581.116.1:581.821.1

MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN, NEDERLAND **61** (7) 1–49 (1961)

THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE TRANSPIRATION OF LEAVES, WITH SPECIAL REFERENCE TO STOMATAL LIGHT RESPONSE

(met een samenvatting in het Nederlands)

by

P. J. C. KUIPER

Laboratory of Plant Physiological Research, Agricultural University, Wageningen, Netherlands, 210th Communication

(Received/Ontvangen 12.5.'61)

CONTENTS

CHAPTER 1.

GENERAL INTRODUCTION

§ 1. ABSTRACT FROM LITERATURE

In the past, a large number of investigations on transpiration has been carried out, as one may see, *e.g.*, from extensive reviews by BURGERSTEIN (1887–1925). This is easily understood, since measurements on transpiration are simple, as compared with other physiological processes as, *e.g.*, photosynthesis, respiration, etc.

Frequently, water loss by transpiration is measured by weighing, while also potometers are often used. In the latter case, water uptake is measured which, generally, is in equilibrium with transpiration. Sometimes the amount of water vapour, produced by the plant, is measured.

It is clear, from numerous investigations, that many environmental factors affect the rate of transpiration. As these factors influence several separate aspects of the transpiration process, a brief outline of the specific effects of different external factors on transpiration will be presented.

With regard to the physical aspect of the problem, it is generally accepted that transpiration is a diffusion process, to a large extent controlled by the stomata (VAN DEN HONERT, 1948; BANGE, 1953). Some environmental factors primarily affect transpiration via physical properties of the diffusion process, whereas others primarily affect the physiology of the stomata.

Under conditions of high transpiration, water supply by the roots sometimes limits transpiration of the shoot. In this case, various factors of the root environment affect transpiration.

It should be noted that many environmental factors also affect the rate of transpiration via changes in root/shoot ratio and growth of the plant (ABD EL RAHMAN *et al.*, 1958).

In general, transpiration increases with an increase in air temperature (and in leaf temperature) and a decrease in humidity of the air. These factors are important in determining the difference in vapour pressure between the air and the intercellular spaces of the leaf (BURGERSTEIN, 1920; CRAFTS, CURRIER, and STOCKING, 1949; STÅLFELT, 1956; YAMAOKA, 1958; KUIPER and BIERHUIZEN, 1958).

Transpiration increases with decreasing atmospheric pressure (BURGERSTEIN, 1920). Since transpiration may be regarded as a transport of water vapour along a concentration gradient (cm^3 water vapour/cm^3 air), the concentration can be expressed as pressure of water vapour/atmospheric pressure (see also LEIGHLY, 1937, CRAFTS *et al.*, 1949).

Increase in wind velocity sometimes causes increase, sometimes decrease in transpiration (STÅLFELT, 1932, 1956; WRENGER, 1935; MARTIN, 1943; YAMAOKA, 1958). Wind velocity affects the resistance to transport of water vapour of the external air layer. The difference between leaf temperature and air temperature decreases with increasing wind velocity. Decrease in stomatal aperture with increase in wind velocity is often observed, especially at limited water supply of the shoot.

Transpiration increases with increasing light intensity. This is due to increase in leaf temperature, and, in addition, to increase in stomatal aperture with in-

creasing light intensity. At very high light intensities, decrease in aperture is sometimes observed (HEATH *et al.*, 1950, 1954; HEATH, 1959; STÅLFELT, 1956; YAMAOKA, 1958; KUIPER and BIERHUIZEN, 1958; ABD EL RAHMAN *et al.*, 1958).

As for light quality, the transpiration rate seems to be correlated with light absorption in chlorophyll and with photosynthesis. The action spectrum of stomatal opening resembles the absorption spectrum by chlorophyll. There is a strong response to blue and red light and a smaller one to green light (PAETZ, 1930; LIEBIG, 1942; MOURAVIEFF, 1958).

Increase in transpiration with decrease in CO_2-content of the air is observed. Stomatal aperture decreases with increasing CO_2-content of the air in the range of 0.01–0.1 % (HEATH *et al.*, 1950, 1954; STÅLFELT, 1957; HEATH, 1959).

Narcotics like ether and chloroform, oxygen-free air, and etherial oils have been observed to reduce transpiration; these factors often promote closure of the stomata. In addition, etherial oils may decrease transpiration by formation of a thin film (BURGERSTEIN, 1920; SCARTH and SHAW, 1951; WILLIAMS, 1954).

As for root environment factors, water uptake and transpiration decrease at low oxygen supply and increasing CO_2-content of the root environment. They also rapidly decrease with decreasing root temperature, especially in plants from warm climates. Low temperature and insufficient aeration of root environment lead to reduced permeation of water into the root cells in connection with reduced metabolism, and sometimes promote closure of the stomata. Viscosity of water increases with decreasing temperature (KRAMER, 1949, 1956; ABD EL RAHMAN *et al.*, 1958).

Water uptake and transpiration increase upon a decrease of osmotic pressure and/or moisture tension. The rate of water uptake is determined to a large extent by differences in moisture tension (and possibly also in osmotic pressure) between soil and plant vessels. Generally, stomatal aperture decreases with increase in moisture stress (MAXIMOV, 1929; CRAFTS *et al.*, 1949).

Water uptake and transpiration are reported to increase with the supply of Ca^{++}, Mg^{++} (sometimes), SO_4^{--}, NO_3^- (sometimes), B^{+++}, Zn^{++}, Cu^{++}, Mn^{++}, and H^+-ions, and to decrease with the supply of Na^+, K^+, Mg^{++}, and Cl^--ions. An opposite reaction is often observed (BIEBL, 1958). Permeability of living cells for water increases under the influence of the different ions as follows: $Li^+ > Na^+ > K^+ > Mg^{++} > Ca^{++} > Al^{+++}$, and $CNS^- > NO_3^- > SO_4^{--}$, in plasmolysis experiments (DE HAAN, 1933). Na^+ and K^+ stimulate opening of the stomata, Ca^{++} inhibits it (ILJIN, 1957).

§ 2. SCOPE OF THE INVESTIGATION

A perusal of the extensive literature on the relation between transpiration and environmental conditions leads to the following statements:

1. Studying the respective relations, several different methods of transpiration measurement have been applied. Sometimes, considerable errors may have occurred, *e.g.*, in the case of transpiration measurements on plants, cultivated in soil, carried out by rapid weighing at short intervals of cut parts of the plant.
2. Most experiments have been carried out under field conditions, glasshouse conditions, or partly controlled climatic conditions. Only a very small number of investigations has been performed under completely controlled con-

ditions of the plant environment, changing each environmental factor independently of the others (*e.g.*, YAMAOKA, 1958).

3. The investigations cover a large number of plant species which may differ in reaction upon the various environmental factors.

It thus is clear that a general conclusion on the influence of the environment on plant transpiration can hardly be derived from the data reported in literature.

In this paper, the effect of several shoot environment factors on transpiration of cut leaves in potometers is described and discussed, while special attention is paid to the stomatal response to light. Experiments on transpiration of cut leaves in potometers have the advantage that limitation of transpiration by water supply rarely occurs.

In a subsequent paper, the effect of some root environment factors will be discussed, and the regulation of water uptake and of transpiration in intact plants described.

CHAPTER 2.

MATERIAL AND METHODS

§ 1. PLANT MATERIAL

In general, leaves of tomato (variety "*Ailsa Craig*"), bean (*Phaseolus vulgaris*, variety "*Wagenaar*"), and of *Hyoscyamus niger* were used in the measurements of the transpiration rate. Beans were most frequently used as experimental material.

If not mentioned otherwise, full-grown leaves were used, especially the first pair of leaves of bean plants (7–14 days after germination of the seeds) and, in the case of tomato, the 4th to 6th leaf from the tip of the plant. Leaves of *Hyoscyamus niger* were taken from vegetative rosettes.

Generally, the plants were cultivated in a light clay soil, the *pF*-curve of which has been determined by BIERHUIZEN (1958, fig. 1). Bean plants were frequently grown in coarse sand or in water culture, in aerated HOAGLAND nutrient solution.

With the exception of beans, the plants were cultivated in a glasshouse until 1 to 6 weeks before the investigation. They then were placed in a growth room at a temperature of $20° \pm 2$ °C and a light intensity of about 2.1×10^4 erg.sec^{-1}.cm^{-2}, obtained from fluorescent daylight tubes, PHILIPS "TL 55". The illumination period generally was 14 hours a day; only in the case of *Hyoscyamus* it was 8 hours a day. The humidity of the air could not be controlled, and varied with the external conditions. Bean plants were grown in this room from germination. The plants were watered every day; all plants showed favourable growth.

Deviations from this brief description occurred in some experiments. They are mentioned in the corresponding sections, together with further details.

§ 2. METHODS

Transpiration was measured in various ways, depending on the requirements of the experiment.

The rate of transpiration of cut leaves was measured with the aid of potometers. Before the potometer experiments, the plants were placed in weak light which lowered the suction tension

in the xylem vessels. Then the leaves were cut under water to prevent the formation of air bubbles in the xylem vessels. Hereafter, they were placed in the potometers with the aid of small pieces of wet cotton wool. An air-tight fit of petiole and potometer was obtained with cocoa butter. Within the range of conditions of our experiments, the water uptake by the petiole, measured with the potometer, was equal to the steady transpiration rate of the leaf, measured as loss of weight of the entire system on an analytical balance. After transfer from one condition to another, about 15–30 minutes are required for adaptation to the changed conditions and for re-establishment of the equilibrium between water uptake and transpiration. This adaptation is finished when steady rates of transpiration and water uptake are again recorded. Under constant conditions, the transpiration rate of cut leaves remained constant for at least 3 hours. The experiments were carried out within this period.

Potometers were also used for experiments with entire bean plants in water culture. These contained about 50 cm^3 of aerated water in which the roots were. An air-tight fit of stem and potometer was obtained with a rubber stopper and cocoa butter. With regard to the adaptation to changed conditions, and the equilibrium between water uptake and transpiration, similar remarks as for the potometer experiments with cut leaves can be made. Under constant conditions, water uptake and transpiration remained constant for at least 4 hours.

In some cases, transpiration was measured almost continuously (2 measurements per minute) by weighing cut leaves in very small potometers on a "METTLER" rapid-weighing balance with an accuracy of 0.1 mg.

Evaporation of wet blotting paper and of petri-dishes with water was measured in the same way.

In some cases, cuticular transpiration of the upper side of a hypostomatous leaf was measured. A small lucite cup was then placed on the leaf. Dry air was pushed through this cup at a constant rate. The released water vapour was absorbed by phosphor pentoxide, and measured by weighing.

Since transpiration depends on the degree of stomatal opening, the width of the slit was measured under the microscope in several experiments. Some qualitative measurements were performed using a 3% collodion-ether solution. This method can hardly be compared with microscopical measurement. Semi-quantitative measurements were carried out with the application of different alcohol-water mixtures and ether. Infiltration was considered to depend on the surface tension of the liquid used. Thus, a range of solutions was used, the surface tension of which varied from about 73 dyne.cm^{-1} (pure water) till about 17 dyne.cm^{-1} (pure ether).

In the microscope studies of stomatal behaviour a water-cooled high pressure mercury vapour lamp, "PHILIPS HO 2000" was used as a light source, and placed below the microscope stage. The condensor was removed in order to obtain a relatively large irradiated area (4.5 cm^2). A bean leaf, placed in a potometer, was put upside down on the microscope stage so that the stomata of the hypostomatous leaf were clearly visible. A shallow lucite cup was placed on the leaf. Air of known humidity and carbon dioxide content was passed through at a constant rate, *viz.* 4.6 cm^3 air.sec^{-1}.cm^{-2} leaf area. The light intensity was varied by paper screens between the light source and the leaf. The width of the stomatal slit was recorded by microphotography. Afterwards, changes in stomatal aperture were determined by enlargement of the negatives.

During the experiments, several environmental factors have been measured, *viz.*, air temperature, leaf temperature, humidity of the air, wind velocity, and light intensity.

Air temperature was measured with ordinary mercury thermometers with an accuracy of 0.1 °C, while leaf temperature was measured with copper-constantan thermocouples. The junctions of the latter were made as small as possible in order to decrease possible effects from direct irradiation. Reference junctions were placed in a water-filled DEWAR flask, together with a mercury thermometer with an accuracy of 0.1 °C. The thermocouples were calibrated against accurate mercury thermometers (accuracy 0.05 °C) within a wide range of temperature differences, and found to be accurate within 0.1 °C.

Leaf temperatures were measured by placing the junctions against the lower side of the leaf, in good contact with the leaf surface. The temperature recorded was considered to be the temperature of the transpiring surface of the leaf. This seems allowed since the heat capacity of a junction is very small as compared with that of a leaf. Owing to the large difference in heat conductivity of air and water, possible irradiation effects of the junction result in an almost quantitative heat transfer from the thermocouple to the leaf (see also GAASTRA, 1959). Another indication is the observation that junctions with a small contact with the leaf (*e.g.*, when only the tip of the junction touches the leaf surface) showed the same temperature as junctions having a good contact with the leaf. All these considerations point to the validity of

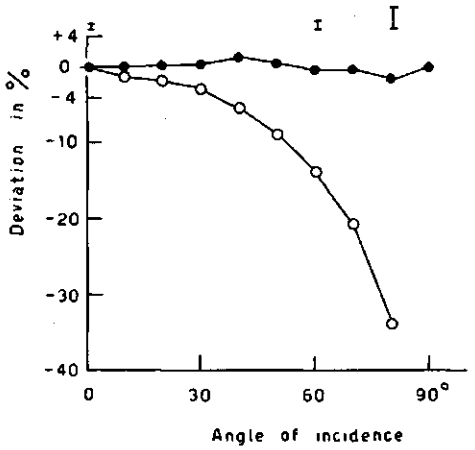


FIG. 1.
Deviation from indication according to cosine rule in percent;
(●——●) flat light meter with a correction after design of HARTIG and HELWIG (1955);
(○——○) flat light meter, covered with a slightly convex opaline glass;
(I) error of measurement, increasing from 0 to 90° as indicated.

these measurements. A mean leaf temperature was calculated from measurements at 5 different places at least on the leaf.

The water vapour pressure of the air was measured regularly during the course of the experiments with the aid of a psychrometer.

Wind velocities between 75 and 300 cm.sec^{-1} were measured with a calibrated anemometer. Lower velocities were determined with a flow meter, which was calibrated by measuring the volume of water, replaced by air per unit time.

The light intensity was measured with a spherical radiation meter or (in most cases) with a flat meter which a selenium barrier layer photo-cell with a diameter of 32 mm ("Elektrocell" 732). The spherical radiation meter, designed by WASSINK and VAN DER SCHEER (1951) contained 2 such selenium cells of 18 mm diameter ("Elektrocell" 718), and was mainly used in preliminary potometer experiments. The light intensity incident on the leaves was measured by exposing the meter just above the surface of cut leaves in the potometers.

Since a flat light meter would appear to answer more appropriately the situation in these particular experiments, this type has been used throughout, except in the very first experiments. In order to approach the theoretical cosine sensitivity, this meter was constructed according to the design of HARTIG and HELWIG (1955). A preliminary calibration of the "cosine-corrected" cell was made by KEMA, Arnhem; subsequent calibrations were carried out at our laboratory. It is clear, from fig. 1, that deviations from the cosine law are small, and negligible for the purpose of our experiments.

Both light meters were calibrated regularly against a MOLL large surface thermopile for each light source used, within a wide range of light intensities. The thermopile was calibrated once at the Physical Laboratory of the University at Utrecht. The photo-current proved to be linear with light intensity in the entire range of intensities from fluorescent tubes, "PHILIPS TL 55 and TL 33", and from sodium lamps, "PHILIPS SO". Since the sensitivity of the photo-electric cells decreases at higher light intensities, as obtained from high pressure mercury vapour lamps, "PHILIPS HO 2000", more extensive calibration curves of the electrical output against the incident light intensity had to be made. An example of such a curve is given in fig. 2. It is evident that the sensitivity of the meter decreases at light intensities above 15×10^4 erg.sec^{-1}.cm^{-2}. Since changes in light emission, owing to ageing of the light sources and changes in the sensitivity of selenium cells by ageing had to be considered, calibrations were repeated several times in the course of the investigation.

The percentage of infrared radiation of each light source was determined with a SCHOTT RG 8-filter. The range of transmission of this filter reaches from about 675 mμ to about 3000 mμ; its transmission is about 95% between 750 and 1800 mμ. Taking into consideration the spectral bands, proposed by the Committee on Plant Irradiation (1953), the light transmitted by a RG 8-filter mainly contains the 1st and 2nd bands (radiation >720 mμ) and a small amount of radiation in the 3rd band (between 720 and 610 mμ).

The percentages of the total radiation transmitted by this filter were:

5– 6%, new fluorescent tubes,
8–10%, aged fluorescent tubes,
6%, new high pressure mercury vapour lamps, with a water filter of about 10 cm.,

Notice

It occurs to us that the reproduction of a few figures, *viz.*, Nos 3, 4, 6, and 12 may be somewhat too small. Owing to the time schedule required for this paper, replacement in the text was no more possible.

Therefore, we supply herewith copies of these figures at a somewhat enlarged scale. Their new size is thus that they may well be glued on in place of the ones printed in the text, if required.

P. J. C. KUIPER
E. C. WASSINK

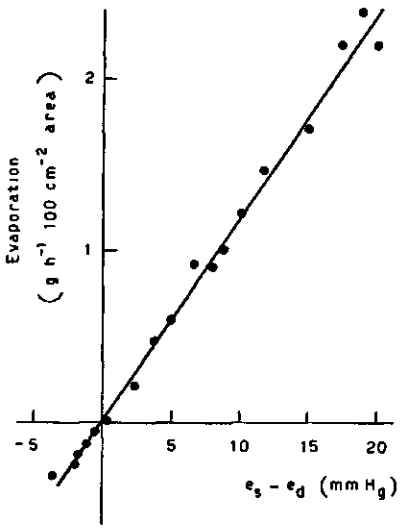


FIG. 3.
Evaporation of a water surface ($g.h^{-1}.100\ cm^{-2}$) in relation to difference in saturation vapour pressure at surface temperature and actual vapour pressure of the air (e_s–e_d, in mm Hg).

FIG. 4.
Evaporation ($g.h^{-1}.100\ cm^{-2}$) and temperature of the evaporating water surface in relation to light intensity;
(●——●) at 29.5 °C air temperature and 29% r.h.;
(+——+) same, corrected for differences between surface temperature and air temperature; e_a-e_d = 22 mm Hg.

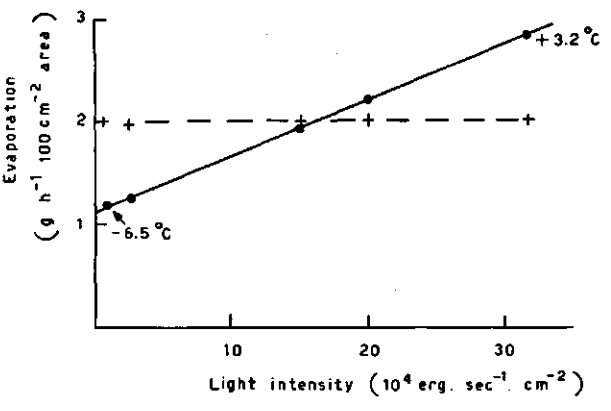
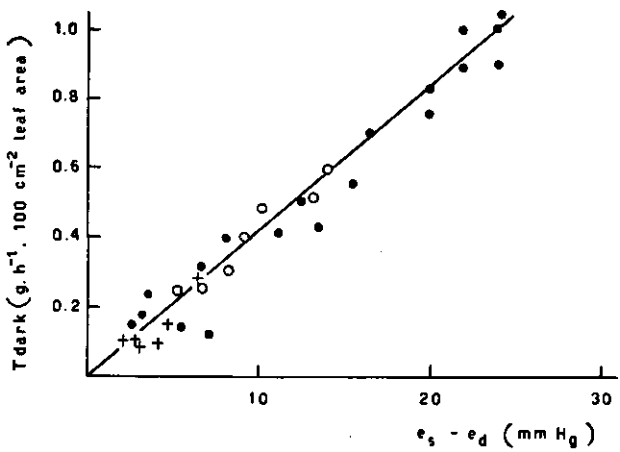


FIG. 6.

Dark transpiration (T_{dark}) of cut leaves of *Hyoscyamus* in potometers ($g.h^{-1}$. 100 cm^{-2} leaf area) as related to difference in saturation vapour pressure at surface temperature and actual vapour pressure of the air (e_s–e_d, in mm Hg) for various ranges of leaf temperature (●) >20 °C, (○) between 10° and 20 °C, (+) <10 °C.



