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Effects of Photoperiod on the Yield and Composition of Peppermint Oil[1]

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Abstract. **Peppermint (*Mentha piperita* L.) was grown under growth room conditions with two photoperiodic treatments, short day and long day. Each treatment received a total of 13 hours light per 24 hour cycle, either continuously (13H) or as an interrupted night treatment (131) with 1 hour of light in the middle of the dark period. In addition to the previously reported changes in dry matter yield of herb, oil yield, growth habit and flowering, the photoperiodic treatments strongly influenced the proportions of several individual monoterpenes in peppermint. The long day treatment resulted in reduced levels of menthofuran, pulegone, menthyl acetate and limonene as well as increased levels of menthone, menthol, neomenthol acetate (+ unknown), trans-sabenine hydrate, cineole and β pinene + sabenine.**

There are many indications that peppermint is affected by photoperiodic treatment. For example, Langston and Leopold (8) demonstrated, by night interruption studies, that a true photoperiod effect was involved in determining the growth habit and flowering in peppermint. These workers reported that short day conditions give rise to peppermint plants that do not flower and that form many stolons with small leaves. In contrast, peppermint plants grown under long day conditions form erect upright lateral shoots and produce large leaves and flowers (1, 3, 11). Values ranging from 14 to 18 hr light per day are quoted as the critical day length requirements of peppermint (1, 2, 8). According to Burbott and Loomis (2) this critical day length is greatly influenced by temperature.

The production of oil of *Mentha piperita* has been related to daylength. The greatest production of oil has been associated with long days (4, 5, 7). However, Burbott and Loomis (2) associated greater oil production under long days to its effect on plant growth.

Several workers have studied the effect of photoperiod on oil composition. In such a study Grahle and Hoeltzel (4) found that leaves of *M. piperita* grown at 20°C constant temperature subjected to long days (18:6), contained relatively small amounts of menthofuran and large amounts of menthol and menthone. Plants subjected to short days (12:12) contained very small quantities of menthone and menthol and large amounts of menthofuran. In order to differentiate between photosynthetic and photoperiodic effects, Grahle and Hoeltzel (4) conducted night interruption studies and the data obtained indicated that the effects were caused by photoperiodic treatment rather than by differences in photosynthate between short day and long day conditions. These workers found that short days (12:12) produced oil high in menthofuran (85%) and low in menthol (10%) and menthone (1%). Plants subjected to a photoperiodic treatment of (12:12) with 1 hr of interrupting light in the middle of the dark period yielded oil which was low in menthofuran (9%) and relatively high in menthol (56%) and menthone (25%). thus resembling plants grown under an 18 hour photoperiod with respect to oil composition.

In contrast to the above work, Burbott and Loomis (2) conducted experiments with interrupted nights and low light intensity and found that photoperiod as such did not directly influence the composition of the essential oil of peppermint. Plants grown under conditions of 8 hours light/day at 25°C constant temperature and plants grown under identical conditions with a 15 min light interruption in the middle of the dark period produced oils which were typical of short day plants since both contained predominantly menthofuran. These workers also found that when the light intensity was reduced, plants grown under daylengths of 18 hr at 25° constant temperature produced oil with a composition typical of short day plants.

It appears from the above discussion that there exists an apparent disagreement between the results of Burbott and Loomis (2) and Grahle and Hoeltzel (4). The experimental work reported here examines the influence of photoperiodic treatments on the monoterpene composition of peppermint.

Materials and Methods

Plant material. Cuttings of 'Black Mitcham' peppermint were propagated vegetatively from plants growing under a 14 hr photoperiod in the greenhouse. Cuttings consisted of short sections of underground stem material that were rooted in a 1 sand:1 peat mix. These cuttings were transplanted into pots in the growth rooms when the plants were about 3cm tall and after they had produced 3 pairs of leaves.

Growing conditions. All experiments were done in 2 identical growth rooms, each 1.5 x 4m in size, lined with aluminum foil and fitted with air conditioners. The air conditioners provided the same air movement in each growth room. Temperatures within the rooms were monitored with a thermometer shielded from the light and with a thermograph. Irradiation for all experiments was provided by 10 Osram MCFER 40W white fluorescent lamps, 2 Mazda 75W incandescent bulbs and 2 Philips HLRG-N mercury vapour lamps. The fluorescent and incandescent lamps were evenly distributed on the ceiling of the rooms, 2m above the plant material, and the mercury vapour lamps were suspended 1.5m above the plant material and 0.5m apart, so as to give an even distribution of irradiance over all plant material. This provided 75$\mu\Sigma$ m^{-2} sec^{-1} at the bottom of the room and 150$\mu\Sigma$ m^{-2} sec^{-1} 1m above the floor, as measured by a Lambda LI-185 meter fitted with a quantum flux sensor.

