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Biosynthesis of Monoterpenes and Norisoprenoids in Raspberry Fruits (*Rubus idaeus* L.): The Role of Cytosolic Mevalonate and Plastidial Methylerythritol Phosphate Pathway

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The biosynthesis of the monoterpenes (−)-α-pinene, linalool, and the norisoprenoids α- and β-ionone in raspberry fruits (*rubus idaeus* L.) was investigated by *in vivo* feeding experiments with [5,5-$^2H_2$]-mevalonic acid lactone and [5,5-$^2H_2$]-1-deoxy-D-xylulose. The volatile compounds were extracted by stirbar sorptive extraction and analyzed using thermal desorption-multidimensional gas chromatography–mass spectrometry (TD-enantio-MDGC-MS). The feeding experiments demonstrate that (−)-α-pinene and (*S*)-linalool are exclusively synthesized via the cytosolic mevalonic acid pathway. In contrast, ^{2}H-labeled (*R*)-(*E*)-α-ionone and ^{2}H-labeled (*E*)-β-ionone are detectable after application of d_2-1-deoxy-D-xylulose and d_2-mevalonic acid lactone, respectively. However, (*R*)-linalool reveals no incorporation of either one of the fed precursors, even though this enantiomer is detectable in the fruit tissue.

KEYWORDS: Rosaceae; fruit aroma; terpenoids; cytosolic and plastidial pathways; stir bar sorptive extraction (SBSE); thermal desorption-multidimensional gas chromatography–mass spectrometry (TD-MDGC-MS)

INTRODUCTION

Because of their flavor and nutritional content, raspberries (*Rubus idaeus* L.), a member of the Rosaceae family, have been a popular fruit for thousands of years (*1*). Moreover, the high amounts of antioxidants and polyphenolics in red raspberries are reported to antagonize cancer, age-related mental decline, and heart and circulatory disease (*2*–*5*). Besides the health benefits of the fruit, raspberry seed oil plays an important role in cosmetic applications since this oil has a skin protection factor of 25–50 and is a rich source of ω-3-fatty acids and vitamin E (*6*).

In more than 70 years of study, over 200 volatile compounds have been identified in red raspberries (*1*). Ten are suggested to be important to red raspberry aroma, including 4-(p-hydoxyphenyl)-2-butanone (raspberry ketone) as the character impact compound. Nevertheless, terpenoids such as α-ionone, β-ionone, or linalool are also known to be important aroma contributors (*7*–*9*). Besides these major aroma compounds, several other terpenes have been investigated, for example, α-pinene, limonene, or β-damascenone (*1*). Their amounts vary depending on the cultivar as well as on the stage of ripeness. Robertson et al. (*10*) showed that certain monoterpenes such as α-pinene, camphene, β-myrcene, and limonene appear to rise steadily throughout the period of maturing, while volatiles normally associated with green leaves, for example, *cis*-β-ocimene and *trans*-β-ocimene, decline with fruit ripening.

However, little is known about the biosynthesis of terpenoids in raspberries and the role of the cytosolic mevalonic acid and the plastidial methylerythritol phosphate pathway. Whereas mevalonic acid is supposed to be generated in the cytosol for isopentenyl diphosphate and dimethylallyl diphosphate supply, 1-deoxy-D-xylulose phosphate is expected to be the counterpart localized in the plastids (*11*–*17*). Although both biosynthetic pathways operate in higher plants, the actual influence on terpene biosynthesis of each distinct route varies between and within species. In general, the cytosolic pathway is predominantly responsible for the synthesis of C_{15}-derived terpenes such as sesquiterpenes, whereas monoterpenes, diterpenes, and carotenoids are generated via the plastidial route (*18*). However, *in vivo* feeding experiments show that in carrots (*Daucus carota* L.) the sesquiterpene β-caryophyllene is synthesized by isoprene units derived from the mevalonic acid as well as from the methylerythritol phosphate pathway (*19*), and findings for

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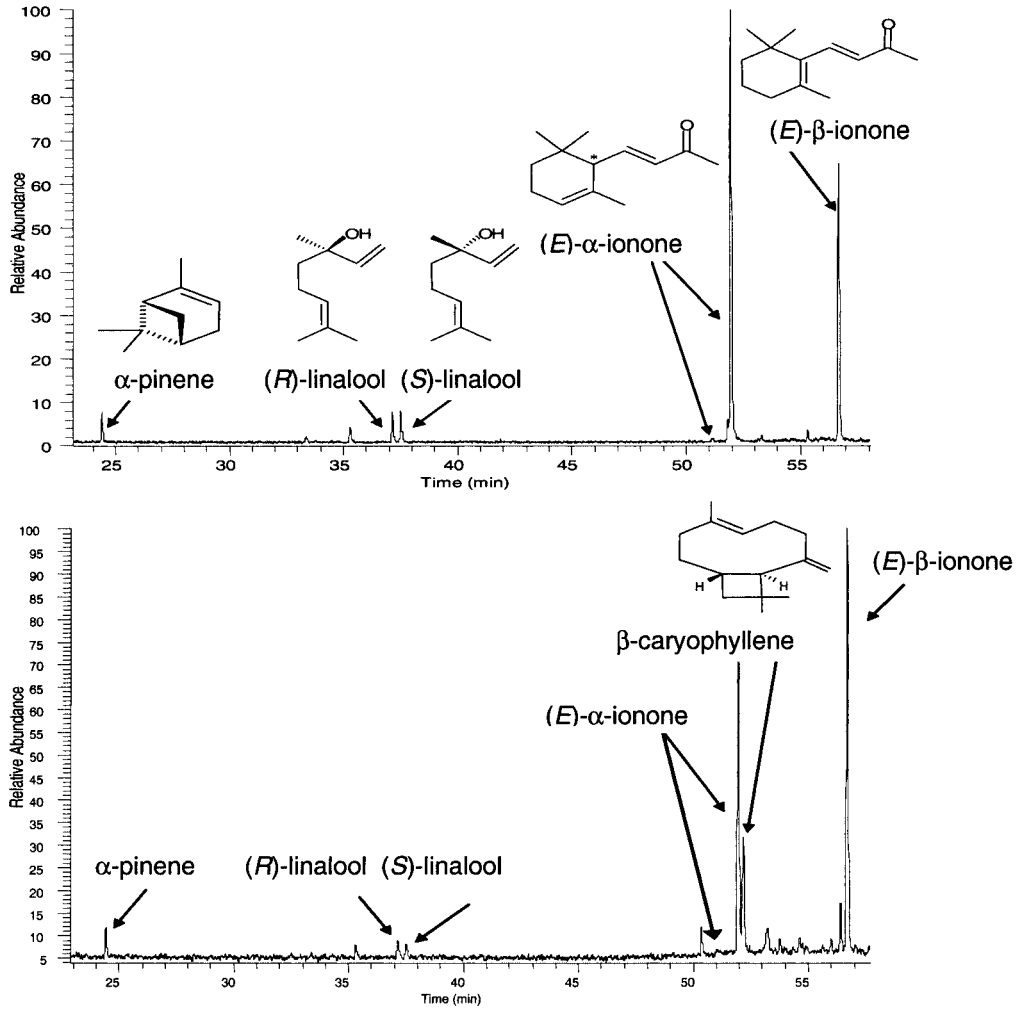

