

Characterization of the Floral Odor of Oregongrape: Possible Feeding Attractants for Moths

Abstract

Moths and other insects at blooms of Oregongrape were sampled with traps during April and May 2001. Moths captured were predominantly the alfalfa looper (43%), and the gooseberry fruitworm (26%) with small numbers of 14 other species in the families Noctuidae and Geometridae. Analyses of samples made from the air around blooms of Oregongrape shrubs revealed the consistent presence of α -pinene, β -pinene, sabinene, E- β -ocimene, β -myrcene, limonene, benzaldehyde, and phenylacetaldehyde. Greatest amounts released per flower per hour were 124.5 ± 40.8 ng phenylacetaldehyde, 87.6 ± 6.7 ng α -pinene, 54.4 ± 17.8 ng E- β -ocimene, and 43.9 ± 7.0 ng limonene. Phenylacetaldehyde and benzaldehyde are known attractants for some moths, including the alfalfa looper. The odor produced by Oregongrape blooms may attract feeding moths for pollination of flowers.

Introduction

Tall Oregongrape (*Berberis aquifolium*) is endemic to the Pacific Northwest of North America (Peck 1961) and is commonly planted as an ornamental in both residential and commercial settings. The shrub blooms in early spring, producing numerous yellow flowers on an upright raceme. The flowers are odorless to the human nose during the day and at night. Observations of moths, including alfalfa looper moths (*Autographa californica*), flying about strong-smelling blooms of tall Oregongrape in Yakima, Washington, led to this investigation of moth visitors at flowers and the odor of those flowers. We hypothesize that volatile chemicals emitted by flowers of Oregongrape attract these moths to the flowers, where they feed on floral nectar.

Volatile chemicals have been identified from flowers of several plants that are frequented by moths, and some of these chemicals attract moths. Examples of plants that possess flowers attractive to moths are bladderflower (*Araujia sericofera*) (Cantelo and Jacobson 1979), glossy abelia (*Abelia grandiflora*) (Grant 1971, Haynes et al 1991), night-blooming jessamine (*Cestrum nocturnum*) (Heath et al. 1992), Japanese honeysuckle (*Lonicera japonica*) (Pair and Horvat 1997; Schlotzhauer et al. 1996), lesser butterfly orchid (*Platanthera bifolia*) (Plethys 2001), and Drummond's gaura (*Gaura drummondii*) (Teranishi et al. 1991, Lopez et al. 2000). Phenylacetaldehyde from flowers of

bladderflower attracts many species of moths (Cantelo and Jacobson 1979). Glossy abelia flowers emit phenylacetaldehyde, benzaldehyde, 2-phenylethanol, and benzyl alcohol, which are attractive to cabbage looper moths: (Haynes et al. 1991). Phenylacetaldehyde, benzyl acetate, and benzyl alcohol, emitted by flowers of night-blooming jessamine, attract cabbage looper moths: (Heath et al. 1992). Cis-jasmone, phenylacetaldehyde, and linalool, which are attractive to several species of moths, are present in extracts of flowers of Japanese honeysuckle (Schlotzhauer et al. 1996, Pair and Horvat 1997). Similarly, five chemicals identified from flowers of Drummond's gaura (phenylacetaldehyde, 2-phenylethanol, limonene, methyl salicylate, and methyl-2-methoxy benzoate) (Teranishi et al. 1991) constitute a blend that attracts corn earworm moths (*Helicoverpa zea*) (Lopez et al. 2000). The gamma moth (*Autographa gamma*) of Eurasia is attracted to lilac aldehydes, methyl benzoate, and benzyl benzoate, which are emitted by flowers of the lesser butterfly orchid (*Platanthera bifolia*) (Plethys 2001).

The alfalfa looper, a close relative of both the gamma moth and the cabbage looper, is a highly polyphagous caterpillar that is a pest of numerous garden and farm crops throughout the western United States. The moth of this species is attracted to some of the same volatile chemicals that are emitted by flowers discussed above. Phenylacetaldehyde and benzyl acetate attract alfalfa looper moths (Landolt et al 2001), cis-jasmone enhances alfalfa looper moth attraction to phenylacetaldehyde, and benzaldehyde enhances

¹ Author to whom correspondence should be addressed. E-mail: landolt@yarl.ars.usda.gov

alfalfa looper moth attraction to benzyl acetate (Landolt et al. 2001). Our general investigation of moth behavior at flowers seeks to understand the roles of different plants as food sources for moths, to understand how moths locate food sources, and to use this information to discover new chemical attractants for management of pest species.

