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## ODORANTS OF THE FLOWERS OF BUTTERFLY BUSH, *BUDDLEJA DAVIDII*, AS POSSIBLE ATTRACTANTS OF PEST SPECIES OF MOTHS

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### ABSTRACT

Flowers of the butterfly bush, *Buddleja davidii* Franch., are visited by butterflies as well as other insects. Night captures revealed also that moths visit butterfly bush flowers. Moths captured in traps over flowers included 12 species of Noctuidae, 6 species of Pyralidae, 2 species of Geometridae, and 1 tortricid species. The majority of moths trapped at these flowers were cabbage loopers, *Trichoplusia ni* (Hübner), and alfalfa loopers, *Autographa californica* (Speyer). Both males and females were captured at butterfly bush flowers. Additionally, butterflies, bees, wasps, flies, and other insects also were captured. Analysis of volatile compounds collected from air over clusters of butterfly bush flowers yielded the consistent presence of nine chemicals: benzaldehyde, 6-methyl-5-hepten-2-one, hexyl acetate, 4-oxoisophorone, (*E,E*)- $\alpha$ -farnesene, (*Z*)-cinnamaldehyde, dihydrooxoisophorone,  $\beta$ -cyclocitral, and oxoisophorone oxide. Emitted amounts of these floral odorants averaged 57 ng per h per floret or 21  $\mu$ g per h per flower cluster (raceme). Five of those floral chemicals, benzaldehyde, 4-oxoisophorone, dihydrooxoisophorone, oxoisophorone oxide, and (*E,E*)- $\alpha$ -farnesene triggered antennal responses in cabbage looper moths, while benzaldehyde, oxoisophorone oxide, and 4-oxoisophorone also stimulated antennal responses in alfalfa looper moths. Some of these compounds may be attractants or co-attractants for moths and play a key role in locating flowers as nectar sources.

Key Words: Lepidoptera, kairomone, floral odor, pollination

### RESUMEN

Las flores del arbusto de mariposa, *Buddleja davidii* Franch., están frecuentemente visitadas por mariposas y otros insectos. Las capturas hechas en la noche revelaron que las polillas también visitan las flores del arbusto de mariposa. Las polillas capturadas en las trampas sobre las flores incluyeron 21 especies de Geometridae, Noctuidae, Pyralidae y Tortricidae. La mayoría de las polillas capturadas en las flores pertenecen al gusano medidor del repollo, *Trichoplusia ni* (Hübner) y al gusano medidor de alfalfa, *Autographa californica* (Speyer). Tanto machos como hembras fueron capturados sobre las flores del arbusto de mariposa. También se capturaron mariposas, abejas, avispas, moscas y otros insectos. El análisis de los volátiles compuestos recolectados del aire sobre los grupos de flores del arbusto de mariposa siempre mostró la presencia de nueve químicos: benzaldehído, acetato hexil 6-metil-5-hepten-2-uno, 4-oxoisoforone, (*E,E*)- $\alpha$ -farnesene, (*Z*)-cinnamaldehído, dihidrooxoisoforone,  $\beta$ -ciclocitral y óxido oxoisoforone. Las cantidades emitidas de estos odorantes de las flores fueron un promedio de 57 ng por hora florecita o 21  $\mu$ g por hora por cada grupo de flores (racimos). Cinco de estos químicos de flores, benzaldehído, 4-oxoisoforone, dihidrooxoisoforone, óxido oxoisoforone y (*E,E*)- $\alpha$ -farnesene estimularon una respuesta en las antenas de las polillas del gusano medidor del repollo, mientras que el benzaldehído, óxido oxoisoforone y el 4-oxoisoforone también estimularon una respuesta en las antenas del gusano medidor de la alfalfa. Algunos de estos químicos compuestos pueden ser atrayentes o co-atrayentes para las polillas que juegan un papel clave en localizar las flores como fuentes de néctar.

