# Evidence for Metabolic Turnover of Monoterpenes in Peppermint[1, 2]

Alice J. Burbott and W. David Loomis
Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

Received July 26, 1968.

*Abstract.* **Two types of experimental evidence are presented which suggest that the monoterpenes of peppermint (*Mentha piperita* L.) are subject to metabolic turnover. In kinetic studies with $^{14}CO_2$, peppermint cuttings rapidly incorporate label into the monoterpenes and then lose most of the label from the monoterpenes, without corresponding changes in the amount of monoterpenes present. When peppermint plants are grown in a controlled environment (16-hr photoperiod, 24° day, 8° night) and analyzed at intervals leaf pair by leaf pair, there is a steady increase in monoterpenes until the time of floral initiation, followed by a rapid decrease. It is suggested that monoterpenes may serve as substrates for energy metabolism in the secretory cells after other stored substrates have been depleted.**

According to the traditional view [see for example Paech (16)] monoterpenes are typical "secondary products" of plant metabolism, and as such they generally have been regarded as waste products or as metabolic "dead ends". Certain plant products very probably are formed irreversibly. For example, tracer experiments with oats (23) indicated that hemicelluloses of stems and leaves are metabolically-inert structural materials. Similar studies with wheat (19) indicated that lignin probably cannot be re-utilized by the plant.

However, evidence is now accumulating which suggests that many secondary products are subject to metabolic turnover. For example, Zaprometov (24) reported extensive catabolism of labeled aromatic compounds of low molecular weight (a mixture of catechins and catechin gallates) fed to tea shoots. The bulk of the label (75 %–80 %) was recovered in respiratory $CO_2$.

There is increasing evidence that alkaloids are metabolically active in the plants which produce them. Fairbairn and co-workers have presented evidence that there is a rapid turnover of alkaloids in the opium poppy (*Papaver somniferum*) (7) and in poison hemlock (*Conium maculatum*) (8). Waller and co-workers (22) have reported that labeled ricinine fed to castor-bean plants (*Ricinus communis*) was degraded to the extent of 75 to 95 %, with synthesis and degradation occurring simultaneously.

Breccia and Badiello (5) fed 2-$^{14}$C-mevalonic acid to horehound (*Marrubium vulgare*) and isolated the diterpene marrubiin at several time-intervals. After 24 hr, 0.84 % of the label administered was in marrubiin. This dropped steadily, and after 96 hr only 0.19 % was in the marrubiin. The authors concluded that marrubiin has a half-life of about 24 hr in the plant.

Aexel *et al.* (1) have recently obtained evidence from experiments with 2-$^{14}$C-mevalonate that the triterpene β-amyrin is subject to metabolic turnover in flower stalks of dandelion (*Taraxacum officinale*). From their data it appeared that β-sitosterol was turned over very slowly, if at all.

In the case of monoterpenes, diurnal fluctuations in the amount of essential oil have been reported in some plants, notably in *Salvia officinalis* (sage) (9, 18). The authors assumed that the decrease in essential oil was due to evaporation and resinification. However, metabolic turnover is an alternate possibility. Sukhov (21) fed $^{14}CO_2$ to a whole pine tree and reported that label in the monoterpenes reached a peak in 13 days and then declined. Nicholas (15) fed 2-$^{14}$C-mevalonate to cuttings of *Ocimum basilicum* (sweet basil) and found that radioactivity in the steam-volatile fraction reached a maximum after 1 hr and then decreased rapidly.

We wish to report evidence for rapid metabolic turnover of monoterpenes in peppermint (*Mentha piperita* L.).

## Materials and Methods

*Plant Material.* Peppermint plants were the Black Mitcham variety of *Mentha piperita* L., propagated vegetatively from the same clone we have used previously (6). Cuttings consisting of the tuft of youngest leaves at the growing tip, plus the

[1] This investigation was supported by Public Health Service Research Grant GM 08818 and Research Career Program Award K3-GM-17,064, both from the National Institute of General Medical Sciences.

[2] A preliminary report was presented at a symposium of the Phytochemical Group in 1966 (14).

next 2 leaf pairs, were used for all of the experiments described here. Fresh weights of the cuttings were between 250 and 300 mg. Cuttings were matched as closely as possible visually for each experiment.