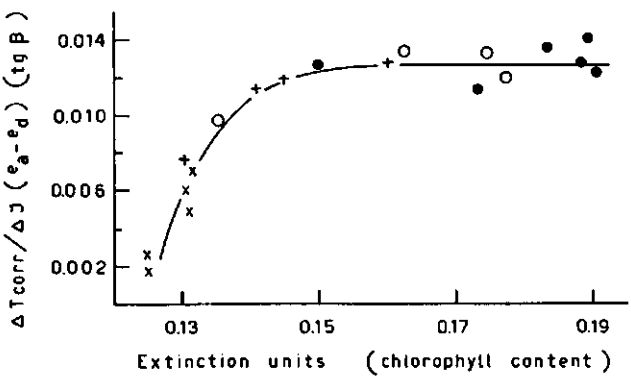
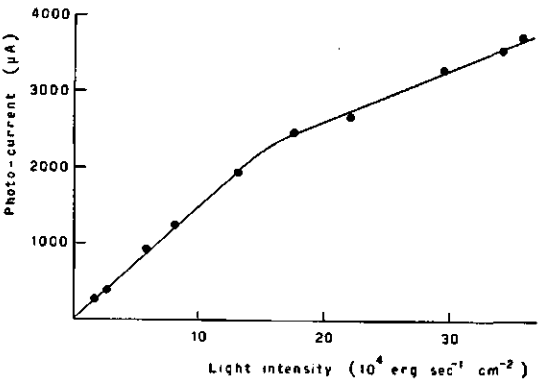


FIG. 12.

The increase in transpiration of bean leaves [$g.h^{-1}.(2.5 \times 10^6$ stomata)$^{-1}$] per unit increase of incident light intensity ($erg.sec^{-1}.cm^{-2}$, sodium light) per unit vapour pressure difference (e_a–e_d, in mm Hg), analogous to tg β of fig. 8, in relation to the chlorophyll content of the leaf, expressed as extinction value at 665 mμ. Symbols represent the light conditions in which the experimental plants were grown:

(●) irradiation from above and from aside, 8×10^4 $erg.sec^{-1}.cm^{-2}$,
(○) „ „ „ „ „ „ 2.5×10^4 $erg.sec^{-1}.cm^{-2}$,
(+) irradiation from above 1×10^4 $erg.sec^{-1}.cm^{-2}$,
(×) „ „ „ 0.5×10^4 $erg.sec^{-1}.cm^{-2}$.

FIG. 2.
Electrical output of flat light meter with a selenium barrier layer cell against incident energy as measured with a large surface thermopile. High pressure mercury vapour lamp, provided with a 10 cm water filter; selenium cell shunted according to incident intensity value.



± 20%, aged high pressure mercury vapour lamps, with a water filter of about 10 cm.,
< 1%, sodium lamps, with a water filter of about 15 cm.,
60%, incandescent lamps (500 W), with a water filter of about 15 cm.,

The light intensity values as given further in the text include the infrared radiation, and represent incident light intensity.

A thermopile was used for intensity measurements of light obtained from incandescent lamps, since selenium cells are hardly sensitive to infrared radiation.

In general, new or little-used lamps were employed.

A comparison was made between measurements with the spherical light meter and with a flat one. For leaves in a horizontal position, 10000 erg.sec^{-1}.cm^{-2} Ø sphere of the spherical meter agrees with 5500 erg.sec^{-1}.cm^{-2} as indicated by the flat meter. In experiments with bean, tomato and *Hyoscyamus* the leaves generally had a horizontal position. It should be stressed that this data is valid only for the light conditions in our experimental compartments. In this paper, the values of light intensity are given in erg.sec^{-1}.cm^{-2}.

It is clear from this description of the light measurements that accurate estimates of relative values of incident light energy can be obtained easily in all cases with both meters.

On the contrary, it is hardly possible to obtain accurate measurements of absorbed light. An approximate measurement was tried for leaves in a horizontal position with regard to light from above. The absorption of a bean leaf as measured in an ULBRIGHT sphere with a sodium lamp as a light source, was 75%. Taking this value into consideration, the absorption of the bean leaf resembles that of the "*Ipomoea* leaf" and the "*Impatiens* leaf" of RABIDEAU *et al.* (1946). From the absorption spectra, one would expect an approximate absorption of about 70–80% within the range of 400–675 mμ for the light sources mentioned earlier. Considering RABIDEAU's data, an approximate absorption of 75% within this range is assumed for the experimental plants, with a possible deviation of 10%. This also agrees with observations of SEYBOLD and WEISSWEILER (1942) and SHUL'GIN *et al.* (1958) on the absorption spectrum of a bean leaf.

Owing to some uncertainty in the position and flatness of the leaves, larger errors may well occur and, therefore, measurements of incident light intensity with the flat meter in a horizontal position, multiplied by 0.75, may be assumed to estimate the absorbed amount of energy, with a possible deviation up to 25%.

For the study of transpiration under controlled conditions, movable light boxes and thermostats were used.

In the range between 5° and 15 °C, the movable light boxes were placed in low-temperature rooms. These boxes were provided with fluorescent tubes. The light intensity was varied by white paper screens placed on a glass panel between the light source and the leaves. Humidity of the air, and air temperature proved to be constant in short-time experiments.

In the range between 15° and 35 °C four thermostats were used, which could be controlled with an accuracy of 0.5 °C by means of a thermo-relay. Each thermostat was divided into two compartments by means of a floor, perforated with holes of 7 mm diameter. The lower compartment contained heating elements, small fans for mixing the air, and a fan introducing cool air from outside the laboratory for temperatures below 23 °C. The upper compartment contained the experimental plants, placed in "still air"; a more exact definition of this "still air" will be given in the next chapter. The air temperature proved to be the same in the entire

thermostat. The walls were covered with filter paper so that a high humidity of the air could be obtained by spraying the walls, if required. Also in this set-up, the relative humidity of the air was constant during short-time experiments. Each thermostat was provided with a sufficient number of thermocouples (*e.g.* five) which touched upon the leaf during an experiment, and were connected to a plurilocal switch so that several leaf temperatures could be measured quickly.

The upper side of the thermostat was covered by a glass panel, above which the light source and the possible water filter was placed. Again, different light intensities were obtained by white paper screens on the glass panel and by white gauze inside the thermostat. The light intensity – including the infrared radiation – thus could be varied between:

0 and 9×10^4 erg. $sec^{-1}.cm^{-2}$, using fluorescent tubes,
0 and 25×10^4 $erg.sec^{-1}.cm^{-2}$, using high pressure mercury vapour lamps with a water filter,
0 and 3.5×10^4 $erg.sec^{-1}.cm^{-2}$, using sodium lamps with a water filter and a solution of 300 $g.m.^{-2}$ copper sulfate,
0 and 50×10^4 $erg.sec^{-1}.cm^{-2}$, using incandescent lamps (500 W) with a water filter.

Experiments of a duration shorter than 15 minutes were sometimes carried out at room temperature, measuring transpiration by weighing. Within this short time the air temperature did not vary appreciably.

Microscopical investigations on stomatal behaviour also were carried out at room temperature (about 21 °C).

Measurements of leaf area (in cm^2) to be mentioned furtheron, refer to one side of the leaf. When referring to both sides of the leaf, the indication "cm^2 cuticle" is used. In general, leaves of about 100 (70 to 130) cm^2 area have been employed.

Further details of the methods applied are given in the subsequent sections.

CHAPTER 3.

PHYSICAL CONSIDERATIONS ON EVAPORATION

Evaporation may be considered from the viewpoint of a heat budget, or from that of a transport of water vapour along a concentration gradient. In many cases, transport of water vapour is governed by a diffusion process, to which FICK's law may be applied. For the simple case of a tube:

$$U = k \cdot \frac{A}{L}(c - c') \tag{1}$$

in which

U = rate of diffusion of water vapour ($cm^3.sec.^{-1}$),
k = coefficient of diffusion ($cm^2.sec^{-1}$),
A = cross section of the tube (cm^2),
L = length of the tube (cm),
c–c' = concentration difference between both ends of the tube (cm^3 water vapour.cm^{-3} air).

In the case of evaporation, and using PENMAN's symbols (1948) $c - c'$ may be replaced by $(e_s - e_d)/P$.

e_s = saturation vapour pressure at surface temperature, in mm Hg,
e_d = actual pressure of water vapour in the air, in mm Hg,
P = atmospheric pressure, in mm Hg.

Usually, the variation of P can be neglected for this purpose and the equation for evaporation may be written as:

$$U = \frac{k}{760} \cdot \frac{A}{L}(e_s - e_d) \tag{2}$$

Similar equations have been given by MAXIMOV, 1929, WELTEN, 1933, PENMAN, 1948, BANGE, 1953, RASCHKE, 1958.

Inside a tube, diffusion is determined by a sequence of flat surfaces of equal vapour concentration. Under circumstances other than those existing in a tube, the surfaces of equal vapour concentration generally are not flat; this holds also for the entire layer of air very near to the evaporating surface where transport of water vapour is determined by diffusion.

So far, evaporation, thus has been treated as a diffusion process. Under various conditions, emphasis shifts from mere molecular diffusion to mass transport by eddies, *e.g.*, if wind velocity plays a role. This, generally, leads to replacement of the factor A in (1) by a surface factor which is a more complicated function of the various parameters involved *(e.g.* length and breath), and which will be symbolized furtheron as O (cm^2). For the boundary layer of air, in which water vapour transport is determined by diffusion, we may introduce the "resistance to diffusion", R:

$$U \cdot R = e_s - e_d \tag{3}$$

in which, according to (2), R equals $\frac{760 \cdot L}{k \cdot A}$ or $\frac{760 \cdot L}{k \cdot O}$.

Since the temperature of the evaporating surface nearly always differs from the air temperature, the diffusion along a vapour pressure gradient and the heat conduction give rise to thermal diffusion. Thus, evaporation can be partly due to a concentration gradient (ordinary diffusion, according to FICK's law) and partly to a temperature gradient (thermal diffusion). This also holds for transpiration of leaves, though the differences between leaf temperature and air temperature mostly are much smaller than those between the temperature of a wet surface and the air.

The coefficient of diffusion, k, in the above equations is about 0.24 $cm^2.sec^{-1}$ for water vapour diffusing into air at 20 °C, and depends on temperature. LEIGHLY (1937) used the relation:

$$k = 0.230\ (T/273)^2\ cm^2.sec^{-1},$$

T in °KELVIN, in tables which also include the effect of atmospheric pressure on evaporation.

MARTIN (1943), in experiments at different wind velocities, derived the following empirical relation:

$$E = 0.73 \cdot k \cdot (e_s - e_d) \cdot B^{-0.2} \cdot C^{-0.3} \cdot V^{+0.5} \tag{4}$$

in which

E = evaporation from a rectangular piece of blotting paper ($g.h^{-1}.100\ cm^{-2}$),
k = coefficient of diffusion ($cm^2.sec^{-1}$),
$e_s - e_d$ = vapour pressure difference in mm Hg,
B = length of the evaporating area at right angles to the wind direction (cm),
C = length of the evaporating area parallel to the wind direction (cm),
V = wind velocity ($cm.sec^{-1}$).

It is clear that L (and also R) is affected by wind velocity; according to (4), L is proportional to $V^{-0.5}$. Other observations indicate a relation between L and V^{α}, α varying between –0.5 and –0.78 (PENMAN, 1948, RASCHKE, 1956, 1960).

According to (4), R, moreover, depends on the shape of the evaporating surface; it is proportional to $B^{+0.2}$ and $C^{+0.3}$ for a rectangular piece of blotting

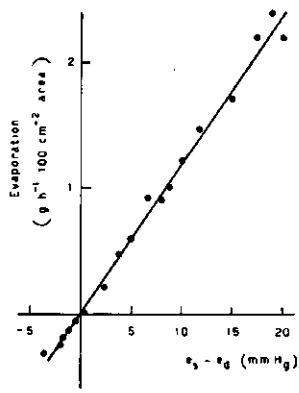


FIG. 3.
Evaporation of a water surface (g.h^{-1}.100 cm^{-2}) in relation to difference in saturation vapour pressure at surface temperature and actual vapour pressure of the air (e_s–e_d, in mm Hg).

paper. Similar relations for blotting papers of various shapes are given by WALTER (1926); RASCHKE (1956), and others.

The validity of equation (3) was tested under the conditions of our experiments. According to general usage, evaporation of a square piece of blotting paper (100 cm^2), and transpiration of leaves, varying in size from 70 to 140 cm^2, were expressed in g.h^{-1}.100 cm^{-2} (E = 1 g.h^{-1} corresponds to U = 0.34 cm^3.sec^{-1} water vapour). Generally, the difference in vapour pressure, $e_s - e_d$, is expressed in mm Hg, while the resistance to diffusion, R, in sec.cm^{-1} per cm^2 area, is calculated from the average transpiration rate per cm^2 area.

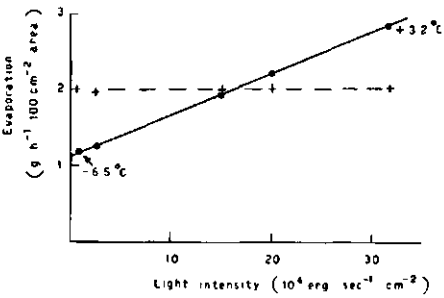
The effect of $e_s - e_d$ on evaporation was measured, and the effect of wind velocity on the diffusion resistance, R, determined for a square piece of blotting paper of 100 cm^2, saturated with water.

Evaporation was measured at different values of e_s and e_d. The saturation vapour pressure at surface temperature, e_s, was varied to a large extent by using a wide range of air temperatures, while the actual vapour pressure of the air, e_d, was varied in short-time experiments by changing the humidity of the air. Surface temperatures were measured with thermo-couples, while evaporation was measured by weighing under "still air" conditions of the experimental room. Some results are presented in fig. 3, and demonstrate a linear relation between evaporation and $e_s - e_d$, within a wide range of vapour pressure differences. In addition, the figure shows that this relation also holds for the condensation rate at negative values of vapour pressure difference. The resistance to diffusion of the boundary air layer derived from this curve is 2.9 sec. cm^{-1} per cm^2 area (under the conditions prevailing in our room).

It should be noted that, since the diffusion coefficient k is temperature dependent (k = 0.230 (T/273)^2 cm^2.sec^{-1}), a small variation of the diffusion resistance with temperature exists. Within the range of temperatures studied, 5° to 30 °C, k varies between 0.234 and 0.255 cm^2.sec^{-1}. Considering the errors of measurement of evaporation and surface temperature, this small variation of k, and therefore of R has not been taken into account.

Since light intensity is one of the most important environmental factors in connection with plant transpiration, its effect on evaporation was also studied. Water in an evaporation pan of 100 cm^2 was blackened with a drop of India ink in order to obtain light absorption, comparable to that of leaves. An example of the effect of light intensity upon evaporation, obtained in the experimental

FIG. 4.
Evaporation ($g.h^{-1}.100\ cm^{-2}$) and temperature of the evaporating water surface in relation to light intensity;
(●——●) at 29.5°C air temperature and 29% r.h.;
+——+) same, corrected for differences between surface temperature and air temperature; $e_a - e_d \simeq 22$ mm Hg.



thermostats, is given in fig. 4. Irradiation has a large effect both on the evaporation rate and on surface temperature, and, thus, on $e_s - e_d$. Correction of the results for differences between surface temperature and air temperature shows that the increase in surface temperature is the only effect of the irradiation. This experiment thus confirms the results of the previous one, and serves as an introduction into the analysis of the effect of light intensity on transpiration of leaves, as will be presented in the following chapter.

The diffusion resistance of the boundary layer of air as found in our experimental thermostats is given in table 1, together with some values, calculated from literature. With the exception of those of BANGE, the data show reasonable mutual agreement. Probably, BANGE's experiments were carried out at a higher degree of air motion.

TABLE 1. Evaporation and resistance to diffusion of the boundary layer of air under the conditions of experimental compartments and rooms. Evaporation of an area of 100 cm^2 is expressed in $g.h^{-1}$ (mm Hg)$^{-1}$, the average resistance to diffusion (R) in $sec.cm^{-1}$ per cm^2 area, and the average length of the diffusion path (L) in cm.

	$E/(e_s - e_d)$	R	L
Thermostats (this paper)	0.090	3.74	0.90
Experimental room (this paper)	0.118	2.94	0.70
MARTIN (1943)	0.103	3.36	0.80
BAUMBACH (1952)	0.086	4.02	0.96
BANGE (1953)	0.182	1.90	0.46

Experiments at different wind velocities were carried out with wet blotting paper in a small wind tunnel (diameter 25 cm), the air moving parallel to the paper surface. The temperature of the evaporating surface under "still air" conditions decreases rapidly with increasing wind velocity, due to increase in heat exchange between the evaporating surface and the air. From measurements of the evaporation rate, of e_s and e_d, the diffusion resistance of the boundary layer of air has been calculated for a wide range of wind velocities. The resistance to diffusion, as related to wind velocity, is given in fig. 5, together with some data calculated from MARTIN's experiments (1943), concerning an evaporating area of similar shape. Three different ranges of wind velocity (V) may be distinguished, *viz.*, *1)* $V < 18$ $cm.sec^{-1}$, *2)* V between 18 and 40 $cm.sec^{-1}$, and *3)* $V > 40$ $cm.sec^{-1}$. The first range seems characterized by laminar streaming of the air; in range *3* air movement is turbulent, while range *2* represents the transition zone. Some characteristics of these three ranges are given in table 2.

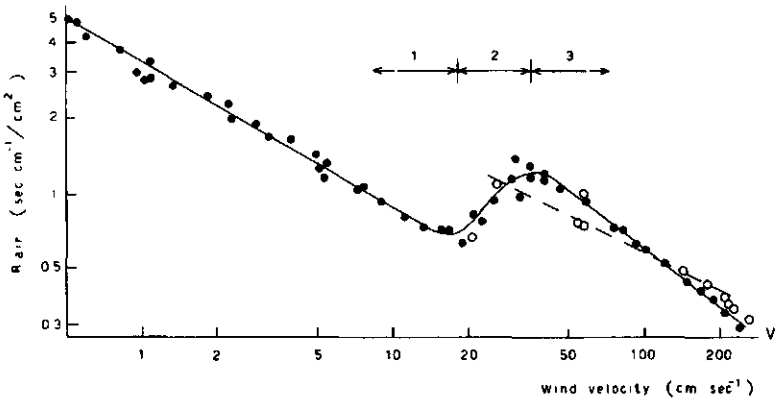


FIG. 5. The effect of wind velocity (V, in cm.sec^{-1}) on the average resistance to water vapour transport of the boundary layer of air (R_{air}, in sec.cm^{-1} per cm^2 area);
(*1*) laminar air movement, (*2*) semi-turbulent air movement, (*3*) turbulent air movement;
(o——o), some data of MARTIN (1943) and his suggested curve.

TABLE 2. The effect of different conditions of air movement on evaporation of a one mm thick layer of blotting paper with an area of 100 cm^2 (see also fig. 5)

Range	1	2	3
Wind velocity (cm.sec^{-1}) Air movement	$V < 18$ laminar	$18 < V < 40$ semi-turbulent	$V > 40$ turbulent
Transport of water vapour	molecular transport by laminar diffusion	partly molecular transport by laminar diffusion, partly mass transport by eddies	mass transport by eddies
Relation between R (in sec.cm^{-1}, per cm^2 area) and wind velocity (V, in cm.sec^{-1})	$R = 3.46\ V^{-0.58}$	$R = 0.052\ V^{+0.95}$	$R = 20.8\ V^{-0.76}$

Transport of water vapour in range *3* is often considered as molecular diffusion through a laminar layer, the thickness of which depends on wind velocity. The values of wind velocity, characterizing the transition between the different ranges, vary with the thickness of the evaporating object, according to the REYNOLDS equation (SCHLICHTING, 1951). One would expect that, for the boundary layer of air around leaves, the transition velocities shift to higher values with decreasing leaf thickness. In general, however, this is more than compensated by the variable position of the leaves with regard to the direction of air movement.

Under different conditions of air movement, as represented in the ranges *1*, *2*, and *3* of fig. 5, the relation between R and V may be represented by $R = b\ V^{-\alpha}$. In turbulent air, the value of α is 0.76. RASCHKE, assuming a similarity in transport of heat and water vapour under turbulent air conditions, derived similar relations between wind velocity and both $1/R$ and the coefficient of heat transfer h (= "Wärmeübergangszahl", see RASCHKE, 1956, 1960). In both cases, α mostly was between 0.68 and 0.78 (see PENMAN, 1948; DE VRIES and VENEMA, 1954).

