samples.
[f] Compounds with asterisk were identified by comparison of mass spectrum and retention data with those of authentic standards.

fatty acid derivatives, phenylpropanoids and nitrogen-containing compounds. The most commonly occurring compounds found in all samples were lilac aldehyde isomers and benzaldehyde. Lilac aldehydes are oxygenated monoterpenes with three chirality centres, and therefore eight different isomers are possible (lilac aldehydes A, B, C, D each with two enantiomeric isomers). Burkhardt and Mosandl (2003), kreck and Mosandl (2003) and Kreck et al. (2003) studied the lilac aldehydes in detail and found only four of the eight possible isomers in the nocturnally pollinated *Syringa vulgaris* L. Commonly occurring compounds in the flower scent of *S. latifolia* were also the fatty acid derivative *cis*-3-hexenyl acetate, the benzenoids phenyl acetaldehyde and methyl salicylate, and the isoprenoid *trans*-β-ocimene. Most of these compounds have been repeatedly found by several authors in species pollinated by noctuids or hawkmoths (Jürgens et al., 2002; Kaiser, 1993; Knudsen and Tollsten, 1993; Miyake et al., 1998), and at least for some of these components it is shown that they attract potential *S. latifolia* pollinators, especially noctuid Lepidoptera species (Bruce and Cork, 2001; Fraser et al., 2003; Haynes et al., 1991; Heath et al., 1992; Meagher, 2001, 2002; Plepys et al., 2002b). Plepys et al. (2002b) e.g. tested authentic samples of methyl salicylate and lilac aldehydes on the generalistic noctuid moth *Autographa gamma* L. and found both substances to be attractive. Moreover, responses of *A. gamma* to a mixture of lilac aldehyde isomers were similar as to a mixture of in total 9 attractive compounds (including lilac aldehydes), and the lilac aldehydes elicited significantly more responses than any other compound tested (Plepys et al., 2002b). *A. gamma* can often be found on *S. latifolia* removing nectar (Jürgens et al., 1996; S. Dötterl, personal observation) and may be attracted to the flowers by the lilac aldehydes. These isoprenoids are also most attractive for the nursery pollinator of *S. latifolia*, the noctuid moth *H. bicruris* (Dötterl, 2004). An isomeric mixture of lilac aldehydes was as attractive to the moths as single flowers of *S. latifolia*. All other floral scent compounds of *S. latifolia* that were tested in a wind tunnel were less attractive.

2.1. Quantitative variation in floral scent

S. latifolia is a nocturnal species, with flowers opening at dusk between 8 and 10 p.m., and normally closing in the morning (Jürgens et al., 1996). To determine the amount of volatiles emitted from a single flower during a whole day, volatiles were collected each hour for 2 min during a time period of 28 h. The results obtained from these analyses indicate that opening of flowers is correlated with emission of scent, resulting in a nocturnal scent emission (Fig. 2(a)). This pattern is also known from other night-flowering plants such as *Stephanotis floribunda* (R. Br.) Brongn. (Matile and Altenburger, 1988), and *Hoya carnosa* (L. f.) R. Br. (Altenburger and Matile, 1988). Emission of all scent compounds starts at dusk after flower opening, reaches a maximum between 2200 and 2400 h, decreases until next morning, and starts again at dusk of the next day. During the day almost no volatiles are emitted. To study the pattern of volatile emission at dusk/night with higher temporal resolution, volatiles were collected every 5 min for 2 min from three flowers starting at dusk, during a time period of 67 min (Fig. 2(b)). The amount of emitted volatiles increased very rapidly after flower opening. For example, after 55 min flower 1 emitted about sevenfold more volatiles than after 30 min (30–32 min after the first sampling 89 ng/2 min, and 55–57 min after the first sampling 590 ng/2 min). To our knowledge, a measurement of quantitative change in scent emission over time

