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Water use efficiency and competition between arid zone annuals, especially the grasses Phalaris minor and Hordeum murinum

326 73)

Abstract

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Field and phytotron experiments reveal that arid zone annuals have different strategies to deal with available water. One strategy is to invest little in roots, and concentrate all efforts on rapid development of leaves and flowers. Such plants, for which the grass *Hordeum murinum* serves as an example in this study, are forced to close their stomata as soon as water stress occurs. Thus avoiding the most severe stress, they save water.

Other plants, such as *Phalaris minor*, produce an extensive root system which allows them a certain tolerance to stress. They tend to keep their stomata open and are thus less economical with water.

Although the 'saver' always takes an initial lead in competition, the 'spender' strategy appears to be advantegous in relative wet years, as such plants have better access to the available minerals and eventually succeed in intercepting most of the light energy available. There are good reasons to assume that in relatively dry years the 'savers' have the best chances to survive and produce seed. Hence in a natural vegetation both strategies coexist.

Acknowledgments

Contents

List of abbreviations

RH = relative humidity
RM = mesophyll resistance to CO_2 diffusion
RS = diffusion resistance of the stomata
RTP = relative rates of transpiration and photosynthesis
RWC = relative water content
SRR = shoot/root ratio
TC = transpiration coefficient
T/P_n = ratio of transpiration and net photosynthesis
WCS = water content of shoot
WUE = water use efficiency

H. Loy

Water Use eff. and competition between arid
zone annuals, especially the grasses Phalaris
minor and Hordeum murinum

Centre for Agrobiological Research, Wgn, 1976.
Plant niveau.

- Water use efficiency = reciproke transpi-
ration coefficient

60% gebruik   1   40%

$$\frac{h/h \text{ water transpired}}{h/h \text{ dry matter produced}}$$
verdampt.

Sewashence → efficient gebruik.

1 General introduction

All land-dwelling organisms lose water to the atmosphere. Even if they are effectively protected by an impenetrable epidermis or skin, the necessity to exchange CO_2 and O_2 takes its toll. On the sites of gas exchange, a wet surface is exposed to the air, and evaporation of water is inevitable. In plants, this exposed surface is mainly formed by the cell walls that line the substomatal cavity, the epidermal cell walls being covered with a cuticle that is virtually impenetrable to water.

Certainly transpiration has important functions for a plant like cooling of the leaves and maintaining the transport of minerals, but I generally endorse the arguments of Gale et al. (1966) and consider transpiration for the major part a wasteful process. Particularly in arid environments, some of the most conspicuous adaptations of the plants concern their water economy.

Primary production in arid zone is limited by the rainfall. However, from only the rainfall in a certain year the crop or pasture production can not be quantitatively determined. How much the proportionality of rainfall and pasture production can vary from season to season, is demonstrated in Fig. 1, where the annual rainfall and production data in a field in Israel are plotted against each other. The maximum amount of water needed to produce 1 kg of dry matter is about 1300 l, whereas the minimum is approximately 550 l. In this field, the Migda Experimental farm (34°25'E 31°22''N), the soil is a deep löss mantle, with a water storage of 150 mm per m of soil. It is situated in Israel's 200 mm rainfall zone, and even the maximum expected rainfall
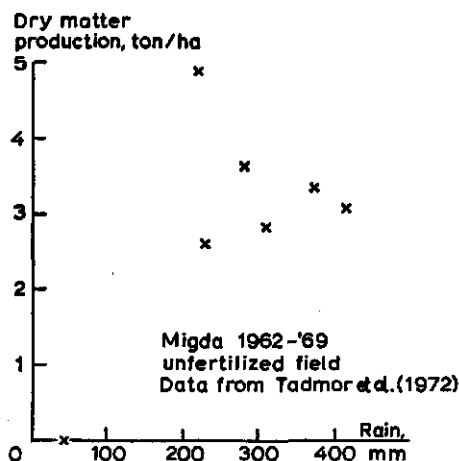

Fig. 1. Relation between production and annual rainfall, for 7 consecutive years.

1

(approx. 400 mm) can be stored in the top soil layer that is within reach of the roots of the (mainly annual) vegetation. Run-off does not occur. Virtually all incoming rain water either evaporates directly from the soil or is taken up and transpired by the vegetation. The fraction of the total incoming water that is lost by evaporation shows variations from year to year (Van Keulen, 1975), but is insufficient to explain the enormous differences in production/rainfall ratio observed. This means that the Water Use Efficiency (WUE) of the vegetation itself must vary considerably from year to year. These are 4 possible causes for this:

1. The potential evaporation of the atmosphere (which is defined as the evaporation from a water surface of infinite extension) differs, owing to yearly differences in average radiation, temperature, relative humidity, and wind speed;

2. temporary droughts between rain showers cause the vegetation to be water stressed, which is liable to have an impact on the efficiency of water use;

3. under the same circumstances, different plant species may show different WUE, so that the composition of the vegetation (which is known to differ considerably for year to year) determines the annual transpiration coefficient.

4. nutrient deficiencies have a distinct effect on the TC, mainly by decreasing the efficiency of photosynthesis.

A joint Dutch-Israeli project was started in 1971 to study primary production under (semi-)arid conditions, and mainly with limited water supply. Models have been developed to simulate this production from climatological input data, using fundamental physical and physiological characteristics of plant, soil and atmosphere. These models, extensively described by Van Keulen (1975), calculate transpiration coefficients for a 'standard plant' under various circumstances but with optimum supply of nutrients and do not account for possible differences between different species. He also assumed that the water potential of the soil only determines the *rate* of water use, but not the water use *efficiency*. It will be the main aim of the present study to deal with these two aspects.

The questions posed were the following:

1. Do differences exist in WUE of different plant species that are growing under apparently the same conditions in the experimental field in Israel?

2. What is the influence of water stress on the WUE of these pasture plants, and does it accentuate differences between species, if any?

3. How can annuals (of which the Migda vegetation practically exists) with different WUE and, possibly, different reactions to stress thrive together, in other words: how do they stand in competition?

4. What kind of simple field techniques are appropriate to study these problems, and which characteristics of the plants can be determined far away from the field, under controlled conditions, and still be relevant for their WUE, reaction to stress and competition in the field?

The last question was raised, because the project was set up to yield methods and results that can be applied in developing countries, where research facilities are limited.

With the 4th question in mind, experiments were set up to answer the first 3 ques-

tions. Since all Migda annuals could not possibly be studied extensively, the 2 most abundant grasses, *Phalaris minor* Retz. and *Hordeum murinum* L., were chosen for detailed study.

The present study has an eco-physiological character, i.e. an attempt has been made to connect field and laboratory observations in a meaningful way. It would be futile to try and separate physiology and ecology, we cannot even distinguish well between them. Purely ecological field observations, without any experimental inter- ference, have been done, but yielded only qualitative data. Purely physiological experiments, with measurements on subcellular phenomena, have not been done. In all experiments described, the plants were left intact and their behaviour was studied under more and less controlled conditions: 'more controlled' in the phytotron in Wageningen, 'less controlled' in the field in Israel, but even there controlled to a certain extent.

Thus, experiments under less controlled conditions have supplied the background for experiments under more controlled conditions, while the latter have clarified some of the results of the former.

2 Theoretical considerations about the transpiration coefficient

The transpiration coefficient (TC) is the ratio of the amount of water transpired to the amount of dry matter produced during a certain period. It is identical to the 'water requirement' of Briggs & Shantz (1913) and the transpiration ratio of De Wit (1958). Inless otherwise stated, the TC will refer to above-ground dry matter only. The term water use efficiency (WUE) will be used to indicate the reciprocal of the TC. The following theoretical considerations will, separately and in combination, treat the processes of transpiration and net photosynthesis. Their ratio is not the same as the TC, since net photosynthesis is not identical to above-ground dry matter production. To link them, dark respiration, distribution of dry matter over shoot and root, and root respiration should be taken into detailed consideration. This has not been done, but it is assumed that for a certain plant species, growing under more or less constant conditions, the amount of carbon ultimately found in the shoot is proportional to the total assimilated carbon, and that the T/Pn ratio can be used to explain some particulars of the TC.

As long as no conclusive evidence has been put forward that the T/Pn ratio is an intrinsic property of the plant, separate treatment of both processes seems to be the best approach to the problem.

2.1 Photosynthesis

The response of photosynthesis to light is represented by the well-known Blackman curve and is interpreted to exist of 2 parts: the first part where photosynthesis is proportional to light intensity, and the second part where it is virtually constant and said to be 'light saturated'. Whether light limited or not, net photosynthesis can be described with a diffusion equation:

$$P_n = \frac{c_{ext} - c_{int}}{R} \tag{1}$$

where c_{ext} and c_{int} are the external and internal (substomatal cavity) CO_2 concentrations, respectively, and $R = RS + RA$ the sum of the diffusion resistances of the stomata and the boundary layer to CO_2.

In 1961, Kuiper published evidence for the hypothesis, that the stomatal opening could be attributed to the CO_2 content of the guard cell chloroplasts, being chiefly determined by their rate of photosynthesis. Recently, the evidence for this theory was reviewed by Raschke (1975). How exactly the regulation takes place, is unknown

but under a broad range of circumstances the CO_2 concentration in the substomatal cavity, c_{int}, seems constant. This yields a constant product of P_n and $(RS + RA)$, constant at least for different light intensities, but not for different external CO_2-concentrations and for different degrees of water stress. This adaptation of the stomatal opening to the rate of photosynthesis leads to appreciable saving of water.

2.2 Transpiration

The transpiration of a leaf can be formulated as:

$$T = \frac{e_{leaf} - e_{air}}{RS + RA} \tag{2}$$

in which e_{air} and e_{leaf} are the H_2O concentrations in $g \cdot m^{-3}$, and RS and RA are the resistances for water vapour diffusion, expressed in $sec \cdot m^{-1}$. The dimension of T is then $g \cdot m^{-2} \cdot sec^{-1}$. $RS + RA$ does not equal the $RS + RA$ used in the expressions for photosynthesis. A constant conversion factor exists, however, slightly depending on the turbulence of the air. Generally, $RA + RS$ for CO_2 is taken to be $1.3 - 1.5$ times $RA + RS$ for H_2O. (For a pure diffusion process, the theoretical ratio is about 1.7).

Since e_{leaf} is a function of leaf temperature, which is very hard to measure independently, the best approach to evaluate evapotranspiration is to account for the whole energy balance of the evapotranspiring body. Treatments of the energy balance have been often given in the literature (Penman, 1948; Rijtema, 1965; Slatyer, 1967), so that it is not necessary to discuss it here.

In its simplest form, storage of energy and energy consumption by photosynthesis are neglected and 3 basic equations are left:

$$ABSRAD = EHL + SHL \tag{3}$$

in words: the absorbed net radiation equals the sum of the evaporative heat loss and the sensible (or convective) heat loss, all expressed in $J \cdot m^{-2} \cdot sec^{-1}$.

$$SHL = \frac{(t_{leaf} - t_{air}) \times \rho_a c_p}{RA} \tag{4}$$

where ρ_a is the density of the air in $g \cdot m^{-3}$, c_p the specific heat of air in $J \cdot g^{-1} \cdot {}^{\circ}C^{-1}$ and RA the resistance of the boundary layer to heat transfer in $sec \cdot m^{-1}$. This RA differs very little from the RA for water vapour transfer (Monteith, 1973)

$$EHL = \frac{SVPL - RH \times SVPA}{RA + RS} \times \frac{\rho_a c_p}{\gamma} \tag{5}$$

with γ being the psychrometric constant in $mmHg \cdot {}^{\circ}C^{-1}$, SVPL and SVPA are the saturated vapour pressures at leaf and air temperature, respectively, in mmHg, RH is the relative humidity, expressed as a dimensionless fraction.

If the atmospheric conditions and RA and RS are known, EHL can be solved from these 3 equations, finding t_{leaf} by means of an iteration procedure.

5

An analytical approximation was proposed by Penman (1948). If SVPL is expressed as $SVPA + \Delta \times (t_{leaf} - t_{air})$, with Δ being the slope of the saturated vapour pressure curve at air temperature (in mmHg $°C^{-1}$), Eqn (5) becomes:

$$EHL = \frac{\Delta \times (t_{leaf} - t_{air}) + (1-RH) \times SVPA}{RA + RS} \times \frac{\rho_a c_p}{\gamma} \qquad (6)$$

From Eqns (3), (4), and (6) SHL and $(t_{leaf} - t_{air})$ can be eliminated, after which rearrangement yields:

$$EHL = \frac{\Delta \times ABSRAD + \dfrac{(1-RH) \times SVPA}{RA} \times \rho_a c_p}{\dfrac{RA + RS}{RA} \times \gamma + \Delta}$$

$$EHL = \frac{\Delta \times ABSRAD + \gamma \times \dfrac{VPHT \times DEFICIT}{RA}}{\dfrac{RA + RS}{RA} \times \gamma + \Delta} \qquad (7)$$

where VPHT is the latent heat of vaporization (2480 J g^{-1}) and DEFICIT is the

saturation deficit of the air in g H_2O m^{-3}. The term $\dfrac{VPHT \times DEFICIT}{RA}$ is called

the wind term of the energy balance. For a freely evaporating surface ($RS = 0$) Eqn (7) becomes:

$$EHL = \frac{\Delta}{\Delta + \gamma} \times ABSRAD + \frac{\gamma}{\Delta + \gamma} \times \text{`wind term'} \qquad (7a)$$

It is easily seem from (3) and (7a) that EHL = ABSRAD = wind term when there is no temperature difference between the air and the evaporating surface. Under such conditions the absorbed radiation exactly replenishes the latent heat lost by evaporation.

If leaf temperature differs considerably from air temperature, using Δ at air temperature causes errors. This situation occurs at very high values of RS and under such circumstances the iteration procedure is preferred. The behaviour of EHL and its dependence on environmental conditions and RS are demonstrated in Figs 2, 3 and 4. The extrapolations to infinite and zero RA have been introduced to make the figures easily surveyable, but they have no practical value. It is notable that with decreasing RA the influence of the relative humidity of the air overrules, while at high values of RA the absorbed radiation is the dominating factor. Whether a change in RA itself causes the evapotranspiration to increase or to decrease, depends fully on the circumstances and cannot be predicted offhand. If RS is very low, decreasing RA increases
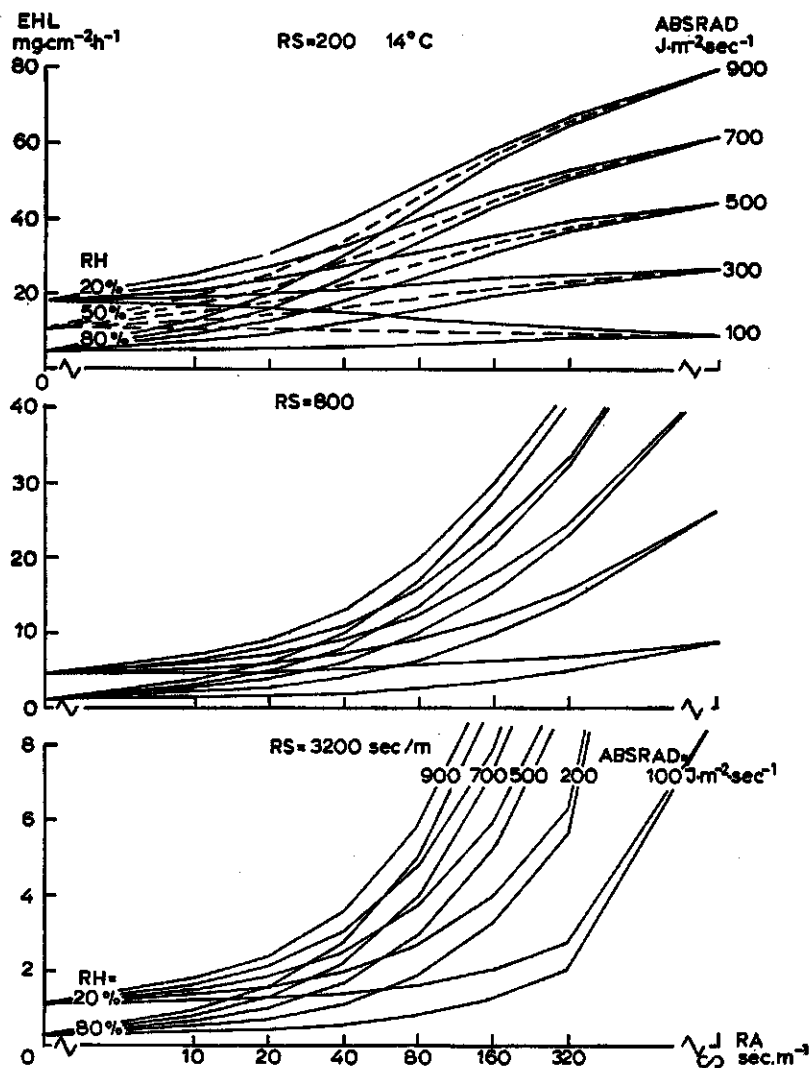
Fig. 2. Relation between evaporation and boundary layer resistance, at different levels of absorbed radiation, relative humidity, and stomatal resistance, calculated according to formula (7). T = 14°C.

evaporation, while generally the reverse holds true for very high values of RS. In the latter case, the direct cooling effect of the wind overrules its stimulation of vapour exchange.

2.3 Advection and continentality

The convection of heat (= SHL) follows a daily course, owing to the natural lag of air temperature behind irradiation. Other things being approximately equal, SHL reaches a maximum in the afternoon. There may even be a flux of sensible heat from

7
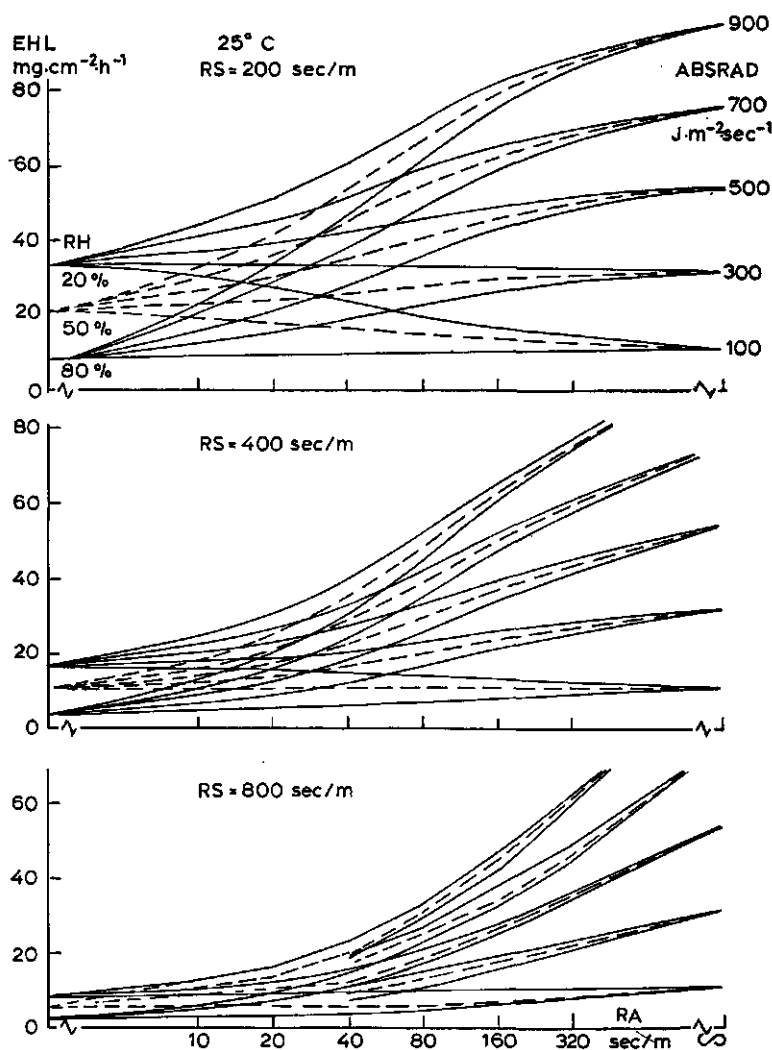
Fig. 3. Relation between evaporation and boundary layer resistance, at different levels of absorbed radiation, relative humidity, and stomatal resistance, calculated according to formula (7). T = 25°C.

the crop to the air in the morning and in the opposite direction during the afternoon. This is clearly demonstrated by the measurements of Lemon et al. (1957), who recorded an evaporative heat loss of the canopy $2\frac{1}{2}$ times in excess of the irradiated energy at 16:30. This phenomenon is sometimes called advection, but we would rather keep this term for those situations where the daily total evaporation is in excess of the daily total absorbed net irradiation. This latter phenomenon is called the 'oasis-effect' by Lemon. It occurs when an irrigated crop is in the midst of dry surroundings, and the wind which reaches the crop has been heated up while passing over the dry area, where irradiated energy is only partly dissipated by the evaporation
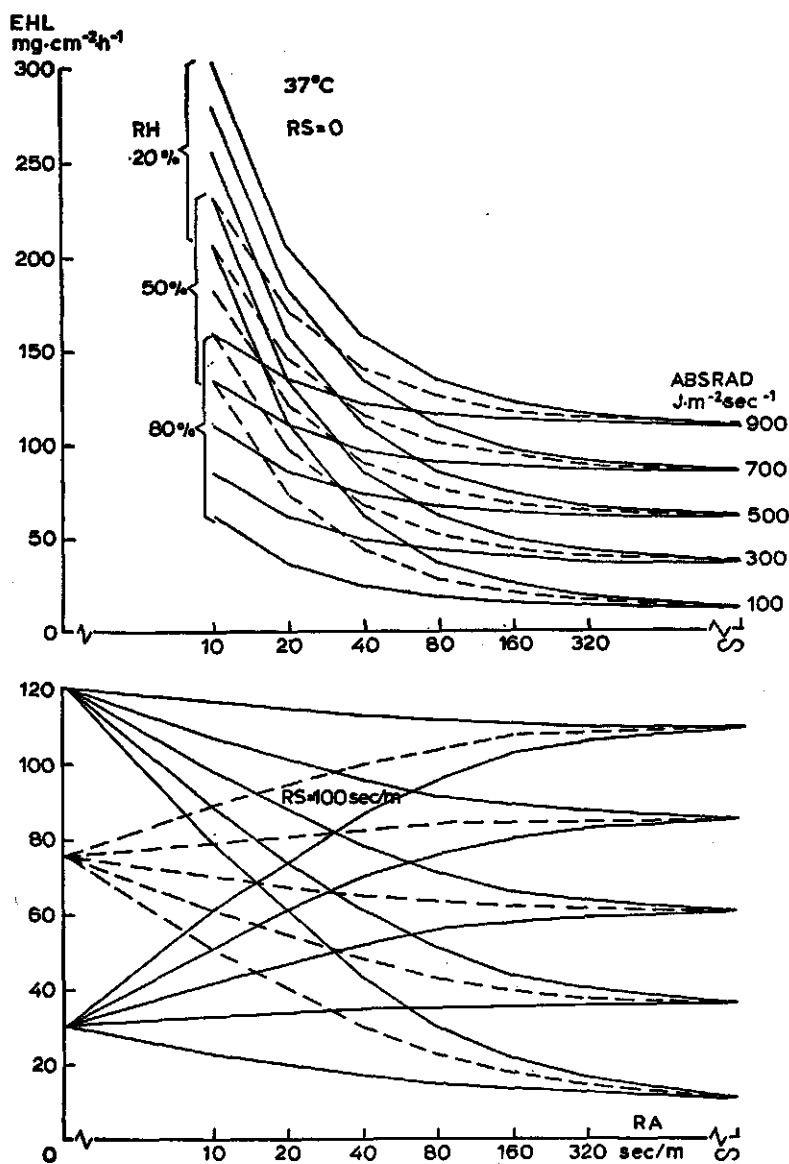
Fig. 4. Relation between evaporation and boundary layer resistance, at different levels of absorbed radiation, relative humidity, and stomatal resistance, calculated according to formula (7). T = 37°C.

of water. Opinions diverge on the extension of this phenomenon. While Stanhill (1961) believed the effect to act only a few hundred metres downwind over the irrigated area, Lemon et al. observed considerable advection in a cotton field, at least 10 miles removed from dry grounds. De Wit (1958), when comparing TC data obtained from containers and irrigated fields, concluded from their close agreement that even large fields gain energy from advection, in arid zones. Rijtema (1965) discriminated

between 'small-scale' advection and 'large-scale' advection. The former is mainly important for container and lysimeter experiments, and for evaporation measurements with a pan. If these containers are not surrounded by a crop, the environment must be kept wet. It does not become clear, how large the scale of the 'large scale' advection is. In our opinion, advection is a rather widespread phenomenon. The saturation deficit of the air is mainly determined by the distribution of the irradiated energy over convected heat and evaporated heat in the area over which the air is passing. This depends on the rainfall, the percentage of the land that is covered with vegetation, and the distance from the sea, in other words: on the continentality. For agricultural purposes, a good measure of continentality would be the quotient EHL/ABSRAD, where EHL must be the potential evaporation, i.e. the evaporation as calculated in a Penman heat balance formula for an open water surface. The ratio is not constant over the day and not even over the year, so that an array of monthly averages would probably be the best representation.

Calculations should be carried out separately for day and night temperatures. Using daily averages in the Penman formula leads to major errors and renders useless results.

In oceanic climates the continentality EHL/ABSRAD approximates unity, since the saturation deficit of the air is in equilibrium with a freely evaporating surface of nearly infinite extension. Then, the wind term of the energy balance, Eqn (7a), is quantitatively equal to the absorbed radiation, at least when averaged over longer periods. In continental climates, however, the saturation deficit of the air allows for more evaporation than is enabled by the irradiation. As a result of this, the temperature of the crop will drop below the air temperature and energy is transported from the air towards the crop. The bigger the proportion of land that lies dry, the more the evaporative demand of the air passing over it will dominate the energy balance. Wallen et al. (1962) observed that in the Penman formula the relative importance of the radiation term and the 'aerodynamic' term depends on the distance from the sea so that in an oceanic country like England the aerodynamic term may be neglected, while on the other hand in typically arid climates the evaporation can well be predicted using only the saturation deficit of the air.

2.4 The transpiration coefficient

Of single leaves The single leaf is supposed to be exposed to meteorological conditions as measured at 2 m height. With minor restrictions, what holds for a single lead holds for a single plant as well, as long as its leaves do not shade each other or otherwise influence each other's microclimate.

With increasing irradiation both a leaf's transpiration and its photosynthesis increase. The T/P_n ratio may be approximately constant in the ascending part of the photosynthesis light-response curve, but after light saturation has been reached the ratio increases proportionally with increasing radiation.

Naturally, the T/P_n ratio will continue to increase with more light, and especially

after photosynthesis has reached light saturation. The stomatal opening is fixed under such conditions, as long as no serious water shortages occur. If they do, their influence may overrule the CO_2 response of the stomata and, in spite of sufficient light, the stomata will close tending to decrease both photosynthesis and water loss. But the former will partly be compensated by lowering of c_{int}, as long as the drought does not hamper the photosynthetic capacity seriously. Depletion of CO_2 in the intercellular space will cause the CO_2 gradient to steepen, so that P_n is liable to decrease less than proportionally at stress-forced stomatal closure. Thus, stress-imposed stomatal closure improves a plant's WUE.

Because the saturation deficit of the air lags behind the radiation (approx. 3 hours, on a sunny day), the daily evaporation curve is asymmetric with its maximum in the afternoon. The T/P_n ratio then, is also highest in the afternoon, and liable to persist close to its maximum for a few hours. Thus stomatal closure in the afternoon will save water, as it will work as a selection between 'cheap' and 'expensive' hours of the day. A plant's stomatal behaviour can thus markedly improve its WUE.

The T/P_n ratio of an extended crop   In a crop that has reached nearly complete light interception, most leaves are in some way influenced by other leaves. The crop has a microclimate, with distinct profiles of light intensity, temperature, relative humidity, and even of CO_2 concentration. The influence of various environmental factors on a crop's transpiration and photosynthesis cannot readily be predicted. If one starts from information obtained on the single leaf level, the best approach is probably the simulation of crop performance with a computer model that keeps track of environmental conditions at different heights in the canopy. Such models have shown to yield applicable results, although many aspects of the crop are as yet unknown.

One such a model for photosynthesis has been described in De Wit's 'Photosynthesis of leaf canopies' (1965). Fig. 5 summarizes the results of his calculations of daily photosynthesis from daily irradiation for a standard canopy with spherical leaf distribution, leaf area index 5, RA = 0.5 sec·cm^{-1}, and not short of water. The represented data are only for very clear days, and for 5 northern latitudes. At very high latitudes the efficiency of photosynthesis is much better than around the equator, but between 20 and 60°N.L. no great differences exist, and it is seen that from 100 to 400 cal cm^{-2}·day^{-1} the photosynthesis responds virtually linearly to irradiation. As a matter of fact, the photosynthetic efficiency of using diffuse light shows an even greater similarity, at different places on earth. Still, the increase of P_n is nowhere proportional to the increase in light, but always less. Light saturation does not occur in well-developed canopies.

Evapotranspiration of the canopy can best be understood from the energetic point of view. In absence of advection, the daily irradiated energy provides the upper limit for the evapotranspiration. A crop of infinite extension will convect heat towards the air in the morning, and get part of it back in the afternoon. As in single plants, the T/P_n ratio follows a daily course with a broad peak in the afternoon, and effective stomatal closure at that time is liable to improve the crop's WUE. In con-

11
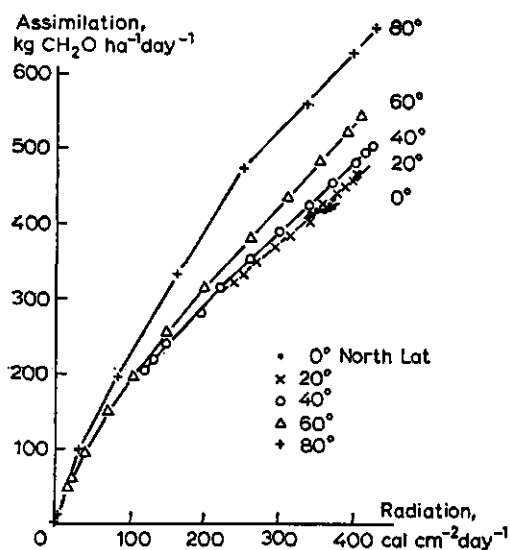
Fig. 5. Relation between net assimilation and daily total radiation on 5 latitudes. Only clear days are considered. A canopy with LAI 5 and density 0.1 is assumed. Data from De Wit (1965).

tinental areas, water loss is drastically increased by advected energy, which has no use for production. Eqn (7) shows that a high saturation deficit of the air renders the radiation factor relatively less important. In the denominator, the influence of RS on the evaporation depends on the relative values of γ and Δ, such that at increasing temperature the effect of RS on water loss decreases. Therefore in cool, humid climates the evaporation is closely linked to the radiation level and the stomatal resistance, while in hot, dry climates this is not so. Since photosynthesis is always strongly dependent on ABSRAD and RS, we may conclude that in cool, humid climates transpiration and photosynthesis are linked, while in hot, dry climates they respond to different parameters. Under comparable conditions of irradiation, the TCs of different sites are proportional to their continentalities. Although corrections for differences in photosynthetic efficiency must be made, this theory generally explains why, as has been observed by De Wit (1958), in climates with a small percentage of bright sunshine production is proportional to water loss, and in climates with a high percentage of bright sunshine the TC is proportional to the pan-evaporation. In my opinion, it is better to talk about continentalities than to discriminate on bright sunshine percentage. That pan-evaporation yields a good measure of advection, is not amazing. The evaporation pans generally used are set up on platforms, and gain a considerable part of their energy through direct advection. On the other hand, the sensitivity for irradiation is reduced by storing heat during the day and dissipating it at night.

The connection between continentality and T/P_n ratio is relevant to artificial climates as well. At least it mainly explains the differences in TC that we found between phytotron and greenhouse. Eqn (7) was applied, assuming RS to be zero and the transpiring body to have the width of a grass leaf: 6 mm. RA was calculated

12

to be:

$$RA = 92.5 \times \sqrt{\frac{WD}{WS}}$$

where WD is the width of the vaporizing surface, in m, and WS is the wind speed at the vaporizing surface, in m·sec^{-1}. Then, for our greenhouse in summer (conditions: 25°C, 70% rel.hum., WS = 0.2 m·sec^{-1}, ABSRAD is 50% of 0.7 cal·cm^{-2}·min^{-1}) the continentality turns out to be 1.9, while it amounts up to 5.2 under the conditions of the phytotron that we used (25°C, 70%, 0.25 m·sec^{-1}, and 50% of 0.2 cal·cm^{-2}·min^{-1}). These values are very high, although not directly comparable to field data, due to differences in RA and % of light absorption. Under conditions of a pot trial outside, with all leaves exposed to similar environment, this approach can be used. The climatic data available for the pot triats in Gilat (Chapter 4) are rather limited. During a trial in April 1974, however, some climatic records were collected. During daylight, the average temperature was 25°C and the average relative humidity about 40%. If now the wind speed is estimated to average 1 m·sec^{-1}, and the ABSRAD 300 J m^{-2}·sec^{-1}, then we find a continentality of 3.8 for a 6 mm wide leaf with zero RS. For a trial in February 1974, using standard meteorological records, about half of this value was found. Wind speed was again assumed to equal 1 m·sec^{-1}, but unfortunately the result was very sensitive to the value of the wind speed used. The continentalities thus estimated for a freely evaporating body of leaf dimensions, give a fair representation of the magnitude of the differences found in TC under the conditions described. Although a more detailed treatment should take the efficiency of the photosynthesis under the various light conditions into account, the present simplified approach clarifies much of the differences in TC observed.

3 Transpiration coefficients of Migda species, determined in Wageningen

Because different species in the Migda vegetation might have different efficiencies of water use, even under controlled conditions, seeds were collected in April 1972 in the field and taken to Wageningen, where, after a few months at low temperature, most of them germinated and grew well.

3.1 Materials and methods

Most measurements, described below, were carried out at the Institute for Field Crops and Herbage (IBS) in Wageningen, mainly using the phytotron facilities, while for some experiments the greenhouse was used. The phytotron has been extensively described by Alberda (1958), and, in short, offers the following options: light intensity and wind speed are kept constant at 0.08 cal·cm^{-2}·min^{-1} and 0.25 m·sec^{-1}, respectively, while air temperature and humidity can be varied between, roughly, 10 – 35°C and 60 – 100% relative humidity. The greenhouse has natural light, reduced about 25% by the glass, while temperature and relative humidity are controlled. The daylength in the phytotron is 17 hours, in the greenhouse it is natural, but can be extended with artificial light.

Seeds were germinated on moist perlite, and after 2 weeks transferred to 1 litre jars, containing nutrient solution ($\frac{1}{2}$ strength Hoagland). After another 2 or 3 weeks the plants were generally big enough to be used, except when they were grown at low temperature (15°C). The grasses were in the vegetative stage at the start of the experiment. Nutrient solution was replenished twice or three times a week, depending on plant size. Transpiration was determined by weighing the pots + plants every time before and after replenishing, and integrating the differences. Evaporation directly from the solution was estimated from blanks, and substracted. The measurements were extended over a period of 2 or 3 weeks, after which the plants were harvested and fresh and dry weight of shoot and root were determined. The dry weight was corrected for the dry weight at onset of the measurements, the difference yielding the net production. TC and TC$_{tot}$ were obtained by simply dividing transpiration by shoot dry weight and total dry weight, respectively. From fresh and dry weight of the shoot the Water Content Shoot was calculated as % of fresh weight:

$$\text{WCS} = \frac{\text{fresh} - \text{dry weight}}{\text{fresh weight}} \times 100$$

Shoot/Root Ratio is abbreviated as SRR, and was calculated from dry weights.

Relative Growth Rates (RGR) were determined by periodic harvesting at 7 day intervals. After 4 or 5 harvests of 10 random plants had been analysed, the shoot dry weights were plotted against time on semilog paper, and the RGR was calculated from the slope of the straight part of the line obtained.

