EFFECTS OF SOME ESSENTIAL OIL VAPORS ON THE STOMATA OF EUPATORIUM AND MENTHA[1]

M. G. A. HAFEZ

DEPARTMENT OF BOTANY, A'IN SHAMS UNIVERSITY, ABBASSIA, CAIRO

Following Tyndall's observations in 1862 (8) on the great absorptive power shown by certain essential oils of plants for thermal radiations, a number of theories have been put forward concerning the possible function of such oils in the water relations of plants secreting them.

Detto (2) tested the effect of menthol vapor on the rate of water loss from twigs of Syringa. He found that the rate was depressed, and that this was accompanied by the death of a large area of leaf surface. He also tested the effects of other oils on other plants, and came to the conclusion that essential oils played no part in regulating water loss from plants.

Dixon (3) found that the vapors given off from leaves of *Artemisia Absinthium* brought about a reduction in the transpiration rate of leafy branches of Syringa and Cytisus. He was of the opinion that such oil vapors retarded the rate of water loss either by depressing the activity of the protoplasm or by retarding the rate of diffusion of water vapor from the mesophyll cells.

The author (4) compared the effects on transpiration by some plants and evaporation from a moist filter paper of eucalyptus, peppermint and lemon oil vapors. These were found to bring about a slight depression in the rate of evaporation, but a more pronounced decrease in the rate of transpiration.

The author (5) also tested the effects of lemon, eucalyptus, peppermint and citronella oil vapors on water loss from atmometers. Vapors of fresh samples of all these oils materially reduced the rate of water loss. After a short time, however, these oils decreased in effectiveness, apparently because the more volatile fractions have been evaporated. It was suggested that essential oil vapors might cause surface tension effects leading to a reduction in the rate of evaporation.

Audus and Cheatham (1) tested the effects of some essential oil vapors on the transpiration rate, taking the rate of water absorption by cut shoots of cherry laurel as a direct measure of transpiration. On exposure of shoots to air saturated with oil vapors, they failed to detect any measurable effect on the absorption rate. The authors, however, reported a small depression which they regarded as presumably due to a toxic action of the vapor. They concluded that essential oils play no part in regulating water loss.

Again, in view of the conflicting reports and conclusions concerning the effects of essential oils on water relations of plants, the author (6) reinvestigated the effects of eucalyptus, lemon, peppermint and citronella oil vapors simultaneously on transpiration and absorption by plants. On applying these oil vapors to cut branches of Mentha and Eupatorium—one plant which did and one which did not produce essential oils themselves—the transpiration and absorption rates were significantly depressed.

On the grounds of the observation that the depressions were, however, greater in transpiration by plants (6) than in water loss by atmometers (5), it was suggested that oil vapors might have, in addition to their physical effects on evaporation of water, a possible effect on the stomatal mechanism, and that such effect would in turn affect transpiration.

The present investigation is an attempt to test this suggestion by studying the effects of the same oil vapors on the stomata of the same species of plants used in the foregoing studies (6).

MATERIAL, METHOD AND EXPERIMENTAL RESULTS

In the following experiments, two glass porometer cups of 12.5 and 7 mm internal diameters respectively were used with *Eupatorium granulosum* and *Mentha spicata* leaves. The cups were of the thistle funnel shape (fig 1), the mouth being surrounded by a rubber tube. Immediately behind the tube was inserted a side-tube which served as an inlet for a steady current of dry air. The air within the cup was therefore not subject to changes in humidity or in oxygen or carbon dioxide tensions. In order to keep the air passing evenly over the leaf surface without dead spaces, the inside nozzle of the side-tube was drawn out and bent towards the mouth of the cup.

Air was drawn through the leaf into the porometer cup by connecting the free end of the latter to a head of water in a narrow-bore horizontal tube with a short vertical arm dipping into a reservoir of cooled boiled water. Thus a constant pressure difference of 7.5 cm of water was obtained throughout the present

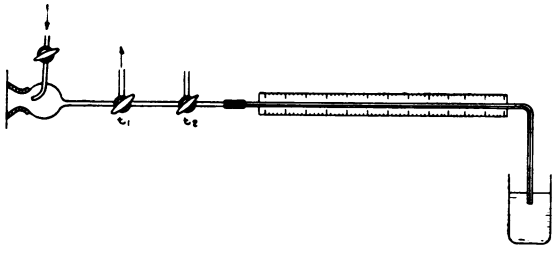

FIG. 1. Porometer cup, see description in text.

