Measurement of Enantiomer Percentages for Five Monoterpenes From Six Conifer Species by Cartridge Tube-Based Passive Sampling Adsorption/Thermal Desorption (ps-ATD)

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June 13, 2022

Abstract

Many monoterpenes have at least two different stereochemical forms, and many biosynthetic pathways are known to favor one product over the other(s). A rapid method was developed and used in the determination of the (-/+) enantiomeric distributions for *α*-pinene, *β*-pinene, camphene, limonene, and *β*-phellandrene as emitted by plant material from six conifer species. The six species included two pine species *Pseudotsuga menziesii* and *Pinus ponderosa*, and four cypress species, *Chamaecyparis lawsoniana*, *Thuja plilcata, Juniperus chinensis, and Thuja occidentalis.* The method involved passive sampling adsorption/thermal desorption (ps-ATD). During sampling, the cartridge tube was placed in a 60 mL glass vial with plant material for 1 h. Sample analytes were thermally transferred to a chiral gas chromatography (GC) column. Detection was by mass spectrometry (MS). The six species exhibited different emission patterns for the five monoterpenes in the –/+ totals, although within a given species the distributions among the five monoterpenes were similar across multiple plants. *β*-pinene dominated in *P. menziesii* and *P. ponderosa*, and *α*-pinene dominated in *T. plicata* and *T. occidentalis*. The chiral separations revealed differences in the –/+ enantiomeric distributions among the species. The (-)-enantiomers of *α*-pinene and *β*-pinene dominated strongly in *P. menziesii* and *P. ponderosa*; the (-)-enantiomer of *β*-phellandrene dominated in *C. lawsoniana*. The dependence of the method precision on percent enantiomer abundance is discussed.

Key words: monoterpenes, enantiomers, chiral distributions, conifers, passive sampling, ATD, ps-ATD

Introduction

Atmospheric emissions of gaseous non-methane organic compounds from plants are both substantial and chemically complex (Guenther et al., 1995, Pankow *et al.,* 2012; de O. Piva et al., 2019). Plant emissions are greater than those from animals, and are believed to be related to a variety of purposes, including repulsion of herbivorous insects and attraction of pollinators and parasites of herbivores (Dicke and Loon, 2000). Isoprene (C_5H_8) and compounds derived from isoprene are particularly prominent in plant emission profiles. Guenther et al. (1995) has estimated that isoprene and monoterpenes constitute approximately 11 and 55%, respectively, of global non-methane emissions. Their oxidation in the atmosphere leads to products that promote formation of ozone (Porter et al., 2017) and which condense as secondary organic aerosol particulate matter (Pankow 1994a; Pankow, 1994b; Zhang et al., 2018).

Monoterpenes that possess chiral carbons can exist in two mirror-image "enantiomeric" forms; for α-pinene, (-) α-pinene and (+) α-pinene. For a given compound, different biochemical synthesis pathways in different plants can favor one enantiomer over the other, and many biochemical interaction loci are chiral (López et al., 2011). An example pertains to carvone. The form predominantly found in carraway seeds (*Carum carvi*) is *S* (+) carvone while the form predominantly found in spearmint (*Mentha spicata*) is *R* (-) carvone.

In forests, where legion species are emitting innumerable compounds for which many have multiple enantiomers, the matter is obviously exceedingly complex. For example, it required careful study by Williams et al. (2007) just to be able to conclude that in tropical forests, emission of (-) α-pinene is light-dependent, and that in boreal forests emission of (+) α-pinene is temperature-dependent. Stephanou (2007) has argued that careful and data driven studies of chirality will be required to fully understand the mechanisms of atmospheric emission of volatile organic compounds by plants. Accordingly, improvements in the requisite analytical methods will be useful.

Table 1 provides a summary of the methodologies used to carry out chiral determinations of plant monoterpenes. Analyte collection has occurred using solvents in various ways, and by sorption of volatilized (gaseous) analytes in air. Following sampling, analytes are subjected to quantitation of the enantiomer forms using chiral gas chromatography (GC). The acquisition of terpenoid analytes from plant samples can be accomplished in different ways. With **solvent extraction** as executed in the examples cited in Table 1 (e.g., with hexane), there are the disadvantages of the: 1) difficulties posed by large organic solvent signals; 2) generally negative implications that solvent injection carries for peak sharpness in GC; and 3) sensitivity problems when the analytes in the extract are not sufficiently concentrated. With **sorptive sampling and "SPME"**, gaseous monoterpenes can be acquired using passive diffusion-limited transfer into the coatings of solid phase microextraction (SPME) fibers. With **sorptive sampling and "ATD"**, sample gas flow is pulled through an "adsorption/thermal desorption" (ATD) cartridge tube (Pankow, 1988). For sampling and placement of analytes on a GC column, SPME can lead to better chromatographic resolution than ATD: less time/gas volume is needed to thermally transfer the analytes from the sorption phase to the column. When optimized, automated SPME can be cost-effective if the main goal is the accurate determination of chiral ratios. Automated SPME, however, is less prevalent and more complicated than automated ATD, the latter being well optimized and available on multiple commercial instrument platforms. Since ATD interfaced with chiral GC in our laboratory has been found to give adequate enantiomeric resolution for monoterpenes of interest, the goal of this work was to develop and test passive-sampling ATD (ps-ATD) as a simple and low-labor method for carrying out enantiomeric analyses of monoterpenes emitted by plant materials. The method is based on passive-sampling with ATD cartridges followed by automated ATD. Since only enantiomeric *fractions* and not actual enantiomer *concentrations* were sought in this work, use of passive diffusion sampling carried no drawbacks (*e.g.*, diffusion coefficients of enantiomer pairs are identical as indicated by Fuller's Equation (Tang et al., 2014)).

2 Materials and Methods

2.1 Percent (-) Enantiomer Format

Two distinctly different formats are available for presenting enantiomer data. The first is the abundance ratio for the two forms (or the log of the ratio); the second is as a percent of one form, *e.g.*, the percent of the (-) form. The ratio format has advantages in the study of the molecular specificity of biosynthetic pathways; the percentage format complements source apportionment work wherein abundances of 0.5% and 1% of a given (-) molecule would not likely lead to meaningfully different model conclusions. This work will use the (-) enantiomer format.

