

STOMATAL RESISTANCE, TRANSPIRATION, AND RELATIVE WATER CONTENT AS INFLUENCED BY SOIL MOISTURE STRESS

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SUMMARY

The effect of increasing soil moisture stress on stomatal resistance, transpiration, and relative water content of cucumber, beans and tomato has been investigated under controlled conditions.

The results demonstrated that stomatal resistance can be used as a tool by which the soil-water-plant relationship can be predicted. Transpiration rates exhibited an initial rise as the soil moisture decreased, followed by a steady reduction. No significant changes in the relative water content of the leaves were observed until a severe soil moisture stress took place.

For each species a linear relationship was obtained between the stomatal diffusive resistance (r_{sd}) and the ratio of the vapour pressure difference between leaf surface and bulk air (Δe) over transpiration (E). The external resistances to water vapour (r_a) obtained from this relationship were 7.2, 4.4 and 2.8 sec cm^{-1} for cucumber, tomato and bean leaves, respectively.

An essentially linear relationship was obtained between r_{sd} and viscous air flow. The intercellular resistance (r_i) obtained from extrapolation of the curves, were 12.0, 8.7 and 6.6 sec cm^{-1} for cucumber, bean and tomato, respectively.

The external resistance calculated from the sensible heat transfer is well below r_a values in all species.

The relatively high values of r_a and r_i have been discussed.

1. INTRODUCTION

In arid and semiarid regions water stress is considered as the most important factor of yield decrements. Extensive studies have been made on the various plant responses towards decreasing soil moisture content and on the amelioration of stress effects through various cultural processes.

It is generally accepted that water stress induces a progressive reduction in photosynthesis and transpiration (SLATYER 1967). It seems evident that this reduction in transpiration and photosynthesis can be mainly attributed to a stomatal closure, which increases the resistance in the gaseous pathway for water vapour and CO_2 (VAADIA *et al.* 1961, TROUGHTON 1969, TROUGHTON & SLATYER 1969, SLATYER 1970). At severe stresses beyond permanent wilting, the mesophyll resistance might also increase and incipient drying may occur. It should be mentioned here that cell elongation is often more sensitive to water stress than photosynthesis or transpiration.

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The water stress effect on stomatal aperture is believed to take place partly by causing changes in the internal CO_2 concentration through an effect on respiration and photosynthesis, and partly via the processes directly concerned with energy flow and guard cell turgor (SLATYER 1967).

Our aim in the present investigations is to study quantitatively the effect of increasing soil moisture stress on stomatal resistance, transpiration, and leaf water content of three vegetable species. In addition the internal resistance to vapour diffusion and the external resistance to latent heat of transpiration and sensible heat transfer were estimated from the above parameters.

2. MATERIALS AND METHODS

Plants of bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Mill.) were utilized in the present investigations. The seeds were germinated in a greenhouse under natural daylight during autumn. After emergence the seedlings were transplanted into small plastic pots (top diam. 9.0 cm, bottom diam. 6.5 cm and depth 7.5 cm) containing a mixture of garden peat and loamy soil, the pF-curve of which was determined. One plant per pot was used. The pots were placed in a climate room three days before the water stress treatments were imposed. The environmental conditions in the climate room were as follows: temperature $25 \pm 1^\circ\text{C}$, relative humidity $55 \pm 10\%$, light intensity at the plant level $2.55 \times 10^4 \text{ erg sec}^{-1} \text{ cm}^{-2}$ obtained from TL 55 fluorescent tubes during a 16 hours photoperiod. The average wind speed was approximately 5 cm sec^{-1} .

The water stress treatments were imposed by discontinuing the watering. The soil was covered with inert perlite in order to reduce evaporation. At the onset of the treatment the plant ages were 17, 21 and 46 days for bean, cucumber, and tomato, respectively.