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TABLE I

HALF-HOURLY MEANS OF 5-MINUTE POROMETER READINGS SHOWN BY EUPATORIUM GRANULOSUM WITHIN 1ST AND 2ND HALF-HOUR EXPOSURES TO OIL VAPORS, EXPRESSED AS PERCENTAGES OF INITIAL RATES JUST BEFORE TREATMENTS *

EXPERIMENT NO.	OIL CONC MG/L	FLOW RATE WITHIN 1ST ½ HR	FLOW RATE WITHIN 2ND ½ HR	EXPERIMENT NO.	OIL CONC MG/L	FLOW RATE WITHIN 1ST ½ HR	FLOW RATE WITHIN 2ND ½ HR
	Eucalyptus				*Lemon*		
1	12.0	49.0	42.8	5	10.6 (F) **	53.2	41.7
2	15.0 (F) **	62.8	56.2	6	7.6	73.0	41.6
3	10.5	59.5	....	7	8.4	35.0	....
4	12.5	61.0	....				
Totals	...	232.3	99.0	..	...	161.2	83.3
Means	...	58.1	49.5	..	...	53.7	41.7
% Depression	...	41.9	50.5	..	...	46.3	58.3
	Peppermint				*Citronella*		
8	7.7	61.0	55.0	12	15.3 (F) **	42.8	61.2
9	2.0 (F) **	80.5	....	13	5.9	82.5	65.0
10	11.5 (F) **	41.5	60.2				
11	6.1	56.0	....				
Totals	...	239.0	115.2	..	...	125.3	126.2
Means	...	59.8	57.6	..	...	62.7	63.1
% Depression	...	40.2	42.4	..	...	37.3	36.9

* Data from 13 experiments.
** (F) = A fresh sample of the oil is applied.

series of experiments. The rate of flow of the air was measured by the rate of movement of the water meniscus, and this rate was taken as an indication of the degree of opening of the stomata.

Two three-way taps were inserted between the cup and the horizontal capillary tube. One of these (t_2) served to place the capillary tube in communication either with the cup (while a porometer reading was to be taken) or with the exterior (as when the water column in the capillary tube needed to be sucked back to its original position after readings). By means of the other tap (t_1), the porometer cup could

TABLE II

HALF-HOURLY MEANS OF 5-MINUTE POROMETER READINGS SHOWN BY MENTHA SPICATA WITHIN 1ST AND 2ND HALF-HOUR EXPOSURES TO OIL VAPORS, EXPRESSED AS PERCENTAGES OF INITIAL RATES JUST BEFORE TREATMENTS *

EXPERIMENT NO.	OIL CONC MG/L	FLOW RATE WITHIN 1ST ½ HR	FLOW RATE WITHIN 2ND ½ HR	EXPERIMENT NO.	OIL CONC MG/L	FLOW RATE WITHIN 1ST ½ HR	FLOW RATE WITHIN 2ND ½ HR
	Peppermint				*Eucalyptus*		
14	4.8	96.3	91.4	17	13.2	99.7	56.0
15	10.1 (F) **	72.6	73.0	18	12.0	65.5	61.5
16	4.7	83.0	47.2	19	14.4 (F) **	41.2	49.3
Totals	...	251.9	211.6	..	...	206.4	166.8
Means	...	84.0	70.5	..	...	68.8	55.6
% Depression	...	16.0	29.5	..	...	31.2	44.4
	Lemon				*Citronella*		
20	15.6	35.2	39.1	25	12.4	31.7	....
21	16.3	52.0	....	26	13.5 (F) **	68.0	56.0
22	13.2 (F) **	111.2	111.4	27	5.5	97.3	79.0
23	8.1	62.5	....				
24	9.1 (F) **	70.5	31.2				
Totals	...	331.4	181.7	..	...	197.0	135.0
Means	...	66.3	60.6	..	...	65.7	67.5
% Depression	...	33.7	39.4	..	...	34.3	32.5

* Data from 14 experiments.
** (F) = A fresh sample of the oil is applied.

be introduced into the air-sweep circuit between readings.

By a suitable arrangement, the circulating dry air could be charged, at will, with a known weight of the oil vapor tested.