The butterfly bush, *Buddleja davidii* Franch. (Loganiaceae), is native to China and Japan, but is a common ornamental shrub planted throughout much of North America and Europe. The shrub has abundant and colorful flowers that occur as 15 to 40 cm long racemic clusters. Flower color varies from white to pink, red, blue, and purple. The flower is strongly fragrant. It is attractive to butterflies (Corbett 2000) and is recommended in horticultural plantings as a source of nectar for butterflies.

Moths were initially observed orienting to butterfly bush flowers on the campus of Washington

State University, Pullman, Washington, USA in late afternoon (P. J. Landolt, unpublished data). We hypothesize that moth visitation at these flowers may result from their response to flower odor. The odor chemistry of these flowers, reported by Andersson et al. (2002), is very complex and includes 31 compounds. Antennal responses for several species of butterflies to *B. davidii* floral scent were reported, narrowing down the list of compounds that could be butterfly attractants (Andersson 2003; Andersson & Dobson 2003). It is not yet known which chemicals in the butterfly

bush flowers might be detected by or attractive to moths.

Some species of moths often visit flowers for nectar, and some of these species arrive at flowers following upwind attraction to floral odors. This behavior is particularly pronounced and well studied for pest species of noctuids in the subfamily Plusiinae. The cabbage looper moth *Trichoplusia ni* (Hübner), for example, visits or responds to odors of flowers of bladderflower, *Araujia sericifera* (Brot.) (Cantelo & Jacobson 1979), glossy abelia, *Abelia grandiflora* (Andre) (Grant 1971), night blooming jessamine, *Cestrum nocturnum* (Heath et al. 1992), and Japanese honeysuckle *Lonicera japonica* (Thunb.) (Pair & Horvat 1997). The alfalfa looper *Autographa californica* (Speyer) responds to flowers of Oregon grape, *Berberis aquifolium* (Prursh.) (Landolt & Smithhisler 2003), and the silver Y moth *Autographa gamma* (L.) is attracted to odors of flowers of creeping thistle, *Cirsium arvense* (L.), butterfly orchid, *Platanthera bifolia* (L.), soapwort, *Saponaria officinalis* (L), and others (Plepys 2001; Plepys et al. 2002b). Isolation and identification of the principal constituents of the odors of these flowers have provided a set of attractive chemicals for several major pest species of moths. For example, phenylacetaldehyde from flowers of bladderflower (Cantelo & Jacobson 1979) is attractive to a number of species of moths, and phenylacetaldehyde, benzyl alcohol, 2-phenylethanol, and benzaldehyde, isolated and identified from flowers of *A. grandiflora*, are attractive to cabbage looper moths (Haynes et al. 1991). The use of some of these floral attractants as lures holds promise for monitoring and surveying moths (Lopez et al. 2000; Landolt et al. 2006) or for management of pest species of moths (Camelo et al. 2007).

We report here studies to determine if moths are consistently visiting butterfly bush flowers and, if so, to identify those moths. We also determined the compounds that consistently make up the odor of those flowers and report antennal responses of cabbage looper and alfalfa looper moths to floral compounds emitted. This work addresses in part the hypothesis that moths are visiting butterfly bush flowers as a result of attraction to flower odor, and provides the identification of a set of chemicals that are putative attractants for moths that can be evaluated as such in future studies.

#### METHODS AND MATERIALS

In May 2002, butterfly bush plants were purchased from Richard Owen Nursery (Bloomington, IL) and were planted on the grounds of the USDA, ARS Yakima Agricultural Experiment Station, near Donald, Yakima County, Washington, USA. These plants received regular irriga-

tion from lawn sprinklers from mid Apr to mid Oct. Plants were 1 to 2 m tall when studies were initiated in Jul, 2003. Blooming occurred from Jun into Oct of 2003 and 2004. Bushes did not receive fertilizer or pesticide applications.