2.2 Plant Samples

Purchased Nursery Plants (Six Species). Six coniferous species were purchased as ~1 m high potted (~8 L pots) saplings from a local nursery in January of 2018. These included the two pine species *Pseudotsuga menziesii* (4 plants) and *Pinus ponderosa* (3 plants), and the four cypress species *Chamaecyparis lawsoniana*, *Thuja plicata*, *Juniperus chinensis* and *Thuja occidentalis* (4 plants each). The saplings were placed on the roof of the SRTC Building on the PSU campus, and were watered daily. The high/low temperature ranges for Portland during 2018 were: March, 19.4/4.3 ºC; April, 30.0/6.7 ºC; May, 31.7/12.3 ºC; June, 34.4/13.1 ºC; July, 35.6/16.7 ºC; August, 35.0/16.6 ºC. The elevation of the PSU campus is 52 m (above sea level). A foliage sample was collected from each plant at mid height in March 2018 and again in June/July 2018 using a clean pruning shears. The samples were taken immediately to the laboratory for processing.

Purchased Nursery *T. occidentalis* – Time of Day Samples. Foliage samples from the purchased *T. occidentalis* plants were collected at mid height with clean shears on August 20, 2018 at 6 AM, 1 PM, 7 PM, and 9 PM. The temperatures and light intensities were recorded. The samples were taken immediately to the laboratory for analysis.

Established Residential *T. occidentalis*. Samples from 6 to 7 established (5+ years), ~3+ m tall) specimens of *T. occidentalis* were collected between February 13-26, 2018 from residential locations in each of three suburban vicinities in Oregon (Hillsboro, Seaside, and Sandy). The approximate time of day for the sampling, the annual mean high/low temperatures, the annual mean precipitation, and the elevation above sea level for each were as follows: Hillsboro, 6:30 to 7:30pm, 17.2 ºC/6.7 ºC , 97.0 cm, 52 m; Seaside, 8:30 to 10:00am, 13.9 ºC/6.7 ºC , 191.4 cm, 8 m; and Sandy, 2:00 to 3:30pm, 15.6 ºC/6.1 ºC, 198.9 cm, 299 m. For each sample, a 15 to 20 cm branch of foliage at ~1.5 m above ground was clipped using a clean shears. The cut end of each sample was wrapped with a wet paper towel at the cut. Each sample was stored in an unzipped ziplock bag with the cut end inside of the bag. The samples from Hillsboro arrived within 14 h and were analyzed immediately. The samples from Seaside and Sandy arrived at the laboratory within 2 h and were processed immediately.

2.3 Sample Preparation

Plant samples were rinsed with deionized water; surface water was removed by blotting with a clean paper towel. Sample material was cut into ~1 cm pieces with a clean laboratory scissors. Each plant replicate subsample of ~0.3 g were transferred to clear 60 mL "VOA" vials (Restek Corporation, Bellefonte, PA). Each vial was sealed with a 0.125 in. thick PTFE lined septum (Restek Corporation, Bellefonte, PA) and held at 20±0.5 °C for 60 min. Passive sampling with an ATD cartridge then GC/MS analysis proceeded as described below.

2.4 Chemical Standards

The five monoterpenes examined here were *α*-pinene, *β*-pinene, camphene, limonene, and *β*-phellandrene. Authentic chiral and racemic standards were purchased from Sigma Aldrich Inc. (St. Louis, MO) at ≥98% purity.

2.5 Gas Chromatography (GC)

Relative total amounts of the monoterpenes (total (+/-) *α*-pinene, total (+/-) *β*-pinene, etc.) and the enantiomeric fractions for the (-) forms were determined by GC. The elution order was established by analysis of standards. The chiral column stationary phase was Supelco Beta DEX™ 120 (Supelco Inc., Bellefonte, PA) with 0.25 μm film thickness, 0.25 mm i.d., and 30 m length. After gaseous introduction of each sample into the column, the GC oven temperature program was: 1) hold at 60 °C for 2 min; 2) ramp to 90 °C at 1 °C/min; 3) ramp to

105 °C at 3 °C/min; 4) ramp to 220 °C at 10 °C/min; then 5) hold at 220 °C for 2 min. The gas flow rate (helium) through the column was approximately 1.0 mL/min. Figure 1 provides an example of a chromatogram for a sample.

2.6 Headspace Sampling, Analyte Transfer to GC, and Mass Spectrometric (MS) Analysis

The "VOA" vials used were from Restek Corporation (Bellefonte, PA). The 40 mL standard vials contained ~1 mg of neat liquid standard. As noted below, the 60 mL vials were loaded with ~0.3 g of plant material. In all cases, sampling proceeded in a passive manner by exposing the inlet end of an ATD gas sampling cartridge to the vial headspace. Before exposure, each cartridge was otherwise wrapped with clean aluminum foil. For standards, sampling of the gas phase involved a 2 s exposure with the cartridge held in the inlet in the headspace of an open vial. For samples, each cartridge was placed in its vial for 2 h with the vial capped. No flow through into the cartridge was required to acquire adequate analyte mass for any given analysis (~0.05 ng of an enantiomer on an ATD cartridge (or ~0.01 ng on-column) was required to obtain a signal to noise (S/N) ratio of 50:1). Passive sampling was used because the primary interest was the enantiomeric percentages of the subject compounds, and not emission rates or consequent ecosystem concentrations. The ATD cartridges were from Camsco Inc. (Houston, TX), as packed with 100 mg of 35/60 mesh Tenax TA on the inlet side followed by 200 mg of 60/80 mesh Carbograph 1 TD.

ATD cartridges were auto-processed using a TurboMatrix 650 ATD (PerkinElmer Inc., Waltham, MA) unit interfaced to a Leco Pegasus 4D GC×GC-TOFMS (Leco Corporation, St. Joseph, MI) used in 1-D GC mode (*i.e.*, without application of a secondary column). (TOFMS

= time of flight mass spectrometer.) In the Turbomatrix 650 unit, the analytes on each ATD cartridge were thermally desorbed (270 °C, 10 min, 40 mL/min He, backflush mode (outlet to inlet) direction) onto an intermediate Tenax-TA focusing trap held at −10 °C. 25 mL/min of the 40 mL/min desorption flow was discarded as “split” flow. The focusing trap was then thermally desorbed at 280 °C for 5 min at 16 psi constant He pressure. About 2 mL/min of the flow passed onto the GC column in the TOFMS unit via a 225 °C transfer line; the remaining ~20 mL/min split flow was discarded. MS data acquisition began upon initiating thermal desorption of the focusing trap.

