Effects of Light and Temperature on the Monoterpenes of Peppermint

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Received June 17, 1966.

Summary. Peppermint (Mentha piperita L.) was grown in a growth chamber under several combinations of temperature and illumination, and the monoterpenes of each leaf pair were analyzed by gas chromatography. Effects on the monoterpenes could be seen in the new leaves after a few days in the growth chamber. Long-day conditions enhanced growth, with a corresponding increase in the total amount of monoterpenes. Either short nights or cool nights, combined with full light intensity during the day, enhanced the formation of menthone and depressed the accumulation of menthofuran and pulegone. Experiments with interrupted night and with low light intensity indicated that photoperiod, as such, does not directly influence the terpene composition. It is suggested that the oxidation-reduction level of the monoterpenes reflects the oxidation-reduction state of the respiratory coenzymes of the terpene-producing cells, and that this, in turn, depends on the concentrations of respiratory substrates in the cells. This suggestion is based on the likelihood that warm nights cause depletion of respiratory substrates, resulting in oxidizing conditions, while cool nights preserve high levels of respiratory substrates, and thus maintain reducing conditions.

There are many indications that the biosynthesis and metabolism of monoterpenes in peppermint (Mentha piperita L.) are influenced by environmental factors. For example, peppermint oil of acceptable commercial quality (containing principally menthone, menthol, and menthyl esters, with little or no pulegone or menthofuran) can be produced only in certain geographical areas. There are many reports (e.g., 1, 3, 12, 18, 19, 23, 28, 31) that peppermint oil varies from one growing region to another, from year to year, and as a function of such factors as shading, fertilization, water balance of the plants, and date of harvest.

It may be assumed that the reported variability in peppermint oil is not due to genetic differences, since most of the commercial plantings, at least in North America and Europe, have been propagated vegetatively from plants of the Black Mitcham variety, which originated in England (11, 12, 16, 28). (Oil of Mentha arvensis L., which is produced largely in Asia and South America, is sometimes referred to as peppermint oil but is distinctly different from the oil of Mentha piperita. In this paper peppermint will refer only to Mentha piperita.)

Peppermint is affected by day length, long days inducing flowering, and short days bringing on a characteristic winter growth habit with many stolons. Langston and Leopold (20) demonstrated, by night interruption studies, that a true photoperiod effect is involved. Under their conditions, plants grown on photoperiods of 14 hours or less produced only traces of essential oil. According to Guenther (12), production of peppermint oil requires a day length of 15 to 16 hours. Grahle and Hölzl (10, 17) reported recently that the proportions of individual monoterpenes in peppermint oil are strongly influenced by day length.

Biggs and Leopold (5) showed that temperature greatly influences growth, flowering, and essential oil yield in peppermint. Steward and coworkers (25, 26) carried out extensive investigations of the influence of environmental factors on growth and metabolism of peppermint. Effects of day length, temperature, and nutrition were found to be intimately related.

Reitsema in 1958 (21) proposed a scheme of terpene interconversions, mostly reductive, to account for the formation of the principal monoterpenes of peppermint oil. Subsequent investigations (4, 8, 9, 13, 22) have supported the main features of this scheme. Figure 1 is an extension of Reitsema's scheme, taking account of stereoisomerism. This revised scheme could account for the formation of isomenthone and of the several isomers of menthol which are found as minor components in peppermint oil (23, 28, and F. J. Cramer, personal communication).

One line of evidence for interconversions of monoterpenes in peppermint was obtained by an-
Fig. 1. Biochemical relationships of monoterpenes in peppermint. Dotted arrows indicate postulated reactions.

alyzing the essential oil from leaves of different ages. Analyses of the monoterpenes of individual leaf pairs from plants grown in the greenhouse (4, 8) showed a consistent trend, from unsaturated ketones and menthofuran in the upper (young) leaves to menthol and methyl esters in the lower (old) leaves. However, there was considerable variation from one shoot to another. It seemed likely that this variation was due to variations in the environmental factors, which could not be closely controlled in the greenhouse. Similar analyses have now been carried out using peppermint grown in controlled environments. The results which are reported here confirm that the metabolism of monoterpenes in peppermint is strongly influenced by environmental conditions.

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 (4). The plants were grown in the greenhouse under photoperiods of 14 hours or longer. Cuttings, consisting of the tuft of youngest leaves at the growing tip, plus the next 3 leaf pairs, were rooted in perlite in the greenhouse for 7 days before being transferred to the growth chamber.