*Kinetic Studies With $^{14}CO_2$.* Kinetic studies with $^{14}CO_2$ (fig 1 and 2) were carried out in a light chamber in the laboratory. The chamber was made of plywood lined with crinkled aluminum foil, and was 40 × 127 cm, × 60 cm high. Illumination was provided by eight 100 watt fluorescent tubes, Westinghouse Super High Output cool white, and two 40 watt incandescent bulbs. Light intensity on the floor of the chamber was approximately 1400 ft-c (General Electric No. 213 light meter with Se photocell). The lights were separated from the rest of the chamber by 2 thicknesses of glass. Temperature control was obtained by blowing air from the laboratory through the chamber. Temperatures were therefore about 25° to 30° during the light period and about 20° to 22° during the dark period.

Cuttings were taken from plants grown in the greenhouse with supplementary fluorescent lights turned on 14 hr per day. The photoperiod was thus 14 hr or more. These cuttings were used immediately, after testing to be sure that they were able to take up water through the cut stem. For exposure to $^{14}CO_2$ the cuttings were supported in small vials of water in a sealed desiccator, around a central vial containing $Na_2{}^{14}CO_3$. Carbon dioxide was generated by injecting a solution of perchloric acid into the carbonate solution through a rubber-capped inlet tube. Before sampling, the vessel was flushed with air which had been passed through 2 NaOH-traps to minimize the amount of unlabeled $CO_2$ entering the chamber. The chamber was resealed and flushed with NaOH-washed air after each sample was removed. Each sample consisted of 2 cuttings.

*Periodic Analyses of Peppermint Plants.* In these experiments matched peppermint cuttings were rooted and grown in a controlled environment, and the monoterpenes were analyzed periodically, node by node, in triplicate, during the course of plant development. The growth chamber and lighting have been described elsewhere (6). Illumination was from a combination of Sylvania Gro-Lux fluorescent lamps and incandescent bulbs and was between 700 and 1000 ft-c as measured by a light meter with Se photocell. In this case the light energy available to the plants was greater than the measured value, since the Se photocell is relatively insensitive in the red and blue regions of the spectrum, where Gro-Lux lamps have their greatest output.

Cuttings were taken from plants placed in the growth chamber, under the same environmental conditions that were to be used in the experiment, at least 3 weeks prior to the start of the experiment. These cuttings were rooted in perlite in 250 ml plastic beakers with drain holes, and were given 50 ml of Hoagland and Arnon nutrient solution No. 2 (13) 3 times a week, and water on the other days. Roots began to appear within 3 days after the cuttings were made, and developed rapidly.

Two experiments were performed, with different photoperiod and temperature regimes. In the experiment represented by table I and figure 3, plants were grown with a 16-hr day at 24° and an 8-hr night at 8°. In the other experiment the temperature was constant at 24°, with a 14-hr day and 10-hr night.

Branches and stolons were removed from the plants as soon as they formed, in order to simplify the analyses.

Harvesting of samples was begun in the morning, about 3 hr after the beginning of the light period. Leaf pairs were removed node by node from the stem starting at the top, weighed, measured, and extracted as quickly as possible. Very little time elapsed between harvesting and extraction of a given leaf pair, but when the plants became large the total sampling procedure required as much as 3 to 4 hr. This difference in time should not affect the analyses. As a control, shoots were harvested and extracted at half-hourly intervals over a 24-hr period, and analyzed. There was the usual variability in essential oil content, but no evidence of diurnal fluctuation.

*Extraction and Analysis of Terpenes.* Plant tissue was extracted immediately after a sample was taken, since it appeared that some enzymic transformations of monoterpenes continued in frozen tissue when it was stored in a freezer. The tissue was extracted by grinding in a mortar with hexane (Skellysolve B) in the presence of anhydrous sodium sulfate. When old fibrous leaves were to be extracted they were frozen with liquid $N_2$ before grinding or else SiC (Carborundum) powder was added as an abrasive. The extracts were decolorized with charcoal (Norit A) and dried over anhydrous sodium sulfate. The extracts were then concentrated under a stream of dry air, and the total extract was separated by gas chromatography in a Beckman GC-2A gas chromatograph with thermal conductivity detector. The procedures are described in detail elsewhere (6). The column used for the periodic analyses was the improved Quadrol-SAIB (sucrose acetate isobutyrate)-KOH column as described (6). The $^{14}CO_2$ kinetic experiments described here were done with an earlier version of the column, which was packed with 5 parts Quadrol and 2 parts SAIB on 93 parts (by weight) of firebrick. This column was operated at about 135°.