3.2 Results and discussion

The most striking feature with this kind of experiment is, that differences in TC of the same species in different experiments are always much bigger than differences between species within one experiment. This is partly explained by the fact that wild plants are used, propagated by seed. Cultivated species, particularly when propagated vegetatively, show much less variation. The differences between experiments cannot be completely accounted for in terms of differences in temperature and relative humidity of the air. The general impression is that interspecific differences in water use efficiency are small, under the conditions described, and that the values for the TCs obtained from the phytotron are about twice as high as the values from the greenhouse, in summer. Table 1 gives the data from 5 annual grasses and 3 cultivated crops. Most TCs range from 300 – 500, but much lower figures are obtained for: 1. plants growing at 15°C; 2. plants growing in the greenhouse; 3. Rhodes grass. The latter is a tropical grass with C_4 type photosynthesis, which accounts fully for its low TC. The greenhouse, as already pointed out, has in summer a much lower 'continentality' than the phytotron, which approximately accounts for the differences in TC observed. That TC in the phytotron should be lower at 15°C than at higher temperatures, can also be understood from the energy balance Eqn (7). But the difference is considerably bigger than one would expect from the corresponding continentalities (EHL/ABSRAD should at 15°C be about 30% lower than at 25°C, other things being equal). Respiratory losses must be much lower at 15°C than at 25°C.

The 'normal' TCs of Table 1 can only be compared within the scope of the same experiment. The data from the 5 Migda grasses have, therefore, been properly ordered in Table 2, where also relative values have been attributed to the absolute figures, taking the TC of *Hordeum* as unity. When these relative values are averaged, the TCs of *Stipa* and *Lophochloa* are approximately the same as *Hordeum*'s, but those of *Phalaris* and *Avena* are consistently about 25% higher. The differences for TC_{total} are slightly smaller because of compensation in the SRRs.

In Table 3 a more detailed comparison is made for the species *Hordeum, Stipa, Avena* and *Phalaris*, in one experiment. They are put in order of increasing TC. It is seen that the trend in TC is accompanied by WCS and SRR, species with higher TC having generally also a higher WCS and a lower SRR. This negative correlation between SRR and WCS is remarkable. In Fig. 6 the SRRs from Table 1 are plotted against the corresponding WCSs. The data of *Stipa, Hordeum, Avena* and *Phalaris* have been summarized in average and standard deviations. As far as Hordeum is concerned, the WCSs obtained in the 1972 measurements deviate considerably from the ones obtained in 1973. If the former are not considered, the average H' is ob-

Table 1. Some characteristics of relevant Migda grasses, and 3 cultivated species as measured plants, grown on nutrient solution in the phytotron. Figures are averages of 10–20 observations.

Species	Date	t °C	RH %	Age days	TC g/g	TC shoot	RGR day^{-1}	WCS %	SRR
Phalaris	September '71	20	70	40	387	519	.	90	2.94
minor	September '71	20	70	50	360	487	.	89.2	2.84
	June '72[1]	20	80–90	35	195	261	0.215	91.6	2.94
	720508–720606	10	80	45	.	.	0.153	88.8	2.57
	730131–730614	25	.	60	255	767	0.196	87.7	2.62
	730201–730215	20	.	40	281	364	0.254	89.3	3.38
	730223–730308	15	.	30	226	295	0.191	87.8	3.27
	730524–730608	15	55	50	196	252	0.190	86.7	3.50
	730524–730606	20	.	50	417	547	0.148	88.7	3.21
Stipa	730320–730403	15	.	60	.	.	0.109	86.3	5.35
capensis	730320–730403	20	80	50	360	425	0.195	81.0	5.51
	730320–730403	25	.	40	.	.	0.195	82.3	4.08
	730524–730608	15	55	30	145	166	0.157	78.7	6.80
	730524–730606	20	.	50	424	488	0.113	80.3	6.59
	720508–720606	15	60	50	.	.	0.140	82.9	5.37
	720508–720606	10	80	50	.	.	0.100	81.3	2.57
Lophochloa	720508–720606	15	60	50	.	.	0.182	80.8	4.28
phleoides	720508–720606	20	70	50	.	.	0.199	81.1	4.82
	730524–730608	15	55	50	181	230	0.157	80.5	3.69
	730524–730608	20	65	50	292	347	0.174	80.2	5.28
Hordeum	Jan./Febr. '72	22	50	40	483	573	0.148	89.0	5.38
murinum	Febr./Mar. '72	22	90–95	45	344	399	.	89.0	6.25
	June '72[1]	21	85	35	187	244	0.199	90.0	5.09
	730131–730214	25	.	40	459	546	0.215	85.5	5.27
	730201–730215	20	.	40	248	291	0.247	84.6	5.72
	730209–730223	15	.	45	260	305	0.215	82.3	5.73
	730524–730608	15	55	30	154	182	0.182	82.3	5.43
	730524–730608	20	65	50	368	439	0.144	82.6	5.18
Avena	720208–720223	23	50	40	483	630	0.165	86.7	3.28
wiestii	720322–720405	22	90–95	45	442	561	.	87.9	3.70
	June '72[1]	20	80–90	30	225	272	0.207	87.4	4.78
	730223–730307	15	.	40	.	.	0.178	84.7	4.48
	730223–730307	20	80	40	409	492	0.174	85.3	4.91
	730223–730307	25	.	40	.	.	0.166	86.4	3.23
	730524–730608	15	55	30	230	292	0.131	85.1	3.70
	730524–730606	20	65	50	420	502	0.118	84.8	5.13
Rhodes	710603–710616	25	75	50	159	187	.	89.1	6.83
grass	710826–710908	25	75	50	148	172	.	88.0	6.28
	710826–710915	25	75	60	180	214	.	86.7	5.35
	730524–730606	20	.	50	146	171	0.157	87.3	5.93
Wheat 1177	710820–710909	20	70	40	327	399	.	87.9	4.53
florence	710820–710909	20	70	40	295	353	.	88.1	5.08
opal	720616–720630	20	80–90	25	149	175	0.199	88.4	5.70
Maize	1968	20						91.5	3.82
	1968	20						91.5	3.82
	1969	20						90.9	2.87
	1969	25						90.6	4.91

1. This experiment was carried out in the greenhouse.

Table 2. Comparison of the transpiration coefficients of 5 grasses in 9 experiments. Within the scope of each experiment the figures are comparable, being related to plants that were growing during the same period in the same environment. Between parenthesis are relative figures with respect to $TC_{Hordeum}$

Period and temperature	Species				
	Stipa capensis	*Hordeum murinum*	*Lophochloa phleoides*	*Avena wiestii*	*Phalaris minor*
June 1972, 20°C					
TC_{total}	.	187 (1)	.	225 (1.2)	195 (1.04)
TC_{shoot}	.	224 (1)	.	272 (1.21)	261 (1.17)
Febr./March 1973, 25°C					
TC_{total}	.	459 (1)	.	.	555 (1.21)
TC_{shoot}	.	546 (1)	.	.	767 (1.41)
Febr./March 1973, 20°C					
TC_{total}	.	248 (1)	.	.	281 (1.13)
TC_{shoot}	.	291 (1)	.	.	364 (1.25)
Febr./March 1973, 15°C					
TC_{total}	.	260 (1)	.	.	226 (0.87)
TC_{shoot}	.	305 (1)	.	.	295 (0.97)
February 1973, 20°C					
TC_{total}	360 (1.06)	.	.	409 (1.2)	.
TC_{shoot}	425 (1.04)	.	.	492 (1.2)	.
May/June 1973, 15°C					
TC_{total}	145 (0.94)	154 (1)	181 (1.18)	230 (1.49)	196 (1.27)
TC_{shoot}	166 (0.91)	182 (1)	230 (1.26)	292 (1.60)	252 (1.39)
May/June 1973, 20°C					
TC_{total}	424 (1.15)	368 (1)	292 (0.79)	420 (1.14)	417 (1.13)
TC_{shoot}	488 (1.11)	439 (1)	347 (0.79)	502 (1.14)	547 (1.25)
February 1972					
TC_{total}	.	483 (1)	.	483 (1)	.
TC_{shoot}	.	573 (1)	.	630 (1.1)	.
March 1972					
TC_{total}	.	344 (1)	.	442 (1.29)	.
TC_{shoot}	.	399 (1)	.	561 (1.41)	.
Averages of relative values					
TC_{total}	1.05	1	0.99	1.22	1.11
TC_{shoot}	1.02	1	1.03	1.28	1.24

Table 3. Comparison of 4 grasses in one experiment.
May/June 1973, phytotron, 15/20°C, 65% RH.

| | 20°C | | | |
	Hordeum	Stipa	Avena	Phalaris
TC_{total}	368	424	420	417
TC_{shoot}	439	488	502	547
RGR	0.114	0.113	0.118	0.148
SRR	5.18	6.59	5.13	3.21
WCS	82.6	80.3	84.8	88.7
	15°C			
	Hordeum	Stipa	Avena	Phalaris
TC_{total}	154	145	230	196
TC_{shoot}	182	166	292	252
RGR	0.182	0.157	0.131	0.190
SRR	5.43	6.80	3.70	3.50
WCS	82.3	78.7	85.1	86.7

tained, which fits the general trend much better. A pronounced influence of the temperature on the SRR is observed in Fig. 6, demonstrated by the points of *Lophochloa* and the single point of *Stipa* at 10°C. The influence of temperature on the WCS is small, if any. The data for wheat, maize and Rhodes grass are not on the line, but clearly on the right hand side of it, suggesting that these cultivars have been selected for such favourable conditions, that they can afford to have both a high SRR and a high WCS. The advantages are clear: little material is invested in a root system, and a high water content in the shoot supports a fast development of the crop. Apparently, wild plants, growing in a natural unirrigated field, have to make a choice between both advantages, an extended root system being the condition to maintain a high water content in the shoot. The coincidence of high WCS with high TC is an obvious example of 'those that have plenty of butter can spread it thickly', but in this report an attempt will be made to arrive at a more physiological formulation.

A similar relationship between SRR and WCS exist in some experiments of Brouwer (1964, 1965), where differences in SRR are obtained by pretreatment at different temperatures and extirpation of various parts of the root system, respectively. With both treatments a change in WCS accompanies the change in SRR, as is shown in Fig. 7. Particularly in the latter case, the causality of the relation is evident. Brouwer suggested that stresses occur as a result of the root extirpation, causing reduced turgor in the shoot, which hampers shoot extension more readily than photosynthesis. This automatically causes the dry matter content of the shoot to increase. Analogous to his experiments and their interpretation, differences in SRR observed in my experiments may result from innate differences in priorities at the distribution of

SRR

S = Stipa capensis
H = Hordeum murimum
H'= idem, 3 extreme
    observations omitted
A = Avena wiestii
P = Phalaris minor
+ = Lophochloa phleoides, 20°C
* = idem, 15°C
x = Rhodesgrass
▽ = Maize
o = Wheat

80   85   90 % WCS
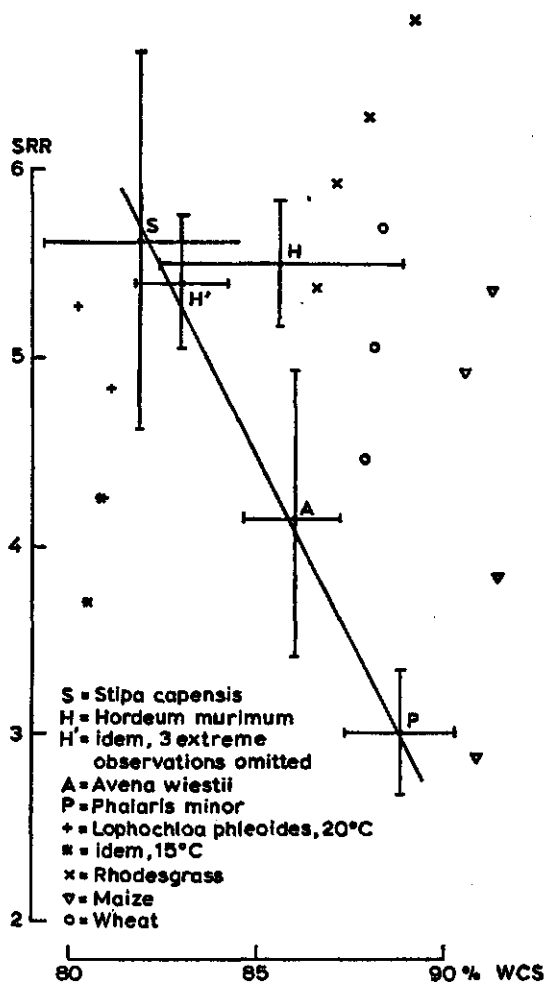
Fig. 6. Relation between SRR and WCS of 5 Migda grasses and 3 cultivated species, grown on nutrient solution, under controlled conditions. For Stipa, Hordeum, Avena, and Phalaris averages and standard deviations are plotted.

assimilates over leaves and roots. One could imagine that species with relatively few roots suffer low turgor most frequently, and hence end up with a lower WCS. Of course, these plants differ from Brouwer's in that they have had time to adapt, and consequently do not suffer 'intolerable' stress. But their adaptation does not go so far as to eliminate *any* stress, so that some of their morphological characteristics may be determined by those conditions where the root system is insufficient to maintain maximum turgor in the leaves. In those circumstances they face basically the same situation as artificially stressed plants.

It can be concluded from this chapter that differences up to 25% in WUE of different Migda grasses are found under well controlled conditions. Such differences seem to be correlated with morphological differences, species with the highest water content and most roots exhibiting the highest TC. This higher investment in the root system only partly explains the higher TC of *Phalaris* and *Avena*. Other possibilities are: a.

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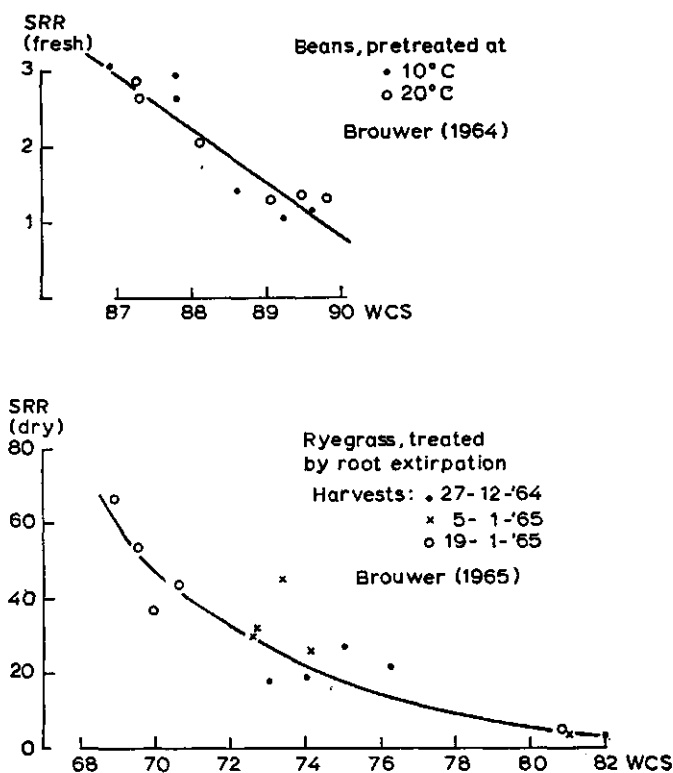
Fig. 7. Relation between SRR and WCS of beans and ryegrass, after different pretreatments. Data from experiments of Brouwer (1964, 1965).

difference in stomatal behaviour, resulting in 'selection' of more or less favourable hours of the day. This explanation must be discarded, since the conditions of the present experiment were constant; b. differences in photosynthetic capacity .Direct measurements of photosynthesis (Chapter 7) show, however, that *Phalaris* can reach a higher level of photosynthesis than *Hordeum*, so that this argument does not hold either; c. differences in stomatal resistance. At the same photosynthetic capacity, differences in RS would influence P_n less than transpiration, owing to the compensatory effect by c_{int}, as supposed in Section 2.4. The fact that *Phalaris* has a higher maximum photosynthesis than *Hordeum*, would involve a higher C_{int} (at equal efficiency), and hence require a lower RS for the same rate of photosynthesis, resulting in a higher TC.

20

4 Transpiration coefficients of plants growing on pots in the open, Gilat 1974

Aim To bridge the gap between field trials and measurements under controlled conditions, transpiration coefficients were determined on plants in pots, with the root environment controlled, but otherwise under natural environmental conditions in Israel.

4.1 Materials and methods

Plastic 12 litre buckets were used as plant pots and were filled with 10 kg of Gilat loam, taken from the top 30 cm of soil that had never been cultivated or fertilized. The soil was sieved before use and was slightly compacted by hand at filling. Samples were taken to determine the initial water content. The pots of 'fertilized' treatments got 10 g NPK fertilizer, containing 1 g pure nitrogen, mixed with the soil at filling.

Young vegetative plants were taken from the field, rinsed, and selected for homogeneity. About half of the leaf area was removed to reduce water losses during the establishment period. Each pot contained 5 plants. 2 kg of uniform and clean gravel was added to each pot to form a 5 – cm layer on top of the soil. The plants were watered and left for 1 or 2 weeks to establish, during which period dying specimens were replaced. On hot days in this period, the pots were covered with jute to shelter them from the sun.

For the fourth trial plants were used that had been sown in Gilat soil 6 weeks before the start of the experiment. They were transplanted to the pots 2 weeks before the start.

After establishment of the plants, the experiment was started. Water was added to bring the pots to the initial water content required, 15% for the dry treatments and 17.5% for the wet treatments (water contents are always given in % of dry soil weight). This 17.5% is close to field capacity for the soil used, but well below pot capacity, which is estimated to be about 22%. At rewatering, however, an overdose was given, so that the indicated water content served rather as an average than as a maximum value. Care was taken that the water content of the buckets could not exceed 22%, lest waterlogging might occur. For the herbs a maximum of about 20% was guarded. From each series of 5 pots, one was harvested at start and served as a blank throughout the experiment. The pots were placed in trenches to minimize heat load, due to direct irradiation of the pots. During the 3rd and the 4th experiment the sunside of the pots was additionally shaded by jute.

Four consecutive trials were carried out, each of 80 buckets: 4 treatments × 4

species × 5 replicates, of which one replicate served as a blank. The 4 trials will be referred to as Trial 1, 2, 3 and 4, and they approximately coincide with the months January, February, March and April, respectively. The 4 species studied are the grasses *Phalaris minor* and *Hordeum murinum*, and the herbs *Erucaria boveana* and *Centaurea iberica*. The four treatments were: fertilized/unfertilized × wet/dry. The wet treatment was watered on top of the pots 2 or 3 times a week, and kept at an average water content of 17.5%. The dry treatment was designed to be watered only at the start, and to dry out gradually, starting from 15% and ending at the permanent wilting point (5%). Only Trial 2 followed this ideal, not receiving any rain, and the available 1 litre of water being just sufficient for 4 weeks of growth under the conditions that prevail in February. The pots were covered during rain, and if occasionally a shower found the pots uncovered, the amount of rain water that each pot had gained was estimated both by calculation from the rain gauge registration and the pot area, and by weighing the blanks. When a check was possible (the buckets having just been weighed the day before the rain), both estimates differed less than 5%. Trial 1 was rained on twice, and the second time enough to make us give up the dry treatment: it was from then on treated as wet. When it rained on Trial 3, both the time and the quantity were very suitable, as the dry treatment was losing water too fast anyway. The dry treatment of the Trial 4 got no rain, but had to be purposely watered nearly once a week, because of the extremely high water losses. Fig. 8 shows the courses of the water content of the *Hordeum* and *Phalaris* series during the 4 trials (dry treatments only).

Water losses by transpiration and evaporation were recorded by periodic weighing and rewatering with calibrated beakers. At the end of trial, shoots were cut off at soil surface, and fresh and dry weights were determined. Sometimes the roots were
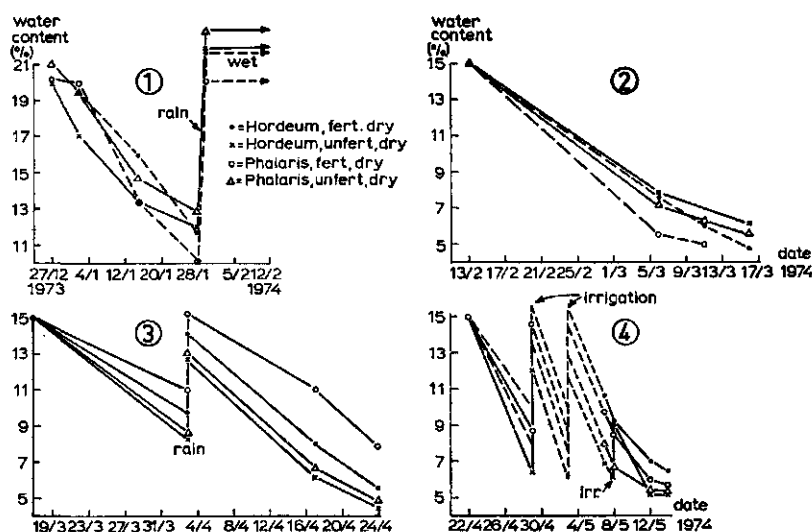

Fig. 8. Courses of water content in the 4 Gilat pot experiments.

collected as well by shaking the soil through an iron gauze. This method was easy enough for the dry treatment pots ,but the pots of the wet treatments had to be left without water about 4 days before the harvest, so that the soil dried to approximately 10%. The roots thus collected were carefully rinsed, to remove the rest of the soil and organic debris. Only the dry weight of the roots was determined.

4.2 Evaporation

The blanks were treated in exactly the same way as the pots with plants, which means that the blanks of the dry treatment were not watered, and watering of the wet blanks was as frequent as watering of the corresponding pots. Still, the blanks can only partly represent the planted pots, and application of the blanks' evaporation to the planted pots is subject to errors. In the first place, plants influence the microclimate at the pot surface. Shading may be of minor importance, as the gravel layer diminishes its effect. However the wind profile is definitely changed, and markedly influences the soil evaporation. On the day of watering, when the gravel is wet, much water may evaporate directly, if the gravel is not shaded by plants. On the other hand, the planted pots naturally get much more water than the blanks, which mostly causes the surface of the blanks to be only partly wetted, and of the planted pots completely. This error counteracts the error due to wind.

The dry treatment has the disadvantage that the blanks contain, throughout the trial, more water than the planted pots. Since the top layer of soil becomes completely dry, the main barrier to evaporation may not be the gravel layer, but the dry top layer of soil. This layer was probably much thinner in the blanks than in the planted pots, where the roots start to withdraw water from the top layers. Thus, evaporation from dry treatments is always overestimated.

4.3 Calculations

One can design and defend several ways to calculate the transpiration coefficient. I used the following method: free soil surface evaporation was taken as the average of total water loss from comparable blanks. Here, fertilization was considered irrelevant, so that only 'wet' and 'dry' blanks were distinguished. Transpiration by the plants was now taken as the total water loss from a pot, diminished by $\frac{2}{3} \times$ free evaporation. The factor $\frac{2}{3}$ has been rather arbitrarily introduced to account for the overestimation of evaporation. Production was taken as total dry shoot weight or total dry shoot + root weight. Initial dry weight (at the start of the experiment) was neglected, because the plants vegetation at harvest were all new tillers, and the leaves and stems of the original plants were considered to have died off at an early stage and to have disappeared at the time of harvesting. The 'net' transpiration figures were plotted against the production figures, for each series, and a line was fitted through these points and the origin, Figs. 9 to 12. The slope of this line is the transpiration coefficient required. If both shoot and root weights had been determined, the S/R

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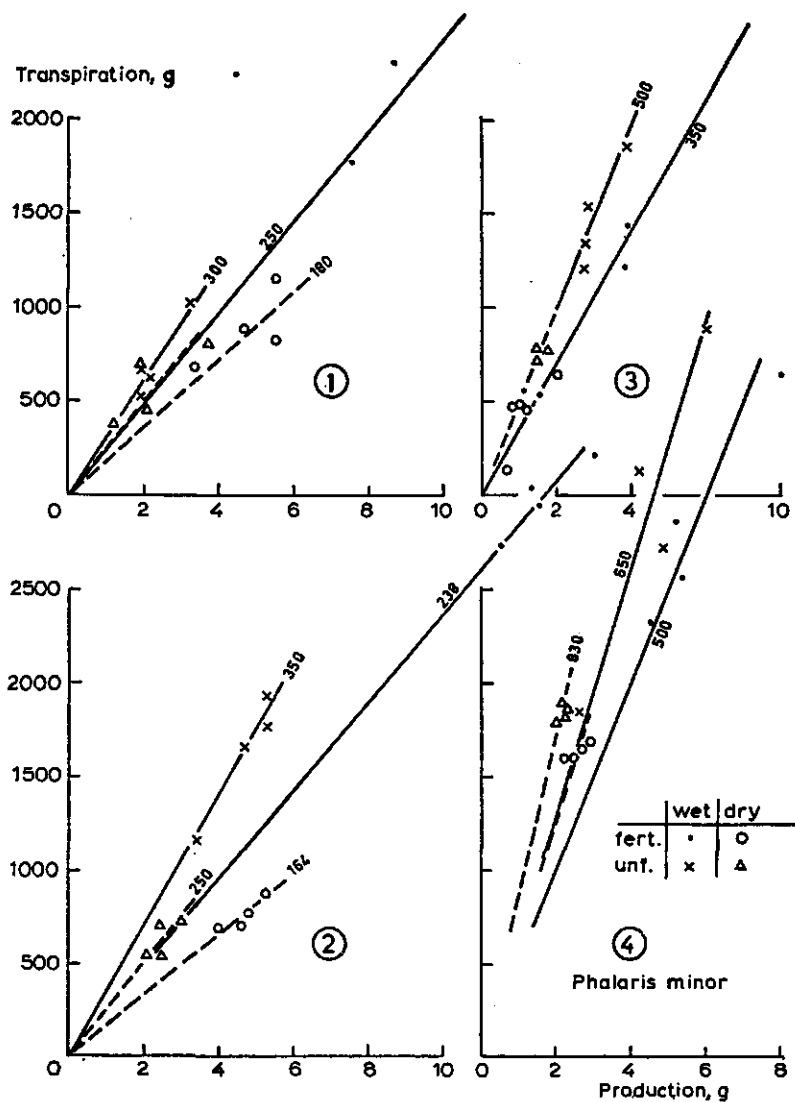
Fig. 9. Relation between transpiration and production for Phalaris minor. The numbers on each line give the slope, which is the transpiration coefficient.

ratio was calculated as the quotient for each pot, and then averaged for the whole series. Water content of the shoot was calculated as

$$100 \times \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}}$$

and averaged over the series.

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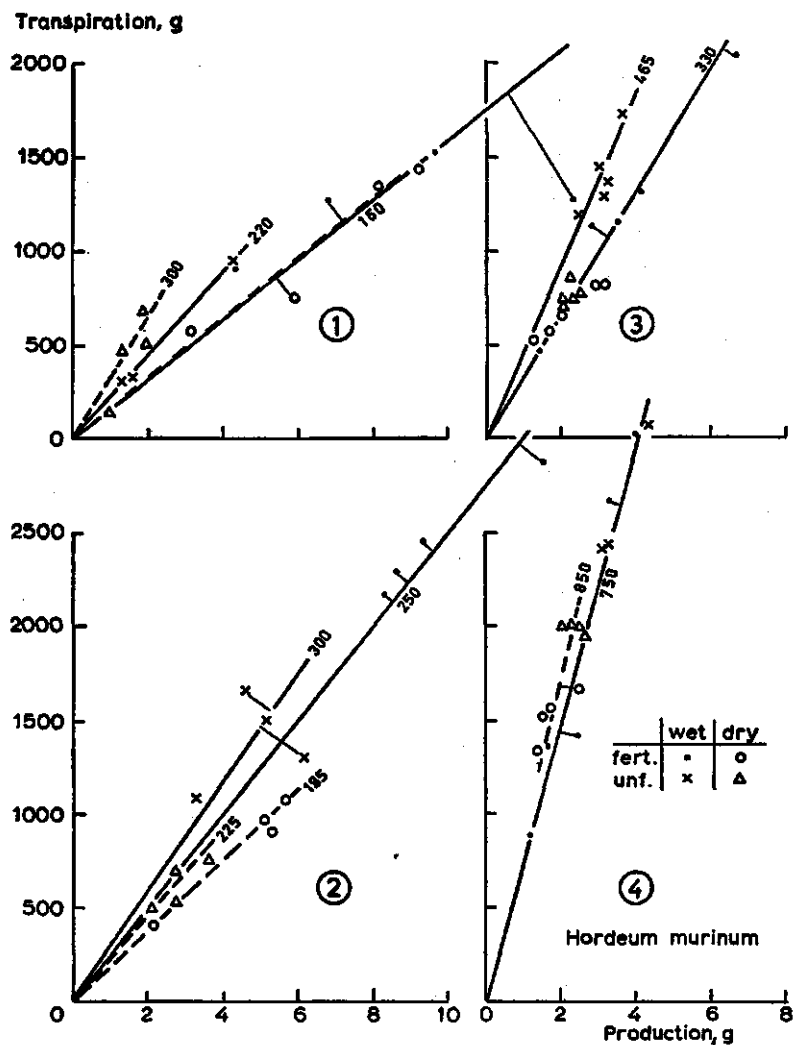
Fig. 10. The same as fig. 9 for Hordeum murinum.

4.4 Results

The results of this experiment are given in Table 4. Many data for *Erucaria* and *Centaurea* are lacking, mainly in Trials 3 and 4 fourth trials. These plants did not take, apparently because they were too old.

The transpiration coefficients with respect to shoot dry weight are plotted against the data in Fig. 13. The most striking feature is that TC rapidly increases as the season proceeds. Differences between consecutive months generally overrule any differences between species and treatments, especially later in the season. The second general feature is that fertilized series show a lower TC than unfertilized ones. Under the prevailing conditions, fertilization improved total production with limited water

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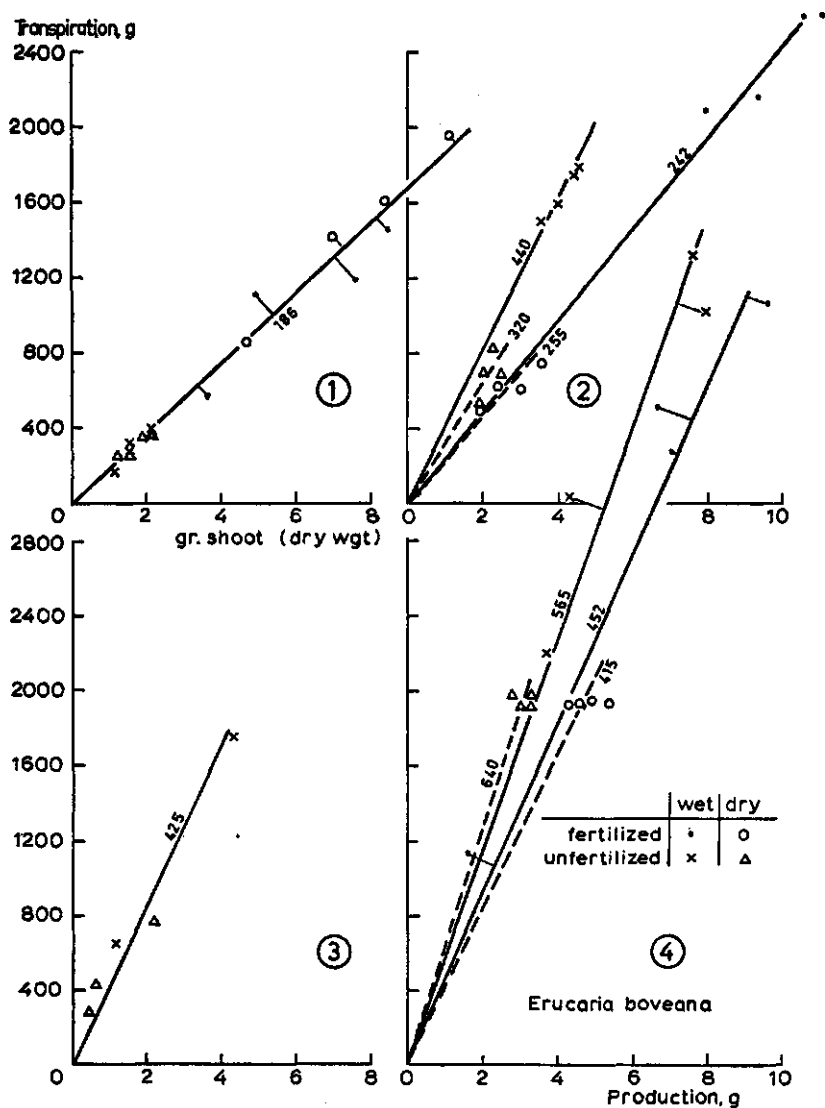
Fig. 11. The same as fig. 9 for Erucaria boveana.

supply by 30 – 50%. The water saving effect of fertilizer was more pronounced in *Phalaris* than in *Hordeum*, and decreased as the season proceeded, i.e. with increasing evaporative demand. The results for *Erucaria* and *Centaurea* were less clear, because of the missing data, but the water saving effect of fertilization seemed more persistent in *Erucaria*. In general, the effect of fertilizer was similar for wet and dry treatment, but for *Hordeum* the effect on the dry treatment had disappeared in March and April. It is interesting to compare this result with the effect of fertilization on the rate of dry matter production. In Fig. 14 the ratios of harvested shoot dry weights under fertilized and unfertilized treatment are plotted against the month of the experiments, separ-

26
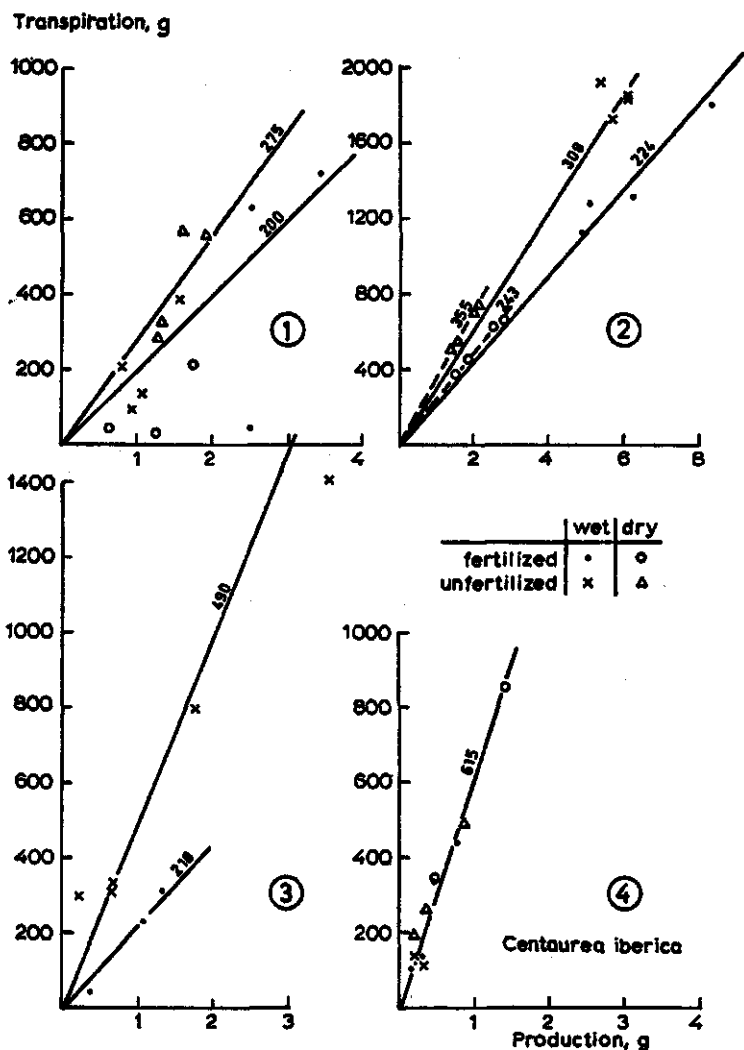
Fig. 12. The same as fig. 9 for Centaurea iberica. In the 3rd and 4th experiment many data were lacking, due to bad reestablishment after transplanting.

ately for wet and dry treatment. The effect on *Hordeum* is most striking: irrespective of the amount of water available, the effect of fertilizer decreases markedly with increasing evaporativity, fertilized/unfertilized yield being 4 in January, 2 in February, unity in March, and even less than 1 in April, indicating an adverse effect of fertilization on growth rate. This could be attributed to the suppressing of root formation at high nitrogen content, causing the fertilized plants to be more readily stressed than the unfertilized ones. In *Phalaris* the same effect is seen although fertilization never suppresses growth as in *Hordeum*. Here a marked difference exists between wet and dry treatment. Again, for *Centaurea* and *Erucaria* the data are not conclusive, but the

27

Table 4. Results of Gilat pot trial, Spring 1974.