Twelve or more rooted cuttings were used in each experiment. Since a certain amount of variability in the cuttings was found to be unavoidable, each cutting to be placed in the cold-night chamber was paired with a cutting, identical in the number and size of leaves, placed in the warm night chamber.

Growing Conditions. All experiments were done in a single controlled environment room, 2.4 m by 2.4 m, but by enclosing the plants in boxes with clear plastic tops it was possible to obtain 2 night temperatures simultaneously. These boxes were approximately 50 x 50 x 50 cm and were lined with aluminum foil and fitted with blowers. During the light period the temperature of both boxes was that of the growth chamber. During the dark period, when the temperature of the growth chamber was lowered, a higher temperature was maintained in one of the boxes by means of a thermostatically controlled warm air blower (210 w electric hair dryer). The blowers provided approximately the same air movement in both boxes. Temperatures within the boxes were checked periodically with a thermometer shielded from the light or with a Belfort Instrument Company hygrometer.

Illumination, for most of the experiments, was from 32 Sylvania F96T12 8-foot VHO Gro-Lux fluorescent lamps, 200 watts each, plus 24 100 watt incandescent bulbs, approximately 150 cm above the floor of the boxes. This provided 700 ± 50 foot candles at the floor of the boxes and 1000 foot candles at the top, as measured with a General Electric No. 213 light meter (Se photo-cell). The actual light energy available to the plants was greater than the measured value, since the selenium photocell is relatively insensitive in the red and blue regions of the spectrum, where Gro-Lux lamps have their greatest output.

The same daytime lighting was used in the experiments with interrupted nights. In addition, a 60 watt incandescent bulb was suspended above each box, centered 63 cm above the floor of the box. These bulbs were turned on in the middle of the dark period and stayed on for 15 minutes.

For the experiment with low light intensity, illumination was provided by twelve 60 watt incandescent bulbs, distributed uniformly on the ceiling of the room, at the same height as before. The measured light intensity was 50 to 60 foot candles at the floor of the boxes and 75 to 80 foot candles at the top.

Hoagland and Arnon nutrient solution No. 2 (15) was supplied every second day, and water on the alternate days.

Sampling. For each analysis, a pair of plants matched at the beginning of the experiment were harvested on the same day. Due to differences in growth rate the plants were no longer identical in leaf size and number at the time of sampling. Samples were taken in the morning, approximately 3 hours after the beginning of the light period. Leaf pairs were removed node by node from the central stem of the plant, weighed and measured, and extracted with hexane. Branches formed in some cases but were not analyzed.

The first analyses were made after 2 to 4 new leaf pairs had developed in the growth chamber (from 4-14 days, depending on the conditions); samples were taken thereafter at intervals of 4 to 6 days, depending on the rate of growth. Consistent results were obtained from the 6 or more pairs of plants thus analyzed for each set of conditions.

Dotted arrows indicate postulated reactions.
conditions. A second series of plants were grown and analyzed for all sets of conditions except the 18 hour day with low light intensity and the 12 hour day. Consistent results were again obtained in all cases.

Extraction and Analysis of Terpenes. The leaves were ground in a mortar containing hexane (Skellysolve-B) and anhydrous sodium sulfate and extracted 4 times with hexane to give a total volume of 10 ml of yellowish extract. A small amount of Norit A charcoal, just sufficient to remove the yellow color, was added to each extract, and removed by centrifuging at low speed. Tests with the principal peppermint terpenes indicated that they were not adsorbed by the charcoal. The clear solutions were concentrated under a stream of air at room temperature, taking care not to remove quite all of the solvent. Evaporation of the oxygenated terpenes is negligible if the hexane is not removed completely.

The extracts were analyzed by gas chromatography in a Beckman GC-2A gas chromatograph with thermal conductivity detector. The column was made of 8 feet (2.4 m) of one-fourth inch (6.35 mm) O.D. aluminum tubing, packed with about 20 g of 5 % KOH-5 % Quadrol-3 % SAIB (sucrose acetate isobutyrate)-87 % firebrick (100 to 120 mesh). The firebrick was coated first with KOH dissolved in 95 % ethanol, and then with the mixture of Quadrol and SAIB, dissolved in methylene chloride. Separations were carried out at 140° to 148°. The column is a modification of one designed for peppermint oil by Wilkens Instrument and Research, Incorporated (32), and the column packing materials were obtained from them. Retention times on this column are shown in table I. Addition of KOH improved the separation of menthol and pulegone, with little effect on the other components. This method separates the principal components of peppermint oil, but is not completely satisfactory for the minor components. Isomenthone has the same retention time as menthone.