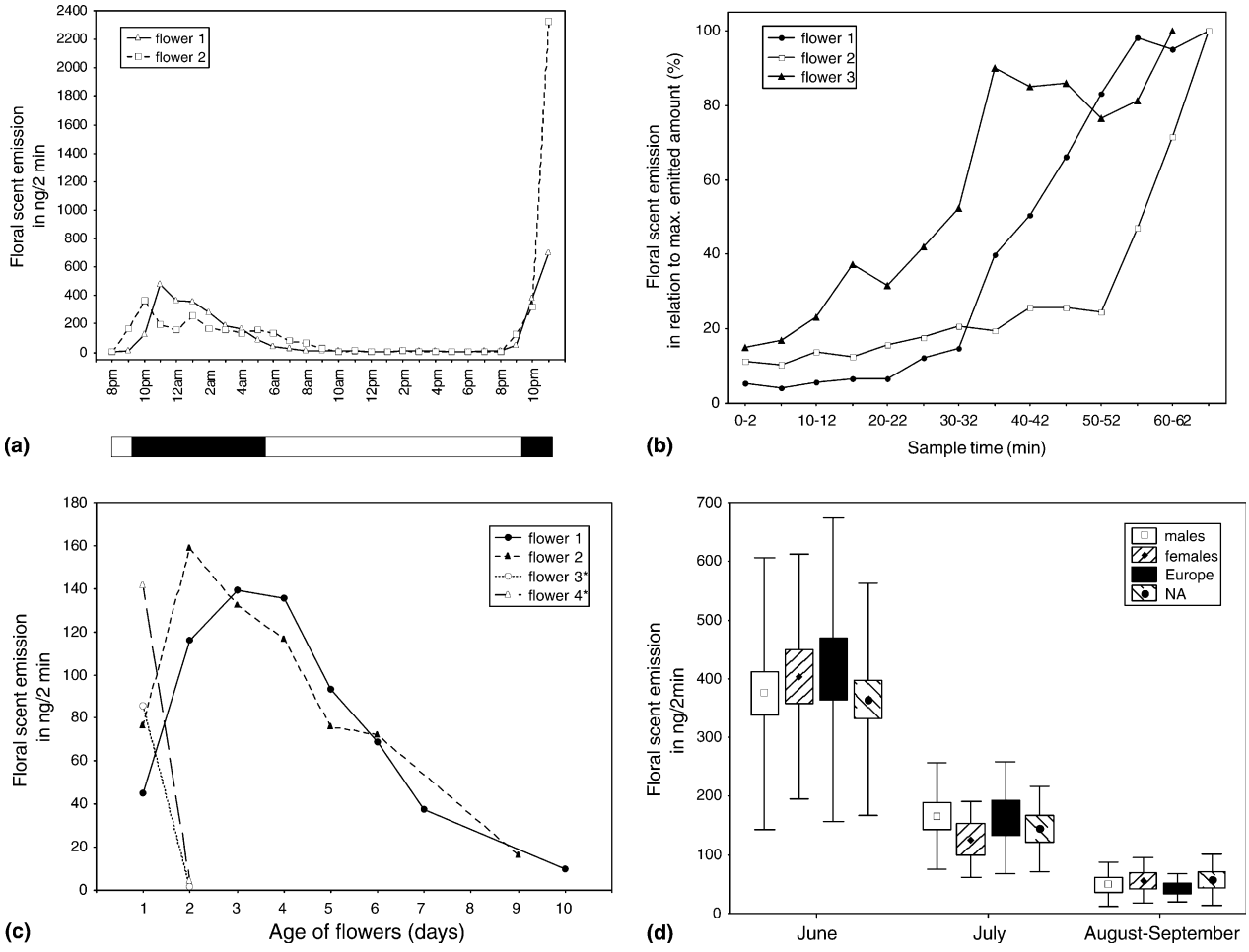

Fig. 2. Quantitative variation in scent of single *S. latifolia* flowers. (a) Rhythm of floral scent emission; (b) detailed pattern of floral scent production starting at dusk; (c) floral scent emission during the life of flowers (*: flowers 3 and 4 were handpollinated on 1st day); (d) emission of volatiles during the flowering season (mean, SE, SD).

with such a high temporal resolution is only reached with one other technique, the so called zNose™ (see Kunert et al., 2002).

In Fig. 2(c) the floral scent emission during the lifespan of four female flowers is presented. Flowers 3 and 4 were hand-pollinated in the first night of anthesis. These pollinated flowers wilted before the next evening and did not emit volatiles during the second night. Several authors have described post-pollination effects, such as permanent flower closure or colour changes (see review by van Doorn, 1997), however, only few data are available describing post-pollination changes in floral scent. In *Platanthera bifolia* (L.) Rich. (Orchidaceae) small changes in composition of volatiles are described, while the total amount of emitted compounds significantly decreased one, two, and five days after pollination (Tollsten, 1993; Tollsten and Bergström, 1989). In the sexually deceptive orchid, *Ophrys sphegodes* Mill. ssp. *sphegodes*, pollination changes the composition of the scent bouquet (Schiestl et al., 1997; Schiestl and Ayasse, 2001). Flowers of this orchid are pollinated by males of the bee *Andrena nigroaenea* (Kirby). The bees are attracted to the flowers especially by different alkenes together with alkanes (Schiestl et al., 2000). While these attractive compounds decrease slightly after pollination, the amount of farnesyl hexanoate increases, resulting in a reduced attractiveness of the pollinated flowers. Therefore, increasing farnesyl hexanoate may be a strategy of the plant to increase their reproductive success by guiding pollinators to unpollinated flowers of the inflorescence (Schiestl and Ayasse, 2001).

In *S. latifolia* the rapid decline of scent emission could be a strategy to avoid or to reduce the parasitism rate by *H. bicruris*. *S. latifolia* relies on different nocturnal Lepidoptera species for pollination (Altizer et al., 1998; Jürgens et al., 1996; Shykoff and Bucheli, 1995; Young, 2002), and at least for some pollinators, among them *H. bicruris*, it has been shown that they are attracted by floral scent (Brantjes, 1976a,b,c; Dötterl, 2004). *H. bicruris* can be found in 90% of European populations, and is responsible for the destruction of about 25% of all *S. latifolia* fruits produced (Wolfe, 2002). Be-

cause risk of parasitism by *H. bicruris* increases with female flower life-span, an immediate arrest of floral scent emission within 24 h might be a mechanism to lower predation risk.