Figure 1. MDGC-MS main column chromatograms of SDE extracts obtained from raspberry fruits from (**top**) March, 2006 and (**bottom**) August, 2005.

tobacco cells reveal that under rather restrictive conditions a complementation of plastidial isoprenoid synthesis by the cytosolic mevalonic acid pathway seems possible (*20*). However, strawberry fruits (*Fragaria x ananassa* Duch.), also belonging to the Rosaceae family, completely lack the capability of exporting plastid derived isopentenyl diphosphate and dimethylallyl diphosphate. Consequently, both mono and sesquiterpenes in strawberry fruits are synthesized exclusively via the cytosolic pathway (*21*). Furthermore, Aharoni et al. (*22*) showed that nerolidol synthase 1 (*FaNES 1*) is the only enzyme present for mono- and sesquiterpene biosynthesis in cultivated strawberry species. The recombinant enzyme synthesized the expected sesquiterpene *trans*-(*S*)-nerolidol from farnesyl diphosphate but also generated the monoterpene (*S*)-linalool from geranyl diphosphate. Moreover, this particular terpene synthase does not contain a targeting signal at its N-terminus. Hence, because of deletions and the introduction of a premature stop codon, the plasmid-targeting sequence from the coding region is excluded. Thus, *FaNES 1* remains in the cytosol (*22*).

To investigate the terpene biosynthesis of another Rosaceae family member in greater detail, raspberry fruits (*Rubus idaeus* L.) were chosen for *in vivo* feeding experiments with [5,5-$^2H_2$]-mevalonic acid lactone and [5,5-$^2H_2$]-1-deoxy-D-xylulose to gain knowledge about pathway utilization and to verify similarities and differences between terpene production in different species within the same family.

EXPERIMENTAL PROCEDURES

Plant Material. For feeding experiments, samples of raspberry fruits (*Rubus idaeus* L.) from Spain were purchased at the local grocery store in March, 2006. SDE extracts were obtained from those samples as well as from a frozen raspberry stock from the Research Centre Geisenheim (Geisenheim, Germany) received in August, 2005. All fruits were at fully ripe stage. The variety was unknown.

Chemicals. (+/−)-linalool, (−)-linalool, (−)-α-pinene, α-humulene, and (*E*)-α(*β*)-ionone were obtained from Fluka (Taufkirchen, Germany), whereas *β*-caryophyllene was obtained from Berje (Bloomfield NJ, USA). [5,5-$^2H_2$]-Mevalonic acid lactone was prepared according to Simpson et al. (*23*), [5,5-$^2H_2$]-1-deoxy-D-xylulose was prepared according to Jux and Boland (*24*). Spectroscopic data of the labeled compounds were in all cases in good agreement with the data given in the references cited above.

SDE Extracts. To screen for terpenoids in raspberry fruits, 250 g of the distinct raspberry samples were subjected to simultaneous distillation and extraction (SDE) according to Schulz et al. (*25*). The fruits were extracted for 6 h with 60 mL of diethyl ether/pentane (1/1, v/v).

Sample Preparation. Raspberry fruits were cut in half. One gram of the plant material was incubated with 1 mL of an aqueous feeding solution (d_2-mevalonic acid lactone, 2 mg/mL; d_2-1-deoxy-D-xylulose, 2 mg/mL) for 4 days at room temperature in darkness. After removing the feeding solution, the pieces were ground up in 2 mL of phosphate buffer (pH 7) to obtain a suspension, which was used for SBSE analysis. Blank values were done by incubation with pure water and grinding up pieces in pure buffer. Each experiment was repeated twice.

SBSE Sampling. A stir bar consisting of a magnetic core sealed inside a glass tube with a length of 1.2 cm, 1.2 mm o.d., and coated

Table 1. Relative Amounts of Genuine and Labeled Compounds after Incubation with d_2-Mevalonic Acid Lactone and d_2-1-deoxy-D-xylulose[a]

	d_2-mevalonic acid lactone		d_2-1-deoxy-D-xylulose	
terpenes	genuine [%]	labeled [%]	genuine [%]	labeled [%]
(−)-α-pinene	39	61	100	nd
(*R*)-linalool	100	nd	100	nd
(*S*)-linalool	48.6	51.4	100	nd
(*S*)-(*E*)-α-ionone	100	nd	100	nd
(*R*)-(*E*)-α-ionone	99.6	0.4	99.7	0.3
(*E*)-β-ionone	99.9	0.1	99.9	0.1

[a] Results are given as the average of at least two independent determinations. nd, not detectable. The labeling degree was determined by integration of the signals of the different labeled isotopomers in the corresponding ion traces as previously described (*21*).

with 55 μL of polydimethylsiloxane (PDMS) was used. Stir bars are manufactured and offered by Gerstel (Mülheim/Ruhr, Germany). The SBSE conditions were as follows: stirring time 30 min at room temperature at approximately 400 rpm for homogeneous distribution of the components on the PDMS phase.