Table I. Retention Times of Monoterpens

The values are relative to the retention time of menthone which is 20 to 25 minutes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Constituent</th>
<th>Relative Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Pinene</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>β-Pinene</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Limonene</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>Cineole</td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td>Menthofuran</td>
<td>0.68</td>
</tr>
<tr>
<td>6</td>
<td>Menthone</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>Menthyl acetate</td>
<td>1.33</td>
</tr>
<tr>
<td>8</td>
<td>Neomenthone</td>
<td>1.50</td>
</tr>
<tr>
<td>9</td>
<td>Pulegone</td>
<td>1.55</td>
</tr>
<tr>
<td>10</td>
<td>Neoisomenthe</td>
<td>1.64</td>
</tr>
<tr>
<td>11</td>
<td>Menthol</td>
<td>1.84</td>
</tr>
<tr>
<td>12</td>
<td>Isomenthe</td>
<td>2.09</td>
</tr>
<tr>
<td>13</td>
<td>Piperitone</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Published analyses of peppermint oil (23, 28) indicate that the ratio isomenthone/menthone ranges from about 0.15 to 0.3. The isomers of menthol appear between menthol and pulegone in the chromatograms and may cause small inaccuracies in the estimation of both of these compounds.

The extracts were injected into the gas chromatograph with a Hamilton Microliter gas-tight syringe with cemented needle. The cemented needle was found to be essential for quantitative transfers.

Peak areas were integrated by means of a Disc Integrator on the gas chromatograph recorder. Integration factors and retention times for the individual compounds were determined with known terpenes. These terpene standards were obtained from several sources. Menthofuran, menthol, and piperitone were donated by Dr. F. J. Cramer of the A. M. Todd Company, Kalamazoo, Michigan. Pulegone and (+)-limonene were donated by Dr. Paul Tornow of I. P. Callison and Sons, Incorporated, Chehalis, Washington. Piperitenone was donated by the Glidden Company, Southern Chemical Division, Jacksonville, Florida. 1,8-Cineole (Eastman White Label grade) and (-)-menthone (practical grade) were obtained from Distillation Products Industries, Rochester, New York. Menthyl acetate and isomenthone were obtained from K & K Laboratories, Jamaica, New York. Samples of isomenthol, neomenthol and neoisomenthol were donated by Dragoco, Gerbering and Company, Holzminden, Germany.

Results

Environmental effects on the monoterpenes of peppermint were observable in the new leaves as soon as they had developed, usually within 5 to 10 days in the growth chamber. Representative analytical data for pulegone, menthofuran, menthone (plus isomenthone), and menthol, from plants grown under several combinations of temperature and illumination, are shown in figures 2 to 7. The compounds shown made up approximately 80 to 90 % of the total monoterpenes, and no obvious trends were observed in the other components. None of the tissues analyzed here were old enough to have produced substantial amounts of menthyl esters.

Each figure compares the terpene values from a pair of matched shoots grown simultaneously, with all factors constant except night temperature, and harvested on the same day. The data shown are selected to represent plants at approximately the same stage of growth, and therefore represent varying periods of time in the growth chamber. A series of at least 6 analyses were made for each set of conditions, and all results were consistent with those shown.

The data have been plotted with the youngest tissues at the left, and the data points are connected by lines for ease in reading. The resulting curves
are analogous to a time-course of the development of the essential oil. Environmental effects on monoterpenoid metabolism are best seen by comparing the patterns of these curves. It is impossible to pick any specific leaf pair and say that it is exactly equivalent to a specific leaf pair of another plant.

Tip indicates the tuft of very small leaves at the growing point, except in figure 5, where the tips were expanding inflorescences. Leaves below the fourth pair had developed before the cuttings were rooted and placed in the growth chamber. They were analyzed in the initial experiments and found to contain predominantly menthol. Since these leaves had developed under somewhat variable conditions in the greenhouse, and since their monoterpenoid content appeared to be more a function of age than of environmental conditions in the growth chamber, they were not analyzed in later experiments.

