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# Effect of Water Stress on Ethylene Production by Detached Leaves of Valencia Orange (*Citrus sinensis* Osbeck)

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## ABSTRACT

**Detached leaves of Valencia oranges, *Citrus sinensis* Osbeck, emanated ethylene at markedly higher rates and contained more endogenous ethylene when placed under water stress at 55% relative humidity than when placed in water-saturated air. Water stress induced defoliation from detached branches. Relieving the water stress of such leaves by transfer to a mist chamber resulted in lowering of the rates of ethylene emanation to the level occurring in leaves maintained continuously in a mist chamber. This ability to recover from the water stress was evident for only up to 10 to 20 hours of stress, when the relative turgidity of the leaves was 50 to 60%. Beyond that time the level of ethylene emanation of stressed leaves was not lowered by rehydration in a mist chamber; these overstressed leaves could not reabsorb their original water content. Ethylene emanation was in high correlation with the relative turgidity of detached leaves of oranges.**

Water stress is known to affect the hormonal status of various plant organs (9). Itai and Vaadia (6) reported that a short period of water stress, induced by enhanced evaporative demands, caused the reduction of about half of the cytokinin activity of root exudate as well as a smaller reduction in the leaves themselves. They concluded that the biosynthesis of cytokinin in the root ceased when water tension in the leaf was enhanced. The cytokinin activity was partly restored after an additional 18 hr in a humid chamber. Several authors (12, 14) showed that levels of ABA increased in wilted leaves, the increase being related to degree of wilting. Recently, McMichael *et al.* (11) showed that a brief period of water deficit of potted cotton plants enhanced the ethylene emanation of intact petioles. Jordan *et al.* (7) and Lipe and Morgan (8) showed that this enhanced ethylene is involved in the enhanced defoliation induced by water stress.

The present paper reports that water stress raises markedly the rates of both ethylene emanation and endogenous ethylene, and that after relief of this stress the ethylene level returns to the original level.

## MATERIALS AND METHODS

Branches and leaves of Valencia orange, *Citrus sinensis* Osbeck, were detached in various orchards in Israel. The branches were immersed in water immediately after detachment and kept at 20 C and a relative humidity of 55 ± 5% or in a continuous flow of water-saturated air. The leaves were kept at 20 C under one of the following conditions: (*a*) at 55% relative humidity to bring about water stress; (*b*) in a mist chamber with water-saturated air; or (*c*) in a continuous flow of water-saturated air. Some of the experiments were carried out in the light and others in the dark; light did not affect the response to water stress.

Ethylene emanation was measured with a Packard gas chromatograph equipped with a 2-m × 6-mm activated Alumina column and a flame ionization detector. In order to measure ethylene emanation, the leaves were enclosed in sealed jars of different volumes or kept in jars continuously ventilated with water-saturated air, as previously described by Ben-Yehoshua and Eaks (4). Before measurements were taken, the leaves maintained in the mist chamber were dried gently with Whatman filter paper.

Endogenous ethylene was determined using the method of withdrawing the gas by vacuum. The method was adapted from our colleague, Mr. I. Adato. Two leaves, together weighing about 2 g, were placed in 34-ml vials sealed with a serum cap. Two 50-ml syringes were inserted into this cap. Four ml of air were added to the vial so that when a gas sample was taken later, no vacuum would be produced in the sampling syringe. The plungers were rapidly pulled to extend the volume of the vials from 34 to 134 ml. This change in volume reduced the pressure to 0.21 atm and extracted the internal atm of these leaves. After 5 min of vacuum, the plungers were returned to their original position and the ethylene content of the vial was immediately determined. This ethylene represented both the endogenous ethylene which was withdrawn from the leaf and the ethylene emanated during the period of measurement. Results were satisfactory for comparing the endogenous ethylene of leaves under water stress with that of nonstressed leaves.

Water stress was measured by determining the relative turgidity of the leaves (2). Fully turgid weight of leaves was measured by weighing 20 individual leaves placed in a mist chamber with their petioles in a beaker filled with water. Maximal weight was reached about 4 hr after introduction to the mist chamber. Changes in weight of these leaves later were smaller than the experimental error. Thus, the original fully turgid weight was utilized throughout the experiment. Ethylene emanation was measured at various levels of relative turigidity both during drying and after rehydration of stressed leaves.