^{1}H-NMR. The spectra of the synthesized products were recorded on a Bruker AMX 300 spectrometer at 300 MHz, at room temperature in $CDCl_3$/TMS or D_2O. The chemical shifts are given in δ (ppm).

Gas Chromatography–Mass Spectrometry (GC-MS). The GC-MS analyses of the SDE extracts were carried out on a Varian GC 3400 instrument, coupled to a Finnigan MAT Magnum ITD mass spectrometer, equipped with self-prepared 30 m × 0.25 mm i.d. fused silica capillary coated with a 0.25 μm film of 4‰ heptakis (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in OV1701 (50%). GC conditions: carrier gas, helium at 36 kPa; split, 20 mL/min; injector temperature, 250 °C; oven temperature, 60 °C (5 min isothermal), then 5 °C/min to 220 °C (10 min isothermal); ion source temperature, 200 °C; mass range, 40–300 amu; EI, 70 eV. The molecular ions (M^+) and fragment ions are given as *m*/*z* with relative peak intensities in % of the most abundant peaks.

The GC-MS analyses of the synthesized products were done on a Fisons Instruments 8065 GC, coupled to a Fisons Instrument MD 800 mass spectrometer equipped with a self-prepared 30 m × 0.25 mm i.d., 0.23 μm fused silica capillary column coated with SE-52. GC conditions were as follows: carrier gas, helium at 69 kPa; split, 20 mL/min; injector temperature, 230 °C; oven temperature, 40 °C (5 min isothermal), then raised at 5 °C/min to 260 °C (20 min isothermal); ion source temperature, 200 °C; mass range, 40–300 amu; EI, 70 eV.

Thermal Desorption-Enantioselective Multidimensional Gas Chromatography–Mass Spectrometry (TD-Enantio-MDGC-MS). The TD-enantio-MDGC-MS (*26*) consisted of a Gerstel TDS thermal desorption system, mounted on a Siemens SiChromat 2-8, with two independent column oven programs and a live T-switching device, coupled to the transfer line of a Finnigan MAT GCQ mass spectrometer, using an open split interface. For the thermal desorption, the following conditions were applied: desorption temperature program, 10 °C at 60 °C/min to 250 °C, 2 min isothermal (6.0 min); flow mode TDS, splitless; and transfer line temperature set at 250 °C. A Gerstel CIS-3 PTV injector was used for cryogenic focusing of the released analytes.

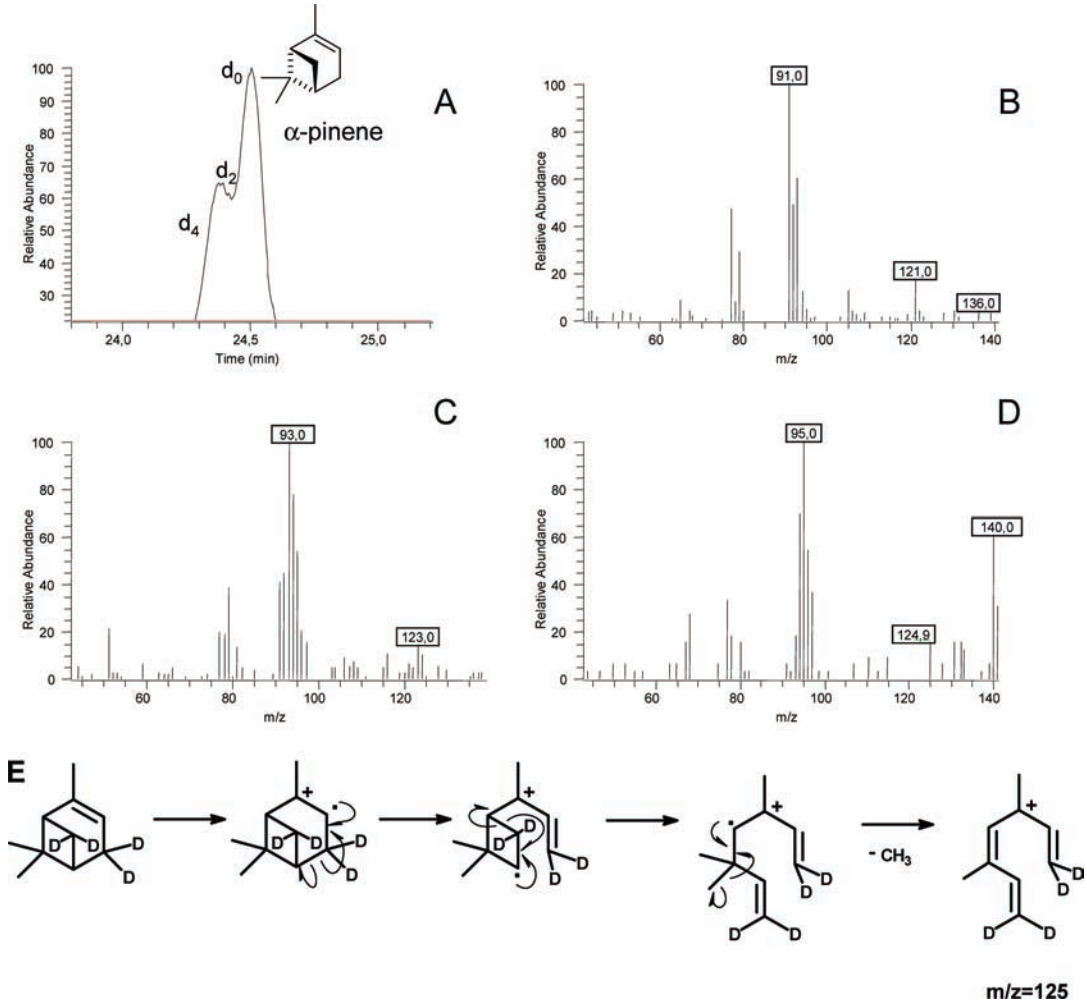

Figure 2. (**A**) Main column chromatogram and mass spectra of (**B**) genuine and (**C** and **D**) labeled (−)-α-pinene after d_2-mevalonic acid lactone was administered. The mass spectra **C** and **D** correspond to the d_2 and d_4 isotopomers, respectively. (**E**) illustrates the fragmentation mechanism that gives rise to the mass peak at *m*/*z* = 125 of the d_4 isotopomer (*31*).