The data are presented here in terms of the quantity of each monoterpenoid per leaf pair, because this presentation best shows the formation of the individual compounds as the leaves develop. Table II gives the fresh weights of the leaf pairs and the amount of monoterpenes per gram of leaf tissue for all of the experiments except the experiment with 14-hour day (fig 2). The values shown are the sum of all monoterpenes except those with very short retention times (i.e. all except hydrocarbons and cineole) and represent approximately 85 to 95% of the total monoterpenes. It is apparent from table II that the amount of total monoterpenes per gram of tissue in the expanded leaves is relatively constant; differences in the amount of total monoterpenes per leaf pair largely reflect differences in leaf size. In the young tissues terpene synthesis proceeds relatively more rapidly than growth, and these tissues in some cases contain very large amounts of terpene per gram of tissue.

The lowest ratio of terpene to tissue was always found in the 4th, 5th or 6th leaf pair (table II). These were the leaves which were expanding during the time the cuttings were developing roots. It appears that in unrooted cuttings conditions are unfavorable for the accumulation of monoterpenes. Rapid incorporation of label from 14CO2 into monoterpenes by such unrooted cuttings (4) indicates that synthesis of monoterpenes is occurring, but in further experiments (8) it was found that a large part of the label disappeared with time, suggesting metabolic turnover of the monoterpenes. 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 there is evidence that the active agent may be a kinin produced in the roots (27).

A day length of 14 hours was taken as the starting point for these experiments because experience with greenhouse-grown peppermint indicated that this intermediate photoperiod was near optimal for obtaining vigorous and prolonged vegetative growth, with a minimum of flowering. Shorter days decreased growth and caused the pro-

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**Table II. Fresh Weights and Monoterpene Contents of Leaf Pairs from Peppermint Grown under Several Conditions**

<table>
<thead>
<tr>
<th>Leaf pair</th>
<th>Day 25°, night 25°</th>
<th>Day 25°, night 8°</th>
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<tbody>
<tr>
<td>Tip</td>
<td>9</td>
<td>Tip</td>
</tr>
<tr>
<td>Fr wt of tissue (mg)</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>µMoles terpene/g</td>
<td>9.7</td>
<td>9.0</td>
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<th>Day 25°, night 8°</th>
</tr>
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<tbody>
<tr>
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<td>Tip</td>
</tr>
<tr>
<td>Fr wt of tissue (mg)</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>µMoles terpene/g</td>
<td>11.0</td>
<td>11.0</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Leaf pair</th>
<th>Day 25°, night 25°</th>
<th>Day 25°, night 8°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>9</td>
<td>Tip</td>
</tr>
<tr>
<td>Fr wt of tissue (mg)</td>
<td>122</td>
<td>122</td>
</tr>
<tr>
<td>µMoles terpene/g</td>
<td>21.0</td>
<td>21.0</td>
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</table>

<table>
<thead>
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<th>Leaf pair</th>
<th>Day 25°, night 25°</th>
<th>Day 25°, night 8°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>9</td>
<td>Tip</td>
</tr>
<tr>
<td>Fr wt of tissue (mg)</td>
<td>32</td>
<td>32</td>
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<tr>
<td>µMoles terpene/g</td>
<td>7.4</td>
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<table>
<thead>
<tr>
<th>Leaf pair</th>
<th>Day 15°, night 15°</th>
<th>Day 15°, night 8°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>7</td>
<td>Tip</td>
</tr>
<tr>
<td>Fr wt of tissue (mg)</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>µMoles terpene/g</td>
<td>8.2</td>
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duction of horizontal runners instead of erect stems. Longer days hastened flowering. With 14-hour days there was a striking effect of night temperature on the monoterpene composition (fig 2). The warm night temperature (25°C) favored the relatively oxidized compounds pulegone and menthofuran, while the cold night temperature (8°C) greatly favored production of the more reduced compound menthone.

In experiments with 8-hour days, growth was poor, and there was a correspondingly low yield of essential oil (fig 3). Again, warm nights produced oxidized terpenes and cold nights produced menthone. If a 15-minute light flash was added in the middle of the dark period, typical long-day growth resulted, with larger leaves and therefore much more terpene produced, but menthone still predominated in the cold nights, and menthofuran in the warm nights (fig 4).

When the day length was increased to 18 hours, the night temperature had very little effect on the composition of the essential oil (fig 5). Menthone predominated in the leaves at either 8° or 25° night temperature. However, an 18-hour photoperiod at low light intensity (about 70 ft-c) gave very poor growth, and produced predominantly menthofuran on warm nights and menthone on cold nights (fig 6). The light intensity used in this experiment was adequate for photoperiod effects but provided very little energy for photosynthesis.