#### Moth Sampling

In order to sample night-flying insects visiting butterfly bush flowers, a large, white, mesh cone shaped trap (Scentry Heliothis trap, Gempler's, Madison, WI) was attached to a vertical pole, and positioned so that the trap opening was above but overlapped the top of a cluster of flowers (Landolt & Smithhisler 2003). Moths visiting these flowers and then departing might then fly up into the trap and be unable to escape the top chamber. Traps were positioned over flowers in late afternoon (1500 to 1600 h P.S.T.) and trapped insects were collected the following morning (0800 to 0900 h P.S.T.). Insects at flowers of 5 butterfly bushes were sampled intermittently, providing a total of 32 trap samples between 2 Jul and 22 Aug 2003, by sampling 2 bushes per night with 1 trap per bush. Insects in traps were captured with plastic vials, generally when alive, and were then killed in a freezer where they remained until identified.

#### Volatile Collections

Chemical odorants emitted by flower clusters on butterfly bushes growing outside were collected with a portable volatile collection system. This volatile collection system consisted of an electric air suction pump (model E2 M1.5, BOC Edwards, Wilmington, MA), flow meters (Gilmont #13, 0-13 L/min, Cole-Palmer, Vernon Hills, IL), Tedlar gas sampling bags (#40046, 10 L, 12 x 18" Tedlar snap with septum port, Alltech), and Super Q adsorbent traps (Analytical Research Systems, Gainesville, FL). Airflow into the suction pump was from 2 latex air lines provided by a glass T-shaped tube. One air line pulled air through a gas sampling bag holding a flower sample at the end of the air line, while the other air line pulled air through a gas sampling bag holding foliage and no flowers. The bag was placed over a branch with a flower raceme or with foliage only and was closed up with clamps around the stem of the branch. Air was pulled from the gas sampling bag through a septum port and then through the Super Q adsorbent trap and lastly through the flow meter at a rate of 2 L per min. The trap of the volatile collection system contained 30 mg of Super Q (400 mesh) adsorbent in a 0.635 cm x 6.67 cm long (1/4" x 2 5/8") borosilicate glass tube. A backup adsorbent trap of the same construction, but containing 10 mg of Super Q, was connected between the first trap and the vacuum pump to check for breakthrough of chemicals passing through the primary first adsorbent

trap. These collections were made for a period of 1 h, following a 15 min purge period of the system. Adsorbent traps were removed, sealed with Teflon tape and stored in a freezer overnight until they were extracted the following day. For each collection from a floral raceme, the number of open florets was counted so that chemical amounts per floret could be computed. This procedure was followed 9 times in 2003 over 4 different plants, providing the sampling of odorants from 9 butterfly bush branches with flowers and 9 branches without flowers. Volatile collections were conducted at sunset, between 18:30 and 20:30 h P.S.T.

#### Chemical Analysis

Adsorbent traps were extracted with 600  $\mu$ L of 10% ether in hexane. One  $\mu$ L aliquots of extracts from these Super Q traps were analyzed by gas chromatography mass spectrometry (GC-MS; Agilent 6890 GC) with a 5973 electron impact mass selective detector, and a 7683 series autosampler. The GC was equipped with a DB-1 (J & W Scientific, Folsom, CA) fused silica capillary column, 0.25 mm (ID)  $\times$  60 m (length) with 0.25  $\mu$ m film thickness. The temperature program used was 40°C for 1 min, increasing 15°C per min to 200°C. All 9 collections were first analyzed as above, and then 6 of the extracts were analyzed on a DB-Wax (J & W Scientific) capillary column of the same dimensions with a temperature program of 40°C for 2 min, increasing at 10°C per min to 180°C. Mass spectra of eluting peaks were matched to those in the NIST 98 library of compounds to obtain preliminary structural assignments. These assignments were confirmed by comparing the retention times of butterfly bush compounds with retention times of known standards, in both types of GC columns, and by comparing mass spectra of butterfly bush compounds with spectra of known standards. Pentadecane at 20  $\mu$ g/mL was used as an external standard for quantification of the identified chemicals.