Transpiration was measured by means of weighing the pots every 24 hours. Two pots without plants, placed under the plant cover, were used to measure soil evaporation. The relationship between evaporation and soil moisture stress was obtained and correction for transpiration was made accordingly. The stomatal resistance (at the lower surface of the leaves) was measured by a diffusion porometer which was built and calibrated as described by KANEMASU *et al.* (1969), and by an air-flow porometer connected to a sphygmo-manometer (BIERHUIZEN *et al.* 1965). In the latter case the length of time for the air pressure to drop from 250 to 150 mm Hg was measured. Leaf temperature was measured with copper-constantan thermocouples pressed against the lower surface of the leaves. In combination with air temperature and relative humidity, the leaf temperature minus the air temperature was recorded with a potentiometric multi-channel recorder. The relative water content of leaves was determined by the technique described by BARR & WEATHERLEY (1962). The leaf area was estimated with a leaf-area-meter (Technical Physical Service of Agricultural University of Wageningen 66-2014). The term "leaf area" in this paper means the area of a single surface of leaves.

Except for transpiration, all other measurements were performed on the unifoliolate leaves of bean and the third and fourth leaf from the base of cucumber and tomato, respectively. The measurements were carried out every day between 9.00–10.00 A.M. until wilting symptoms appeared. Five plants from each species were sampled for the daily measurements.

3. RESULTS

The effect of increasing soil moisture stress on stomatal diffusive resistance (sec cm^{-1}), transpiration ($\text{mg day}^{-1} \text{cm}^{-2}$), and relative water content is shown in *fig. 1*. Each point is an average of five observations. It is evident that stomatal diffusion resistance (r_{sd}) is extremely sensitive towards the reduction in soil moisture content, particularly above pF 3.0. A substantial increase in r_{sd} took place at severe water stress. For instance, r_{sd} values of 103.6 and 86.5 sec cm^{-1} at pF values near 4.0 were observed in case of bean and tomato, respectively. These points are not shown in the figure. The relative water content (RWC) of the leaves did not exhibit any apparent change until the stage of wilting. It is clear from such results that RWC is not a reliable parameter from the agronomic point of view since its response to increasing soil moisture deficit is negligible until wilting, at which abrupt reduction took place. The transpiration rate,

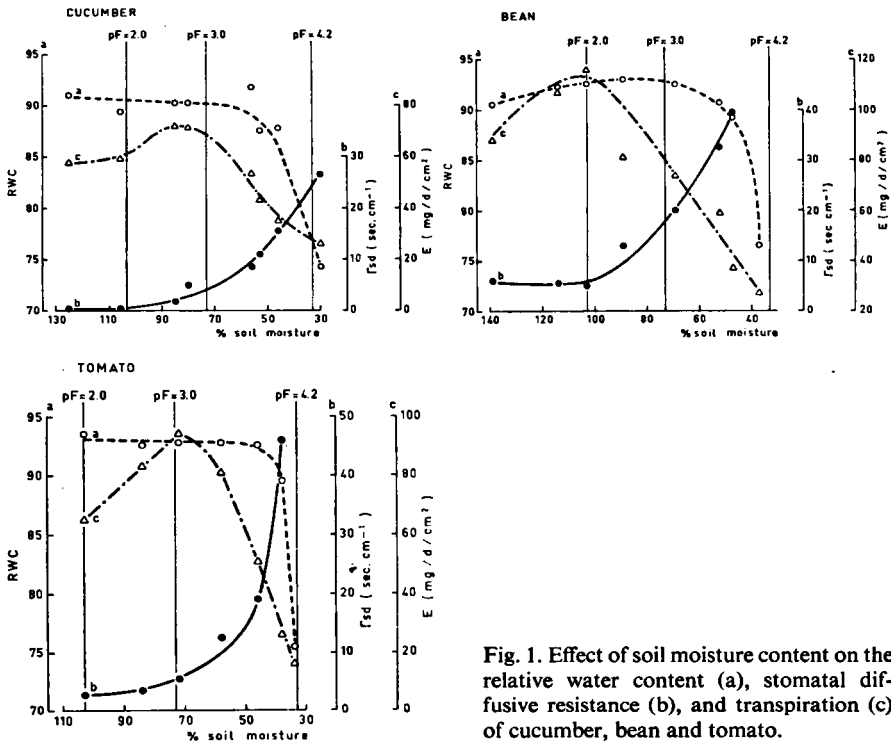


Fig. 1. Effect of soil moisture content on the relative water content (a), stomatal diffusive resistance (b), and transpiration (c) of cucumber, bean and tomato.

on the other hand, exhibited an initial increase followed by a progressive decrease with increasing soil moisture stress. The lower transpiration rate at a high soil water content may be attributed, at least in part, to the lack of oxygen in the ambient root media.