For α-pinene, camphene, limonene and β-phellandrene, for the percent enantiomer determinations, the MS quantitation ion used was $m/z = 93$. For β-pinene, $m/z = 69$ was used. For each compound in a given sample, the percent of each enantiomer was calculated using the area for each deconvoluted peak (in any case of co-elution) for the enantiomer quantitation ion divided by the corresponding sum for both enantiomers. Note here that both enantiomers in a given pair will have exhibited the exact same: 1) diffusion coefficient during sampling; 2) transfer efficiencies during analysis; and 3) detector sensitivities.

The fractional mass distribution among the five monoterpenes was calculated for each sample using the peak pair sums, each of which was normalized using total ion chromatogram (TIC)-based relative response factors relative to α-pinene ($\mathrm{RRF}_{\alpha\text{-pinene}}$). Obtained from analyses of replicate ATD cartridges onto which known amounts (~10 ng) of each monoterpene in 4 μL of methanol/acetone had been loaded (by syringe), the measured TIC $\mathrm{RRF}_{\alpha\text{-pinene}}$ values were α-pinene, 1:00; β-pinene, 0.83; camphene, 0.93; limonene, 0.83; and β-

phellandrene, 0.44. Inherent in these calculations of the fractional mass distributions among the five monoterpenes are the assumptions that: 1) the passive sampling rate by gaseous diffusion was essentially the same for all of the compounds (per Fuller's Equation); and 2) the desorption transfer efficiencies to the analytical unit were very similar for all of the compounds.

The average of the above five TIC RRF$_{\alpha\text{-pinene}}$ values (0.81) was used to obtain an estimate of the mass percentage for each sampling of the sum of the five monoterpenes (10 enantiomers) relative to all detected monoterpenes (=($\Sigma^5/\Sigma^{\text{all}}$)×100%). The LECO software was used to deconvolute: 1) each of the 10 enantiomer TIC peaks for the five compounds; and 2) each of the other compound TIC peaks identified (based on mass spectral matching and GC retention time window) as probable monoterpenes. The most abundant of these were sabinene and myrcene. The deconvoluted TIC peak areas (A) were integrated then used with the TIC response factors with

$$\sum\nolimits^{5} = \frac{A_{\alpha\text{-pinene}}}{\text{RRF}_{\alpha\text{-pinene}}} + \frac{A_{\beta\text{-pinene}}}{\text{RRF}_{\beta\text{-pinene}}} + \frac{A_{\text{camphene}}}{\text{RRF}_{\text{camphene}}} + \frac{A_{\text{limonene}}}{\text{RRF}_{\text{limoene}}} + \frac{A_{\beta\text{-phellandrene}}}{\text{RRF}_{\beta\text{-phellandrene}}} \qquad (1)$$

$$\sum\nolimits^{\text{all}} = \sum\nolimits^{5} + \sum\nolimits_{i}^{\text{other}} \left(\frac{A_{\text{other}}}{0.81} \right)_{i} \qquad (2)$$

2.7 Statistical Analyses

One-way ANOVA was used to analyze variables such as proportion of monoterpenes and enantiomeric ratios among six species, as well as enantiomeric ratios in *T. occidentalis* under different conditions. Multiple comparisons among different species, different sampling time and different positions were detected using the least significant difference (LSD) test, with a critical significance level of $p = 0.05$. All analyses were performed using SPSS statistical software (version 27.0, IBM Inc., Armonk, NY, USA).

3 Results and Discussion

3.1 Proportion of Monoterpenes Among Different Nursery-Purchased Species

Mass percent values among the five target monoterpenes for the six nursery-purchased species and their $(\Sigma^5/\Sigma^{all})\times100\%$ values are given in Figures 2.a and 2.b. (and Tables 2.a and 2.b). These values were obtained using the combined (enantiomer pair) deconvoluted TIC peak area data for each monoterpene together with the corresponding $RRF_{\alpha\text{-pinene}}$ values. *α*-pinene and *β*-pinene were found to be the dominant monoterpenes in the two pine species *P. menziesii and P. ponderosa*, and *α*-pinene and limonene dominated in *C. lawsoniana.* Limonene represented more than 90% of the five compounds for *J. chinensis.*

3.2 Enantiomer Percentages among Different Nursery-Purchased Species

The percentages of the (-) form for the five compounds in the six species for March and June/July are given in Figures 3.a and 3.b (and Tables 3.a and 3.b). For all species, the results were similar for the two sampling times. The results for the two pine species (*P. menziesii* and *P. ponderosa*) were similar, but the results varied among the four cypress species (*C. lawsoniana*, *T. plicata*, *J. chinensis*, and). In the two pine species, the percentages of the (-) form were >90%, >90%, and >50% for *α*-pinene, *β*-pinene and limonene, respectively. The lowest percentages of the (-) form for *α*-pinene and limonene were observed in *C. lawsoniana* and *J. chinensis*. The lowest percentages of the (-) form for *β*-pinene were observed in *C. lawsoniana* and *T. plicata*. The (-) form of camphene strongly dominated in *C. lawsoniana*. The (-) form of *β*-phellandrene was highest in *C. lawsoniana*.

3.3 Enantiomer Percentages in Nursery-Purchased *T. occidentalis* from 6 AM to 9 AM

The percentages of the (-) form for the five compounds in the nursery-purchased *T. occidentalis* plants in one day in August 2018 are given in Figure 4 (and Table 4). The enantiomeric profiles were very similar for the four different sampling times.

3.4 Enantiomer Percentages in Nursery-Purchased vs. Residential *T. occidentalis*

The percentages of the (-) form for the five compounds in nursery-purchased and residential *T. occidentalis* plants (sampled in March 2018 and February 2018, respectively) are given in Figure 5 (and Table 5). The enantiomeric profiles were all remarkably similar.

