Volatile Constituents from the Flowers of Japanese Honeysuckle (*Lonicera japonica*)

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The flowers of Japanese honeysuckle (*Lonicera japonica*) have been shown by field observation to be attractive to a variety of economically important adult lepidoptera. The present study was performed to identify and quantify the volatile chemical constituents of *L. japonica* flowers, at different stages of development, to provide a basis for systematic evaluation of insect attraction. Methylene chloride extracts of flowers obtained at three stages of development (freshly opened, overnight, and 24 h) were subjected to vacuum-steam distillation/hexane extraction using a modified Likens−Nickerson apparatus. Volatile constituents were identified and quantitated using gas capillary chromatography/mass spectrometry. Twenty-seven compounds, 13 of which have not been reported in *Lonicera* spp. flowers, were identified among the three developmental stages. Germacrene D was a major component at all stages; linalool and α-farnesene appeared in high concentrations in fresh and 24 h flowers but were greatly reduced in overnight flowers. The latter, however, contained elevated levels of phenylpropanoid biosynthesized compounds, suggesting a marked diurnal influence on the biosynthesis of volatile flower constituents involving two modes of action: phenylpropanoid and lipoxygenase derivation.

Keywords: *Lonicera japonica; volatile flower constituents; cis-jasmone; diurnal variations*

INTRODUCTION

The flowers of Japanese honeysuckle (*Lonicera japonica*) have been shown to be an important larval host of the tobacco budworm (*Heliothis virescens*) and the corn earworm (*Helicoverpa zea*) (Pair, unpublished data, 1994). Further field observations by Pair (1994) of traps baited with *L. japonica* flowers demonstrated attraction for a variety of economically important adult lepidoptera; budworms, loopers, armyworms, and hornworm moths were among the most prevalent species captured. Identification and quantitation of the principal volatile chemical constituents of *L. japonica* flowers would provide data for systematic entomological studies to establish a chemical basis for these observed attractions.

The chemistry of *Lonicera* spp. has been partially described in several previous papers. Flavonoids and anthocyanins were identified in the fruit of *Lonicera ataica* by Feedosova (1971). The fruits of this species and those of *L. edulis* and *L. caerulea* were shown by Shapiro et al. (1981) to be high in anthocyanins, leucoanthocyanins, and catechols. Several studies have reported floral headspace analyses of various *Lonicera* spp. DePooter et al. (1985) identified linalool, *cis*- and *trans*-linalool oxide, and α-terpineol as principal constituents in the headspace of *Lonicera periclymenum*. Lamparsky (1985) performed headspace analyses on flowers of *L. periclymenum* and *L. japonica* and reported 10-fold higher levels of linalool oxides in the latter as compared to the former. This study also identified germacrene D as the principal sesquiterpene hydrocarbon in *L. japonica* floral headspace. Floral headspace from *Lonicera caprifolium* was analyzed by Joulain (1986); linalool was the major constituent (75%), and germacene D, indole, α-farnesene, nerolidol, and jasmone derviatives were reported in lesser but significant quantities. Analysis of the essential oil from flowers of *L. japonica* by Wu and Fong (1981) showed that linalool and linalool oxide C were the major identified constituents, and pinene, 1-hexene, *cis*-3-hexen-1-ol, α-terpineol, geraniol, benzyl alcohol, *β*-phenylethanol, carvacol, eugenol, and some substituted furans were reported in lesser amounts.

In studies of pheromones and volatile plant attractants for the honeysuckle aphid, Hedin et al. (1991) analyzed hexane-extractable volatiles of a 25 g sample each of leaves and flowers of *Lonicera* spp.; methyl esters, two ketones, seven alcohols, and three terpenoids [Δ-carene, (*E*)-*β*-farnesene, and (*E*,*E*)-farnesol] were identified in the plant samples. These authors suggested that additional terpenoids might have been identified if larger quantities of plant material had been analyzed.