Differences in sensitivity of transpiration and stomatal control to water stress between the various species may be attributed partly to the species themselves and partly to the plant ages (BROWN & ROSENBERG 1970, SLATYER & BIERHUIZEN 1964b).

Transpiration can be written as a function of vapour pressure deficit and total resistance in the vapour pathway according to:

$$E = \frac{\Delta e}{r_{sd} + r_a} \tag{1}$$

in which E is transpiration rate in $g\ cm^{-2}\ sec^{-1}$ (the daily transpiration was considered to be a 16 hour period because dark transpiration was assumed to be negligible), Δe is the difference between maximal water vapour pressure at the leaf surface, which depends on leaf temperature, and the actual vapour pressure of the ambient air in mm Hg, r_{sd} the stomatal diffusive resistance in $sec\ cm^{-1}$, and r_a the boundary layer resistance to diffusion in $sec\ cm^{-1}$. The value of Δe could be converted from mm Hg to $g\ cm^{-3}$ by multiplying Δe by $0.623\ \rho/p$ where ρ is air density in $g\ cm^{-3}$ and p is atmospheric pressure in mm Hg. Equation 1 can then be written as

$$r_{sd} + r_a = \frac{0.623\rho}{p} \cdot \frac{\Delta e}{E} \tag{2}$$

Calculated values of $\Delta e/E$ ($sec\ cm^{-1}$) at various water stresses were plotted against their respective r_{sd} values ($sec\ cm^{-1}$) obtained from the diffusion porometer (fig. 2).

The relationships are essentially linear. Values of r_a obtained from extrapolation of the curves were 7.2, 4.4 and 2.8 $sec\ cm^{-1}$ for cucumber, tomato and bean, respectively. These values seem relatively high. It should be noted, however, that a single side of the leaves was considered for the calculation of E, whereas

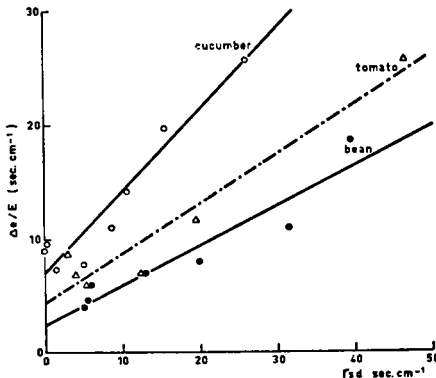


Fig. 2. Relationship between the ratio of vapour pressure difference from surface to air (Δe) to transpiration (E) and the stomatal diffusive resistance of three species. Cucumber: $y = 0.71 x + 7.2$ (σ_a 0.09, σ_b 1.1, $r = 0.95$), Bean: $y = 0.34 x + 2.8$ (σ_a 0.06, σ_b 1.3, $r = 0.93$), Tomato: $y = 0.44 x + 4.4$ (σ_a 0.06, σ_b 1.3, $r = 0.96$).

Table 1. Stomatal frequency number of stomata per mm² of the upper and lower surfaces of three vegetables.

| | lower surface | upper surface |
|----------|---------------|---------------|
| Cucumber | 341 | 328 |
| Bean | 263 | 75 |
| Tomato | 188 | 8 |

r_{sd} of the lower side of the leaves is measured, which does not have to be equal to r_{sd} of the upper side. Moreover, the wind speed in the climate room is low. The same reasons could account for the fact that no parallel curves with a tangent of 1 were obtained, since cucumber leaves are amphistomatous whereas tomato and bean leaves are hypostomatous, as shown in *table 1*. The data of *table 1* were obtained by nail polish replica. Differences in r_a values between the various species may be attributed to the variation in the leaf surface, hairiness, and shading.

The relation between diffusive and viscous flow resistance of the leaves has been theoretically investigated by WAGGONER (1965); however, no experimental evidence with a diffusive porometer was available. More recently JARVIS *et al.* (1967) developed a theory permitting the comparison between the resistance for viscous flow of air and that for diffusive gas flux. Moreover, a comparison was made between experimental data and calculated ones from anatomical dimensions. The authors also calculated the internal resistance (r_i) for a cotton leaf, from data obtained with a diffusion porometer and from the anatomical dimensions. It was in the order of 2.9 sec cm⁻¹ from one surface to the other.