In conducting an experiment, the lower surface of a selected leaf detached under water and with its petiole dipping in water was attached to the porometer cup with rubber grease. The leaf was held in position by merely pressing it lightly against the greased edge. Dry air was circulated through the cup at a rate of four liters per hour. After a period of time, with the leaf in the diffuse daylight of the laboratory, the rate of flow of water meniscus in the porometer tube was measured at 5-minute intervals. For a state of equilibrium to be reached before taking a reading, 20 seconds were allowed to elapse after placing the column of water in communication with the porometer cup. The time required for the meniscus to move 1 cm was then determined. The air current was thus interrupted for periods of roughly one half minute while the porometer readings were taken. The leaf was next illuminated by a 500-watt lamp with the filament about 30 cm from the leaf. A cooling screen of water, changed every 15 minutes, was placed between the lamp and the leaf in order to cut off the greater part of the heat. The light intensity at the leaf surface, as measured by a photo-electric meter, was found to be 1200 ft-c.

When the new porometer readings seemed to have

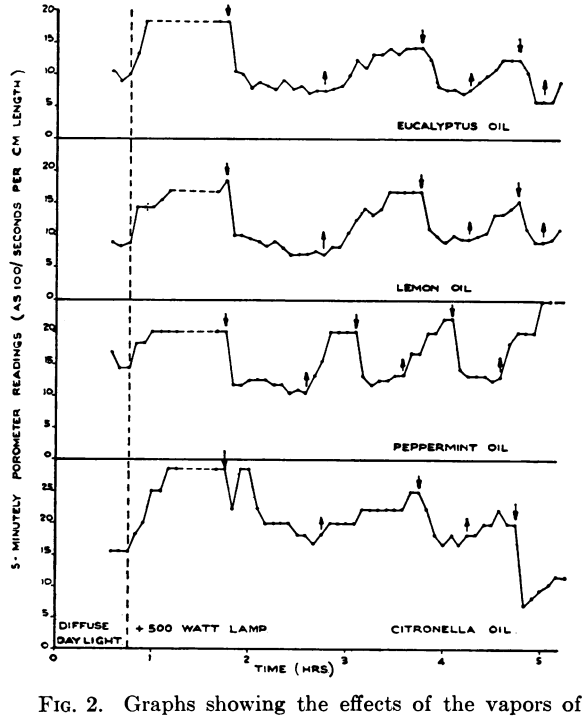

FIG. 2. Graphs showing the effects of the vapors of the four different oils upon the stomata of *Eupatorium granulosum*. (Data from experiments 1, 5, 8 and 13.) N.B. ↓ = Oil vapor on; ↑ = Oil vapor off.

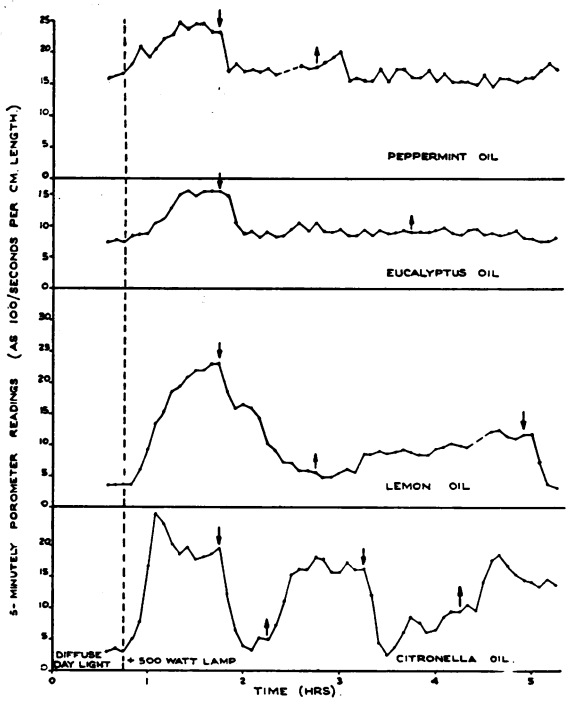

FIG. 3. Graphs showing the effects of the vapors of the four different oils upon the stomata of *Mentha spicata*. (Data from experiments 15, 18, 24 and 25.)

attained a reasonably steady value, the circulating air current was charged with oil vapor at a known concentration. After a further period of time during which 5-minute readings were taken, the oil vapor was cut off and pure air again allowed to circulate through the cup.

The temperature in the immediate vicinity of the leaf was determined by a thermometer placed very close to it.