Peaks were identified by comparing their retention times on these columns and on a 20-foot (6.1 m) column of 10% Carbowax 1000 with the retention times of standard monoterpenes known to occur in peppermint (6). In addition, individual components of standard peppermint oil were re-chromatographed on silicic acid thin layer plates (Silica gel G, E. Merck A. G., Darmstadt, Germany) after separation by gas chromatography. The compounds were trapped by holding a silicic acid plate against the outlet of the gas chromatograph as each individual

peak was eluted. The plates were then developed with 8% ethyl acetate in Skellysolve B. The $R_F$ values of the trapped materials, and their reactions with spray reagents, were compared with the $R_F$ values and reactions of known monoterpenes (3).

With the Quadrol-SAIB-KOH column the "menthone" peak contains both (−)-menthone and (+)-isomenthone, with the former predominating (6). The "menthol" peak contains almost entirely (−)-menthol with probably some (+)-isomenthol. The other menthol isomers cannot be distinguished from pulegone on this column and are very minor components as evidenced by the complete disappearance of the pulegone peak with time in the periodic analyses. In the description of the results, "menthone" or "menthones" will refer to the menthone-isomenthone peak, and "menthol" will refer to the menthol-isomenthol peak.

*Measurement of Radioactivity.* Labeled components from the gas chromatograph were trapped on silicone-coated anthracene in glass cartridges, using a Packard Tri-Carb Model 830 gas chromatography fraction collector attached to the sample effluent port of the gas chromatograph. The cartridges were then counted in a Packard Model 314 liquid scintillation counter at 900 V, with window settings of 10 to 100 and 100 to infinity. These settings provide balance point conditions for this system. The anthracene cartridges gave a counting efficiency of 75% when aliquots of a $^{14}C$-labeled standard (geraniol) were added directly to the cartridges. However, when aliquots of the same standard were carried through the combined separation and counting procedure (gas chromatography, trapping, and counting) the overall efficiency proved to be 25%. This test was repeated several times, with consistent results.

## Results and Discussion

*Kinetic Studies With $^{14}CO_2$.* A number of time course experiments were carried out, in which young peppermint shoots, matched as carefully as possible, were exposed to $^{14}CO_2$ and harvested at intervals, and the monoterpenes analyzed and counted. Consistently in these experiments monoterpenes incorporated $^{14}C$ rapidly, and subsequently lost a large part of the label, without corresponding changes in the amount of monoterpene present. However, there was frequently a considerable amount of apparently random variation in the labeling, so that the details of time courses were difficult to reproduce with the number of shoots that it was feasible to use in an experiment.

This variability in labeling patterns appeared to be due to difficulty in obtaining cuttings which were exactly matched. There was considerable variability in the cuttings, even though clonal material was used and the cuttings were matched as carefully as could be done visually. Variations were apparent even when cuttings were taken from plants grown in a growth chamber. When visually matched cuttings were harvested simultaneously and extracted and analyzed immediately, 2-fold differences in the amount of essential oil per cutting were not uncommon.

Young peppermint tissues, such as were used here, usually have a much higher content of monoterpenes on a weight basis than do older tissues (6, 10), indicating a high rate of monoterpene production compared to the growth rate. It seems likely that analysis of the monoterpenes will provide a more sensitive index of the physiological age or condition of such tissues than does visual matching of leaf size. In any case, the time courses with the least variation in the amount of essential oil between cuttings also showed the least amount of seemingly random fluctuation of $^{14}C$-label. We have concluded that uniformity is best judged by comparing the essential oil analyses of the cuttings as well as their appearance. Unfortunately the terpene analysis can only be made after an experiment is completed. For these reasons, the data presented here have been selected from experiments in which uniformity of cuttings was indicated both by visual matching and by relative uniformity in the amount of monoterpene per cutting.