In *fig. 3*, the r_{sd} values in sec cm⁻¹ were plotted against the corresponding values of resistance to viscous-air flow across the leaf in seconds. The relationships are essentially linear for the three species studied. It is obvious that with a diffusive resistance of zero the viscous flow resistance still exhibits a value of

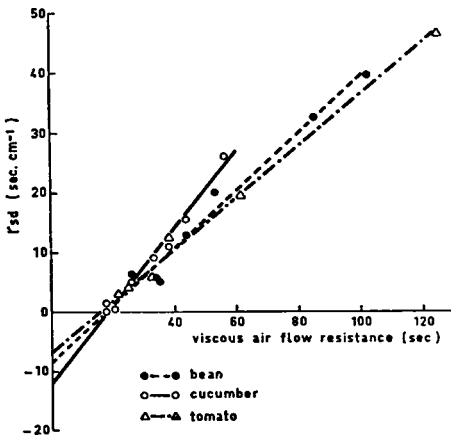


Fig. 3. Relationship between stomatal diffusive resistance and viscous air flow resistance of three species.

Cucumber: $y = 0.65x - 12.0$ (σ_a 0.04, σ_b 1.4, $r = 0.99$), Bean: $y = 0.48x - 8.7$ (σ_a 0.04, σ_b 2.2, $r = 0.99$), Tomato: $y = 0.43x - 6.6$ (σ_a 0.02, σ_b 1.1, $r = 0.99$).

20 seconds for the internal resistance (r_i). Assuming the relationship to be linear at all values, estimates of r_i values in sec cm^{-1} were obtained by extrapolation of the curves since the resistance to viscous-air flow mainly represents $r_{sd} + r_i$. They are 12.0, 8.7, and 6.6 sec cm^{-1} for cucumber, bean, and tomato, respectively. The relatively high values of r_i and the validity of their estimation will be considered in detail in the following section.

It would be of value to examine also the sensible heat transfer from leaf to air of the experimental plant species. The energy balance of a leaf can be written as

$$R_n = E + H \tag{3}$$

$$\text{or } H = R_n - E \tag{4}$$

where R_n is the net radiation absorbed by the leaf, E is the latent heat transfer, H is the sensible heat transfer, all terms expressed in $\text{cal cm}^{-2} \text{min}^{-1}$. A conversion of 600 cal g^{-1} water transpired was applied for E . The net metabolic energy exchange (which is mainly consumed in photosynthesis) was neglected since it rarely exceeds 2% of the absorbed, photosynthetically active radiation (WASSINK 1948).

When Δt (leaf temperature, t_l , minus ambient air temperature, t_a) is zero, R_n is equal to E . By plotting E against Δt , R_n can be estimated at $\Delta t = 0$. It is then possible to estimate the sensible heat transfer and heat transfer coefficient from eq. 4 and the measured Δt . The data are given in *fig. 4*, in which H values as $R_n - E$ are plotted against Δt . Each value is a mean of five observations. Some extreme negative values of H (Δt more than -1.0°C) were not included, because the lower leaf temperature will result in an underestimation of R_n and thus of H . At higher leaf temperatures, on the other hand, R_n and thus H will be overestimated, so that more or less S-shaped curves exist.

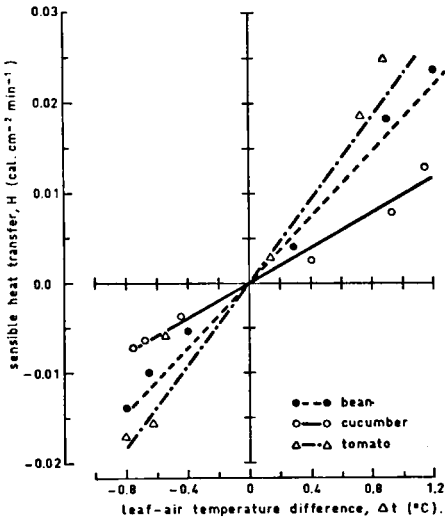


Fig. 4. Relationship between sensible heat transfer, H , and leaf-air temperature difference, Δt , of cucumber ($a = 0.0097$, $\sigma_a 0.007$, $r = 0.99$), bean ($b = 0.0186$, $\sigma_b 0.001$, $r = 0.99$), and tomato ($b = 0.0234$, $\sigma_b 0.002$, $r = 0.98$).