Trial	Phalaris minor				Hordeum murinum			
	unfertilized		fertilized		unfertilized		fertilized	
	wet	dry	wet	dry	wet	dry	wet	dry
1. January								
TC^1_{shoot}	300	250	250	180	220	300	160	160
TC_{total}	.	.	.	.	.	.	.	.
SRR^2	.	.	.	.	.	.	.	.
2. February								
TC_{shoot}	350	250	238	164	300	225	250	185
TC_{total}	220	185	170	135	210	190	225	164
SRR	1.66	2.9	2.66	4.7 (?)	2.7	5.3	7.2	6.9
3. March								
TC_{shoot}	500	500	350	350	465	330	330	330
TC_{total}	391	380	295	296	376	283	281	260
SRR	4.02	3.05	4.48	3.14	4.46	4.57	5.04	4.06
4. April								
TC_{shoot}	650	830	500	650	750	850	750	850
TC_{total}	472	594	380	504	566	550	637	702
SRR	2.53	2.27	3.3	3.67	3.16	1.89	5.16	3.30
1. January								
$T–E^3$	700	573	2333	923	533	465	1250	1038
DW^4_{shoot}	2.31	1.97	8.25	4.76	2.15	1.56	8.51	6.61
DW_{total}	.	.	.	.	.	.	.	.
WCS^5	.	.	.	.	.	.	.	.
2. February								
T–E	1634	635	2980	760	i400	627	2460	846
DW_{shoot}	4.67	2.73	12.62	5.0	4.8	2.8	9.7	4.6
DW_{total}	7.69	3.55	17.5	6.0	6.67	3.32	11.06	5.27
WCS	83.5	64.5	82	.	77	60	78	62
3. March								
T–E	1525	757	1240	440	1425	771	1230	684
DW_{shoot}	3.10	1.50	3.81	1.14	3.08	2.24	3.70	2.19
DW_{total}	3.93	2.00	4.60	1.50	3.79	2.74	4.41	2.72
WCS	.	.	.	.	.	.	.	.
4. April								
T–E	2905	1840	2850	1630	2650	1990	1590	1521
SW_{shoot}	4.44	2.16	5.73	2.57	3.56	2.34	2.10	1.72
DW_{total}	6.30	3.1	7.5	3.2	4.57	3.61	2.54	2.25
WCS	79	69.5	81	69.5	77.5	61	75.5	67

1. TC = transpiration coefficient, $g\,g^{-1}$
2. SRR = shoot root ratio
3. T–E = transpiration $- 2/3 \times$ evaporation, g
4. DW = dry weight, g
5. WCS = water content shoot, % of fresh weight

| Erucaria boveana | | | | Centaurea iberica | | | |
| unfertilized | | fertilized | | unfertilized | | fertilized | |
wet	dry	wet	dry	wet	dry	wet	dry
186	*186*	*186*	*186*	*275*	*275*	*200*	*200*
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
440	*320*	*242*	*235*	*308*	*303*	*224*	*243*
6.86	4.68	10.3	6.36	8.05	7.53	11.68	8.43
425	*425*	.	.	*490*	.	*218*	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
565	*640*	*452*	*415*	*615*	*615*	*615*	*615*
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
297	319	1759	1464	205	433	348	120
1.55	1.67	6.13	7.56	1.06	1.52	2.62	1.19
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
1652	682	2420	616	1831	620	1380	521
4.120	2.19	9.81	2.75	5.78	1.76	6.13	2.14
82.67	64.41	85.11	69.93	82.32	56.78	82.62	69.43
1173	413	.	.	627	.	193	.
2.712	1.095	.	.	1.366	.	9.08	.
.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.
3408	1943	3008	1926	125	315	266	597
5.89	3.10	6.221	4.80	0.26	0.47	0.41	0.932
82.64	70.12	84.07	68.82	76.1	74.81	80.64	71.64
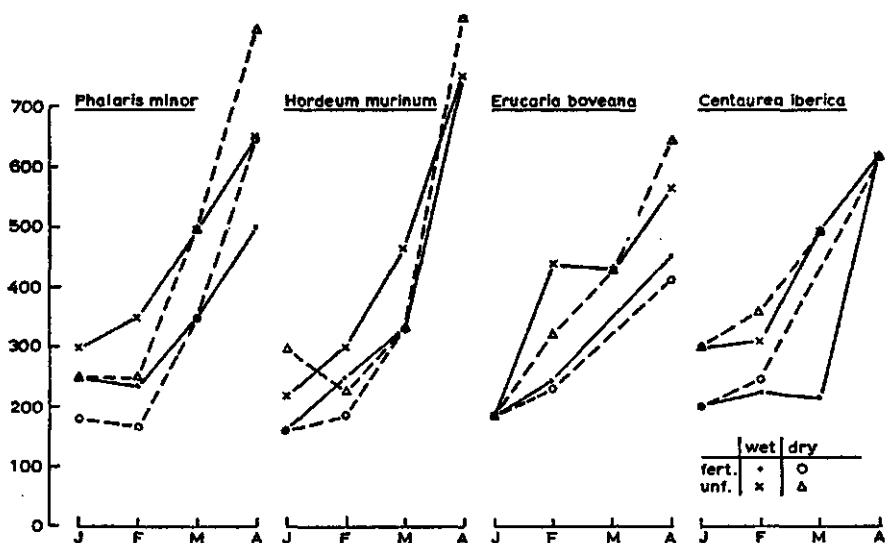
Fig. 13. Transpiration coefficients of 4 species, plotted against the months of the 4 Gilat pot experiments: January, February, March and April 1974.
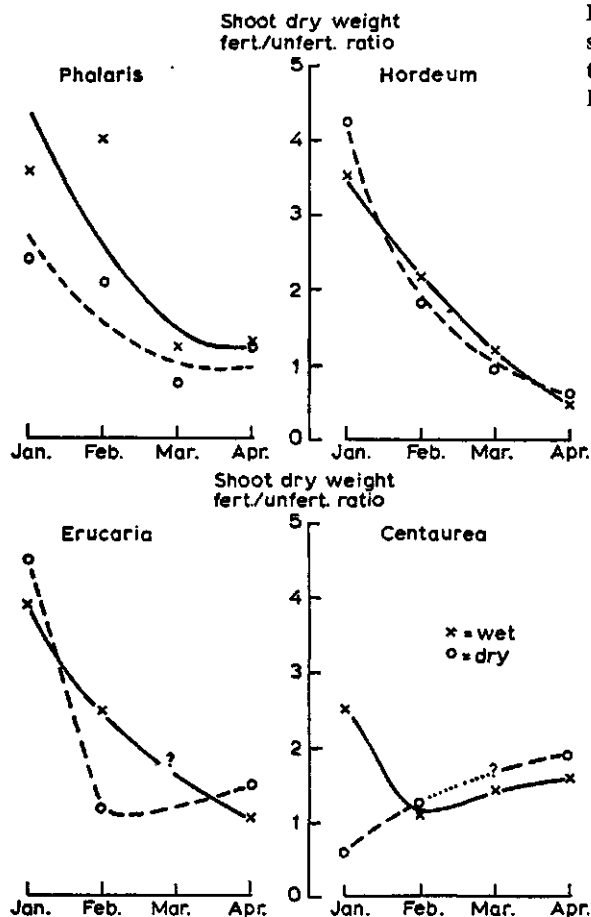
Fig. 14. Quotients of dry matter productions with and without fertilization, during each of the pot experiments, Gilat, 1974.

effect on *Erucaria* seems to be similar to the grasses, while the growth rate of *Centaurea* does not seem to be very much influenced by fertilization.

The third feature of Fig. 13 to be considered with respect to its effect on the TC and its course in the season, is drought. It is, at the same time, the most difficult factor to be discussed. Effects in Trial 1 should be considered with care, and better be forgotten, because this trial was rained on. Trial 2 was exemplary, and in the grasses a water saving effect was noticed, whereas in *Erucaria* there was hardly any effect with the fertilized treatment, and in *Centaurea* drought seemed to influence the TC adversely. Figs. 9 to 12 show the consistency of this phenomenon in the replicates. In Trial 3 drought seemed to have no appreciable influence on TC, except in *Hordeum* unfertilized, where it saved nearly 30% of water. In April most series under dry treatment had higher TCs than under wet treatment, and replicates were good in *Phalaris*, fair in *Hordeum*, and not too bad in *Erucaria*.

The last variable in Trial 3 is the plant species. Differences between species were small, as compared with differences between consecutive months, and differences caused by fertilization. Taking again the second experiment as example – it was the only trial that went according to plan –, the similarity of TCs of fertilized plants is striking. Without fertilization differences did occur, *Hordeum* particularly being more economical with water than any of the others, *Erucaria* appearing to be most wasteful under these circumstances. In Trial 4 *Hordeum* was least and *Erucaria* most economical with water. In the field *Hordeum* has long finished its cycle before such harsh conditions occur, and the plants on the buckets, though looking all right, were abnormal in that they stayed vegetative throughout this trial. In the first three trials *Hordeum* always was the first species to flower.

Furthermore in Table 4 the water content of the shoot and the shoot/root ratios confirm what could be expected from other experiments: plants grown with ample water have a considerably higher WCS than those suffering drought. Fertilization has little, if any, effect on WCS. *Phalaris* leaves contain more water than *Hordeum* leaves, and *Centaurea* is dryer than *Erucaria* (except in the Trial 4 under dry treatment, but here *Centaurea* grew to slowly, that it never used up all the water, whereas *Erucaria* did).

The S/R ratios also show differences between species: *Phalaris* always had considerably more roots than *Hordeum*, except in Trial 4, dry treatment, where *Hordeum* made relatively more roots. Under wet regime SRR was nearly always higher than under drought, and also the effect of fertilization was quite apparent. In Trial 2, the SRR ranged from 1.66 for *Phalaris*, unfertilized and wet to 7.2 for *Hordeum* fertilized and wet.

4.5 Discussion

Although slightly disappointing for the botanist, the most important conclusion from this section, and perhaps from the whole report, is that differences in TC between different species are insignificant when compared with differences between con-

secutive months and the effect of fertilization. If management cannot include fertilization, then differences between species are worth considering. However if proper fertilization can be applied, the only factor that seems to matter is, that the available water is used up as early in the season as possible. Therefore one should select for species that tolerate low temperatures. High fertilizer concentrations might suppress root development and thus eventually be detrimental to growth and water uptake, but this phenomenon has not been shown to occur in the field.

Maximum evapotranspiration per day per pot under wet treatment was approximately 200 g in the Trials 1 and 2, 150 in Trial 3, and 300 in Trial 4. The cover was close to 100% in Trials 2 and 4, but considerably less in Trial 3, due to poor establishment and a fast switch to the reproductive phase. Since the surface of the pot was 500 cm², water losses were estimated to be 4 and 6 mm/day for Trials 2 and 4 respectively, which is in general agreement with the pan-evaporation in those periods. As the plants on the buckets were not part of an extended crop, much advective heat gain could be expected, possibly more than in the field, which would tend to increase TC. Fortunately, data from parallel experiments in the Migda field (which is less than 20 km from Gilat) are available and permit comparison. From Van Keulen's simulation of dry matter production and water withdrawal in Field 13 NPK, TCs for January, February and March 1974 could be calculated to be 240, 213 and 421, respectively. These figures agree well with mine. For April no field observations exist, as growth had stopped. This agreement is rather encouraging, and indicates that even in an extended field in semi-arid zone the vegetation is subject to considerable advection.

The effect of fertilization on TC has long been known and was thoroughly reviewed by Viets (1962). On a leaf area basis, it is thought to be mainly a direct effect of protein content of the leaves on their efficiency of photosynthesis (cf. Fig. 50), while transpiration rate is not affected and follows in principle evaporative demand. In the field, there is also a clear effect of fertilization on leaf development, which would in all cases lead to improved light interception and higher production rate, but not in all cases to increased water loss. Pot trials and fields in arid zone are expected to gain much advective heat, but even under such conditions application of fertilizer improves the TC. This improvement was up to 30% in the present experiment, but indications exist that in the Migda fields it can be up to 50%. This is shown in Van Keulen's Fig. 51 (my Fig. 15), where the results of *simulated* dry matter yields in a *fertilized* field are compared with the *observed* yields in an *unfertilized* field for the years 1961-1974. The simulated data could be validated in the last 3 seasons and showed good agreement (Van Keulen, 1975). With sufficient rainfall, the simulated productions of fertilized were twice as high as the actual production of unfertilized. In low rainfall years, however, productions under fertilized and unfertilized conditions were equal, which seems to confirm my observation that under severe stress, application of fertilizer has no enhancing effect on crop growth rate, and hardly on TC. Still, the possibility must not be excluded that in such cases there may be a significant influence of fertilization on the protein content of the crop.
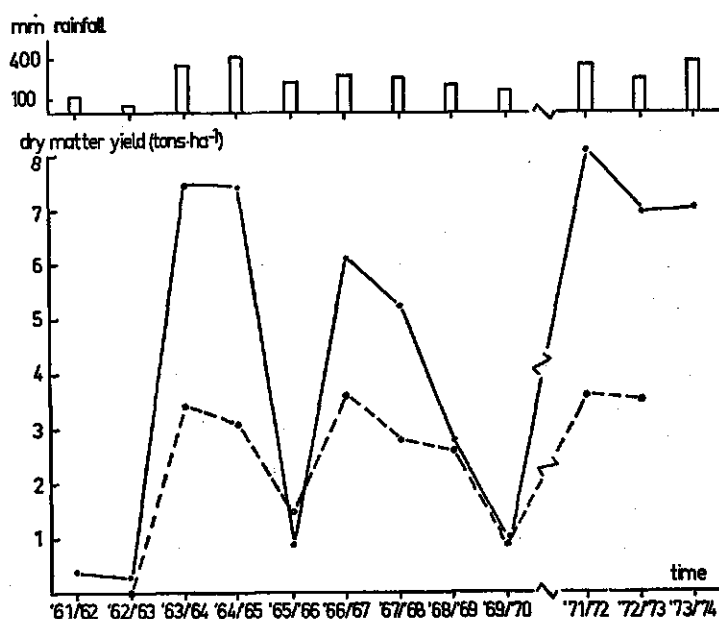
Fig. 15. Calculated, water limited (optimally fertilized), dry matter yields of Migda natural vegetation for the period '61/'62 to '73/'74 (solid line), and measured dry matter production, without fertilization, for the same period (broken line). Taken from Van Keulen (1975).

The water saving effect of dry treatment, as has been concluded from the results of Trial 2, requires further discussion. The TC of all pots were calculated, neglecting the initial weight of the plants, and estimating evaporative water loss to be $\frac{2}{3} \times$ the water loss from the appropriate blanks. There could be conflicting opinions on these assumptions, but that would not change the results of the *irrigated* pots very much. However it is unfortunately decisive for the interpretation of the results from the *dry* treatments. Initial weights were always less than 10% of ultimate weights for the wet treatment, they were up to 25% of the ultimate weights in the unfertilized dry treatment, however. Not neglecting initial weights would tend to decrease the difference in TC between wet and dry treatment. We have already stated that the evaporation from the blanks is an overestimation of that from the planted pots, and accounts for a reduction factor of $\frac{2}{3}$. If one takes an extreme position, claiming that evaporation from the planted pots under dry treatment is completely negligible, because of the expected fast drying out of the top layer, then this would also minimize the difference in TC between wet and dry. Assuming *both* alternatives: initial dry weight should be substracted and evaporation should be neglected, the pots under dry and wet treatment will have virtually the same TC. We still believe that the observed savings are realistic, but at the same time we have to admit that the evidence supplied is not conclusive.

If my approach is correct, then the conclusion must be that water shortage leads to more efficient water use under low evaporative demand, and to wasting of water

33

under high evaporation. Besides the difference in evaporative demand between trials, the difference in treatment could also play a role: gradually drying out in Trial 2 as opposed to suboptimum watering in Trial 4. With experiments on the Avdat farm, with basically the same set-up, suboptimal watering also resulted in increased TC (Evenari, pers. commun.), but no experiment was done with drying out, in this case. At suboptimum watering the water supplied does not evenly distribute over the soil, but fills the top soil to field capacity, leaving the bottom of the bucket dry. Under high evaporation, such a treatment could lead to a situation, where the plants either have abundant water, or no water at all. This is the more true, if the soil has a steep pF curve: the water potential of the soil increases then very rapidly at a certain water content, and an appreciable traject of moderate stress does virtually not exist. When the soil is, however, gradually drying out, moderate stress is the rule and extreme situations are exceptional, not because the water potential of the bulk soil is 'moderate', but because at decreasing water in the soil the resistance to water transport increases and at high transpiration stress situations in the plant can develop. Examples of stomatal behaviour under 'moderate' stress conditions are given in Fig. 28.

I think that plants under mainly *extreme* conditions, respond in an either-or way, having either their stomata completely open and showing then normal TC, or completely closed without any photosynthesis, but with still considerable losses of water (cuticular) and dry matter (respiration). Thus, their TC would be higher than normal. Under mainly *moderate* conditions, plants would react in a more flexible way, increasing stomatal resistance with increasing evaporation ('hydroactive' closure). Such behaviour should be water-saving, because C_{int} is lowered, and also because gas exchange is most intensive during the most favourable periods of the day.

Both ways of applying drought have their equivalent in the field, the suboptimum watering representing what happens in the beginning of the growing season, with scattered rain showers, while the gradual drying of the soil reflects the situation after the last heavy rains have fallen and the crop is growing on storage water.

5 Direct measurements on RS with a porometer

5.1 Introduction

Water transport in the plant from soil to atmosphere is most effectively controlled by the resistance of the stomatal pores to water vapour diffusion. As has been suggested in the introduction, differences in stomatal behaviour of species in the field might well have an important influence on the transpiration coefficient (TC). Particularly an increase in stomatal resistance (RS) during the hottest hours of the day would restrict gas exchange to the moderate conditions of the morning and late afternoon, thus promoting a more favourable proportionality between assimilation and water loss. For determination of RS in the field a Wallihan-type diffusion porometer is the best tool.

5.1.1 Transpiration measurement

In 1972 a Wallihan-type diffusion porometer was kindly supplied by B. Bravdo. Its construction and calibration are described in Bravdo (1972). During the spring seasons of 1973 and 1974 I used a copy of his model made out of teflon, because of the low water absorption of this material. The apparatus exists of a chamber that can be clamped onto the leaf. The air inside the chamber is continuously stirred by a fan and can be dried by shifting a plunger, holding dry $MgC10_4$, into it. The porometer contains a LiCl humidity sensor (Hygrodynamics, USA), the electrical resistance of which varies with the relative humidity of the air. By determining with a stopwatch the time necessary for the sensor's resistance to proceed from one previously fixed value to another, the transpiration rate is measured. If, air and leaf temperature during the measurement are recorded simultaneously, the diffusion resistance of the leaf can be calculated with the calibrations of the sensor supplied by the manufacturer. This was done in 1972 and Bravdo's computer program was used to calculate the results.

With my copy of the porometer, some modifications of this procedure were introduced. The work of Stigter (1973) made it clear, that use of LiCl sensors, particularly in dynamic response, cause some complications, because of the slowness of this sensor type, its hysteresis, and the water absorption of the system, mainly of the sensor itself. Calibrations with resistances of known value are necessary to deal with such problems. Stigter showed that the effects connected with lagging of the sensor response and the water absorption can be caught in one calibration term: the *theoretical* volume (V)

of the porometer. This V considerably exceeds the *geometrical* porometer volume (25 cm³ in our case) and is sensitive to porometer temperature. Fortunately, V could be shown to be independent of the temperature of the vapourizing surface. However, V changes with the age of the sensor. The hysteresis of the sensor can be dealt with by a strict measuring procedure: each measurement is started from exactly the same point on the sensor indicator scale, and each measurement should be preceded by approximately 2 minutes of rest at this point. Thus, a combination of adapted calibration and measuring procedure enables reproducible use of the apparatus.

Since proper calibration was omitted in the first and third year of using the porometer, no absolute value should be set on the data obtained. In 1973 calibrations were carried out, as described below, but field conditions differed so much from calibration conditions, that even here the results must be considered with caution. Since I always followed a strict measuring procedure, and took care that the temperature of the apparatus did not vary too much over the day, mutual comparison of data, obtained during each set of measurements, is admissible. With the calibration data obtained, absolute values of RS can only be estimated.

5.1.2 Photosynthesis measurement

Bravdo's porometer had been designed for simultaneous measurement of water vapour diffusion resistance and rate of photosynthesis. Just before rate of photosynthesis is measured, the air in the chamber is flushed with air from a pressure cylinder, containing a known concentration of $^{14}CO_2$. As the volume of the flush exceeds the porometer volume five-fold, it is assumed that all the air in the porometer chamber is replaced. During the measurement the leaf is exposed to sunlight (the chamber being clamped onto the underside) and absorbs CO_2. After the measurement the exposed part of the leaf is punched out and kept in a vial containing 70% alcohol. Later on, extraction is done at 70°C, the chlorophyll is bleached in strong light, and scintillation liquid is added. The radioactivity is then determined in a liquid scintillation counter, correction for counting efficiency being made by the internal standard method. This simultaneous determination of RS and P_n has, in spite of its elegancy, two drawbacks: first, the starting humidity of the transpiration measurement is now determined by the water content of the $^{14}CO_2$-enriched air and, secondly, the measurement has to be performed in full sunlight, which influences the leaf temperature.

The apparatus was used for measurements in 3 consecutive years: in 1972 it was tested on Rhodes grass in Beit Dagan, in 1973 it was used for direct measurements on various species in the Migda field, in 1974 it was used to determine stomatal behaviour of the plants of the Gilat pot trial. The experiments are treated in chronological order, the last section being the most interesting for this report. So many differences exist between aims, set-ups and procedures in the 3 consecutive years, that a separate treatment of each experiment's particulars is preferred.

5.2 Experiment June 1972, Beit Dagan, Israel

Aim   To test the porometer's performance on grass leaves, measurements were carried out in a field of Rhodes grass (*Chloris gayana*). Stomatal resistance to water vapour diffusion (RS), and net photosynthesis (Pn) were determined on leaves at different heights in the canopy and at different times of the day, and were related to climatological data. Some attention was paid to light dependence of RS and Pn, and to the influence of leaf age.

5.2.1 Materials and methods

All measurements were carried out in an established Rhodes grass field, well supplied with water and nitrogen, where stands of different heights were available, ranging from 10 to 100 cm, due to an experimental mowing regime. Measurements were done on cloudless days with W wind. Bravdo's porometer was used, and adapted for measuring narrow leaves by fitting a rubber insert with a 4×19 mm slit in the porometer's circular opening, Fig. 19. This width was sufficient to cover more than 90% of the leaves of the grass. If shaded leaves were measured, their position was in principle left unchanged, but sun leaves were purposely turned towards the sun to prevent them from being shaded by the sides of the slit.

Between consecutive measurements, the porometer's humidity was kept at a fixed point for about 1 minute. After a leaf was clamped onto the apparatus, a flush of $^{14}CO_2$-enriched air was given. The leaf was exposed to this gas by opening the tap, and the time measurement was started. The $^{14}CO_2$-enriched air was so dry that it took the porometer about 20 s before the fixed starting point of the transpiration measurement was reached. The transient time ranged from 10 to 20 s, after which the leaf was left in the porometer till for at least 45 s of exposure to the $^{14}CO_2$. The leaf was then removed and the exposed part was put in a vial containing Alc-70. Temperatures of porometer air and leaf surface were recorded at regular intervals, with a needle thermistor, but not during the measurements. The temperatures recorded can only yield an approximation of the situation existing during the RS measurement. Parallel with the porometer measurements, record is held of the temperature and relative humidity of the surrounding air were recorded with an aspirated psychrometer. In addition, radiation levels were measured with a temperature-corrected radiometer.

5.2.2 Results

Table 5 gives the results of RS and Pn measurements on the leaves of a horizontally growing stolon, a so-called 'runner'. It had 11 relatively short leaves, looking all very similar, except that the 7th and the 8th leaf served as axillary leaves for young tillers. As all these leaves were very similar in their exposition to environmental conditions, they were expected to differ only in age. Table 5 shows that an age effect is completely

Table 5. Porometer measurements on consecutive leaves of a runner.

		Transpiration transient time (s)	Photosynthesis (mg $CO_2/dm^2/h$)	Leaf resistance to water vapour diffusion (s/cm)
Leaf No.	1 (youngest	15	80.6	4.41
	2	14.5	130	4.3
	3	13	137	3.86
	4	15	124	4.45
	5	14.5	137	4.3
	6	14.5	101	4.3
	7	22	57	6.53
	8	19.6	82	5.82
	9	14.6	123	4.33
	10	10	139	2.97
	11	13	176	3.57

absent. Only the leaves Nos. 7, 8 and 10 have RS and Pn values that are different from the others. Possibly, the special function of 7 and 8 has something to do with this. Leaf No. 10 was bigger than the other leaves, but no reason can be given why its RS should be lower than normal.

In Table 6 the transient times are given as measured on consecutive leaves of another runner and a lodging plant, both characterized by the fact that their leaves are all similar in exposure to sun and wind. Considerable variations between leaves occur, but do not seem to bear any systematic relation to their age.

Table 6. Porometer transpiration measurements on leaves of of horizontally growing plants, a runner and a 'normal' plant.

		Transient time (s)	Replicate
Runner			
leaf No.	1 (youngest)	50	
	2	22	
	3	22.5	
	4	17	
	5	12	
	6	23	
	7	23	
Normal plant			
leaf No.	1 (youngest)	11	11.5
	2	15.5	14.5
	3	16.5	14
	4	12.5	12.5

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37

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	2	15.5	14.5
	3	16.5	14
	4	12.5	12.5
	5	11	12
	6	13	11
	7	13	12.5

The profiles of RS and Pn, measured in stands of 100, 80, and 60 cm are shown in Fig. 16. The Pn profiles are similar to the radiation profiles, any differences in pattern occurring in the top 20 cm layer, where, or course, light distribution is far from homogeneous and something like a representative leaf is virtually non-existent. The RS profiles show that a decrease of Pn is accompanied by an increase of RS. Differences between profiles in various stands are partly due to the different times at which they were measured.
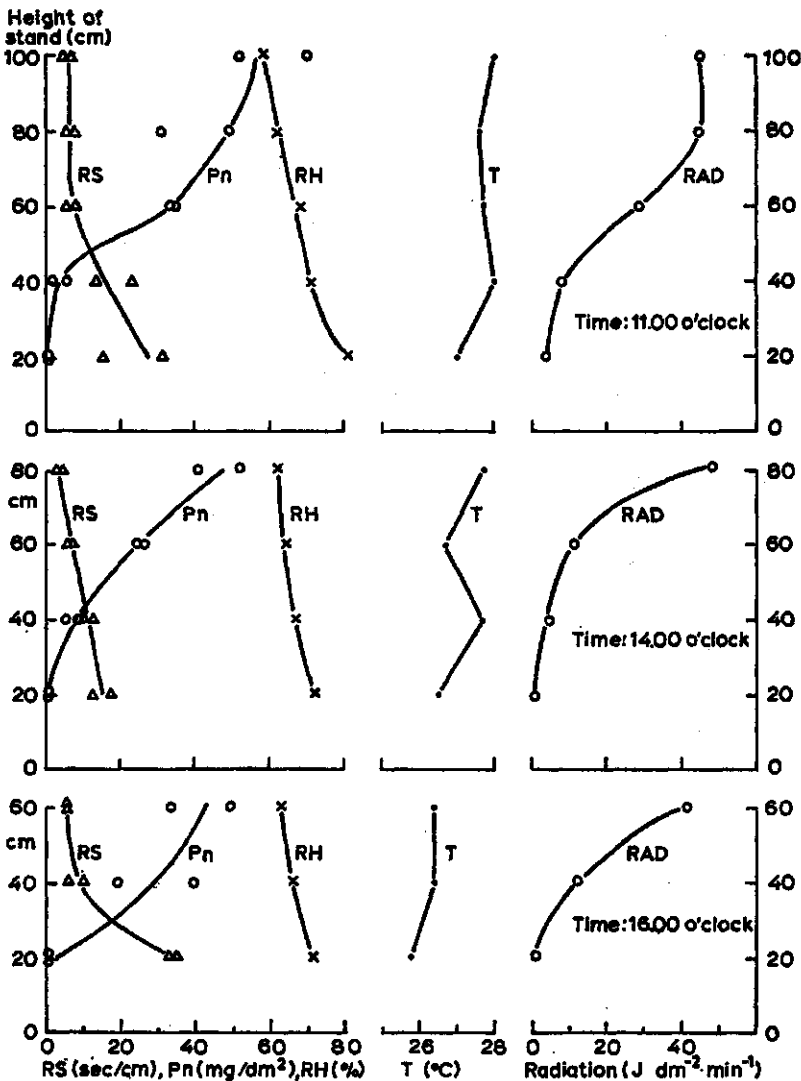

Fig. 16. Profiles of stomatal resistance and net photosynthesis with some environmental parameters: relative humidity, temperature of the air, and net radiation in stands of Rhodes grass.

In Fig. 17 daily courses are shown of RS, Pn, and environmental variables. At 80 cm 3 replicates were averaged, at 40 cm and 10 cm single leaves were measured. The product of Pn and RS was nearly constant over a broad range of observations. The radiation just above the canopy was measured in 2 ways: keeping the radiometer horizontal, and directing it towards the sun. As the sun leaves were also directed towards the sun, during measurement, the radiation to which they were exposed is represented by the upper curve. The photosynthesis at 40 cm showed major oscilla-
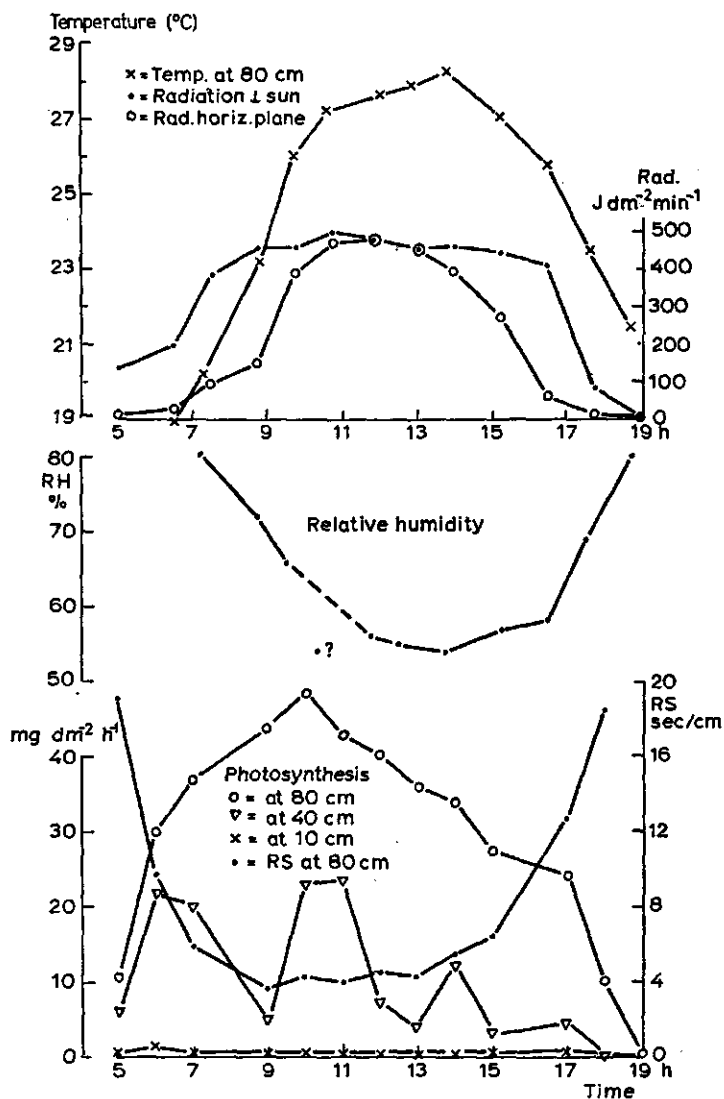

Fig. 17. The daily course of stomatal resistance and net photosynthesis on a cloudless day. The measurements at 80 cm (top layer of the canopy) consist of 3 replications, at 40 and 10 cm single leaves were measured.

40

tions, which are not to be ascribed to stomatal movements, but rather to local differences in microclimate, mainly irradiation.

A light response curve (Fig. 18) was obtained by intercepting different amounts of light with screens of frosted glass and nylon netting, placed over a metal frame, and measuring RS and Pn after adaptation. The points given are averages of 3 or 4 observations each. These measurements were carried out between 11:00 and 13:00. The product of net photosynthesis and RS was virtually constant.

5.2.3 Conclusions and discussion

It can be concluded from the data presented, that net photosynthesis and stomatal resistance (SR) to water vapour diffusion are closely linked. In most cases, the relation seems to be causal, Pn being the cause and RS the effect, particularly where Pn is clearly following the radiation intensity, like in the light response curve and during the ascending and descending part of the daily course. However, Fig. 17 shows the
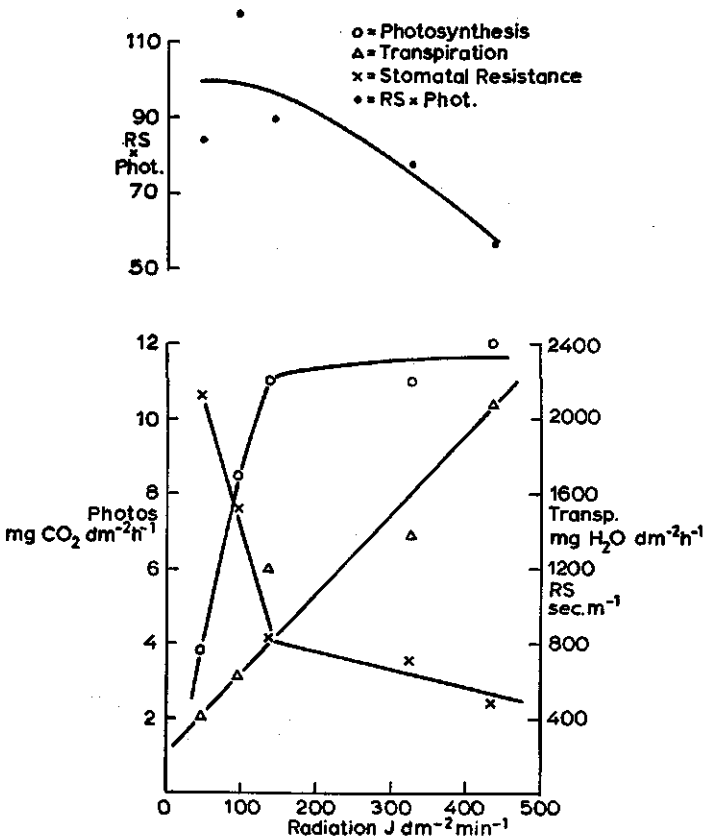
Fig. 18. Light response curves of photosynthesis and stomatal resistance. The product of RS and Pn, in arbitrary units, is also shown.

41

opposite to be true between 10:00 and 15:00: there Pn is decreasing in spite of the increasing light intensity. The calculated RS increases less rapidly than would explain the decrease in photosynthesis, probably because of the underestimation of RS in this period: the high level of irradiation is liable to cause leaf temperatures to be higher during the measurement than in between, which leads to high transpiration and accordingly underestimation of the stomatal resistance. Apparently the high rate of water loss causes a high water deficit to develop in the leaves, which results in increasing stomatal resistance.