The mechanisms regulating the emission of floral scents after pollination are mostly unknown, but were studied in snapdragon and petunia flowers in case of methyl benzoate (for details see Negre et al., 2003). The postpollination decrease in emission of this benzenoid is mediated by ethylene, and began only after pollen tubes reached the ovary, a process which took 32 h in petunia, and between 35 and 40 h in snapdragon. In *S. latifolia*, this process normally takes less than 24 h (E. Mayer, personal communication). Thus, pollen tubes reaching the ovary may also be a trigger for female flowers to stop scent production.

The unpollinated flowers (flower 1 and flower 2 in Fig. 2(c)) emitted volatiles until they were 9 or 10 days old. The maximum amount of compounds was emitted on day 2 or 3 of anthesis, followed by a continuous decrease until they wilted. A decrease of emitted volatiles during a flower's life-span was also found in other plants such as *P. bifolia* (Tollsten, 1993), and *H. carnosa* (Matile and Altenburger, 1988).

In *S. latifolia*, the total emitted amount of volatiles, which is independent from the sex ($F_{df = 96;1} = 0.02$, $p = 0.9$), and the origin (Europe, North America, $F_{df = 96;1} = 0.01$, $p = 0.9$) of the plants, changed during the season ($F_{df = 95;2} = 23.2$, $p < 0.001$, see Fig. 2(d)). In June, flowers produced significantly more volatiles than in July, August and September. Weather conditions were probably responsible for this phenomenon, because from the beginning of July it was very hot and dry. A single 1st day flower produced about 400 ng/2 min in June indicating a high investment of the plant in production of floral volatiles. The emission of high amounts of volatiles may increase the attractiveness to pollinators and may be important to ensure high reproductive success in *S. latifolia*. Comparably high amounts of emitted volatiles were found in *Berberis aquifolium* Prursh (Landolt and Smithhisler, 2003), Hybrid Tea rose 'Fragrant Cloud' (Kaiser, 1994), *Rosa hybrida*, cv. Honesty (Helsper et al., 1998), *Geonoma macrostachys* (Knudsen et al., 1999), *Grias peruviana* (Knudsen and Mori, 1996), and *Anthirrinum majus* L. (Kolosova et al., 2001). However, it is hard to directly compare the data of *S. latifolia* with other species because of different sampling methods. We applied a thermal desorption method (Micro SPE), while in most other papers solvent extraction methods (with some sample loss during preparative treatment) were used.

2.2. Qualitative variation between samples

Qualitative differences of the chemical composition between individual samples, based on the CNESS index

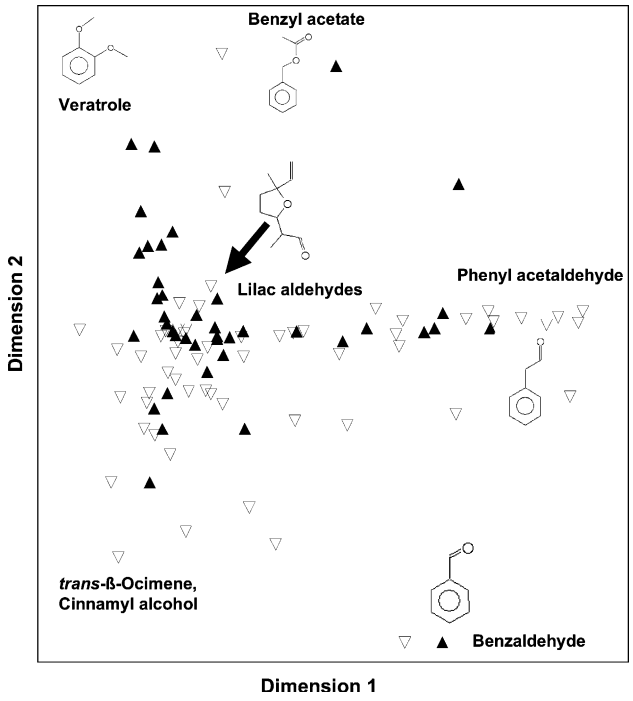

Fig. 3. Nonlinear multidimensional scaling (NMDS) of the floral scent profile of 41 European (filled triangles) and 57 North American (open triangles) samples based on the CNESSm1 index.

($m = 1$) are visualised using non-linear multidimensional scaling (stress = 0.16) in Fig. 3. Variation between the samples was very high and we found several chemotypes. The dominant chemotype was characterised by high amounts of lilac aldehyde isomers. We also found samples with almost no lilac aldehydes, but high amounts of phenyl acetaldehyde (phenyl acetaldehyde chemotype). We also found benzaldehyde, veratrole, and benzyl acetate dominated chemotypes. Another group of samples was characterised by *trans*-β-ocimene or cinnamyl alcohol.