Synthetic chemical standards used in establishing GC retention times and for comparing spectroscopic data were purchased from Aldrich Chemical Co. or Sigma-Aldrich Flavors and Fragrances (Milwaukee, WI). These were hexyl acetate, 6-methyl-5-hepten-2-one, benzaldehyde,  $\beta$ -cyclocitral, 4-oxoisophorone, and (*Z*)-cinnamaldehyde. These were 98-99% pure except for  $\beta$ -cyclocitral which was 90% pure. (*E,E*)- $\alpha$ -farnesene was extracted with pentane from apples and purified on silica gel columns to attain 98% purity.

#### Gas Chromatography with Electroantennographic Detection Analysis

One  $\mu$ L aliquots of extracts obtained from volatile collections were analyzed by gas chromatography coupled to electroantennographic detection

(GC-EAD) with a Hewlett Packard 5890 Series II gas chromatograph and an IDAC-232 data acquisition interface with a micromanipulator assembly type IRN-5 (Syntech, The Netherlands). The gas chromatograph was equipped with a DB-1 (Agilent Technologies, Palo Alto, CA) fused silica capillary column 0.25 mm (ID)  $\times$  60 m (length) with 0.25  $\mu$ m film thickness and samples were injected manually in splitless mode. The injector temperature was 250°C and helium was the carrier gas. The column temperature program started at 40°C for 2 min, increased to 200°C at a rate of 10°C/min and held at 200°C for 12 min. The effluent from the column was split by a OSS-2 splitter (SGE Analytical Science, Austin, TX) between the flame ionization detector (FID) and the electroantennographic detector (EAD) with a ratio FID:EAD of 1:2. The make-up gas connected to the splitter was nitrogen delivered at a flow rate of 40 mL/min.

#### Insects

Eggs of cabbage looper moths were provided by the Entomology Department at the University of Georgia, Athens, GA, USA. Larvae were reared on artificial cabbage looper diet (Southland Products Inc., Lake Village, AR). Males and females were separated at the pupal stage, and kept (as pupae and emerged adults) in separate rooms on reversed light cycle; L16:D8, at  $26.0 \pm 0.1$  °C and 65-70% relative humidity. Cabbage looper moths used for GC-EAD analyses were 2-6-d-old virgin females. Male alfalfa looper moths were captured in a walk-in light trap at the Yakima Agricultural Research Laboratory, during Jun and Jul 2007. These were kept for 1 to 2 d in the laboratory on a L16: D8 light cycle, at 24.8°C and 65-70% RH. GC-EAD analyses were conducted on male alfalfa looper moths because of light trap captures. One antenna per insect was excised at the base of the antennal scape and fixed between 2 silver electrodes with electrically conductive gel (Spectra 360 electrode gel, Parker Laboratories, Fairfield, NJ). GC-EAD analyses on volatile collections of butterfly bush were conducted on 5 separate antennae from 5 different moths and for each moth species. Antennal responses of identified compounds were confirmed through GC-EAD analysis by exposing 5 cabbage looper antennae to 1  $\mu$ L aliquots of a mixture of 20 ng/ $\mu$ L solutions of the corresponding synthetic standards.

#### RESULTS

Two hundred ninety three moths were captured in traps over butterfly bush flowers (Table 1). The greatest numbers were cabbage looper (52%) and alfalfa looper moths (24%). Other pest moths captured were the true armyworm *Pseudaletia unipuncta* (Haworth), the corn earworm,

TABLE 1. NUMBERS OF MOTHS CAPTURED IN HELIOTHIS TRAPS PLACED OVER FLOWER RACEMES OF BUTTERFLY BUSHES AT THE USDA, ARS YAKIMA AGRICULTURAL RESEARCH LABORATORY, WAPATO, WA. JUL AND AUG 2003.