Another situation, where RS and Pn seem to be uncoupled, occurs in Fig. 16 in the lowest level of the canopy. Pn decreased to very low levels, while there was still a considerable water loss. It is suggested, that this water loss is mainly cuticular, and should, in fact, not be expressed as RS. Thus, the hypothesis, that RS follows net photosynthesis by a stomatal control mechanism responding to CO_2 concentration in the substomatal cavity is generally supported, but the effect can be overruled by stresses which can develop even at optimum water supply and which cause stomata to close, at least partly.

Pure ageing effects could not be observed. Where leaves of different age were exposed to identical environmental conditions, they roughly showed the same RS and Pn values.

No absolute value can be set on the calculated data. First, the RS calculations were done, excluding water absorption by the chamberwalls and the sensor. For the calculations the geometrical porometer volume of 25 cm^3 was used. No calibrations with known resistances were performed, but the calibrations of 1973 indicated that the theoretical porometer volume was at least 3 $\times$ bigger than the geometrical volume. Secondly, the photosynthesis figures were inaccurate, due to differences in the relative radioactivity of the gas used. The maximum levels of Pn should be virtually the same on different days. That they were not, proves that the content of $^{14}CO_2$ in the enriched air was subject to variations, and that the balloon could not be filled in a reproducible way. Therefore, only values obtained with the same balloon can be compared, which means that the observations on 4 – 6 and 11 – 6 (Figs 16 and 17) are comparable with AMAX = 50 mg dm^{-2} h^{-1}, while on 15 – 6 (Fig. 18) it is 12 mg$\cdot dm^{-2}\cdot h^{-1}$, and in Table 5 even 180 mg$\cdot dm^{-2}\cdot h^{-1}$.

5.3 Experiment spring 1973, Migda, Israel

*Aim*   To compare leaf diffusion resistances and photosynthesis of various plant species, that naturally occur at the experimental site. Their stomatal behaviour during the day, under different conditions of nitrogen and water supply, was studied to trace differences that would explain differences in WUE.

5.3.1 Materials and methods

The measurements were carried out with an exact copy of Bravdo's instrument,

made from teflon. As leaves of different form and size were to be measured, a slit could not be designed that would fit them all. Therefore, the opening outwards was closed with a watchglass, allowing sunlight to reach the enclosed leaf and exposing *both* sides of the leaf to the porometers atmosphere, as is seen in Fig. 19. On the watch glass a grid of dots was made, each dot forming the centre of a 4 mm^2 area. This allowed an estimate of the leaf area enclosed in the porometer to be made, by counting the number of dots coinciding with the leaf.

RS and P_n were not measured simultaneously for three reasons: First the RS measurement could be carried out in the shade giving the advantage of a nearly constant leaf temperature. Secondly, the starting point of the wetting cycle could be chosen
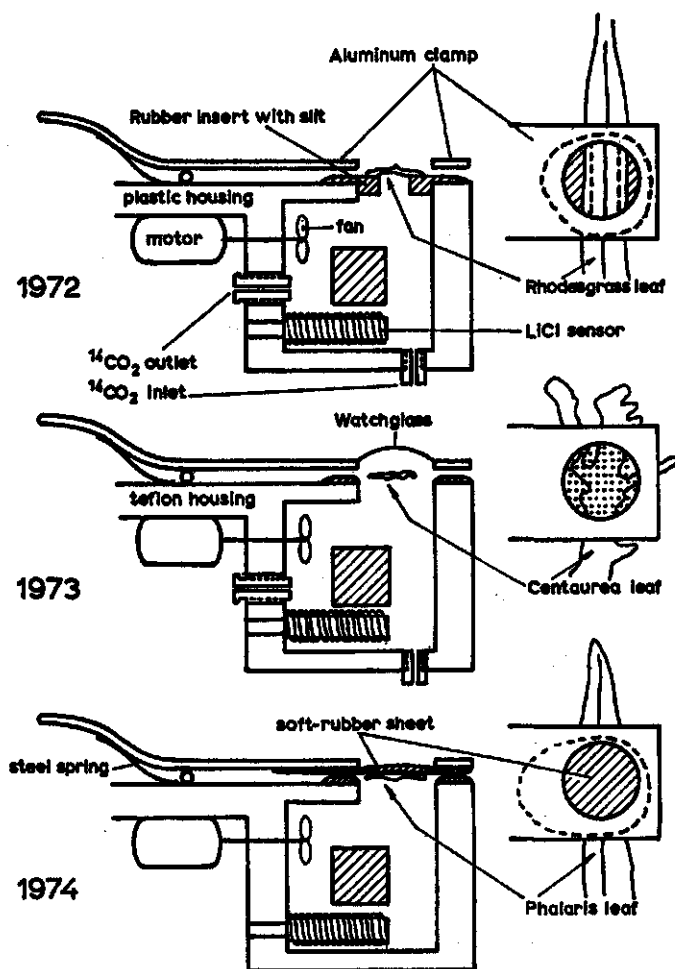
Schematical cross-section Porometer

Fig. 19. Schematical cross-sections of the porometer, as it was used in the 3 consecutive years of our measurements. On the right-hand side, the clamp with leaf is shown from above.

and fixed which enhanced reproducibility and generally shortened measuring time. Thirdly, many RS measurements could be done, without being left with an equal number of radioactive samples and without having to refill the cylinder with $^{14}CO_2$-enriched air every day. As a rule, 3 replicate transpiration measurements were followed by 1 photosynthesis measurement. If the 3 replicates were not well comparable, more measurements were taken till 3 comparable leaves had been found. The photosynthesis of the last of these leaves was then determined and did not need replication, as it had been performed on a representative lef. A preliminary experiment had shown that previous shading for about a minute does not influence the leaf's photosynthesis after bringing it back to sunlight, as is demonstrated in Fig. 20. The whole measurement took no more than 1 minute, and if it did, the RS measurement was shortened by taking a shorter interval on the scale, for which separate calibrations were done. Between the photosynthesis measurement and the next series, a 'dummy' measurement was made to prepare the sensor for the transpiration measurements. Between consecutive RS measurements a pause of about 1 minute at a fixed point was inserted.

In the field the porometer did not operate free of leaks, which thoroughly disturbed the RS measurement. Usually the outside air contained much more water vapour
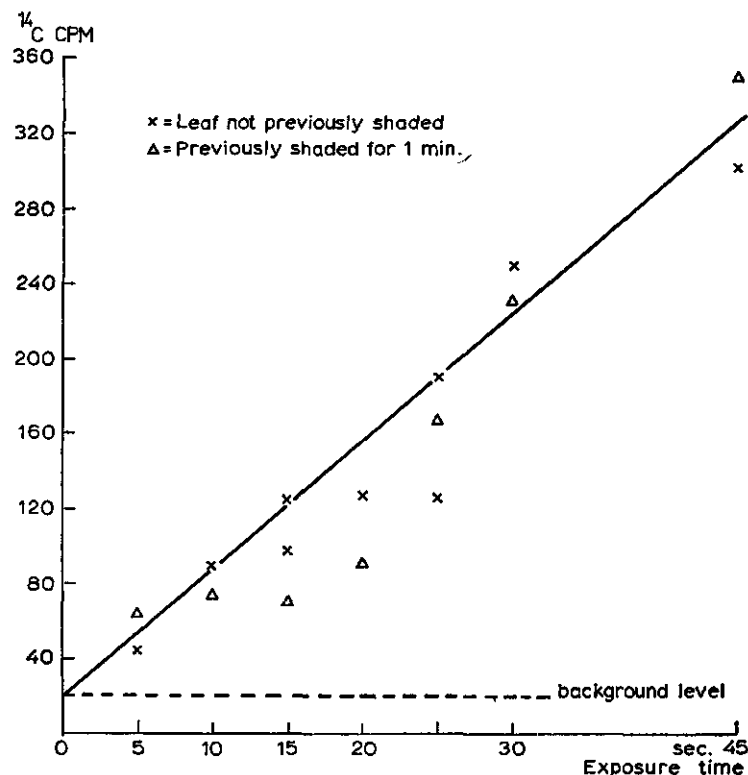

Fig. 20. Linear relation between the radioactivity of the leaf sample and the time it was exposed to $^{14}CO_2$ in the porometer, and the influence of shading the leaf previous to the exposure.

44

than the porometer air and a draft of outside air would have visible effect on the results.

This effect could not be completely ruled out, but was minimized by shielding the porometer from the wind, keeping it on the observer's lap, while the plants were picked and brought towards the apparatus. Leaves were not taken at random, but selected: young fullgrown leaves were chosen that were fully exposed to the sun. Flag leaves of grasses were avoided. Sometimes stomata of *Hordeum* and *Stipa* closed rather quickly after picking so that they were measured in situ, whenever this was considered necessary (at midday) and possible (not too much wind). During the RS measurement the porometer was kept in the shade of the observer's body and leaf area and leaf temperature were determined. The thermistor that reads leaf temperature was fixed in such a way that the leaf was pressing against it, when positioned in the porometer. Both leaf and (porometer) air temperature were measured at the beginning and the end of the RS determination, and, if they differed, averages were used.

The part of the leaf that had been exposed for 30 s to $^{14}CO_2$ was collected in a scintillation vial, containing 5 ml of alcohol. After extraction at 70°C and bleaching of chlorophyll in high light, scintillation solution (BBOT) in a mixture of toluene and Triton-x-100) was added and the radioactivity counted in the same vial, with the leaf left inside. Such countings were usually subject to rather high quenching, the amount of which could however be accurately determined by adding 1 ml of a standard ^{14}C-acetate solution and counting again. In the beginning $Na_2^{14}CO_3$ was used as a standard, but this compound is not recommended, as it easily dissociates and releases $^{14}CO_2$.

As the preparation of the $^{14}CO_2$-enriched air was not reproducible with the available set-up, the relative radioactivity of the applied air had to be determined every time a cylinder was newly filled. A piece of blotting paper was folded several times, drenched in ethanolamine (which is well-known for its CO_2 binding properties) and exposed for 2 min to the gas mixture in the porometer after flushing. After 2 minutes no further increase in radioactivity could be detected, so that this time was assumed to be sufficient for the ethanolamine to catch quantitatively all the CO_2 in the chamber. This piece of blotting paper was then treated as if it were a leaf, except that methanol had to be used, because ethanolamine does not mix with ethanol. The counting efficiency of this sample was again determined with the internal standard method.

In the calculation of P_n from the uptake of $^{14}CO_2$, it has to be taken into account that the concentration of CO_2 decreases during the measurement. The gas, however, is far from depleted in the 30 s the leaf is exposed to it: if 1 cm^2 of a leaf is photosynthesizing in the porometer at the high rate of 40 mg CO_2 $dm^{-2} \cdot h^{-1}$, then an exposure of 30 s would reduce the CO_2, present in the porometers 25 ml volume, by about 20%. Assuming the concentration in the enriched air to be about 330 ppm, 300 ppm will be a fair estimate of the average CO_2-concentration during the measurement.

5.3.2 Calibration of the RS measurement

To calculate the theoretical volume of the porometer and its own resistance to vapour diffusion, calibrations were done with known resistances. The dummy resistances used were stainless steel perforated membranes with a thickness of 0.01 cm and 4 pores per cm² of various dimensions. These membranes were kindly supplied by Dr. S. Moreshet. The resistance of such a membrane equals, according to Penman & Schofield's equation:

$$R = \frac{4L}{\pi d^2 nD} + 2 \times \frac{1}{2dnD}, \text{ in which}$$

R = diffusion resistance in s/cm
L = length tube = thickness plate (cm)
d = diameter of perforations, cm
n = density of perforations, cm^{-2}
D = diffusion coefficient of water vapour in air, being at $20°C : 0.257 \text{ cm}^2/s$

The first term of this equation is the proper resistance of a number of parallel tubes, the second term is the 'end correction', applied twice if the water surface is separated from the plate, and only once if the evaporating surface touches the membrane. If the pore length is small compared with the pore $\varnothing$, the end correction becomes rather important. In fact, if $d > 4L/\pi$, the end term exceeds the 'proper' term in value, as with the membranes used. Still, it follows from the work of Stigter (1974) that even under such conditions the formula may be applied. Table 7 gives the dimensions of the perforations of the membranes used and the calculated resistances.

A comparison between calculated and measured resistances was made in two preliminary experiments:

In Experiment A aluminium tins were partly filled with about 100 ml of distilled water and sealed with the membranes. The tins were, after initial weighing, placed in

Table 7. The dimension of the perforations of the membranes used and the calculated resistances.

Diameter of perforations (cm)	$R, s \cdot cm^{-1}$ (+ 2 end corrections)	$R, s \cdot cm^{-1}$ (+ 1 end correction)	$R, s \cdot cm^{-1}$ (only end corrections)
0.2	4.95	2.63	2.43
0.1	10.9	6.1	4.85
0.056	21.2	12.6	8.67
0.036	36.5	23	13.5
0.029	48	31	16.8
0.024	62	42	20.2
0.021	74	51	23.1
The resistances given are at 20°C			
Temperature (°C)	10	20	30
$D (cm^2 \cdot sec^{-1})$	0.241	0.257	0.273

a ventilated oven at about 20°C and 50% relative humidity, and left there for 40 h. The water loss was determined by weighing. In Fig. 21A total water loss is plotted against 1/R of the perforated plates used. R was calculated with the formula, using both end corrections. A good correlation is obtained.

In Experiment B the plates were used as epidermis of 'imitation leaves', consisting of a few layers of wet blotting paper, covered with the membrane and for the rest wrapped in aluminium foil. They were left in a ventilated oven at 22°C and 40% relative humidity for a few hours, and evaporation was determined by weighing every hour. I tried to calculate R from the rate of water loss and the vapour gradient, assuming the temperature of the evaporating surface to equal air temperature.

This assumption is justified, at least for the high-resistance membranes, because of the excellent contact between the aluminium envelope and the steel mass of the oven, guaranteeing good conduction of heat directly from the oven to the perforated membrane. These resistances are plotted in Fig. 21B against R-calculated, using the formula with one end correction. It can be seen that the measured resistances deviate from the calculated ones. This deviation can be explained from 2 errors: first, the temperature of the vapourizing surface differs from the air temperature, and especially as R becomes smaller. Secondly, if the water surface touches the membrane, capillary rise into the pores is likely, reducing the resistance, and especially as R becomes bigger. Apparently this second effect is (pre)dominant and causes most of the deviation observed. As the diameter of the biggest pores is still only 2 mm, and a tube of such a
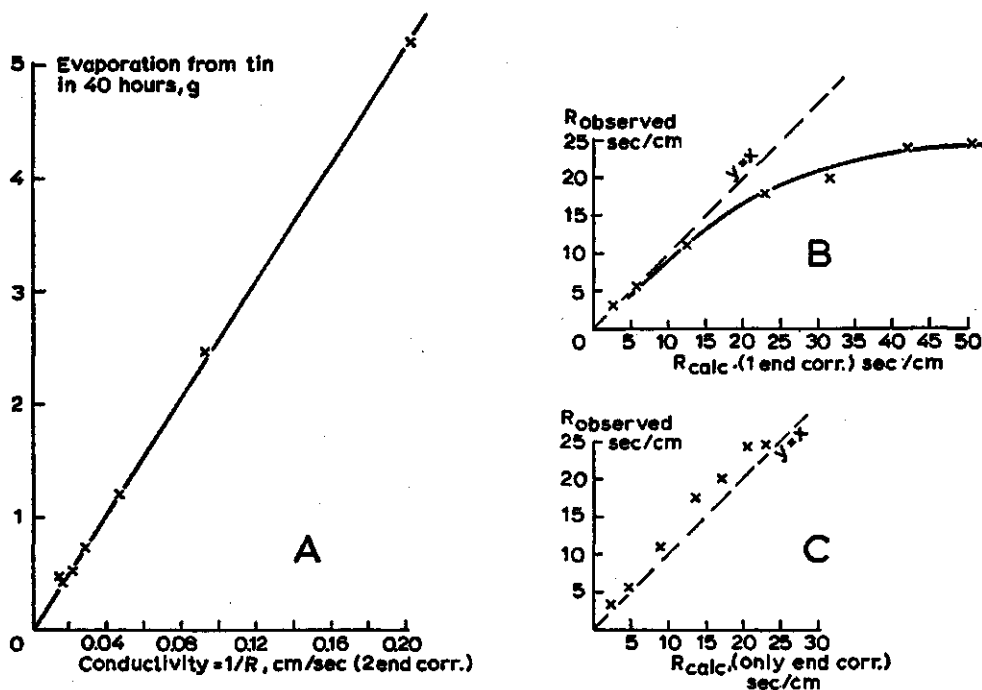

Fig. 21. Calibration of the RS measurements. Explanation in section 5.3.2.

diameter is small enough to cause about 1 cm of capillary rise, it is fair to assume that in the set-up used the water level does not touch the underside of the membranes, but the upper side, so that the resistance of the tube itself is negligible and only the single end correction counts. In fig. 21C this assumption appears to be the best under the given conditions. The porometer was calibrated using the membranes in the 'imitation leaf' way, and according to the result of the preliminary experiment their resistance was calculated as $\dfrac{1}{2dnD}$.

The calculations now proceed as follows:

$$\text{Transpiration} = \frac{\text{VOLUME}}{\text{AREA}} \times \frac{\Delta RH \times SVPA}{\Delta time} = \frac{SVPL - RH \times SVPA}{(R_{por} + R_{air}) + RS} \qquad (8)$$

in which the saturated vapour pressures (SVP) and the relative humidity (RH) are taken at the average air (A) (inside the porometer) and leaf (L) (or membrane) temperature. The second expression is similar to Eqn (5), and the first expression says in words, that the increase per unit time of the humidity in the porometer volume equals the trainspiraton rate of the vaporizing area involved. Here, ΔRH is the increase in relative humidity of the air inside the porometer, during the transient time $\Delta time$, and VOLUME is the *theoretical* volume of the apparatus. For calibration purposes Eqn (8) is rewritten:

$$V = \frac{1}{R_{total}} \times \Delta time \times AREA \times \frac{SVPL - RH \times SVPA}{\Delta RH \times SVPA} = \frac{1}{R_{total}} \times \text{'EXPR'} \qquad (9)$$

If EXPR is plotted against the calculated R of the calibration plates, a straight line is obtained with slope V and intercept $R_{por} + R_{air}$ on the abscissa. The values of RH and ΔRH are estimated from the manufacturer's calibration graphs as a function of porometer temperature. Their exact value is irrelevant, as they influence calibration and real measurements in the same way. Fig. 22 shows the results of the calibration carried out on 18 April 1973 inside the laboratory, at an average porometer temperature of 20°C. The theoretical volume was 70 cm³ when the normal interval was used, and 110 cm³ for the short interval, that was used in the field in case the measurement was going to last longer than 1 min. The discrepancy between V at normal and short transient time must be ascribed to errors in the values of ΔRH that were used. Theoretically, it is inconceivable that V should vary with transient time.

The resistance of the porometer, including the laminar air layer, equals 1.25 s/cm. The calibrations that were performed outside were always considerably worse than this one. The influence of the wind was obviously responsible for this. The calibration measurements in the field, e.g. Fig. 23, were always disturbed more than the leaf measurements because no pressure could be applied to the clamp, lest water droplets be forced out of the 'imitation leaf', which would greatly disturb the measurement. For this reason, the indoor calibration is assumed to be the best one, and to be relevant to the leaf measurements in the field. It is known that the volume is influenced by the
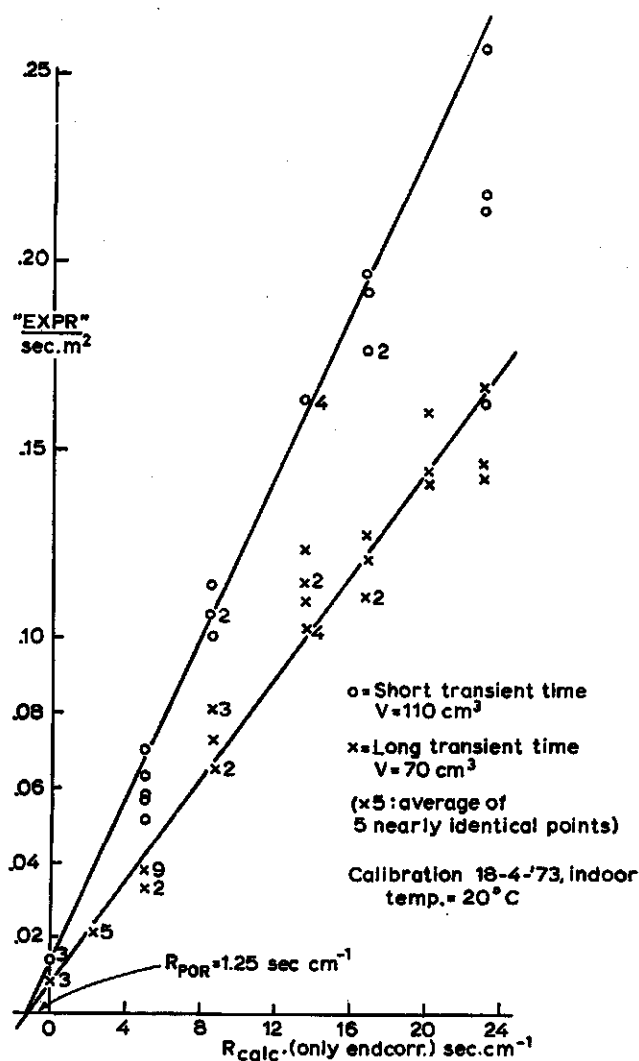
Fig. 22. Indoor calibration of the porometer, using perforated plates of known resistance. Explanation in section 5.3.2.

temperature, but as no reliable observations exist, this influence is assumed to be negligible. Anyway, the temperature of the porometer, when used in the field, was rarely far away from 20°C. Due to the sweeping generalizations that have been made here, the stomatal resistance obtained are liable to errors as big as 100%, and comparisons between data collected on different days can be no better than that.
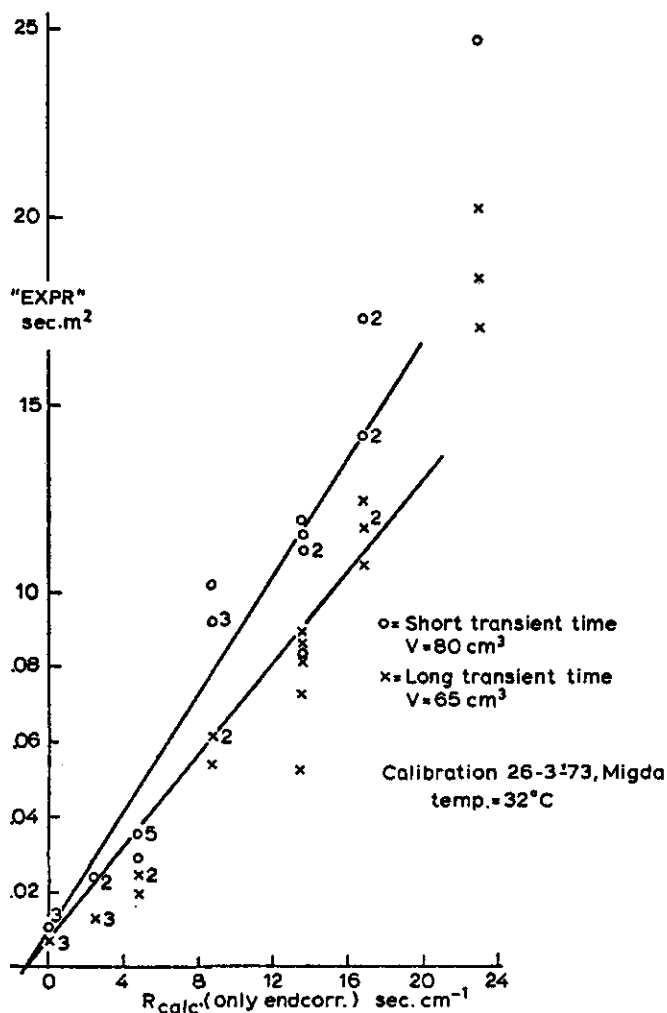
Fig. 23. Field calibration of the porometer. See text for explanation.

5.3.3 Calculation of RM

By combining the results of the P_n and the RS measurements, an estimate can be made of the mesophyll resistance to CO_2 diffusion, RM. It is assumed that at light-saturated photosynthesis the CO_2 concentration at the sites of fixation has dropped to the compensation point Γ, about (50 ppm).

$$P_n = \frac{C_{ext} - C_{int}}{RS' + RA'} = \frac{C_{int} - \Gamma}{RM}$$

where $RS' + RA'$ (for CO_2) = $1.7 \times$ (RS + RA) (for H_2O) = $1.7 \times \dfrac{e_{leaf} - e_{air}}{T}$

50

Elimination of C_{int} yields:

$$P_n = \frac{C_{ext} - RM \times P_n - \Gamma}{RS' + RA'}$$

so that $\quad RM = \frac{C_{ext} - \Gamma}{P_n} - (RS' + RA')$

or $\qquad RM = \frac{C_{ext} - \Gamma}{P_n} - 1.7 \times \frac{e_{leaf} - e_{air}}{T}$

5.3.4 Results and discussion

A representative sample of the calculated values of P_n and RS is given in Table 8, as well as the values of RM (mesophyll resistance to CO_2 diffusion) derived from them. Each RS value is the average of 3 observations; the P_n figures are single observations on a representative leaf. So much scatter exists in the figures, that sometimes negative values of RM are obtained, which are obviously erroneous. The photoseynthesis figures are generally more consistent than the RS figures, indicating that exchange of water vapour with the outside air is responsible for most of the inaccuracy. On cold, windy, and humid days the effect is most conspicuous. Thus it is useless to compare RM values obtained on different occasions. Comparison of values, obtained at the same day and hour, however, is feasible. Within each group of measurements, all species involved were compared in pairs, the value 1 being attached to the species with the higher RM, and –1 to the lower. For each species these amounts were added, and then divided by the total number of times that species had been involved in a comparison. Thus, each species was granted a value between -1 and $+1$, which yielded the following order:

Species:	Phalaris	Erucaria	Stipa	Trigonella	Centaurea	Hypocrepis	Hordeum	Anthemis
Rank:	−0.64	−0.52	−0.47	0	0.027	0.21	0.47	0.68
N:	22	54	68	8	74	61	71	38

(N is the total number of times that the species was compared with another)

The species *Hordeum*, *Hypocrepis* and *Anthemis* have a considerably higher RM than the species *Phalaris*, *Erucaria* and *Stipa*, with *Centaurea* and *Trigonella* being somewhere in between. The magnitude, in which the species' RM values differ, cannot be given. As far as *Hordeum* and *Phalaris* are concerned, this observation is in agreement with *Phalaris*' higher rate of net photosynthesis, under controlled conditions (Chapter 7). The relatively low TC of *Hordeum* can be explained, if *Hordeum*'s RS is considerably higher than that of *Phalaris*. From Figs 24 to 26, where transient times are plotted against the time of the day, it can be seen that this condition is generally fulfilled in the field. On each day, considerable differences between species exist, but

Table 8. Results of porometer measurements in the field.

730403, Migda, Field 11					730407, Migda, Field 11				
time	species	RS¹	P	R^1_{mes}	time	species	RS¹	P	R^1_{mes}
9:30	Hordeum	560	14.5	917	10:00	Erucaria	415	7.26	1934
	Stipa	189	23.5	520		Hordeum	1744	.	.
	Hypocrepis	151	14.5	1221	11:00	Stipa	1237	9.96	−673
	Anthemis	46	19.3	959		Hypocrepis	1241	6.35	1008
10:00	Hordeum	357	18.6	460		Centaurea	506	10.4	1037
	Stipa	161	30.1	297	12:00	Erucaria	535	23.7	38
	Hypocrepis	145	11.6	1459		Hordeum	2269	9.63	−1018
	Anthemis	67	19.7	890		Stipa	2277	21.9	− 759
10:40	Hordeum	331	10.7	1361		Hypocrepis	971	6.61	2127
	Stipa	266	22.8	536		Centaurea	473	11.7	724
	Hypocrepis	303	16.8	933		Anthemis	1254	2.42	6723
	Anthemis	190	10.6	1546	14:00	Erucaria	320	16.8	585
11:05	Hordeum	451	15.7	492		Hordeum	629	6.15	2226
	Hypocrepis	250	12.8	1118		Stipa	952	11.8	262
	Stipa	289	23.6	348		Hypocrepis	1856	4.04	2804
	Anthemis	192	5.38	3524		Centaurea	587	12.05	758
	Hordeum	289	7.73	2071	15:00	Anthemis	234	6.09	2852
11:50	Stipa	152	23.3	603	16:00	Erucaria	591	5.57	2445
	Hypocrepis	316	6.16	2597		Hordeum	567	3.76	4375
	Anthemis	89	18.8	902		Stipa	1055	6.49	1921
	Hordeum	355	9.14	1592		Hypocrepis	772	2.86	5614
	Stipa	284	22.2	510	17:00	Anthemis	534	2.22	7952
	Hypocrepis	202	15.2	964		Centaurea	991	4.22	3005
	Anthemis	303	11.8	1195					
12:50	Centaurea	283	14.4	891					
	Avena	168	18.9	761					
	Erucaria	41	32.2	879					
	Stipa	228	13.2	1107					
	Hordeum	286	11.5	1239					
	Hypocrepis	235	17.1	871					
	Anthemis	156							

1. RS and R_{mes} are expressed in $s \cdot m^{-1}$, P in $mg \cdot dm^{-2} \cdot h^{-1}$.

when different days are compared, little consistency is found. On 12 March (Fig. 24), which was a day with a dry desert wind blowing, the effect of previous irrigation was studied, and appears to markedly influence stomatal resistance.

During the measurements I observed that species showing rapid stomatal closure do not usually show any signs of wilting, whereas species that keep their stomata open do sometimes wilt, and look droopy at the end of a hot day. This is a typical example of 'tolerant' vs 'avoiding' behaviour (terminology will be discussed in Chapter
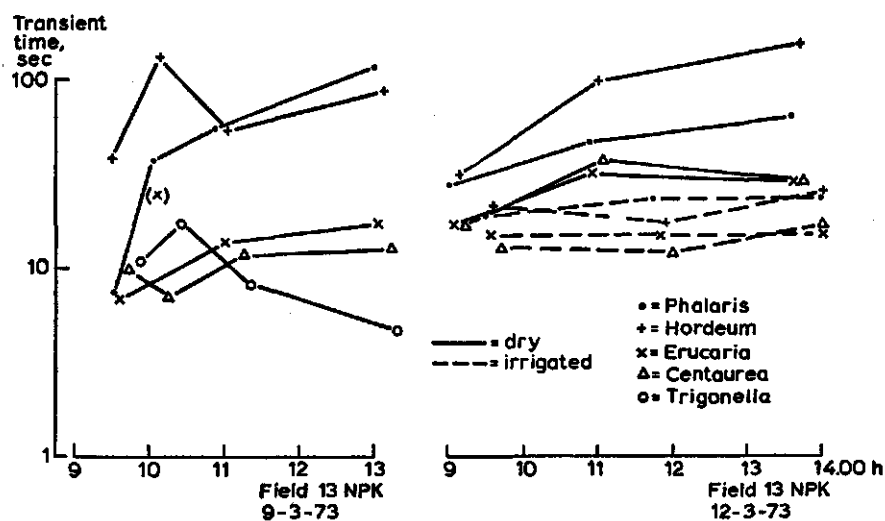
Fig. 24. Daily course of transient time (a measure of stomatal resistance) of some species in the Migda field.
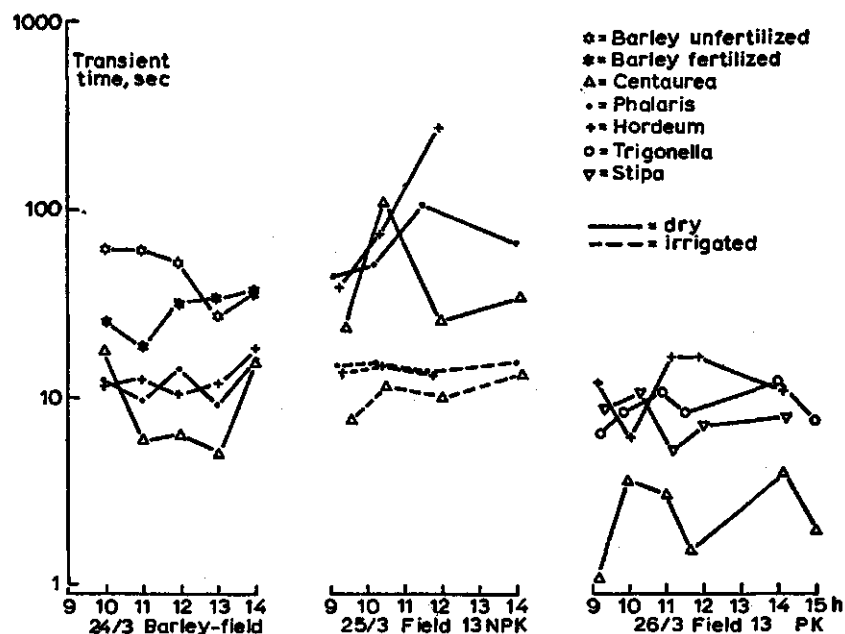

Fig. 25. Daily course of transient times of some species.

8). Avoiding plants escape from wilting by closing their stomata preventively. In the species that carry on transpiration longest, hydropassive closure of stomata is sometimes observed.
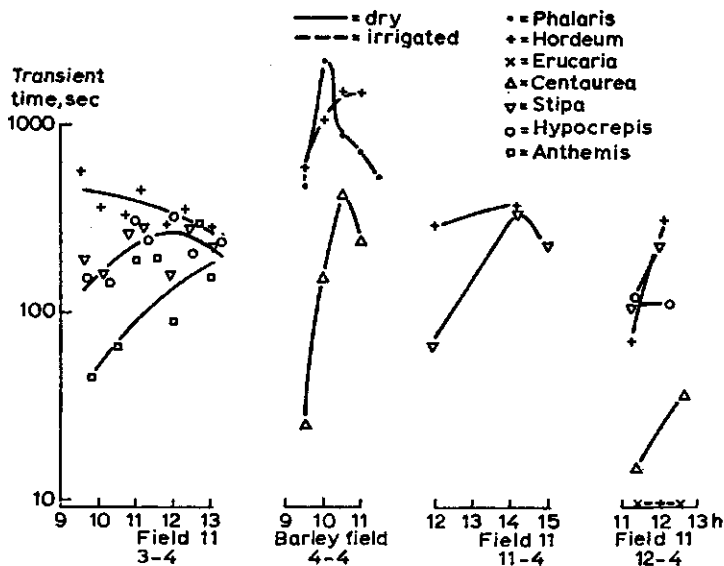
53

Fig. 26. Daily course of transient times of some species.

5.4 Experiment April/May 1974, Gilat, Israel

Aim   To determine the daily course of stomatal diffusion resistance in *Hordeum* and *Phalaris* leaves, under controlled conditions of nutrient and water supply.

5.4.1 Materials and methods

The measurements were carried out on young sunlit leaves of *Hordeum murinum* and *Phalaris minor* plants of Trials 3 and 4 in Gilat (see Chapter 4). On the days, on which the measurements were done, the water content of the pots was known, while temperature and relative humidity of the air were recorded. Clear days were chosen for the measurements, at a stage of the experiment that the plants under dry treatment could be expected to suffer from water stress during the hottest hours of the day, but had not yet reached the permanent wilting point.

The same porometer and sensor as in 1973 were used. No special precautions had been taken with regard to their storage. The sensor had not been used since the previous measurements and had been stored in a plastic vial, without any hygroscopic substance. According to Stigter this should have greatly influenced its properties, but there was no opportunity to repeat the previous year's calibrations. With only the short traject of the sensor scale, I assumed V to be again around 100 cm^3 and the porometer's own resistance to equal 1.25 s · cm^{-1}. No conclusions will be drawn with respect to the absolute values of RS thus obtained: only comparisons of the stomatal behaviour and the magnitude of its variations will be made.