Most compounds listed in Table 1 were found in both, European and North American specimens, and only some minor compounds were detected either in European or North American specimens (e.g. 4-oxoisophorone, myrcene). Variation of samples within Europe was significantly lower than variation of samples within North America, and also lower than variation between European and North American samples (Kruskal–Wallis-ANOVA: H (2;4753) = 39.1; $p < 0.001$). Mean dissimilarity (CNESS) within European samples was 0.76 (quartile: 0.52–1.03), within North American samples 0.86 (quartile: 0.59–1.13), and between European and North American samples 0.82 (quartile: 0.59–1.08). These results indicate that the variability in North America is higher than the variability in Europe, and that there are in total no clear differences between European and North American samples. Many of the populations sampled here were also studied by Blair and Wolfe (2004), who used *S. latifolia* to determine if

there has been genetically based change in life history characters, and by Wolfe et al. (2004), who compared susceptibility of *S. latifolia* to enemies, between Europe and North America. In both studies, and contrary to the floral scent analyses, clear differences between European and North American plants were found. Nevertheless, there is also in floral scent some variation between these two regions, and when comparing the relative abundances of compounds between European and North American populations in a variance component analysis, we found four substances, explaining 97% of total variation between these two regions: veratrole (37%), phenyl acetaldehyde (35%), the coeluting lilac aldehydes B + C (18%), and lilac aldehyde A (6%). Veratrol was characteristic for some European populations (see Fig. 3) and was more abundant in Europe than in North America (*U* test: $Z_{df1 = 40,df2 = 56} = 2.7$; $p = 0.007$; see Fig. 4). Phenyl acetaldehyde was frequently found in European and North American populations, and there was no difference between the mean abundances (*U* test: $Z_{df1 = 40,df2 = 56} = -0.67$; $p = 0.50$). However, there were some North American samples where phenyl acetaldehyde was the most abundant compound, reaching up to 83% (see Fig. 4). The lilac compounds lilac aldehyde A and lilac aldehydes B + C were more abundant in Europe than in North America (see Fig. 4, *U* tests: $Z_{df1 = 40,df2 = 56} = 2.03$; $p = 0.04$ resp. $Z_{df1 = 40,df2 = 56} = 2.20$; $p = 0.03$).

The observed high variability of the flower scent of *S. latifolia* is comparable to other plant species, like the Orchidaceae species *P. bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Tollsten and Bergström, 1993), *Epipendrum ciliare* L. (Moya and Ackerman, 1993), *Stanhopea* Frost ex Hook. species (Whitten and Williams, 1992), the Magnoliaceae *Magnolia kobus* DC. (Azuma et al., 2001), and the Apiaceae

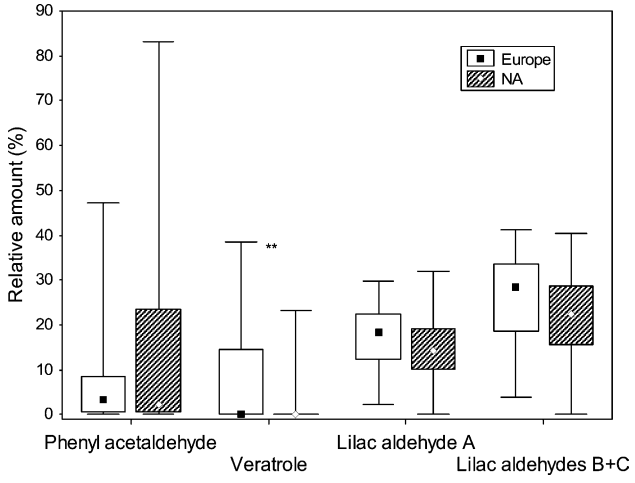

Fig. 4. Compounds most responsible for variation in floral scent between European and North American populations (Median, Quartile, Min–Max).

Conopodium majus (Gouan) Loret (Tollsten and Øvstedal, 1994), and different explanations were offered for this variability. It was discussed that different chemotypes may be equally effective in attracting pollinators (Azuma et al., 2001; Whitten and Williams, 1992), that different chemotypes may be an adaption to different pollinators (Tollsten and Bergström, 1993; Tollsten and Øvstedal, 1994; Whitten and Williams, 1992), or that different chemotypes may be the result of random genetic drift in isolated populations (Tollsten and Bergström, 1993). It has also been argued that high variability in floral scent chemistry may be due to the importance of visual cues in the reproductive biology of the studied species (Azuma et al., 2001), or in the case of the non-rewarding moth-pollinated orchid *E. ciliare*, a pollination strategy was suggested that disrupts associative learning processes and inhibits pollinators ability to recognise non-rewarding flowers (Moya and Ackerman, 1993).