3.5 Enantiomer Percentage Method Precision

When relative enantiomer abundance is expressed in terms of the percent of one of the forms, a decreasing abundance will tend to be accompanied by an increasing coefficient of variation CV = (standard deviation of abundance/mean abundance) × 100%. For example, in the hypothetical data in Table 6, for both monoterpene 1 and monoterpene 2 the standard deviation of the (-) abundance is 0.28%; for monoterpene 1 at 99% abundance the CV value is much smaller than for monoterpene 2 at 1% abundance. For the data in Tables 3.a and 3.b obtained here, the effect of decreasing percent for the (-)-enantiomer on the CV (%) is shown in Figure 6. Nevertheless, CV < 20% for 75% of the data points.

4 Conclusions

The method allowed differences to be discerned in the relative abundances of the enantiomers for multiple monoterpenes in six plant species. The relative precision values tended to deteriorate at low percent values for the (-) enantiomer; since the replicates analyses were carried out on plant sample replicates (i.e., each with a different plant subsample), that deterioration was due at least in some measure to biological variability in the subsamples.

Acknowledgements

Support for this work was provided to Y.W. by the Education Department of Jilin Province under Awards JJKH2016058 and JJKH20210273KJ. Additional support for the project was provided by the Maseeh Foundation.

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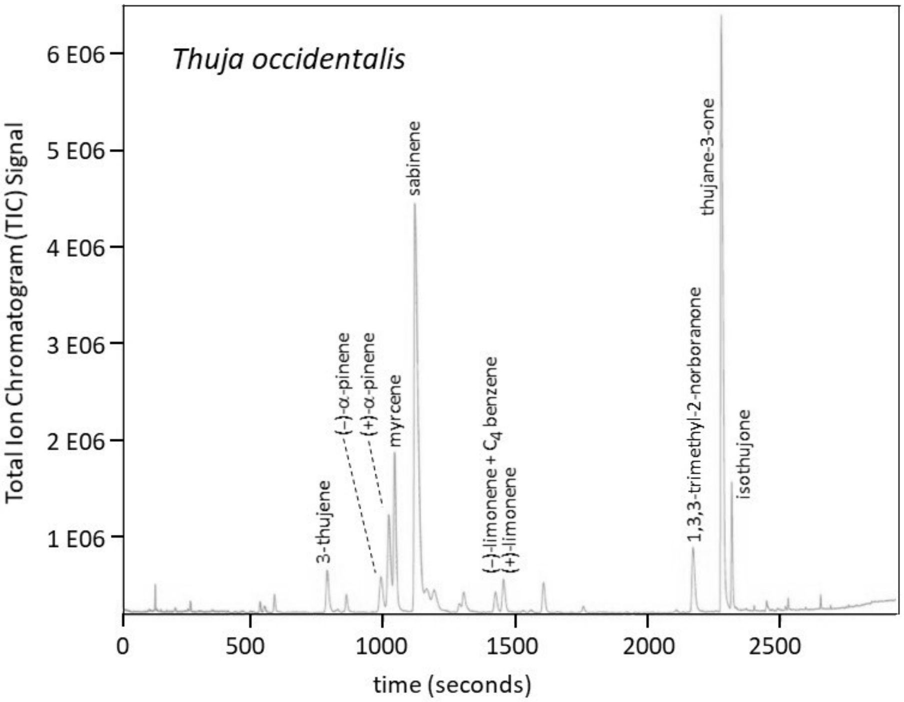

Figure 1. Total ion chromatogram (TIC) by GC/MS (gas chromatography/mass spectrometry) using a Supelco Beta DEX™ 120 chiral capillary column (0.25 µm film thickness, 0.25 mm i.d., and 30 m long; Supelco Inc., Bellefonte, PA) for a *T. occidentalis* sample. The peak marked for (-)-limone contains a contribution from an unidentified C_4-benzene. The two α-pinene enantiomers and the two limonene enantiomers were quantitated using the ion $m/z = 93$.

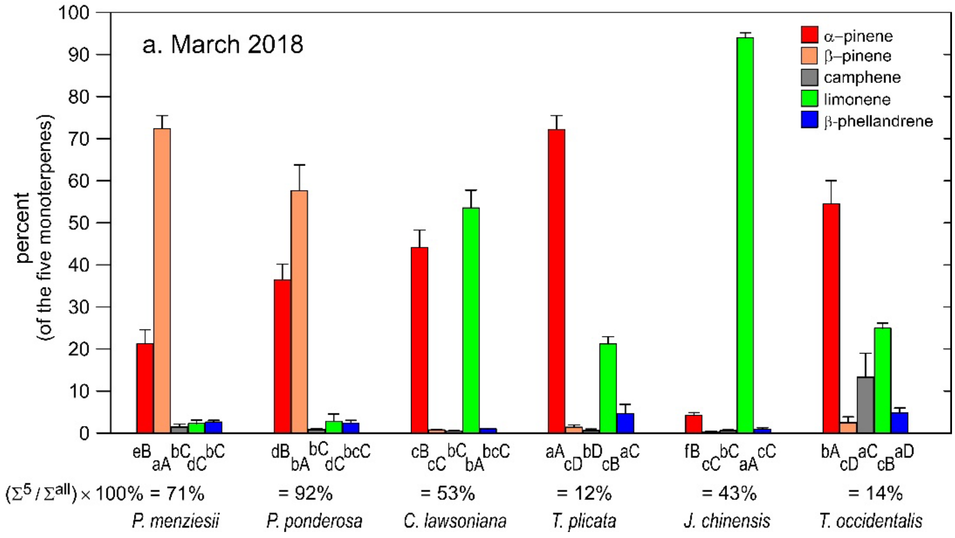

Figure 2.a. Bar graph showing percentages among five monoterpenes in March 2018 for six nursery-purchased conifer species. Within a given species, the largest value is designated "A"; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated "a"; thereafter, a different lower case letter indicates a significant difference between the species. The percentage values that the five monoterpenes represent as a sum relative to the sum of all detected monoterpenes (=(Σ^5/Σ^{all})×100%) are given. The error bars are ±1s. The data values are given in Table 2.a.