Figures 1 and 2 show the total monoterpene label (sum of the individual compounds) and the monoterpene contents of the cuttings as a function of time in 2 of these experiments. The cuttings

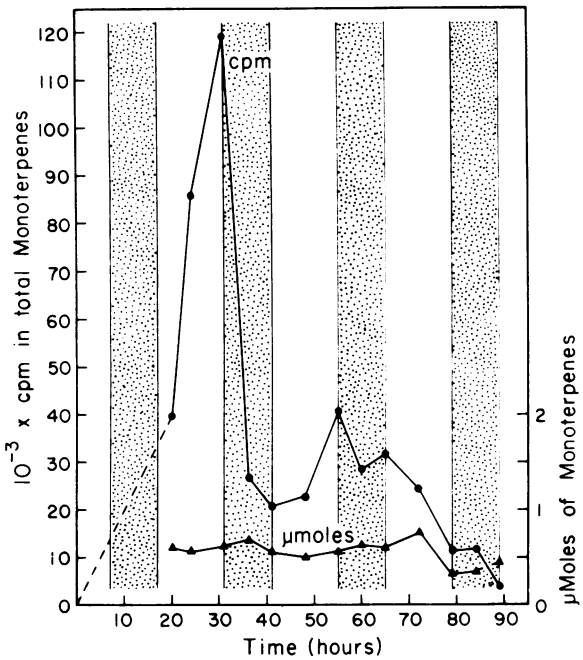


FIG. 1. Time course of labeling of peppermint monoterpenes from $^{14}CO_2$ in alternating light and darkness. Stippling indicates dark periods. Twenty-six cuttings were exposed to 400 μc of $^{14}CO_2$ for 20 hr before sampling was started. Each sample consisted of 2 cuttings, and the values given are per 2 cuttings.

used in these experiments were obtained from plants grown in the greenhouse in winter, with a photoperiod of 14 hr but with rather low light intensity. The plants grew slowly under these conditions, and this may have made it easier to select uniform cuttings visually. Cuttings for most of the other experiments were from faster-growing plants.

In the experiment represented by figure 1, 26 peppermint cuttings were exposed to 400 $\mu$c of $^{14}CO_2$ for a period of 20 hr, in alternating light and dark. (At the time this experiment was done we felt that this long exposure period was needed in order to obtain a high level of $^{14}C$ in the monoterpenes. Later we found that incorporation of label is much faster than we had supposed.) Samples were taken at intervals for 3 days. For the first 2 days after sampling started, the monoterpenes gained label in the light and lost it in the dark. The failure of the terpenes to regain label in the third light period may mean that the $^{14}C$ had by this time been converted largely into metabolically inactive materials. However, it should be noted that the shoots used for the last 3 samples (at 79, 84, and 89 hr) were badly wilted, even though the stems were still immersed in water, and that they also contained much less total monoterpenes than the other cuttings.

In this experiment the cuttings contained approximately equal amounts of menthones and menthofuran as the principal monoterpenes, and the bulk of the monoterpene label was divided between the menthones and menthofuran. An essential oil containing menthofuran in amounts equal to or greater than the menthones is characteristic of plants grown with short to medium length days and warm nights (6).

Light is required for the initial incorporation of label from $^{14}CO_2$ into monoterpenes (12, and W. D. Loomis, unpublished). Since these experiments were done in a closed chamber, which was opened only when samples were to be removed, a large part of the respiratory $^{14}CO_2$ released during each dark period would be available for photosynthesis during the following light period. Thus it is not surprising that the time courses tended to show, as in figure 1, a gain of label during the light periods and a loss during the dark periods. However, the loss of label is not, as at first appeared, directly related to lack of light. In several of the time course experiments decreases in monoterpene labeling during the light period were observed. Figure 2 shows the results of a shorter time course, in continuous light. Twenty cuttings were exposed to 800 $\mu$c of $^{14}CO_2$ for 1 hr and then sampled hourly for the next 8 hr. Label was incorporated into the monoterpenes for the first 6 hr and then nearly disappeared from them during the next 3 hr. The 1-hr and 2-hr values in this experiment probably should be rejected, as these cuttings contained much less monoterpene than the others, indicating physiological non-uniformity. Rejection of these 2 points does not affect the overall trend. In this experiment, as in most of the other time courses, menthone (plus isomenthone) was the predominant monoterpene and contained most of the label.