Important pollinators or at least flower visitors of *S. latifolia* in Central Europe are different noctuids, like *H. bicruris*, *A. gamma*, *Diachrysia chrysitis* L., and *Cucullia umbratica* L., or different sphingids, such as *Deilephila porcellus* L., *D. elpenor*, and *Proserpinus proserpina* Pall. (Jürgens et al., 1996). North American *S. latifolia* specimens are pollinated or visited at night by noctuids like *Amphipoea americana* Speyer, *Autographa californica* Speyer, *A. precationis* Guenée, *Hadena variolata* Smith, and *Leucania multilinea* Walker, by sphingids such as *Hyles lineata* (Fabricius), *H. gallii* Rottenburg, and *Sphinx kalmiae* J.E. Smith, and by geometrids like *Euchlaena serrata* (Drury), and *Itame pustularia* Guenée (Altizer et al., 1998; Young, 2002). However, with the exception of *A. gamma* and *H. bicruris*, which are strongly attracted to lilac aldehydes (Dötterl, 2004; Plepys et al., 2002b), and *Hyles lineata*, which elecrophysiologically responds to several volatiles (Raguso et al., 1996) occuring in *S. latifolia*, nothing is known about the importance of single floral scent compounds of *S. latifolia* for the attraction of these pollinators and/or flower visitors. One explanation for the variability of the floral scent in *S. latifolia* thus may be, that these moth species are attracted by different compounds of *S. latifolia*, and that different chemotypes are therefore equally effective in attracting pollinators. The flower visitors in North America are differing from the flower visitors in Europe, and it would be very interesting to know, whether North American flower visitors of *S. latifolia* are more effectively attracted by phenyl acetaldehyde than by veratrole or the lilac aldehydes, because phenyl acetaldehyde is more abundant in North American than in European populations, and veratrole and lilac aldehydes are more abundant in Europe than in North America (see Table 1, Fig. 4). These differences may be the result of adaption to different flower visitors.

Also random genetic drift could be responsible for the high variability especially in North America, where *S. latifolia* was introduced about 200 years ago (McNeill, 1977), and where the floral scent profile is more variable than in Europe. These introductions resulted almost certainly in small, isolated populations favouring genetic drift.

3. Conclusions

We have shown that the variability in the floral scent of *S. latifolia* is very high. However, the factors responsible especially for the qualitative variability are not yet clear. Pollinators as well as genetic factors could influence floral scent evolution. Behavioural studies with pollinators testing the attractivity of different floral scent chemotypes, and analyses combining floral scent data with genetic data would be very helpful to understand the variability of floral scent.

4. Experimental

4.1. Plant material

Floral scent samples of *S. latifolia* were collected from 41 European and 57 North American specimens belonging to 15 European and 19 North American populations (Fig. 1). The plants were grown in the greenhouse for about two months until they built up a rosette, and the pots were then placed in flower beds in the field. Vouchers of all studied populations are housed in the University of Bayreuth (UBT). Of each population we sampled in Europe one (one population), two (five populations), three (six populations), or four (three populations) individuals, and in North America one (four populations), two (four populations), three (two populations), four (six populations), five (two populations), or six (one population) individuals. The localities of the different populations are shown in Fig. 1.

4.2. Volatile collection

Floral scent was collected outdoors using dynamic head-space methods. A single, newly opened flower was enclosed within a polyethylene oven bag (Toppits®) and the emitted volatiles were trapped in an adsorbent tube through the use of a membrane pump (ASF Thomas, Inc.). The flow rate was adjusted to about 200 ml/min using a power supply and a flow meter. To get data on qualitative variation, samples were collected for 2 min at night within a short period of time (1–1.5 h), when *S. latifolia* was emitting most of its floral volatiles (see also Fig. 2(a)). These samples were also used to get information about the amount of emitted volatiles during seasons (see Fig. 2(d)). When analyzing temporal variation over a day, a single flower was enclosed within an oven bag at beginning of measurements, and the oven bag was not removed before the end of the experiment. Volatiles were trapped for 2 min each hour. To avoid accumulation of flower scent from one measurement to the next, volatiles were sucked out of the bag by the use of a membrane pump between measurements. We took ChromatoProbe quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), cut the closed end, filled them with a mixture (1:1) of 3 mg Tenax-TA (mesh 60–80) and Carbotrap (mesh 20–40), and used them as adsorbent tubes. The adsorbents were fixed in the tubes using glass wool. Simultaneous collections of the surrounding air were used to distinguish between floral compounds and ambient contaminants.

4.3. Chemical analysis

The samples were analysed on a Varian Saturn 2000 System using a 1079 injector, that had been fitted with the ChromatoProbe kit. This kit allows the thermal desorption of small amounts of solids or liquids contained in quartz microvials (Micro-SPE, Amirav and Dagan, 1997; Wilkinson and Ladd, Varian Application note), or in our case the thermal desorption of the trapped volatiles. The adsorbent tube was loaded into the probe, which was then inserted into the modified GC injector.

The injector split vent was opened (1/20) and the injector heated to 40 °C to flush any air from the system. The split vent was closed after 2 min and the injector was heated at 200 °C/min, then held at 200 °C for 4.2 min, after which the split vent was opened (1/10) and the injector cooled down.

A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m long, inner diameter 0.25 mm, film thickness 0.25 μm, Phenomenex). Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 1.8 ml min^{-1}. The GC oven temperature was held for 7 min at 40 °C, then increased by 6 °C per min to 250 °C and held for 1 min. The MS interface was 260 °C and the ion trap worked at 175 °C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 $scan^{-1}$ from *m*/*z* 30 to 350. The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 02 mass spectral data base (NIST algorithm), or MassFinder 2.3, and confirmed by comparison of retention times with published data (Adams, 1995; Davies, 1990). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards.

For quantification of compounds known amounts of lilac aldehydes, *trans*-β-ocimene, *cis*-3-hexenyl acetate, benzaldehyde, phenyl acetaldehyde, and veratrole were injected, and the mean response of these compounds was used for quantification.