To avoid troubles with leakage of air into and out of the chamber, the watch glass was replaced by a sheet of soft-rubber, covering the opening, sealing the clamp and preventing the upper side of the leaf from transpiring during the measurement as is shown in Fig. 19. The leaf temperature was assumed to equal the porometer temperature, because of the leafs contact with the rubber sheet and because the plant was shaded for about 30 s. before the measurement, as was recommended by Morrow & Slatyer (1971). As it was not possible to measure photosynthesis under these circumstances, the access and exit tubes for the enriched air could be removed. The entrances for the thermistors were also sealed, and the porometer temperature was only recorded twice an hour, which was enough, provided the apparatus was kept in the shade during and between measurements. With these modifications there were no signs of interaction between the porometer atmosphere and the outside air and the accuracy and reproducibility of the measurements increased considerably compared with previous years. The porometer could then be moved towards the plants, instead of the reverse. A measuring procedure was adopted with no pause between the measurements other than the time necessary to bring the sensor resistance back to the fixed starting point. This time lapse was utilized to move the porometer from one plant to the other, clamp it onto a leaf, and even give the leaf some time to adapt to the porometer's temperature. Each time the wetting cycle started from exactly the same point, and if the choosing of a new leaf and clamping on took more time than needed for the drying cycle, a dummy wetting cycle was made, in order not to disturb the routine of wetting and drying. Each series of 24 measurements took about 45 min, after which a 15 min pause was inserted. At the starting of a new series of measurements a few dummy cycles were performed, to acclimatize the sensor. This routine worked very satisfactorily and I feel that entering a hysteresis loop every time at the same point and at the right moment, may be just as good for reproducibility as equilibrating a few minutes at a fixed starting point of each loop. Generally the leaves were no longer than about 30 s in the porometer, and no effect of the method on RS is expected. If the transient time was to be much longer than 1 min, an estimate was often made, the disadvantage of inaccuracy being considered less serious than the disadvantage of having the leaf so long in the porometer that its RS might change. Each pot was measured once an hour and a random leaf was chosen that fitted the condition of being young, full-grown and fully exposed to sunlight. The pots under wet treatment had many of these leaves, and the chance to measure the same leaf twice was small. The dry treatment, however, had a very limited number of 'good' leaves, and some leaves were used for measurement several times a day. No adverse effect was detectable for either treatment: different leaves had much the same RS if they looked comparable, and the same leaf measured repeatedly showed no stomatal closure due to the handling.

Twenty-four pots were measured each hour: three replicates of eight groups, with all possible combinations of *Hordeum* and *Phalaris*, fertilized and unfertilized, wet and dry. The wet treatments had always recently been irrigated and were assumed to be at field capacity, dry pots were weighed on the day of measuring and their water content could be exactly calculated.

RWC determination On May 12th and 14th leaves were sampled at midday from all pots measured, to determine their relative water content. Stoppered flasks with water had been prepared and accurately weighed previously. The cut-off leaves were put into these flasks, which at the end of the sampling were weighed again, the difference giving the fresh weight of the leaves at the moment of sampling. After being left overnight in the flasks, the leaves were assumed to be fully turgescent, and they were taken out, carefully blotted and weighed. Then they were dried 24 h in the oven and weighed again. From the 3 figures obtained, % water at picking, % water turgescent, and RWC could be calculated, the latter being:

$$\text{RWC} = \frac{\%\text{ water at picking}}{\%\text{ water turgescent}} \times 100$$

5.4.2 Results and discussion

The transient times of the measured leaves are plotted against the hour of the day in Figs 27 to 30. RS values could not be calculated, since leaf temperatures had not been measured and the porometer-sensor combination had not been calibrated this season. It is estimated, that RS is about $\frac{1}{2} \times \Delta$time (RS in s·cm^{-1}, Δtime in s), assuming V to be 100 cm^3 and t^2 to equal t^2. In the figures the individual observations are shown, the lines drawn are usually averages of 3 observations for the wet treatments, for the dry treatments each pot is represented with a separate line. Differences in soil water content (indicated between parenthesis) sometimes cause marked differences in transient time, though more in the amplitude than in the daily pattern. Particularly Fig. 30 shows this. The difference between 'moderate' and 'stress' days is also clearly demonstrated by comparing Fig. 28 with Fig. 27 or Fig. 29 with Fig. 30. The day's evaporativity is characterized by the temperature and relative humidity at the hottest hour, for 7th and 8th May the daily courses of relative humidity are given. For both wet and dry treatments, *Hordeum* generally had slightly longer transient times than *Phalaris*. A comparison of the dry treatments was often not possible, as the humidities of the soil differed from pot to pot. But on 7th May the fertilized pots could be compared and showed no striking differences. On 8th May, again some fertilized pots had about 8 – 9% of water content for both species. Here the difference between the species seems to be significant, and also if the wet treatments on that day are compared. Small differences have to be regarded with care, as leaves of *Hordeum* are slightly narrower than *Phalaris* leaves, and this alone suffices to explain a difference in transpiration of 20 – 30%. Then only the results of Fig. 30 fertilized demonstrate *Hordeum*'s greater sensitivity to stress.

Hardly any differences could be observed between fertilized and unfertilized, partly because a direct comparison is in all cases of the *dry* treatment impeded by differences in water content. Looking at the *wet* treatments, one notices that Δt for unfertilized plants is always a few seconds shorter, a small but significant difference, which cannot be attributed to differences in leaf width: fertilized plants have leaves of the same

56

Fig. 27. Stomatal behaviour of plants, growing in pots. The points referring to wet treatment are averages of 3 observations.

width as unfertilized ones, or even slightly broader. This means that the lower TC of the fertilized plants is not only due to a higher production rate, but also to a lower rate of water loss, so that it is justified to talk about a water 'saving' effect of fertilization.

Closely observing the stomatal behaviour, an answer can now be formulated to the question, whether some plants 'select' the most favourable hours of the day by adjusting their stomatal resistance to the stress applied, and thus improve their transpiration coefficient. From these results such a conclusion cannot be drawn. Often the curves have 2 or 3 peaks so that at the hottest hours of the day the stomata are often found to be open, while at more moderate hours they are closed. Under moderate stress conditions the one-peak type of stomatal behaviour seems to occur, like on 18 April with unfertilized plants, on 19 April, and with some pots on 8th May. Very severe conditions, such as prevailed on 12th May (not shown), when in addition the
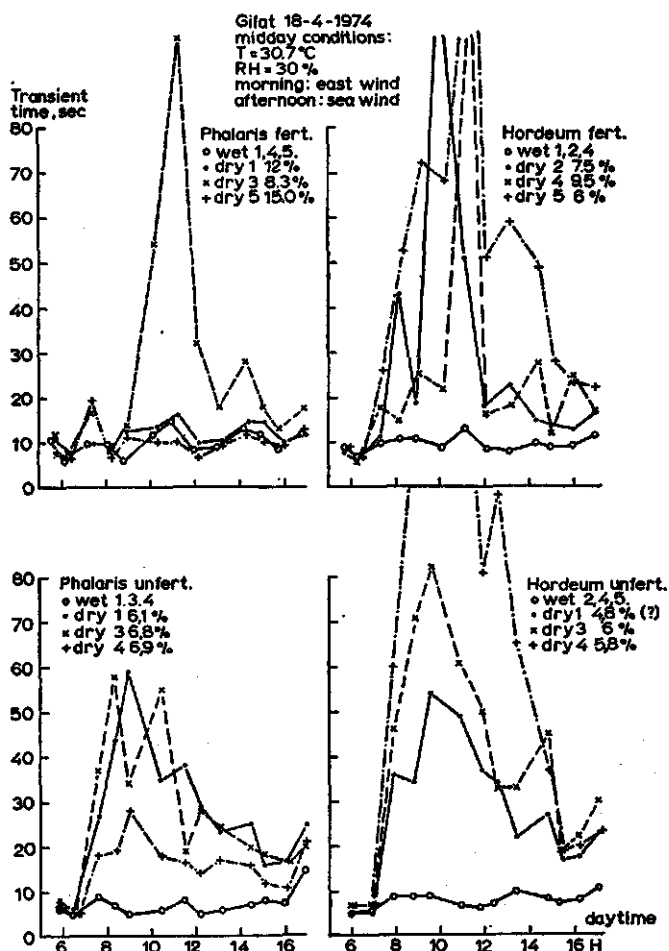
57

Fig. 28. Stomatal behaviour of plants, growing in pots. The points referring to wet treatment are averages of 3 observations.

water content of the pots was very low, keep stomata closed during most of the day-time. In the cases between 'moderate' and 'extremely severe', stomatal oscillations seem to form the normal behaviour pattern, as is best seen in Figs 29 and 30 (7 and 8 May). The latter type will be called the 'collapse-recovery' type of regulation, as against the 'flexible response' type, which is observed under more moderate condi-tions. One is tempted to think that the collapse-recovery type is possibly an artifact of the pot trial, being indirectly caused by the limited extension of the root system. In the field, where the plants root to a depth of 1.5 m, one could imagine the water supply to be more steady, so that stress cannot develop speedily. Measurements on the same plants in the field, Section 5.3, are by no means conclusive, but do not show clear indications of a 'peaky' behaviour of the stomata. However, in the literature such behaviour has been described frequently in field measurements on desert plants. Evenari & Richter (1937) observed all sorts of stomatal behaviour on plants growing
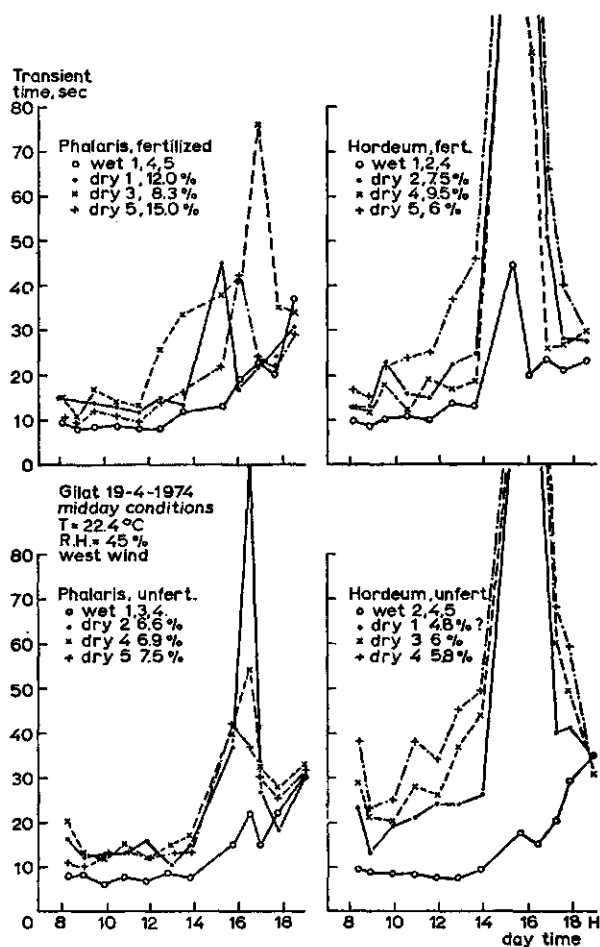
58

Fig. 29. Stomatal behaviour of plants, growing in pots. The points referring to wet treatment are averages of 3 observations.

in the Judean Wilderness. They were the first to describe daily courses with 3 peaks and considered them characteristic for desert plants. Stocker (1954) said that desert plants can be subdivided into stabile and labile types. Examples of the latter type show several peaks on a day, which Stocker characterized as 'Seiltanzerei' (rope-walkery). Other authors found the same phenomenon (Schulze et al., 1972; Hellmuth, 1971; Lemee, 1962). Lemee observed a relationship between a plant's stomatal behaviour and its shoot/root ratio: the more roots a plant has, the more stabile is in general its stomatal behaviour. From my observations, no support can be given to this attractive theory.

RWC measurements The relative water contents of *Hordeum* and *Phalaris* leaves are plotted in Fig. 31 against the transient times, measured on those leaves just before harvesting. On 12th May, 24 different buckets were sampled once, at noon, on 14th May 4 buckets (2 *Phalaris* wet, 1 *Phalaris* dry, and 1 *Hordeum* wet) were each sampled
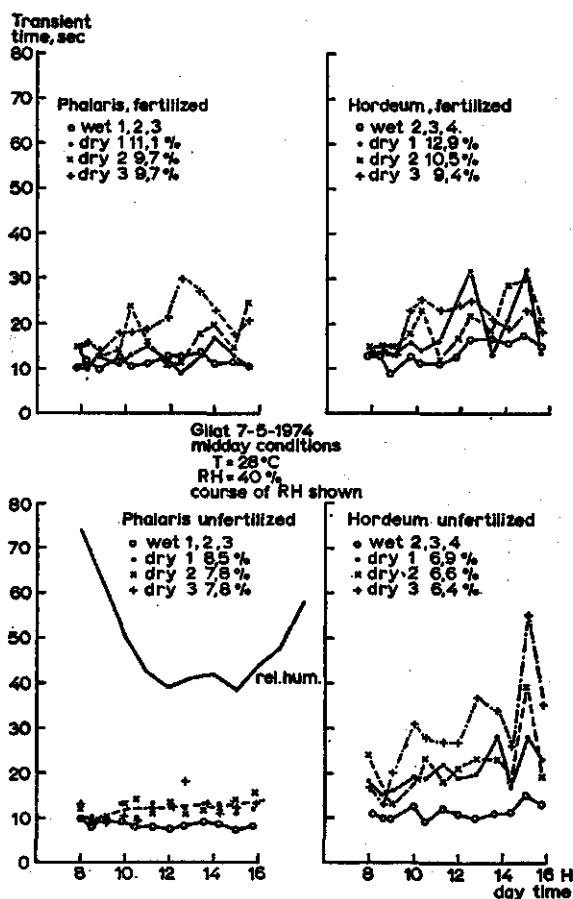
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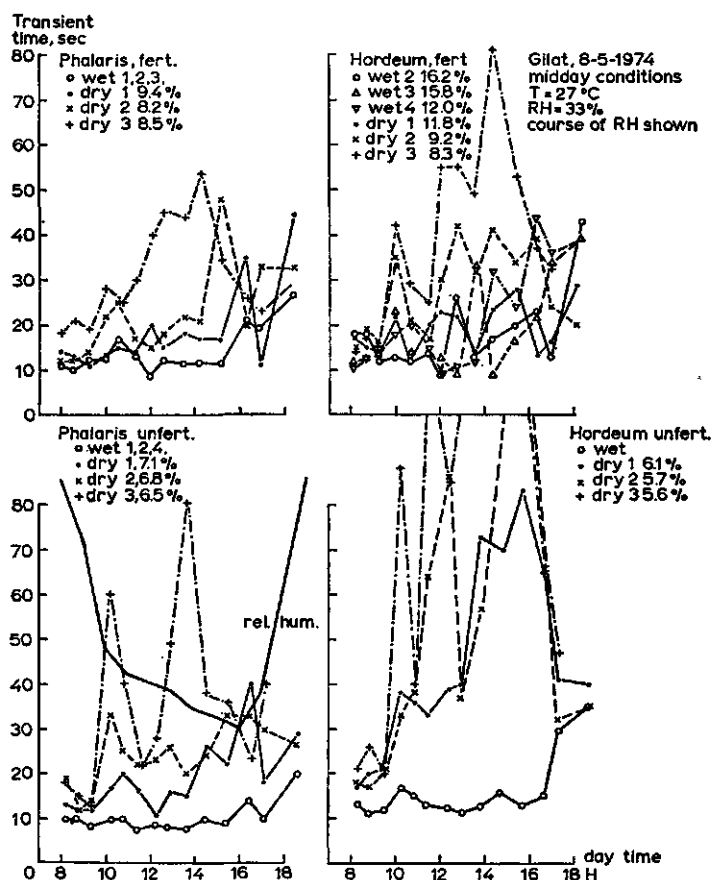
Fig. 30. Stomatal behaviour of plants, growing in pots. The points referring to wet treatment are averages of 3 observations, except in Hordeum fertilized.

5 times during the day. As can be expected, the latter observations show much less scatter than the former. For the plants under wet treatment, a uniform relationship appears to exist between RS and RWC, irrespective of the species, an RWC of about 85% being the lowest point reached. Under dry treatment, *Hordeum* has its stomata closed very rapidly, when RWC falls below 70%. *Phalaris'* stomata generally react slower, less radically, and at a lower RWC. Remarkably, all leaves of a species returned to the same water %, when left overnight in water: around 82% for *Hordeum* and around 84% for *Phalaris*, in spite of enormous differences in daily behaviour.
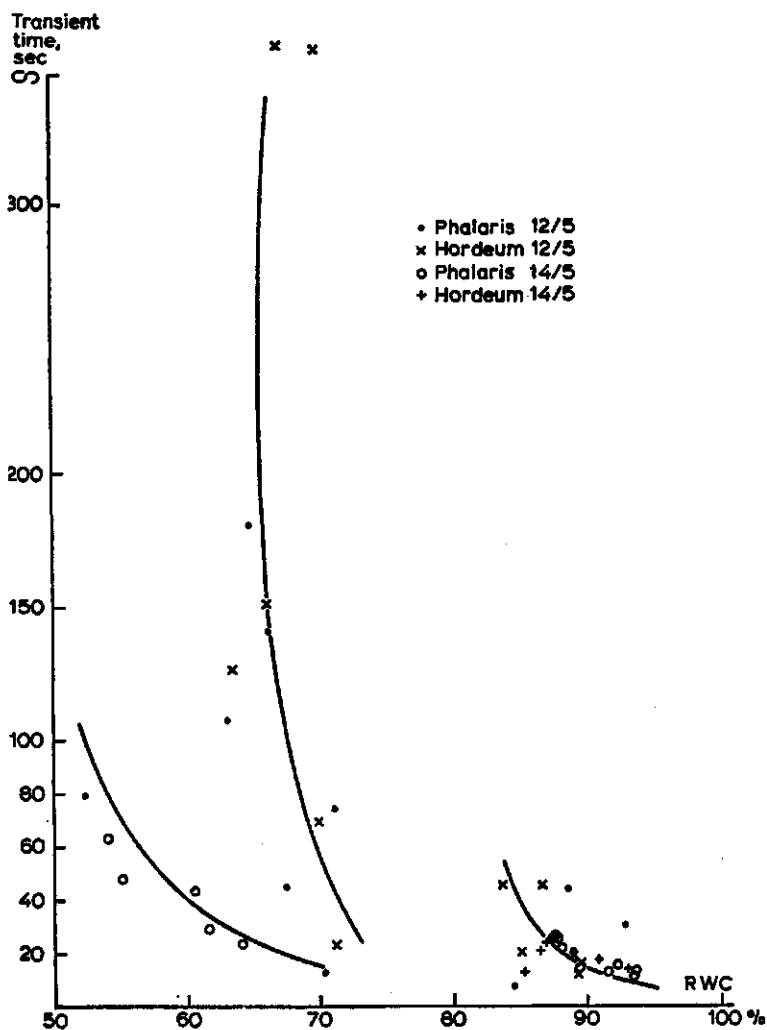
Fig. 31. Relation between the transient time (which is proportional to RS) and the relative water content of Phalaris and Hordeum leaves. The observations between 80 and 100% RWC have been taken from plants under wet treatment, the others are suffering from drought.

61

6 Experiments with osmotic stresses, under controlled conditions

6.1 Introduction

The plant's reactions to environmental stresses are believed to determine where and when they can thrive. Most plant species, coexisting in the same biotope, behave similarly under optimal conditions, but react very differently to all kinds of stress. Stresses are hard to characterize, and generally the plant's reaction is taken as a measure for the stress it undergoes. Although for practical use this is generally sufficient, it is of little value in scientific experiments.

With respect to the supply of nutrients and water, all stresses that can be applied are mainly determined by what takes place at the root-soil interface, a site where accurate registration of parameters is impossible. Another drawback is, that such stresses can rarely be kept constant. Particularly suboptimal availability of water is hard to maintain or even define. Often water stresses are applied by a watering regime and at each moment in time the stress is defined as the water potential of the bulk soil. It is either measured with tensiometers or calculated from the water content of the soil. Between field capacity and wilting point no water potentials can be kept constant, as any suboptimal watering will result in an uneven distribution of the water applied, field capacity being reached on the spot where the water is given and other places getting no water at all. Several methods have been proposed to achieve constant water stress in an experimental soil-plant-atmosphere system. The best way is to hang a water column under a homogeneous sandy soil, the length of which must be limited, however, since air penetration can break the column.

It has been proposed to water the soil with osmotic solutions, mainly NaCl, which has the desired effect, but many side-effects as well. An elegant solution was recently proposed by Ben-Zur (1966). In his approach the soil's water potential is kept in equilibrium with an osmotic solution of controlled potential, from which the soil is separated by semi-permeable membranes. Although theoretically sound, this method encounters numerous practical problems, like limited size of the soil compartments, and rapid disintegration of the membrane. No results have been reported, as far as I know.

A method which is theoretically questionable, but practically feasible, is the injection method of Vaclavik (1966) where a sort of 'statistical stress' is obtained by restoring transpired water by small random injections in the soil. Thus pockets of soil at field capacity are created, but as their sites vary, no concentration of roots on wet places occurs, and always a constant portion of the root system is in contact

with free water, whereas the rest is not. However this situation is totally unnatural with no equivalence in nature.

The method which I preferred to use, was growing the plants on nutrient solution to which polyethylene glycol (PEG) has been added to any desired osmotic potential. Of course, this method does not have its equivalent in nature either, but it has great advantages over all other methods, being simple, reproducible, and the result being fairly well definable. If gradients near the root surface are disregarded (which exist even in nutrient solution, but can be expected to be small) the situation at the root surface is well represented by the level in the bulk solution, and thus can be accurately quantified. PEG is a very suitable osmotic, as it is not taken up by the plant if the roots are handled with care, and it is not easily decomposed by bacteria (Lawlor, 1970). The viscosity of the solution inhibits proper aeration, so that I had to limit the experiments to grasses, which have no apparent need for aeration of the root environment.

6.2 Materials and methods

Plants were grown on normal half-strength Hoagland nutrient solution until about 3 weeks after germination. Then the required amount of the osmotic was added at once. The relationship between concentration and osmotic potential of PEG_{6000} was taken from Lawlor (1970), and proved correct when, at the end of one experiment, samples were tested in an osmometer. To prevent osmotic stress from building up too rapidly, the PEG was allowed to dissolve slowly, while the plants were kept in a dark, humid climate room. It took the PEG about 3 days to dissolve completely. After that period the plants were placed back in a constant environment room as described in Section 3.1, and the experiment started. The experiment lasted for 2 weeks, during which water losses were determined by periodic weighing of the pots, and replaced by double-strength Hoagland solution (concentration $4 \times$ normal half-strength Hoagland). It was assumed that the nutrient consumption was 4 times the water consumption. The NO_3 concentration of the nutrient solution was measured at the end of one experiment, and it appeared that the assumption holds for plants which are growing at 5 atm, whereas water use was relatively higher at lower stresses and NO_3 consumption relatively higher at higher osmotic stresses. At 0 atm stress (= normal $\frac{1}{2}$ Hoagland) this practice resulted in a doubling of the NO_3 concentration during the 2 weeks of the experiment, while at 9 atm the NO_3 level dropped to about 60% of its original level. These data were considered to support the method used, and as the plants did not show any signs of deficiency, it was assumed that of all required nutrients sufficient amounts were available throughout the experiment. Record was held of the rate of elongation of growing leaves, during the experiment, by taking measurements with an ordinary ruler 3 times a week. The length from the leaf tip to the base of the plant was taken, assuming no stem elongation. If a plant showed signs of turning reproductive, these measurements were stopped.

After 2 weeks the plants were harvested and fresh and dry weights of shoot an root

determined. Root fresh weight was taken after ample blotting with filter paper. Leaf samples were frozen, to be squeezed later on, and the osmotic potential of the press sap was determined with a Knauer osmometer (Type M). Transpiration was calculated as total water loss, corrected for the average water loss from blanks during the same period. Dry matter production was measured as final dry matter minus initial dry matter, being the average weight of ten plants, harvested at the onset of the experiment.

Two such experiments were done, the first with 4 species: *Phalaris, Hordeum, Stipa* and *Chloris*, the second with only *Phalaris* and *Hordeum*. In both experiments the treatments were: nutrient solution + 0, 3, 5, 7 and 9 atm PEG_{6000} per litre (concentrations: 0, 100, 150, 190 and 230 g PEG/l, respectively). The first experiment had 5 replicates per series per treatment, the second 10.

6.3 Results

The results of the first experiment are summarized in Fig. 32, where various parameters are plotted against the osmotic potential of the rooting medium. In all 4 species both dry matter production and transpiration decrease with increasing stress, and so does RGR. They decrease with different rates in different species, most in *Hordeum* and least in *Phalaris*. In Rhodes grass the decrease of production and transpiration are proportional, so that the transpiration coefficient is constant for all treatments. In the other species transpiration decreases more rapidly than production, causing the TC to drop with increasing stress. More clearly the same phenomenon is observed in Fig. 33, where the results of the second experiment are shown.

Osmotic stress has another effect, that seems to be general: it decreases the water content of the plant. *Hordeum* and *Stipa* always had a lower water content than *Phalaris* and *Rhodes* grass, as has been pointed out in Section 3.2, and it may be that the water content of *Phalaris* and *Rhodes* grass decreased more when stress was applied. But the data are not conclusive in this respect, due to their variability, and the fact that *Hordeum* and *Stipa* had only a few survivors at 9 atm. In the first experiment 2 plants of *Phalaris* died at the highest osmotic stress, leaving 3 survivors, while none of the *Hordeum* plants survived this stress. In the second experiment 7 plants of *Phalaris* survived 9 atm, against only 3 of *Hordeum*. No treatment had any detrimental effect on *Rhodes* grass.

The osmotic potential of the press sap of the leaves generally followed the osmotic potential of the nutrient solution, but not in Rhodes grass, and hardly in *Hordeum* in the second experiment. The evidence from both experiments is conflicting on this point.

Remarkably, the Shoot/Root Ratio of all species involved was either unaffected by the treatment, or showed a minor increase with increasing potential, quite contrary to what one might expect.

Finally, in the second experiment the dry weight·cm^{-2} of leaf area increased with increasing stress in *Phalaris*, and less so, if at all, in *Hordeum*. In both species the
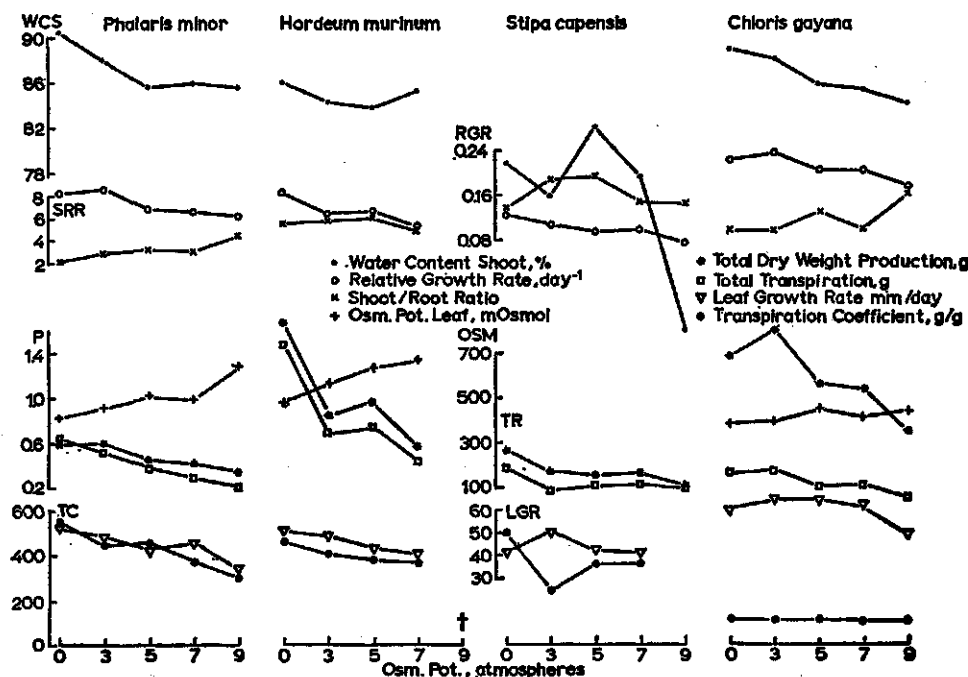
Fig. 32. Results of the first PEG experiment, with 4 species.

fresh weight per unit leaf area decreased. Thus dry weight increase in *Phalaris* was not so much hampered by stress as was leaf area expansion, and water content decreased so much that leaf fresh weight was most radically changed by osmotic stress.

6.4 Discussion

A comparison of *Hordeum* and *Phalaris* confirms that *Hordeum*'s SSR was higher and its water content and TC lower. Its leaf area ratio was also higher than that of *Phalaris*, at least as far as fresh weight is concerned.

The most conspicuous difference effected by continuous stress on *Hordeum* and *Phalaris* was the more radical reduction in *Hordeum*'s growth rate. At 9 atm this growth rate even became zero so that the plants starved to death (the leaves turned yellow gradually, starting with the oldest, without the plants showing any visible sign of wilting).

These experiments clearly show that the stress applied leads to a more efficient use of water, with the single exception of Rhodes grass. In the Gilat pot trial the same phenomenon was observed, and there I argued that saving of water is a result of the stomatal behaviour, which limits gas exchange to the most favourable hours of the day. Here, this argument is invalid, because the plants are growing under constant conditions and all hours are similar. The observed improvement of the plant's water use efficiency can be explained by either a relative increase of the CO_2 diffusion, or a

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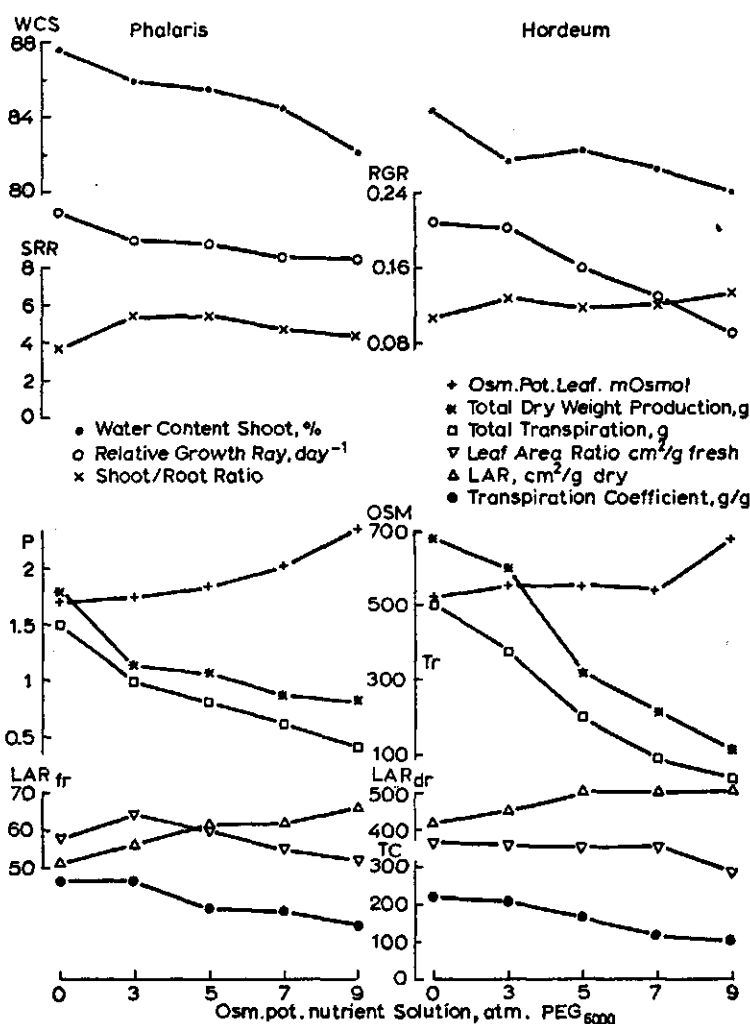
Fig. 33. Results of the second PEG experiment, with 2 species.

decrease of the mesophyll resistance. The former is liable to occur, since any stress that leads to increased stomatal resistance, without affecting directly the photosynthetic capacity, would cause the internal CO_2 concentration to drop, and hence the gradient of CO_2 over the stomata to grow bigger. This is already sufficient to explain how osmotic stress leads to saving of water. The magnitude of the saving can, however, not be indicated. A decrease of the mesophyll resistance would also lead to a lowering of the internal CO_2 concentration, and hence to an increased CO_2 gradient over the stomata. The mesophyll resistance has several components, of very diverging character: the ratio mesophyll cell surface/leaf area, the length of the diffusion path between mesophyll cell wall and the sites of chemical CO_2 fixation, the concentration of carboxylation enzymes. The effect of water stress on each of these constituents of

66

the mesophyll resistance is not evident, but cell size could influence the length of the diffusion path, and also the relative mesophyll area. I observed a decrease in mesophyll cell size with increasing osmotic stress. Mesophyll cells of both *Hordeum* and *Phalaris* were observed under the microscope, and their diameter appeared to be approximately 30 μm at zero stress, decreasing to about 20 μm at 9 atm. In the samples that I observed, however, the transition was not gradual, but showed a major jump at 7 atm. Because of its very efficient fixation of CO_2, Rhodes grass always has a very low mesophyll resistance. This serves to explain why the TC of this species shows no change under stress.

In Section 5.3.3, it was found that *Hordeum*'s mesophyll resistance is higher than that of *Phalaris*, and in Chapter 7 this result will be confirmed. If *Hordeum* still displays a lower TC, then its stomatal resistance must be appreciably higher than that of *Phalaris*, and its internal CO_2 concentration much lower. Under stress, this is apparently so, as is demonstrated by the more rapid decrease of production and transpiration in *Hordeum*. But under non-stress conditions, and even at 3 atm of osmotic stress, *Hordeum* produced and transpired more than *Phalaris* did in the same period, in spite of its lower TC. I am unable to explain this satisfactorily, but presumably it has something to do with the differences in shoot/root ratio between the species: under optimum conditions *Hordeum* develops more leaves than *Phalaris*, and so gets a lead in dry matter production. The extended root system that *Phalaris* develops and maintains requires apparently not only the investment of much assimilates for structural material, but also leads to considerable respiratory losses.

The osmotic potential of the cell sap generally increased with increasing osmotic potential of the nutrient solution. It does not completely meet the environment's challenge, but approximately half-way. Only the response of Rhodes grass was much smaller and can be quantitatively accounted for by the relative decrease of the plant's water content. In the other species the relative increase in osmotic potential mirrors the relative increase of the dry matter content of the shoot. It is not very clear, whether these statements have any practical meaning. Water and dry matter in the cell are spatially separated: most of the water is located in the vacuole and most of the dry matter in the cell wall, the protoplast taking an intermediate position. If the rise in osmotic potential of the cell sap (which is mainly vacuole liquid) is proportional to the drop in water content, then the amount of solutes in the vacuole remain virtually unchanged, while water is removed. If, on the other hand, the increase of the osmotic potential follows the increase in dry matter content, then this indicates that transport of materials into the vacuole has taken place to the point where the solute content of the vacuole again reflects the dry matter content of the whole cell. An appropriate mechanism cannot be proposed at present. It has been suggested by Slavik (1963), that part of the cell water is 'bound', and that therefore the osmotic potential rises more rapidly than could be expected theoretically from the water deficit. In the second experiment, *Hordeum* does not seem to increase its osmotic potential very much, in spite of an appreciable drop in the shoot's water content.

Looking at the results of the second experiment only, I am tempted to call *Phalaris*'

response to osmotic stress a good example of tolerance, while *Hordeum* shows the characteristics of an avoider. Under prolonged stress conditions, as in this experiment, the tolerant type of behaviour is apparently the best one, since it helps to keep up an appreciable rate of growth, while avoidance results in starvation, at high stress.

7 Direct measurements on the transpiration and photosynthesis of Hordeum and Phalaris, under various circumstances. Effect of desiccaiton on RS

It is well-known, and appears also from the previous chapters, that plants undergo morphological changes when subjected to environmental stresses. Such adaptations to stress have an impact on the plant's metabolism. In this chapter we will discuss whether, and how adaptations at various pretreatments influence the stomatal response to rapid desiccation.