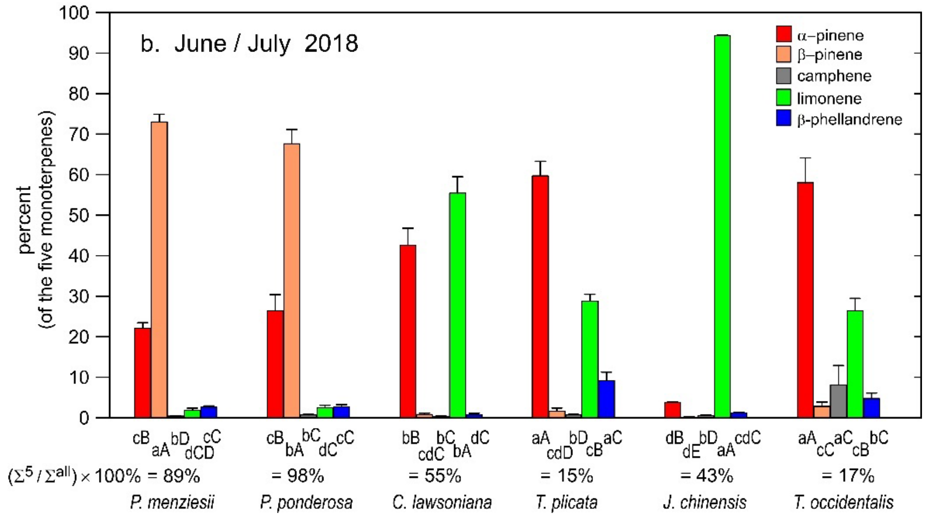

Figure 2.b. Bar graph showing the percentages among five monoterpenes in June/July 2018 for six nursery-purchased conifer species. Within a given species, the largest value is designated "A"; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated "a"; thereafter, a different lower case letter indicates a significant difference between the species. The percentage values that the five monoterpenes represent as a sum relative to the sum of all detected monoterpenes (=(Σ^5/Σ^{all})×100%) are given. The error bars are ±1s. The data values are given in Table 2.b.

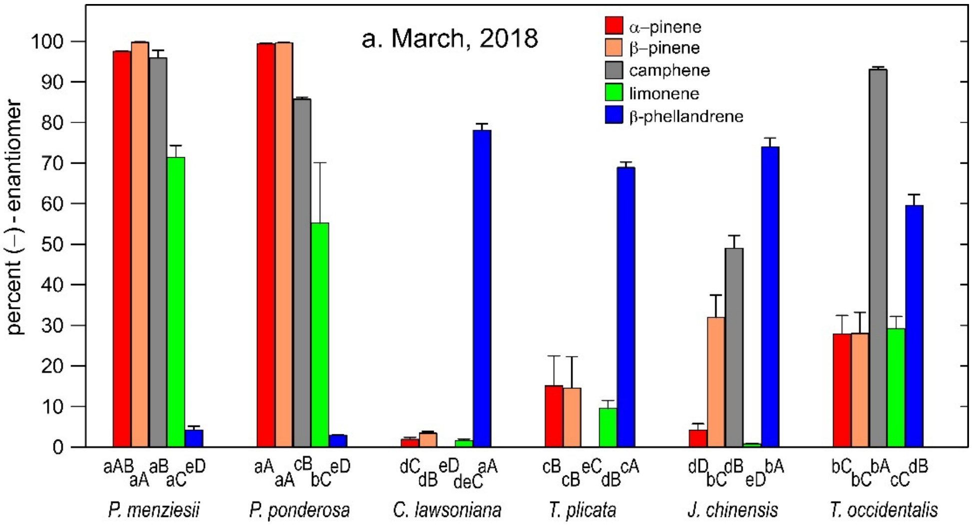

Figure 3.a. Bar graph showing the percentage values for the (-)-enantiomer for five monoterpenes in March 2018 for six nursery-purchased conifer species. Within a given species, the largest value is designated “A”; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated “a”; thereafter, a different lower case letter indicates a significant difference between the species. The error bars are ±1s. The data values are given in Table 3.a.

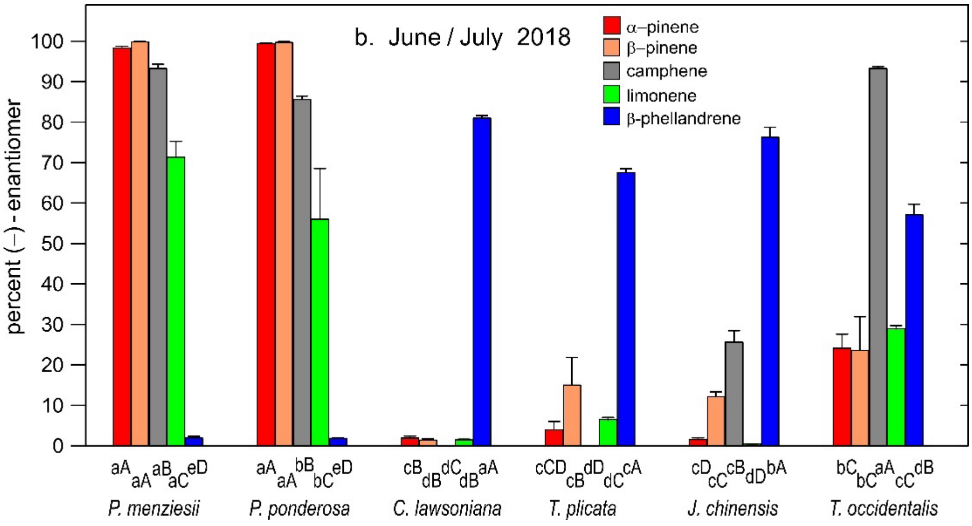

Figure 3.b. Percent of the (-)-enantiomer for five monoterpenes in June/July 2018 for six nursery-purchased conifer species. Within a given species, the largest value is designated “A”; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated “a”; thereafter, a different lower case letter indicates a significant difference between the species. The error bars are ±1s. The data values are given in Table 3.b.