The final potting medium consisted of a 50:50 sand:peat mix to which basal nutrients were added. Hoagland and Arnon (6) nutrient solution was supplied weekly and tap water daily.

Sampling and oil extraction. Prior to distillation, all peppermint samples were dried at 20°C under greenhouse conditions for 1 to 2 days until they had reached a moisture content of about 30%. All herb samples were comminuted and immediately distilled. The distillation apparatus consisted of a modified 20 liter S.E.B. aluminum pressure cooker with condenser.

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Gas chromatographic technique. Gas chromatographic analysis of oil samples was conducted using a Pye Unicam Series 104 Chromatograph, fitted with a 56 M x 0.5mm I.D., FFAP., SCOT Capillary Column. The optimum operating conditions for such a capillary column were considered to be as follows: carrier gas (N_2) flow rate 2ml/min, air pressure 0.90kg/cm^2 and hydrogen pressure 1.25kg/cm^2. The column oven temperature program was as follows: 80 to 160°C at 5° per min with no injector head heating being used.

For analysis, 0.1μl of an oil solution (1:1, oil in redistilled n-hexane), was injected into the G.C. The identification of peaks eluting from the SCOT column was made by comparing the retention time of peaks to a sample chromatogram run on a similar column. In addition, authentic samples of all compounds were added to an oil sample to confirm the identity of these peaks. Peak areas of all components were determined using a Pye Unicam D.P. 88, computing integrator.

Experimental. The experimental work consisted of 2 photoperiodic treatments, a short day and a long day treatment. The first of these treatments involved 13 hr light per day (13H) and the second treatment consisted of 12 hr of light followed by a 1 hr light break in the middle of the dark period (13I). Temperature was maintained constant at 20°C (±1°C), and the relative humidity at about 50% during the day and night.

Each photoperiodic treatment consisted of 3 replications with 10 plants in each replicate. As well as having replications within photoperiodic treatments, the actual treatments were repeated and consistent results were obtained.

Both treatments were harvested after 62 days in the growth rooms.

Results

The growth habit of the plants receiving 13I and 13H photoperiods is shown in Fig. 1. Plants grown under a 13I inductive photoperiod were erect and formed inflorescences during the course of the experiment. In contrast, growth under a 13H non-inductive photoperiod was poor, with plants being decumbent with many stolons and few erect stems.

The mean dry matter yield of herb per plant, yield of oil per plant, and percentage oil on dry matter basis are listed in Table 1 and from these results it appeared that plants grown under long day (13I) conditions had significantly higher yields of herb and oil and an increased percentage oil, relative to that produced under short day (13H) conditions. Both an increase in percentage oil and an increase in dry matter production per plant seemed to contribute to the increase in oil yield per plant.

Table 1. The effect of photoperiod on dry matter yield of herb, yield of oil, and percentage oil.

Mean values from 3 replications	Photoperiod treatments		Variance ratio
	13I	13H	
Dry herb yield (g/plant)	4.32	2.16	27.87**
Oil yield (mg/plant)	76.94	27.32	207.8***
Yield (%, dry matter (basis)	1.78	1.26	11.12*

*, **, ***Significant at 5% (*), 1% (**), or 0.1% (***) level.

The influence of the photoperiod on oil composition is illustrated in Fig. 2 and 3. The mean value for percentage of total peak area represented by each of the 12 major compounds is listed in Table 2. The twelve compounds selected represent about 97% of the total peak area and no other compounds were observed to vary with photoperiod. From these results it appeared that the photoperiodic treatments imposed have several effects on oil composition. The most significant of these changes in oil composition is the increase in menthofuran, limonene, menthyl acetate and pulegone and the decrease in amount of cineole, menthone and menthol comparing

Fig. 1. Peppermint *(Mentha piperita)* grown under 2 photoperiodic treatments: 12 + 1 (13I) indicates 12 hr light per day, plus 1 hr of light in the middle of the dark period; 13H indicates 13 hr light per day.

Fig. 2. Gas chromatogram of peppermint oil extracted from plants growing under long day conditions provided by a 13 hr interrupted photoperiod (13I). (Peak numbers on the chromatogram correspond to those shown in Table 2.)