7.1 Materials and methods

Transpiration and photosynthesis were measured in plants of 5 – 6 weeks old, which had been pretreated at: (a) different light intensities (duration of the pretreatment: 4 weeks); (b) different osmotic potentials (pretreatment lasted 2 weeks); (c) different N-level (pretreatment lasted 7 to 11 days).

Treatment a   These measurements were carried out in winter, when light intensity in the greenhouse was considerably lower than in the phytotron. Light intensity in the phytotron is referred to as normal light (NL). High light (HL) and low light (LL) conditions were created in the greenhouse, LL being the normal light intensity there, and HL the light intensity under a battery of HPL mercury lamps. The daylength under all treatments was the same, the lamps in the greenhouse being on for 17 hours a day, which is the standard daylength in the phytotron as well. The LL plants were placed in the same greenhouse as the HL plants, but 5 m away from the lamps, so that they received only a little additional light.

Treatment b   Two weeks before measurement, appropriate amounts of PEG_{6000} were added to the nutrient solution and allowed to dissolve slowly. Stresses of 0, 3, 5, 7 and 9 atm were applied. Water losses were replenished by $4 \times$ concentrated nutrient solution, to prevent exhaustion of minerals.

Treatment c   Seven days before the first measurements, all plants, except a control group, were transferred to N-free nutrient solution. As the measurements themselves proceeded for 5 days, the plants that were measured on consecutive days had been deprived of nitrogen for 7, 8, 9, 10 and 11 days, respectively.

The actual measurements were carried out with the photosynthesis measuring equipment as described by Louwerse & Van Oorschot (1969). A part of the plant's leaf

area was enclosed in a leaf chamber and exposed to well-controlled environmental conditions, while photosynthesis and transpiration were recorded simultaneously. The rest of the plant's leaves were exposed to virtually the same light intensity and temperature as the measured leaves, but wind speed and relative humidity may have been different outside the chamber. First a light response curve was obtained by exposing the leaves to stepwise increasing light intensities up to 0.4 $cal \cdot cm^{-2} \cdot min^{-1}$. Then the plant was equilibrated at 50% of maximum light intensity, and the roots were taken out of the solution, adhering water being blotted off. The plant was then allowed to dry out for about 3 hours. A leaf sample was taken at the onset and at the end of the desiccation period, the first sample being taken from the leaves that were outside the measuring chamber. All the leaf material for the second sample was taken from inside the chamber. Fresh and dry weight of both samples were determined, as well as leaf area of the second sample. Water content was calculated on fresh weight basis. The course of the relative water content during desiccation was calculated as follows: measured transpiration rates were integrated with time, and the total transpired water loss set equal to the observed difference in water content between onset and end of desiccation. If RWC at the beginning is 95% of full turgescence, the RWC at any moment during desiccation can be calculated with the formula:

$$RWC_t = (\frac{\int_{t_0}^{t} T dt}{\int_{t_0}^{t_e} T dt} \times \frac{\text{water \% } t_e - \text{water \% } t_0}{\text{water \% } t_0} + 1) \times 0.95$$

in which T = transpiration rate (in arbitrary units), and t_0 and t_e are the times of onset and end of desiccation, respectively.

It has been assumed that the relative water distribution over the plant did not change during the experiment. However this assumption was incorrect, since the root system lost relatively most water and the stubble relatively least, during desiccation, and thus it is not certain that the relation between total water loss and water content shoot is linear. The approach followed was considered the best possible, under the given circumstances.

7.2 Results

Treatment a In Figs 34 and 35 plots are shown of relative transpiration and photosynthesis (referring to rates at onset of drying) against RWC, for the 3 pretreatments HL, NL, and LL. No separate points for photosynthesis and transpiration are given, since they usually coincide, therefore the abbreviation RTP is used for the average of both. During desiccation, different light intensities were applied: 30, 50, 75 and 100% of the maximum light intensity in the photosynthesis chamber (30% is approximately the light intensity in the phytotron = pretreatment NL). Differences in light intensity

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Fig. 34. The relation between relative transpiration and photosynthesis and relative water content of Hordeum, during desiccation. The plants have been pretreated at 3 levels of light intensity. Water content at onset of desiccation is indicated between parenthesis.
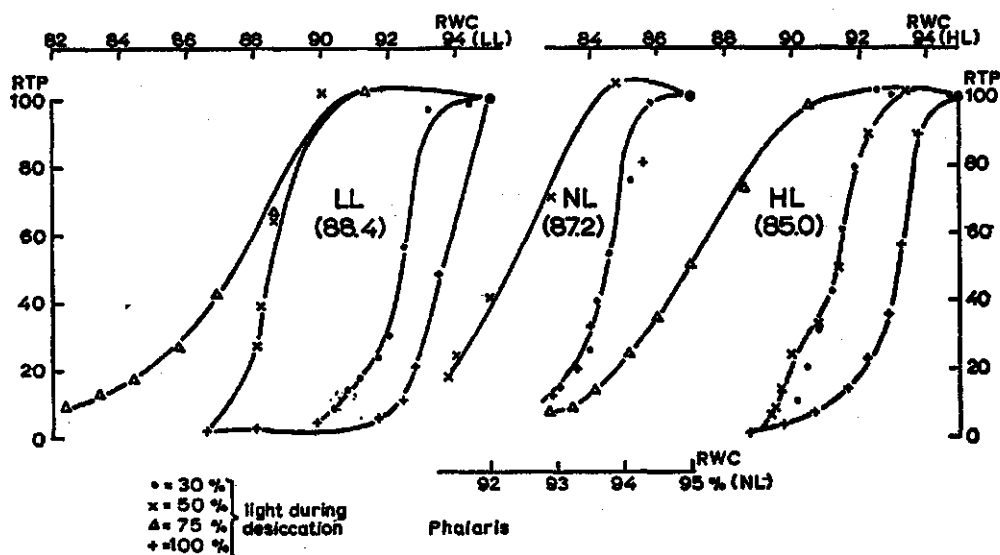
Fig. 35. Like fig. 34 for Phalaris.

during desiccation had much more effect on the stomatal behaviour of *Phalaris* than on *Hordeum*. In Fig. 34 the curves for HL and NL pretreatment are similar, but plants pretreated at LL close their stomata slightly earlier (i.e. at a higher RWC) than the others. The (absolute) water contents at onset, which are displayed between parentheses near to the curves, are correlated with this behaviour, being virtually equal for HL and NL, but higher for LL.

71

Conclusions cannot be made about the behaviour of the *Phalaris* stomata in this experiment: they seemed to respond both to the RWC and to the light intensity, but in an incomprehensible manner, causing differences that completely overrule any effects of the pretreatment. The water contents at onset (between parentheses) reflected the differences in light intensity during the pretreatment.

Treatment b During the measurement of the light response curve, the plants were exposed to different osmotic potentials, not necessarily the same as during pretreatment. In Fig. 36 the RTP of *Hordeum* is plotted against RWC. The indication 7 → 3 means that the plant was pretreated at 7 atm, and measured at 3 atm. For *Phalaris* the pattern is similar to that for *Hordeum* (Fig. 37), but leaves dried out further before

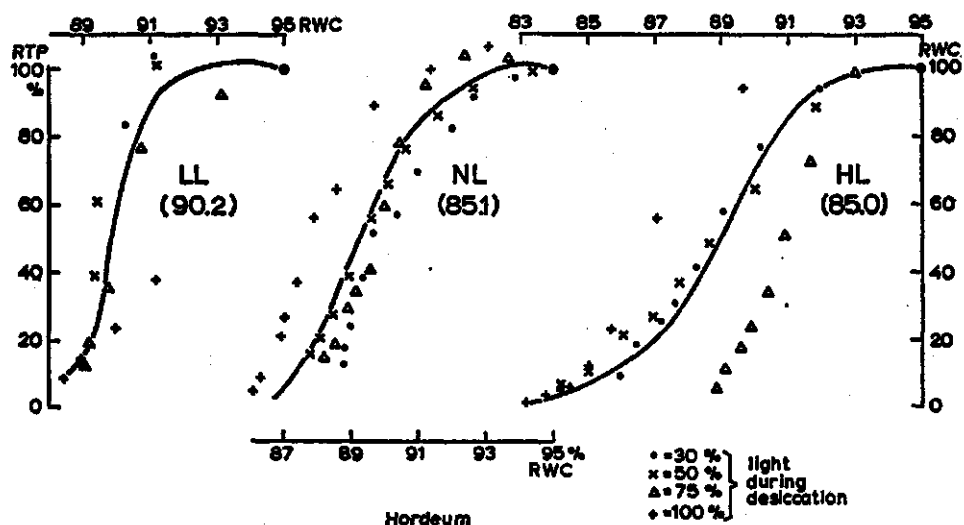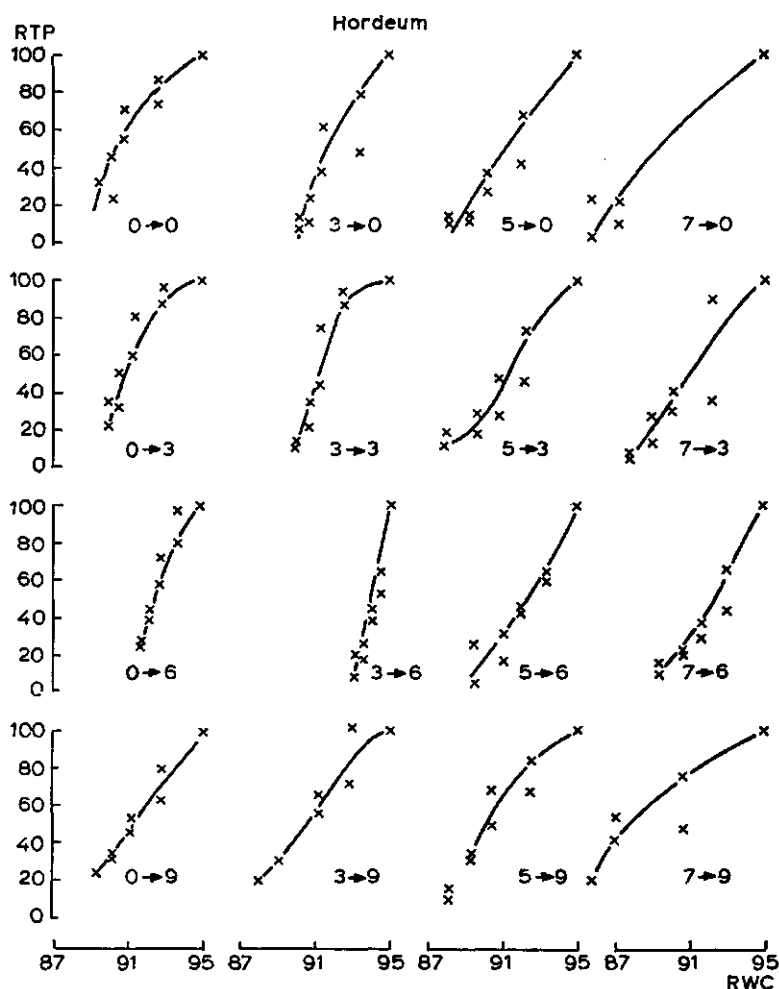Fig. 36. Influence of different osmotic pretreatments on the relation between relative transpiration and photosyntesis and relative water content of Hordeum during desiccation.

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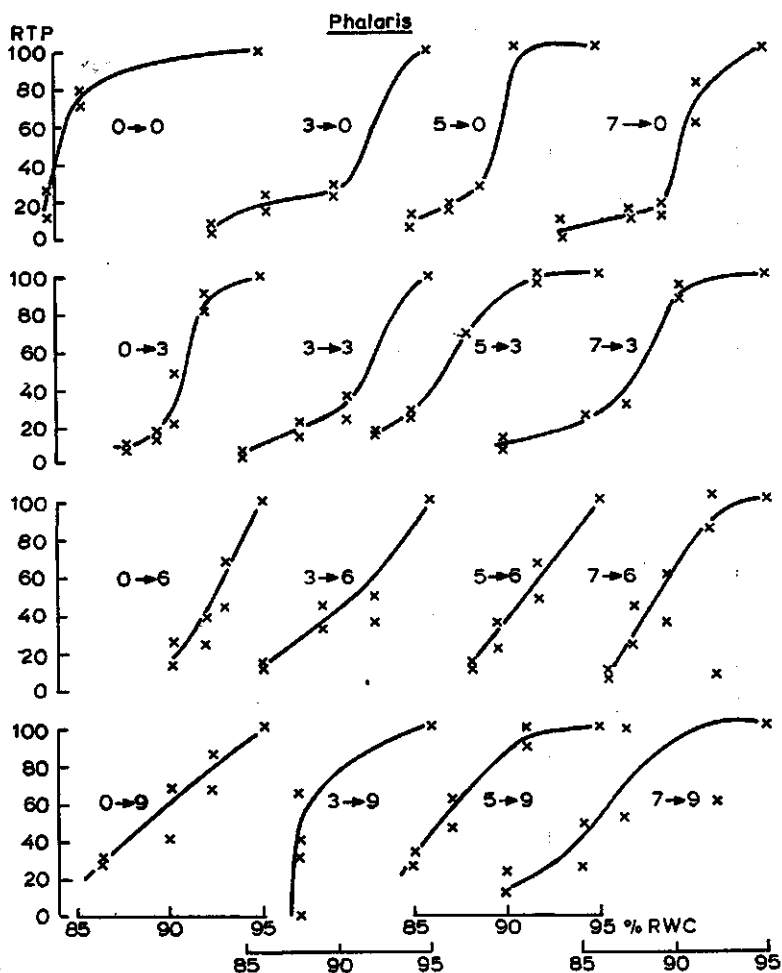
Fig. 37. Like fig. 36 for Phalaris.

stomatal closure was effective. Although the scatter is considerable, (each curve represents a single plant), stomata of plants that were pretreated at low stress close more rapidly at desiccation than do stomata of plants that were pretreated at high stress. This indicates that an adaptation to stress takes place. More clearly, this effect is shown in Figs 38 and 39, where the RWC at which RTP is 80, 50 and 20% respectively is plotted against the osmotic stress during pretreatment. The curves for *Phalaris* are most consistent, but no explanation can be given for the deviating behaviour of the plants that were transferred to zero stress. The *Hordeum* curves demonstrate the same effect, although considerably weaker. Only at the highest stress (9 atm) and at nearly complete stomatal closure (20% RTP) was the influence of the pretreatment obvious.

Fig. 40 shows that a relation exists between the water content of the leaf at onset of desiccation, and the RWC at which transpiration and photosynthesis have dropped to 50% of their initial rates. The correlation is, however, not statistically significant,

73

Fig. 38. Effect of pretreatment on the RWC, at which relative transpiration and photosynthesis are 80, 50 and 20% of their initial value. The same data were used as for fig. 36.

probably because of a few extreme points, at least for *Phalaris*.

In Fig. 41 the maximum rate of photosynthesis, observed before the desiccation, is plotted against the osmotic stress during the measurement, for plants pretreated at 4 different osmotic potentials. *Hordeum* hardly shows any signs of adaptation, due to pretreatment, whereas *Phalaris* does. In *Phalaris* the rate of photosynthesis was about the same at all osmotic stresses up to the one at which the plant was pretreated. But in *Hordeum* the actual situation during the measurement seems to be the main thing that counts. Under non-stress conditions *Phalaris*' net photosynthesis was much higher than that of *Hordeum*.
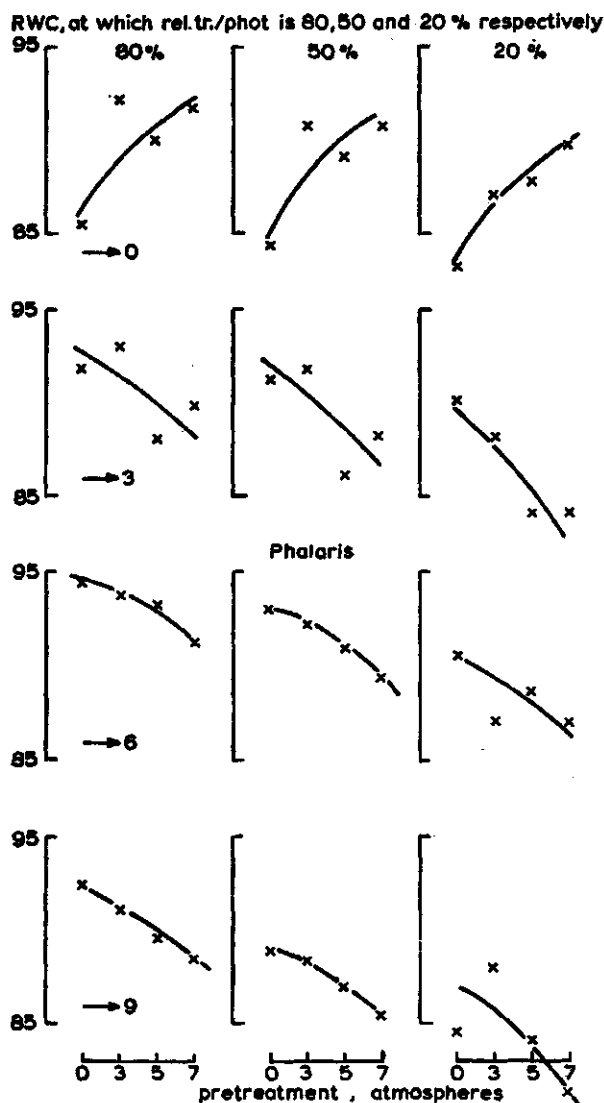
Fig. 39. The same as fig. 38 for Phalaris. Data of fig. 37 were used.

Treatment c The data were treated in the same way as those of Treatment b. The curves of RTP against RWC are not shown themselves, but they are summarized in Fig. 42, where, like in Fig. 38, the RWCs at 80, 50 and 20% RTP are plotted against the pretreatment, for both *Hordeum* and *Phalaris*. The stomatal response of *Phalaris* to RWC shows adaptation, whereas *Hordeum* is only slightly affected by the nitrogen deprivation, as far as its RTP/RWC relationship is concerned. Fig. 43 shows that with increasing tolerance to desiccation, the water content of the leaves decreases. Again, the correlation is not statistically significant.
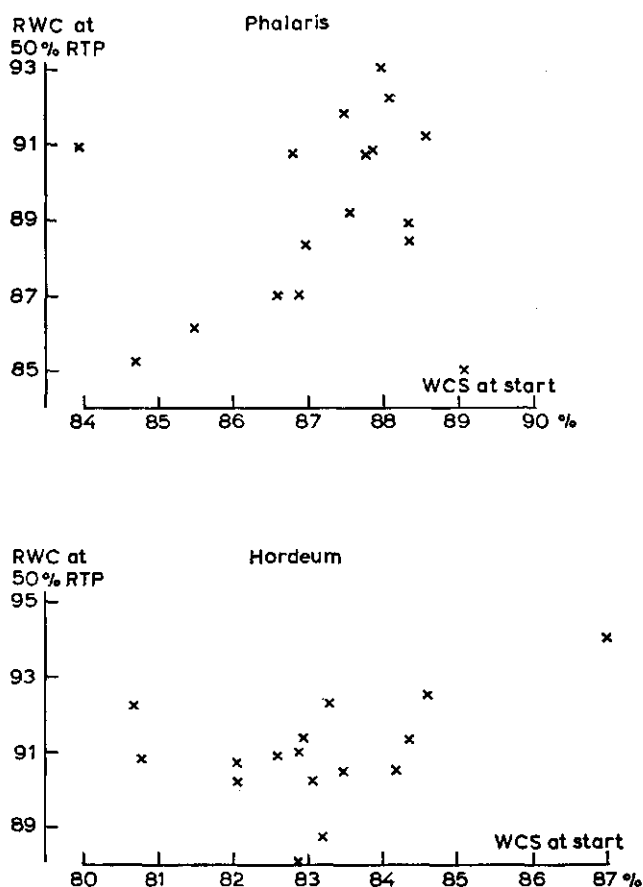
Fig. 40. Relation between the RWC, at which relative transpiration and photosynthesis have been reduced to 50%, and the water content of the shoot at onset of desiccation.

7.3 Discussion

As has already become apparent in the presentation of the results, the relationship between relative transpiration and photosynthesis and relative water content is meaningful. Stomatal behaviour is determined both by light intensity and internal water status of the plant. The relative weight of each of these factors cannot be quantitatively derived from indoor experiments, like the ones reported on. Raschke's work made it clear, that the pretreatment of the plant is important in determining the sensitivity of its stomata to light and water status. From indoor experiments it can be derived whether, and in which way, a plant adapts to certain environmental conditions. The results from the experiments, reported on in this section, show that *Phalaris* and *Hordeum* adapt differently to various stresses.

Phalaris adapts most conspicuously, and its adaptations do not seem to be easily reversible. The stresses that we applied invariably caused the plant to change its
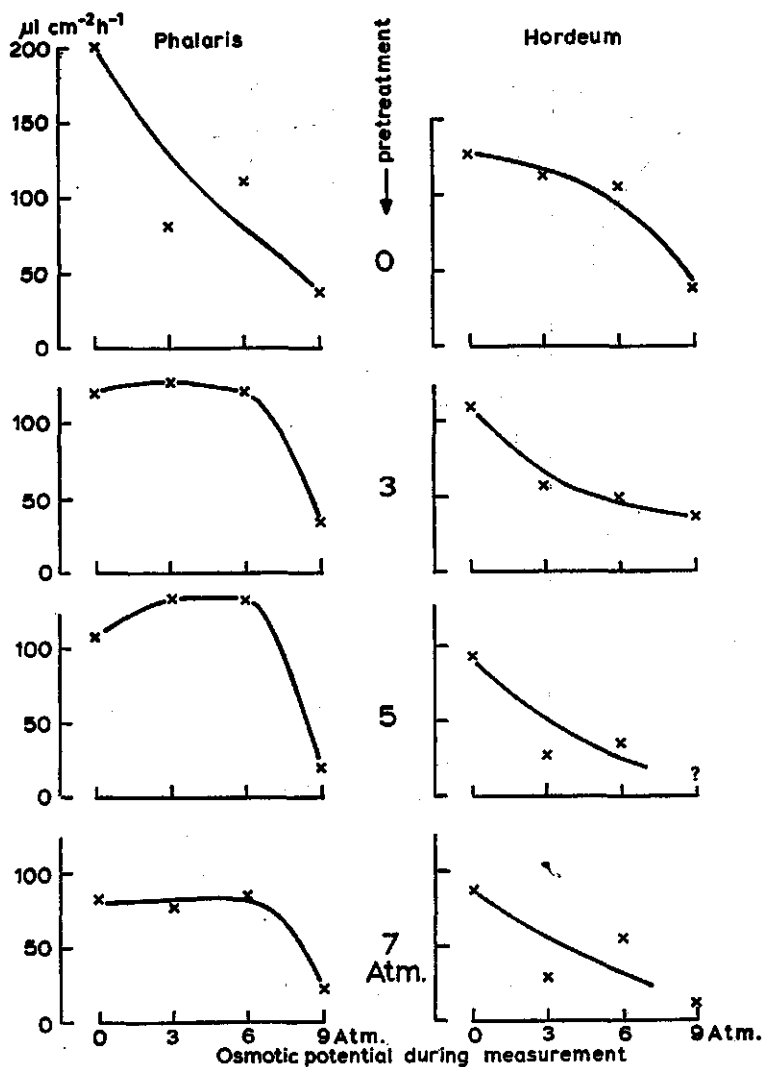
Fig. 41. Effect of osmotic potential during pretreatment on the relation between the maximum rate of photosynthesis and the osmotic potential during measurement.

stomatal behaviour, a change that has been connected in all cases to a change in the water content of the plant. Generally, the stress caused the plant to increase its dry matter content, while stomata responded more slowly to water loss, a behaviour which I have qualified as 'tolerant'. One could visualize that stresses that interfere with growth tend to hamper cell elongation more readily than dry matter production, leading to an increase of the cell's dry matter content. If this means that both the solute content of the vacuole liquid and the density of the cell wall are increased, then one could imagine that not only the plant's osmotic potential is increased, but also its matric potential rises rapidly when water is withdrawn. Thus, a positive turgor pressure can be main-
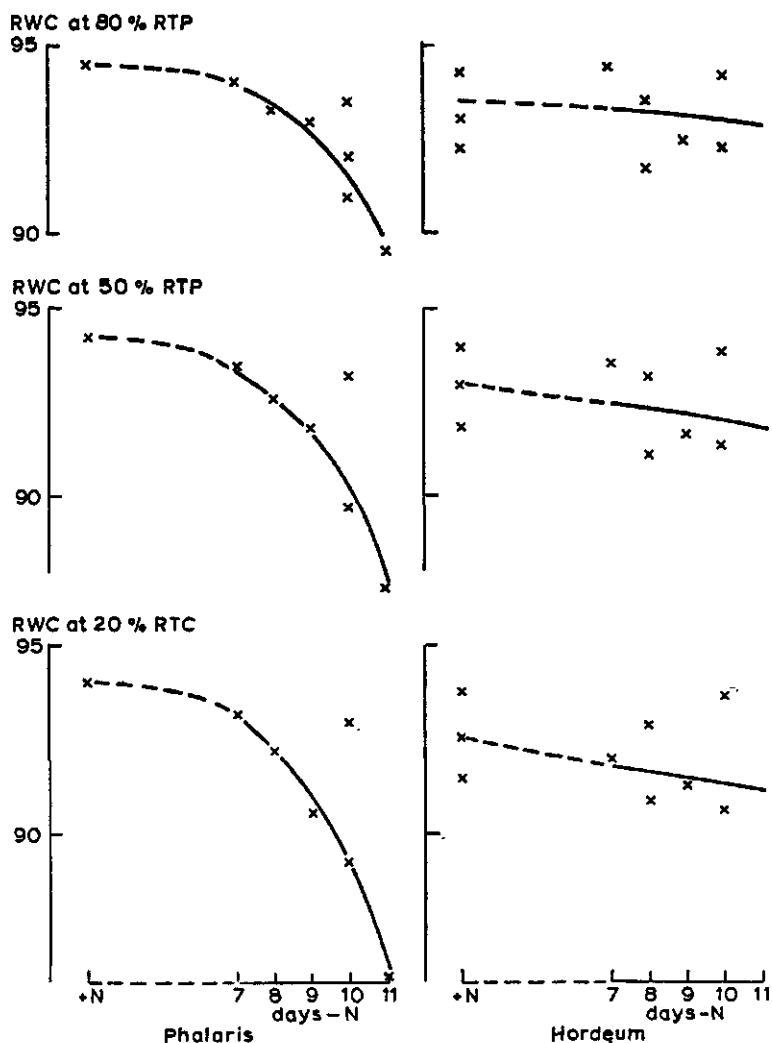
Fig. 42. The influence of nitrogen deprivation on the relation between RWC and RTP.

tained at higher water potentials, so that more water can be lost before the plant loses turgor. It is generally accepted that the leaf turgor is a major determinant of stomatal opening, and not leaf water potential.

Hordeum hardly shows such adaptations, and in all cases studied its stomatal conductivity is much more determined by the actual conditions of the moment than by the previous history. Apparently it keeps its 'milieu interne' as constant as possible, and does so by 'avoiding' to be exposed to stress conditions. This avoidance is realized by a rapid stomatal closure and resulting retardation of the growth.
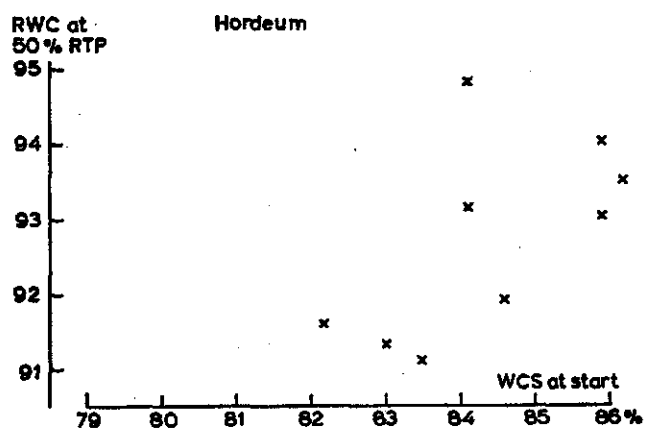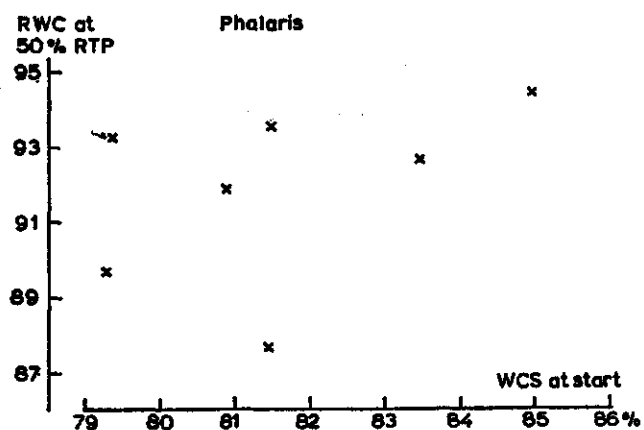
Fig. 43. Like fig. 40. Plants were pretreated by withholding nitrogen.

8 Competition between Hordeum murinum and Phalaris minor

8.1 Introduction into the problem and terminology

For a good understanding of the place of a competition experiment within the framework of questions treated in this report, a better definition is required of the terms used to describe drought resistance of *Hordeum* and *Phalaris*. Most of these terms were originally conceived in studies on xerophytism, so that they have only relative significance for mesomorphic ephemerals.

In the previous chapters, the contrasting properties of *Hordeum* and *Phalaris* were characterized as 'avoiding' and 'tolerant'. Quantitative definitions of these terms do exist (Levitt, 1972), but they require measurement of the plant's and the environment's water potentials in a steady state situation at a stress that kills 50% of the plants. Such definitions look more exact than they are; (it depends for how long the observations are made, whether survivors are measured or the 'dyers', what is 'steady state', in a situation where 50% is dying.). Therefore I did not try to quantitatively determine the degree of tolerance and avoidance in the species studied. A species is called 'tolerant' if it comes to thermodynamic equilibrium with the stress, without suffering injury. An 'avoider' resists the stress by avoiding such equilibrium; it keeps the stress outside. Neither of the species studied will ever reach equilibrium with the environment, and thus they must be classified as avoiders. But, within the group of avoiders, it is possible to distinguish between more and less tolerant avoiders, and this difference is meant when speaking about 'tolerant' and 'avoiding' species. When subjected to water stress, the more tolerant species keeps its stomata open as long as possible, resulting in a considerable drop in its water content. The avoider closes its stomata relatively rapidly, and keeps its 'milieu interne' as constant as possible. In the steady state that is reached, the water potential of the tolerant species is closer to the water potential of the environment, but in the species studied it always remains a steady state, and equilibrium is never approached. The relationship between RWC and stomatal resistance yields a good measure of tolerance and avoidance. Such data as I found (Fig. 31), indicated that under irrigated conditions the differences were small, but under drought conditions the greater tolerance of *Phalaris* was evident. In the PEG experiment (Fig. 33) a good indication for *Hordeum*'s avoiding character was obtained from its rapidly decreasing production and water loss, with increasing stomatal resistance.

Another model that describes plants' reactions to drought is the 'savers-spenders' model. Savers generally have a high water use efficiency, as compared with spenders,

but WUE is not suitable for a definition of saving and spending. Differences in WUE may be caused by physiological or morphological differences, and have nothing to do with saving or spending. The use of these terms will be restricted to adaptations and behaviour that have to do with the course of the water utilization. If a plant transpires water at a potential rate and continuously, then it is called a *spender*. What characterizes the *saver* is that it discontinues the process of water use, or keeps it at a low level. Generally, the saver will show a higher WUE, since it restricts the exchange of CO_2 and H_2O to periods of low stress. On the other hand, respiratory losses of assimilates and water loss by cuticular transpiration during the periods of stomatal closure may sometimes annul the advantage of avoiding unfavourable conditions. In the strict use of the terminology, savers and spenders both belong to the avoiders, as both types of behaviour are different strategies to avoid themodynamic equilibrium between the plant's 'milieu interne' and its environment. In the relative sense of the terms, as used in this report, there is a high degree of coincidence between avoiders and savers on one side, and spenders and tolerant species on the other.

Since savers discontinue their metabolic activities, they can be expected to grow more slowly than spenders. Thus spenders have an advantage when both types are competing. Obviously, a slow-growing saver will be rapidly eliminated, if a neighbouring fast-growing spender uses up all the water. Such considerations led Cohen (1970) to the conclusion that a high efficiency of water utilization is to be expected in nature only on sites where competition is absent. Saving is only advantageous if the saved water is at the saver's disposal. In arid zone plenty of examples can be found to support the general validity of this rule: water saving is found, where plant growth is dispersed, but where plants are competing in a canopy, the water is consumed with a relatively low efficiency. Still, as we have seen, differences in WUE between plant species that are growing together have been found under experimental conditions, and there can be little or no doubt that such differences exist in the field as well. As it is also evident that the root systems of *Phalaris* and *Hordeum* intermingle in the field, as they do in the laboratory, the corollary is inescapable that *Hordeum*'s relatively drought-avoiding properties must sometimes be to the advantage of *Phalaris*. *Hordeum* is saving for the neighbour, nevertheless it has not been ruled out by 'natural selection', but is, on the contrary, a widely occurring, successful species.

Hordeum stands competition with *Phalaris* quite well, in spite of their differences in WUE, from which *Phalaris* profits. Partly, this is because *Hordeum* has a high shoot/root ratio, which means an estimated 20 – 30% more shoot growth with the same quantity of assimilates. (Estimated, because the respiration rate of a root system is not known, but it seems to be much higher than the respiration of an equal weight of shoot). The experiments show that under non-stress conditions *Hordeum* exhibits a higher RGR than *Phalaris*, although the former has a lower potential photosynthesis. Thus, *Hordeum* can afford to have a higher stomatal and mesophyll resistance, and still keep pace with *Phalaris*. However a plant's success in competition is not only determined by its RGR. Temperature tolerance, reproduction capacities, etc. are major factors as well. Differences in morphology may sometimes by much more im-

portant than differences in maximum photosynthesis. The heterogeneity of a natural vegetation mirrors the heterogeneity of the environment, and serves to demonstrate

that under some conditions some species do better, while others thrive under other conditions.

In this chapter, a competition experiment is described, which was carried out in the Migda field, during Spring 1974. It is preceded by a preliminary experiment that was done in Wageningen.

8.2 Theoretical backgrounds

The terminology and models developed by De Wit (1960) will be used. The sum of the requirements of competing species is called the available 'space' and the final yield of each of the competing species is assumed to be proportional to the share each of them conquers in that available space. If we consider 2 competing species, then at any moment in time their respective yields in the mixture can be compared with their yields in monocultures. The quotient of plant frequency in the mixture to that in the monoculture is the relative plant frequency. If the sum of the relative plant frequencies of both species is 1, then the set-up of 2 monocultures and 1 or more mixtures is called a 'replacement series'. In a replacement series the quotient of yield in mixture to yield in monoculture is called 'relative yield' of that species at a certain moment. If 2 species are competing for exactly the same space, the sum of their relative yields (RYT) at each moment will be 1, and they are said to completely exclude each other. If, on the other hand, there is no overlap at all between the spaces that both species try to occupy, then $RYT = 2$. If the desired spaces are partly overlapping, then $1 < RYT < 2$, and the species are said to be partly exclusive. Usually 2 grasses in competition are completely exclusive (Van den Bergh, pers. commun.).

The competitional situation at any moment in time (t_2) can be compared with that at any earlier moment (t_1) by the 'relative crowding coefficient' of the one species over the other, defined as:

$$\frac{RYA(t_2) / RYB(t_2)}{RYA(t_1) / RYB(t_1)}$$

where A and B are the competing species. The relative crowding coefficient is symbolized as k_{AB}.