In the experiments described here, as well as in others not described, peppermint cuttings rapidly incorporated label from $^{14}CO_2$ into monoterpenes and then rapidly lost label from the monoterpenes. Although there were variations in the amount of essential oil per cutting these variations did not appear to parallel the variations in label, and in any case they were of a considerably lower magnitude than the 5- to 10-fold fluctuations of label that were commonly observed. The results therefore cannot be due simply to evaporation unless one assumes preferential evaporation of the newly formed labeled terpenes. Furthermore, there is evidence that very little evaporation of monoterpenes occurs in peppermint. We have observed intact glands, still filled with oil, in peppermint acetone powders which had been stored for several years in the freezer. Hefendehl (11) found less than 10 % loss, and no change in the composition, of the monoterpenes over a period of 14 months when dried peppermint leaves were kept in the air at room temperature. Reitsema and co-workers (17) fed $^{14}CO_2$ to peppermint plants and used a toluene trap to collect any essential oil that might evaporate. Their data indicated that over

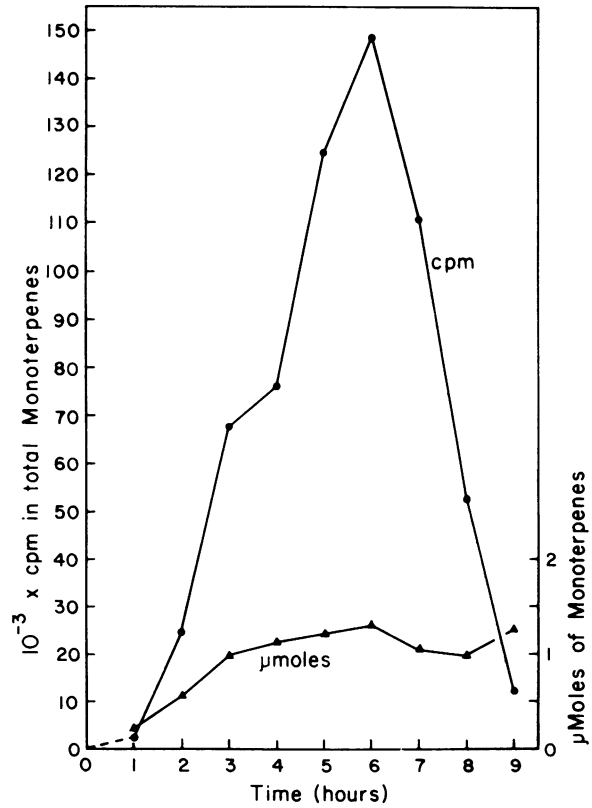


FIG. 2. Time course of labeling of peppermint monoterpenes from $^{14}CO_2$ in continuous light. Twenty cuttings were exposed to 800 $\mu$c of $^{14}CO_2$ for 1 hr and then sampled hourly. Each sample consisted of 2 cuttings, and the values given are per 2 cuttings.

a period of 23 hr only 1 % of the radioactivity originally present in the essential oil was lost by evaporation. The losses of label observed in our experiments are much greater than this, and it seems unlikely that evaporation of monoterpenes played a major role.

The values shown in figures 1 and 2 represent the total label in monoterpenes, obtained by adding the counts in the individual components. Time course curves for the individual compounds generally had very much the same form as the total-monoterpene curve. In the 9-hr time course, however, the time intervals were sufficiently short to reveal differences in the rate of labeling of the individual terpenes. Pulegone label reached its maximum in 3 hr, menthofuran in 5 hr, and menthone only after 6 hr. These results are consistent with the finding (2,4) that pulegone is a precursor of both menthofuran and menthone.

*Periodic Analyses of Peppermint Plants.* Further evidence for metabolic turnover of monoterpenes was obtained from analyses of peppermint plants grown in a controlled environment. Table I shows the monoterpene content of each leaf pair through the growing period for plants grown on a 16-hr photoperiod with 24° days and 8° nights. The values given are the sum of all peaks except the most volatile components (hydrocarbons and cineole), and represent at least 90 to 95 % of the total monoterpenes. Under these conditions, the plants were unusually vigorous in appearance and produced 2 to 3 times the quantity of monoterpenes per leaf as under usual greenhouse conditions. With age the leaves became increasingly fibrous and red-pigmented. Menthone was the predominant monoterpene, even in very young leaves. As the leaves aged, menthol appeared. The inflorescences contained predominantly menthofuran and pulegone, consistent with earlier observations (6).

The intermediate and lower leaves (up to node 11) reached their highest essential oil content at approximately the time when macroscopic observation of the growing tips revealed that the plants were going to bloom. These leaves lost monoterpenes rapidly thereafter. This pre-blooming peak of monoterpenes contained predominantly menthones; there appeared to be a burst of menthone synthesis in these leaves just before, or at the time of, floral initiation. After floral initiation there was a rapid loss of menthones, and then a second smaller peak due largely to menthol, which coincided closely with the opening of the first flowers. Previous evidence (2, 17) has indicated that menthone can be converted metabolically to menthol, but in the present experiment the amount of menthol formed was not sufficient to account for the menthone that disappeared. Subsequently there was a decrease in menthol as well.