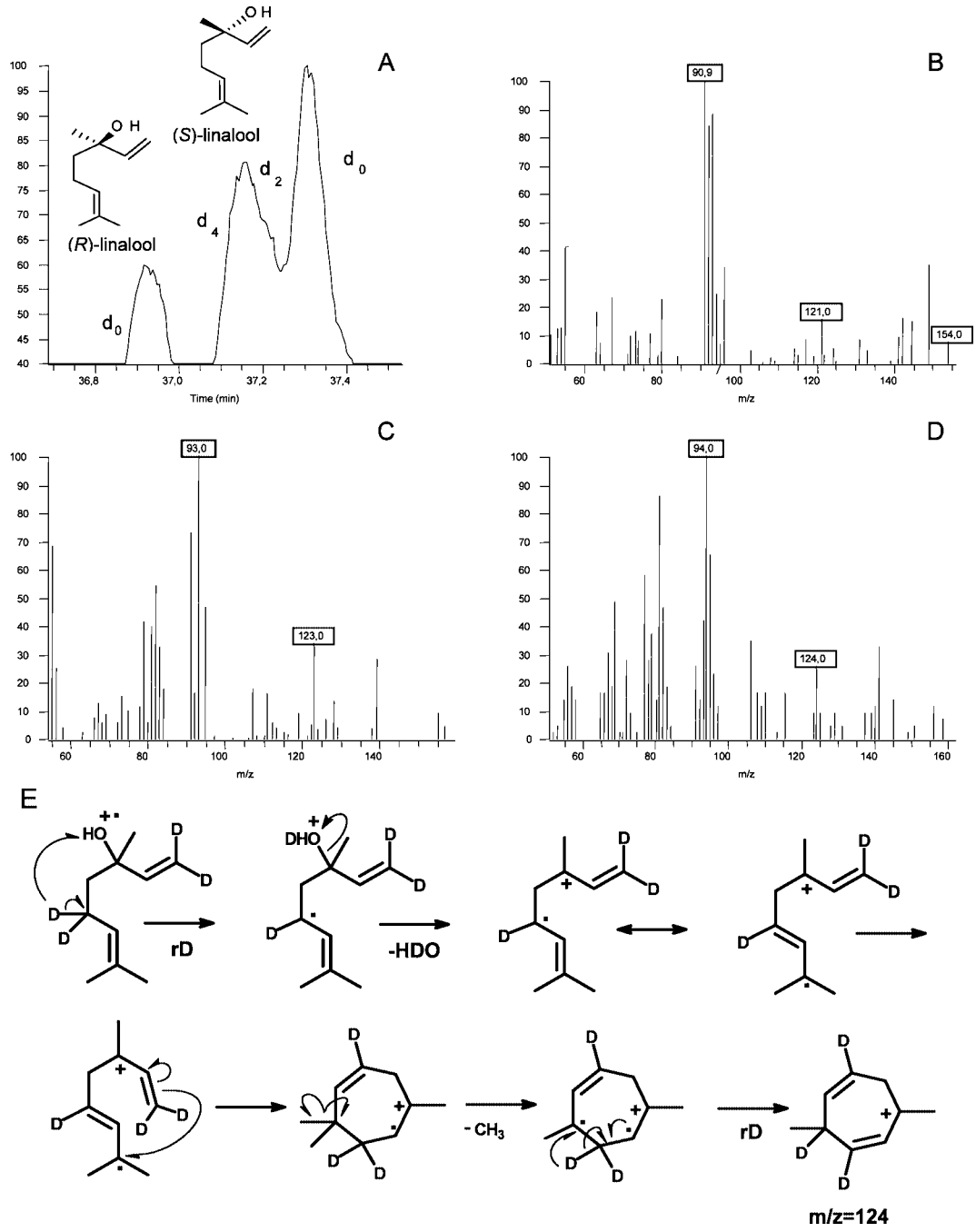

Figure 3. (**A**) Main column chromatogram and mass spectra of (**B**) genuine and (**C** and **D**) labeled (*S*)-linalool after d_2-mevalonic acid lactone was administered. Mass spectra **C** and **D** correspond to the d_2 and d_4 isotopomers, respectively. (**E**) Fragmentation mechanism that gives rise to the mass peak at $m/z = 124$ of the d_4 isotopomer (*31*).

The PTV was cooled to −150 °C using liquid nitrogen. The PTV was programmed from −150 °C at 12 °C/s to 250 °C; 2 min isothermal (2.5 min). Flow mode CAS was splitless (1 min). The liner was filled with Tenax TA (Alltech, Deerfield, IL).

GC conditions were as follows: precolumn, self-prepared 30 m × 0.25 mm i.d.; 0.25 μm, fused silica capillary coated with SE-52; carrier gas helium, 1.8 bar; detector, FID, 250 °C. Main column: self-prepared 30 m × 0.25 mm i.d. fused silica capillary coated with a 0.23 μm film of 4‰ heptakis (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (30%) in SE-52 (70%); detector, ITD 800; transfer line temperature, 250 °C; open split interface, 250 °C; helium sweeping flow, 1 mL/min; ion trap manifold, 200 °C; EI, 70 eV; oven temperature program, precolumn, 60 °C (5 min isothermal), raised at 4 °C/min to 250 °C (30 min isothermal); main column, 60 °C (5 min isothermal), then 2 °C/min to 200 °C (20 min isothermal); cut times, (−)-α-pinene, 14.3–16.0; (*R*/*S*)-linalool, 21.5–23.1; (*E*)-α(β)-ionone, 34.6–38.4. The labeling degree was determined by integration of the signals of the different labeled isotopomers in the corresponding ion traces as previously described (*21*).