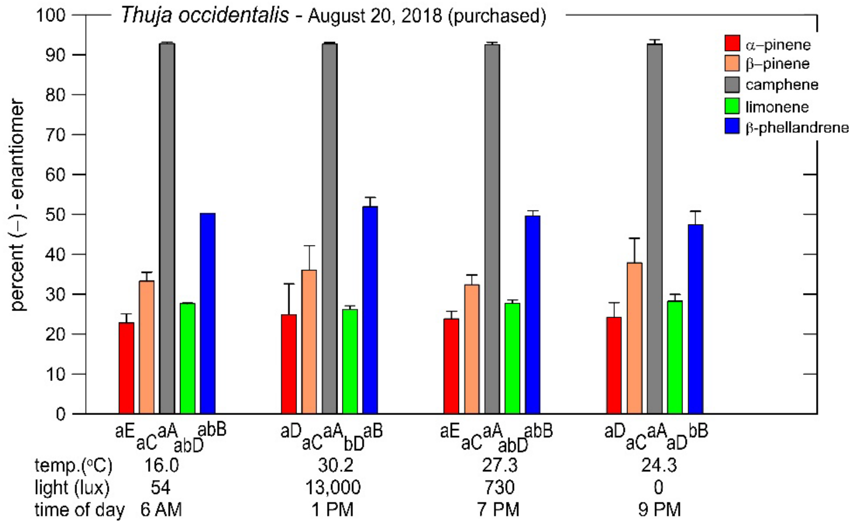

Figure 4. Percent of the (-)-enantiomer for five monoterpenes in nursery-purchased samples of *Thuja occidentalis* on August 20, 2018. For a given time, the largest value is designated "A"; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated "a"; thereafter, a different lower case letter indicates a significant difference between the times. The error bars are ±1s. The data values are given in Table 4.

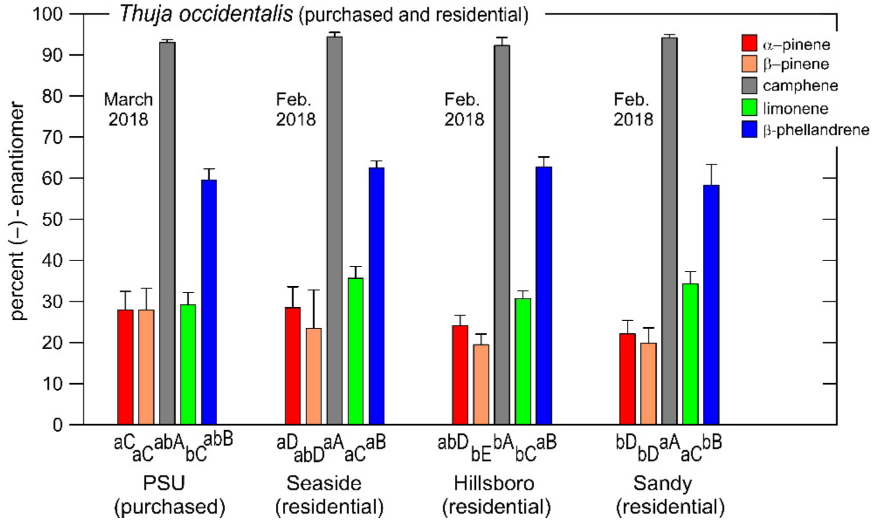

Figure 5. Percent of the (-)-enantiomer for five monoterpenes in nursery-purchased (March 2018) and residential (February 2018) samples of *Thuja occidentalis*. For a given sample location, the largest value is designated "A"; thereafter, a different capital letter indicates a significant difference between the monoterpenes. For a given monoterpene, the largest value is designated "a"; thereafter, a different lower case letter indicates a significant difference between the locations. The error bars are ±1s. The data values are given in Table 5. The data for the "PSU (purchased)" plants also appear in Figure 3.a.

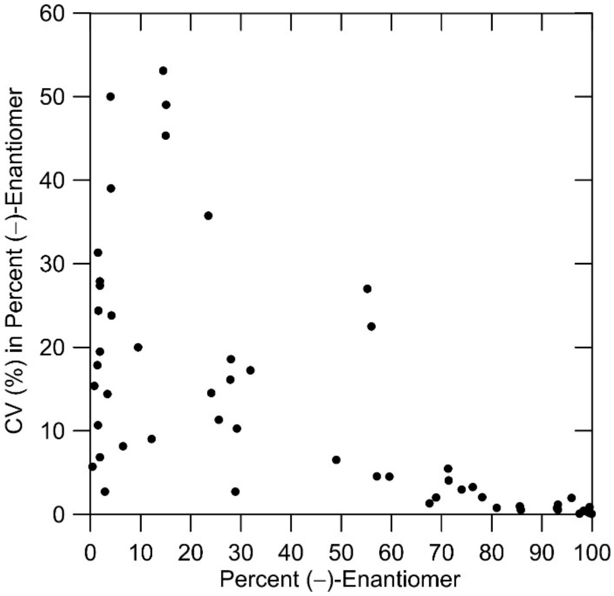

Figure 6. Coefficient of variation (CV, %) values for the percent (-)-enantiomer vs. percent of the (-)-enantiomer (based on data in Tables 3.a and 3.b). CV < 20% for 75% of the data points.

Table 1. Summary of methods used to sample then analyze plant-derived chiral biogenic volatile organic compounds.

A. Solvent Extraction then Injection

Citation - Plant/System(s)	Summary
Persson *et al*., 1993 *Picea abies*	Method. Extraction of plant material with hexane, silica gel clean-up, then two-dimensional heart-cut GC-FID (GC phases: DB-WAX then permethylated *β*-cyclodextrin). Analytes: α-pinene, camphene, *β*-pinene, sabinene, limonene, *β*-phellandrene.
Persson *et al.* (1996) *Picea abies*	Method: Extraction of plant material with hexane, silica gel clean-up, then two-dimensional heart-cut GC-FID (GC phases: DB-WAX then permethylated *β*-cyclodextrin) for most chiral separations. For 3-carene, a dipentylbutyryl-γ-cyclodextrin phase was used; the constituents of the monoterpenes were identified by mass spectroscopy (MS). Analytes: *α*-pinene, camphene, *β*-pinene, sabinene, limonene, *β*-phellandrene, and others (23 total enantiomers).
Sjödin *et al.* (1996) *Pinus sylvestris*	Method: Same as in Persson et al. (1996). Analytes: *α*-pinene, camphene, *β*-pinene, sabinene, limonene, *β*-phellandrene, myrcene, 3-carene.
Wibe *et al.* (1998) *Picea abies*, *Pinus sylvestris*, *Juniperus communis*	Method: Following Wibe and Mustaparta (1996), headspace volatiles were sampled with flow through an adsorbent (Porapak Q). Analytes were recovered with hexane. Two-dimensional heart-cut GC/FID followed using the GC phases DB-WAX and permethylated *β*-cyclodextrin. Analytes: *α*-pinene, camphene, *β*-pinene, sabinene, 3-carene, limonene, *β*-phellandrene.
Ložienė and Labokas (2012) *Juniperus communis L*	Method: Steam distillation collection of essential oils, then dilution in a solvent mix (diethyl ether/pentane), then GC/FID (GC phase: HP-Chiral-20B). Analyte: *α*-pinene.
Southwell *et al.* (2017) *Melaleuca alternifolia and M. linariifolia*	Method: Steam distillation collection of essential oils, then dilution with ethanol, then GC/FID (GC phase: cyclodextrin). Analytes: Terpinen-4-ol, limonene, *α*-terpineol.
Inoue *et al.* (2018) *Lindera umbellata var. membranacea*	Method: Hexane extraction of plant material, then GC/MS analysis (GC phase: CycloSil-B). Analytes: *α*-pinene, camphene, *β*-pinene, sabinene, limonene, *β*-phellandrene, and others (29 total, including enantiomeric variations).