Figure 3 shows the analytical values for individual monoterpenes in leaf pair number 9 of these plants. This pair were the largest leaves and produced the largest amounts of monoterpenes, but qualitatively all of the leaves from node 4 through 11 behaved similarly.

The upper leaf pairs, from about pair 12 upward, completed their development after floral initiation. They showed a menthone peak at about the time of first bloom, followed by accumulation, and subsequent loss, of menthol.

The lowest leaf pairs (3 and 4), which were expanding at the time the cuttings were made,

Table I. *Development of Essential Oil in Peppermint*

The plants were grown on 16-hr photoperiod, 24° day and 8° night. The data are presented in mμmoles of monoterpenes per leaf pair (or tip) during development of the plant and are the average of triplicate analyses.

| Leaf pair[1] | Days A[2] | | | | | | B | | C | D |
|---|---|---|---|---|---|---|---|---|---|---|
| | 16 | 21 | 28 | 35 | 42 | 49 | 56 | 64 | 70 | 91 | 105 |
| Inflorescence | | | | | | 810 | 4560 | 5960 | 21520 | *24500*[3] | 7180 |
| 15 | | | | | | | | *550* | 470 | 110 | 50 |
| 14 | | | | | | | 200 | *3380* | 1520 | 300 | 330 |
| 13 | | | | | | 940 | 2720 | *3780* | 3220 | 2290 | 840 |
| 12 | | | | | 1530 | 3600 | 3990 | *5170* | 4830 | 4120 | 2510 |
| 11 | | | | 530 | 3320 | *6840* | 5400 | 4520 | 5590 | 4220 | 1330 |
| 10 | | | | 1040 | *8560* | 7040 | 4760 | 5640 | 3250 | 4530 | 2350 |
| 9 | | | 330 | 3930 | *10300* | 7740 | 4520 | 5950 | 2960 | 4080 | 2720 |
| 8 | | 40 | 970 | 4960 | *9210* | 3610 | 3720 | 4120 | 2570 | 3420 | 1160 |
| 7 | | 180 | 2270 | 3770 | *5680* | 3120 | 2240 | 3120 | 2070 | 2280 | 920 |
| 6 | 80 | 450 | 2520 | 2910 | *4360* | 2070 | 1520 | 2220 | 1190 | 1310 | 550 |
| 5 | 330 | 760 | 1470 | 1460 | *2980* | 1560 | 1710 | 1100 | 610 | 1020 | 420 |
| 4 | 400 | 570 | 740 | 760 | *1230* | 570 | 480 | 550 | 800 | 520 | 320 |
| 3 | 410 | 550 | 660 | *800* | 750 | 400 | 660 | 450 | 710 | 580 | 330 |

[1] Leaf pairs are numbered from the base of the plant. The uppermost "leaf pair" in each case represents the tip of the plant: the tuft of very small leaves at the growing point or, where indicated, an inflorescence.

[2] A = Time at which floral initiation could be recognized macroscopically. B = Time at which first flowers opened. C = Full bloom. D = End of bloom.

[3] Italicized values indicate the maximum monoterpene content reached in the respective leaf pairs.