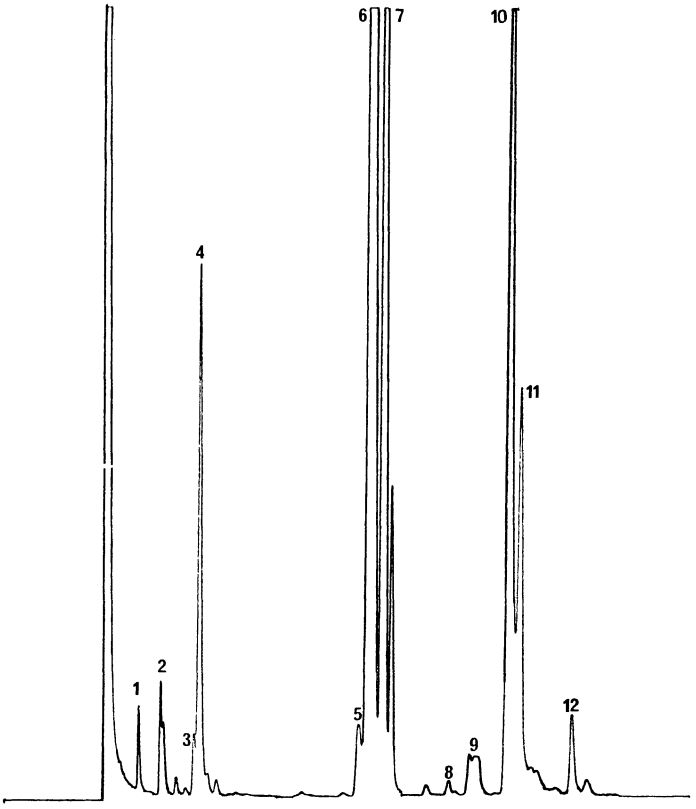

Fig. 3. Gas chromatogram of peppermint oil extracted from plants growing under a short day photoperiod (13H). (Peak numbers on the chromatogram correspond to those shown in Table 2.)

treatment 13I to 13H. Other changes in oil composition are decreases in β pinene + sabinene, trans-sabinene hydrate, neomenthol acetate (+ unknown) and the unknown (peak 12) in treatment 13H relative to 13I.

Discussion

Photoperiod clearly has an effect on vegetative growth and flowering in *M. piperita*, both being promoted by long days or by interrupted nights. This observation is in agreement with several workers (1, 2, 8, 9).

The amount of essential oil accumulated in plants receiving a 13H non-inductive photoperiod was about 1/3 that found in the plants exposed to a 13I inductive photoperiod.

Burbott and Loomis (2) state that photoperiod, as such, does not directly influence the terpene composition of peppermint. These results were obtained by using interrupted night and low light intensity studies. They go on to suggest that the oxidation-reduction level of the monoterpenes reflects the oxidation-reduction state of the respiratory co-enzymes of the terpene producing cells, and that this, in turn, depends on the concentration of respiratory substrates in the cells. Therefore, any factors which influence the concentration of respiratory substrates in such cells would effect terpene composition. Factors such as night temperature and length of the photosynthetic period are seen as being important in this context.

The present study does not substantiate the oxidation-reduction hypothesis put forward by Burbott and Loomis (2). In addition, it does not support the claim that photoperiod as such has no direct influence on monoterpene composition in peppermint. In contrast, it appears that photoperiodic treatments imposed in the present work have a profound influence on the monoterpene composition of peppermint oil.

Table 2. The effect of photoperiod on the composition of peppermint oil.

Peak no.	Compound	Photoperiod treatments (% total peak area) 13I	13H	Variance ratio
1	α Pinene	0.703	0.404	4.788 n.s.
2	β Pinene + Sabinene	1.568	0.770	15.061*
3	Limonene	0.541	1.612	183.844***
4	Cineole	6.371	0.877	228.500***
5	Trans-Sabiene Hydrate	1.325	0.487	24.877**
6	Menthone	43.77	8.135	631.55***
7	Menthofuran	21.098	64.340	884.60***
8	Menthyl Acetate	0.356	2.144	11752.05***
9	Neomenthol (+ Unknown)	2.077	1.360	9.059*
10	Menthol	13.869	9.545	86.86***
11	Pulegone	7.075	10.146	79.804***
12	Unknown	1.268	0.241	64.497**

The results obtained agree with the work of Grahle and Hoeltzel (4) who report that the proportions of individual monoterpenes in peppermint oil are strongly influenced by day length.

In addition to the reported change in proportions of compounds such as menthofuran, menthone and menthol (4) with photoperiod, the present work indicates that several other compounds are significantly altered by the photoperiodic treatments. For example, the large change in the ratio of limonene to cineole which results from the photoperiod treatments, is previously unreported. Smith and Levi (10) consider that a ratio of 0.2-0.7 is characteristic of *M. piperita* and a ratio greater than 2 is characteristic of *M. arvensis*. From their observations, they suggest that such a ratio is genetically controlled and could offer a means of identifying authentic oils. The wide variation in this ratio obtained in the present work suggests that such a ratio is strongly influenced by environmental conditions.