B. Diffusion Sampling by Exposure of SPME Fiber to Air Containing Plant Emissions then Thermal Desorption

Citation - Plant/System(s)	Summary
Ruiz del Castillo *et al.* (2004)	Method: SPME with 100 µm polydimethylsiloxane (PDMS) phase, then GC/MS (GC phase: permethylated β-cyclodextrin or 2,3-di-acetoxy-6-*O*-

Mentha piperita	*tert*-butyl dimethylsilyl γ-cyclodextrin). Analytes: α-pinene, camphene, β-pinene, sabinene, limonene, β-phellandrene, and others (19 total, including enantiomeric variations).
Yassaa and Williams (2007) *P. sylvestris chemotype A and B* (boreal coniferous forest)	Method: SPME with PDMS/DVB phase, then GC/MS (GC phase: permethylated β-cyclodextrin). Analytes: α-pinene, camphene, β-pinene, sabinene, limonene, β-phellandrene, and others (17 total, including enantiomeric variations).
Yassaa *et al*. (2010) *Quercus ilex*	Method: SPME with PDMS/DVB phase, then GC/MS (GC phase: β-cyclodextrin). Analytes: α-pinene, camphene, β-pinene, sabinene, limonene, myrcene, 3-carene, 1,8-cineol, cis-β-ocimene.

C. Active Flow Sampling of Air Containing Plant Emissions Through an ATD Sorbent Cartridge Tube then Thermal Desorption

Citation - Plant/System(s)	Summary
Williams *et al.* (2007) tropical and boreal forests	Method: ATD with Carbograph I/Carbograph II adsorbent, then GC/MS (GC phase: β-cyclodextrin). Analytes: α-pinene, camphene, β-pinene, limonene, myrcene, 3-carene.
Song *et al.* (2011) *Pinus pinea L.* (forest canopy)	Method: ATD with Tenax TA/Carbograph I, then GC/MS (GC phase: β-cyclodextrin). Analytes: α-pinene, β-pinene, limonene, camphor, and others (12 total including enantiomeric variations).
Song *et al.* (2014) *Quercus ilex L., Rosmarinus officinalis L., and Pinus halepensis Mill.*	Method: ATD with Carbograph I/II or Tenax/carbograph, then GC/MS (GC phase: β-cyclodextrin). Analytes: α-pinene, β-pinene, limonene, camphor, isoprene, and others (13 total including enantiomers).
Staudt *et al.* (2019) Maritime pine (forest canopy)	Method: ATD with Tenax TA/Carbograph 1 adsorbent, then GC/MS (GC phase: dimethyl TBS β-cyclodextrin). Analytes: α-pinene, β-pinene.
Zannoni *et al*. (2020) Amazon rain forest	Method: ATD with Carbographs 1 and 5, then GC/MS (GC phase: dimethyl TBS β-cyclodextrin). Analyte: α-pinene.

D. This Work - Passive Diffusion Sampling of Air Containing Plant Emissions Into Open End of ATD Sorbent Tube the Thermal Desorption

Citation - Plant/System(s)	Summary
This Work *Pseudotsuga menziesii, Pinus ponderosa, Chamaecyparis lawsoniana, Thuja plilcata, Juniperus chinensis, Thuja occidentalis*	Method: ATD with Tenas TA/Carbographs 1 adsorbent, then GC/MS (GC phase: β-cyclodextrin). Analytes: α-pinene, camphene, β-pinene, limonene, β-phellandrene.

Table 2. Mass fraction values (including both enantiomers) for each of five chiral monoterpenes over those five monoterpenes, and average values of (Σ^5 / Σ^{all}) × 100% (= mass fractions for the mass sum for those five terpenes over all detected monoterpenes). The nursery-purchased plants were located at PSU and sampled in March 2018 and again in June/July 2018. Number of plant sample replicates $N = 4$ for all species, except $N = 3$ for *P. ponderosa*. For each plant sample replicate, a separate sample of plant material was analyzed once.

Table 2.a. March 2018 (see Figure 2.a).

	percent of the monoterpene over the five monoterpenes (total = 100%)					
species	α-pinene	β-pinene	camphene	limonene	β-phellandrene	(Σ^5 / Σ^{all}) × 100%
P. menziesii	21.2 ± 3.3	72.4 ± 3.1	1.4 ± 0.8	2.3 ± 0.8	2.7 ± 0.4	71.2
P. ponderosa	36.4 ± 3.8	57.6 ± 6.1	0.80 ± 0.22	2.8 ± 1.7	2.4 ± 0.7	92.3
C. lawsoniana	44.1 ± 4.1	0.78 ± 0.1	0.50 ± 0.10	53.5 ± 4.2	1.0 ± 0.1	53.2
T. plicata	72.2 ± 3.3	1.4 ± 0.5	0.59 ± 0.37	21.2 ± 1.7	4.6 ± 2.2	11.5
J. chinesis	4.2 ± 0.7	0.30 ± 0.15	0.59 ± 0.17	93.9 ± 1.2	1.0 ± 0.3	43.0
T. occidentalis	54.5 ± 5.6	2.5 ± 1.4	13.3 ± 5.7	25.0 ± 1.2	4.8 ± 1.2	14.4

Table 2.b. June/July 2018 (see Figure 2.b).