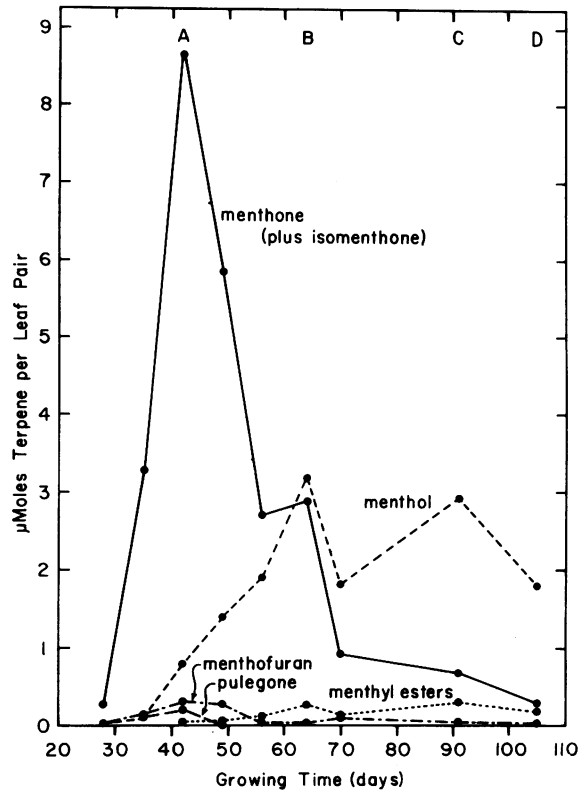


FIG. 3. Individual monoterpenes of leaf pair number 9 of peppermint plants grown on a 16-hr photoperiod with a 24° day and 8° night. (Average of triplicate analyses; same experiment as table I. For explanation of A, B, C, and D, see footnote 2 of table I.)

showed very little accumulation of monoterpenes. This is consistent with previous observations (6). Yet studies with $^{14}CO_2$ presented in figures 1 and 2, as well as those reported previously (4, 17) show rapid synthesis of monoterpenes in such rootless detached shoots. This paradox could possibly be due to an especially rapid turnover of monoterpenes in the unrooted cuttings. These observations may be related to the observation that in many leaves, detachment from the plant results in a decline in protein and chlorophyll content. This decline is prevented if roots form, and it appears that the active agent may be a cytokinin produced in the roots (20).

The periodic analyses described here show monoterpene synthesis continuing longer, after the leaves have reached full size, than did earlier evidence based on incorporation of label from $^{14}CO_2$ (4). This difference may be due to differences in the environmental conditions. It may also indicate that after a certain stage of development the secretory cells are cut off from outside carbon sources but continue to produce monoterpenes from stored substrates. The later loss of monoterpenes could be due to their being used as metabolic energy sources after other stored substrates are depleted. Evidence that the secretory cells are relatively isolated from the other tissues of the plant has been discussed elsewhere (6, 14).

In the periodic analyses of peppermint plants grown on 14-hr days with warm nights there was a similar trend to that described above, but the accumulation of menthones was never so great, and the menthol peak was more or less equivalent in size to the menthone peak. In general there was some decrease in total monoterpenes with time, but not as striking as in the plants grown on 16-hr days with cold nights. However, the kinetic data shown in figure 1 indicate that both synthesis and degradation of monoterpenes occur rapidly with 14-hr days and warm nights. Apparently these conditions are unfavorable for accumulation of monoterpenes, and turnover can only be observed by use of tracers (comparable to the observations on unrooted cuttings).

In both of these experiments an increasing number of empty or partially empty oil glands were noted during the period of monoterpene loss. These glands were sometimes browned, suggesting death. There was no sign of external injury or rupture. They appeared rather to have been partially or fully deflated as though the contents had been transported away or metabolically depleted. The extreme stability of oil glands of dried peppermint leaves, cited above, suggests that the breakdown of the glands observed here has a metabolic basis.

Grahle (10) several years ago carried out detailed node-by-node analyses of field-grown peppermint shoots over a period of 66 days, up until the beginning of bloom. With the tedious microdistillation methods available at that time much more plant material was required than for the analyses described here, and it is understandable that samples were taken less frequently than in these experiments. Her results agree completely with the results shown in table I, showing a steady increase in essential oil as the leaves developed, and a decrease in the essential oil content of all leaves below the tenth node at the time of blooming. However, since her last 2 samples corresponded approximately to "A" and "B" respectively of table I, the decrease in essential oil was seen only in the last sample, and it was not clear that this was the beginning of a downward trend. She emphasized, however, that there was no further increase in the essential oil content of these leaves after blooming started. The ninth leaf pair contained the largest quantity of essential oil in her experiments, just as in ours.

## Literature Cited

1. AEXEL, R., S. EVANS, M. KELLEY, AND H. J. NICHOLAS. 1967. Observations on the biosynthesis and metabolism of β-sitosterol, β-amyrin and related methyl sterols. Phytochemistry 6: 511–24.
2. BATTAILE, J., A. J. BURBOTT, AND W. D. LOOMIS. 1968. Monoterpene interconversions: Metabolism

of pulegone by a cell-free system from *Mentha piperita*. Phytochemistry 7: 1159–63.