Tables I and II show sets of half-hourly mean flow rates, as percentages of initial reference rates immediately before applying oil vapors, of 5-minutely porometer readings within the 1st and 2nd half-hour exposures of Eupatorium and Mentha respectively to eucalyptus, lemon, peppermint and citronella oil vapors.

To illustrate the action of each vapor on each plant representative experiments have been chosen and shown in graphical form in figures 2 and 3. In these figures, the individual 5-minute readings, rather than their respective half-hourly mean rates, of the representative experiments are plotted against time. Porometer readings taken at 5-minute intervals give rates of flow of meniscus in the porometer tube in arbitrary units as 100/seconds per cm length. Broken lines indicate that some readings were not taken. Allowance was made for this in calculating half-hourly mean rates.

Preliminary experiments on Eupatorium and Mentha in the absence of any oil treatment have shown

that the drift of porometer readings with time within the period of an experiment was not significant at the 5 % probability level.

Considering the data of tables I and II, it is evident that the stomata of Eupatorium and Mentha leaves are responsive to the application of the vapors of all four oils tested, the oil treatment bringing about, in each case, a distinct closing effect.

In the case of Eupatorium, a one hour exposure of the leaf to eucalyptus or lemon vapor resulted in a reduction of the stomatal pores to as little as one half or even less of their original area. The vapor effects were fully exercised even upon exposure to the lowest concentrations, obtained by merely passing the air over the oil surface (table I, experiments 3 and 6). The treatment with peppermint and citronella oils also brought about similar, though relatively lesser, closing effects. These could be detected even upon exposure to as low a concentration as 2 mg of vapor per liter of circulating air, obtained, in experiment 9, by passing the air over only one drop of peppermint oil.

In the case of Mentha, the stomata again showed the usual closing effect upon exposure to any oil vapor, the closing response being most with eucalyptus and lemon, then citronella and peppermint least. In experiment 22, the treatment with a relatively high vapor concentration (13.2 mg/liter) of a fresh sample of lemon oil gave peculiar results. The stomata immediately closed but within 10 minutes reopened to slightly more than their original areas, the apertures then remaining constant throughout the rest of the period of the one hour treatment. On cutting off the vapor, a slight still further opening of the stomata occurred followed after two hours by a gradual closing movement. In experiment 21, with still higher vapor concentration (16.3 mg/liter) but with a used sample of the same oil applied for only one half hour, a distinct closing response occurred, followed by a partial recovery upon cutting off the vapor. But, after 1.5 hours, on reapplying the vapor for another half an hour, an appreciable further opening rather than closing of the stomata occurred. It is probable that such effects are due to a toxic action of the vapor.

Concerning the post-treatment effects, the two plants behaved differently. In the case of Eupatorium, stomatal recovery of the oil-treated leaf usually started immediately on cutting off the vapor and was, in many cases, eventually almost complete. On the other hand, in the case of Mentha the closing response due to applied vapors was usually maintained either indefinitely or for a time after the vapors were cut off. This implies that a lasting or a temporary effect of oil treatment may occur. Recovery was in no case complete.

In order to test the significance of the observed responses to oil treatments, it seemed that, owing to the differences between the half-hourly means of each set (tables I and II), the best estimate of the population variance is that based on the "residual" sum of squares of deviations, i.e., the deviations of the individual (5-minute) observations about their respective (half-hourly) means. The t test of significance was applied to the following differences: (a) The difference between the grand mean of the 1st half-hour observations and the mean of all half-hourly means (adjusted to 100) just before applying the oil vapor. (b) The difference between the grand mean of the 2nd half-hour observations and the mean (= 100) just before treatment.

The conclusion was that the observed depressions in the rates of air flow through the stomata of Eupatorium and Mentha leaves within the 1st and 2nd half-hour treatments with vapors of any of the four oils tested are highly significant at the 1 % probability level. They cannot, therefore, be ascribed to chance or experimental error.

As far as any generalization can be made with reference to these experiments only, it would appear that the stomata of the plant producing essential oil itself (Mentha) are less affected by treatment with oil vapors than those of the plant which does not produce essential oil (Eupatorium). Moreover, the stomata of the plant producing an essential oil (Mentha) are less affected by treatment with its own oil vapor than by treatment with the vapors of oils produced by other plants.