## RESULTS

**Effect of Water Stress on Pattern of Ethylene Emanation.** Immediately after detachment, the leaves exhibited a rise in ethylene emanation which leveled off after 2 to 4 hr. The pattern of ethylene emanation of orange leaves maintained under

conditions of relatively low humidity (55%) was then very different from those of leaves kept in a mist chamber with water-saturated air or when water-saturated air was flowing over leaves (Fig. 1). Detached leaves kept at 55% relative humidity showed a continuous marked rise in the rate of ethylene emanation up to levels 40- to 60-fold higher than the original one. Leaves in the mist chamber increased in weight for a period of several hr, reaching 105 to 111% of their original fresh weight, and then lost little to no weight during a period of 50 hr. However, leaves kept at 55% relative humidity lost weight rapidly (Fig. 2), reaching 33% of their relative turgidity 50 hr after detachment.

Leaves attached to branches, the ends of which were immersed in water, responded to water stress in a pattern similar to that of detached leaves. During a period of 3 hr after detachment, leaves kept at 55% relative humidity emanated 1.94 ± 0.03 μl/kg·hr and branches emanated 0.35 ± 0.02 μl/kg·hr. Under these conditions the leaves lost 0.85 ± 0.08% and the branches 0.48 ± 0.03% of their fresh weight. After 2 days at 55% relative humidity, leaves still on branches emanated 5 to 15 μl/kg·hr and detached leaves emanated 30 to 50 μl/kg·hr.

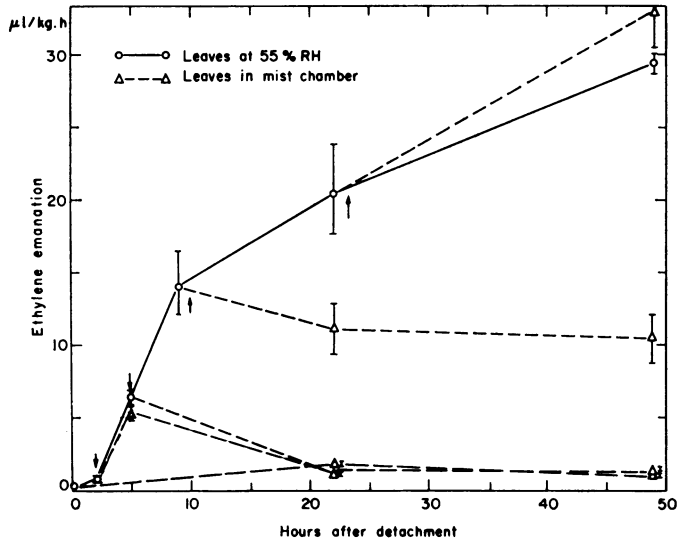


FIG. 1. Effect of water stress and rehydration upon ethylene emanation of Valencia orange leaves. Water stress was achieved by placing detached leaves at 55% relative humidity. Rehydration and no stress conditions were achieved by placing the leaves in a mist chamber. Arrows indicate the transfer of leaves from 55% relative humidity to the mist chamber. These curves represent one experiment out of five. Leaves under a flow of water-saturated air behaved the same as leaves kept in a mist chamber.

Water stress induced abscission of most leaves from detached branches kept at 55% relative humidity, within the first week after detachment. Leaves that did not abscise within a week seemed to dry out before abscission took place. In water-saturated air, little to no abscission occurred during a period of over a month.

**Relief of Water Stress.** The marked difference in ethylene emanation between leaves under conditions of high and low humidity was related to water stress. Accordingly, the effect of relieving this water stress was investigated by placing the stressed leaves in a mist chamber. Figure 1 shows that such rehydration affected greatly the rates of ethylene emanation. The rise in ethylene emanation evoked by water stress did not stop immediately upon transfer to the mist chamber. Leaves that were 2 hr at 55% relative humidity and then transferred to the mist chamber showed, after 3 more hr in the mist chamber, much higher levels of ethylene emanation. However, after 20 hr in the mist chamber, the rate of ethylene emanation of these stressed leaves declined to the level of the leaves which remained continuously in the mist chamber. The effect of rehydration on ethylene emanation depended also on the length of the water stress period. After 9 hr at 55% relative humidity, ethylene emanation following rehydration declined to a level intermediate between that of leaves in the mist chamber and that of detached leaves at 55% relative humidity. After 22 hr at 55% relative humidity, rehydration did not affect the leaves further.

Figure 2 shows also that the amount of water absorbed by the stressed leaves depended upon the duration of the stress at 55% relative humidity. After 2 hr of stress, leaves that were placed in the mist chamber absorbed enough moisture to attain 100% of their relative turgidity, similar to leaves placed in the mist chamber immediately after being detached. However, leaves that were stressed for a longer period at 55% relative humidity did not recover their original turgidity. This lack of ability to absorb water is more marked after 20 hr of stress (Fig. 2).

A very high negative correlation was obtained between the relative turgidity of leaves and the rates of ethylene emanation.