The present investigation was performed to identify and quantitate the volatile components of *L. japonica* flowers by vacuum-steam distillation/hexane extraction of kilogram quantities of material obtained at differing stages of development. The objective was to provide a comprehensive survey of flower volatiles, with consideration for diurnal changes involved in component formation, thereby forming a basis for informed design of entomological studies on insect attraction.

MATERIALS AND METHODS

Collection of Flowers. Honeysuckle (*L. japonica*) flowers were collected between May 27 and June 30, 1993, at locations in Lane and Boswell, OK. Flowers opened from 7:00 to 9:00 p.m. each night. The freshly opened flowers (1.0 kg = approximately 10 000 individual blooms) were excised within

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30 min of opening (7:30−10:00 p.m. that evening). Flowers opened for 12 h (1.0 kg) were collected between 9:00 a.m. and noon the following morning. Flowers opened for 24 h were collected from 5:00 to 10:00 p.m. the second evening. The pooled samples of each flower stage were immersed in approximately 3 L of methylene chloride (distilled in glass; Burdick and Jackson) and shipped in sealed containers, surrounded by dry ice, to Athens, GA. Samples were maintained at 0 °C prior to initiation of analytical procedure.

Isolation of Volatile Flower Constituents. The methylene chloride extracts were removed from cold storage and allowed to attain room temperature (21 °C); solids and flower residues were removed by filtration through prepleated filter paper (Schleicher and Schuell, grade 588). The filtrates were shaken in 4 L separatory funnels, and methylene chloride layers were separated from aqueous layers. The latter, observed to comprise approximately 10% of total volume in each case, were stored at 5 °C for further analyses. Methylene chloride layers were dried over anhydrous sodium sulfate (Sigma Chemical Co.), filtered, and concentrated to 5 mL in Kuderna-Danish concentrators. Vacuum-steam distillation/hexane extraction of volatile flower constituents was accomplished using a modified Likens−Nickerson extraction apparatus, as described by Schultz et al. (1977). Methylene chloride concentrates were transferred to a 3 L round-bottom flask containing 900 mL of distilled water and connected to the Likens−Nickerson extractor heads. The extracting solvent was 120 mL of *n*-hexane (Baker Chemical Co.; HPLC grade). Continuous extraction was performed at 110 mmHg for 4 h. The recovered hexane extracts were initially concentrated to 2 mL according to the Kuderna-Danish method and then to 50 μL under a stream of dry purified nitrogen at 40 °C.

Glass Capillary Gas Chromatographic (GC) Analyses. GC analyses were performed on a Hewlett-Packard Model 5890 gas chromatograph, equipped with a flame ionization detector. Injector and detector temperatures were 240 and 280 °C respectively. The column was a 15 m × 0.2 mm (i.d.) fused silica capillary column coated with DB-1 methylsilicone (J&W Scientific, Deerfield, IL). The column oven temperature was initially held at 60 °C for 1 min, then programmed to 90 °C at 3 °C/min, held for 0.5 min, programmed to 180 °C at 5 °C/min, held for 0.5 min, and then programmed to 240 °C at 8 °C/min. Samples (5 μL/50 μL total concentrate) were injected at a split ratio of 50:1. Yields of individual constituents were determined by response factors for peak areas as measured by a Hewlett-Packard Model 3396A computing integrator.

Gas Chromatographic/Mass Spectral (GC/MS) Analyses. GC/MS analyses were performed on a Perkin-Elmer Sigma 300 gas chromatograph (Norwalk, CT), interfaced with an Extrel Model C50/400 mass spectrometer (Pittsburgh, PA). Samples were introduced by cold on-column injection. The column was a 30 m × 0.32 mm (i.d.) fused silica capillary (J&W Scientific) coated with DB-1. Helium was used as carrier gas at 7.0 psi. The column oven temperature was held for 1.0 min at 50 °C, then programmed to 100 °C at 2 °C/min, held for 0.5 min, and then programmed to 220 °C at 3 °C/min, with a final hold time of 40 min. The mass spectrometer conditions were as follows: interface temperature, 240 °C; ion source temperature, 150 °C; ionization voltage, 70 eV; and scan rate 200 emu/s. Initial identifications of individual constituents were based on comparison of mass spectra obtained with those in the National Institute of Standards and Technology, standard reference data program (Stein, 1990), and final identifications on mass spectra and retention data of authentic compounds, where available.