Our fig. 5 demonstrates that lower values of α, *e.g.*, 0.5, as reported by MARTIN and RASCHKE must be due to the fact that some of their experimental data apply to the transition zone beween laminar and turbulent flow (range *2)*, and others tu turbulent flow only (range *3)*.

CHAPTER 4

OBSERVATIONS ON TRANSPIRATION

The effect of light intensity on transpiration is the major object of our study. The relation between light intensity and transpiration will be discussed as follows: dark transpiration (§ 1), transpiration at low light intensity (§ 2), and transpiration in strong light (§ 3).

§ 1. DARK TRANSPIRATION

Measurements of transpiration in dark were carried out with *Hyoscyamus* leaves. The variation in leaf area was from 70 to 140 cm². In general, the leaves were found to be at temperatures slightly below those of their surroundings, but the difference between leaf and air temperature never exceeded 0.7 °C. Transpiration rates were calculated for a leaf area of 100 cm², $e_s - e_d$ was calculated from measurements of the leaf temperature and the humidity of the air. The results of several measurements are presented in fig. 6, showing a linear relation between the transpiration rate and $e_s - e_d$. Dark transpiration seems to be temperature-independent to a large extent, considering the large variation in temperature at low values of $e_s - e_d$. It should be noted, however, that also in dark transpiration a small variation with temperature must exist since the diffusion coefficient k is temperature dependent [$k = 0.230\ (T/273)^2$ cm².sec^{-1}]. In relation to the errors of measurements, the small variation of k has not been taken into account.

Several data on dark transpiration of bean and tomato leaves have been obtained by extrapolation of transpiration versus light intensity curves to zero light intensity. This seems acceptable, since there is a simple relation between light intensity and transpiration, and the observations included measurements in weak light (see, *e.g.*, fig. 7). A relation similar to that in *Hyoscyamus* was found for bean and tomato when the transpiration rate in darkness is plotted against $e_s - e_d$. (KUIPER and BIERHUIZEN, 1958).

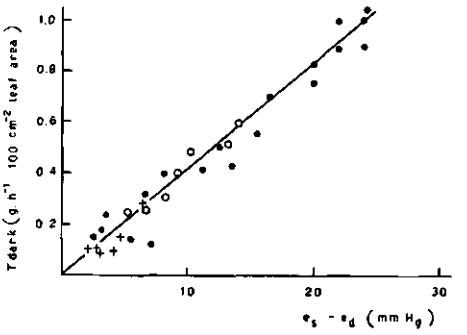


FIG. 6.
Dark transpiration (T_{dark}) of cut leaves of *Hyoscyamus* in potometers (g.h^{-1}.100 cm^{-2} leaf area) as related to difference in saturation vapour pressure at surface temperature and actual vapour pressure of the air (e_s–e_d, in mm Hg) for various ranges of leaf temperature (●) >20 °C, (○) between 10° and 20 °C, (+) <10 °C.

Table 3 contains some values calculated for the diffusion resistance of leaves in darkness. It is clear that considerable differences exist between various species, ranging from 4.6 *(Hyoscyamus)* to 17.0 sec.cm^{-1} per cm^2 leaf area *(Myrica rubra)*. Moreover, it seems reasonable to assume that for a given plant species, the value is dependent upon culture conditions.

TABLE 3. Transpiration and resistance to diffusion of cut leaves in potometers in dark. Transpiration is calculated in g.h^{-1}.(mm Hg)$^{-1}$.100 cm^{-2} leaf area $[T/(e_s-e_d)]$ and the average resistance to diffusion in sec.cm^{-1} per cm^2 leaf area. In the case of *Myrica rubra* the diffusion resistance of the air at wind velocity "zero" was supposed to be 3.7 sec.cm^{-1} per cm^2 area, just as in the experiments described.

1	2	3	4	5
Plant species	Leaf type	$T/(e_s-e_d)$	R_{total}	R_{leaf}
Tomato	hypostomatous	2.56×10^{-2}	13.5	9.8
Bean	hypostomatous	2.60×10^{-2}	13.3	9.6
Hyoscyamus	amphistomatous	4.15×10^{-2}	8.3	4.6
Myrica rubra (YAMAOKA, 1958)	?	1.67×10^{-2}	20.7	17.0

Some experiments were made with hypostomatous leaves of bean and tomato, in which dark transpiration was measured, while one of the leaf sides was covered with vaseline. In either case this treatment reduced dark transpiration to about fifty percent of the rate for the untreated leaf. This shows that the stomata do not contribute appreciably to dark transpiration so that the latter is primarily cuticular. In potometer experiments with cut leaves of bean and tomato, the stomatal resistance to diffusion in darkness is very large, and the diffusion resistance of 1 cm^2 cuticle is twice that of R_{leaf}, given in Table 3.

It is more difficult to determine cuticular transpiration in amphistomatous leaves. The lower diffusion resistance of *Hyoscyamus* leaves may indicate that stomata contribute a little to dark transpiration. This seems likely, since infiltration with pure ether in darkness sometimes was possible. Also in *Hyoscyamus*, dark transpiration of both sides of the leaf was the same.

§ 2. TRANSPIRATION AT LOW LIGHT INTENSITY

The effect of light intensity was studied in relation to air temperature and humidity, using fluorescent tubes as a light source. Fig. 7 gives some examples of these experiments, which were carried out with *Hyoscyamus*, bean and tomato.

Within the range of light intensities studied, the transpiration rate shows a linear increase with light intensity at each air temperature and at each degree of humidity of the air. This increase may be due to increase in leaf temperature resulting in a higher value of e_s. In addition, an increase in stomatal opening with increasing light intensity, causing a lower stomatal resistance to diffusion, may be expected.

With increase in light intensity, only small differences in temperature between leaf and air were found, *viz.*, from –0.7 °C (in dark) to +1.6 °C (at 4.5×10^4 erg.sec^{-1}.cm^{-2} incident energy). This temperature effect of the irradiation contributes only little to the increase in transpiration at high values of $e_a - e_d$ (e_a = saturation vapour pressure at air temperature, e_d = actual vapour pressure of

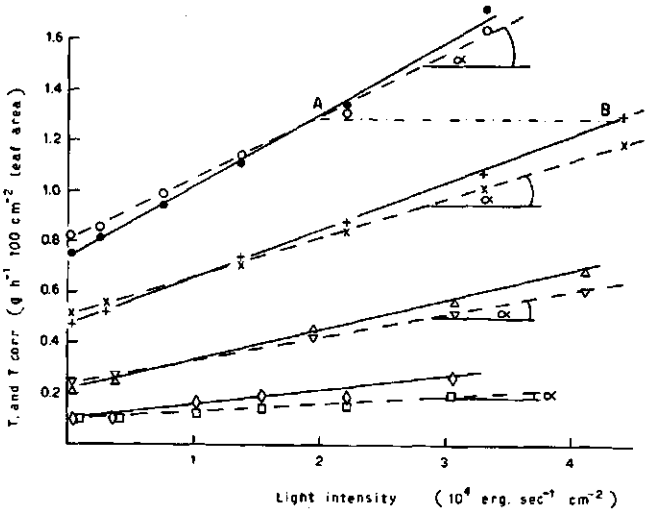


FIG. 7. The effect of light intensity (erg.sec^{-1}.cm^{-2}, fluorescent tubes) on the rate of transpiration of cut leaves of *Hyoscyamus* in potometers (g.h^{-1}.100 cm^{-2}) at various air temperatures and various relative humidities; ●——● 29 °C, 33% r.h.; +——+ 24 °C, 45% r.h.; △——△ 15.2 °C, 50% r.h.; ◇——◇ 5.2 °C, 60% r.h.
Broken lines with corresponding symbols the same, corrected for differences between leaf temperature and air temperature (T_{corr}).

the air). At low values of $e_a - e_d$, *e.g.*, at 5.2 °C air temperature, this effect is more important (fig. 7).

Equal transpiration rates may occur at different light intensities as, *e.g.*, indicated by A..... B in fig. 7. The additional amount of energy absorbed at B as compared with A, *viz.*, 1.8×10^4 erg.sec^{-1}.cm^{-2} leaf area (absorption being 75 %, see p. 7), is mainly converted into thermal emmission. Taking into consideration values from literature for the coefficient of heat transfer under "still air" conditions (BROWN and WILSON, 1905; HUBER, 1935), ranging from 1.6×10^4 to 4.8×10^4 erg.sec^{-1}.cm^{-2} cuticle for 1 °C difference in leaf and air temperature, differences between leaf and air temperatures of the order of 1 °C may be expected. As stated above, this has been confirmed by measurement of the leaf temperature.

The effect of light intensity on stomatal opening has been studied by microscope observations (see § 6) and by infiltration experiments. The infiltration at increasing light intensity of liquids of increasing surface tension demonstrates that the stomata regularly increase in opening with increasing light intensity. This was observed between 5° and 30 °C in *Hyoscyamus*, and between 10° and 30 °C in bean and tomato. Infiltration of bean and tomato leaves at temperatures lower than 10 °C was impossible, indicating inhibition of stomatal opening at these temperatures. In bean and tomato, the increase in transpiration at temperatures lower than 10 °C, as reported earlier (KUIPER and BIERHUIZEN, 1958), thus appears mainly due to increase in $e_s - e_d$.

In fig. 7, the data of each separate curve have been reduced to the value of $e_a - e_d$, so that the effect of light intensity on $e_s - e_d$ is excluded, in order to study other light effects on transpiration. If the evaporation formula holds for the transpiration of leaves under our experimental conditions, the increase in

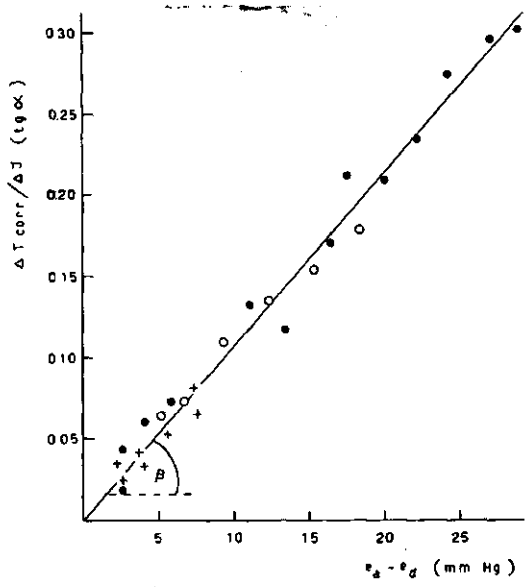


FIG. 8.
Increase in transpiration ($g.h^{-1}$. 100 cm^{-2}) of *Hyoscyamus* leaves per unit increase inlight intensity (10^4erg. $sec^{-1}.cm^{-2}$, fluorescent tubes) in relation to the difference in vapour pressure (e_a–e_d, in mm Hg), *viz.*, tg α obtained from the corrected transpiration/light intensity curves of fig. 7, and similar ones. The corrected leaf temperatures are indicated by ● (>25 °C), ○ (between 15° and 25 °C), and + (between 5° and 15 °C)

transpiration with light intensity along these corrected curves should be proportional to the decrease in the total diffusion resistance, $R_{air} + R_{leaf}$, since in that case $\Delta T_{corr}. \Delta R_{total} = e_a - e_d$. One then may expect a linear relation between tg α (tg $\alpha = \Delta T_{corr}/\Delta I$, in which α represents the slope of the corrected curves of fig. 7), and $e_a - e_d$. This has been plotted in fig. 8, from the corrected curves of fig. 7 and similar ones, and indeed, a linear relationship is found.

In fig. 8, the value of tg β, *viz.*, $\Delta T_{corr}/\Delta I$ per mm Hg, is 1.09×10^{-2} $g.h^{-1}$. $(mm\ Hg)^{-1}$.100 cm^{-2} leaf area. $(10^4\ erg.sec^{-1}.cm^{-2})^{-1}$, or 1.09 "units". In an earlier publication (KUIPER and BIERHUIZEN, 1958), tg β for bean and tomato was 1.33 and 1.36 units respectively, from uncorrected curves of transpiration *versus* light intensity. In the case of bean 1.18 units is found after correction of the transpiration curves to $e_a - e_d$. Thus, the transpiration rates of these three species appear to be about equally affected by light intensity. Since the leaves have about equal numbers of stomata per unit area, *viz.*, 250 per mm^2, the stomata probably will show very similar reactions to light intensity in these species.

Changes of R_{leaf} must be responsible for the decrease in R_{total} with increasing light intensity, since R_{air} is light-independent (see fig. 4). The change in R_{leaf} may be due to a change in cuticular resistance to diffusion, to a decrease in stomatal resistance to diffusion, and to changes in other diffusion resistances, as, *e.g.*, an "incipient drying" resistance of the mesophyll cell walls.

Cuticular transpiration of the (hypostomatous) bean leaves was determined at different light intensities by measuring the amount of water vapour, produced by the upper leaf side. Although these measurements showed some variation, no influence of light intensity was apparent. Since R_{air} was very small in this experiment, it is improbable that the cuticular resistance to diffusion is affected by light intensity in our experiments.

Under the conditions of our potometer experiments it is difficult to prove that no "incipient drying" resistance exists. One would expect similar reactions to light intensity of an "incipient drying" resistance and of the cuticular resistance, since the water content of the leaf – an important factor in these resistances – decreases rapidly with increasing light intensity. A hypothetical "incipient drying" resistance is assumed to be independent of light intensity in our potometer experiments, since the cuticular transpiration was not affected by light intensity, and since both cuticular transpiration and tg α (tg $\alpha = \Delta T_{corr}/\Delta I$) show a linear relation with the difference in vapour pressure between leaf and air. A discussion on "incipient drying" is given in chapter 5, § 2.

The observed linear increase with light intensity of the corrected transpiration curves of fig. 7 thus appears to be completely due to the change in the stomatal diffusion resistance. In the next section it will be shown that a hyperbolic curve relates stomatal resistance to diffusion and light intensity.

Fig. 8 shows that the tg α-values are largely independent of temperature, considering the large variation in temperature at low values of $e_a - e_d$ (*e.g.*, $e_a - e_d = 2.5$ mm Hg at 5.5 °C, 63 % rel. humidity, and at 27.1 °C, 90 % rel. humidity). A small variation in tg α still might be expected, owing to variation in the diffusion coefficient k. This small variation of k apparently doet not surpass the errors of measurement, as was also observed with respect to dark transpiration. Since the increase of T_{corr} with increasing light intensity is due only to the decrease in stomatal diffusion resistance, we may conclude that this decrease, and the stomatal diffusion resistance as such, are independent of temperature. This shows that the stomatal resistance to diffusion is regulated by a photochemical process under the conditions of these experiments.

§ 3. Transpiration in strong light

In this section, experiments will be discussed in which the transpiration rate of cut leaves in potometers was measured in a wide range of light intensities.

An example is given in fig. 9, with fluorescent tubes as a light source. Two different ranges of light intensities may be distinguished with respect to the rate of transpiration, *viz.*, below and above 5×10^4 erg.sec^{-1}.cm^{-2} incident energy. In the lower range, transpiration increases linearly with light intensity, as has been discussed in the previous section. At light intensities above 5×10^4 erg. sec^{-1}.cm^{-2}, also a linear increase in the rate of transpiration exists, but less pronounced. In the same figure, a transpiration curve, reduced to the value of

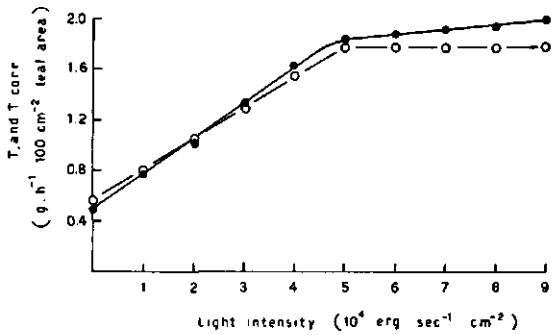


FIG. 9.
Effect of light intensity (erg. sec^{-1}.cm^{-2}, fluorescent tubes) on transpiration of bean leaves at 30.1 °C air temperature, 31% r.h. (●——●); o——o same, corrected for differences in leaf temperature and air temperature along the curve (e_a–e_d = 22 mm Hg)

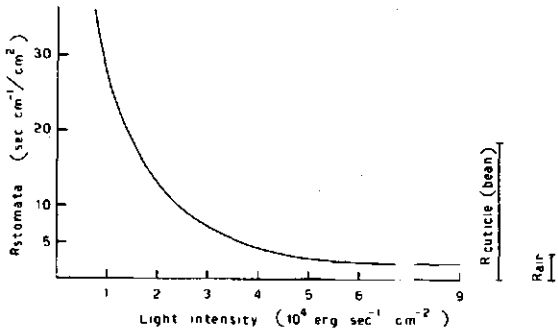


FIG. 10.
The relation between stomatal resistance to diffusion (sec.cm^{-1}, per cm^2 leaf area) and light intensity (erg.sec^{-1}.cm^{-2}) compiled from fig. 7 and 9 and similar ones. Fluorescent tubes; area of leaves used about 100 cm^2. For comparison the diffusion resistance of the boundary layer of air and of the cuticula are also presented.

$e_a - e_d$, is presented. It is clear that the increase in transpiration rate above 5×10^4erg.sec^{-1}.cm^{-2} is completely due to the increased leaf temperatures, caused by the irradiation, which results in increased values of e_s and thus of $e_s - e_d$.

It was shown in the previous section that the increase in transpiration rate along the corrected curve is due to a decrease in stomatal resistance to diffusion. This resistance reaches its lowest value at a light intensity of about 5×10^4 erg. sec^{-1}.cm^{-2}, where stomatal opening is maximal (see § 6). In our experiments, this light intensity varied from 4×10^4 to 6×10^4 erg.sec^{-1}.cm^{-2}.

Fig. 10 demonstrates that stomatal diffusion resistance is related to light intensity by a hyperbolic curve, assuming that no "incipient drying" of the mesophyll cell walls occurs. The stomatal resistance to diffusion is calculated according to the formula $T_{corr}\,(R_{air} + R_{leaf}) = e_a - e_d$, in which R_{leaf} consists of $R_{cuticle}$ and $R_{stomata}$, the latter only being affected by light intensity. The minimum value of $R_{stomata}$ is somewhat below that of R_{air}, *viz.*, 2.60 and 3.74 sec.cm^{-1} per cm^2 area respectively. The minimum value of $R_{stomata}$ varied between 2.4 and 3.4 sec.cm^{-1} per cm^2 leaf area, and was found to be about equal in *Hyoscyamus*, bean and tomato leaves under evaporation conditions with different values of $e_s - e_d$.

It is remarkable that the corrected transpiration curve of fig. 9 – which shows the changes of $1/R_{total}$ – is a nearly ideal curve of the theoretical BLACKMAN-type, showing a strict limitation. The sharp transition in the curve, as represented in this figure, was generally found in these experiments, while similar curves were obtained from experiments with intact bean plants with their roots in aerated tap water.

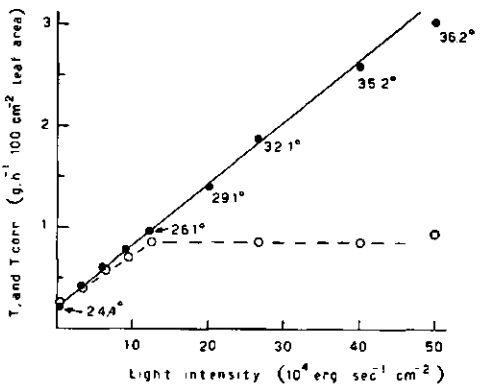
§ 4. THE EFFECT OF LIGHT FROM DIFFERENT SOURCES ON TRANSPIRATION

Experiments on cut bean leaves in potometers were made with different light sources. Transpiration versus light intensity curves were determined while measurement of the leaf temperature allowed correction for differences between leaf temperature and air temperature.

There was a marked difference between transpiration under incandescent lamps, even provided with a water filter, and that observed with other light sources.

With incandescent lamps, the transpiration rate increases linearly with incident energy, as shown in fig. 11. The leaf temperature rises considerably above the air temperature with increase in light intensity, *viz.*, from –0.5° to +12°C

FIG. 11.
The effect of light intensity (erg.sec^{-1}.cm^{-2}, incandescent lamps with a water filter, containing 60% infrared radiation) on transpiration and leaf temperature of bean leaves; (●——●) at 25°C air temperature, and 60% r.h.; (○——○) same, corrected for differences between leaf temperature and air temperature along the curve; e_a-e_d = 9.5 mm Hg.



in the range studied. Correction of the transpiration curves for differences in $e_s - e_d$, by reduction to $e_a - e_d$, shows that a large part of the increase in transpiration along this curve is due to increase in $e_s - e_d$. The transpiration curve is straight, in contrast to the broken curve in fig. 9, in which the increase in $e_s - e_d$ along the transpiration curve is small.