The objectives of this study were to determine what moths might visit flowers of Oregongrape plants, determine if known moth attractants are part of the odor of those flowers, and characterize the floral odor for possible further investigation of these chemicals as insect attractants. We collected and identified moths at flowers of Oregongrape plants, and characterized the volatile chemicals emitted by those flowers. The results support the hypothesis that moth visitation at flowers of this plant is in part a response to floral odor chemistry.

Methods

Insect visitors to flowers of Oregongrape plants were sampled using large white mesh cone shaped traps (*Heliothis* traps) placed directly above clusters of open flowers. Insects are trapped when they fly up from flowers into the cone of the trap and cannot escape the upper chamber. Traps were placed over flower clusters in late afternoon (1500 to 1600 hrs P.S.T.) and were checked the following morning (800 to 900 hrs P.S.T.) for insects. We sampled 37 times during April and May 2001: at the Yakima Agricultural Research Laboratory near Wapato ($n = 3$), Randall Park in the city of Yakima ($n = 5$), a residence in Yakima ($n = 4$), Oak Creek Wildlife Preserve ($n = 9$), Mud Lake ($n = 15$) west of Yakima, and the Valley Mall in Union Gap ($n = 1$). All trap sites were in Yakima County, Washington.

Volatiles emitted by flower clusters on Oregongrape shrubs at the USDA-ARS Yakima Agricultural Research Laboratory were collected by a portable volatile collection system, which included an electric air suction pump, flow meter, a gas sampling bag, and a SuperQ trap. Air was pulled through a septum port in the 10 liter Tedlar gas sampling bag housing the flowers, through the flowmeter at a rate of 2-3 L of air per minute, and then through the trap in which odor chemicals were adsorbed. The bag was placed over a branch with flowers and was loosely tied off at

the branch stem. Air was pulled through the volatile collection system for 1 hr, during late afternoon or early evening. The volatile collection trap contained 30 mg of SuperQ adsorbent in a 0.635 cm x 6.67 cm long borosilicate glass tube. The trap was extracted with 600 μ L of 10% ether in hexane. As a control, similar volatile collections were made over Oregongrape plants that did not possess flowers. The open flowers within volatile collection bags were counted and recorded for computations of chemical amounts emitted per flower per unit time. This volatile collection procedure was followed five times for plants with flowers and nine times for plants without flowers. All collections were made over plants on the grounds of the USDA, ARS, Yakima Agricultural Research Laboratory.

One-microliter aliquots of the extracts of SuperQ traps were analyzed by gas chromatography-mass spectrometry (GC-MS), using a Hewlett Packard 6890 Plus gas chromatograph with a model 5973 electron impact mass selective quadrupole detector. The gas chromatograph was equipped with an HP-1 MS fused silica capillary column (60 m long, 0.25 mm i.d., 25 μ m film thickness) and then a DB Wax fused silica capillary column of the same dimensions. Analyses were run at an initial temperature of 40°C for 2 min, increasing 15°C per min to a maximum of 200°C. Mass spectra of eluting peaks were matched to those in the Wiley 275 and NIST98 libraries of compounds to obtain preliminary structural identifications. Structures were confirmed by comparing retention times of eluting peaks with known standards, using both types of GC columns, and by comparing mass spectra of eluting peaks and of known standards to those in the Wiley and NIST data bases.

Additional analyses were conducted to determine the enantiomeric makeup of α - and β -pinene and limonene. Commercial sources of compounds were used as standards that were compared with α -pinene, β -pinene, and limonene from Oregongrape odorants (volatile collection trap extract) and standards for (R)- and (S)- α -pinene, (R)- and (S)- β -pinene, and (R)- and (S)-limonene were analyzed on a Hewlett Packard 5890 II Plus GC using a 0.25 mm x 30 m Cyclosil B fused silica capillary column. Temperature program was 40°C for 2 min, increasing 10°C per min to a maximum of 180°C. Kovat's retention indices were

calculated using a homologous series of hydrocarbons to bracket each compound (nonane through dodecane). One extract sample was spiked with the hydrocarbon series before analysis, while another sample was compared to the hydrocarbons in a parallel GC analysis. A sample of racemic α -pinene was also analyzed following the addition of the hydrocarbon series to the sample, for precise determination of retention indices of the two enantiomers and to verify that the two enantiomers were adequately separated using those methods.