RESULTS AND DISCUSSION

Besides the *in vivo* feeding experiments, the samples were subjected to simultaneous distillation and extraction (SDE) to gain information about the terpene profile of the fruit. Furthermore, a frozen raspberry fruit stock, obtained in August, 2005, was used for terpene screening as well. Since the main objectives were to study the biosynthesis of terpenoids, the SDE extracts were not used to verify the complete terpene profile but to prove the presence of the representatives of each distinct terpene class of interest. Thus, the extracts were screened for mono and

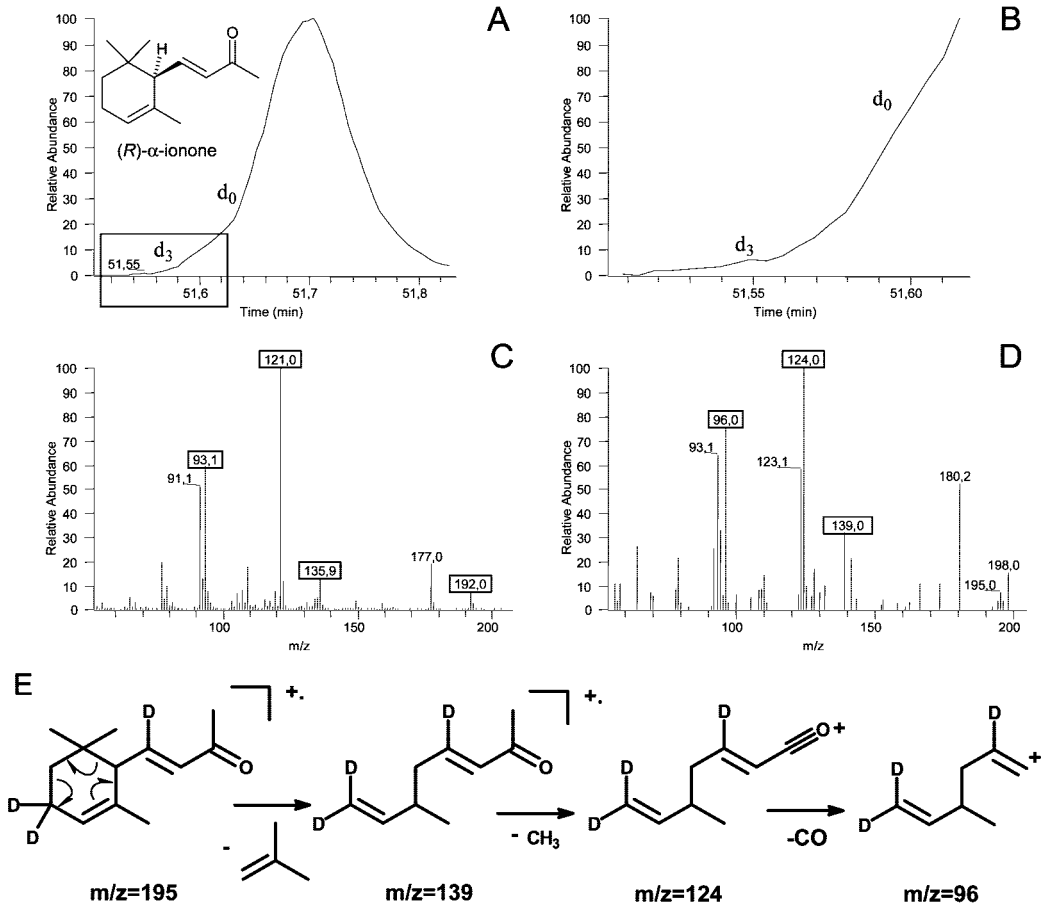

Figure 4. (**A** and **B**) Main column chromatogram and mass spectra of (**C**) genuine and (**D**) labeled (*R*)-(*E*)-α-ionone after d_2-mevalonic acid lactone was administered. Mass spectrum **D** corresponds to the d_3 isotopomer. (**E**) Fragmentation mechanism that gives rise to the mass peaks at $m/z = 96$, 124, 139, and 195 of the d_3 isotopomer (*32*).

sesquiterpenes. Besides these terpene classes, it is well known that α- and *β*-ionone are important constituents in raspberry flavor (*27*) and that they are frequently chosen to assess the authenticity of processed raspberry fruit and raspberry products (*28*). Thus, both norisoprenoids were included in the investigation.

Figure 1 illustrates the main column chromatogram of SDE extracts derived from the raspberry samples from March, 2006 and August, 2005. As a result, the monoterpenes (−)-α-pinene and (*R*)- and (*S*)-linalool, and the norisoprenoids (*R*)-(*E*)-α-ionone, (*E*)-*β*-ionone, and traces of (*S*)-(*E*)-α-ionone were detectable in both extracts. All compounds were identified by comparison with commercial standards. As expected, the amount of (*R*)-(*E*)-α-ionone and (*E*)-*β*-ionone as major aroma contributors was significantly higher than the monoterpene concentration. The (*R*)-enantiomer of α-ionone appeared with high enantiomeric purity (>> 99%) in raspberry fruits (*28*), thus (*S*)-(*E*)-α-ionone occurred only in trace amounts. Moreover, in the extract of fresh raspberries, no sesquiterpene was detectable, as opposed to the frozen stock in which the sesquiterpene *β*-caryophyllene is clearly visible (**Figure 1** B). As already pointed out, the terpene profile changes during fruit maturation and differs from cultivar to cultivar (*10*). Since the two different raspberry samples were harvested in different seasons (March, 2006 and August, 2005) and in different locations (Spain and Germany), it is conceivable that either *β*-caryophyllene is not produced in every cultivar or it does not appear in early seasonal fruits and that the responsible sesquiterpene synthase activity is up-regulated during the summer because of climatic conditions. In their studies of aroma extract dilution analysis of red raspberries cv. Meeker, Klesk et al. (*1*) did not identify *β*-caryophyllene in the cultivar used, whereas earlier investigations concerning raspberry aroma in several cultivars have proved the presence of this sesquiterpene in raspberry fruits (*10*, *29*).