Since the irradiation from incandescent lamps, even provided with a 15 cm water filter, contains a large amount of infrared radiation – 60 %, as measured with SCHOTT RG 8 filter – the effect of the infrared radiation transmitted by this filter on transpiration of bean leaves was measured separately. Within the range of intensities of infrared radiation, *viz.*, from zero up to 10×10^4 erg.sec^{-1}.cm^{-2}, the increase in transpiration with the intensity of infrared radiation was completely due to increase in $e_s - e_d$, and no change in the stomatal resistance to diffusion was evident. Also, infiltration of the leaf with pure ether was impossible. It is evident that stomata are insensitive to infrared radiation. At least 60 % of the irradiation, obtained from the incandescent lamps, is inactive with respect to stomatal opening.

It is clear, therefore, that for a comparison of different radiation sources with regard to their effect on stomatal opening, only the spectral range between 400 and 700 m μ should be taken into consideration. The results of such a comparison are summarized in table 4. Within the spectral range of 400 to 700 mμ the effect of light intensity on transpiration is about the same for each light source used (column 5). Only in the case of mercury vapour lamps, a somewhat lower value is obtained. Accordingly, in this case, the corrected transpiration curve reaches its saturation level at a higher light intensity (column 7). Thus, the nearly equal effectivity of the various light sources does not reflect the large differences in spectral composition of the irradiation. The absorbed radiation from the respective light sources by the leaf in quanta per incident erg is represented in table 4, column 4 (taken from GAASTRA, 1959). These figures show at first sight probably only surprisingly small differences (about 20 % maximally). Obviously, the specific absorption of the receptive pigment is smoothed out by the absorption of the leaf as a whole under the conditions of the present experiments. Taking into consideration the errors of calibration measurements of light intensity, the similarity of the figures in column 4, 5, and 7 moreover indicates that there is no important inactive absorption at specific wavelengths with the spectral region considered.

TABLE 4. The effect of light from different sources on transpiration of cut bean leaves in potometers. Mean values of at least 10 curves of transpiration *versus* light intensity for each source. Column 4: absorbed radiation in 10^{-13} EINSTEINS per incident erg; values taken from GAASTRA (1959, table 5, "average leaf"), except in the case of sodium lamps, where the figure was calculated from an absorption measured in the ULBRIGHT sphere (75%). Columns 5 and 6: increase in transpiration per unit light intensity per mm vapour pressure difference in g water.h^{-1}. 100 cm^{-2} leaf area. (mm Hg)$^{-1}$.[10^4 erg.sec.$^{-1}$.cm^{-2} incident energy]$^{-1}$ (is tg β in fig. 8). Column 7: light intensity in erg.sec^{-1}.cm^{-2}, at which corrected transpiration reaches light saturation

1	2	3	4	5	6	7
Light source	Filter	Infrared percentage	Absorbed radiation (400–700 mμ)	$\frac{T_{corr}}{I(e_a-e_d)}$ (400–700 mμ)	$\frac{T_{corr}}{I(e_a-e_d)}$ (+ infrared)	Light intensity at saturation level (400–700 mμ)
Incandescent lamps (500 W)	water filter, 15 cm	60	4.02 (100)	1.18×10^{-2}	0.47×10^{-2}	5×10^4
Sodium lamps	water filter + $CuSO_4$-filter	1	3.72 (93)	1.24×10^{-2}	1.24×10^{-2}	not reached
Fluorescent tubes (TL 55)	colourless glass panel	6	3.61 (89)	1.16×10^{-2}	1.12×10^{-2}	5×10^4
High pressure mercury vapour lamps (HO450)	water filter, 15 cm	7	3.20 (80)	1.00×10^{-2}	0.93×10^{-2}	6.5×10^4

§ 5. The effect of light intensity on transpiration of leaves, grown under different light intensities, and of leaves of different age.

Experiments with cut leaves of bean and tomato in potometers were carried out, using sodium lamps or fluorescent tubes as a light source. For each leaf the transpiration versus light intensity curve, reduced to the same value of $e_s - e_d$, *viz.*, $e_a - e_d$, was determined from measurements of transpiration and leaf temperature at four or five different light intensities. The experimental plants were grown at about 20 °C under the following light intensities, obtained from fluorescent tubes and irradiation from above only: 0.5, 1, and 2.5×10^4 erg.sec^{-1}.cm^{-2}; with irradiation from above and from aside: 2.5 and 8×10^4 erg.sec^{-1}.cm^{-2}.

The colour of the leaves varied from pale green to dark green with the light intensity at which the plants were grown. The dark green leaves, grown at high light intensity, were rather thick as compared with leaves, grown in weak light. Relative values of the chlorophyll content per unit leaf area were determined in punched-out leaf discs by extraction with 85 % ethanol. The extinction of the chlorophyll solution was measured at wavelength 665 mμ. Estimation of the chlorophyll content in absolute figures was not attempted.

The number of stomata per unit leaf area varies from 150 to about 400 per mm^2 leaf area, according to age and light regime during growth. However, the number of epidermis cells per stoma was constant in each species.

In order to determine whether leaves, grown under different light intensities, differ in stomatal response to light the increase in transpiration per unit light intensity and per unit vapour pressure difference, *viz.*, $\Delta T_{corr} / \Delta I (e_a - e_d)$ (is tg β in fig. 8) was calculated in the range from zero to 3×10^4 erg.sec^{-1}.cm^{-2}, applied in the transpiration measurements. Since these leaves have different numbers of stomata per unit leaf area, the transpiration rate was calculated per 2.5×10^6 stomata – corresponding to 100 cm^2 leaf area with 250 stomata per mm^2 – assuming that stomatal transpiration is proportional to the number of stomata.

In this assumption, the diffusion resistance of the air layer is left out of consideration, which is not allowed, since different surface areas are considered, related to the number of stomata. Therefore, the average diffusion resistance of the air layer for the leaf area with 2.5×10^6 stomata ranges from 2.4 sec.cm^{-1} (400 stomata per mm^2) to 6.4 sec.cm^{-1} (150 stomata per mm^2) per cm^2 area, as may be calculated from the value of 3.74 sec.cm^{-1} per cm^2 blotting paper area (see table 1). Since, especially at the low light intensities of these experiments, the stomatal resistance to diffusion is much larger than that of the air layer, this variation of R_{air} was left out of consideration.

Some results, referring to chlorophyll content, obtained with bean leaves and sodium lamps as a light source, are given in fig. 12. It is evident that within the range from 0.15 to 0.19 extinction units no difference in stomatal reaction upon light intensity (as expressed by tg β in fig. 8) exists, although the leaves were grown under strongly different light intensities. This is confirmed in experiments with tomato leaves, even in a wider range of chlorophyll contents of the leaves, *viz.*, between 0.15 and 0.37 extinction units, the leaves varying in colour from light green to dark green (fig. 13). Below the extinction value of 0.15 units, the effect of light intensity upon stomatal opening, as expressed above, clearly is smaller in bean as well as in tomato. However, the ordinates of fig. 12 and of fig. 13 indicate the increase in transpiration per unit increase in incident energy.

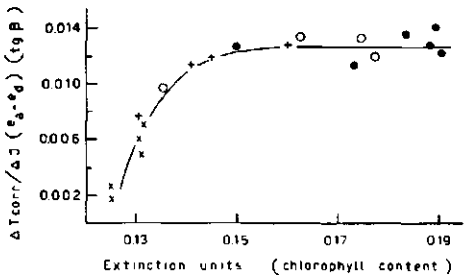


FIG. 12.
The increase in transpiration of bean leaves [g.h^{-1}.(2.5 × 10^6 stomata)$^{-1}$] per unit increase of incident light intensity (erg.sec^{-1}.cm^{-2}, sodium light) per unit vapour pressure difference (e_a–e_d, in mm Hg), analogous to tg β of fig. 8, in relation to the chlorophyll content of the leaf, expressed as extinction value at 665 mμ. Symbols represent the light conditions in which the experimental plants were grown:

(●) irradiation from above and from aside, 8 × 10^4 erg.sec^{-1}.cm^{-2},
(○) „ „ „ „ „ „ 2.5 × 10^4 erg.sec^{-1}.cm^{-2},
(+) irradiation from above 1 × 10^4 erg.sec^{-1}.cm^{-2},
(×) „ „ „ 0.5 × 10^4 erg.sec^{-1}.cm^{-2}.

The effect of the lower light absorption connected with the pale green colour of these leaves is manifest between the extinction values of 0.125 and 0.15. The absorption of some of these pale green leaves was determined in an ULBRICHT sphere in sodium light, and was between 20 and 55 %, while leaves grown under more favourable light conditions showed an absorption of about 75 %. Clearly, the observed values of tg β in fig. 12 and 13 are fairly proportional to the relative light absorption, values of tg β of 0.003, 0.008, and 0.010 corresponding to 20, 55, and 75 % light absorption respectively. Thus, the increase in transpiration per unit absorbed energy evidently is about the same in all leaves and independent of chlorophyll content and of the light conditions in which the plants were grown. It is, therefore, probable that the stomatal light response depends on the total quantity of light absorbed by the leaf. In addition, it is observed that it does not matter from which side the hypostomatous leaves are irradiated.

In tomato plants, irradiated from above, the young leaves grow under a

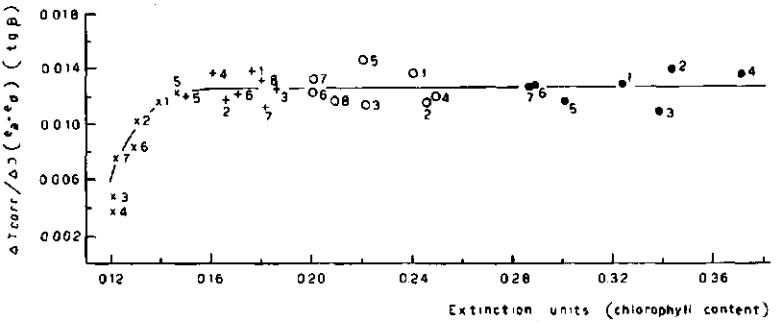


FIG. 13. The increase in transpiration of tomato leaves (g.h^{-1}.(2.5 × 10^6 stomata)$^{-1}$) per unit increase of incident light intensity (10^4 erg.sec^{-1}.cm^{-2}, sodium light), per unit vapour pressure difference (e_a–e_d, in mm Hg) in relation to the chlorophyll content of the leaves, expressed as extinction value at 665 mμ (cf also fig. 12). Symbols represent the light conditions in which the experimental plants were grown and the age of the leaves.

(●) irradiation from above and from aside, 8 × 10^4 erg.sec^{-1}.cm^{-2},
(○) „ „ „ „ „ „ 2.5 × 10^4 erg.sec^{-1}.cm^{-2},
(+) irradiation from above 2.5 × 10^4 erg.sec^{-1}.cm^{-2},
(×) „ „ „ 1 × 10^4 erg.sec^{-1}.cm^{-2}.
(1) = youngest leaf, (8) = eldest leaf of a plant.

higher light intensity than the older ones. In studies of the effect of age on the curve of transpiration *versus* light intensity, young tomato plants were grown under different light intensities while irradiated from above and aside, so that all leaves of a plant grew up under similar light conditions. It is evident from fig. 13 that the age of the leaves (1 = youngest leaf, 8 = oldest leaf) has no visible effect on the increase in transpiration per unit light intensity per equal number of stomata.

Since it was observed that the number of stomata per unit leaf area decreases with age, the increase in transpiration rate per unit leaf area, per unit light intensity, is higher in young leaves than it is in older ones.

Dark transpiration of very young, still somewhat folded leaves is higher than that of full-grown leaves. Infiltration of the young leaves with ether was sometimes possible in dark. Since dark transpiration of the lower leaf side of this hypostomatous leaf was about twice that of the upper leaf side, one may conclude that the stomata of young tomato leaves are not completely closed in darkness.

§ 6. Microscope observations on stomatal light response

It has been shown in the previous sections that the stomata strongly affect the transpiration rate of a leaf, by influencing the resistance to diffusion. For this reason, some microscope observations on the effect of light on the stomatal aperture of bean leaves were made.

In the first experiment, the effect of the intensity of light from a high pressure mercury vapour lamp on the width of the stomatal slit was determined. The leaf was exposed to the "still air" conditions of the experimental room. Stomatal aperture increases rapidly between 3×10^4 and 6×10^4 erg.sec^{-1}.cm^{-2}, and more slowly so in weaker and stronger light (fig. 14A). The maximal aperture reached was 5.5 μ at an incident light intensity of about 7×10^4 erg.sec^{-1}.cm^{-2}. These figures for light intensity refer to the region from 400 to 700 mμ, since the stomata are insensitive to infrared radiation (§ 4). The relation between width of stomatal slit and stomatal resistance to diffusion is given in fig. 14B, based on the relation of stomatal aperture to light intensity (fig. 14A) and on that of

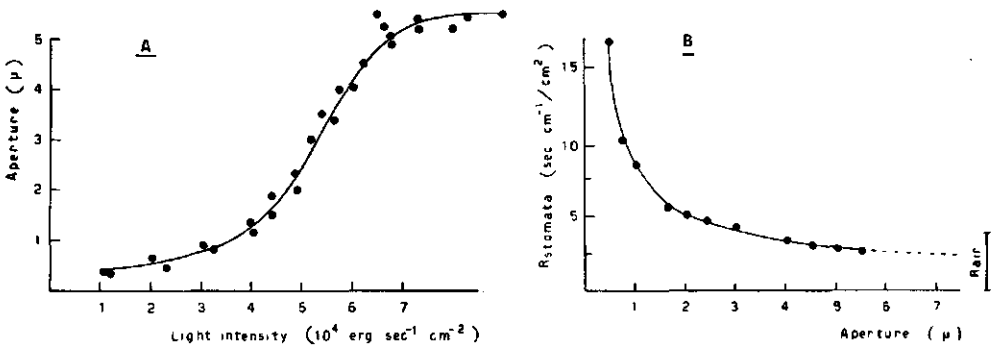


FIG. 14. A. Width of the stomatal slit (in μ, average of 20 measurements) in relation to light intensity (erg.sec^{-1}.cm^{-2}, mercury lamp); microscope observation, bean leaf.
B. Stomatal resistance to diffusion (sec.cm^{-1}, per cm^2 leaf area) in relation to slit width (μ), computed from fig. 14A and an analogue of fig. 10;
(———) extrapolation to larger apertures.

stomatal diffusion resistance to light intensity; the latter type of relation is shown in fig. 10 (other light source). It is clear that changes in the width of the stomatal slit below 3 μ have a large effect on the stomatal resistance to diffusion, whereas at about 5 μ, the the stomatal resistance to diffusion is affected only little. A pronounced regulation of transpiration by changes in stomatal resistance to diffusion, therefore, only obtains at small apertures.

In the following experiments, a flat cup of lucite was placed upon the leaf, and air of known CO_2-content (0 %, 0.03 %, 1 %, and 5 % CO_2) blown through. The humidity of the air was maintained at about 12 mm Hg; air temperature was 21 °C. The turbulent movement of air through the cup, *viz.*, 4.6 cm^3 air. $sec^{-1}.cm^{-2}$ leaf area provided sufficient gas exchange.

No opening of stomata has been observed in air containing 1 % CO_2 or 5 % CO_2.

The opening reaction of the stomata was studied in normal air (0.03 % CO_2), and in CO_2-free air. Previously, the leaf was exposed to normal air at a light intensity of 4×10^4 erg.$sec^{-1}.cm^{-2}$, resulting in a stomatal aperture of 1.7 μ. Then, four different treatments were given, the leaves being exposed to normal air or to CO_2-free air at the low light intensity mentioned, or at a higher one, *viz.*, 20×10^4 erg.$sec^{-1}.cm^{-2}$.

It is evident from fig. 15 that stomata increase their aperture within five minutes after the stronger irradiation has started, at a mean rate of increase in width of the stomatal slit of about 1 μ in 6 minutes, in normal air as well as in CO_2-free air. However, the maximal aperture in normal air is about 5.5 μ width, reached after 30 minutes, whereas the width of the stomatal slit continues to increase up to 7.5 μ in CO_2-free air, reached after 45 minutes. It is clear that in normal air an increase in aperture above 5.5 μ is prevented by the carbon dioxide content of the air.

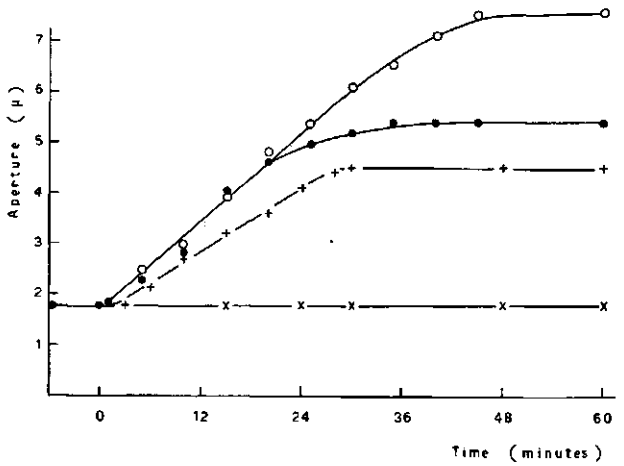


FIG. 15. Opening reaction of the stomata of bean leaves at 21 °C air temperature (slit width in μ, average of 30 as observed by microscopy).
(● —— ●), upon shift from normal air (0.03% CO_2) and weak light (4×10^4 erg.$sec^{-1}.cm^{-2}$) to normal air and strong light (20×10^4 erg.$sec^{-1}.cm^{-2}$);
(o —— o), same for shift to CO_2-free air and strong light, and
(+——+) for shift to CO_2-free air and weak light;
(×——×), continued exposure to normal air and weak light.

Transfer to CO_2-free air under the light conditions, applied in the pretreatment, yields an increase in aperture at a rate of about 1 μ in 9 minutes, the maximal width is 4.5 μ, and is reached after 30 minutes. The degree of opening of the stomata in weak light and in normal air remains constant for a long time. It should be noted that in complete darkness the width of the stomatal slit was 4.4μ in CO_2-free air.

It is quite evident from fig. 15 that stomatal opening strongly reacts upon decrease in CO_2-content of the air from 0.03 % CO_2 to CO_2-free by changing from 1.8 μ to 4.5 μ in weak light and from 5.5 μ to 7.5 μ in strong light. There also exists an effect of light intensity, *viz.*, from 1.8 μ to 5.5 μ in normal air and from 4.5 μ to 7.5 μ in CO_2-free air. The data on the relation between width of stomatal slit and stomatal resistance to diffusion *(vide* fig. 14B*)* is summarized in table 5. The stomatal resistance to diffusion is about the same in strong light – both in CO_2-free air and in normal air – and in weak light or in darkness in CO_2-free air. Regarding the stomatal diffusion resistance, the effect of strong light is nearly quantitatively equal to that of CO_2-free air.

TABLE 5. The effect of light intensity and carbon dioxide content of the air on stomatal opening in bean leaves, represented as width of stomatal slit in μ, and as stomatal resistance to diffusion in sec.cm^{-1} per cm^2 leaf area (with 2.5 $\times$ 10^4 stomata)

Light intensity (erg.sec^{-1}.cm^{-2})	Normal air (0.03 % CO_2)		CO_2-free air	
	Slit width (μ)	Diff. resistance	Slit width (μ)	Diff. resistance
0	(closed)	(very large)	4.4	2.98
4 $\times$ 10^4	1.8	5.54	4.5	2.94
20 $\times$ 10^4	5.5	2.60	7.5	2.08

The closing reaction of the stomata upon a decrease in light intensity from 20 $\times$ 10^4 erg.sec^{-1}.cm^{-2} to 4 $\times$ 10^4 erg.sec^{-1}.cm^{-2} was followed in normal air

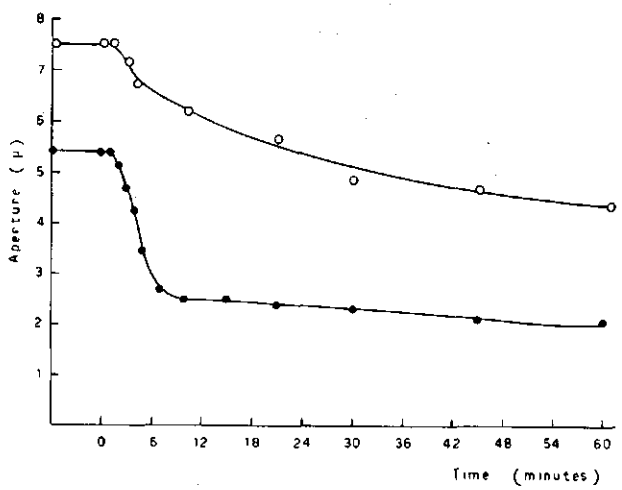


FIG. 16. Closing reaction of the stomata of bean leaves at 21 °C air temperature (slit width in μ, average of 30 as observed by microscopy).
(● ——●) upon shift from strong light (20 $\times$ 10^4 erg.sec^{-1}.cm^{-2}) to weak light (4 $\times$ 10^4 erg.sec^{-1}.cm^{-2}), in normal air;
(o —— o) same in CO_2-free air.