4.4. Statistical analyses

Multi-way ANOVA was used to determine factors (season, sex, origin) responsible for quantitative variability in floral scent. Unequal N HSD was used as post hoc test.

We used the CNESS (chord-normalised expected species shared) distance index, ranging between 0 and the square root of 2, to determine the qualitative differences between the single samples. Therefore, relative quantities (percentage) of compounds were used, because the total amount of emitted volatiles varied during the year. CNESS is a metric version of Grassle and Smith's (1976) NESS similarity index, which was originally built to compare faunal samples. NESS and CNESS can be regarded as more generalised forms of the Morisita index (Morisita, 1959), and these indices are the most appropriate ones for analysing quantitative data (Wolda, 1981, 1983; Trueblood et al., 1994). We calculated CNESS indices using the updated version of the COMPAH (Combinatorial Polythetic Agglomerative Hierarchical Clustering) program (Boesch, 1977), provided by Gallagher at UMASS/Boston (http://www.es.umb.edu/edgwebp.htm).

We also tried other multivariate methos, such as principal component analysis (PCA) or correspondence analysis (CA), but did not get good separations of the samples. The reason is probably the structure of our dataset, where lots of compounds were found only in few samples, and were absent in the other samples. PCA and CA seems to be susceptible to this zero values.

Though multivariate methods are not often used by chemical ecologists, they are powerful tools for analyzing floral scent data.

We used nonlinear multidimensional scaling (NMDS) to detect meaningful underlying dimensions and to visualise similarities between samples (see Borg and Lingoes, 1987). To evaluate how well (or poorly) the particular configuration produces the observed distance matrix the stress value is given. The smaller the stress value, the better is the fit of the reproduced ordination to the observed distance matrix (Clarke, 1993).

A variance component analysis was used to estimate the contribution of single compounds to the obtained variation between European and North American samples.

We used the Mann–Whitney *U* test to compare the mean relative abundances of the compounds most responsible for the variation between European and North American samples. The *p*-values of the latter tests were Bonferroni corrected (sequential Bonferroni procedure, Hochberg, 1988) to assess the significance of differences.

Kruskal–Wallis-ANOVA was used to compare the pair-wise dissimilarities within and between European and North American populations. The Tukey–Kramer test for non-parametric data was used as post hoc test (Siegel and Castellan, 1988).

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References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corporation, Carol Stream, IL.

Altenburger, R., Matile, P., 1988. Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R. Br. Planta 174, 248–252.

Altizer, S.M., Thrall, P.H., Antonovics, J., 1998. Vector behavior and the transmission of anther-smut infection in *Silene alba*. Am. Midl. Nat. 139, 147–163.

Amirav, A., Dagan, S., 1997. A direct sample introduction device for mass spectrometry studies and gas chromatography mass spectrometry analyses. Eur. Mass Spectrom. 3, 105–111.

Azuma, H., Toyota, M., Asakawa, Y., 2001. Intraspecific variation of floral scent chemistry in *Magnolia kobus* DC. (Magnoliaceae). J. Plant Res. 114, 411–422.

Blair, A.C., Wolfe, L.M., 2004. The evolution of an invasive plant: an experimental study with *Silene latifolia*. Ecology 85, 3035–3042.

Boesch, D.F., 1977. Application of numerical classification in ecological investigations of water pollution. Special Scientific Report, Institute of Marine Science, Virginia.

Bopp, S., 2003. Parasitismus oder Symbiose? Beziehungen zwischen einem parasitischen Bestäuber (*Hadena bicruris* Hufn., Lepidoptera Noctuidae) und seinen Wirtspflanzen. (*Silene* -Arten, Caryophyllaceae). Zoologica 152, 1–140.

Borg, I., Lingoes, J., 1987. Multidimensional Similarity Structure Analysis. Springer, Berlin.

Brantjes, N.B.M., 1976a. Riddles around the pollination of *Melandrium album* (Mill.) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn., (Noctuidae, Lepidoptera), I. Proc. Koninkl. Ned. Akad. Wetensch., Ser. C 79, 1–12.

Brantjes, N.B.M., 1976b. Riddles around the pollination of *Melandrium album* (Mill.) Garcke (Caryophyllaceae) during the oviposition by *Hadena bicruris* Hufn. (Noctuidae, Lepidoptera), II. Proc. Koninkl. Ned. Akad. Wetensch., Ser. C 79, 125–141.

Brantjes, N.B.M., 1976c. Senses involved in the visiting of flowers by *Cucullia umbratica* (Noctuidae, Lepidoptera). Entomol. Exp. Appl. 20, 1–7.

Bruce, T.J., Cork, A., 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. J. Chem. Ecol. 27, 1119–1131.

Burkhardt, D., Mosandl, A., 2003. Biogenetic studies in *Syringa vulgaris* L.: Bioconversion of O-18(H-2)-labeled precursors into lilac aldehydes and lilac alcohols. J. Agric. Food Chem. 51, 7391–7395.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.

Davies, N.W., 1990. Gas-chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 503, 1–24.