| Moth species                                    | # Males | # Females | Total |
|-------------------------------------------------|---------|-----------|-------|
| <b>Geometridae</b>                              |         |           |       |
| <i>Digrammia curvata</i> (Grote)                | 1       | 0         | 1     |
| <i>Pero hubneraria</i> (Guenee)                 | 1       | 0         | 1     |
| <b>Noctuidae</b>                                |         |           |       |
| <b>Catocalinae</b>                              |         |           |       |
| <i>Melipotis januaris</i> (Guenee)              | 1       | 0         | 1     |
| <i>Caenurgina erecta</i> (Cramer)               | 0       | 1         | 1     |
| <b>Hadeninae</b>                                |         |           |       |
| <i>Dargida procincta</i> (Grote)                | 0       | 2         | 2     |
| <i>Discestra trifolii</i> (Hufnagel)            | 5       | 0         | 5     |
| <i>Lacanobia subjuncta</i> (Grote and Robinson) | 1       | 0         | 1     |
| <i>Leucania farcta</i> (Grote)                  | 1       | 0         | 1     |
| <i>Mamestra configurata</i> (Walker)            | 0       | 1         | 1     |
| <i>Pseudaletia unipuncta</i> (Walker)           | 9       | 1         | 10    |
| <b>Heliothinae</b>                              |         |           |       |
| <i>Helicoverpa zea</i> (Boddie)                 | 3       | 2         | 5     |
| <b>Plusiinae</b>                                |         |           |       |
| <i>Autographa californica</i> (Speyer)          | 41      | 28        | 69    |
| <i>Anagrapha falcifera</i> (Kirby)              | 2       | 2         | 4     |
| <i>Trichoplusia ni</i> (Hübner)                 | 83      | 69        | 152   |
| <b>Pyralidae</b>                                |         |           |       |
| <i>Crambus cypridalus</i> (Hulst)               | 11      | 3         | 14    |
| <i>Loxostege sticticalis</i> (L.)               | 6       | 2         | 8     |
| <i>Nomophila nearctica</i> (Munroe)             | 1       | 0         | 1     |
| <i>Prorasia simalis</i> (Grote)                 | 1       | 0         | 1     |
| <i>Dioryctria</i> sp.                           | 3       | 0         | 3     |
| <i>Udea rubigalis</i> (Guenee)                  | 4       | 6         | 10    |
| <b>Tortricidae</b>                              |         |           |       |
| <i>Choristoneura occidentalis</i> (Freeman)     | 2       | 0         | 2     |

bertha armyworm, *Mamestra configurata* (Walker), *Lacanobia subjuncta* (Barnes & MacDunnough), clover looper, *Caenurgina erecta* (Cramer), and the celery leaf-tier, *Udea rubigalis* (Guenee). In total, 12 species of Noctuidae, 6 species of Pyralidae, 2 species of Geometridae, and 1 tortricid species were captured in the traps. In addition, we caught 28 butterflies, 33 honeybees *Apis mellifera* L., 22 other bees and wasps, 277 syrphid flies, 133 other Diptera, 17 Hemiptera, 13 lacewings, 5 beetles, 9 earwigs, 3 damsel flies, and 10 spiders.

Nine compounds were present consistently in all 9 flower odorant samples and not in foliage odor samples (Table 2). Those compounds are 4-oxoisophorone (2,6,6-trimethyl-2-cyclohexene-1,4-dione); benzaldehyde; (*E,E*)- $\alpha$ -farnesene; 6-methyl-5-hepten-2-one; hexyl acetate; oxoisophorone oxide (1,3,3-trimethyl-7-oxabicyclo [4.1.0]-heptan-2,5-dione); dihydrooxoisophorone (2,6,6-trimethyl-1,4-cyclohexadione),  $\beta$ -cyclocitral, and (*Z*)-cinnamaldehyde (Fig. 1, chemical structures). These 9 compounds were initially identified by mass spectral data and the structures of 6 of these were then confirmed by compar-

ing retention times and spectral data of those compounds with those of synthetic standards. Synthetic samples were not available for oxoisophorone oxide and dihydrooxoisophorone and those structural assignments are tentative. Mean amount of odorants emitted per floret was  $57.4 \pm 6.0$  ng/h (mean  $\pm$  S.E.). Flower clusters or racemes averaged  $371 \pm 38.7$  florets, and mean amount of volatiles per raceme was then  $21.3 \pm 2.2$   $\mu$ g/h (mean  $\pm$  S.E.). Amounts of chemicals collected were quite variable, indicated by the large standard errors for mean amounts of individual chemicals in Table 2. The limit of detection was estimated to be 0.1 ng per analysis, which was 60 ng per collection (0.1 ng/ $\mu$ L  $\times$  600  $\mu$ L/sample).