RESULTS AND DISCUSSION

Volatile constituents from *L. japonica* flowers at three stages of development are shown in GC profile (Figure 1) and as quantitative yields (Table 1). Twenty-seven compounds were identified, accounting for 96.4, 85.2, and 93.5% of the total GC volatile profiles of fresh and 12 and 24 h flowers, respectively. Thirteen of the identified compounds have not been previously reported in *Lonicera* spp. floral analyses.

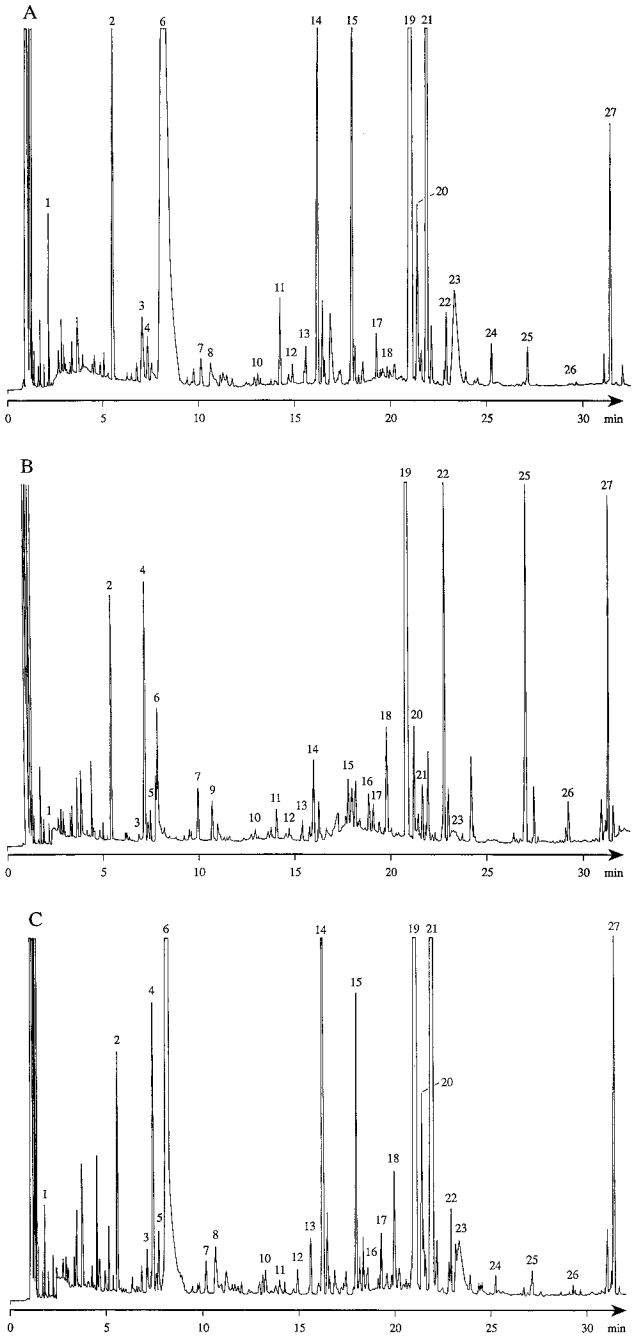

Figure 1. Gas chromatographic profiles of volatile constituents of *L. japonica* flowers: (A) freshly opened flowers; (B) 12 h flowers; (C) 24 h flowers. Peak identification numbers correspond to those in Table 1.