However, since no sesquiterpene was identified in the fresh fruit sample, no data were obtained for sesquiterpene biosynthesis in this study. To investigate the pathway utilization of the detected monoterpenes and isoprenoids, d_2-1-deoxy-D-xylulose or d_2-mevalonic acid lactone in aqueous solution was directly applied to raspberry fruit tissue. After incubation, the plant material was homogenized using a mortar and pestle. The volatiles were extracted by stir bar sorptive extraction (SBSE) and analyzed by thermal desorption-enantioselective multidimensional gas chromatography–mass spectrometry (TD-enantio-MDGC-MS), allowing the simultaneous detection of genuine and labeled terpenes.

The application of ^{2}H-labeled MVL revealed the incorporation into monoterpenes (−)-α-pinene and (*S*)-linalool as well as into (*R*)-(E)-α-ionone and (*E*)-*β*-ionone (**Table 1**). **Figures 2–5** show the SBSE-enantio-MDGC-MS analyses of both (−)-α-pinene and (*S*)-linalool, and (*R*)-(E)-α- and (*E*)-*β*-ionone, respectively after labeled mevalonic acid lactone was administered. Besides unlabeled genuine terpenes, labeled d_2- and d_4-(−)-α-pinene, d_2-and d_4-(*S*)-linalool, and d_3-(*R*)-(E)-α-ionone and d_2-(*E*)-*β*-ionone are clearly detectable and separated because of the inverse isotope effect of deuterium labeled compounds. However, it should be noted that the mass spectra presented for nonlabeled compounds do not match NIST library spectra because the used ion trap detector was tuned for maximum sensitivity operating at the limit of sensitivity. However, the

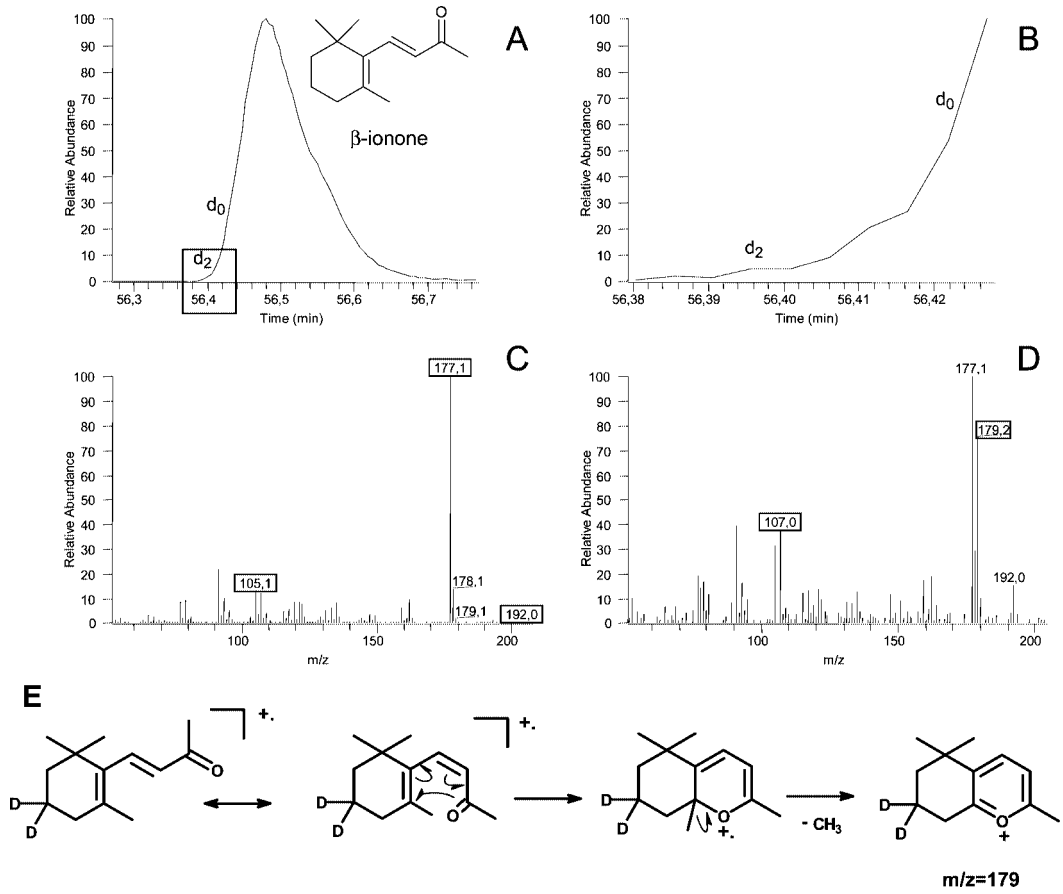

Figure 5. (**A** and **B**) Main column chromatogram and mass spectra of (**C**) genuine and (**D**) labeled (*E*)-*β*-ionone after d_2-mevalonic acid lactone was administered. Mass spectrum **D** corresponds to the d_2 isotopomer. (**E**) Fragmentation mechanism that gives rise to the mass peak at $m/z = 179$ of the d_2 isotopomer (*32*).