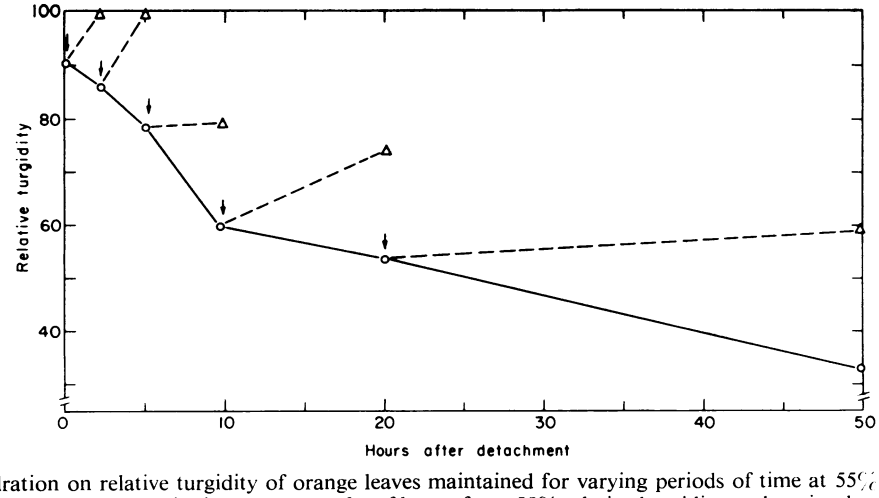


FIG. 2. Effect of rehydration on relative turgidity of orange leaves maintained for varying periods of time at 55% relative humidity. The SE of measurements were 0.5 to 3.0. The arrow indicates the transfer of leaves from 55% relative humidity to the mist chamber. Symbols are the same as in Figure 1.

Table I. *Effect of Water Stress on Ethylene Emanation and Endogenous Ethylene of Orange Leaves*

Values represent one typical experiment out of three with five replicates in each treatment.

| Treatment | Relative Turgidity | Endogenous Ethylene | Rate of Ethylene Emanation |
|---|---|---|---|
| | % | μl/kg fresh wt | μl/kg·hr |
| Immediately after detachment | | 1.504 ± 0.088 | 0.814 ± 0.047 |
| 24 hr at 55% relative humidity | 57.5 | 2.225 ± 0.824 | 2.581 ± 0.526 |
| 24 hr at 100% relative humidity | 100 | 0.282 ± 0.031 | 0.055 ± 0.034 |

The r in the range of relative turgidity down to 50% was 0.93, 0.83, and 0.97 in 3 experiments.

**Endogenous Ethylene and Water Stress.** As water stress might affect the resistance of plant tissue to gas diffusion (3), the mere measurement of ethylene emanation is not adequate and determination of endogenous ethylene is desirable. Table I shows that water stress raised the ethylene level endogenously as well as its rate of emanation.

## DISCUSSION

Water stress promoted markedly the ethylene emanation of orange leaves. The high correlation found between rates of ethylene emanation and the relative turgidity of detached leaves, confirms and extends the findings of McMichael *et al.* (11), that attached petioles of potted cotton plants emanated more ethylene after a period of brief water deficit. The fact that endogenous ethylene is affected, as well as rate of emanation, proves that the high rate of ethylene emanation is due to production and is not merely related to a rapid rate of diffusion of ethylene out of its endogenous reservoirs.

Relief of water stress by placing the stressed leaves in a mist chamber resulted in lowering of the rates of ethylene emanation to the level of nonstressed leaves. However, the capacity to recover from water stress declined as the relative turgidity or water content of the stressed leaves dropped. When a relative turgidity of 55% was reached, leaves kept emanating higher rates of ethylene even after transfer to the mist chamber. Thus, beyond a certain level of stress, the enhanced activity was irreversibly induced. Furthermore, water-stressed leaves, with time, also lost their capacity to reabsorb their full water contents. The promotive effect of water stress on ethylene production was not found in preliminary experiments with attached leaves.

The promotion of ethylene emanation and abscission of orange leaves under conditions of water stress might explain the previously unexplained "effect of ventilation" reported by Ben-Yehoshua and Eaks (see Fig. 3 of Ref. 4). Leaves and fruit which were under periodic ventilation in their report were exposed for a long period to an atmosphere containing 50% relative humidity and thus were water-stressed.

Various forms of stress are known to induce greater activity in ethylene emanation (1). However, water stress has so far been related mainly to increased levels of ABA. Recently, various reports showed that ethylene application raised the level of ABA in various tissues (5, 10, 13). Consequently, it is possible that the rise in ethylene brought about by the water stress induced, in turn, the rise in ABA.

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