Synthetic chemical standards used in establishing GC retention times and for comparing spectroscopic data were purchased from chemical supply companies. These were benzaldehyde, phenylacetaldehyde, β -myrcene, S-(-)- β -pinene, R-(+)- β -pinene, racemic α -pinene, (1R)-(+)- α -pinene, R(+)- and (S-(-)- limonene, sabinene, and (S)-(-)- α -pinene. Synthetic E- β -ocimene was not commercially available. A 93% pure sample of E- β -ocimene was then obtained by HPLC fractionation of pure essential oil of basil which possesses this compound (Özek et al. 1995, Fleisher and Fleisher 1992). The E- β -ocimene was isolated from basil oil using an Agilent 1100 series HPLC equipped with an Agilent Eclipse semi-prep column (#XDB-C18, 9.4 mm x 250 mm). A standard flow cell was used with a column-switching valve for collection of fractions from elution with 50% acetonitrile: 50% water at 4 ml/min. Identification of a compound in basil oil as E- β -ocimene was verified by GC-MS analysis under the conditions indicated above.

Results

Ninety-two moths were captured in traps over Oregongrape flowers, 41 males and 51 females (Table 1). Most moths captured were either alfalfa looper moths (42%), or the gooseberry fruitworm (*Zophodia grossulariella*) (26%). While both male and female alfalfa looper moths were captured, only female and no male gooseberry fruitworm moths were captured in the traps. Eleven other species of noctuid moths and three species of geometrid moths were captured. In addition, 125 honeybees (*Apis mellifera*), 137 queen bumblebees (*Bombus* sp.), 71 other unidentified bees, 23 queen German wasps (*Vespula germanica*), 3 female golden paper wasps (*Polistes aurifer*), and 152 unidentified flies were trapped.

Eight compounds were present in chromatograms from all five air collections made over blos-

TABLE 1. Number of moths captured in traps over clusters of flowers of Oregongrape shrubs. March-May 2001, Yakima County, Washington.

Species	Males	Females
Noctuidae		
Plusiinae		
<i>Autographa californica</i>	26	13
<i>Trichoplusia ni</i>	1	0
Amphipyriinae		
<i>Apamea cariosa</i>	7	5
<i>Apamea cincta</i>	2	1
<i>Apamea spaldingi</i>	1	1
Hadeninae		
<i>Discestra trifolii</i>	0	2
<i>Discestra mutata</i>	0	1
<i>Discestra oregonica</i>	1	0
<i>Dargida procincta</i>	1	0
<i>Leucania insueta</i>	0	1
Noctuinae		
<i>Diarsia rosaria</i>	0	1
<i>Agrotis volubilis</i>	0	1
Geometridae		
Ennomiinae		
<i>Digrammia californiaria</i>	1	0
<i>Synaxis cervinaria</i>	1	0
<i>Eupithecia</i> sp.	0	1
Pyralidae		
Phycitinae		
<i>Zophodia grossulariella</i>	0	24

soms of Oregongrape shrubs (Figures 1,2). β -pinene and limonene were present in volatile collections as both enantiomeric forms (Figure 2). These compounds were not detected in samples collected from nonflowering Oregongrape shrubs. The limit of detection was estimated to be about 30 ng per collection, which is a limit of 0.05 ng as a GC peak, and with one μ L of the 600 μ L sample injected for analysis (0.05 ng/ μ L x 600 μ L/sample = 30 ng/sample).

The largest amounts of chemicals collected were phenylacetaldehyde and S-(-)- α -pinene, followed by E- β -ocimene and limonene (Table 2). The amounts collected were also quite variable, as indicated by the standard errors of the means (Table 2), but the proportions were relatively stable, as is indicated by the smaller standard error values for mean percents of total emissions calculations (Table 2).

Discussion

Results of this study show that several species of moths, bees, wasps, and flies, visit flowers of