Comparison of the constituent profiles and yields for the three stages of flower development examined suggests that the biosynthesis of individual constituents can be markedly influenced by diurnal changes. Two principal biosynthetic pathways were suggested: phenylpropanoid and lipoxygenase derivation. Twelve hour flowers, sampled after overnight development, produced significant levels of phenylpropanoid biosynthesized compounds methyl benzoate (GC peak 5), methyl salicylate (GC peak 9), hexenyl benzoate (GC peak 22), benzyl benzoate (GC peak 25), and benzyl salicylate (GC peak 26) either undetected or present in minor amounts in fresh flowers and/or flowers developed over a full night/day cycle. These results are similar to those observed by Loughrin et al. (1990) for volatiles emitted in the headspace of flowers of the vesperescent plant

Table 1. Volatile Compounds Identified in *L. japonica* Flowers

GC peak	compound	yield (μg/kg) fresh	12 h	24 h
1	(*Z*)-3-hexen-1-ol	70	tr[a]	23
2	phenylacetaldehyde[d]	366	279	272
3	linalool oxide (I)[b]	120	tr	72
4	linalool oxide (II)[b]	49	365	425
5	methyl benzoate[d]	nd[c]	39	nd
6	linalool	6854	186	3858
7	methyl phenylacetate[d]	42	74	146
8	α-terpineol	81	nd	102
9	methyl salicylate[d]	nd	66	nd
10	β-citronellol[d]	tr	tr	tr
11	*trans*-geraniol	107	48	tr
12	2,4-decadienal[d]	tr	tr	tr
13	indole	52	tr	87
14	(*Z*)-3-hexenyl tiglate	640	108	1056
15	*cis*-jasmone	602	80	442
16	β-bourbonene[d]	nd	62	tr
17	β-caryophyllene[d]	49	47	85
18	geranylacetone[d]	tr	172	182
19	germacrene D	4806	3876	6179
20	germacrene B[d]	186	149	275
21	α-farnesene	2058	68	5850
22	hexenyl benzoate[d]	72	561	108
23	nerolidol	476	tr	87
24	methyl jasmonate	42	nd	tr
25	benzyl benzoate[d]	46	602	51
26	benzyl salicylate[d]	tr	65	tr
27	methyl palmitate	242	442	546
	total	16960	7289	19884

[a] trace, <20 μg/kg. [b] *cis* and *trans* isomers not distinguished. [c] nd, not detected. [d] Not previously reported in *Lonicera* spp. flowers.

Nicotiana sylvestris. These authors reported that night headspace emissions were high in benzaldehyde, benzyl acetate, and benzyl alcohol when compared to day emissions of that species. In *L. japonica*, two aromatic compounds, phenylacetaldehyde (GC peak 2) and methyl phenylacetate (GC peak 7), were not observed to follow a diurnal pattern, suggesting a biopathway differing from the other benzenoid products identified.

(*Z*)-3-Hexen-1-ol (GC peak 1), a known product of the action of lipoxygenase on unsaturated fatty acids in many plant species (Tressl et al., 1981; Robinson, 1983), occurred, but in trace amounts, in night developed flowers, as compared to fresh or night−day flowers. Phytochrome control of lipoxygenase synthesis has been demonstrated by Oelze-Karnow et al. (1970) in mustard cotyledon, and suppression of night emissions of this compound and (*Z*)-3-hexenyl acetate in headspace of apple flowers was observed by Loughrin et al. (1990).

A major component of fresh and 24 h flowers of *L. japonica* as determined in this study is (*Z*)-3-hexenyl tiglate (GC peak 14), previously identified in *L. caprifolium* by Joulain (1986). Tiglic acid [(*E*)-2-methyl-2-butenoic acid] has been found to be widely distributed among plant species in the form of various esters (Buckles et al., 1955). The presence of the (*Z*)-hexenyl ester of tiglic acid in the overnight developed flowers of *L. japonica* suggests esterification of tiglic acid with the hexenyl alcohol, possibly arising through enzyme action.