The statement that 2 species compete for the same space, implies that their interspecific competition is similar to their intraspecific competition. In such a situation much can be learnt about the competitive behaviour from the behaviour of each species in a spacing experiment. If yield is plotted against planting density in a monoculture, a saturation curve is obtained because of intraspecific competition. The result of such a spacing experiment can be described by the formula:

82

$$M_s = \frac{\beta}{\beta + s} \times \Omega \qquad (10)$$

in which M_s = yield at plant density $1/s$ (s is the available area per plant in cm²)

Ω = the extrapolated yield at infinite plant density, and

β = a characteristic constant for the crop, equalling M_{sinf}/Ω

where M_{sinf} is the yield of one plant on an infinitely large field, that is, basically, the yield of a plant whose is undisturbed. Since the stand density curve is completely determined by the parameters β and Ω, only a few field measurements are necessary to establish that curve: the yield-curve of an infinite dense field supplies the saturation level and the yield-curve of an undisturbed plant gives the slope of the stand density curve at each time. Of course, both extremes must be approximations, but that does not change the result very much.

If 2 species are completely exclusive, and their growth curves follow similar courses in time, then the results of the competition experiment can be linked with the results of spacing experiments by 2 simple expressions:

$$M_s = \frac{\beta}{\beta + s} \times \Omega$$

as we have already seen, and

$$k_{AB} = \frac{\beta_B + s_B}{\beta_A + s_A} \qquad (11)$$

The condition of similarity of the growth curves is fulfilled if the relative rates of change of the βs of both species are always the same (Baeumer & De Wit, 1968). If the Ωs are similar, it makes no difference whether the βs or the undisturbed growth curves are compared, since $M_{sinf} = \beta \times \Omega$ (12). Fig. 46 shows that *Hordeum* and *Phalaris* fit this condition reasonably well. The connection between the undisturbed growth and the behaviour in competition is made clear, if one realizes that from Eqn (10) it follows that $\beta + s = \beta \times \Omega/M_s = M_{sinf}/Ms$. Substituting this in Eqn (11) yields:

$$k_{AB} = \frac{M_{sinf}(A)}{M_s(A)} \bigg/ \frac{M_{sinf}(B)}{M_s(B)}$$

When starting densities are equal and the initial relative yield of A and B is 0.5, then

$$k_{AB} = RY_A/RY_B = \frac{Y_A(\text{mixture})}{M_s(A)} \bigg/ \frac{Y_B(\text{mixture})}{M_s(B)}$$

from which it follows that

$$\frac{Y_A}{Y_B} (\text{mixture}) = \frac{M_{sinf}(A)}{M_{sinf}(B)}$$

or, in words, that the relative shares of A and B, planted in a 50/50 mixture, are

proportional to the yields of their undisturbed plants at each moment. The potential rate with which a species can occupy the available space, determines its share in competition.

8.3 Experimental

8.3.1 Preliminary pot trial in the phytotron at IBS, Wageningen, Nov. 1973

8.3.1.1 Materials and methods Mitscherlich pots were filled with 8 kg of a light sandy loam, containing 17% (on dry weight basis) of water. Per pot 15 g of fertilizer, containing 2 g of pure N, was added. The soil was covered by a polystyrene disc with at equal distances 8 holes ($\varnothing$ 8 mm) for plants and 3 holes (17 mm) in which a piece of plastic pipe was fitted for watering.

There were 2 treatments, a wet and a dry treatment. The pots of the wet treatment were watered 3 times a week till 17.5%; the dry treatment, initially 17.5%, was allowed to dry out.

Plants were germinated on perlite and after 2 weeks transferred to the pots. *Hordeum murinum* and *Phalaris minor* were planted in a replacement series, each series consisting of 19 pots in the following manner:

Code	0a, b, c	1	2a, b, c	3	4a, b, c	5	6a, b, c	7	8a, b, c
number of *Hordeum*	0	1	2	3	4	5	6	7	8
number of *Phalaris*	8	7	6	5	4	3	2	1	0
number of *replicates*	3	1	3	1	3	1	3	1	3

sum = 19

No special system was adopted to compose the mixtures, as a general rule the species were alternated as much as possible for maximum interaction. The experiment was continued until the dry treatment had reached the wilting point, being approximately 6%. This was reached after 22 days, under the environmental conditions: phytotron t = 23°C, RH = 80%.

At harvesting, the plants were cut at soil level, and fresh and dry weight determined. Evapotranspirational water loss was followed throughout the experiment by periodic weighing of the pots. A correction was made for free soil surface evaporation, which was measured on blanks, amounting to approximately 10% in the dry treatment. No correction was applied for the fresh weight of the plant material, which was about 2% of the transpiration, for both wet and dry treatment.

After the first week of the experiment a technical error caused the phytotron to run out of control during the weekend. A maximum temperature of 50°C was reached, while the RH dropped to as low as 20%! This situation lasted for about 4 hours, and caused considerable damage to Phalaris, particularly under dry treatment, while Hordeum appeared to be completely unaffected. About 25% of all *Phalaris* plants were replaced, but several leaves of the plants that were left because they looked

undamaged, died afterwards.

8.3.1.2 Results The transpiration, fresh and dry weights, and the TC of both *Hordeum* and *Phalaris* are given in Table 9. Under the wet treatment *Hordeum* is appreciably more economical with water than *Phalaris*, the average TCs of the mono-cultures being 317 and 394, respectively. The TC of the mixtures gradually changes with the proportion of H and P, as is shown in Fig. 44a. This seems to demonstrate that each species grew with its own TC in the mixtures, and competition apparently did not influence the efficiency of water use. In the dry treatment the rate of survival was too low to allow conclusions about the TC.

As the variability of the pots is considerable, the data had to be smoothed before any statement on the competition was possible. This was done by assuming RYT to be 1, so that the total dry weight in each mixture should be a linear interpolation be-tween the dry weights of the monocultures. Fig. 44b demonstrates how this was done, the yields of the monocultures being taken as standard, and their averages connected with a line. All mixtures are then connected to the origin, and where these lines cross the standard line, the extrapolated standard yields of both competitors is read from the graph. These figures are used for the replacement diagrams, Fig. 44c, where dry matter yields are plotted against the relative planting rates. Thus, it has been assumed that any adverse or favourable conditions, determining the growth on a pot, influenced both species in the same way. For the replacement diagrams, it is seen that neither

Table 9. Results of the competition experiment in Wageningen.

Series		Total Transpiration –Evaporation (g)	*Hordeum* shoot (g) fresh	dry	*Phalaris* shoot (g) fresh	dry	Transpiration coefficient shoot
0	a	2702	.	.	51.45	6.364	424.6
	c	2417	.	.	58.35	6.657	363.1
1		1358	6.55	1.003	13.53	2.060	443.4
2	a	2089	16.2	2.168	32.18	3.915	343.4
	c	1339	12.02	1.669	8.11	1.294	451.9
3		2558	12.5	1.622	41.77	4.867	394.2
4	a	2315	25.74	3.664	15.85	2.004	408.4
	c	1590	24.76	3.270	5.9	0.965	375.4
5		1366	32.3	4.265	2.05	0.411	292.1
6	a	2162	39.25	5.269	13.1	1.473	320.7
	b	2366	42.42	5.927	10.27	1.431	321.6
	c	1910	28.32	3.928	13.7	1.655	342.1
7	a	2226	43.82	5.506	3.64	0.487	371.4
	b	1471	27.5	3.808	1.9	0.298	358.3
8	b	2046	45.9	6.405	.	.	319.4
	c	2165	52.06	6.864	.	.	315.4
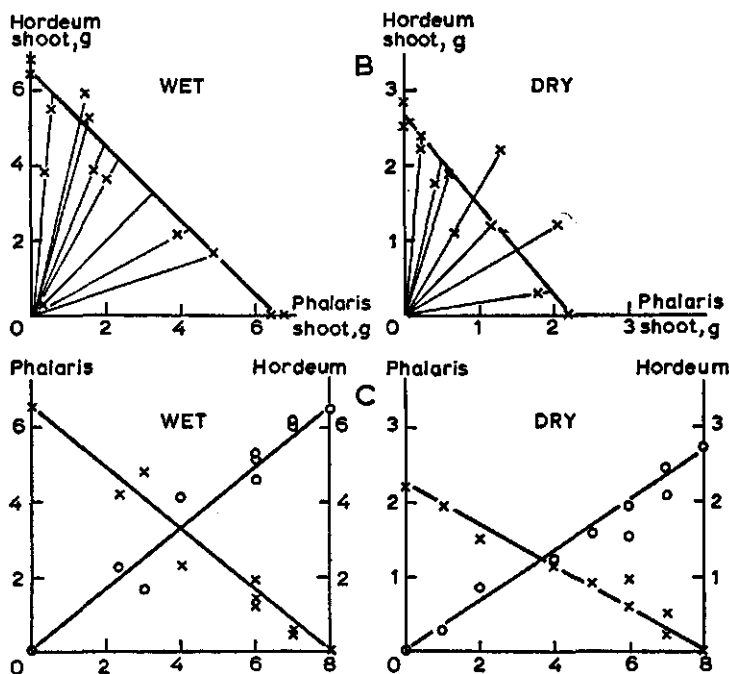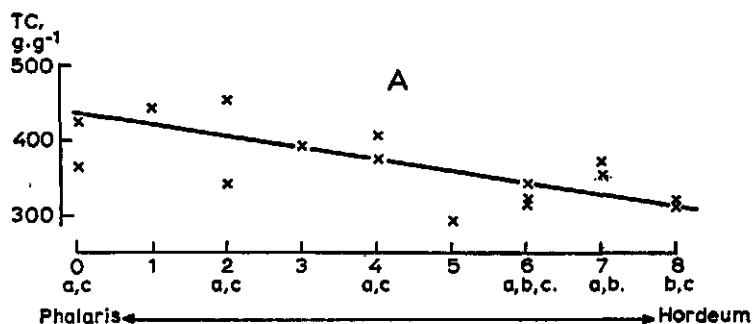
Fig. 44. Results of preliminary indoor competition experiment.
A: Transpiration coefficients of monocultures and mixtures of the replacement series.
B: 'Smoothing' of the data, explanation see text.
C: Replacement diagrams of the smoothed data.

Hordeum nor *Phalaris* gains from the competition, neither in the dry, nor in the wet treatment. Under the prevailing conditions, the species seemed to be a perfect match for each other. Notice, however, that under dry treatment *Phalaris* produced less in an absolute sense, which may of course be due to damage, caused by the extremely severe conditions during the weekend of 4th November.

This experiment shows us 3 things:

86

1. Neither *Hordeum* nor *Phalaris* has a clear advantage over the other, under the conditions described;
2. *Hordeum* has a lower TC than *Phalaris* under wet treatment, and the TC of neither species seems to be influenced by competition;
3. A short period of very severe conditions is most destructive for *Phalaris* and has no visible impact on *Hordeum*.

8.3.2 Competition of Hordeum and Phalaris in the field, Migda, Israel, Spring 1974

8.3.2.1 Materials and methods In January, 20 replacement series were set out in Field 13 of the Migda site, 12 in an NPK fertilized part of this field, and 8 in a part that received only PK and which will be referred to as 'unfertilized'. The replacement series were of the simplest possible type: each consisted of two monocultures and one 50/50 mixture of *Hordeum* and *Phalaris*. I first tried to realize this layout by weeding, so that the experiment could be started with well established and undisturbed plants. This attempt had to be given up because it required too much time and labour, and still the species could not be separated with sufficient accuracy. The following approach proved satisfactory: for each replacement series a 5×2 m part of the field was selected for homogeneity and occurrence of both species. In such a plot 3 squares of 1.30×1.30 m, with a 40 cm strip in between, were prepared by removing the top 5 cm layer with a spade, and planting *Hordeum* and *Phalaris* on rows. The distance between the rows and between the individual plants in each row was 7 cm, so that a density of 200 plants/m^2 was obtained. The plants used were taken from the same field, except that the unfertilized plots had to be planted with plants from the fertilized field, since there were not sufficient of them in the unfertilized part. Young vegetative plants were used with enough tillers, at least 3 for *Phalaris* and 10 – 20 for *Hordeum*, which makes more tillers. To make sure that the mixture would be an almost ideal representation of each monoculture, rows were alternately planted inmixture and monoculture. Immediately after the plots were finished, a sample of 25 plants was removed from each of them, and replaced. This sample gave the initial weight/m^2 on the plots. No open space was left between the plots and the rest of the field, so that the plots became part of the field, and could in March only be recognized by the posts.

In the 10 weeks following preparation, growth in mixture and monocultures was studied by sampling each plot 2 or 3 times. At each harvest a strip of 50 plants was removed on the north or east side of each plot. Every time 3 rows of plants were skipped to avoid border effects. The sample that was taken from the mixture was split up in 25 *Hordeum* and 25 *Phalaris* plants. Often a subsample was taken to determine % leaf (sometimes also % green and % dead) and % stems + flowers (later on separately). Usually, leaf area was determined as well, being estimated as $0.5 \times \Sigma(L \times W)$ for *Hordeum* and $0.67 \times \Sigma(L \times W)$ for *Phalaris*. Length and width were measured with a ruler, the correction constants being calibrated with an automatic leaf area meter (Hayashi Denko). The whole sample was dried 24 hours in a ventilated oven at 80°C and dry weight was determined on a Mettler analytical balance.

8.3.2.2 Results Dry weights were calculated per m² and so was leaf area, if determined. In Table 10 the results are shown. The k values are calculated with respect to the starting situation, 50/50, so that $k_{PH} = RY_P/RY_H$ each time. With only few exceptions the values of RYT, based on weight, do not differ very much from 1, confirming the idea that both grasses can be considered to compete for the same space. It is also seen that the situation is different, when leaf areas are considered. Apart from the fact that leaf area measurements are, of course, subject to much bigger errors than are weight determinations, the available space in this case is not chiefly determined by available light. Once more, it has to be stressed that de Wit's notion of 'space' is by no means identical to the geometrical notion of space. Available 'space' in competition can imply any requisites for plant growth, the most apparent of which were, in my situation, light, water, and nitrogen.

The day of planting markedly influenced the course of competition, but there was

Table 10. Dry weights in g/m² and leaf area in m²/m², all harvests of competition experiment Spring 1974, Migda.

Plot Code	Date	Hordeum mono	Hordeum mixture	Phalaris mixture	Phalaris mono	RY_H	RY_P	RYT	k^1_{HP}	k_H
Fertilized (NPK)										
FW2	14/1	32.8	16.4	12.8	25.5	0.5	0.5	1.0		
	9/3	105.0	58.5	58.2	114.8	0.56	0.51	1.06	1.10	0.91
	2/4	309.2	98.1	137.8	288.7	0.32	0.48	0.79	0.67	1.51
	LA	0.10	0.04	0.37	0.52	0.40	0.71	1.11	0.64	1.56
FW4	8/1	42.4	21.2	9.9	19.8	0.5	0.5	1.0		
	8/3	194.7	94.4	103.4	179.4	0.49	0.58	1.06	0.84	1.19
	LA	1.12	0.48	1.33	2.13	0.43	0.63	1.05	0.68	1.47
	29/3	310.6	132.9	345.5	601.7	0.43	0.57	1.00	0.75	1.34
	LA	0.60	0.22	1.29	3.07	0.37	0.42	0.79	0.88	1.14
FW5	9/1	66.2	33.1	11.6	23.2	0.5	0.5	1.0		
	4/3	162.3	83.5	88.3	155.3	0.52	0.57	1.08	0.91	1.10
	LA	0.83	0.37	0.87	2.20	0.44	0.40	0.84	1.10	0.91
	28/3	426.3	228.2	264.5	428.6	0.54	0.62	1.15	0.87	1.15
	LA	0.44	0.43	0.88	1.63	0.98	0.54	1.53	1.82	0.55
	26/4	532.8	221.6	296.8	548.0	0.41	0.54	0.95	0.77	1.31
FWT2	14/1	31.8	15.9	8.9	17.8	0.5	0.5	1.0		
	9/3	92.9	61.6	53.2	126.7	0.66	0.42	1.08	1.58	0.63
	2/4	297.1	119.4	190.6	320.9	0.40	0.59	1.00	0.68	1.48
FWT3	13/1	47.3	23.6	18.0	36.1	0.5	0.5	1.0		
	12/3	199.2	69.4	147.8	265.3	0.35	0.56	0.91	0.63	1.60
	LA	0.72	0.23	1.52	3.14	0.32	0.48	0.81	0.67	1.50
	28/3	307.6	102.0	253.9	495.4	0.33	0.51	0.85	0.65	1.55
	LA	0.49	0.09	0.79	2.30	0.18	0.34	0.52	0.53	1.89

Continuation Table 10.

Plot Code	Date	Hordeum mono	Hordeum mixture	Phalaris mixture	Phalaris mono	RY_H	RY_P	RYT	$\dot{k}_{HP}$	k^s_{PH}
FWT4	8/1	27.2	13.6	12.1	24.2	0.5	0.5	1.0		
	9/3	109.0	58.3	67.2	93.4	0.54	0.72	1.25	0.74	1.34
	29/3	259.6	166.9	230.0	333.7	0.64	0.69	1.33	0.93	1.07
FWT5	9/1	49.4	24.7	16.6	33.1	0.5	0.5	1.0		
	9/3	338.3	151.2	120.7	216.1	0.45	0.56	1.00	0.80	1.25
	LA	1.12	0.77	1.01	1.97	0.68	0.51	1.19	1.33	0.75
	23/3	560.7	270.4	240.5	408.4	0.48	0.59	1.07	0.82	1.22
	26/4	440.4	222.4	299.6	584.0	0.51	0.52	1.02	0.98	1.02
FWT6	13/1	34.2	17.1	15.2	30.4	0.5	0.5	1.0		
	23/2	71.4	25.0	44.6	100.5	0.35	0.44	0.79	0.73	1.36
	29/3	267.7	139.9	246.5	356.5	0.52	0.69	1.22	0.76	1.32
	LA	0.27	0.14	1.13	0.87	0.53	1.30	1.83	0.41	2.46
FD2	14/1	38.1	19.0	13.0	26.0	0.5	0.5	1.0		
	13/3	162.9	76.1	71.5	136.4	0.47	0.53	0.99	0.89	1.12
	LA	0.59	0.24	0.77	1.33	0.41	0.58	0.99	0.71	1.42
	2/4	374.9	145.2	202.9	371.5	0.39	0.55	0.93	0.71	1.41
	LA	0.86	0.10	0.81	1.68	0.12	0.48	0.60	0.25	4.00
FD4	11/1	41.0	20.5	9.1	18.2	0.5	0.5	1.0		
	10/3	140.0	59.4	70.1	130.0	0.43	0.54	0.96	0.79	1.27
	29/3	338.0	132.0	259.2	372.1	0.39	0.70	1.09	0.56	1.79
	LA	0.55	0.11	0.95	2.15	0.20	0.44	0.64	0.46	2.20
FD5	11/1	40.8	20.4	10.0	20.1	0.5	0.5	1.0		
	9/3	153.2	66.6	79.4	157.0	0.44	0.51	0.94	0.86	1.16
	28/3	330.1	145.8	293.5	588.9	0.44	0.50	0.94	0.89	1.13
	26/4	575.6	188.4	398.4	572.8	0.33	0.70	1.02	0.47	2.14
FD6	13/1	33.8	16.9	9.7	19.4	0.5	0.5	1.0		
	9/3	127.8	46.4	56.1	126.9	0.36	0.44	0.81	0.82	1.22
F1	28/1	–no harvest on day of transplanting–				0.5	0.5	1.0		
	3/3	44.4	18.4	39.3	65.8	0.41	0.60	1.01	0.68	1.46
	LA	0.13	0.05	0.58	0.77	0.39	0.76	1.15	0.51	1.95
	27/3	123.4	33.4	140.7	265.8	0.27	0.53	0.80	0.51	1.95
	LA	0.36	0.05	0.95	1.30	0.14	0.73	0.87	0.19	5.18
	3/4	137.0	45.5	240.1	368.8	0.33	0.65	0.98	0.51	1.95
	LA	0.31	0.05	0.91	1.49	0.15	0.61	0.77	0.25	4.07
	26/4	294.0	43.5	332.5	442.0	0.15	0.75	0.90	0.20	5.00

Plot Code	Date	Hordeum mono	Hordeum mixture	Phalaris mixture	Phalaris mono	RY_H	RY_P	RYT	k^1_{HP}	k^2_{PH}
F2	28/1					0.5	0.5	1.0		
	24/3	116.8	34.5	152.1	263.1	0.30	0.58	0.87	0.51	1.95
	3/4	197.6	56.9	277.0	394.8	0.29	0.70	0.99	0.41	2.44
	26/4	260.0	44.8	373.6	422.8	0.17	0.88	1.06	0.19	5.17
Unfertilized (PK)										
UW2	27/1	15.3	7.6	11.8	23.6	0.5	0.5	1.0		
	14/3	36.9	19.0	24.9	44.8	0.52	0.56	1.07	0.93	1.08
	31/3	86.9	20.1	71.2	99.0	0.23	0.72	0.95	0.32	3.11
UW4	16/1	15.2	7.6	9.3	18.6	0.5	0.5	1.0		
	14/3	35.2	19.6	38.2	49.4	0.56	0.77	1.33	0.72	1.39
	31/3	70.5	20.3	79.6	107.5	0.29	0.74	1.03	0.39	2.57
UW5	27/1	22.8	11.4	10.6	21.2	0.5	0.5	1.0		
	14/3	48.9	31.1	21.5	34.8	0.64	0.62	1.26	1.03	0.97
	31/3	80.8	46.8	43.8	74.4	0.58	0.59	1.17	0.98	1.02
UW6	16/1	27.0	13.5	14.7	29.4	0.5	0.5	1.0		
	14/3	42.3	19.9	39.8	49.9	0.47	0.80	1.27		
	31/3	80.6	23.8	68.2	92.4	0.30	0.74	1.03	0.40	2.50
UD2	25/1	29.2	14.6	6.8	13.6	0.5	0.5	1.0		
	14/3	92.5	34.0	22.7	34.2	0.37	0.66	1.03	0.55	1.81
	31/3	119.5	56.6	41.4	75.1	0.47	0.55	1.03	0.86	1.17
UD4	20/1	27.0	13.5	6.6	13.2	0.5	0.5	1.0		
	14/3	62.9	28.9	15.5	50.3	0.46	0.31	0.77	1.49	0.67
	31/3	131.8	48.6	53.0	104.9	0.37	0.51	0.87	0.73	1.37
UD5	20/1	19.3	9.6	6.7	13.4	0.5	0.5	1.0		
	14/3	64.5	28.3	22.1	47.0	0.44	0.47	0.91	0.93	1.08
	31/3	88.6	62.3	58.1	87.4	0.70	0.67	1.37	1.06	0.95
UD6	17/1	36.1	18.0	11.2	22.5	0.5	0.5	1.0		
	14/3	104.1	38.5	30.5	52.3	0.37	0.58	0.85	0.65	1.55
	31/3	148.5	38.7	70.1	117.1	0.26	0.60	0.86	0.44	2.30

1. $k_{HP} = RY_H/RY_P$
2. $k_{PH} = RY_P/RY_H$
3. LA = Leaf area

no systematic influence of age of the plants used, as is seen from Fig. 4,5 where RYs and RYT are plotted against the date of planting. Major deviations from the ideal situation (RYT = 1) were observed, but no systematic deviations. A comparison of Fig. 45a with Fig. 45b, being the situations at the first (beginning of March) and the last (end of March) green harvest, respectively, shows that the RYT tends to come closer to 1 as the growing season proceeds. Also, it is noteworthy that the original disadvantage of *Phalaris* in some series switches to a benefit in nearly all cases during the month of March, which was the month of peak growth.

Phalaris suppressed *Hordeum* in competition, with the crowding coefficient K_{PH} from planting to the last green harvest averaging 1.36 in the fertilized and 1.88 in the unfertilized field (F1 and F2 were not considered, because of their late planting date).

8.3.3 Stand density in the field, Migda, Israel, Spring 1974

8.3.3.1 Materials and methods The experiment was designed to obtain the 2 characteristic parameters of the stand density curve: Ω and β. An estimate of Ω was obtained by taking periodic harvests from the densest nearly-pure stands of each species, that naturally occurred in the field. They were always of the same magnitude and compared well with the highest observations obtained in the random clipping routines, used in an experiment of Van Keulen (loc. cit.). It was assumed that Ω is virtually the same for everything that is green, being limited only by the irradiated energy and sometimes by shortage of water. Fig. 47a shows the line for Ω that was used for the fertilized field, and the observations that support it. This estimate is certainly arbitrary but cannot possibly be far out. As far as the unfertilized field is concerned, the matter
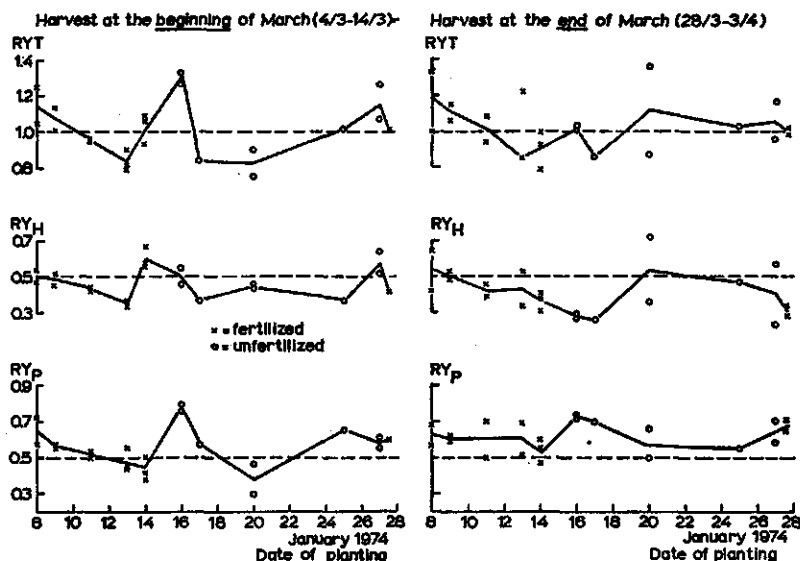

Fig. 45. Relative yields of Hordeum and Phalaris in the first and the last green harvest of the competition experiment, plotted against the day of planting.

was more complicated, as in that field only closed stands of *Trigonella* could be found, which do not suffer shortage of nitrogen as the grasses do. Since there were no pure stands of plants suffering shortage of nitrogen in the unfertilized field, a rather arbitrary estimate for Ω was adopted: $\frac{1}{4} \times \Omega$ of fertilized. This figure is based on the fact that the final yield/m² in unfertilized competition plots was also about $\frac{1}{4}$ of that in fertilized plots. It is realized that now the competition experiment and the stand density experiment are no longer completely independent, as far as the unfertilized treatment is concerned.

β was calculated from Ω and the yield curve of 'undisturbedly' growing plants. Such plants were obtained in 2 ways:

1. 'Strips'. Both in the fertilized and the unfertilized field a strip of a few hundred meters long, and 2 m wide, was prepared by grinding the 10 cm top soil with vegetation. At a distance of about 1 m apart *Hordeum* and *Phalaris* plants were transplanted from the field, and it was assumed that 1 m² per plant satisfied the condition of 'infinite space', especially as consecutive harvest would increase the available space per plant considerably. From time to time weeding was necessary, to keep the strip free from other plants. Every 3 weeks about 30 plants were harvested, in a systematic, rather than a random way. Plants that looked extremely poor were excluded and thrown away.

2. 'Dukatalon'. Plots of 15 × 15 m were selected for homogeneity and one young plant per m² was covered with a plastic cup, after which the plot was sprayed with a mixture of paraquat and diquat (sold in Israel under the trademark DUKATALON), to which some detergent had been added. After the cups had been removed, plants immediately around the protected ones were cut away to prevent the protected plants coming in contact with herbicide. Sometimes this weeding around the protected plants was not done in time or not effectively enough, so that damage due to herbicides was observed. Where such damage was seen, the plants were removed. The other plants now remained as single plants with approximately 1 m² of available space, gradually increasing as harvesting proceeded. Every three weeks 20 plants were taken for harvest. The plots were weeded by hand periodically and very poorly growing plants were discarded. The plants never grew big enough to form an above- ground canopy; in addition rainfall was abundant and ample fertilizer was applied. Therefore it was felt that in the fertilized plots intraspecific competition did not play an important role, and that the plants could be considered to have infinite space available. It cannot, however, be ruled out that in the unfertilized field shortage of nitrogen caused interference of the plants.

8.3.3.2 Results   The growth curves of the average *Hordeum* and *Phalaris* single plant under the conditions described are given in Fig. 46. It is clearly seen that in the fertilized field the Dukatalon plants grew much bigger than the plants in the strips, while for unfertilized plants the reverse holds true. Furthermore in the fertilized plots *Phalaris* took more advantage of not having been transplanted than *Hordeum*. In unfertilized plots, *Hordeum* grew better and had the greatest advantage over *Phalaris*
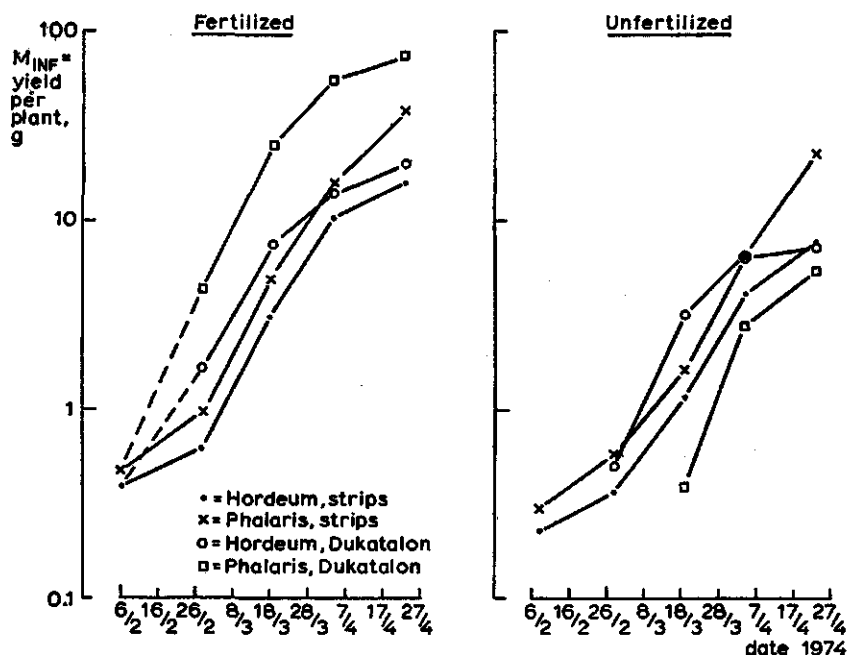
Fig. 46. Growth curves of Hordeum and Phalaris plants.

in the Dukatalon plots. The situation in the fertilized plots is qualitatively under-
standable: transplanting can be expected to have an adverse effect on growth, espec-
ially as the transplanted plant has a low S/R ration and thus has to restore a extensive
root system, from which it has been completely cut off. Still, it is amazing that the
differences observed are so big and that they did not level out during the growing
season. In unfertilized plots, the transplanted plants probably had the advantage of a
high initial nitrogen content, since they were taken from the fertilized field. This ex-
plains why the plants in the strips grew better than the Dukatalon plants. However,
this argument is far from sufficient to quantitatively explain the enormous difference
between *Phalaris'* growth in strips and on Dukatalon plots. Neither does it make
clear, why both species behave so differently. Figs 47 and 48 show the results of the
comparison of the observed yields of the *Hordeum* and *Phalaris* monocultures in the
replacement series with the predicted course of the yields, derived from the un-
disturbed growth in strips. The fit is good in all cases, except for unfertilized *Phalaris*
(Fig. 48b). In the other cases, the predicted line represents the upper level of the ob-
servations rather than their average, but these differences are unimportant, since the
exact values of Ω are not known. The undisturbed growth in the Dukatalon plots
gave quite different predictions, which did not fit the observed values, except for un-
fertilized *Phalaris*, where the fit was better. In Fig. 49 a comparison is made between
the k_{PH} values, as calculated from the competition experiment and from the un-
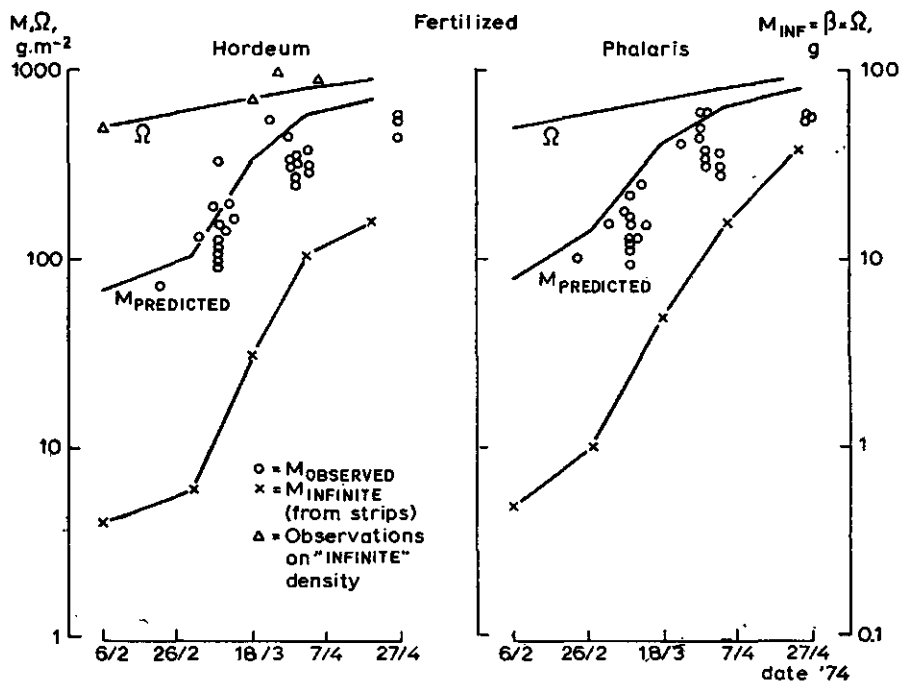disturbed growth, respectively. Again, the prediction from the strips is the best.

93

Fig. 47. Yield of monocultures of Hordeum and Phalaris, as observed in the replacement series, and as predicted from the 'undisturbed' growth and the growth at 'infinite' density.
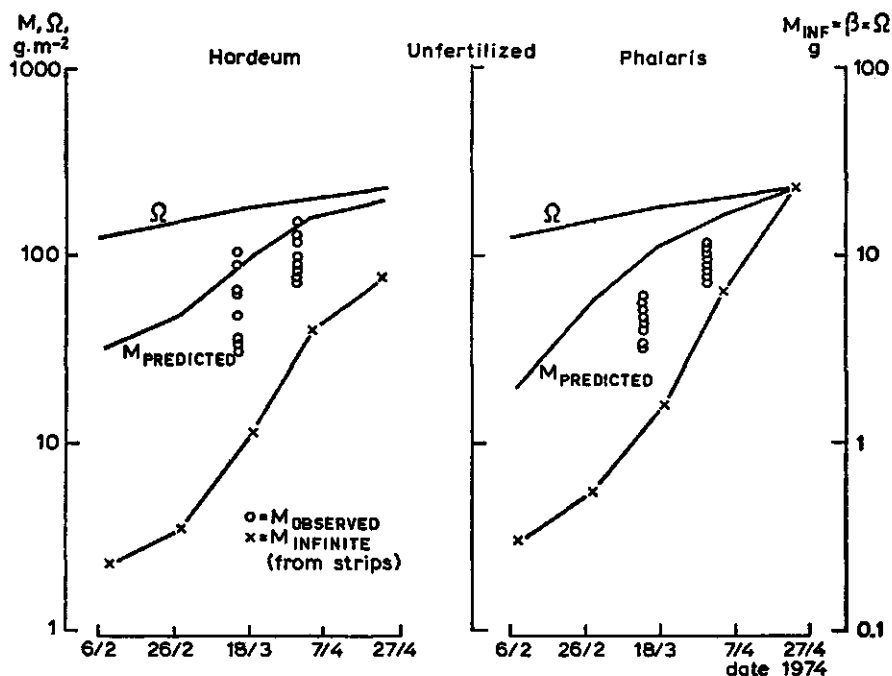

Fig. 48. The same as fig. 47 for the unfertilized plots.