Figure 7 shows the results of an experiment with 12-hour days, and temperatures of 15° and 8°. Under these conditions menthone predominated with either 8° or 15° night temperature, but 15° nights resulted in more vigorous growth and increased essential oil production. With the cooler temperature regime used in this experiment, a 12-hour day produced essentially the same effects on monoterpene metabolism in the leaves as previously observed with an 18-hour day (fig 5). No inflorescences developed on the 12-hour day in the 31 days that the plants were in the growth chamber.

Peppermint plants grown under continuous light at room temperature (20° to 25°) produced predominantly menthone in the leaves (the data are not shown).

Inflorescences, whenever they appeared, were found to contain high levels of menthofuran and pulegone, even with cold nights. This is seen in figure 5 for plants grown on 18-hour days with full light intensity for 21 days. The same effect was also observed with 14-hour days and constant temperature of 25°, when inflorescences finally developed after 63 days in the growth chamber (data not shown). The plants grown in continuous light also formed inflorescences, which contained predominantly menthofuran and pulegone, though the leaves contained predominantly menthone.

Qualitative observations indicated a close parallelism in these experiments between the formation of menthone and the development of pigments, presumably anthocyanins. Generally the leaves of warm-night plants were pale green, while those of cold-night plants were dark green to reddish. However, in those cases where the warm-night plants formed predominantly menthone (fig 5,7) they also had dark colored leaves, scarcely distinguishable from those of the cold-night plants.

Growth of the plants was also influenced strongly by the environmental conditions. It is apparent from figures 2 to 7 and table II that warm nights stimulated growth. This can be seen both in the number of leaf pairs produced, and in the weights of the leaves and inflorescences produced. The fast-growing plants were frequently the plants which produced high levels of menthofuran and pulegone.

Discussion

The effects of environmental factors on terpene metabolism in peppermint are complex, and caution is called for in interpreting the data. There are clearly photoperiod effects on flowering and vegetative growth in peppermint; both are promoted either by long light periods or by interruption of the dark periods. However, photoperiod probably has no direct influence on monoterpene metabolism.

The increased amounts of essential oil formed under long-day conditions appear to be largely a reflection of increased growth. Grahle and Hölzel (10,17) have reported that long photoperiods result in high levels of menthones and menthols in peppermint leaf oil, while short photoperiods produce high levels of menthofuran. Their plants were grown at a constant temperature of 20°, and the results

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Fig. 2*. Monoterpene of peppermint grown on 14-hour day for 10 days.
Fig. 3*. Monoterpene of peppermint grown on 8-hour day for 13 days.
Fig. 4*. Monoterpene of peppermint grown on 8-hour day with interrupted night for 12 days.
Fig. 5*. Monoterpene of peppermint grown on 18-hour day for 21 days. Tips were inflorescences. Leaf pair 8 of the cold night leaves were very small leaves immediately below the inflorescence.
Fig. 6*. Monoterpene of peppermint grown on 18-hour day with low light intensity for 26 days.
Fig. 7*. Monoterpene of peppermint grown on 12-hour day for 19 days.

* Abbreviations for figures 2 to 7: mf = menthofuran; ml = menthol; mn = menthone (plus isomenthone); pu = pulegone. Leaf pairs are numbered from the base of the plant upward.

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therefore cannot be compared directly to ours. However, their findings are for the most part similar to the results we obtained at 25°. It appears from our results that these are not true photoperiod effects. If a direct photoperiod effect on monoterpene metabolism were involved, the experiment with interrupted night (fig 4) and the experiment with 18-hour day and low light intensity (fig 6) should have given results similar to the 18-hour day with full light intensity (fig 5), i.e. they should have produced predominantly menthol at either 25° or 8° night temperature.

In general, in our experiments, either short nights or cool nights, combined with full light intensity during the day, enhanced the formation of menthol and depressed the accumulation of menthofuran and pulegone in the leaves. Continuous light had the same effect. It is possible that the oxidation-reduction level of the monoterpens reflects the general oxidation-reduction state of the respiratory coenzymes of the terpene-producing cells, and that this depends on the balance between daytime photosynthesis and nighttime utilization of photosynthetic.

In the light, photosynthesis produces reducing conditions. In the dark, the products of photosynthesis CO₂ fixation serve as respiratory substrates, and as long as these substrates are available in abundance, one might expect the respiratory coenzymes to remain in a relatively reduced state. However, these products may be depleted by respiration, by growth, or by translocation. Warm nights were found to stimulate growth of peppermint, and they very likely stimulate respiration as well, and thus bring on oxidizing conditions. In particular, one might expect to find strongly oxidizing conditions during the later part of a long warm night.