	percent of the monoterpene over the five monoterpenes (total = 100%)					
species	α-pinene	β-pinene	camphene	limonene	β-phellandrene	(Σ^5 / Σ^{all}) × 100%
P. menziesii	22.1 ±1.3	73.0 ± 1.9	0.38 ± 0.15	1.9 ± 0.5	2.6 ± 0.3	88.7
P. ponderosa	26.5 ± 3.9	67.7 ± 3.5	0.71 ± 0.11	2.5 ± 0.6	2.7 ± 0.6	98.2
C. lawsoniana	42.6 ± 4.2	0.83 ± 0.31	0.33 ± 0.09	55.4 ± 4.0	0.82 ± 0.27	55.2
T. plicata	59.7 ± 3.6	1.6 ± 0.8	0.72 ± 0.15	28.8 ± 1.7	9.2 ± 2.1	15.1
J. chinesis	3.8 ± 0.15	0.13 ± 0.15	0.54 ± 0.10	94.3 ± 0.09	1.2 ± 0.2	43.3
T. occidentalis	58.0 ± 6.1	2.8 ± 1.1	8.1 ± 4.8	26.4 ± 3.1	4.7 ± 1.4	16.5

Table 3. Percent (-)-enantiomer values ± 1 standard deviation (*s*) for five chiral monoterpenes in six conifer species in nursery-purchased plants located at PSU and sampled in March 2018 and again in June/July 2018. (The data were obtained from the same set of analyses carried out to generate the data in Table 2.)

a. March 2018 (see also Figure 3.a).

species	*α*-pinene	*β*-pinene	camphene	limonene	*β*-phellandrene
P. menziesii	97.5 ± 0.085	99.7 ± 0.14	95.9 ± 1.9	71.4 ± 2.9	4.2 ± 1.0
P. ponderosa	99.3 ± 0.23	99.6 ± 0.14	85.8 ± 0.46	55.2 ± 14.9	2.9 ± 0.079
C. lawsoniana	1.9 ± 0.53	3.4 ± 0.49	0.0 ± 0.0	1.6 ± 0.39	78.1 ± 1.6
T. plicata	15.1 ± 7.4	14.5 ± 7.7	0.0 ± 0.0	9.5 ± 1.9	68.9 ± 1.4
J. chinesis	4.1 ± 1.6	31.9 ± 5.5	49.0 ± 3.2	0.78 ± 0.12	74.0 ± 2.2
T. occidentalis	27.9 ± 4.5	28.0 ± 5.2	93.0 ± 0.71	29.2 ± 3.0	59.6 ± 2.7

b. June/July 2018 (see also Figure 3.b).

species	*α*-pinene	*β*-pinene	camphene	limonene	*β*-phellandrene
P. menziesii	98.3 ± 0.43	99.9 ± 0.084	93.2 ± 1.1	71.3 ± 3.9	1.9 ± 0.37
P. ponderosa	99.5 ± 0.87	99.7 ± 0.17	85.6 ± 0.84	56.0 ± 12.6	1.9 ± 0.13
C. lawsoniana	1.9 ± 0.52	1.4 ± 0.25	0.0 ± 0.0	1.5 ± 0.16	81.0 ± 0.64
T. plicata	4.0 ± 2.0	15.0 ± 6.8	0.0 ± 0.0	6.5 ± 0.5	67.6 ± 0.90
J. chinesis	1.5 ± 0.47	12.2 ± 1.1	25.6 ± 2.9	0.42 ± 0.024	76.2 ± 2.5
T. occidentalis	24.1 ± 3.5	23.5 ± 8.4	93.2 ± 0.52	28.9 ± 0.79	57.1 ± 2.6

Table 4. Percent (-)-enantiomer values ± 1 standard deviation (*s*) for five chiral monoterpenes in *Thuja occidentalis* in four nursery-purchased plants located at PSU and sampled once each (*N* = 4) in March 2018 and once each in June/July 2018 (see also Figure 4.)

time	*α*-pinene	*β*-pinene	camphene	limonene	*β*-phellandrene
6 AM	22.8 ± 2.3	33.3 ± 2.2	92.8 ± 0.43	27.6 ± 0.15	50.2 ± 0.065
1 PM	24.8 ± 7.7	36.1 ± 6.1	92.7 ± 0.35	26.2 ± 0.93	51.9 ± 2.4
7 PM	23.9 ± 1.8	32.4 ± 2.4	92.5 ± 0.58	27.7 ± 0.88	49.6 ± 1.3
9 PM	24.2 ± 3.7	37.9 ± 6.1	92.6 ± 1.2	28.3 ± 1.7	47.5 ± 3.3

Table 5. Percent (-)-enantiomer values ± 1 standard deviation (*s*) for five chiral monoterpenes in *Thuja occidentalis* in four nursery-purchased plants located at PSU and sampled once each (*N* = 4) in March 2018, and in residentially-planted samples found in a field trip to three suburban areas in Oregon (Seaside, *N* = 7 plants sampled once each; Hillsboro, *N* = 6 plants sampled once each; and Sandy, *N* = 7 plants sample once each). (Data are plotted in Figure 5.)

location	*α*-pinene	*β*-pinene	camphene	limonene	*β*-phellandrene
PSU (purchased)	27.9 ± 4.5	28.0 ± 5.2	93.0 ± 0.7	29.2 ± 3.0	59.6 ± 2.7
Seaside (residential)	28.4 ± 5.1	23.5 ± 9.3	94.4 ± 1.1	35.6 ± 2.9	62.5 ± 1.7
Hillsboro (residential)	24.1 ± 2.5	19.5 ± 2.6	92.2 ± 2.0	30.7 ± 1.9	62.7 ± 2.5
Sandy (residential)	22.1 ± 3.3	19.8 ± 3.8	94.1 ± 0.78	34.2 ± 3.0	58.3 ± 5.1

Table 6. Hypothetical enantiomer peak area data with associated percent (-)-enantiomer values and associated statistical values.

	(-)-enantiomer peak area	(+)-enantiomer peak area	percent (-)-enantiomer
monoterpene 1			
replicate 1	95,000	1,300	98.7%
replicate 2	99,000	1,000	99.0%
replicate 3	103,000	700	99.3%
			99.0% ±0.28%
			CV = 0.28%
monoterpene 2			
replicate 1	1,300	95,000	1.35%
replicate 2	1,000	99,000	1.00%
replicate 3	700	103,000	0.68%
			1.01% ±0.28%
			CV = 27.3%