Five of the floral compounds identified triggered consistent antennal responses in female cabbage looper moths (Table 2). These 5 compounds were benzaldehyde, 4-oxoisophorone, dihydrooxoisophorone, oxoisophorone oxide, and (*E,E*)- $\alpha$ -farnesene. Antennal responses to benzaldehyde, 4-oxoisophorone, and (*E,E*)- $\alpha$ -farnesene were confirmed by exposing female cabbage looper antennae to the corresponding standards. Three of the floral compounds, benzaldehyde, ox-

TABLE 2. MEAN ( $\pm$  SEM) AMOUNTS OF ODORANT COMPOUNDS PER FLORET OBTAINED IN VOLATILE COLLECTIONS FROM FLOWER RACEMES OF BUTTERFLY BUSHES AND ANTENNAL RESPONSE THROUGH GC-EAD ANALYSIS (EAD) OF CABBAGE LOOPER (CL) AND ALFALFA LOOPER (AL) MOTHS TO THESE ODORANT COMPOUNDS OBTAINED IN VOLATILE COLLECTIONS (EXTRACT) AND TO THE CORRESPONDING SYNTHETIC CHEMICALS (SYNTHETIC).

| Compound                   | Nanograms per floret | Percent of Blend | CL EAD to extract | CL EAD to synthetic | AL EAD to extract |
|----------------------------|----------------------|------------------|-------------------|---------------------|-------------------|
| Benzaldehyde               | 12.8 $\pm$ 1.5       | 22.4 $\pm$ 2.7   | +                 | +                   | +                 |
| 6-Methyl-5-hepten-2-one    | 1.2 $\pm$ 0.2        | 2.0 $\pm$ 0.3    | —                 |                     | —                 |
| Hexyl acetate              | 0.8 $\pm$ 0.1        | 1.4 $\pm$ 0.2    | —                 |                     | —                 |
| Oxoisophorone oxide        | 1.8 $\pm$ 0.4        | 3.2 $\pm$ 0.6    | +                 | nt                  | +                 |
| 4-Oxoisophorone            | 29.8 $\pm$ 3.4       | 52.0 $\pm$ 6.0   | +                 | +                   | +                 |
| Dihydrooxoisophorone       | 1.1 $\pm$ 0.3        | 1.8 $\pm$ 0.5    | +                 | nt                  | —                 |
| $\beta$ -Cyclocitral       | 1.4 $\pm$ 0.3        | 2.5 $\pm$ 0.6    | —                 |                     | —                 |
| (Z)-Cinnamaldehyde         | 1.3 $\pm$ 0.4        | 2.3 $\pm$ 0.7    | —                 |                     | —                 |
| (E,E)- $\alpha$ -Farnesene | 7.1 $\pm$ 2.0        | 12.4 $\pm$ 3.5   | +                 | +                   | —                 |
| Total                      | 57.4 $\pm$ 6.0       | 100%             |                   |                     |                   |

+: EAD response in at least 3 antennae out of 5 antennae exposed to either the extract or the synthetic chemicals;  
-: no EAD response; nt: not tested.

oisophorone oxide, and 4-oxoisophorone, elicited consistent antennal responses with male alfalfa looper moths (Table 2).