Ulrich (29, 30) reported that the accumulation of sucrose in sugar beet roots is enhanced by long days, high light intensities, or low night temperatures. He suggested that under these conditions sucrose is produced in excess of that required for ordinary growth and metabolism, and the excess is diverted into storage. We suggest that under similar environmental conditions peppermint may also produce an excess of sucrose, or equivalent products of photosynthesis, which maintains reducing conditions in the oil glands.

Data of Rabson and Steward (25, part III: 26, part VI) indicate that warm nights do cause depletion of tricarboxylic acid cycle intermediates and related amino acids and amides in peppermint. Plants subjected to a 10° night temperature had much higher levels of these respiratory substrates in stems and leaves than did plants subjected to 27° nights. This was true at all photoperiods which they tested: 8, 12, and 15 hours. The concentration of α-ketoglutarate was found to decrease during the night, and this decrease was more rapid at high night temperature.

Stahl (24) was able to show reduction of triphenyltetrazolium chloride within the secreting cells of the glandular hairs of yarrow. The reducing activity of these cells was very strong on warm sunny days and was weak on cold cloudy days. Stahl mentioned that he had made similar observations on other plants, and that the 3-celled glandular hairs of peppermint had especially strong reducing power, which was also influenced by environmental conditions.

Guayule grown on 8-hour days has been found to accumulate rubber only if the nights are cold (6). The optimum night temperature for rubber production was 5° to 7°. Since rubber is a terpenoid polymer, the parallelism to monoterpene metabolism in peppermint is especially interesting.

There is considerable evidence indicating that the site of monoterpene formation and metabolism in plants is isolated, both from the air and from the rest of the plant. Morphological studies, with the light microscope (7) and with the electron microscope (2), show that in peppermint oil glands the only access is through a heavy cuticle or through a single-celled stem, presumably through the protoplasm of the stem cell. Evidence of another type is available from an experiment performed by Hefendehl (14) in which dried peppermint leaves were left exposed to the air and the essential oil analyzed monthly. Over a period of 15 months there was only 10 % loss of essential oil, and no change in its composition. This is in sharp contrast to the lability of the oil after it is removed from the plant. We have observed oil glands, still filled with oil, in peppermint acetone powders which had been stored for 4 years in a freezer.

Tracer studies with several species of plants also suggest that the site of monoterpene synthesis is not readily accessible. ¹⁴CO₂ has usually been the best substrate, and mevalonic acid a very poor substrate, for monoterpene production (e.g. 4, 8, 9). Label from ¹⁴CO₂ or ¹⁴C-glucose is incorporated slowly and steadily into monoterpens over a period of several hours after the substrate is administered to the plant. One cannot completely discount the possibility that monoterpenses are formed by some unknown metabolic pathway, different from the pathway to other isoprenoid compounds, but physical isolation of the site of synthesis seems a more likely explanation of the results.

It appears then that the formation and metabolism of monoterpenses occurs at a site which is not readily accessible either to carbon substrates or to oxygen. It seems likely that only certain metabolites, such as sucrose, can reach the secreting cells, and that mevalonic acid for monoterpene synthesis must be produced in the secreting cells. Such a situation would make the cells of the oil gland extremely sensitive to the types and amounts of substrates available to them from the adjacent cells.
The fact that inflorescences respond to environmental influences quite differently from leaves is unexplained, though not unexpected. Analyses of commercial peppermint oil (12, 31) suggested that inflorescences produce large amounts of menthofuran. Grahle and Höltzel (10, 17) also found large amounts of menthofuran in flowers under conditions which produced high percentages of menthol and menthones in the leaves. There are of course complex physiological changes in the plant at the time of flowering. The fact that vegetative growth of the main shoot of peppermint ceases and formation of branches increases when blooming starts may be important in this respect. Inflorescences also contain a large amount of non-photo-synthetic tissue, which may modify their metabolism by producing a shortage of respiratory substrates in the terpene-producing cells. The appearance of flowers on 14-hour days contrasts with Langston and Leopold’s (20) observation that the critical day length for flowering in peppermint was between 16 and 18 hours. However, Biggs and Leopold (5) later observed flowering on 16-hour days and quoted Allard to the effect that he had observed a few flowers on peppermint grown on 14-hour days. Biggs and Leopold found that the time of flowering was greatly influenced by small changes in temperature, and they suggested that temperature may modify the critical day length for photoperiodic effects. Our results are consistent with this suggestion.

Literature Cited