94
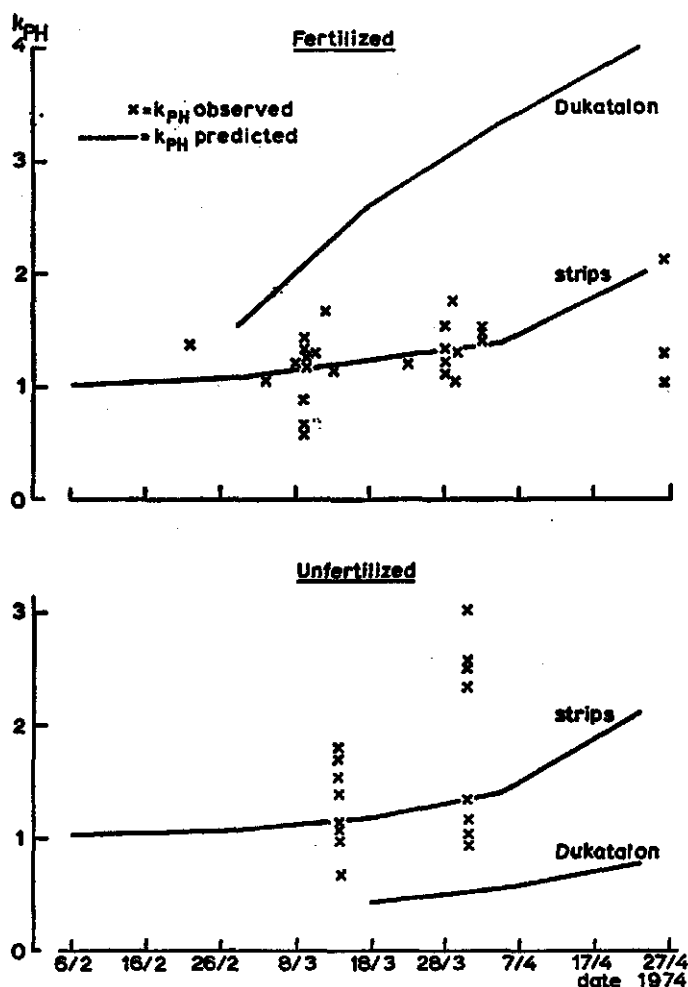
Fig. 49. Crowding coefficients of Phalaris over Hordeum, as calculated from the replacement experiment ('observed'), and as predicted from the undisturbed growth.

8.3.3.3 Discussion   Although the data are considerable scattered, as is to be expected from field trials, they indicate clearly enough that the plants involved in the experiments, 'feel' the presence of their own species as they do that of other species. Thus, the results of a competition experiment could in this case be fairly well described from the results of a spacing experiment in its simplest concept: growth at infinitely high and at infinitely low density. It is conditional that the plants to be compared have had the same treatment, in this case: transplanting. If we extrapolate from these considerations, the corollary is attained that the undisturbed growth of plants that have *not* been transplanted, i.e. in the Dukatalon-plots, should yield a prediction of what happens in the field. Quantitatively, this theory cannot be tested from the available data, but it has been observed that *Phalaris* predominates over *Hordeum* more in

95

the fertilized field, than in the replacement plots. In January, the amount of *Hordeum* in the fertilized field was estimated to exceed the amount of *Phalaris*. This impression was obtained during an attempt to prepare some replacement series by mere weeding. Also for transplanting, it was always easier to find sufficient *Hordeum* plants than *Phalaris*. From Table 11 it is seen that by the end of the growing season *Phalaris'* share in the total dry matter production is considerably higher than *Hordeum's*. However, here *Phalaris* takes over mainly in the last month, as on 9th March *Hordeum* predominates. According to the curve of k_{PH}, as derived from the plants in the Dukatalon plots (Fig. 49), a dramatic take-over of *Phalaris* in the last month of the growing season is not to be expected. From the growth curves (Fig. 46) it can be seen that the last month's growth of both species is not comparable, and does not allow for quantitative extrapolations. *Hordeum* aged earlier than *Phalaris*, especially in the field and the competition plots.

The relative crowding coefficient is not sufficient to describe competition. Under conditions of highly variable rainfall, survival not only depends on the quantity of seeds formed, but equally on how quickly they can be formed. All my observations, both in the field and in the pot trials in Gilat, indicate clearly that *Hordeum* is considerably faster in producing ripe seeds than *Phalaris*, and does so with less water. Thus *Hordeum* should have an appreciable advantage over *Phalaris* in relatively dry years.

Another complicating factor is the stratification of the canopy. The assumption that *Hordeum* and *Phalaris* are competing at the same time for the same space is only an approximation of the reality. In their competition for light, they may be comparable when growing vegetatively, and it is certainly in this stage that complete light interception is reached and the shares are claimed. But when the grasses start producing a stem, a redistribution takes place, the pattern of which varies with age. *Hordeum* is the first to turn reproductive and probably profits from having all its youngest leaves in full sunlight for some time. When *Phalaris* follows in producing a stem, it soon overtakes *Hordeum*, both because it has much more leaf area per unit weight

Table 11. Vegetation composition on 3 days during the 1974 growing season in Migda, Field 13 NPK. The experiments were carried out in NPK1, but on 10/13 no sample was taken from this plot. Figures are % of total weight.

	740310 NPK2	740319 NPK1	NPK2	740414 NPK1	NPK2
Phalaris minor	30.4	22.4	27.6	71.2	68.0
Hordeum murinum	9.9	55.3	10.0	13.3	2.6
Other grasses	5.1	2.6	6.3	0.8	0.7
Erucaria boveana	42.4	14.1	33.7	5.1	18.5
Centaurea iberica	0.1	0.2	.	5.2	0.7
Trigonella arabica	4.4	5.0	17.2	4.2	6.8
Asphodelus	7.7	0.5	5.3	0.3	2.7
Other herbs	.	.	.	0.01	.

(see Table 10), and because its stem is considerably longer. But by that time *Hordeum* has sent up flower heads, that, balancing on slender stalks, form an extensive layer on top of the highest *Phalaris* leaves. Each ear has an estimated area of 7 cm². Observations with an inclined point quadrat (Warren Wilson, 1963) showed that at the end of March, 25 – 50% of the *Hordeum* hits were flowers, while in *Phalaris* the flower-heads got only 5 – 10% of the hits. Thus, *Hordeum* seeds are probably filled directly with assimilates from the spikes, which are in a very favourable position as far as competition for light is concerned. Hence in the fertilized field *Phalaris* produces more total dry matter than *Hordeum*. This does not mean that *Hordeum* will be ruled out by *Phalaris*. Apparently it concentrates its efforts more on survival than on production, and does so successfully. In semi-arid zones, defining competition between annuals in terms of dry matter production is risky, and seed yield ratios would be a better measure for success.

The situation in the unfertilized field is rather unclear. From the curves of undisturbed growth, one learns that in the Dukatalon plots *Hordeum* grows much better than *Phalaris*, but in the strips the situation is reversed. In most replacement series, *Phalaris* wins the competition, but the undisturbed growth predicts that without transplantation *Hordeum* would have more success. Indeed, *Phalaris* is hardly found in the unfertilized fields, where *Hordeum* is abundant. It is hard to vizualize how transplanting could be in favour of *Phalaris*. In fertilized fields this is apparently not so, and there it was argued that *Phalaris* suffers the most serious loss when cut from its root system. It is well possible that the plants, being introduced from the fertilized field, had a high initial content of nitrogen, and that *Phalaris* took most profit out of that. That competition for light cannot play a significant role in these unfertilized plots, follows from the observation that total cover never exceeded some 25% of the soil area.

8.3.4 Competition for Nitrogen

From 7 replacement series in fertilized field and all 8 in the unfertilized, field samples were used to determine total nitrogen and nitrate content, as percentage of dry matter. The samples used were subsamples from the last green harvest (between 29 March and 3 April), split up into green leaves, dead leaves, stems and flowers. The determinations were carried out by the chemical analytical laboratory of the IBS, Wageningen. The protein content, in percentage of dry matter, was calculated as: protein $\% = 6.25 \times$ (total N% $-14/62 \times NO_3^-\%$). As the harvests are expressed in m^{-2}, so is the protein yield.

The results are shown in Table 12. The samples from unfertilized plots were so small that they were put together in two groups of four. The unfertilized, wet group received about 100 mm of extra irrigation, which not only turned out to be unnecessary, because of sufficient rainfall, but was even detrimental for growth – particularly for *Hordeum*.

Considering the fertilized plots first, we notice that the amount of protein per m²

Table 12. Competition, in terms of protein.

	Fertilized									Unfertilized	
	FW4	FWT4	FD4	FWT6	FW2	FWT2	FD2	Average	σ	UW 2, 4,5,6	UD 2, 4,5,6
Phalaris mono											
protein (%)	11.27	9.74	12.27	7.40	6.79	10.65	10.00	9.73	1.99	9.50	10.35
protein (g/m²)	67.7	32.4	45.3	26.3	19.6	43.1	37.3	37.53	15.60	8.9	10.0
Phalaris mixed											
protein (%)	8.49	10.58	10.66	11.98	7.83	12.29	12.21	10.58	1.80	12.40	11.37
protein (g/m²)	29.6	24.3	27.6	29.6	10.7	23.5	24.7	24.28	6.49	8.2	6.3
Hordeum mixed											
protein (%)	9.53	12.38	9.47	10.46	7.42	12.22	10.50	10.28	1.72	9.07	8.69
protein (g/m²)	12.7	20.7	12.5	14.8	7.3	14.6	15.3	13.99	4.01	2.5	4.5
Hordeum mono											
protein (%)	12.51	11.19	13.59	10.86	8.69	12.38	13.49	11.82	1.72	11.34	9.33
protein (g/m²)	39.0	29.1	45.9	29.1	27.0	37.0	50.5	36.80	9.04	9.1	11.4
RY_P								0.64[1]		0.63[a]	
RY_H								0.38[1]		0.39[a]	
RYT								1.03		1.02	

1. k_{PH} = 1.68.
2. k_{PH} = 1.62 (only UD).

98

is equal for monocultures and mixture: 36 – 38 g. This is equivalent to 6 g of nitrogen, which is probably all that was available to the plants. In the beginning of the growing season 10 g N/m² had been applied, and to this another 4 g was added after preparation of the replacement series, to compensate for the N taken away by cleaning the plots. It was not realized, at that time, that considerably more than 4 g N/m² had been removed with the sod. From data of Van Keulen & Seligman (pers. comm.), given in Table 13, it appears, that 8 – 10 g is a better estimate as the standing crop at that time was equivalent to 200 g dry matter/m², and it contained 4 – 5% nitrogen.

From these data, it can be concluded that the plants in the fertilized competition plots had about 4 – 6 g more N available than those in the unfertilized ones. In unfertilized, the available N was approximately 1.5 g/m². It is hereby assumed that in the fertilized field there was no appreciable level of N left from previous years. This assumption has been proven by Van Keulen (1975), who found that in the growing season 1972 – 1973 the vegetation in the fertilized field was suffering from shortage of nitrogen. Thus, it can be concluded that all available N had been taken up by mixtures and monocultures, both in fertilized and unfertilized plots, and, the data of Van Keulen & Seligman show that this was so long before the final yields were reached.

Differences between *Hordeum* and *Phalaris* in yield of the monocultures can now be completely interpreted in terms of different protein % in both species. It is seen from the data that at this harvest *Hordeum* had a higher protein content than *Phalaris*, always in fertilized plots and on average in unfertilized ones. Interestingly, in the mixed cultures *Hordeum* contains less protein than *Phalaris*, and it becomes apparent that, in terms of N uptake, *Phalaris* profits from the competition with *Hordeum*. Obviously, the faster and broader extension of its root system allows *Phalaris* to claim the biggest part of the N in the soil, and to fix it before *Hordeum* can reach it. In the monocultures it does not matter that *Hordeum* develops its root system slowly because it will take up all the N anyway. But in the mixture the rate of root develop-

Table 13. N content of grasses in Migda,
Field 13 NPK % of dry matter.

Date	% N in *Hordeum* and *Phalaris*
720127	4.4
720218	3.6
720407	1.9
730207	4.11
730325	1.65
730418	0.72
731202	6.42
731220	4.92
740104	3.84
740125	3.53
740321	2.03

ment determines at an early stage the results of the competition, at least in a situation where N eventually becomes the limiting factor, as is apparently the case here. In principle competition for nitrogen can be treated with De Wits model, but as we have data from only one harvest, assumptions have to be made. If we assume that *Hordeum* and *Phalaris* start the competition for N in a 50/50 situation, then a coefficient of 1.6 – 1.7 is obtained for the relative crowding coefficient R_{PH}, both for fertilized and unfertilized plots. In the latter case, only the dry group is considered, because in the wet group the RYT differs considerably from 1. In fertilized plots, a fraction of 1.36 of this 1.7 is accounted for by dry matter competition, and 1.25 by protein %. The few harvests at the end of April (see Table 10) indicate that 1.7 is about the value that dry matter competition reaches at the very end of the growing season. Apparently, *Hordeum* and *Phalaris* have by that time both dropped to the same nitrogen level.

The available data are detailed enough to be able to say which parts of the plants suffer most from competition and from shortage of fertilizer in general. Comparing *Hordeum* mono culture with mixed cultures in the fertilized in Table 14, we can see

Table 14. Distribution of protein over different parts of the plant.

	Average of ferti- lized plots	σ	UW 2, 4,5,6	UD 2, 4,5,6
Phalaris mono				
stem	6.61	1.31	6.3	6.7
flower	13.2	1.75	12.5	13.1
green leaves	16.64	5.27	17.3	18.0
dead leaves	7.96	1.14	5.3	8.1
Phalaris mixed				
stem	7.41	1.49	7.4	7.4
flower	14.06	1.03	19.2	13.3
green leaves	18.07	4.14	19.2	20.7
dead leaves	8.46	1.27	6.5	8.7
Hordeum mixed				
stem	8.16	1.81	7.3	6.8
flower	11.59	1.36	9.3	8.8
green leaves	18.99	4,23	19.8	21.0
dead leaves	10.51	1.20	6.7	7.8
Hordeum mono				
stem	9.5	1.59	9.9	7.6
flower	12.34	1.35	10.0	9.2
green leaves	23.06	3.43	26.0	22.3
dead leaves	13.6	2.66	7.4	8.5

that all organs share the set-back, but flower heads relatively least. If fertilized and unfertilized are compared, it is striking that the protein contents of the plants differ so little (which indicates that the plants in fertilized are close to their starvation level), and that the green leaves contain even more N in unfertilized than in fertilized plots. The latter phenomenon is not statistically significant, but may be related to the fact that in the unfertilized field virtually all green leaves were in full sunshine, whereas in the fertilized field the mutual shading was considerable. It could be that the minimum % of protein that a leaf must contain is determined by the light level to which it is exposed.

8.3.5 *Effect of N-deprivation on photosynthesis*

For a better understanding of the impact of low nitrogen level on transpiration and photosynthesis, an experiment was carried out under controlled conditions in Wageningen:

Plants of *Hordeum* and *Phalaris* that had been growing on full nutrient solution, were suddenly transferred to a nutrient solution without nitrogen. From the 7th to the 11th day after stopping N-supply, transpiration and net photosynthesis were measured every day. On the first and the eleventh day a sample of shoots was harvested, and the percentage of nitrogen determined. The results of this experiment are shown in Fig. 50. In both species net photosynthesis decreased considerably, when N was withheld, and this decrease in photosynthesis was accompanied by a drop in protein content. Surprisingly, the transpiration did not decrease very much, so that stomatal resistance was hardly influenced, and the reduction in photosynthesis must be mainly ascribed to a drop in photosynthetic efficiency, which apparently is closely linked to the protein content of the green leaves. The levels of protein, after 11 days of withholding nitrogen, were remarkably similar to the levels found in the monocultures in the field. Apparently, this is about the maintenance level. This level was lower for *Phalaris* than for *Hordeum*, while the level at optimum N supply (the 'saturation-level') was higher for the former. The levels of photosynthesis are related accordingly, being higher for *Phalaris* at optimum N supply and higher for *Hordeum* at N-starvation.

8.4 General discussion of competition

In the previous sections, it has become clear that several factors are involved in competition; undoubtedly more factors could have been traced. Of course, if I had known that nitrogen would become limiting, more would have been supplied. Intensive irrigation would have completely eliminated water as a limiting factor. But then other factors would have become limiting, and it is doubtful whether a situation could be obtained in which only one factor is determining competition and only one relative crowding coefficient is describing the process. A good approach seems to be that of Hall (1974), who measured as many factors as possible (mainly nutrients) and said that
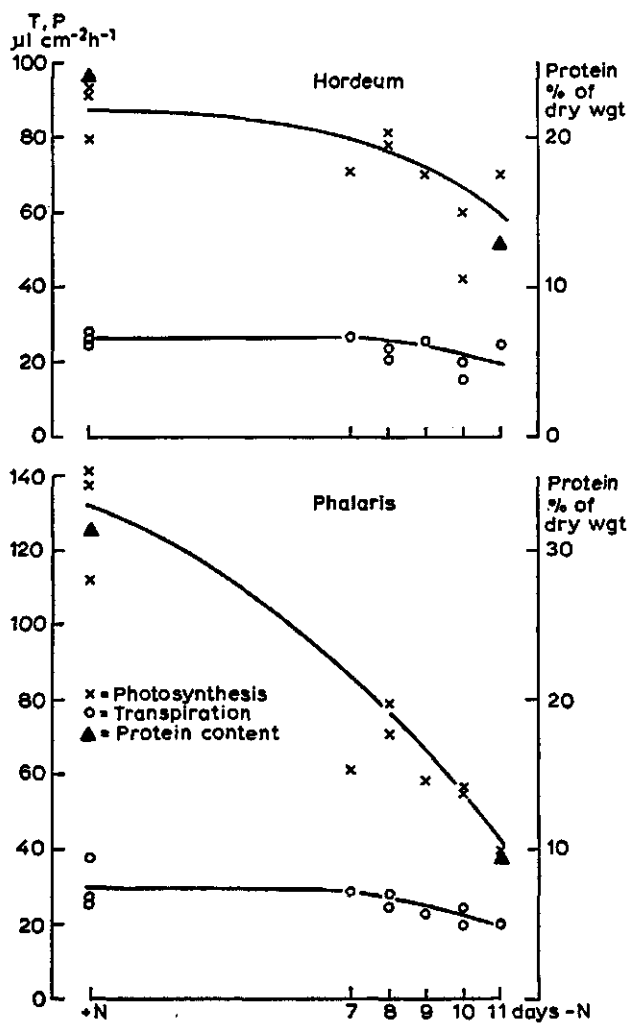
Fig. 50. The effect of nitrogen deprivation on photosynthesis, transpiration, and protein content of Hordeum and Phalaris.

the factor with the highest relative crowding coefficient determines the course of the competition. Such an approach, however, assumes independence, and does not account for the interaction of environmental parameters. I found that shortage of nitrogen has an impact on the transpiration coefficient, so that water may become used up at a lower yield than would have occurred with an ample supply of nitrogen. There are probably many such interactions with different minerals.

It is not feasible, and probably not even possible, to deal with all factors and their interactions, which influence competition. Therefore, a choice has to be made, and at the beginning of experiments the aspects of the crop in which one is interested should be defined. If one is just interested in protein production, then it is sufficient

to look at the competition for nitrogen. It has been shown in Section 8.3.4 that the vegetation stopped taking up nitrogen at a surprisingly early stage: in the beginning of the grand period of growth. In that stage the amount of protein seems to be fixed, and the rest of the available water is used up, while the plants are only forming carbohydrates and cell wall material and thus 'diluting' the proteins. The digestibility of the vegetation for animals is decreasing at that time. If one is interested in total dry matter, then it is not sufficient to consider light and water. It must be kept in mind that the efficiency of the photosynthetic process depends on the availability of nutrients, N and Fe being the best known examples. From a viewpoint of palatability, one could even be interested in the percentage of leaves or seeds that the dry matter will yield. Certainly, then, the influence of environmental factors on the plant's morphology and physiological age must be considered. For example it is well known that in dry farming of a grain crop, one should take care not to give so much nitrogen that the crop consumes all available water in the vegetative stage.

The question, how the relatively economical species *Hordeum* can live together with the water spending *Phalaris*, can now be answered. Indeed, the saver is suppressed by the spender, the latter consuming not only most of the available water, but most of the nitrogen as well. Still, *Hordeum* has two advantages over *Phalaris*, which make it stand the competition quite well: first, it exhibits a rapid early growth, when conditions are favourable, investing little in the production of roots, and covering the soil with many nearly horizontal tillers. This gives it a lead over *Phalaris* in claiming the available geometrical space. Secondly, *Hordeum* completes its phenological cycle much faster than *Phalaris*: its seeds are ripe when *Phalaris* is only starting to flower. When flowering, most of *Hordeum*'s photosynthetic activity takes place in its awns. *Phalaris* reaches much higher productions, but its relatively 'solid' behaviour renders it unable to eliminate a rapid and efficient opportunist, like *Hordeum*'s.

The last question to be answered is, whether behaviour in the mixture can be predicted from stand density experiments with each of the competing species. If several factors play a role in competition, there is little, if any chance that they will all be traced in monoculture experiments. Often, in competition, the limiting character of a factor appears much more dramatically than in monocultures. On the other hand, I think that factors that are known to be limiting at any stage of growth, can be studied in simple situations, and their behaviour predicted for more complicated situations. If the complicated situation has different properties that were not foreseen, then in general the components were not sufficiently studied. In my experiments, it was not foreseen that N would play an important role in competition, because in undisturbed plants N shortage is not liable to take place. But, if the monocultures had been infinitely' dense with respect to nitrogen, this influence would certainly have been traced, and could have been included quantitatively in the predicted competition.

9 Conclusions and general discussion

The questions posed in the general introduction can now be answered. When annuals that coexist in the Migda fields are studied under controlled condition, differences in transpiration coefficient are observed between some species. It is possible to distinguish between 'savers' and 'spenders', the latter showing a higher 'tolerance' than the former. Under appropriate stress conditions, the tolerant species displays a broader range of dry matter contents and of nitrogen contents in the shoot; also the osmotic potential of its cell sap varies more. The more tolerant species thus shows more adaptation as a result of stresses. The less tolerant species 'avoids' such adaptations. I have suggested that in the avoider the metabolic rates vary in approximately the same way as the cell elongation rate, so that cell composition hardly changes with stress. The tolerant species reduces its metabolism less than its (elongation) growth, under stress, causing cell composition to change considerably. This seems to depend mainly on the reaction of the stomatal diffusion resistance to reduced turgor pressure in the mesophyll.

Water stress leads to an improved water use efficiency, both in the saver and the spender, but with more improvement in the saver. I have suggested 3 possible causes for this saving:
1. increased stomatal resistance leads to a drop in the internal CO_2 concentration, so that photosynthesis is reduced less than transpiration;
2. stomatal resistance is highest during the hottest hours of the day, so that the most favourable hours are 'selected' for metabolic gas exchange;
3. decreased mesophyll cell size may cause mesophyll resistance to CO_2 diffusion to be lower.
To this we may add:
4. under stress a mesophyll resistance to water vapour exchange may arise, as has been suggested in the literature (Jarvis & Slatyer, 1970), although the exact character of such a resistance is as yet unknown.

In competition the spender profits from the saver, consuming most of the water and nutrients available. Although the spender has the higher dry matter production, it is unable to eliminate the saver, since the latter exhibits a more rapid development under favourable conditions, and invests little of its dry matter in roots and stems. Hence the saver has a high survival value, at least in a vegetation of animals where the available space is not permanently occupied. In fact, the saver, *Hordeum* is known to be more suited to more arid conditions than the spender, *Phalaris*, and, in addition to that, it can stand grazing and shortage of nitrogen better.

From the production point of view, the fast early development of the saver (which invests little in roots) yields a double advantage: first it produces more with the same amount of water, owing to its lower TC. Secondly, it produces more with the same water, since it covers the soil more rapidly and uses up the water in an earlier stage of the season. As appears from Section 4.4, this advantage can be considerable, seasonal differences in TC being generally bigger than differences between species and treatments.

The validity of these conclusions for the field situation depends on the resemblance of plant response in the phytotron, the pot trial outside, and the field. In some respects there is a close resemblance, so that we may extrapolate for other characteristics.

The following characteristics were found to be similar indoors and in the field:

1. The shoot water content was under all conditions lower for *Hordeum* than for *Phalaris*, although the absolute values differed with different environmental condidions. In the field, much water was lost between picking and weighing, but since *Hordeum* has been observed to close its stomata rather rapidly after picking, the real differences are underestimated by the observations, rather than exaggerated.

2. In all experiments under controlled conditions a striking difference in shoot/root ratio between *Hordeum* and *Phalaris* has been observed. The results of the competition for nitrogen in the field yielded strong evidence that here a similar difference in SRR exists. Both *Hordeum* and *Phalaris* withdraw water from the whole wetted soil profile (unpublished data, collected in the monocultures of the replacement series in the field), so that the difference must be in rooting density, rather than in vertical extension.

3. Both in the field and in Wageningen the mesophyll resistance of *Hordeum* was higher than that of *Phalaris*.

4. The protein contents of both species were similar under field and laboratory conditions.

The Gilat pot trials take an intermediate position between field observations and indoor experiments. Fortunately, a number of characteristics of *Hordeum* and *Phalaris* were observed both in Gilat and in Wageningen: SRR, WCS, and its reaction to drought, the RWC-RS relation that is comparable with the RWC-RTP relation, and last but not least the TC, which in virtually all trials was found to be 25 – 30% higher for *Phalaris* than for *Hordeum*, and to decrease with stress.

A comparison of the Gilat trials with field measurements is difficult. Porometer measurements were done in both cases, but in different periods. The porometer measurements in the Migda field are not very good, but clearly the patterns observed show no resemblance to the ones obtained during the pot trials. The peaky stomatal behaviour observed in the stressed plants of Trial 4 ('collapse-recovery type') is not likely to save water, and we have seen that indeed water is spent under the dry treatment, in this trial. Thus, I expect the stomatal behaviour in Trial 2, where the dry treatment did save water, to be different, in fact more like the patterns found in

Migda in 1973. There is no proof, however, that this is so. The gap between the field situation and the pot situation is too big to allow for extrapolations, because in the field a much deeper soil profile is rooted, and water stress is not likely to develop so rapidly as in a pot of limited size. The only clear resemblance between pot and field is the evident difference in RS between irrigated and unirrigated plants.

One is tempted to extrapolate the differences in TC, as found under controlled conditions, including the Gilat trials, to the field. But better than mere extrapolation would be to try and understand some of the processes and causalities on one level, for which much information is available, and apply this to the other level, for which there is less information. In Section 3.2, I related *Phalaris'* higher TC to its higher WCS. Because of its better water supply (low SRR) *Phalaris* has a higher WCS and can also afford to have its stomata wider open than *Hordeum*, resulting in a higher photosynthesis and transpiration. Since the CO_2 concentration in the stomatal cavity adapts to the better supply, the response of Pn to RS will be less than proportional. The response of T is expected to be nearly proportional, since under the prevailing conditions of high continentality the energy balance of the leaf is dominated by the saturation deficit, and the leaf temperature is insensitive to a change in stomatal resistance. Thus, under conditions of high continentality a lower RS means a higher T/Pn ratio. In this respect, at least, the phytotron conditions represent well the conditions in a field in an arid zone. From direct simultaneous measurements of Pn and T, however, a higher T/Pn ratio was found for *Hordeum*. Calculating RM values according to Gaastra (1959), I found a much higher RM for *Hordeum*. Indeed in the Gilat trials, *Hordeum* was found to have a higher RS than *Phalaris*, but the differences generally found between their rates of photosynthesis were more impressive than these differences in RS. The evidence from these data seems to be contradictory.

We arrive now at the slightly paradoxical conclusion, that *Hordeum* has a lower TC than *Phalaris*, in spite of its apparently higher T/Pn ratio. If this is true, it must mean that *Hordeum's* respiratory losses are considerably less than those of *Phalaris*. Particularly the root respiration deserves attention, since *Hordeum* is known to have less roots, and the respiration of a root system may be considerable. Having no data whatsoever on root respiration, we are left with an unsatisfactory situation. In the field, measurements of the WCS may give a fair indication about the SRR, but no direct relation with the TC can be assumed. Possibly, a correlation can be found between protein content and maximum photosynthesis, so that in comparison of some species in the field the combination of protein content and dry matter content gives an indication of the transpiration coefficient and the shoot/root ratio.

The effects of stress were most conveniently studied in the PEG experiment (Chapter 6). Stomatal resistances were not recorded directly, but the decrease of photosynthesis and transpiration with increased stress indicated that RS increased. *Hordeum* reacted more sharply than *Phalaris* did. Shoot/root ratios did not respond very much to osmotic stress, so that in this experiment the observed saving of water cannot be ascribed to differences in respiratory losses. As in the comparison of different species

106

(cf. Table 3), increased RS coincided with increased dry matter content and decreased TC. But the comparison with different species was faulty because plants growing at higher osmotic stress combined saving of water with a higher tolerance and adaptation (Chapter 7). It is regrettable that with my our data it is not possible to propose identical models describing on one hand the differences between *Phalaris* and *Hordeum*, and the effects of stress on the other.

Some conditions appear to be fatal for *Hordeum*, others for *Phalaris*. As we have seen, *Hordeum* cannot stand a prolonged moderate stress, while Phalaris is very sensitive to a rapidly developing severe stress. It is probable that in the field some years are unfavourable for tolerant species, other years for avoiders, although during most years both types of annuals will coexist. In extreme years, it must be possible to detect at an early stage whether it is going to be a year for savers or spenders. With this knowledge, the expected annual production could be predicted more accurately. Still, most of the conclusions arrived at in the present study are of mere theoretical interest, and have no direct impact on range management.

References

Alberda, Th., 1958. The phytotron of the Institute for Biological and Chemical Research on Field Crops and Herbage at Wageningen. Acta Bot. Neer. 7: 265–277.

Baeumer, K. & C. T. de Wit, 1968. Competitive interference of plant species in monocultures and mixed stands. Neth. J. agric. Sci. 16: 103–122.

Ben-zur, 1966. Osmotic control of the matric soil water potential. Soil Sci. 102: 394–398.

Bravdo, B. A., 1972. Photosynthesis, transpiration, leaf stomatal and mesophyll resistance measurements by the use of a ventilated diffusion porometer. Physiol. Plant. 27: 209–215.

Briggs, L. J. & H. L. Shantz, 1913. The water requirements of plants. I. Investigations in the Great Plains in 1910 and 1911. U.S. Dept. Agr. Bur. Plant Ind., Bull. 284.

Brouwer, R., 1964. Responses of been plants to root temperatures. I. Root temperatures and growth in the vegetative stage. Jaarb. I.B.S. 1964: 11–22.

Brouwer, R. & A. Kleinendorst, 1965. Effect of root excision on growth phenomena in perennual ryegrass. Jaarb. I.B.S. 11–20.

Cohen, D., 1970. The expected efficiency of water utilization in plants under different competition and selection regimes. Isr. J. Bot. 19: 50–54.

Evenari, M. & R. Richter, 1937. Physiological-ecological investigations in the Wilderness of Judaea. J. Linnean Soc. 51: 333–381.

Gaastra, P., 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. Meded. Landbouwhogesch. Wageningen 59: 1–68.

Gale, J. & R. M. Hagan, 1966. Plant antitranspirants. Annu. Rev. Plant Physiol. 17:269-282.

Hall, R. L., 1974. Analysis of the nature of interference between plants of different species. I. Nutrient Relations in a Nandi Setaria and Greenleaf Desmodium association with particular reference to potassium. Aust. J. Agric. Res. 25: 749–756.

Hellmuth, E. O., 1971. Eco-physiological studies on plants in arid and semi-arid regions in Western Australia. III. Comparative studies on photosynthesis, respiration and water relations of 10 arid zone and 2 semi-arid zone plants under winter and late-summer climate conditions. J. Ecol. 59: 225–259.

Jarvis, P. G. & R. O. Slatyer, 1970. The role of the mesophyll cell wall in leaf transpiration. Planta 90: 303–322.

Keulen, H. van, 1975. Simulation of water use and herbage growth in arid regions. Simulation Monographs, Pudoc, Wageningen.

Kuiper, P. J. C., 1961. The effects of environmental factors on the transpiration of leaves, with special reference to stomatal light response. Mede. Landbouwhogesch. Wageningen 61: 1–49.

Lawlor, D. W., 1970: Absorption of Polyethylene Glycols by plants and their effects on plant growth. New Phytol. 69: 501–513.

Lemee, G., 1961. Sur l'économie d'eau des plantes annuelles aux confins septentrionaux du Sahara. Unesco Arid Zone Research Series, Vol. XVI: 127–135.

Lemon, E. R., A. H. Glaser & L. E. Satterwhite, 1957: Some aspects of the relationship of soil, plant and meteorological factors to evapotranspiration. Proc. Soil Sci. Soc. Am. 21:464-468.

Levitt, J., 1972. Responses of plants to environmental stresses. Academic Press, London.

Louwerse, W. & J. L. P. van Oorschot, 1969. An assembly for routine measurements of photosynthe-

sis, respiration and transpiration of intact plants under controlled conditions. Photosynthetica 3: 305–315.

Monteith, J. L., 1973. Principles of environmental physics. Edward Arnold, London.

Morrow, P. A. & R. O. Slatyer, 1971. Leaf temperature effects on measurements of diffusive resistance to water vapor transfer. Plant Physiol. 47: 559–561.

Penman, H. L., 1948. Natural evaporation from open water, bare soil and grass. Proc. R. Soc. A193: 120–145.

Raschke, K., 1976. Stomatal action. Annu. Rev. Pl. Physiol. 27 (in press).

Rijtema, P. E., 1965. An analysis of actual evapotranspiration. Agric. Res. Rep. 659, Wageningen.

Schulze, E. D., O. L. Lange & W. Koch, 1972. Ökophysiologische Untersuchungen an Wild- und Kulturpflanzen der Negev-Wüste. III. Tagesläufe von Netto-photosynthese und Transpiration am Ende der Trockenzeit. Oecologia 9: 317–340.

Slatyer, R. O., 1967. Plant-water relationships. Academic Press, London.

Slavik, B., 1963. Relationship between the osmotic potential of cell sap and the water saturation deficit during the wilting of leaf tissue. Biol. Plant. 5: 258:264.

Stanhill, G., 1961. A comparison of methods calculating potential evapotranspiration from climatic data. Isr. J. agric. Res. 11: 159–171.

Stigter, C. J., J. Birnie & B. Lammers, 1973. Leaf diffusion resistance to water vapour and its direct measurement. II. Design, calibration and pertinent theory of an improved leaf diffusion resistance meter. Meded. Landbouwhogesch. Wageningen, 73–15.

Stigter, C. J. & B. Lammers, 1974. III. Results regarding the improved diffusion porometer in growth rooms and fields of Indian corn (Zea Mays). Meded. Landbouwhogesch. Wageningen, 74–21.

Stocker, O., 1954. Der Wasser- und Assimilationshaushalt südalgerischer Wüstenpflanzen. Ber. Dtsch. bot. Ges. 67: 289–299.

Tadmor, N. H., E. Eyal & R. Benjamin, 1972. Primary and secondary production of arid grassland. In: Ecophysiological Foundation of Ecosystems productivity in arid zone. Int. Symp. USSR, June 7–19, NAUKA, Leningrad.

Vaclavik, J., 1966. The maintaining of constant soil moisture levels, lower than max. capillary capacity, in pot experiments. Biol. Plant. 8: 80–85.

Viets Jr., F. G., 1962. Fertilizers and the efficient use of water. Adv. Agron. 14: 223–264.

Wallen, C. C. & G. Perrin de Brichambaut, 1962. A study of agroclimatology in semi-arid and arid zones of the Near East. FAO/Unesco/WMO interagency project on agroclimatology, Technical Report, Rome.

Warren Wilson, J., 1963. Estimation of foliage denseness and foliage angle by inclined point quadrats. Aust. J. Bot. 11: 95–105.

Wit, C. T. de, 1958. Transpiration and crop yields. Versl. landbouwkd. Onderz. 64 (6), Wageningen.

Wit, C. T. de, 1960. On competition. Versl. Landbouwkd. Onderz. 66 (8), Wageningen.

Wit, C. T. de, 1965. Photosynthesis of leaf canopies. Versl. Landbouwkd. Onderz. 663, Wageningen.