DISCUSSION

Although known for “attractiveness” to day-active butterflies (Corbet 2000), the flowers of the butterfly bush also were visited consistently by night-flying Lepidoptera. The list of moths trapped indicates that a diversity of species of moths visits these flowers. However, most of the moths trapped were 2 pest species of Noctuidae in the subfamily Plusiinae; the cabbage looper and the alfalfa looper. Because our sampling was conducted in Jul and Aug and at 1 site, we expect that various numbers and additional species of moths would visit butterfly bush flowers at other times of the season and at other locations. Several species of Plusiinae, including the cabbage looper, alfalfa looper, and silver Y moth have been reported as frequent visitors at flowers of other plants. Cabbage looper moths have been observed at flowers of *A. grandiflora* (Grant 1971), alfalfa looper moths were collected at flowers of Oregon grape (Landolt & Smithhisler 2003), and the silver Y moth is an abundant visitor at flowers of *Silene latifolia* (Brantjes 1976).

Other pestiferous moth species that were trapped over butterfly bush flowers were the corn earworm and the true armyworm. The corn earworm moth feeds at flowers (Hendrix et al. 1987; Lindgren et al. 1993) and responds to odors of flowers such as Japanese honeysuckle *Lonicera japonica*, and *Gaura drummondii* (Beerwinkle et al. 1996; Pair & Horvat 1997; Lopez et al. 2000) and is also attracted to odors of fermented sugar baits (Ditman & Cory 1933). The true armyworm has not been reported as a frequent flower visitor, but was captured in traps baited with acetic acid and 3-methyl-1-butanol, a feeding attractant derived from fermented molasses solutions (Utrio & Eriksson 1977; Landolt & Higbee 2002).

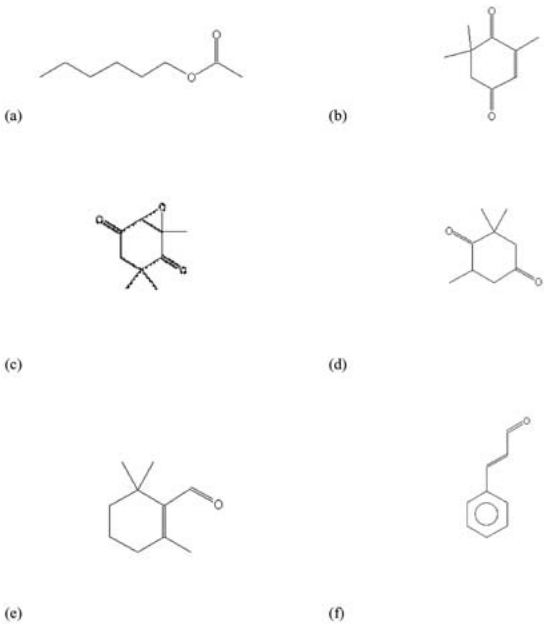


Fig. 1. Structures of compounds identified from volatile collections made over open flowers of *Buddleja* bushes. (a) Hexyl acetate CAS 142-92-7 (Acetic acid, hexyl ester); (b) 4-Oxoisophorone CAS 1125-21-9 (2,6,6-trimethyl-2-cyclohexene-1,4-dione); (c) Oxoisophorone oxide (1,3,3-trimethyl-7-oxa-bicyclo[4.1.0]heptane-2,5-dione); (d) Dihydrooxoisophorone CAS 20547-99-3 (2,2,6-trimethyl 1,4-cyclohexadione); (e)  $\beta$ -Cyclocitral CAS 432-25-7 (2,2,6-trimethyl 1-cyclohexene-carboxaldehyde); (f) (Z)-Cinnamaldehyde CAS 104-55-2 (2-Propenal, 3-phenyl-).