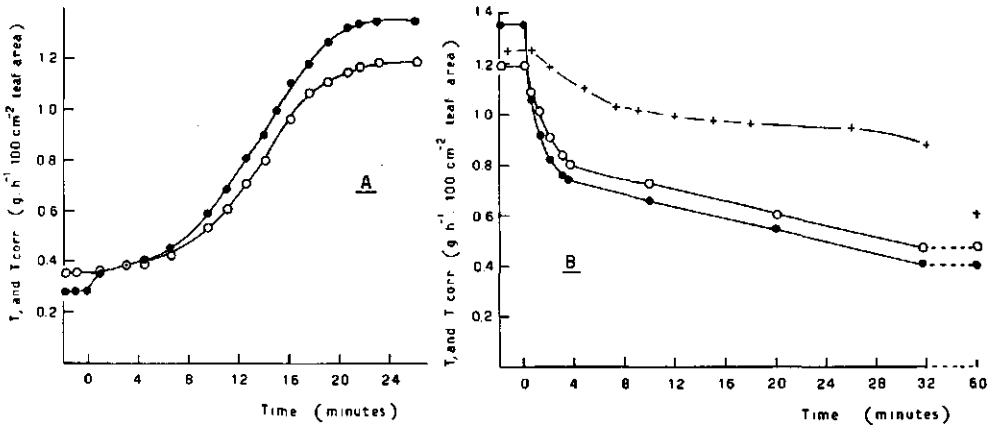


FIG. 17. A. Transpiration rate of a bean leaf (● —— ●) as observed by rapid weighing at short intervals, after transfer from darkness to strong light (20×10^4 erg.sec^{-1}.cm^{-2}, mercury lamp);
(o —— o) same, corrected for differences between leaf temperature and air temperature along the curve (e_a–e_d = 15 mm Hg, air temperature 26 °C, 41% r.h.).

B. Transpiration rate of a bean leaf (● —— ●) as observed by rapid weighing at short intervals, after transfer from strong light (20×10^4 erg.sec^{-1}.cm^{-2}, mercury lamp) to darkness;
(o —— o) same, corrected for differences between leaf temperature and air temperature (e_a–e_d = 15 mm Hg, air temperature 26 °C, 41% r.h.);
(+——+) corrected transpiration curve of a bean leaf, which previously had taken up 0.3 cm^3 of a 0.01 N sodium azide solution through the petiole in light (e_a–e_d = 18 mm Hg, air temperature 28 °C, 36% r.h.).

and in CO_2-free air (fig. 16). In normal air, stomata decrease their aperture rapidly, at a rate of 1 μ in 1.5 to 2 minutes, which decrease becomes very slow after 10 minutes, when an aperture of 2.5 μ is reached. In CO_2-free air, a more gradual decrease in aperture is observed, though also in this case the decrease is more pronounced immediately after the exposure to the lower light intensity.

In addition to the microscope observations discussed above, stomatal opening and closure was followed indirectly by measuring transpiration by repeated rapid weighing at short intervals after transfer from darkness to strong light (fig. 17A), and *vice versa* (fig. 17B). It was observed that leaf temperature rises to its maximum within 10 seconds after exposure to strong light had started, and then gradually decreases to a constant value, 1.5 °C higher than the air temperature. The transpiration curve, corrected for differences between leaf temperature and air temperature, rises gradually to a maximum after 25 minutes, when the resistance to diffusion of the leaf has reached its minimum.

Transpiration falls directly after transfer to darkness, which is partly due to a decrease in leaf temperature, partly to a rapid increase of the stomatal resistance to diffusion. After five minutes, the decrease in transpiration is slower, corresponding to the observed smaller rate of decrease in aperture *(vide* fig. 16).

The remarkably rapid closing reaction in normal air may be explained by DECKER's observation of a CO_2-gush in a tobacco leaf after transfer to darkness. In order to study this effect in a bean leaf, we found Ir. G. A. PIETERS kind enough to perform some measurements on CO_2-exchange at different light in-

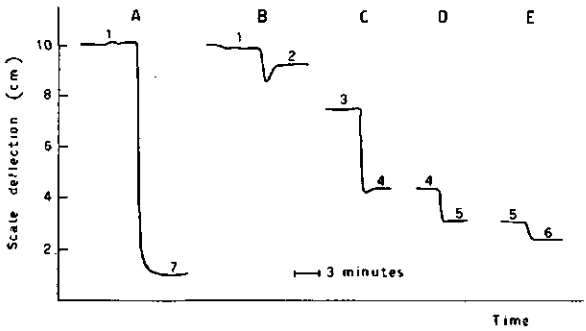


FIG. 18.
Time course of CO_2-exchange of a bean leaf in mm scale deflection of diaferometer upon a sudden decrease in light intensity at various intensity levels; (*1*) 18, (*2*) 8.1, (*3*) 6.3, (*4*) 3.2, (*5*) 1.8, (*6*) 1.5, and (*7*) 0×10^4 erg.sec^{-1}.cm^{-2} of radiation between 400 and 700 mμ; incandescent lamp, provided with a water filter; CO_2-content of the air 5%; air temperature 23 °C.

tensities with the diaferometer technique. However, air of 5 % CO_2 had to be used in this experiment. Some of the results are given in fig. 18. Upon transition from strong light to darkness (A: 1 →7), CO_2-absorption of the leaf ceased immediately. Transition from strong light to a lower light intensity (B: 1 →2) only gave a small ultimate decrease of the CO_2-absorption of the leaf. However, an initial CO_2-gush appeared directly after lowering of the light intensity. Such a CO_2-gush, though less pronounced, is also apparent at a similar transitions in a lower light intensity range (C: 3 →4). At transitions at still lower light intensities (D: 4 →5, E: 5 →6), the CO_2-outburst gradually disappears. Taking into consideration DECKER's results with a tobacco leaf, it seems very probable that such a CO_2-gush also is present in normal air in bean leaves (see also p. 39).

It may be concluded that a decrease in light intensity causes a rapid decrease in stomatal aperture in normal air (fig. 16). Between 10 and 30 minutes after transfer to weak light, a small further decrease in the width of the stomatal slit is observed, corresponding to a rather considerable increase in stomatal resistance to diffusion (fig. 17B). In normal air, there is a remarkable difference between the opening and closing reactions. After transfer from strong light to a lower light intensity, the initial CO_2-gush observed (in air with 5 % CO_2, see fig. 18, in normal air, see DECKER, 1955, 1959) may provide an explanation for the rapid closing reaction upon transfer to weak light or darkness.

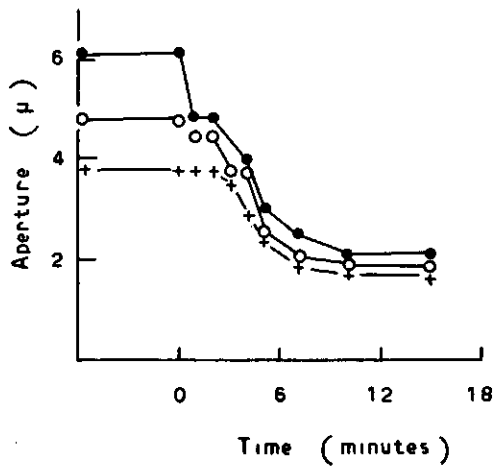


FIG. 19.
Closing reaction of three different stomata of a bean leaf at 21 °C air temperature, followed by microscopy, after transfer from strong light (20×10^4 erg.sec^{-1}.cm^{-2}) to weak light (4×10^4 erg.sec^{-1}.cm^{-2}) in normal air as representative examples.

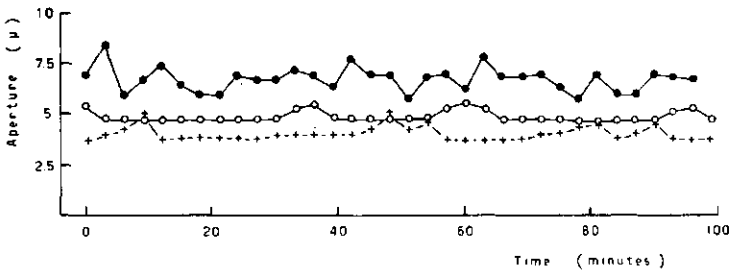


FIG. 20. Time course of variation in slit width in μ of three stomata of a bean leaf at 21 °C air temperature in continuous strong light (20×10^4 erg.sec^{-1}.cm^{-2}) and normal air as observed by microscopy.

Some additional remarks may be made on the behaviour of individual stomata. Contrary to figures 14, 15, and 16 which summarize mean values of 30 measurements on the width of the stomatal slit, figures 19 and 20 contain curves of three representive examples of individual stomata. It is clear from fig. 19 that the wider the stomatal aperture, the more rapidly the closing reaction starts. Taking into account the intitial CO_2-gush, observed at transition from strong light to weak light (fig. 18; DECKER, 1955), it may be supposed that, the wider the stomatal opening, the more sensitive the guard cell is to this CO_2-gush and to changes in CO_2-content in general.

In continuous strong light, fluctuations in the width of the stomatal slit occur (fig. 20). These fluctuations are most frequent and most pronounced at large aperture values, and may be related to a large sensitivity of the guard cells to changes in CO_2-content, just as in the closing reaction. Under the controlled conditions of this experiment, the rate of CO_2-fixation by photosynthesis is supposed to be constant as long as the conditions remain the same. Since the rate of diffusion of CO_2 from the air to the substomatal cavity depends on the stomatal resistance to diffusion, the CO_2-content in the substomatal cavity will vary according to fluctuations in the stomatal aperture and the stomatal resistance to diffusion. This variation in CO_2-content of the air in the substomatal cavity may affect the rate of diffusion of CO_2 from the substomatal cavity to the guard cell chloroplasts, resulting in fluctuations of photosynthesis of the latter, and consequently of the stomatal aperture. These oscillations of stomatal apertures thus would be due to two coupled processes, *viz.*, photosynthesis of the guard cell chloroplasts, and the response of the guard cells to CO_2, operating as a feed back with regard to the regulation of the stomatal aperture and of the stomatal resistance to diffusion.

Summarizing, the following conclusions can be drawn. The opening of the stomata brought about by strong light is almost quantitatively equal to that brought about by transfer to CO_2-free air in weak light or darkness (table 6). This suggests that the effect of light on opening of the stomata is primarily due to a decrease in CO_2-content of the guard cells by photosynthesis. It should be observed, moreover, that an increase in aperture from 4.4 μ to 7.5 μ by transfer from darkness to strong light in CO_2-free air is noticed, corresponding to a decrease in the stomatal resistance to diffusion from 2.98 sec.cm^{-1} per cm^2 leaf area (100 %) to 2.08 sec.cm^{-1} per cm^2 leaf area (70 %). It seems possible to ascribe this phenomenon to the consumption of respiratory CO_2 of the guard

cells in photosynthesis. Some further discussion on this aspect will be given later (chapter 5, § 3).

Taking into consideration the observed CO_2-gush upon a decrease in light intensity (fig. 18; DECKER, 1955), the rapid closing reaction may well be explained as a reaction upon the sudden increase in CO_2-content of the stomatal guard cells. At large apertures, stomata seem to be more sensitive to this CO_2-gush (fig. 19). For these reasons, it may be supposed that, in the experiments described, the main regulating factor in opening and closing of the stomata is the carbon dioxide content of the guard cells.

§ 7. THE EFFECT OF SOME CHEMICALS ON TRANSPIRATION AND ON STOMATAL LIGHT RESPONSE

A brief description of some preliminary experiments on the effect of some substances on transpiration and stomatal opening may follow. Cut tomato leaves of about 100 cm^2 area were allowed to absorb, via the petiole, about 0.3 cm^3 of the following 0.01 N solutions, in dark and in strong light (20×10^4 $erg.sec^{-1}.cm^{-2}$): sodium azide (NaN_3), hydroxylamine hydrochloride ($NH_2OH.HCl$), 0-phenanthroline, phenazine ($C_6H_4NC_6H_4N$), N-phenyl urethan ($C_6H_5NHCOOC_2H_5$), and potassium cyanide (KCN).

The leaves which had taken up one of these substances in dark were exposed to strong light two hours afterwards. The reaction of the stomata was followed by measuring transpiration by repeated rapid weighing at short intervals, while the results were corrected for differences between leaf temperature and air temperature. Increase in transpiration, due to increase in stomatal opening was found in leaves, treated with hydroxylamine or phenanthroline. Only a slight increase in transpiration, due to the irradiation effect on the leaf temperature, occurred in leaves treated with the other substances. Thus, stomatal opening in strong light is inhibited by sodium azide, phenyl urethan, and potassium cyanide, taken up in dark before.

Measurements on CO_2-fixation by photosynthesis in the same leaves showed that the last three substances had strongly inhibited photosynthesis. No effect of hydroxylamine and phenanthroline on photosynthesis was observed at the concentrations applied. Obviously, these substances either did not reach the chloroplasts or were used in too low a concentration. It should be noted that in leaves which had taken up 0.2 cm^3 of a 0.2 N hydroxylamine solution before, stomatal opening and photosynthesis were strongly inhibited.

The leaves which had been allowed to take up the chemicals in strong light showed a different behaviour. Rapid decrease in transpiration, due to closing of the stomata, appeared in leaves, treated with phenyl urethan and potassium cyanide. Decrease of transpiration to about 70 % of the preceding level after one hour was observed in leaves, treated with sodium azide, while no decrease in transpiration was detectable in leaves, treated with hydroxylamine or phenanthroline (0.01 N-solutions).

Leaves, treated with sodium azide in strong light, were then transferred to darkness. Only a small decrease in transpiration was observed, instead of the rapid decrease occurring in normal leaves (see fig. 17B). It may be concluded that leaves, treated with sodium azide, do not show the normal stomatal opening reaction upon dark-light transition, nor the rapid closing reaction

upon light-dark transition. It seems that sodium azide prevents changes in stomatal aperture, irrespective of the light conditions of the experiment.

CHAPTER 5.

DISCUSSION

§ 1. CUTICULAR TRANSPIRATION AND DARK TRANSPIRATION

In the present paper the transpiration rate of cut leaves in darkness and under various light conditions has been considered as determined by a diffusion process, to which the formula $T \cdot R_{total} = e_s - e_d$ may be applied (p. 8). Recently, MILTHORPE (1958, 1960) made a theoretical analysis of transpiration of plants, using a similar formula.

In the hypostomatous leaves of bean and tomato, the resistance to diffusion in the dark appears to be mainly determined by the cuticula; in the amphistomatous *Hyoscyamus* leaves the value of R_{leaf} ($R_{total} - R_{air}$) in dark was lower which may indicate that stomata contribute to dark transpiration. A more exact determination of the stomatal resistance to diffusion in the dark in amphistomatous leaves is extremely difficult (STÅLFELT, 1956).

According to various data from literature, cuticular transpiration is only slightly raised by wind, *viz.*, 21 % (WRENGER, 1935), 23 % (STÅLFELT, 1932), 10–20 % (FIRBAS, 1931), 20 % (YAMAOKA, 1958). Since wind velocity affects R_{air}, probably $R_{cuticle}$ is mostly large as compared to R_{air}. Support for this could be derived from observations in bean and tomato (Chapter 4, § 1).

The cuticular resistance to diffusion did not show appreciable variation with light intensity (Chapter 4, § 2) nor with the evaporation conditions of the air, as one may conclude from STÅLFELT (1932), KUIPER and BIERHUIZEN (1958), and from Chapter 4, § 1 of this paper. Taking into consideration the variation in water content of the leaf at different light intensities (MELVILLE, 1937; GREGORY *et al.*, 1950), the existence of a resistance caused by drying of the epidermis cell walls and of the cuticula (cuticular "incipient drying") could not be demonstrated in our experiments with cut leaves in potometers. However, it is possible that it exists in leaves short of water (see *e.g.* STÅLFELT, 1932).

§ 2. STOMATAL AND NON-STOMATAL REGULATION OF TRANSPIRATION

Several investigators, *e.g.*, LIVINGSTON and BROWN (1912), SHREVE (1914), MAXIMOV (1929), and KLEMM (1956) have discussed the influence of "incipient drying" of the mesophyll cell walls, surrounding the intercellular spaces, on transpiration, also with respect to the stomata. However, their experimental data often are difficult to interprete, owing to less adequate techniques (LLOYD, 1908; KLEMM, 1956) or uncontrolled environmental conditions.

Numerous observations, both from literature and in this paper, tend to show that transpiration under several conditions is quantitatively regulated by variation in stomatal aperture, and we have found no evidence for the existence of an "incipient drying" resistance. On the other hand it seems that under conditions of water stress an "incipient drying" resistance may become manifest. Below, we will present data pertaining to these statements.

LIVINGSTON (1906) introduced the "relative transpiration" T/E, in order to

eliminate the effect of environmental factors equally affecting transpiration *(T)* and evaporation *(E)*. According to the notation in chapter 3, of the present paper, the relative transpiration may be expressed as follows:

$$\frac{T}{E} = \frac{R_{air}}{R_{leaf} + R_{air}} \cdot \frac{e_{leaf\ surface} - e_d}{e_{evaporimeter\ surface} - e_d}.$$

This ratio is affected by numerous external factors. Wind velocity affects it via changes in R_{air}; $e_{leaf\ surface}$ and $e_{evaporimeter\ surface}$ depend on the respective surface temperatures, which vary with many factors (wind velocity, light absorption, etc.). It is not surprising, therefore, that a certain discrepancy between relative transpiration and stomatal aperture is often found, especially in experiments under natural conditions, so that such experiments do not give direct information about the existence of "incipient drying". On the contrary, in some experiments, a clear relation between relative transpiration and stomatal aperture is observed (STÅLFELT, 1929). Also SHREVE (1914) observed a rather good agreement between T/E and stomatal aperture in experiments during the morning. The experiments of STÅLFELT and SHREVE probably were carried out under better controlled conditions. When no large differences between the temperatures of the leaf and the evaporimeter surface occur, a good agreement between T/E, which, in this case, represents $R_{air}/(R_{air} + R_{leaf})$, and stomatal aperture may be expected, and has indeed been found (STÅLFELT, 1932).

DARWIN (1916) observed a rather good correlation between stomatal aperture as derived from porometer readings and transpiration rate, especially at small apertures. STÅLFELT (1932), GREGORY and coworkers (1950), and MILTHORPE and SPENCER (1957) found transpiration largely independent of the water content of the leaf. They conclude that, in these experiments, incipient drying of the mesophyll cell walls did not contribute to the regulation of transpiration. More convincing is STÅLFELT's observation that relative transpiration – at a certain stomatal aperture – is largely independent of the evaporation conditions in the range between 146×10^{-3} and 505×10^{-3} g.h^{-1}.25 cm^{-2} area of wet blotting paper (see also BANGE, 1953). This could be confirmed in our experiments. The diffusion resistance of the leaf, in dark and under various conditions of illumination, is completely independent of the difference in vapour pressure between the leaf and the air, $e_s - e_d$, since transpiration was found proportional to $e_s - e_d$.

In a thorough investigation of the problem of stomatal regulation of transpiration, BANGE (1953) computed the relation between transpiration per unit vapour pressure difference and stomatal slit width. This estimate was based on the calculation of the successive diffusion resistances of substomatal cavity, stomatal porus, and micro-vapour-cups over the stomata. The fourth resistance, that of the adhering layer of air ("macro-vapour-cup"), was determined by measuring the evaporation of a wet blotting disc. BANGE obtained experimental results with leaf discs of *Zebrina pendula* in rather good agreement with his theoretically determined curve of transpiration *versus* stomatal aperture, in still air as well as in wind. At large apertures, between 8 and 20 μ, the stomatal resistance to diffusion no longer decreases appreciably so that effective regulation of transpiration by this resistance is possible only at small apertures (BANGE, fig. 20; STÅLFELT, 1932, fig. 12; this paper, fig. 14B); at large apertures the other diffusion resistances become apparent.

STÅLFELT (1932) found a relationship between transpiration and stomatal aperture in *Picea excelsa* needles. He concluded that transpiration is regulated by the stomata, which are small in this species.