3. BATTAILE, J., R. L. DUNNING, AND W. D. LOOMIS. 1961. Biosynthesis of terpenes. I. Chromatography of peppermint oil terpenes. Biochim. Biophys. Acta 51: 538–44.
4. BATTAILE, J. AND W. D. LOOMIS. 1961. Biosynthesis of terpenes. II. The site and sequence of terpene formation in peppermint. Biochim. Biophys. Acta 51: 545–52.
5. BRECCIA, A. AND R. BADIELLO. 1967. The role of general metabolites in the biosynthesis of natural products. I. The terpene marrubiin. Z. Naturforsch. 22b: 44–49.
6. BURBOTT, A. J. AND W. D. LOOMIS. 1967. Effects of light and temperature on the monoterpenes of peppermint. Plant Physiol. 42: 20–28.
7. FAIRBAIRN, J. W. AND S. EL-MASRY. 1967. The alkaloids of *Papaver somniferum* L. – V. Fate of the "end-product" alkaloid morphine. Phytochemistry 6: 499–504.
8. FAIRBAIRN, J. W. AND P. N. SUWAL. 1961. The alkaloids of hemlock (*Conium maculatum* L.) – II. Evidence for a rapid turnover of the major alkaloids. Phytochemistry 1: 38–46.
9. FLÜCK, H. 1963. Intrinsic and extrinsic factors affecting the production of secondary plant products. In: Chemical Plant Taxonomy. T. Swain, ed. Academic Press, London. p 167–86.
10. GRAHLE, A. 1955. Zur Lokalisation des ätherischen Oels im Spross von *Mentha piperita*. Pharmazie 10: 494–500.
11. HEFENDEHL, F. W. 1963. Das Verhalten des ätherischen Oels von *Mentha piperita* L. bei Lagerung getrockneter Droge. Pharmazie 18: 777–80.
12. HEFENDEHL, F. W., E. W. UNDERHILL, AND E. VON RUDLOFF. 1967. The biosynthesis of the oxygenated monoterpenes in mint. Phytochemistry 6: 823–35.
13. HOAGLAND, D. R. AND D. I. ARNON. 1950. The water-culture method for growing plants without soil. Calif. Univ. Agr. Expt. Sta. Circ. 347.
14. LOOMIS, W. D. 1967. Biosynthesis and metabolism of monoterpenes. In: Terpenoids in Plants. J. B. Pridham, ed. Academic Press, London. p 59–82.
15. NICHOLAS, H. J. 1962. Biosynthesis of β-sitosterol and certain terpenoid substances in *Ocimum basilicum* from mevalonic acid-2-$C^{14}$. J. Biol. Chem. 237: 1485–88.
16. PAECH, K. 1950. Biochemie und Physiologie der sekundären Pflanzenstoffe. Springer-Verlag, Berlin.
17. REITSEMA, R. H., F. J. CRAMER, N. J. SCULLY, AND W. CHORNEY. 1961. Essential oil synthesis in mint. J. Pharm. Sci. 50: 18–21.
18. SCHIB, R. 1958. Untersuchungen über tageszeitliche Schwankungen des Wirkstoffgehaltes in Arzneipflanzen. 3. Mitteilung. Die Tagesperiodizität des Gehaltes an ätherischem Oel im Laubblatt von *Salvia officinalis* L. Pharm. Acta Helv. 33: 32–50.
19. STONE, J. E. 1953. Studies of lignin biosynthesis using isotopic carbon. I. Long-term experiment with $C^{14}O_2$. Can. J. Chem. 31: 207–13.
20. STREET, H. E. 1966. The physiology of root growth. Ann. Rev. Plant Physiol. 17: 315–44.
21. SUKHOV, G. V. 1958. The use of radiocarbon in the study of the biosynthesis of terpenes. In: Radioisotopes in Scientific Research. Vol. IV. Research With Radioisotopes in Plant Biology and Some General Problems. R. C. Extermann, ed. Pergamon Press, London. p 535–47.
22. WALLER, G. R., M. S. TANG, M. R. SCOTT, F. J. GOLDBERG, J. S. MAYES, AND H. AUDA. 1965. Metabolism of ricinine in the castor plant. Plant Physiol. 40: 803–07.
23. WHISTLER, R. L. AND J. R. YOUNG. 1960. The role of hemicelluloses in the oat plant. Arch. Biochem. Biophys. 89: 1–5.
24. ZAPROMETOV, M. N. 1959. The ability of higher plants to split the benzene ring. Deep oxidation of $C^{14}$-catechins in tea sprouts. Dokl. Biochem. Sect. (English Transl.) 125: 123–26. [From Dokl. Akad. Nauk SSSR 125: 1359–62 (1959)].