The nature of the closing response of stomata to oil treatments is obscure, but these responses, at least as far as relatively high concentrations of the oil vapors are concerned, might be due to toxic action of the vapor on the leaf cells. No visible change of color was observed in the present series of experiments, but the earlier observations made by the author (4, 6), Detto (2), and Audus and Cheetham (1) may be recalled. All these authors reported that vapors of certain essential oils, when applied in high concentrations to plants, exercise harmful effects, including discoloration, drop of turgor and death of large areas of treated leaves. On the grounds of these observations, there is reason to suggest that the closing responses and failure, under certain conditions, to recover might be due to alterations in the turger pressure of the guard cells resulting in turn from toxic effects of the vapors on the guard cell membranes.

These findings on the responses of stomata to oil treatments are, however, not in line with the general conclusions arrived at by Audus and Cheetham (1). Nevertheless, these authors reported a slight closing of stomata on exposure of a cherry laurel leaf to the saturated vapor of thyme oil. Except for one experiment with rosemary oil, which also showed a transitory closing of the stomata, all the other experiments with rosemary and anise oil vapors failed to elicit any stomatal response. The reason for this failure seems likely to be due to toxic action on the leaf cells following prolonged exposures to saturated oil vapors. Reference should be made here to the abnormal results of experiments 21 and 22 (table II) of the present work. In these experiments, a one hour exposure to relatively high concentrations of

lemon vapor resulted in slight further opening of the stomata of Mentha rather than the usual closing response following brief exposure or exposure to relatively lower concentrations of the same oil. Moreover, the experiments of Audus and Cheetham are seemingly examples of stomata in a confined space, where their behavior cannot be regarded as normal. It has been shown (Williams (9); Heath and Williams (7)), that the stomata of illuminated leaves open much wider than elsewhere if the leaf surface is enclosed in a small volume of still air, as in the classical type of porometer cup used by these authors.

Summary

1) Experiments were performed to investigate the effects of eucalyptus, lemon, peppermint and citronella oil vapors upon the stomata of Eupatorium and Mentha.

2) On charging the air current circulating through the porometer cup with oil vapors, significant closing responses by the stomata of both plants could be detected.

3) Presumably the closing responses, at least as far as relatively high vapor concentrations are concerned, may be due to toxic action of the vapors on leaf cells.

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EFFECTS OF ROSEMARY AND THYME OIL VAPORS ON THE STOMATA OF CHERRY LAUREL[1]

M. G. A. HAFEZ

Department of Botany, A'in Shams University, Abbassia, Cairo

The possible effects of essential oil vapors upon stomata were first investigated by Audus and Cheetham (1), and later by the author (2). Audus and Cheetham tested the effects of rosemary, thyme and anise oil vapors on the stomata of cherry laurel leaves, measuring their resistance to mass flow of air within an ordinary permanently attached porometer cup. They reported a slight closing of the stomata on exposure to thyme oil, but failed to detect any stomatal response to anise and rosemary vapors. Their conclusion was that the vapors of all these oils had no significant effects on the stomata of cherry laurel.

Using a modified type of porometer cup through which air was kept circulating, the author (2) studied the effects of eucalyptus, lemon, peppermint and citronella oil vapors upon the stomata of *Eupatorium granulosum* and *Metha spicata,* Linn. The rates of air flow through the stomata of both plants were shown to be significantly depressed on charging the air stream with oil vapors. This depression was a clear indication of a closing stomatal response following oil treatments.

There existed, however, the possibility that lack of accord between the general conclusions arrived at separately by Audus and Cheetham and the author was due to a difference in the technique by which the results of both were obtained. It is now known that the behavior of stomata within the usual form of porometer cup used by Audus and Cheetham cannot be regarded as normal (Heath and Williams (3)). Consequently it was decided to reinvestigate the effect of some of these oil vapors on the same plant used by these workers, but employing modified forms of porometer cups.

Material and Method

All experiments were carried out on leaves of cherry laurel (*Prunus laurocerasus* Linn.) leaves. A selected leaf was fitted to a water supply and illuminated by an electric lamp, the light intensity at its surface being 1200 ft-c. The oils tested were rosemary and thyme. Two forms of porometer cups, the mouth in both being 7 cm internal diameter, were used for measuring the rate of flow of air through stomata. One of these was the sweep-type previously used and described by the author (2), and the other was a new slightly modified form of it (fig 1). The cups were permanently attached to the leaf and swept, either periodically or throughout the whole period of experiment, by a stream of dry air circulating at a rate of four liters per hour. The air cur-

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