In the experiments, described in Chapter 4 of the present paper, R_{leaf} remained constant at light intensities above 6.5×10^4 erg.sec^{-1}.cm^{-2}. Since the maximal degree of stomatal opening is reached at about the same light intensity (microscope observations), it is clear that the diffusion resistances R_{air}, $R_{cuticle}$, $R_{stomata}$, and also $R_{incipient\ drying}$ are constant above this level of light intensity. Between 0 and 6.5×10^4 erg.sec^{-1}.cm^{-2}, R_{total} decreases together with the increase in aperture of the stomata.

Taking into account the experimental results, presented in Chapter 4, it is clear that an incipient drying resistance of any importance would have interfered with the stomatal resistance to diffusion at least under some of the experimental conditions, and thus would have been noticed as a deviation in the observed relationship. Such deviation has never been observed and, therefore, it seems unlikely that an incipient drying resistance of any importance exists under our experimental conditions.

A theoretical analysis of the resistance of the water flow from the soil through the plant into the surrounding air has been carried out by GRADMANN (1928) and by VAN DEN HONERT (1948). Water transport was considered as a catenary process along a vapour pressure gradient. A drop in suction tension of the order of 10 atmospheres may be expected in the liquid phase of the plant, *i.e.* the cells of the root parenchyma, the xylem vessels, and the parenchyma cells of the transpiring leaf. However, in the gas phase, *i.e.* the intercellular spaces of the leaf, the stomata, and the adhering air layer, a fall of the order of 1000 atmospheres may be calculated, according to the following equation, derived from the gas law:

$$P = \frac{R\,T}{V} \ln(e_s/e_d)$$

P = suction tension or "diffusion pressure deficit" (atm.),
R = gas constant,
T = absolute temperature
V = molecular volume (18 cm^3),
e_s = saturation vapour pressure at surface temperature,
e_d = actual vapour pressure of the air.

Values of suction tension P have been given by HOMAN, YOUNG and SHULL (1934), CRAFTS, CURRIER and STOCKING (1949), and others.

GRADMANN and VAN DEN HONERT concluded that the liquid phase of the plant hardly contributes to the resistance for water transport from the soil through the plant into the surrounding air. Effective regulation of transpiration is only possible in the gas phase of the plant, and, for this reason, its magnitude can be controlled by the stomata.

To our opinion, the above formula only applies for equilibrium conditions, and its validity for natural processes, as, *e.g.*, transport of water, is questionable. The protoplasma membrane and the cell membranes of the parenchyma cells, surrounding the intercellular spaces of the leaf, may influence evaporation of water by their structure. Imbibition forces and osmotic pressure certainly have an effect on the vapour pressure of the parenchyma cells of the leaf. LIVINGSTON and BROWN (1912), and MAXIMOV (1928) have suggested that the mesophyll

cell walls, surrounding the intercellular spaces, are provided with submicroscopical pores. The water meniscii in these pores may retreat when the water supply through the xylem vessels decreases. Owing to the increased curvature of the water surface (GREGORY *et al.*, 1950), the resistance of these submicroscopical pores might well vary with the conditions of water supply of the leaf. However, there is no experimental evidence herefor.

Several investigators point to a certain discrepancy between transpiration and stomatal aperture. LIVINGSTON and BROWN (1912) observed a decrease in relative transpiration before any stomatal closure was detectable. Similar results are reported by SHREVE (1914), TRELEASE and LIVINGSTON (1916), and KNIGHT (1922), who generally assumed the possibility of regulation of transpiration via incipient drying of the mesophyll cell walls, especially at wide stomatal apertures. Since discrepancy between T/E and stomatal aperture often occurs in the afternoon, it is possible that the water supply to the leaves in some experiments becomes a limiting factor for transpiration via the effects mentioned above, and an incipient drying resistance becomes manifest. However, the experimental results do not allow a final conclusion regarding this question. Another possible explanation of a discrepancy between stomatal aperture and transpiration is the effect of several environmental factors on the T/E ratio when measured under various climatic conditions.

The existence of a possible incipient drying resistance is more plausible from anatomical observations. FREY-WEYSSLING and HÄUSERMANN (1941) and HÄUSERMANN (1944), in experiments with lipophilic and hydrophilic liquids ascending in narrow strips of *Dianthus* leaves conclude that the mesophyll cell walls, surrounding the intercellular spaces, are provided with a submicroscopical cutine layer. HUBER and coworkers (1956) observed a wrinkled cuticle-like layer, extending from the stomatal porus far into the substomatal cavity. Similar observations have been published by SCOTT (1950), who finds an "internal suberization" in various xerophytic, mesophytic, and hydrophytic leaves; it is especially obvious in xerophytes. In this connection, it is not surprising that early American investigators often found a disagreement between relative transpiration and stomatal aperture in desert plants in soil. It is quite possible that under such conditions incipient drying is effectively controlling transpiration, and we would not quite follow STÅLFELT (1956) who goes so far as to conclude that an effect of incipient drying on transpiration hardly exists.

It is known that stomata decrease their aperture when the water supply through the petiole is reduced (see STÅLFELT, 1956). It remains undecided whether at small apertures, an incipient drying resistance may become manifest by shortage of water.

Measuring the amount of water vapour, produced by leaves of *Vicia faba*, STÅLFELT (1932) found no relation between stomatal aperture and transpiration at large aperture values. It is evident that, at large apertures, a diffusion resistance other than the stomatal one is important in determining the transpiration rate. Taking into consideration the large variation of transpiration at large stomatal apertures, the observations of DARWIN (1916) and BANGE (1953) are in general agreement with those of STÅLFELT.

In BANGE's figures 13 and 14, representing transpiration per unit vapour pressure difference at apertures above 8 μ, the number of data above and below his theoretical curve are 51 and 17 respectively in still air, and 48 and 32 in wind. The points above the curve generally are at larger deviations than those below the curve. It may be concluded that a certain discrepancy

between the calculated resistance to diffusion and the experimental data exists above 8 μ, especially in still air. This discrepancy may be ascribed either to systematic errors of measurement, due to BANGE's experimental technique, or to small deviations of the supposed diffusion resistances from the actually existing ones. It is possible that a leaf disc punched out of a leaf, attached to a plant, shows a temporary rise in transpiration. IWANOFF (1928) and KAMP (1930) observed such a rise in leaves of in many plants, about 3 to 5 minutes after disturbing the water transport through the xylem vessels, by cutting the petiole in air. Both authors supposed this to be due to the sudden annihilation of the negative suction tension in the xylem vessels. Theoretical considerations lead BANGE to postulate micro-vapour-cups over the stomata. It is possible that the shape, proposed by BANGE, does not hold for large apertures, owing to air convections or mutual interference of the stomata. A choice between these possibilities and still other ones, *e.g.* the validity of the experimental value of k, is difficult.

A summary of this discussion shows that in experiments with cut leaves or with leaf discs, transpiration is regulated quantitatively by the stomatal aperture below a slit width of about 7 μ, and BANGE's experiments and calculations support this conclusion. At large stomatal apertures, transpiration may vary largely, and considerable deviations from the calculated relation between stomatal aperture and transpiration have been reported in literature. A satisfactory explanation cannot be given at the moment. Under conditions of water stress, transpiration may well be limited by the water supply through the petiole, causing an incipient drying resistance to become of importance. This is supported by anatomical observations on the cutine layer of the mesophyll cell walls, surrounding the intercellular spaces, which is especially obvious in several xerophytes.

§ 3. MECHANISM OF STOMATAL RESPONSE TO LIGHT

It is clear from the results, presented in Chapter 4, § 2, that the variation with light intensity of the stomatal resistance to diffusion is independent of temperature, leaving out of consideration a small temperature effect on the diffusion coefficient. Inhibition of stomatal opening was observed at temperatures below 10 °C in leaves of bean and tomato. In numerous experiments, STÅLFELT, 1928, 1956 could not detect any direct temperature effect on stomatal aperture. In his opinion, temperature may indirectly influence stomatal aperture by affecting the water balance of the leaf. WILSON (1948), in porometer experiments on cotton plants in soil, observed an increase in aperture between 5° and 25 °C, and a decrease above 30 °C. However, this temperature effect may well be due to an effect on water supply to the leaves, since water uptake by cotton roots strongly depends on root temperature (ARNDT, 1937). We thus arrive at the conclusion that stomatal aperture is independent of temperature under optimal conditions of water supply.

Photosynthesis is temperature-independent under light limiting conditions (VAN DEN HONERT, 1930; RABINOWITCH, 1951; GAASTRA, 1959). In normal air, there is a slight temperature effect at the saturation level, owing to changes in the diffusion coefficient. In turnip, GAASTRA (1959) observed a Q_{10} of 1.18 at light saturation.

Measurements with various light sources in our experiments have shown that the stomatal response to light is insensitive to infrared radiation, in agreement with SIERP's observations (1933). Within the range of 400 to 700 mμ, the transpiration response is about equal for different radiation sources; only in the case of mercury vapour lamps it is somewhat smaller, which is due to a somewhat

lower light absorption. It was concluded that there is no important inactive absorption in specific spectral regions.

Several experiments on the effect of light quality on stomatal aperture are reported in literature. In some of them (LIEBIG, 1942; PAETZ, 1930), the stomatal response was found to be weak in green light, and much stronger in red and blue light, whereas in others (HARM, 1936), the stomatal reaction was minimal in red light. Sometimes, the optimal response of the stomata was in blue (LIEBIG, 1942; MOURAVIEFF, 1958), sometimes in red (PAETZ, 1930). The mentioned autors concluded that the action spectrum of stomatal opening resembles that of photosynthesis and the absorption spectrum of chlorophyll.

EMERSON and LEWIS (1943), in *Chlorella*, observed a sharp decrease of the quantum yield of photosynthesis above 690 mμ. The rate of photosynthesis is negligible in infrared radiation above 730 mμ, which may be accepted to be the same for leaves.

Between 400 and 700 mμ, the reports on the relation of photosynthesis to wavelength vary, which can be ascribed to differences in plant species, thickness of leaves, presence of pigments other than chlorophyll, etc. (RABINOWITCH, 1951). At first sight, it may be surprising that absorption spectra of different leaves (SEYBOLD and WEISSWEILER, 1942; SHUL'GIN *et al.* 1958; RABIDEAU *et al.*, 1946) are very similar to the action spectrum of photosynthesis of wheat leaves (HOOVER, 1936). This becomes understandable since, evidently, the absorption spectrum of chlorophyll between 400 and 700 mμ is smoothed out by the optical characteristics of the leaf as a whole, which are due to its complicated structure.

The results, reported in Chapter 4, § 5, show that the response of the stomata to light varies neither with the age of the leaf (senescent leaves excluded), nor with the light conditions under which the plants were grown. These factors only indirectly affect stomatal transpiration via changes in stomatal frequency and leaf area. No literature on this subject was found.

RICHARDSON (1957) showed that photosynthesis of *Acer* and *Quercus* leaves was independent of the stage of leaf development at light intensities below saturation. At light saturation in air, containing 4.65 % CO_2, a strong effect of the stage of leaf development on photosynthesis was apparent. Under natural conditions from July to October, HEINICKE and HOFFMAN (1933) observed a fluctuation of the rate of photosynthesis of apple leaves with the CO_2-content of the air, the cloudiness of the sky, especially at high degrees of cloudiness, etc. (see their table 8). During this period, photosynthesis in bright sunshine or with partly clouded sky did not vary much, except late in the season. In bright sunshine, photosynthesis of *Acer* and *Tilia* leaves did not show much variation per unit leaf area from June to the end of September (WILLSTÄTTER and STOLL, 1918). In air, containing 0.06 % CO_2, photosynthesis of sugar cane leaves showed a small increase with the age of the leaves (SINGH and LAL, 1935). This effect of age is much stronger in air, containing 0.35 % CO_2. It may be concluded that, especially under light limiting conditions in normal air, photosynthesis does not depend much on the age of the leaf, except in senescent and very young, still folded leaves.

In pale green leaves, the response of stomatal transpiration to incident light generally was smaller than in normal green leaves. This decrease accompagnies the decrease in light absorption, and most likely the stomatal response per unit absorbed light is independent of the chlorophyll content. VIRGIN (1957) measured stomatal transpiration in several plant species, studying normal green

leaves and "white" leaves. In general, white leaves only showed little or no increase in transpiration after transfer from dark to strong light. The "white" leaves often contained some chlorophyll, especially in the guard cells, and a correlation was found between the chlorophyll content of the leaf and the response of transpiration to incident light. In an albino mutant of barley, no chlorophyll was detectable at all, nor any increase in transpiration after transfer from dark to light (VIRGIN, 1957). SHAW (1958) neither could find any opening of the stomata upon transfer to light in an albino mutant of barley. These observations strongly suggest that chlorophyll is necessary for the opening reaction of the stomata. It should be noted, however, that the stomata might be unable to open at all in these albino mutants (KETELLAPPER, 1959).

It was shown in Chapter 4, § 7, that opening of the stomata after transfer from dark to strong light is inhibited by sodium azide, potassium cyanide and phenyl urethan, previously taken up by the leaves via the petiole in dark. Similar results have been obtained by MOURAVIEFF (1956), who, moreover, showed that the opening reaction of the stomata was not inhibited by phloridzine nor by hydroxyl amine and phenanthroline. MOURAVIEFF supposed photosynthesis to be inhibited in his experiments. However, no inhibition of photosynthesis by the last two substances at low concentration was apparent in our experiments, and this may perhaps also have been so in those of MOURAVIEFF.

According to the above discussion, both photosynthesis and stomatal response to light are practically temperature-independent in normal air under light limiting conditions and are insensitive to infrared radiation. Between 400 and 700 mμ every incident erg is about equally effective in both processes, while the lower effectivity of green light might be explained by lower light absorption. Both processes are independent of leaf age, and of the light conditions under which the plants were grown, while, moreover, their behaviour towards several inhibitory substances is similar. It is reported that no stomatal opening is brought about by light in chlorophyll-free albino mutants. From these facts it may be concluded that the stomatal light response somehow depends on photosynthesis.

Extensive discussions on the mechanism of the stomatal response to light have recently been given by STÅLFELT (1956), HEATH (1959), and KETELLAPPER (1959). In many hypotheses and theories on the mechanism of the stomatal light response, photosynthesis is rightly regarded as one of the links in the catenary process of stomatal opening and closing upon transfer from dark to light or *vice versa (e.g.* SCARTH and SHAW, 1951; VIRGIN, 1957). The stomatal light response has been ascribed, *e.g.*, to the photosynthetic production of carbohydrates (VON MOHL, 1856), to photosynthetic reduction of the carbon dioxide content of the guard cells (LINSBAUER, 1917), to indirect effects of the photosynthetic reduction of the CO_2-content of the guard cells, *e.g.*, by affecting the starch-sugar equilibrium (SAYRE, 1926; SCARTH, 1932) and the pH of the guard cells (SCARTH, 1932; PEKAREK, 1934).

The fact that in strong light, the stomatal aperture is nearly equal to that in CO_2-free air in dark, strongly suggests that the primary effect of light is due to decrease in CO_2-content of the guard cells, owing to photosynthesis. In CO_2-free air, a further increase in aperture was observed upon transfer from dark to strong light, which probably may be ascribed to the consumption of respiratory carbon dioxide.

Results reported by HEATH and MILTHORPE (1950), SCARTH and SHAW (1951),

HEATH and RUSSELL (1954), and others have been summarized by HEATH (1959). HEATH and RUSSELL distinguish two direct and two indirect light effects on stomatal opening. The "first direct" effect of light is due to photosynthesis of the guard cells themselves and determines the CO_2-content of the guard cells chloroplasts. The "first indirect" effect of light depends on the sensitivity of the guard cells to carbon dioxide as dependent on the CO_2-content of the substomatal cavity, the latter resulting from photosynthesis and respiration of the mesophyll.

The "secondary direct and indirect effects" of light do not act *via* reduction of the carbon dioxide content of the guard cells, a conclusion mainly based on an experiment of HEATH and RUSSELL (1954). In this experiment, the substomatal cavities and the intercellular spaces of the leaf were washed out with CO_2-free air at a rate of 250 cm^3.111 mm^{-2} leaf area. h^{-1}.

Assuming a respiration rate of 10 mm^3 $CO_2.cm^{-2}.h.^{-1}$, as observed by GAASTRA (1959) in turnip (CO_2-free air, at 20.3 °C air temperature), the mean carbon dioxide concentration of the air, forced through the leaf, should be about 0.0045 %. Using the porometer technique, HEATH and RUSSELL determined the effect of light intensity on the stomatal resistance to air flow, and it appeared that the effect of variation in light intensity could be followed in a second cup, kept at constant light intensity on the same leaf. Between these two cups, an area of 1.7 cm width was exposed to a light intensity of 800 foot candles, corresponding to about 3.6×10^4 $erg.sec^{-1}.cm^{-2}$ in the spectral range of 400 to 700 mμ. The differences in stomatal resistance observed in the second cup upon changes in light intensity in the first cup are small but significant, and the authors suggest that a light effect has been transmitted across a "light barrier" by some chemical or electrical stimulus, not travelling in the intercellular space. In this connection it is of interest that SCARTH (1932), in variegated *Pelargonium* leaves, already observed that the stomata in the white area remain about closed, except those near the boundary which followed the reaction in the green part of the leaf. The present author could completely confirm these observations which shows that the stomata of a leaf may mutually interfere. However, in the latter case the distance over which the interference is observable seems much smaller than that apparent in the experiment of HEATH and RUSSELL. Mutual interference of stomata has also been observed in experiments on heat stimuli (LINSBAUER, 1917; WILLIAMS 1948). With a view to this interference, a large and equally irradiated leaf area (4.5 cm^2) was always used in our microscope experiments.

A discussion as to whether changes in the CO_2-concentration in the protoplasm of the guard cells explain all light effects on stomatal movement, reported until now, must be focussed on the effect of photosynthesis of the guard cell chloroplasts upon this concentration.

In experiments in CO_2-free air (HEATH and MILTHORPE; HEATH and RUSSELL) a noticeable effect of light intensity on the stomatal diffusion resistance still exists. HEATH ascribes this effect to a factor other than CO_2-reduction in the guard cell chloroplasts. It seems even more probable, however, that the effect is due to consumption of respiratory products, including CO_2, in the photosynthetic process of the guard cell chloroplasts.

In our experiments in strong light, the maximum stomatal aperture was 5.5 μ in normal air, and 7.5 μ in CO_2-free air. This observation is in good agreement with the very plausible assumption that the CO_2-content of the guard cell chloroplasts in normal air does not quite decrease to zero in strong light, owing to a supply of respiratory CO_2 from the surrounding non-photosynthesizing epidermis cells to the guard cells. In CO_2-free air, a further decrease in CO_2-content of the guard cell chloroplasts appears quite possible in this connection.

At equal light intensities within the range from 90 to 975 foot candles (corresponding to 0.4×10^4 and 4.4×10^4 $erg.sec^{-1}.cm^{-2}$, respectively, in the range from 400 to 700 mμ) no difference in stomatal response between 0 and 0.01 % external CO_2 was detected by HEATH and MILTHORPE, and HEATH and RUSSELL. It may be accepted that at 0.01 % external CO_2 in weak light, and already at

0.017 % external CO_2 in strong light (4.4×10^4 erg.sec^{-1}.cm^{-2}), the CO_2-content of the guard cell chloroplasts reaches its minimum value. The minimum value is determined by the rate of respiration and the resistance of the CO_2 diffusion path between the site of respiration and the chloroplasts. In dark, the stomatal resistance increases rapidly in the range from 0 to 0.01 and 0.017 % external CO_2. In the mentioned experiments, the stomatal resistance increased with decrease in light intensity in the entire range, and with increase in external CO_2 concentration in a range between 0, 0.01, or 0.017 % and 0.084 %, the former values depending on light intensity. The authors did not measure photosynthesis. Data from literature indicate that, probably, saturation of photosynthesis has been reached neither with respect to light, nor to external CO_2 (cf, *e.g.*, HOOVER *et al.*, 1933; GAASTRA, 1959). Therefore, regulation of the stomatal resistance by reduction of the CO_2-content of the guard cell chloroplasts owing to photosynthesis appears well possible in the entire range of light intensities and carbon dioxide concentrations applied. According to HEATH and RUSSELL (1954), it is improbable that the observed large response to light is due to CO_2-reduction in the guard cells, in a region where a reduction of external CO_2 from 0.084 % to 0.049 % hardly had any effect. In our opinion, light saturation of photosynthesis seems improbable in this case, while, moreover, a considerable reduction of the CO_2-content of the guard cell chloroplasts by photosynthesis is likely to occur, taking into account the large resistance to diffusion of CO_2 in the protoplasm.