Dötterl, S., 2004. Importance of floral scent compounds for the interaction between *Silene latifolia* (Caryophyllaceae) and the nursery pollinator *Hadena bicruris* (Lepidoptera: Noctuidae). Ph.D. Thesis, University of Bayreuth.

Dufay, M., Anstett, M.C., 2003. Conflicts between plants and pollinators that reproduce within inflorescences: evolutionary variations on a theme. Oikos 100, 3–14.

Ellis, W.N., Ellis-Adam, A.C., 1993. To make a meadow it takes a clover and a bee: the entomophilous flora of N.W. Europe and its insects. Bijdr. Dierkunde 63, 193–220.

Fraser, A.M., Mechaber, W.L., Hildebrand, J.G., 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. J. Chem. Ecol. 29, 1813–1833.

Grassle, J.F., Smith, W., 1976. A similarity measure sensitive to the contribution of rare species and its use in investigation of variation in marine benthic communities. Oecologia 25, 13–22.

Hacker, H., 1996. A revision of the genus *Hadena* Schrank, 1802 (Lepidoptera). Esperiana 5, 7–696.

Haynes, K.F., Zhao, J.Z., Latif, A., 1991. Identification of floral compounds from *Abelia grandiflora* that stimulate upwing flight in cabbage looper moths. J. Chem. Ecol. 17, 637–646.

Heath, R.R., Landolt, P.J., Dueben, B., Lenczewski, B., 1992. Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. Environ. Entomol. 21, 854–859.

Helsper, J.P.F.G., Davies, J.A., Bouwmeester, H.J., Krol, A.F., van Kampen, M.H., 1998. Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. Planta 207, 88–95.

Hochberg, Y., 1988. A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75, 800–802.

Jürgens, A., Witt, T., Gottsberger, G., 1996. Reproduction and pollination in central European populations of *Silene* and *Saponaria* species. Bot. Acta 109, 316–324.

Jürgens, A., Witt, T., Gottsberger, G., 2002. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochem. Syst. Ecol. 30, 383–397.

Kaiser, R., 1993. Vom Duft der Orchideen. Roche, Basel.

Kaiser, R., 1994. Trapping, investigation and reconstitution of flower scents. In: Müller, P.M., Lamparsky, D. (Eds.), Perfumes: Art, Science and Technology. Blackie Academic & Professional, London, pp. 213–250.

Knudsen, J.T., 1999. Floral scent differentiation among coflowering, sympatric species of *Geonoma* (Arecaceae). Pl. Spec. Biol. 14, 137–142.

Knudsen, J.T., Andersson, S., Bergman, P., 1999. Floral scent attraction in *Geonoma macrostachys*, an understorey palm of the Amazonian rain forest. Oikos 85, 409–418.

Knudsen, J.T., Mori, S.A., 1996. Floral scents and pollination in neotropical Lecythidaceae. Biotropica 28, 42–60.

Knudsen, J.T., Tollsten, L., 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. Bot. J. Linn. Soc. 113, 263–284.

Knudsen, J.T., Tollsten, L., Bergström, L.G., 1993. Floral scents: a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33, 253–280.

Kolosova, N., Gorenstein, N., Kish, C.M., Dudareva, N., 2001. Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitting plants. Plant Cell 13, 2333–2347.

Kreck, M., Mosandl, A., 2003. Synthesis, structure elucidation, and olfactometric analysis of lilac aldehyde and lilac alcohol stereoisomers. J. Agric. Food Chem. 51, 2722–2726.

Kreck, M., Puschel, S., Wüst, M., Mosandl, A., 2003. Biogenetic studies in *Syringa vulgaris* L.: synthesis and bioconversion of deuterium-labeled precursors into lilac aldehydes and lilac alcohols. J. Agric. Food Chem. 51, 463–469.

Kunert, M., Biedermann, A., Koch, T., Boland, W., 2002. Ultrafast sampling and analysis of plant volatiles by a hand-held miniaturised GC with pre-concentration unit: kinetic and quantitative aspects of plant volatile production. J. Sep. Sci. 25, 677–684.

Landolt, P.J., Smithhisler, C.L., 2003. Characterization of the floral odor of Oregon Grape *Berberis aquifolium* Prursh: possible feeding attractants for moths (Insecta, Lepidoptera). Northwest Sci. 77, 81–86.

Levin, R.A., Raguso, R.A., McDade, L.A., 2001. Fragrance chemistry and pollinator affinities in Nyctaginaceae. Phytochemistry 58, 429–440.

Matile, P., Altenburger, R., 1988. Rhythms of fragrance emission in flowers. Planta 174, 242–247.

McNeill, J., 1977. The biology of canadian weeds. 25. *Silene alba* (Miller) E.H.L. Krause. Can. J. Plant Sci. 57, 1103–1114.

Meagher, R.L., 2001. Collection of soybean looper and other noctuids in phenylacetaldehyde-baited field traps. Fla. Entomol. 84, 154–155.

Meagher, R.L., 2002. Trapping noctuid moths with synthetic floral volatile lures. Entomol. Exp. Appl. 103, 219–226.

Miyake, T., Yamaoka, R., Yahara, T., 1998. Floral scents of hawkmoth-pollinated flowers in Japan. J. Plant Res. 111, 199–205.