The principal terpenoid constituents of *L. japonica* were shown in this study to be linalool (GC peak 6), *cis*- and *trans*-oxides (GC peaks 3 and 4), germacrene D (GC peak 19), and α-farnesene (GC peak 21). Terpenoids present in lesser quantities were α-terpineol (GC peak 8), β-citronellol (GC peak 10), geraniol (GC peak 11), β-bourbonene (GC peak 16), β-caryophyllene (GC peak 17), geranyl acetone (GC peak 18), germacrene B (GC peak 20), and nerolidol (GC peak 23). Among the monoterpenes, the diene alcohol linalool, previously reported (Wu and Yang, 1981) as a major component of *L. japonica* flower essential oil, was in this study the largest component of fresh flowers, a major constituent of 24 h flowers, but produced in relatively low amounts in 12 h (overnight developed) flowers. Mookherjee et al. (1990) reported highest levels of linalool above living blossoms of *L. americana* as occurring at night, whereas indole and *cis*-jasmone levels maximized during the day. However, in our study, linalool oxides were seen to increase with time. Linalool, like all monoterpenes in plants, is believed to arise from geranyl pyrophophate (Templeton, 1969). α-terpineol, which is generally reported to be present with linalool in a number of fruit flavors (Morton and MacLeod, 1990), paralleled the diurnal pattern observed for linalool. Additional monoterpenes from *L. japonica*, β-citronellol and geraniol, gave an ambiguous pattern, the former increasing with flower development and the latter showing an opposite trend. Geranyl acetone increased as geranyl alcohol decreased. Loughrin et al. (1990) reported elevated amounts of the monoterpene sabinene during the day cycle, in headspace of flowers of *Nicotiana otophora*, but observed no significant diurnal patterns for production of myrcene and *trans*-β-ocimene, suggesting that floral production of some, but not all, monoterpenes is phytochrome regulated.

The principal sesquiterpenes identified in this study were germacrene D and α-farnesene. Germacrene D has been reported (Peterson et al., 1994) as attractive to the female pickelworm moth (*Diaphania nitidalis*). Levels of this compound in flowers of *L. japonica* appeared independent of diurnal cycle. α-Farnesene, however, was not produced overnight. Sesquiterpenes have been shown to arise through a farnesyl pyrophosphate pathway (Parker et al., 1967). The apparent differences in biosynthetic pathways for these sesquiterpenes require further study. Of the minor sesquiterpene components germacrene B, and β-caryophyllene production paralleled that of germacrene D, and β-bourbonene was present in measurable amounts in night-developed flowers only. The sesquialcohol nerolidol is present in significant quantities in freshly opened flowers only.

cis-Jasmone (GC peak 15), the principal component of the essential oil of flowers of *Jasminium officinale* var. Grandiflorum and widely used in perfume formulations (Saul, 1943), was present in relatively high concentrations in fresh and 24 h *L. japonica* but greatly reduced in flowers developed overnight. Methyl jasmonate (GC peak 24) was present in significant amounts in fresh flowers only. This compound, and the parent acid (not found in this study), have been found to occur ubiquitously in the plant kingdom (Meyer et al., 1984) and have been suggested as a phytohormone (Ueda and Kato, 1982).

In conclusion, the formation of volatile components in flowers of *L. japonica* has been shown to display marked diurnal effects. Phenylpropanoid derived constituents are greatly increased at night; the compounds arising from lipoxygenase action show an opposite trend suggesting phytochrome regulation. Monoterpenes, sesquiterpenes, and jasmone display the latter trend; however, the major sesquiterpene, germacrene D, is found in high levels throughout the night/day cycle. The results of this study suggest that attractiveness of *L.*

japonica flowers for specific insect species will be found to vary markedly with diurnal patterns. These data will be employed as a basis for entomological field studies of individual chemicals, and various combinations of such, to determine potential for attraction of economically important insect species, with the objective of developing effective monitoring and trapping systems.

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