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MECHANISM OF MONOTERPENE VOLATILIZATION IN *SALVIA MELLIFERA*

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Abstract—Monoterpene volatilization in *Salvia mellifera* is primarily dependent on the vapor pressures of the terpenes as they are influenced by temperature, the humidity of the air surrounding the leaf and the surface area of oil present on the leaf.

INTRODUCTION

Plants synthesize organic compounds which are subsequently released into the atmosphere [1–4] and foliar emissions which have been identified include the hemiterpene isoprene and several monoterpenes. The physiology of terpene production has been extensively studied but the mechanism of terpene volatilization is still largely a matter of speculation. Rasmussen and Went [4] observed a correlation between air temperature and levels of atmospheric organics, and Rasmussen [3] noted that terpene volatilization by several tree species was temperature dependent. The objective of our present study was to examine the mechanism of monoterpene volatilization in *Salvia mellifera* and its relationship to the metabolic activities of the plant.

RESULTS

The steady state rate of camphor volatilization from the *Salvia* sample increased with temperature (Fig. 1) although photosynthetic rates reached a maximum at 20° [1]. Camphor volatilization was not light dependent. At a constant temperature the volatilization rate was proportional to humidity but not proportional to transpiration rate (Table 1). The effect of humidity was also examined using an excised branch. The camphor volatilization rate in dry and humid air was measured once after 24 hr and again after 8 days. The branch had ceased CO_2 fixation and transpiration prior to the 24 hr measurements. Although the camphor volatilization rate for the 24 hr excised branch was higher than that for the intact branch or the 8 day excised branch, the rate was proportional to humidity in all cases (Table 1).

The steady state rate of camphor volatilization by *S. mellifera* was determined in the light after

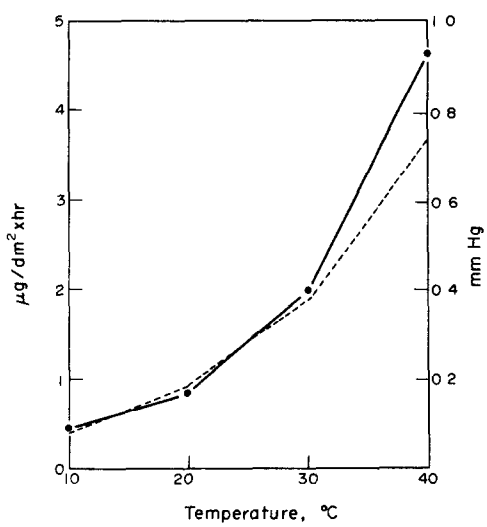

Fig 1 Temperature curve for camphor volatilization by *S mellifera* (solid line) and for camphor vapor pressure (broken line)

Table 1 Camphor volatilization rates from *Salvia mellifera* in $\mu g/dm^2$ hr at 40°

Sample	High humidity ($DP = 22°$)	Low humidity ($DP = 0°$)	High humidity ($DP = 22°$)
Live branch	5 8 (2 4)	3 1 (7 5)	5 7 (2 1)
Excised branch (after 1 day)	13 1 (0)	7 4 (0)	11 9 (0)
Excised branch (*after 8 days*)	6 3 (0)	3 2 (0)	6 1 (0)

Transpiration rates in g H_2O/dm^2 hr are given in parentheses
DP = dew point (°)

12 hr pre-treatment in the dark at 10° and 40°. The plant exhibited a higher steady state volatilization rate when pretreated at a low night temperature (LNT) than at a high night temperature (HNT) (Table 2). Only the 40° LNT volatilization rate showed significant variability which was due to the continuous decline in the LNT volatilization rate to a level approaching that of the HNT steady state volatilization rate. The effect of light was to increase the volatilization rate for the first 30 min and after the initial increase, the rate reached a steady state which was equal to the initial dark rate.

DISCUSSION

Our results indicate that the volatilization rate of monoterpenes in *S. mellifera* is dependent on the vapor pressures of the terpenes. The steady state volatilization rate of camphor and the calculated camphor vapor pressure are proportional to the temperature (Fig. 1). These data, together with the observation that camphor volatilization is not light dependent, indicate that terpene volatilization in *S. mellifera* is not directly dependent on the photosynthetic activity of the plant.

A second factor which affects the monoterpene volatilization rate in *S. mellifera* is humidity. An increase in volatilization rate when the air was changed from dry to humid was observed in both intact, physiologically active branches and excised, physiologically inactive branches (Table 1). Camphor volatilization is not dependent on transpiration but humidity does affect volatilization by a mechanism which may be strictly physical since its effects are observed in both physiologically active and inactive tissue. The high rates observed in the branch which had been excised 24 hr prior to measurement is probably due to breakdown of the structural integrity of the leaf.