The differences in oil composition resulting from the treatments imposed, in general follows previously reported trends (2, 4). Subjecting plants to night-break conditions had similar effects on monoterpene composition as were observed by Burbott and Loomis (2) when cold nights and long photoperiods were employed. These treatments resulted in changes such as increasing menthone and menthol and decreasing menthofuran and pulegone. All such changes fit the scheme of reductive terpene interconversions proposed by Reitsema (9). That is, generally interconversions can be seen to proceed via pulegone either to menthofuran or to the series of components; menthone; menthol and menthyl acetate. However, the biochemical relationships between monoterpenes in peppermint as proposed by Reitsema (9) do not explain how conditions which favor the accumulation of menthofuran also favor the accumulation of menthyl acetate.

Conclusion

As a result of the work conducted by Burbott and Loomis (2) it is generally accepted that the important factors determining the monoterpene composition of peppermint oil are those such as night temperature and day length. Their studies suggest that the changes in monoterpene composition under long-day conditions are of photosynthetic origin rather than a response to photoperiodic treatment. The present work indicates that photoperiodic treatment itself is an important determinant of monoterpene composition.

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The Importance of Harvest Date and Plant Density on the Yield and Quality of Tasmanian Peppermint Oil[1]

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Abstract. **Oil yield of peppermint (*Mentha piperita* L.) per unit area obtained from plant density treatments 30 and 40 plants/m^2, reached a maximum early in the growing season, whereas oil yield from the lower density treatment, 10 plants/m^2, continued to increase even at a menthol content of 50%. The latter density treatment yielded less oil per unit area. At the 2 highest densities, herb harvested at a stage when oil contained 45% free menthol resulted in maximum oil yield and optimum oil quality. Delaying harvest once the above stage had been reached resulted in increased levels of menthol but at the expense of increased levels of menthofuran and decreased oil yields. As the growing season progressed, menthol and menthyl acetate contents of oil increased while menthone decreased. This effect was accelerated at the high plant densities.**

Timing of harvest is of critical importance for both yield and quality of oil extracted from *Mentha piperita* (6). A desirable time to harvest might coincide with maximum oil quality and oil yield per unit area but in practice these may be in conflict.

Numerous workers have found that for optimum oil and menthol contents, plants should be harvested at the full bloom stage (1, 3, 9, 10). Ellis and Gaylord (2) considered such a method unreliable and too dependent on environmental conditions. They found that the oil content of plants increased to a stage at which the oil contained 45% free menthol. If plants continued growing, the yield of oil per plant decreased. This decrease was accompanied by an initial increase in free menthol followed by a decrease in free menthol. Within 10 to 15 days the decrease in oil yield amounted to 30% of the total oil yield. An increase of similar magnitude was found to occur in the period which preceded the time of optimum harvest.

Compositional changes in peppermint oil occur with maturity and these changes are important determinants of oil quality. Maturation of peppermint oil is associated with increased levels of menthol, isomenthol and menthyl acetate, and decreased levels of menthone and isomenthone (6, 7).

Few references appear in the literature on the effect of plant density on the yield or quality of peppermint oil. Guenther (4) considered it advisable to produce bushy plants, since most oil is produced in leaves. Reference is made to the difference in stage of maturity of row and meadow mint (4, 9). Commonly, meadow mint matures earlier than row mint.

Nelson *et al.* (8), found that oil yield was highest when the herb was allowed to grow in solid stands with no thinning or cultivation. Observations by Hamon and Zuck (5), indicated that meadow mint tended to become overcrowded resulting in loss of lower leaves, lowering the oil yield.

It is the normal cultural practice in Tasmania to plant new fields of mint in rows about 75cm apart. In the second and subsequent years an almost uniform stand of herb, commonly referred to as meadow mint is developed. As a consequence of this meadow mint habit, where plant densities of 50 to 70 plants/m^2 are common, competition for light becomes very severe towards the latter part of the growing season. This results in tall plants with leaves present only on the uppermost portion of the stem.

The following experimental work is concerned with the effect of cutting date and plant density on quality and yield of Tasmanian peppermint oil, grown under commercial conditions.

Materials and Methods

Experiment 1. This experiment consisted of a 4 x 4 factorial, arranged in 3 randomized complete blocks. Each block consisted of 16 plots which were 3 x 3m. The trial was located at

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