It may be concluded that the effect of light intensity and external CO_2 on the stomatal resistance to diffusion (HEATH, 1959) can be explained almost quantitatively by the effect of photosynthesis on the CO_2-content of the guard cells. Thus, the latter may be considered as the main regulating factor of the stomatal resistance to diffusion. However, the possibility of a more complicated effect of light intensity on the stomata, as proposed by HEATH (1959), cannot be fully excluded since it has been shown by HEATH and RUSSELL that a light stimulus effect can be transmitted through a leaf, just as a heat stimulus.

Recent reviews on stomatal light response (STÅLFELT, 1956; HEATH, 1959; KETELLAPPER, 1959) suggest several theories for the explanation of the stomatal mechanism. The most common classical theory is the following: Light → photosynthesis → reduction of CO_2 → rise in pH → starch – sugar conversion → increase in osmotic value → increase in turgor → stoma opens (SAYRE, 1926; SCARTH, 1932, see also YIN and TUNG, 1948).

Changes in starch content may well play a role in stomatal movement, but, in general, they are only rather incompletely correlated (SCARTH, 1932; WILLIAMS and BARRETT, 1954; YEMM and WILLIS, 1954). According to STÅLFELT (1957), the rapid closure of stomata is often accompagnied by a slow, and sometimes only small decrease in the osmotic value of the guard cells. For these reasons, also a specific protoplasmic reaction of the guard cells was assumed by SCARTH and SHAW (1951), an idea which has been elaborated by WILLIAMS (1954). WILLIAMS' hypothesis consists of the following points:

"1. The closing movement is the most important movement, opening being a return, probably osmotic, to a resting state.
2. Closure of the stomata is an "active", non-osmotic, energy requiring transfer of water from the guard cells to neighbouring cells, possibly mediated by contractile structures of some type.
3. Carbohydrate changes are secondary "stabilizing" changes, not directly linked to the primary process."

We will now discuss these suggestions in some detail.

Ad. 1. Taking into account the rapid closure of the stomata in dark and their slower opening in light, one may consider the most rapid movement, *viz.* the closing movement, as "active", in analogy with, *e.g.*, the leaf movement of *Mimosa pudica* (WEINTRAUB, 1951). However, another explanation of the difference in speed of closure and opening is possible. It has been shown that, after transfer from strong light to a lower intensity, an initial CO_2-gush occurs which may provide an explanation for the rapid closure. We have observed this outburst in air, containing 5 % CO_2. DECKER (1955, 1959) observed a similar gush in bean and tobacco leaves in normal air after exposure to dark, while VEJLBY (1958) found it in moss plants, and VAN DER VEEN (1949) made it probable in *Sciadopitys* needles.

It seems reasonable to explain opening as well as closure of the stomata as reactions upon CO_2. WILLIAMS states that "CO_2 is metabolically a very active substance; its presence may be expected to have a positive effect and the important movement is thus likely to be (that of) closure". Since the mechanism of CO_2-action is unknown, it is as well possible that the absence of CO_2 is required for the accumulation of a substance, necessary for stomatal movement. For this reason, the validity of postulate 1 appears questionable.

Ad. 2. The suggestion that the stomatal closing movement is based upon an "active", non-osmotic, energy requiring transfer of water from guard cells to neighbouring cells, is very interesting, however, there is not yet much experimental evidence for it. STÅLFELT (1957) observed that sodium azide inhibits the opening reaction of the stomata in light as well as the closing movement upon transfer to dark, which was confirmed in our experiments. According to STÅLFELT, the effect of sodium azide is opposite to that of CO_2; the azide is supposed to inhibit the non-osmotic removal of water required during closure of the stomata. A similar inhibition of non-osmotic water flow by sodium azide was found by PRELL (1955) in plasmolysis experiments.

Taking into account the possible importance of the CO_2-gush in explaining the difference in rapidity between opening and closing movements in normal air, as suggested above, it does not follow that only the closing movement is active. We may assume that the energy supply, required for non-osmotic water transport originates from conversion of light energy. In this connection it is important that stomatal guard cell chloroplasts are capable of photosynthesis (see *e.g.* SHAW and MACLACHJAN, 1954), so that the energy required may be provided by a product of photosynthesis or of photosynthetic phosphorylation. PRELL (1955) suggested that phosphorylation may play a role in non-osmotic water flow. Adenosine triphosphate (ATP) may well be important in mediating the transfer of the energy required for the process. In the light at decreasing CO_2-content, increase in ATP may be expected since ATP-consumption in the photosynthetic carbon cycle then decreases (CALVIN *et al.*, 1958). This would also be in accordance with earlier evidence from this laboratory that phosphate compounds tend to pile in cells, capable of photosynthesis more easily when CO_2 is withdrawn (WASSINK, WINTERMANS and TJIA, 1951; WINTERMANS, 1955; WASSINK, 1955), so that more ATP produced by photo-phosphorylation will then be available as an energy source for non-osmotic water uptake into the guard cells during the opening movement. In a CO_2-free medium in dark ATP-formation by respiratory phosphorylation may be sufficient for purpose.

WILLIAMS assumes the existence of contractile vacuoles in the guard cells in analogy with the contractile vacuoles, observed by WEINTRAUB (1951) in the pulvinar cells of *Mimosa pudica*. BEYER (1929) observed that the rapid closure of the stomata in *Tradescantia zebrina* is accompagnied by the formation of small globules, which observation contains evidence for non-osmotic water output during the closing movement.

In this connection it is of interest that HAYASHI (1953) could demonstrate contraction of surface-spread actomyosine and nucleoprotein after ATP-application in vitro. According to HEILBRUNN (1956) most animal physiologists hold the opinion that ATP is primarily responsible for muscular contraction, though HEILBRUNN himself is not convinced that it could shorten the proteins of the protoplasmic colloid.

Ad. 3. Since starch is the only photosynthetic product, microscopically visible in the guard cells, many experiments have been made on the relation between starch and stomatal aperture. The question whether starch plays a significant role in the mechanism of the stomatal light response, *e.g.*, in stabilizing the effect of the primary movement or whether it is only of minor importance, appears unanswered as yet.

In summary, one has to state that the mode of action of CO_2 is unknown and probably will have to be looked for in the metabolism of the guard cells. It may be suggested that the primary mechanism is of non-osmotic nature, producing protoplasmic contractions and/or non-osmotic water transport, which indirectly affect the turgor of the guard cells. The energy required for this active process may be supplied, *e.g.*, by adenosine triphosphate, photochemically produced by the guard cell chloroplasts, especially at low CO_2-concentrations. Since a negative correlation between CO_2 and ATP in light seems probable, CO_2 may well affect stomatal opening via the ATP-supply.

§ 4. SOME REMARKS ON THE EFFECT OF LIGHT INTENSITY ON TRANSPIRATION

In weak light, up to about 5×10^4 erg.sec^{-1}.cm^{-2}, the rate of transpiration increases linearly with light intensity. This increase in part is a temperature effect, which is especially apparent in experiments with light sources, containing a large amount of infrared radiation, *e.g.*, incandescent lamps. Similar curves with a marked effect of light intensity on leaf temperature have been reported by MARTIN (1943), BROUWER (1956), and YAMAOKA (1958).

In general, the transpiration versus light intensity curves were reduced for differences between leaf temperature and air temperature (see figures 7, 9, and 11), after which nearly ideal "BLACKMAN"-curves result. The corrected transpiration is proportional to $(R_{total})^{-1}$, and increases linearly with light intensity until saturation is reached at 5×10^4 erg.sec^{-1}.cm^{-2} (in light from fluorescent tubes). Stomatal opening then is maximal, and the increase in transpiration rate above this light intensity is completely due to increase in leaf temperature.

The response of the stomata could be attributed to the CO_2-content of the guard cell chloroplasts, the latter chiefly determined by photosynthesis. Analoguous to transpiration, photosynthesis of a leaf may be considered as a diffusion process, characterized by the subsequent resistances $(R_{air})CO_2$, $(R_{stomata})CO_2$, and $(R_{mesophyll})CO_2$. GAASTRA showed that, under light limitation, considerable closure of the stomata is without an appreciable effect

on the rate of photosynthesis of a leaf, since the mesophyll resistance in general is large compared with the stomatal resistance. In view of the small dimensions of the guard cells, it may be expected that the diffusion resistance of their protoplasm is small compared with that of the leaf parenchyma cells. The most simple explanation of the linearity of the corrected curve of transpiration versus light intensity in normal air is that this curve represents the photosynthesis curve of the guard cells. The similarity is most complete if the guard cell resistance to CO_2-diffusion is small compared with stomatal resistance. Evidently, this is the case in the described experiments, so that it may be concluded that, in normal air, transpiration is regulated by photosynthesis of the stomatal guard cells.

VAN DEN HONERT (1930) obtained nearly ideal "BLACKMAN"-curves in experiments on photosynthesis of the alga *Hormidium*, indicating that the equilibrium in the first chemical reaction of CO_2 is largely at the side of the photosynthetic reaction products. The supposition that the same holds for photosynthesis of guard cell chloroplasts is supported by the fact that stomatal transpiration yields similar curves.

Transpiration in strong light of leaves without shortage of water increases with increasing wind velocity to 240 % (STÅLFELT, 1932) or 230 % (WRENGER, 1935, mean value) of its value under "still air" conditions. Leaving out of consideration the effect of wind on the difference between leaf temperature and air temperature, wind velocity only affects R_{air}. This indicates that R_{leaf} in strong light with fully open stomata is somewhat smaller than R_{air} under still air conditions. This is in agreement with our experiments, in which the minimum value of $R_{stomata}$ was about 70 % of the R_{air} under "still air" conditions.

The curves, representing opening and closing of the stomata upon a shift from weak light to strong light or *vice versa* in normal air and in CO_2-free air (obtained by transpirations measurements or by microscope observation), resemble those, given by VIRGIN (1956). Analysis of such curves requires simultaneous measurement of leaf temperature. Leaf temperature changes within 10 seconds after transfer from strong light to weak light (or dark) and vice versa; after correction for such temperature effect, the transpiration curves show a more gradual course. This sort of analysis has not been carried out by VIRGIN (1956), and therefore his conclusion, based directly on transpiration curves, that closing of the stomata sets in within 15 seconds after transfer to darkness, seems premature. Certainly, the heat irradiation effect on transpiration may not be neglected in his experiments, owing to the large amount of

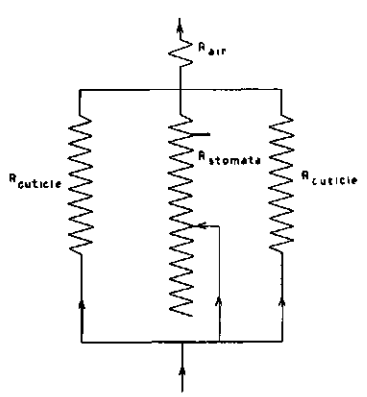


FIG. 21.
The path of water molecules, entering via the petiole, through a hypostomatous leaf. At the upper side of the leaf (left part of the figure) they are subject to the cuticular resistance followed by that of the air. At the lower side of the leaf (right side of the figure) they may leave the leaf either via the variable resistance of the stomata (— indicates the minimum value), or via the cuticular resistance, followed by the resistance of the air.

infrared radiation from his incandescent lamp, even though provided with a 10 cm water filter; according to our data in this case around 60 % of the radiation still will have been between 0.7 and 1 μ. It also seems quite possible that the fluctuations in transpiration after application of intermittent light, observed by VIRGIN, may be ascribed largely to leaf temperature fluctuations and not to the suggested variation in stomatal diffusion resistance.

Fig. 21 presents in a scheme results obtained in the previous sections concerning the various resistances which play a role in regulating transpiration. Water, entering via the petiole, may leave the leaf via the upper cuticula, via the lower cuticula, or through the stomata. In these cases diffusion of water vapour is submitted to the diffusion resistance of the upper cuticula, to that of the lower cuticula, or to that of the stomata, followed by the resistance of the boundary layer of air. The resistances presented in this figure refer to our measurements on transpiration of bean and tomato leaves and the figure shows that application of the diffusion law is valid for transpiration of leaves.

SUMMARY

This paper deals with experiments on transpiration of cut leaves in potometers as affected by light intensity, leaf temperature, and humidity of the air, while special attention is paid to stomatal light response. Leaves of bean, tomato or *Hyoscyamus* were mostly used.

It was proven that FICK's diffusion law can be applied to evaporation of water and to transpiration of leaves under our experimental conditions, leading to the formula $T.R_{total} = e_s - e_d$. The resistance to transport of water vapour of the adhering air layer, R_{air}, and its relation to laminar and turbulent air movement were determined for a wet piece of blotting paper of 100 cm^2. Evaporation was found to be proportional to V^{α} (V = wind velocity), α being 0.76 under turbulent air conditions. Lower values of α, *e.g.*, 0.5, reported in literature may be ascribed to the fact that some of the experimental data apply to the transition zone between laminar and turbulent flow and the others to turbulent flow only.

The transpiration rate shows a linear increase with light intensity below 5×10^4 erg.sec^{-1}.cm^{-2}. This increase is mainly due to increase in leaf temperature by irradiation with incandescent light, containing a high amount of infrared radiation; with fluorescent tubes, sodium lamps, or high pressure mercury vapour lamps, the increase in transpiration is mainly due to increase in stomatal opening.

In general, curves of transpiration versus light intensity were corrected for differences in leaf temperature along each single curve, a procedure justified by experiments on the effect of light intensity on evaporation. After this correction, curves of the "BLACKMAN" type result, with saturation at about 5×10^4 erg. sec^{-1}.cm^{-2}. Increase in transpiration at light intensities above this value is entirely due to increase in leaf temperature. Since no experimental evidence for the existence of an "incipient drying" resistance was found in our experiments, the linear increase in transpiration with increase in light intensity along the corrected curves entirely reflects the change in stomatal resistance to diffusion. The latter, therefore, could be determined accurately from measurements on transpiration, leaf temperature, and humidity of the air.

The stomatal resistance to diffusion was found to be independent of temperature. No effects of the age of the leaves and of the light conditions during growth

were found. The stomata were found to be insensitive to infrared radiation. Within the range of 400 to 700 mμ, the effect of various light sources on transpiration and stomatal resistance to diffusion was about the same, while a somewhat smaller effect of the high pressure mercury vapour lamp could be ascribed to lower light absorption. Photosynthesis and stomatal response to light showed a similar behaviour with regard to several inhibitors, allowing the conclusion that, in normal air, the stomatal response to light depends on photosynthesis.

The relation between stomatal aperture and light intensity was established, moreover, by microscopic observation, and in combination with experiments on transpiration, the relation between aperture and stomatal resistance to diffusion could be established. Regulation of transpiration by small changes in stomatal resistance to diffusion was most pronounced at small apertures, whereas at larger apertures the stomatal resistance to diffusion is only little affected.

The maximal aperture of the stomata was 5.5 μ in normal air and strong light, 7.5 μ in CO_2-free air and strong light, and 4.5 μ in CO_2-free air in darkness and in weak light. The main effect of light on opening of the stomata is supposed to be the decrease in CO_2-content of the guard cell chloroplasts, owing to photosynthesis. The effect of light observed in CO_2-free air has been ascribed to consumption in photosynthesis of respiratory CO_2 in the guard cell chloroplasts.

Oscillations in stomatal aperture were observed; probably they are due to two coupled processes, *viz.*, photosynthesis of the guard cell chloroplasts, and the response of the guard cells to CO_2, indicating the existence of a stomatal feed back. The curve of the "BLACKMAN" type, representing the relation between light intensity and corrected transpiration rate in normal air, is considered as a photosynthesis curve of the guard cell chloroplasts, taking into account the differences between diffusion of CO_2 and H_2O.

In normal air, closure of the stomata was much more rapid than opening. which is explained by an initial CO_2-gush, observed after transfer of the leaf from strong light to a lower light intensity.

It is assumed that the primary mechanism of the action of CO_2 is non-osmotic, via protoplasmic contractions and/or non-osmotic water transfer, interfering with the turgor of the guard cells. CO_2 may affect the energy supply required for stomatal opening, *e.g.*, by affecting the adenosine triphosphate level.

SAMENVATTING

Het doel van het beschreven onderzoek was de meting van de verdamping van bladen, zoals deze beïnvloed wordt door de lichtintensiteit, de temperatuur van het blad en de luchtvochtigheid. Aan de lichtreactie van de huidmondjes is bijzondere aandacht geschonken. Als proefplanten dienden boon, tomaat en *Hyoscyamus*.

Onder de proefomstandigheden kon de diffusiewet van FICK worden toegepast op de verdamping van een vrij wateroppervlak (evaporatie) en van bladen (transpiratie). De verdampingsweerstand van het aan het verdampende oppervlak grenzende laagje lucht werd bepaald bij een laminaire en bij een turbulente beweging van de lucht. De evaporatie bleek evenredig met V^{α} (V = windsnelheid); $\alpha = 0.76$ bij een turbulente luchtbeweging. Lagere waarden van α, bijv.

0.5, zoals die soms in de literatuur worden vermeld, vinden hun oorzaak in het feit dat in die gevallen een deel van de experimentele gegevens klaarblijkelijk verkregen werd in het overgangsgebied tussen laminaire en turbulente luchtbeweging, de overige onder uitsluitend turbulente luchtbeweging.

De verdamping van bladen neemt rechtlijnig toe met de lichtintensiteit tot ongeveer 5×10^4 erg.sec^{-1}.cm^2. Indien de betreffende lichtbron veel infrarood emitteert, zoals een gloeilamp wordt de toeneming van de verdamping in hoofdzaak bepaald door verhoging van de bladtemperatuur. Bij natriumlampen, hoge-druk kwiklampen of fluorescentiebuizen, kon de toeneming van de verdamping met de lichtintensiteit in hoofdzaak worden toegeschreven aan het zich openen van de huidmondjes.

De transpiratie-lichtintensiteit curven werden gecorrigeerd voor de invloed van de lichtintensiteit op de bladtemperatuur. Na correctie werden BLACKMAN-curven verkregen met verzadiging beginnende bij ongeveer 5×10^4 erg.sec^{-1}. cm^{-2}. Toeneming van de transpiratie boven deze lichtintensiteit is geheel te danken aan verhoging van de bladtemperatuur. De rechtlijnige toeneming van de verdamping met de lichtintensiteit langs de gecorrigeerde curve wordt veroorzaakt door verandering in de diffusieweerstand van de huidmondjes. De laatste kan nauwkeurig bepaald worden door meting van de transpiratie, de bladtemperatuur en de luchtvochtigheid.

De diffusieweerstand van de huidmondjes was vrijwel onafhankelijk van de temperatuur. Er werd evenmin invloed van de leeftijd van de bladen en van de belichting gedurende de opkweekperiode waargenomen. Binnen het spectraalgebied van 400–700 mμ was de invloed van de verschillende lichtbronnen op de transpiratie en de diffusieweerstand van de huidmondjes vrijwel gelijk; een iets kleiner effect van de hoge-druk kwiklampen kon worden toegeschreven aan geringere lichtabsorptie van de bladen voor deze lichtsoort. Verschillende onderzochte remstoffen hadden dezelfde invloed op de fotosynthese van de bladen en op de lichtreactie van de huidmondjes. De conclusie was, dat de lichtreactie van de huidmondjes afhankelijk is van het fotosyntheseproces.

De betrekking tussen de spleetbreedte van de huidmondjes en de lichtintensiteit werd microscopisch bepaald, alsmede, met behulp van transpiratiemetingen, de betrekking tussen opening en diffusieweerstand van de huidmondjes. Regeling van de verdamping door veranderingen in de openingstoestand van de huidmondjes was het meest werkzaam bij geringe spleetbreedte; bij grote spleetbreedte worden diffusieweerstand en transpiratiesnelheid slechts weinig beinvloed.

De maximale spleetbreedte van de huidmondjes in sterk licht was 5.5 μ in gewone lucht en 7.5 μ in CO_2-vrije lucht; in CO_2-vrije lucht in het donker of in zwak licht 4.4 μ. In gewone lucht in het donker zijn de huidmondjes gesloten. Verschillen in openingstoestand van de huidmondjes boven 4.4 μ komen overeen met relatief geringere verschillen in diffusieweerstand. De invloed van licht op de openingstoestand van de huidmondjes kan vrijwel geheel worden toegeschreven aan verlaging van het CO_2-gehalte van de sluitcellen door photosynthese. De waargenomen lichtinvloed in CO_2-vrije lucht kan verklaard worden door het verbruik van ademhalings-CO_2 in het fotosyntheseproces van de sluitcelchloroplasten.