The regression coefficients, therefore, were approximate and in the order of 0.0097, 0.0184, and 0.0234 cal cm⁻² min⁻¹ deg C⁻¹ for cucumber, bean, and tomato, respectively. The external resistance to heat transfer (r'_a) could be calculated from these data using the following equation (SLATYER 1967)

$$H = C_p \rho \Delta t / r'_a \quad (5)$$

where C_p is the specific heat of dry air in cal g⁻¹ deg C⁻¹, all other terms were previously defined. The r'_a values are 1.8, 0.94, and 0.74 sec cm⁻¹ for cucumber, bean, and tomato, respectively. These values are far below those obtained from *fig. 2*, although the percentual change between the three species is almost equal.

An attempt was made to examine stomatal opening by infiltration technique. No results, however, could be obtained in the experimental set up of relatively low light conditions. In greenhouses at higher light intensities some differences in infiltration were observed between well watered and water stress plants, although the differences were small and highly variable.

4. DISCUSSION

The results reveal a clear-cut increase in r_{sd} values with increasing soil moisture stress. The effect is more pronounced at pF-values greater than 3.0. However, no apparent effect of water stress on RWC up to severe water stress took place, indicating that RWC is a poor criterion from an agronomic point of view. The transpiration rate showed an initial increase followed by a steady decrease as soil moisture decreased. Similar results were obtained by KUIPER & BIERHUIZEN (1958) with rye plants at light intensities above 1.5×10^4 erg. sec⁻¹ cm⁻². The low transpiration rates at higher soil moisture content may be caused by a poor aeration of the roots. However, no increase in stomatal diffusive resistance was evident. Transpiration rate could be underestimated also with an overestimation of soil evaporation. It is possible also that growth may interfere with the results in case it took place at high soil moisture conditions and ceased when water stress was more pronounced. In general, r_{sd} could be considered an excellent criterion in estimating the water deficit in the plant, at least under the present experimental conditions. It is generally agreed that the effect of water stress on stomatal aperture is achieved by its effect on internal CO₂ concentration through respiration and photosynthesis, and by acting on the process directly concerned with energy flow and guard cell turgor (SLATYER 1967).

The boundary layer resistance to water vapour diffusion, r_a , is usually measured with surface-wetted leaves or wet blotting paper. In the present studies, r_a was calculated (eq. 1 and 2) from transpiration (E) and r_{sd} values obtained by stomatal diffusion porometer (*fig. 2*). The reported values of r_a are higher than those obtained by wet blotting paper (KUIPER 1961, SLATYER & BIERHUIZEN 1964a). These results were discussed in the previous section.

An essentially linear relationship was obtained between stomatal diffusion resistance and viscous air flow resistance for the three species (*fig. 3*). The internal resistances of the leaves were obtained by extrapolations of the curves. The

r_1 value of cucumber was greater than of tomato and bean. It should be noted that, in case of tomato and bean, when the leaves wilted, the r_{ad} values were 86.5 and 103.6 sec cm^{-1} and air-flow resistances were 278.8 and 370.4 sec. These values were not included in the Figure and they shift from the curves due to the high mass-flow resistances. The latter may be brought about by enclosing part of the leaves for a long time which may change the conditions of the measured sites. In addition, the internal resistance of those hypostomatous leaves may be increased due to a more tortuous intercellular pathway caused by shrinkage (MEIDNER 1955). It should be kept in mind, however, that under our conditions the internal resistance depends not only on the thickness of the leaf, the compactness of the mesophyll cells, and the geometry of the intercellular spaces, but probably also on the stomatal geometry and density on both surfaces. Moreover, differences in stomatal opening on both sides of the leaves have to be considered as well. Since only the lower surface of the leaves was measured in the present investigations, the estimated values of r_1 are questionable. This phenomenon is subject to further study at present.

The data of *fig. 4* indicated that $H/\Delta t$ values were 0.0097, 0.0186, and 0.0234 cal. $\text{cm}^{-2} \text{min}^{-1} \text{ } ^\circ\text{C}^{-1}$ for cucumber, bean, and tomato, respectively. The calculated r'_a values (equation 5) were much lower (about 1:4) than the r_a values obtained from *fig. 2*. These results and those obtained by SLATYER & BIERHUIZEN (1964a) may indicate that the boundary layer resistance to sensible heat and water vapour are not similar. However, overestimation of r_a cannot be ruled out.

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