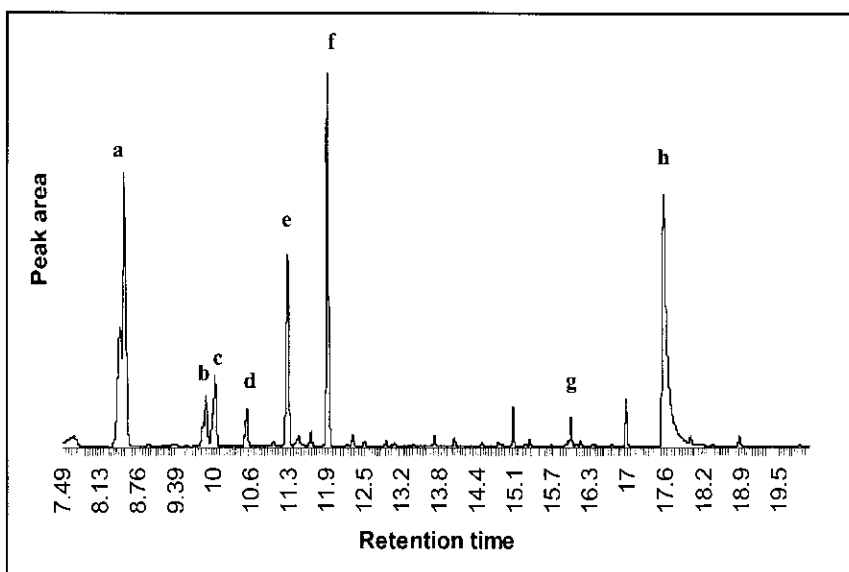


Figure 1. Typical chromatogram from analysis of volatile collection made over flowers of Oregongrape, using a DB wax capillary column. Peaks: (a) α -pinene, (b) β -pinene, (c) sabinene, (d) β -myrcene, (e) limonene, (f) E- β -ocimene, (g) benzaldehyde, and (h) phenylacetaldehyde.

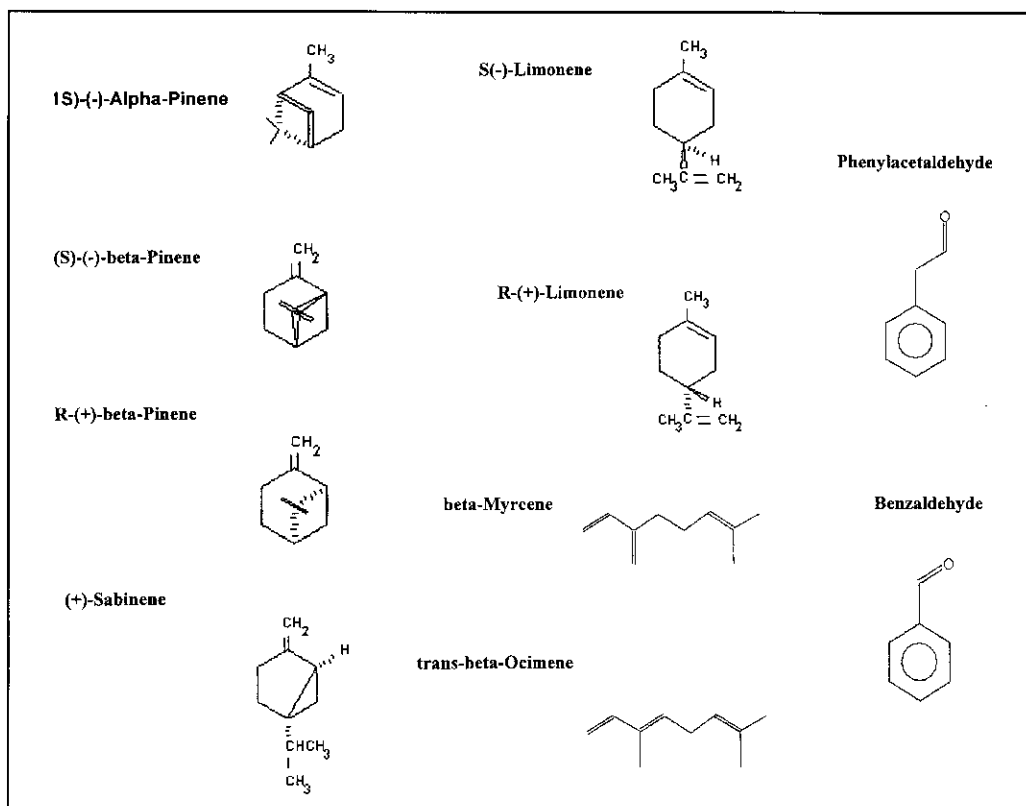


Figure 2. Structures of compounds isolated and identified from volatile collections made over open flowers of Oregongrape.

TABLE 2. Mean (\pm SE) nanograms of compounds emitted per Oregongrape flower per hour. N is the number of volatile collections from flowers from which quantitative data were obtained. α -Pinene and benzaldehyde co-eluted on two of five collections and were not quantified in those samples.