A third factor which affects monoterpene volatilization rates in *S. mellifera* is the amount of oil present on the surface of the leaf. The effect of this factor is demonstrated by the higher volatilization rate of the LNT samples since the HNT regime would be expected to cause high volatilization rates and deplete the surface oil available.

The independence of camphor volatilization and stomatal opening indicates that terpenes are volatilized from the surface of the leaf rather than from its interior. This observation differs from the proposal that terpene volatilization from leaves of conifers occurs via the normal gas exchange pathways [3,5]. Scanning electron micrographs show that the upper surface of *S. mellifera* leaves are covered with glandular structures of two types, these are similar to those found on *Mentha piperita* [6,7]. One consists of a spherical, cuticle-bound structure containing material which is presumably terpene. The other is a three-celled structure with material at the apex. This material resembled an oil droplet when examined under a dissecting microscope. Thus there appears to be a significant surface area of oil on the upper side of the leaf from which the terpenes volatilize.

The HNT and LNT results are consistent with the report of increased terpene accumulation in cool night plants when compared with warm night plants [8]. Loomis and Croteau [8] proposed that these differences were due to the greater availability of photosynthate under cool night conditions. The observed differences in volatilization rates at high and low temperatures could also account for the reported pattern. The volatilization rates for both HNT and LNT experiments converge toward a similar value as the amount of oil on the leaf of the LNT pretreated plant decreases and approaches that of the HNT.

Table 2 Camphor volatilization rates (μg from *Salvia mellifera* dm^2 hr) after HNT and LNT pretreatment*

Pretreatment	20°	30°	40°
HNT	0 40 (0 30–0 50)	1 10 (1 05–1 15)	4 50 (4 30–4 80)
LNT	1 35 (1 25–1 45)	2 20 (2 05–2 35)	5 25 (4 00–6 35)

* The branch was placed in the cuvette under either HNT (40°) or LNT (10°) conditions in the dark at least 12 hr before sample collection. After this pretreatment the cuvette was adjusted to the sampling temperature, the light was turned on and the steady state volatilization rate was determined

pretreated plant. This rapid depletion is apparent only at 40° where the volatilization rate is high. This value may represent either the rate of terpene synthesis or the rate at which terpenes are added to the external pool.

An explanation of the light induced increase in the volatilization rate from HNT pretreated branches comes from an examination of available information on the physiology and biosynthesis of monoterpenes. It is proposed that terpene synthesis occurs in oil glands which are isolated from the rest of the plant [9]. These structures, because of their isolation, may be somewhat energy deficient and rely on exogenous energy sources for active synthesis [10]. Loomis and Croteau [8] suggested that *in vivo* biosynthesis of lower terpenes might be directly influenced by the presence of sucrose or equivalent products of photosynthesis, which might in turn be controlled by the balance between photosynthesis and the utilization of photosynthate. The sudden illumination in the experimental procedure might result in a short term accumulation of photosynthate that could be utilized by the terpene synthetic systems in the oil glands

EXPERIMENTAL

Net photosynthesis (under saturating light) and dark respiration of potted plants of California black sage, *Salvia mellifera* Greene, were measured in an intact branch using a gas analysis system like that previously described [11] The branch was contained within a cuvette where temperature and humidity were controlled Light could be excluded by placing a dark cloth over the cuvette The volatile constituents in the air flowing out of the gas exchange cuvette were trapped in stainless steel loops which were packed with Pyrex wool and immersed in liquid N_2 A cryogenic pump was used to maintain a flow of 1 l./min through the loops. Organic volatiles were separated from the H_2O which accompanied them and analyzed qualitatively by GC–MS [12] GLC was used for quantitative analysis, Only the major volatile component, camphor, was measured quantitatively in this study The only other major component was 1,8-cineol (25%) whose levels correlated closely with those of camphor (50%). The remaining terpene hydrocarbons (α-pinene, β-pinene, camphene, limonene, cymene, myrcene, β-phellandrene and γ-terpinene) were difficult to quantitate and were not monitored

The steady state rate of camphor volatilization was determined at a given temp by placing a branch in the cuvette and taking samples at 30–60 min intervals for a period of 3 hr After 1 hr of equilibration the values obtained showed little variation and they were averaged to give the volatilization rate for that temp Volatilization rates were determined at 10, 20, 30 and 40° The effect of transpiration on the camphor volatilization rate was determined by altering the transpiration rates and measuring the release of camphor while the temp was kept constant Transpiration was altered by changing the humidity in the cuvette

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