In de openingstoestand van de huidmondjes worden schommelingen waargenomen, die vermoedelijk worden veroorzaakt door twee processen met tegengesteld effect, namelijk de fotosynthese van de sluitcelchloroplasten en de reactie

van de sluitcellen op CO_2. Tezamen vormen deze twee processen een terugkoppelingsmechanisme.

In gewone lucht is de sluitingsbeweging van de huidmondjes na de overgang licht-donker veel sneller dan de openingsbeweging na de overgang donker-licht. Deze snelle sluiting kan verklaard worden door de waarneming dat na de overgang licht-donker in het blad een voorbijgaand maximum in CO_2-uitscheiding plaats vindt.

Het onbekende mechanisme, dat de turgor van de sluitcellen en de opening van de huidmondjes regelt, kan in eerste instantie van niet-osmotische aard zijn en bij voorbeeld door middel van protoplasmacontracties en/of niet-osmotisch watertransport functioneren. Men kan zich verder denken dat CO_2 in licht invloed uitoefent op de hoeveelheid adenosine trifosfaat, welke stof bij de energielevering voor de openingsreactie van de huidmondjes, een rol kan spelen.

ACKNOWLEDGEMENTS

This investigation was carried out while I was a member of the staff of the Laboratory of Plant Physiological Research of the Agricultural University, Wageningen, under the direction of Professor E. C. WASSINK, to whom I am much indebted for critical advice and continued interest. I owe also thanks to Dr. P. GAASTRA and to Dr. C. J. P. SPRUIT for useful criticism during this study.

REFERENCES

ABD EL RAHMAN, A. A., P. J. C. KUIPER and J. F. BIERHUIZEN, Preliminary observations on the effect of light intensity and photoperiod on transpiration and growth of young tomato plants under controlled conditions. Meded. Landbouwhogesch. Wageningen **59** (11), 1959: 1–12.

——— and ———, Preliminary observations on the effect of soil temperature on transpiration and growth of young tomato plants under controlled conditions. Meded. Landbouwhogesch. Wageningen **59** (15), 1959: 1–12.

ARNDT, C. H., Water absorption in the cotton plant as affected by soil and water temperatures. Plant Physiol. **12**, 1937: 703–720.

BANGE, G. G. J., On the quantitative explanation of stomatal transpiration. Acta Bot. Neerland. **2**, 1953: 255–297.

BAUMBACH, S., Vergleichsmessungen mit verschiedenen Verdunstungsmessern unter definierten Versuchsbedingungen. Ber. d. Wetterd. U.S.-zone **35**, 1952: 211–216.

BEYER, A. F., Über Tropfenbildung in den Schliesszellen der Spaltöffnungen von *Tradescantia zebrina*. Bot. Archiv. **26**, 1929: 224–256.

BIEBL, R., Der Einfluss der Mineralstoffe auf die Transpiration. *In:* Handbuch der Pflanzenphysiologie, ed. by W. RUHLAND, **4**, 1958: 382–427.

BIERHUIZEN, J. F., Some observations on the relation between transpiration and soil moisture. Neth. Journ. Agric. Sc. **6**, 1958: 94–98.

BROUWER, R., Radiation intensity and transpiration. Neth. Journ. Agric. Sc. **4**, 1956: 43–48.

BROWN, H. F. and W. E. WILSON, On the thermal emissivity of a green leaf in still and moving air. Proc. Roy. Soc. London **76B**, 1905: 112–138.

BURGERSTEIN, A., Materialen zu einer Monographie betreffende die Erscheinungen der Transpiration der Pflanzen. Wien, 1887: 94 p. Teil **2**, 1889: 68 p. Teil **3**, 1901: 60 p.

BURGERSTEIN, A., Die Transpiration der Pflanzen. Fischer, Jena. Teil **1**, 1904: 283 p. Teil **2**, 1902: 264 p. Teil **3**, 1925: 63 p.

CALVIN, M. *et al.*, Photosynthesis. Proceed. 2nd Intern. Conf. peaceful uses of atomic energy, United Nations, **24** (1), 1958: 24–32.

CRAFTS, A. S., H. B. CURRIER and C. R. STOCKING, Water in the physiology of plants. Chronica Bot. Co. Waltham, U.S.A., 1949: 240 p.

DARWIN, F., On the relation between transpiration and stomatal aperture. Philosoph. Transact. Roy. Soc. London, **B207**, 1916: 413–439.

DECKER, J. P., A rapid postillumination deceleration of respiration in green leaves. Plant Physiol. **30**, 1955: 82–84.
DECKER, J. P., Comparative responses of carbon dioxide outburst and uptake in tobacco. Plant Physiol. **34**, 1959: 100–103.
EMERSON, R. and C. M. LEWIS, The dependence of the quantum yield of *Chlorella* photosynthesis on wavelength of light. Amer. Journ. Bot. **30**, 1943: 165–178.
FIRBAS, F., Die Wirkung des Windes auf die Transpiration. Ber. Deutsch. Bot. Ges. **49**, 1931: 443–452.
FREY-WYSSLING, A., and E. HÄUSERMANN, Ueber die Auskleidung der Mesophyllinterzellularen. Ber. Schweiz. Bot. Ges. **51**, 1941: 430–432.
GAASTRA, P., Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Meded. Landbouwhogesch. Wageningen **59** (13), 1959: 1–68.
GRADMANN, H., Untersuchungen über die Wasserverhältnisse des Bodens als Grundlage des Pflanzenwachstumms 1. Jahrb. f. Wiss. Bot. **69**, 1928: 1–100.
GREGORY, F. G., F. L. MILTHORPE, H. L. PEARSE and the late H. J. SPENCER, Experimental studies of the factors controlling transpiration. 2. The relation between transpiration rate and leaf water content. Journ. Exptl. Bot. **1**, 1950: 15–29.
HAAN, IZ. DE, Protoplasmaquellung und Wasserpermeabilität. Rec. Trav. Bot. Neerland. **30**, 1933: 234–335.
HARM, H., Beziehungen zwischen Stomataweite, Lichtstärke und Lichtfarbe. Planta, **25**, 1936: 155–194.
HARTIG, F. and H. J. HELWIG, Ein cos *i*-gerechtes Photometer. Lichttechnik 7, 1955: 181–182.
HÄUSERMANN, E., Über die Benetzungsgrösze der Mesophyllinterzellularen. Ber. Schweiz. Bot. Ges. **54**, 1944: 541–578.
HAYASHI, T., Surface protein as a basis for cell structure and cell movement. Amer. Naturalist **87**, 1953: 209–227.
HEATH, O. V. S., Light and carbon dioxide in stomatal movements. *In*: Handbuch der Pflanzenphysiologie, ed. by W. RUHLAND, **17** (1), 1959: 415–464.
———, The water relations of stomatal cells and the mechanisms of stomatal movement. *In:* Plant Physiology, ed. by F. C. STEWARD, **2**, 1959: 193–250.
——— and F. L. MILTHORPE, Studies in stomatal behaviour. 5. The role of carbon dioxide in the light response of stomata. Journ. Exptl. Bot. **1**, 1950: 227–244.
———, and J. RUSSELL, Studies in stomatal behaviour. 6. An investigation of the light responses of wheat stomata with the attempted elimination of controll by the mesophyll. Part 1. Journ. Exptl. Bot. **5**, 1954: 1–16. Part. 2. Journ. Exptl. Bot. **5**, 1954: 269–292.
HEILBRUNN, L. V., The dynamics of living protoplasm. Acad. Press Inc., New York, 1956: 327 p.
HEINICKE, A. J. and M. B. HOFFMAN, The rate of photosynthesis of apple leaves under natural conditions. Cornell Univ. Agric. Exp. St. Ithaca, New York, bull. 577, 1933: 1–32.
HOMAN, C., T. F. YOUNG and C. A. SHULL, Evaporation in its thermodynamic relation to the cohesion theory and to imbibitional and osmotic pressures of cell constituents. Plant Physiol. **9**, 1934: 653–662.
HONERT, T. H. VAN DEN, Carbon dioxide assimilation and limiting factors. Rec. Trav. Bot. Neerland. **27**, 1930: 149–286.
———, Water transport in plants as a catenary process. Disc. Faraday Soc. **3**, 1948: 146–153.
HOOVER, W. H., E. S. JOHNSTON and F. S. BRACKETT, Carbon dioxide assimilation in a higher plant. Smithsonian Misc. Coll. **87** (16), 1933: 1–19.
HOOVER, W. H., The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. Smithsonian Misc. Coll. **95** (21), 1937: 1–13.
HUBER, B., Der Wärmehaushalt der Pflanzen. Naturwiss. u. Landwirtsch. **17**, 1935: 1–148.
HUBER, B., E. KINDER, E. OBERMÜLLER and H. ZIEGENSPECK, Spaltöffnungsdunstschnitte im Elektronenmikroskop. Protoplasma **46**, 1956: 380–393.
ILJIN, W. S., Drought resistance in plants and physiological processes. Ann. Rev. Plant Physiol. **8**, 1957: 257–275.
IWANOFF, L., Zur Methodik der Transpirationsbestimmung am Standort. Ber. Deutsch. Bot. Ges. **46**, 1928: 306–311.
KAMP, H., Untersuchungen über Kutikularbau and kutikuläre Transpiration von Blättern. Jahrb. f. Wiss. Bot. **72**, 1930: 403–466.
KETELLAPPER, H. J., The mechanism of stomatal movement. Amer. Journ. Bot. **46**, 1959: 225–231.
KLEMM, G., Untersuchungen über den Transpirationswiderstand der Mesophyllmembranen

und seine Bedeutung als Regulator für die stomatäre Transpiration. Planta **47**, 1956: 547–587.

KNIGHT, R. C., Further observations on the transpiration, stomata, leaf water content and wilting of plants. Ann. Bot. **36**, 1922: 361–385.

KRAMER, P. J., Plant and soil water relationships. Mac Graw-Hill Book Co. New York, 1949: 347 p.

———, Physical and physiological aspects of water absorption. *In:* Handbuch der Pflanzenphysiologie, ed. by W. RUHLAND, **3**, 1956: 124–158.

KUIPER, P. J. C. and J. F. BIERHUIZEN, The effect of some environmental factors on the transpiration of plants under controlled conditions. Meded. Landbouwhogesch. Wageningen **58** (11), 1958: 1–16.

LEIGHLY, J., A note on evaporation. Ecology **18**, 1937: 180–199.

LIEBIG, M., Untersuchungen über die Abhängigkeit der Spaltweite der Stomata von Intensität und Qualität der Strahlung. Planta **33**, 1942/43: 206–258.

LINSBAUER, K., Beiträge zur Kenntnis der Spaltöffnungsbewegungen. Flora **109**, 1917: 100–144.

LIVINGSTON, B. E., The relation of desert plants to soil moisture and to evaporation. Publ. Carnegie Instit. Washington **50**, 1906: 1–78.

——— and W. H. BROWN, Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. **53**, 1912: 309–331.

LLOYD, F. E., The physiology of stomata. Publ. Carnegie Instit. Washington **82**, 1908: 1–142.

MARTIN, E., Studies on evaporation and transpiration under controlled conditions. Publ. Carnegie Instit. Washington **550**, 1943: 1–48.

MAXIMOV, N. A., The plant in relation to water. English translation, ed. by R. H. YAPP, London, 1929: 451 p.

MELVILLE, R., The influence of environment on the growth and metabolism of the tomato plant. 2. The relation between water content and assimilation. Ann. Bot. N.S. **1**, 1937: 153–174.

MILTHORPE, F. L., Transpiration from crop plants. Field Crop Abstr. **12**, 1959: 1–9.

———, Plant factors involved in transpiration. Proc. Madrid Symp. on water relations of plants, Unesco, 1960 (seen in manuscript).

——— and the late E. J. SPENCER, Experimental studies of the factors controlling transpiration. 3. The interrelations between transpiration rate, stomatal movement and leaf water content. Journ. Exptl. Bot. **8**, 1957: 413–437.

MOHL, H. VON, Welche Ursachen bewirken die Erweiterung und Verengung der Spaltöffnungen? Bot. Zeitung **14**, 1856: 697–704 and 713–721.

MOURAVIEFF, I., Action du CO_2 et de la lumière sur l'appareil stomatique séparé du mesophylle. 2. Experiences avec les stomates maintenus sur des milieux complexes. Le Botaniste **40**, 1956: 195–212.

———, Action de la lumière sur la cellule vegetale. 1. Production de mouvement d'ouverture stomatique par la lumière des diverses régions du spectre. Bull. Soc. Bot. de France **105**, 1958: 467–475.

PAETZ, K. W., Untersuchungen über die Zusammenhänge zwischen stomatärer Öffnungsweite und bekannten Intensitäten bestimmter Spektralbezirke. Planta **10**, 1930: 611–666.

PEKAREK, J., Über die Aziditätsverhältnisse in den Epidermis- und Schlieszzellen bei *Rumex acetosa* im Licht und im Dunkeln. Planta **21**, 1934: 419–446.

PENMAN, H. L., Natural evaporation from open water, bare soil and grass. Proc. Roy. Soc. London **A193**, 1948: 120–146.

PRELL, H., Die Wasserbilanz plasmolierter Protoplasten. Planta **46**, 1955/56: 272–285.

RABIDEAU, G. S., C. S. FRENCH and A. S. HOLT, The absorption and reflection spectra of leaves, chloroplasts suspensions and chloroplast fragments as measured in an ULBRIGHT sphere. Amer. Journ. Bot. 33, 1946: 769–777.

RABINOWITCH, E. I., Photosynthesis and related processes. Intersc. Publ. Inc. New York, **2** (1), 1951: 604–1208.

RASCHKE, K., Über die physikalischen Beziehungen zwischen Wärmeübergangszahl, Strahlungsaustausch, Temperatur und Transpiration eines Blattes. Planta **48**, 1956: 200–238.

———, Über den Einflusz der Diffusionswiderstände auf die Transpiration und die Temperatur eines Blattes. Flora **146**, 1958: 546–578.

———, Heat transfer between the plant and the environment. Ann. Rev. Plant Physiol. **11**, 1960: 111–126.

RICHARDSON, S. D., The effect of leaf age on the rate of photosynthesis in detached leaves of tree seedlings. Acta Bot. Neerland. **6**, 1957: 445–457.

SAYRE, J. D., Physiology of the stomata of *Rumex patientia*. Ohio Journ. Sc. **26**, 1926: 233–267.
SCARTH, G. W., Mechanism of the action of light and other factors on stomatal movement. Plant Physiol. **7**, 1932: 481–505.
——— and M. SHAW, Stomatal movement and photosynthesis in *Pelargonium*. Part. 1. Plant Physiol. **26**, 1951: 207–225. Part 2. Plant Physiol. **26**, 1951: 581–597.
SCHLICHTING, H., Grenszschicht-Theorie. Braun, Karlsruhe, 1951: 477 p.
SCOTT, F. M., Internal suberization of tissues. Bot. Gaz. **111**, 1949/50: 378–395.
SEYBOLD, A. and A. WEISSWEILER, Spektrophotometrischen Messungen an grünen Pflanzen und an Chlorophyll-Lösungen. Bot. Archiv **43**, 1942: 252–290.
SHAW, M., The physiology of stomata. 2. The apparent abscense of chlorophyll, photosynthesis and a normal light response to light in the stomatal cells of an albino barley. Canad. Journ. Bot. **36**, 1958: 575–581.
——— and G. A. MACLACHLAN, The physiology of stomata. 1. Carbon dioxide fixation in guard cells. Canad. Journ. Bot. **32**, 1954: 784–794.
SHREVE, E. B., The daily march of transpiration in a desert perennial. Publ. Carnegie Instit. Washington **194**, 1914: 37 p.
SIERP, H., Untersuchungen über die Öffnungsbewegungen der Stomata in verschiedenen Spektralbezirke. Flora **128**, 1933: 269–285.
SHUL'GIN, L. A., A. F. KLESHNIN and M. I. VERBOLOVA, A photoelectric method for determining the optical properties of plant leaves. Fiziol. Rastenii (engl. transl.) **5**, 1958: 273–275.
SINGH, B. N. and K. N. LAL, Investigation of the effect of age on assimilation of leaves. Ann. Bot. **49**, 1935: 291–307.
STÅLFELT, M. G., Die Abhängigkeit der photischen Spaltöffnungsreaktionen von der Temperatur. Planta **6**, 1928: 183–191.
———, Die Abhängigkeit der Spaltöffnungsreaktionen von der Wasserbilanz. Planta **8**, 1929: 287–341.
———, Der stomatäre Regulator in der pflanzlichen Transpiration. Planta **17**, 1932: 22–86.
———, Der Einflusz des Windes auf die kutikuläre und stomatäre Transpiration. Svensk. Bot. Tidskr. **26**, 1932: 45–70.
———, M. G., Die stomatäre Transpiration und die Physiologie der Spaltöffnungen. *In*: Handbuch der Pflanzenphysiologie, ed. by W. RUHLAND, **3**, 1956: 351–426.
———, The water output of the guard cells of the stomata. Physiol. Plantarum **10**, 1957: 752–774.
TRELEASE, S. F. and B. E. LIVINGSTON, The daily march of transpiring power as indicated by the porometer and by standardized hygrometic paper. Journ. Ecology **4**, 1916: 1–14.
VEEN, R. VAN DER, Induction phenomena in photosynthesis. 1. Physiol. Plantarum **2**, 1949: 217–234.
VEJLBY, K., Induction phenomenon and CO_2 gush in photosynthesis of *Polytrichum attenuatum*. Physiol. Plantarum **11**, 1958: 866–877.
VIRGIN, H. I., Light-induced stomatal movement in wheat leaves, recorded as transpiration. Physiol. Plantarum **9**, 1956: 280–304.
———, Stomatal transpiration of some variegated plants and of chlorophyll-deficient mutants of barley. Physiol. Plantarum **10**, 1957: 170–187.
VRIES, D. A. DE and H. J. VENEMA, Some considerations on the behaviour of the PICHE evaporimeter. Vegetatio Acta Geobot. **5–6**, 1954: 225–234.
WALTER, H., Die Verdunstung von Wasser in bewegter Luft und ihre Abhängigkeit von der Grösze der Oberfläche. Zeitschr. f. Bot. **18**, 1926: 1–48.
WASSINK, E. C., Phosphate in the photosynthetic cycle in *Chlorella*. Research in photosynthesis (Proc. Gatlinburg Conf., 1955) ed. by H. GAFFRON, Intersc. Publ. Inc. New York, 1957: 333–339.
——— and C. VAN DER SCHEER, A spherical radiation meter. Meded. Landbouwhogesch. Wageningen **51**, 1951: 175–183.
———, J. F. G. M. WINTERMANS and J. E. TJIA, Phosphate exchanges in *Chlorella* in relation to conditions for photosynthesis. Proc. Kon. Ned. Akad. v. Wet. A'dam **C54**, 1951: 41–52.
WEINTRAUB, M., Leaf movements in *Mimosa pudica*. New Phytologist **50**, 1951: 357–383.
WELTEN, M., Physiologisch-ökologische Untersuchungen über den Wasserhaushalt der Pflanzen mit besonderer Berücksichtigung der Wasserabgabe-Widerstände. Planta **20**, 1933: 45–165.
WILLIAMS, W. T., Studies in stomatal behaviour. 1. Stomatal movement induced by heat-shock stimuli and the transmission of such stimuli across the leaves of *Pelargonium zonale*. Ann. Bot. N.S. **12**, 1948: 35–51.

WILLIAMS, W. T., A new theory of the mechanism of stomatal movement. Journ. Exptl. Bot. **5**, 1954: 343–353.
——— and F. A. BARRETT, The effect of external factors on stomatal starch. Physiol. Plantarum **7**, 1954: 298–312.
WILLSTÄTTER, R., and A. STOLL, Untersuchungen über die Assimilation der Kohlensäure. Berlin, 1918: 448 p.
WILSON, C. C., The effect of some environmental factors on the movement of guard cells. Plant Physiol. **23**, 1948: 5–37.
WINTERMANS, J. F. G. M., Polyphosphate formation in *Chlorella* in relation to photosynthesis. Meded. Landbouwhogesch. Wageningen **55**, 1955: 69–126.
WRENGER, M., Über den Einflusz des Windes auf die Transpiration der Pflanzen. Zeitschr. f. Bot. **29**, 1935: 257–321.
YAMAOKA, Y., Experimental studies on the relation between transpiration rate and meteorological elements. Transact. Amer. Geoph. Un. **39**, 1958: 249–266.
YEMM, E. W. and A. J. WILLIS, Stomatal movements and changes of carbohydrates in leaves of *Chrysanthemum maximum*. New Phytologist **53**, 1954: 373–397.
YIN, H. C. and Y. T. TUNG, Phosphorylase in guard cells. Science **108**, 1948: 87–88.