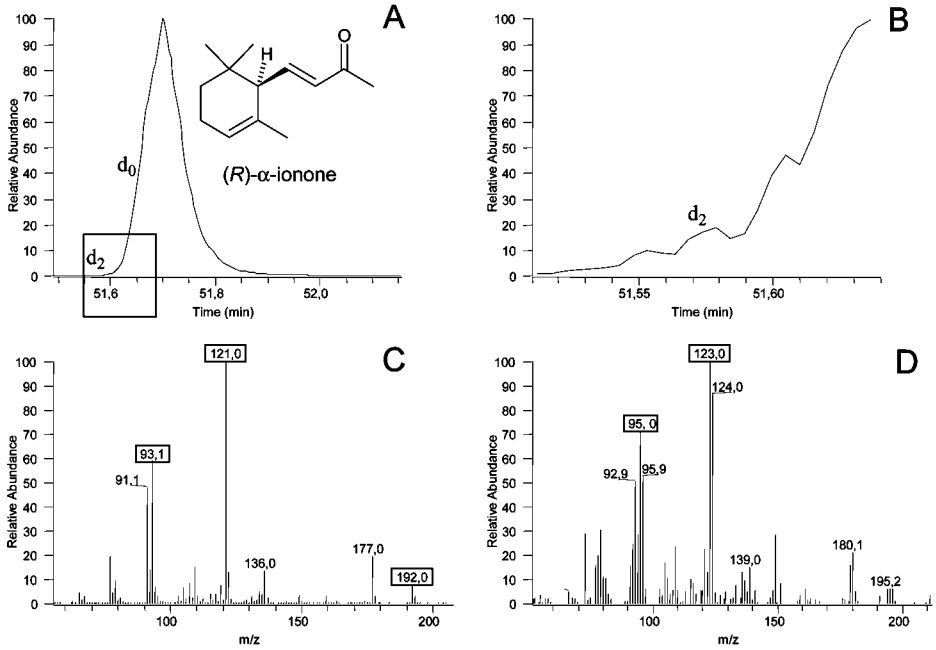

Figure 6. (**A** and **B**) Main column chromatogram and mass spectra of (**C**) genuine and (**D**) labeled (*R*)-(*E*)-α-ionone after d_2-1-deoxy-D-xylulose was administered.

identification of all target compounds was assured by the use of pure reference compounds in combination with a MDGC-system that takes advantage of the combined selectivity of two GC columns with different polarity.

The labeling degree can be deduced from the corresponding mass spectra. Monoterpenoids are assembled from two isoprene molecules. Therefore, a shift up to four mass units in fragments can occur, given that both isoprene units are labeled and no deuterium is lost during the biosynthesis. Indeed, this shift is detectable for the investigated monoterpenes (**Figures 2** and **3**). The fragmentation pattern of the deuterium labeled linalool and α-pinene isotopomers are in agreement with the incorpora-

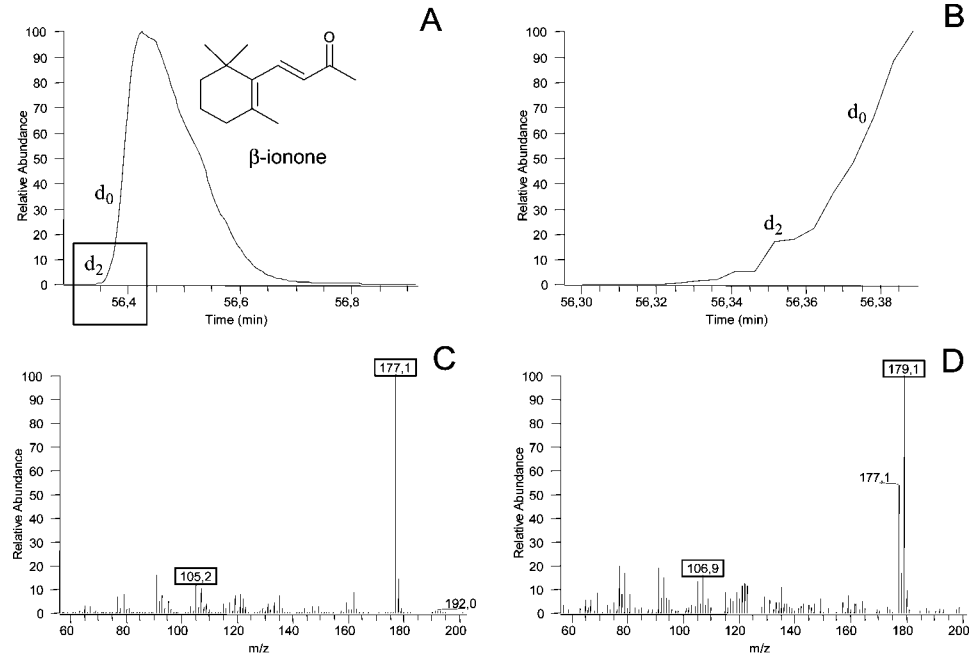

Figure 7. (**A** and **B**) Main column chromatogram and mass spectra of (**C**) genuine and (**D**) labeled (*E*)-β-ionone after d_2-1-deoxy-D-xylulose was administered.

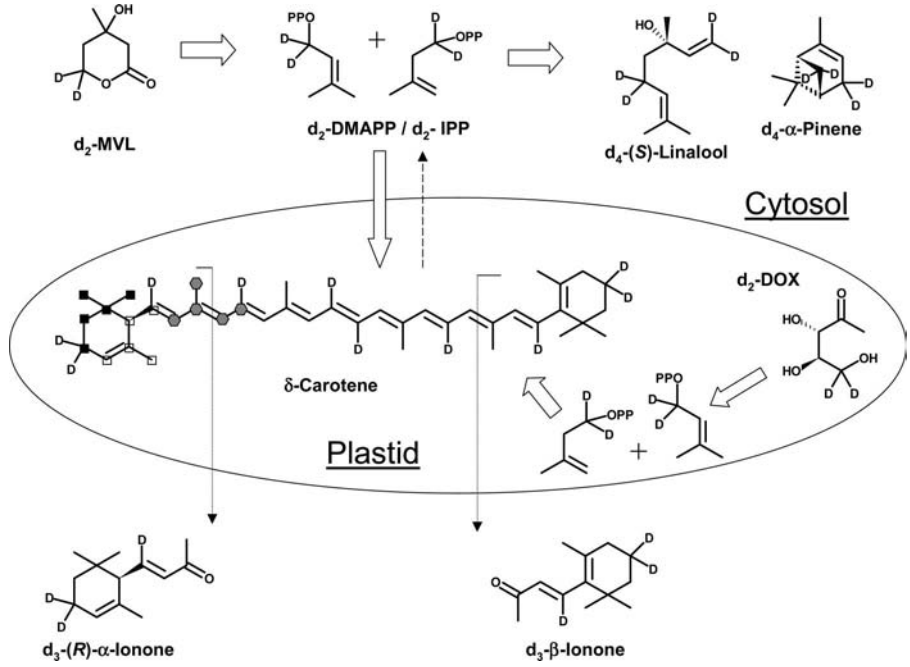

Figure 8. Model for terpene biosynthesis in raspberry fruits. The three labeled isoprene units in the hypothetical precursor δ-carotene that are incorporated into d_3-(*R*)-α-ionone are highlighted with distinct symbols (filled boxes, empty boxes, and gray hexagons).

tion of one or two units of labeled isopentenyl diphosphate and dimethylallyl diphosphate that are generated from administered d_2-1-deoxy-D-xylulose (**Figures 2**, **3**, and **8**).