Chemical	Nanograms	Percent of Total Emission	N
Phenylacetaldehyde	124.5 \pm 40.8	32.9 \pm 2.0	5
α -pinene	87.6 \pm 6.7	25.5 \pm 6.4	3
E- β -Ocimene	54.4 \pm 17.8	13.9 \pm 2.8	5
Limonene	43.9 \pm 7.0	12.5 \pm 0.7	5
Sabinene	17.0 \pm 2.7	4.9 \pm 0.4	5
Benzaldehyde	14.5 \pm 6.7	4.2 \pm 6.7	3
β -pinene	12.3 \pm 2.0	3.6 \pm 0.4	5
β -Myrcene	8.6 \pm 2.1	2.4 \pm 0.3	5

Oregongrape and are potential pollinators. The most common moth visitor collected was the alfalfa looper moth. While both sexes of this moth were captured, the preponderance of alfalfa looper moths in traps were male. This species is generally abundant and flies from early spring into late autumn. Like the related cabbage looper and gamma moths, both male and female alfalfa looper moths visit flowers for nectar. The second most abundant moth collected was the gooseberry fruitworm moth. All collected specimens were female, suggesting that the moth may respond to Oregongrape flower chemicals in search of a place to oviposit, rather than in search of nectar on which to feed. However, this species is not known to infest fruits of Oregongrape. The makeup of the species of moths collected is likely influenced by the early-season blooming of Oregongrape in relation to the phenology of local moths, and by the regional moth community. Moths that emerge later in the season do not coincide with Oregongrape blooming, and the species of moths visiting Oregongrape flowers is probably somewhat different in other areas of the Pacific Northwest.

These data support the hypothesis that Oregongrape releases floral odors attractive to moths. The odorous nature of Oregongrape flowers suggests that volatile chemicals are released that might attract some species of moths. This phenomenon is known for bladderflower (Cantelo and Jacobson 1979), glossy abelia (Haynes et al. 1991), night-blooming jessamine (Heath et al. 1992), Drummond's gaura (Teranishi et al. 1991, Lopez et al. 2000), lesser butterfly orchid (Plethys 2001), and Japanese honeysuckle (Pair and Horvat 1997).

Moths visit flowers of these plants and the flowers of the plants produce volatile chemicals that attract moths. Phenylacetaldehyde, which strongly attracts both sexes of the alfalfa looper (Landolt et al. 2001), is a major component of the floral odor of Oregongrape. This evidence suggests that alfalfa looper moths, and possibly other moth species, locate Oregongrape flowers by tracking the floral odor. It remains to be determined if other Oregongrape odor chemicals attract alfalfa looper moths.

The blend of chemicals collected over Oregongrape flowers includes phenylacetaldehyde and benzaldehyde, which are released by other moth-visited plants (Cantelo and Jacobson 1979, Haynes et al. 1991, Heath et al. 1992, Schlotzhauer et al. 1996). Phenylacetaldehyde is attractive to several species of moths, including the cabbage looper, the soybean looper (*Pseudoplusia includens*), and the alfalfa looper (Cantelo and Jacobson 1979, Haynes et al. 1991, Landolt et al. 1991, Heath et al. 1992, Pair and Horvat 1997). Phenylacetaldehyde and benzaldehyde occur in the hairpencils of males of some moths (Aplin and Birch 1970, Bestmann et al. 1977, Clearwater 1972, Grant et al. 1972). However, they are not known to play any pheromonal role in moth sexual interactions.

The floral bouquet of Oregongrape also includes a set of terpene compounds that are found in plant foliage and as plant odorants, but are not known to be moth attractants or moth pheromones. For example, β -pinene, limonene, (E)- β -ocimene, and myrcene are volatiles emitted from cotton plants (Pare and Tumlinson 1996), α -pinene, β -pinene, sabinene, limonene and (E)- β -ocimene are emitted from maple leaves (Loughrin et al. 1997), and α -pinene, β -pinene, sabinene, myrcene, and limonene are released from foliage of cabbage and nasturtium plants (Geerviliet et al. 1997). These compounds may be pheromones or kairomones for other types of insects. For example, α -pinene, β -pinene, myrcene, sabinene, and limonene are released by some bark beetles and may be part of their aggregation pheromones (Mayer and McLaughlin 1991). Comprehensive field-testing of these compounds, separately and in multi-component blends, will be required to determine any roles they may play in attracting moths or other potential pollinators to Oregongrape flowers.

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