We hypothesize that moths locate flowers of butterfly bush from some distance by flying upwind in response to the flower odor. Initial observations in late afternoon of moths at butterfly bush flowers indicated plume-tracking behavior (zigzagging upwind flights) at distances up to 2 M downwind of flower racemes, but these moths were not identified. We found that these flowers produce a high amount of odorants that might attract moths. Moth attraction to chemicals emitted by flowers is well documented. For example, many moths are attracted to bladderflower, and to phenylacetaldehyde, which is produced by bladderflower (Cantelo & Jacobson 1979). The cabbage looper moth is a frequent visitor at flowers of *A. grandiflora* (Grant 1971), which produces the cabbage looper attractants phenylacetaldehyde, benzaldehyde, 2-phenylethanol, and benzyl alcohol (Haynes et al. 1991). Alfalfa loopers visit flowers of Oregon grape, which produces a blend of odorant chemicals including the alfalfa looper attractants phenylacetaldehyde and  $\beta$ -myrcene (Landolt et al. 2001, 2006; Landolt & Smithhisler 2003). Other examples include moth attraction to flowers of *L. japonica* which produces phenylacetaldehyde, linalool, and cis-jasmone (Pair & Horvat 1997), *C. nocturnum* which produces benzaldehyde, benzyl acetate, and phenylacetaldehyde (Heath et al. 1992), *S. latifolia* which produces phenylacetaldehyde and lilac aldehydes (Dötterl 2004; Dötterl et al. 2006), and *Platanthera bifolia* which produces lilac aldehydes (Plepyš et al. 2002a).

Five of the 9 compounds found in the odor of butterfly bush flowers elicited antennal responses in cabbage looper and 3 compounds in alfalfa looper moths, suggesting differences in antennal receptor sensitivity between moth species. Previous studies with butterflies and butterfly bush scents showed antennal responses to the same chemicals these moths responded to, although selective antennal responses to those compounds also were observed between butterfly species (Andersson 2003; Andersson & Dobson 2003). Differences in antennal responses between moth species could also indicate gender differences, with female cabbage looper being responsive to more compounds than male alfalfa looper, as suggested with the butterfly *Heliconius melpomene* (Andersson & Dobson 2003). Four-oxoisophorone and oxoisophorone oxide were the only 2 compounds that were found to consistently elicit antennal responses in both moth species as well as in the 4 butterfly species when testing butterfly bush scent samples (Andersson 2003; Andersson & Dobson 2003). Four-oxoisophorone is not only eliciting antennal responses in Lepidoptera species, but is active for bees (Dötterl et al. 2005).

Four-oxoisophorone was identified previously and in the present study as the most abundant compound emitted by butterfly bush flowers (Andersson et al. 2002; Andersson 2003). This

compound is found also in floral scents of *Primula farinosa* (Gaskett et al. 2005), *Lumnitzera racemosa* (Azuma et al. 2002), *Salix atrocinerea* (Dötterl et al. 2005), and other plants (Knudsen et al. 2006). Two other compounds in the odor of butterfly bush, benzaldehyde and (*E,E*)- $\alpha$ -farnesene, are odorants for other moth-visited flowers. Benzaldehyde was found in odor of other plant species including *C. nocturnum* (Heath et al. 1992), *A. grandiflora* (Haynes et al. 1991), *S. latifolia* (Jürgens et al. 2002), and *B. aquifolium* (Landolt & Smithhisler 2003). (*E,E*)- $\alpha$ -farnesene was found in the floral odor of Japanese honeysuckle (Schlotzhauer et al. 1996). An additional butterfly bush flower odorant, cinnamaldehyde, which did not stimulate antennal responses in our study, is found in odor of flowers of *S. latifolia* (Jürgens et al. 2002), and *G. drummondii* (Teranishi et al. 1991).

Three to 5 of the 9 compounds produced by butterfly bush flowers are biologically relevant compounds for alfalfa looper and cabbage looper moths. Although it is not completely known which of these compounds may be involved in attracting night-flying moths, some are already known to be behaviorally active for the cabbage looper, alfalfa looper, and other moths. Benzaldehyde was attractive to cabbage looper moths in a flight tunnel assay (Haynes et al. 1991; Heath et al. 1992), but was not attractive to alfalfa looper moths when field-tested by Landolt et al. (2001) in Washington, or to soybean looper moths, *Pseudoplusia includens* (Walker), when field-tested by Meagher (2002) in Florida. Further research will be needed to determine which of these chemicals elicit upwind attraction responses in moths such as the cabbage looper and alfalfa looper.

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