Although (*R*)-linalool represented up to 33% of the total linalool amount in the fruit tissue (**Figure 3**), no incorporation of labeled mevalonic acid lactone was observable. This result is in good agreement with findings obtained by *in vivo* feeding experiments with strawberry fruits. Even though (*R*)-linalool was clearly detectable in strawberry fruit tissue, none of the fed ^{2}H-precursors were incorporated into that specific enantiomer (*21*). Thus, it is conceivable that (*R*)-linalool synthesis, in raspberry as well as in strawberry fruits, occurs exclusively during an early fruit ripening stage and that the expression of the corresponding (*R*)-linalool synthase is completely down-regulated when the fruit attains the fully ripe stage.

The application of d_2-mevalonic acid lactone also showed incorporation into the norisoprenoids (*R*)-(*E*)-α-ionone and (*E*)-β-ionone (**Figures 4** and **5**) but not into (*S*)-(*E*)-α-ionone. However, only low amounts of labeled (*R*)-(*E*)-α-ionone and (*E*)-β-ionone were detectable compared to the genuine compound concentrations (**Table 1**). (*S*)-(*E*)-α-ionone already occurs only in traces. Thus, it is likely that a potential incorporation is not visible because of the marginal amounts of genuine component that could drop a potential amount of labeled (*S*)-(*E*)-α-ionone below the detection limit.

After d_2-1-deoxy-D-xylulose was administered, only the norisoprenoids (*R*)-(*E*)-α-ionone and (*E*)-*β*-ionone showed the incorporation of the precursor (**Figures 6** and **7**). Besides genuine norisoprenoids, d_3-(*R*)-(*E*)-α-ionone and d_2-(*E*)-*β*-ionone were detectable. Ionones are natural degradation products derived from carotenoids, which are thought to be synthesized via the plastidial pathway. Thereby, (*E*)-α-ionone arises from α-carotene, whereas (*E*)-*β*-ionone is obtained from *β*-carotene (*30*). As a result, ionones emerging from ^{2}H-labeled carotenoids show only an incomplete labeling pattern. During the incubation, obviously ^{2}H-labeled as well as nonlabeled isoprenoid precursors are utilized for carotenoid biosynthesis, which leads to partly labeled products. Thus, d_1, d_2, and d_3 isotopomers are expected for both ionones (see **Figure 8**) and were indeed detectable as can be seen in the corresponding mass spectra (**Figures 4**, **5**, **6**, and **7**) by a shift of 1, 2, or 3 mass units of the main mass fragments. The low labeling degrees of the norisoprenoids might demonstrate a rather low turnover rate of these compounds *in planta* when compared to the relatively high labeling degrees of the monoterpenoids.

Unlike the norisoprenoids, no labeled monoterpene was detectable after the application of d_2-1-deoxy-D-xylulose (**Table 1**), even though monoterpenes as well as carotenoids are referred to as terpenoids generated by the classical methylerythritol phosphate pathway. Apparently, raspberry fruits are not able to produce monoterpenoids via the plastidial route, and the responsible monoterpene synthase is located in the cytosol. However, there are other possible explanations for these results: d_2-1-deoxy-D-xylulose must be phosphorylated in plants to be converted to d_2-1-deoxy-D-xylulose phosphate, which is a substrate in the methylerythritol phosphate pathway. The poor incorporation of d_2-1-deoxy-D-xylulose into monoterpenes could simply be due to the weak activity of 1-deoxy-D-xylulose kinase. It is also conceivable that this system is not sensitive enough to rule out the incorporation of 1-deoxy-D-xylulose into any monoterpene. Because of the findings of labeled norisoprenoids after d_2-1-deoxy-D-xylulose application, we could prove the utilization of the plasidial pathway in raspberry fruits. Thus, even though there is detectable terpene synthase activity in plastids, monoterpenoids are almost exclusively generated via the cytosolic route without any detectable participation of plastidial enzymes or substrates. In contrast, norisoprenoids arise from isopentenyl diphosphate and dimethylallyl diphosphate building blocks that are derived from both pathways. Consequently, in raspberry fruits, carotenoids as the natural source of norisoprenoids are synthesized via both biosynthetic routes. However, the present experiments do not give an indication on the major pathway contributing to their biosynthesis. **Figure 8** illustrates a model for the terpene biosynthesis in raspberry fruits that is in agreement with the findings of this study.

Although no sesquiterpene was detectable in the fresh samples used for the feeding studies, *β*-caryophyllene was present in the frozen stock sample (**Figure 1**, **bottom**). Thus, at least several cultivars have the ability to synthesize sesquiterpenes. To date, we have no detailed knowledge about how sesquiterpenes are generated in raspberry fruits. It is possible that in raspberries, as already shown for strawberries, only one enzyme is responsible for mono and sequiterpene synthesis and that this enzyme starts producing sesquiterpenes later in the ripening process.

In conclusion, the work presented offers insight into terpene biosynthesis in raspberry fruits and reveals differences between the utilization of the biosynthetic pathways in the two species of raspberry (*R. idaeus*) and strawberry (*Fragaria* × *ananassa*) plants of the Rosaceae family. Future studies with raspberry fruit tissue and foliage are mandatory to gain more detailed information about a potential intra-plant variation of pathway utilization and to verify the biosynthesis of sesquiterpenes.

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