Morisita, M., 1959. Measuring of the dispersion of individuals and analysis of the distributional patterns. Mem. Fac. Sci. Kyushu Univ., Ser. E (Biol.) 2, 215–235.

Moya, S., Ackerman, J.D., 1993. Variation in the floral fragrance of *Epidendrum ciliare* (Orchidaceae). Nord. J. Bot. 13, 41–47.

Negre, F., Kish, C.M., Boatright, J., Underwood, B., Shibuya, K., Wagner, C., Clark, D.G., Dudareva, N., 2003. Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. Plant Cell 15, 2992–3006.

Pellmyr, O., Thien, L.B., 1986. Insect reproduction and floral fragrances – keys to the evolution of the angiosperms?. Taxon 35, 76–85.

Plepys, D., Ibarra, F., Francke, W., Löfstedt, C., 2002a. Odour-mediated nectar foraging in the silver Y moth, *Autographa gamma* (Lepidoptera: Noctuidae): behavioural and electrophysiological responses to floral volatiles. Oikos 99, 75–82.

Plepys, D., Ibarra, F., Löfstedt, C., 2002b. Volatiles from flowers of *Platanthera bifolia* (Orchidaceae) attractive to the silver Y moth, *Autographa gamma* (Lepidoptera: Noctuidae). Oikos 99, 69–74.

Raguso, R.A., Levin, R.A., Foose, S.E., Holmberg, M.W., McDade, L.A., 2003. Fragrance chemistry, nocturnal rhythmus and pollination syndromes in *Nicotiana*. Phytochemistry 63, 265–284.

Raguso, R.A., Light, D.M., 1998. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and 'green-leaf volatiles'. Entomol. Exp. Appl. 86, 287–293.

Raguso, R.A., Light, D.M., Pichersky, E., 1996. Electroantennogram responses of *Hyles lineata* (Sphingidae: Lepidoptera) to volatile compounds from *Clarkia breweri* (Onagraceae) and other moth-pollinated flowers. J. Chem. Ecol. 22, 1735–1766.

Raguso, R.A., Pichersky, E., 1995. Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. Pl. Syst. Evol. 194, 55–67.

Schiestl, F.P., Ayasse, M., 2001. Post-pollination emission of a repellent compound in a sexually deceptive orchid: a new mechanism for maximising reproductive success? Oecologia 126, 531–534.

Schiestl, F.P., Ayasse, M., Paulus, H.F., Erdmann, D., Francke, W., 1997. Variation of floral scent emission and postpollination changes in individual flowers of *Ophrys sphegodes* subsp. *sphegodes*. J. Chem. Ecol. 23, 2881–2895.

Schiestl, F.P., Ayasse, M., Paulus, H.F., Löfstedt, C., Hansson, B.S., Ibarra, F., Francke, W., 2000. Sex pheromone mimicry in the early spider orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. J. Comp. Physiol. A 186, 567–574.

Shykoff, J.A., Bucheli, E., 1995. Pollinator visitation patterns, floral rewards and the probability of transmission of *Microbotryum violaceum*, a venereal disease of plants. J. Ecol. 83, 189–198.

Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioural Sciences. McGraw-Hill, New York.

Tollsten, L., 1993. A multivariate approach to post-pollination changes in the floral scent of *Platanthera bifolia* (Orchidaceae). Nord. J. Bot. 13, 495–499.

Tollsten, L., Bergström, J., 1989. Variation and post-pollination changes in floral odors released by *Platanthera bifolia* (Orchidaceae). Nord. J. Bot. 9, 359–362.

Tollsten, L., Bergström, G., 1993. Fragrance chemotypes of *Platanthera* (Orchidaceae): the result of adaptation to pollinating moths? Nord. J. Bot. 13, 607–613.

Tollsten, L., Øvstedal, D.O., 1994. Differentiation in floral scent chemistry among populations of *Conopodium majus* (Apiaceae). Nord. J. Bot. 14, 361–367.

Trueblood, D.D., Gallagher, E.D., Gould, D.M., 1994. Three stages of seasonal succession on the Savin Hill Cove mudflat, Boston Harbor. Limnol. Oceanogr. 39, 1440–1454.

van Doorn, W.G., 1997. Effects of pollination on floral attraction and longevity. J. Exp. Botany 48, 1615–1622.

Whitten, W.M., Williams, N.H., 1992. Floral fragrances of *Stanhopea* (Orchidaceae). Lindleyana 7, 130–153.

Wilkinson, S., Ladd, P. The use of a solids inlet system to identify essential oils in anthers and leaves of flowering plants. Varian GC/MS Application Note 65.

Wolda, H., 1981. Similarity indices, sample size and diversity. Oecologia 50, 296–302.

Wolda, H., 1983. Diversity, diversity indices and tropical cockroaches. Oecologia 58, 290–298.

Wolfe, L.M., 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. Am. Nat. 160, 705–711.

Wolfe, L.M., Elzinga, J.A., Biere, A., 2004. Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*. Ecol. Lett. 7, 813–820.

Young, H.J., 2002. Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). Am. J. Bot. 89, 433–440.