Potato Research 32 (1989) 113–121

Genetic differences in water-use efficiency, stomatal conductance and carbon isotope fractionation in potato

J. VOS [1] and J. GROENWOLD

Centre for Agrobiological Research (CABO), P.O. Box 14, 6700 AA Wageningen, the Netherlands
[1] Department of Field Crops and Grassland Science, Wageningen Agricultural University, Haarweg 333, 6709 RZ Wageningen, the Netherlands

Accepted for publication: 31 August 1988

Additional key words: carbon isotope discrimination, drought, stomatal frequency, stomatal resistance, transpiration

Summary

Potato (*Solanum tuberosum* L.) cultivars were grown in pots and containers under a rain shelter to examine differences in stomatal conductance, water-use efficiency, and carbon isotope fractionation. Conductance was measured on abaxial leaf surfaces with a steady state diffusion porometer. Carbon isotopic analyses were made with an isotope ratio mass spectrometer. Water-use efficiency (WUE) was obtained by dividing total dry matter production by cumulative transpiration.

Significant genetic variation was observed for each trait. WUE was positively related to carbon isotope fractionation ($^{13}\epsilon$) and was inversely related to stomatal conductance. Carbon isotope analysis and porometry show potential as indirect screening methods to identify genetic differences in water-use efficiency.

Introduction

The amount of plant dry matter that is produced per unit water transpired, i.e. water-use efficiency (WUE), depends primarily on plant species and the average water vapour saturation deficit of the air (De Wit, 1958; Tanner & Sinclair, 1983). The variation in WUE within species became a subject of study especially after Farquhar et al. (1982) developed theory on the relations between WUE, the ratio between the intercellular (C_i) and external (C_a) carbon dioxide concentration (C_i/C_a), and carbon isotope fractionation. C_i/C_a depends on both stomatal conductance (g_s) and on photosynthetic activity (P). When P increases relative to g_s, C_i/C_a decreases and vice versa. WUE is affected in a similar manner by the relative magnitudes of g_s and P. Plants discriminate against the natural stable isotope ^{13}C, i.e. the molar isotope abundance ratio ^{13}R (= $^{13}C/^{12}C$) of carbon in plant material is lower than ^{13}R of CO_2 in air. This change in ^{13}R during processes from substrate to product is called fractionation. The theory of Farquhar et al. (1982) predicted an inverse relation between ^{13}R of plant carbon and C_i/C_a and a positive relation between ^{13}R and WUE. In other words: a decrease in C_i/C_a is associated with an increase in WUE and a decrease in ^{13}C fractionation. Subsequent studies with wheat (Farquhar & Richards, 1984) and peanut germplasm (Hubick et al., 1986) showed genetic differences in WUE and corroborated the relation between WUE and carbon isotope fractionation.

Indications of genetic variation in WUE and stomatal behaviour were also found in potato. Bodlaender (1986) showed differences in WUE between cultivars ranging from 5.4 g kg^{-1} to 7.9 g kg^{-1}. Vos & Groenwold (1989) confirmed the difference in WUE for two of the cultivars that were included in Bodlaender's study. In addition they observed systematic differences in stomatal conductance (g_s) between those two cultivars (Bintje and Saturna) (cf Dwelle et al., 1981; Levy, 1983).

In this paper we extend observations on genetic variation in water-use efficiency, stomatal conductance and carbon isotope fractionation in potato and we attempt to interrelate these traits.

Materials and methods

Outline of the experiments

Expt 1 (season 1986) had two objectives: (i) to study the genetic variation in carbon isotope composition in the dry matter. For that purpose we grew plants of 15 cultivars and took samples of haulm dry matter 41 days after planting; (ii) to examine the dependence of stomatal conductance on leaf insertion number for two of those cultivars, namely cvs Up-to-Date and Désirée. The plants were grown in 7 l pots under a rainshelter and were optimally provided with water and minerals.

In 1987 we wanted to study genetic differences in WUE, carbon isotope fractionation and g_s. However, because measurement of WUE was possible for only a limited number of cultivars, two experiments (Expts 2 and 3) were run in parallel. In Expt 2 we examined stomatal conductance of 12 cultivars grown in pots with cultural practices similar to those in Expt 1. Between 42 and 69 days after planting five series of porometer measurements were made.

In Expt 3 WUE and carbon isotope fractionation of six cultivars were measured. The plants were grown under a rain shelter in containers 0.8 m by 0.9 m and a soil depth of 0.4 m (6 plants per container). Planting material, planting date, and ambient conditions during growth were similar to those in Expt 2. The containers were arranged to comply with requirements of the 4×4 balanced lattice analysis. To minimize evaporation from the soil, the containers were covered with a plastic sheet, provided with slits to allow the plants to protrude. Each container was weighed twice weekly and the water lost by transpiration was replenished. Water was distributed through perforated tubes 5 cm below the soil surface. For three cultivars a drought treatment was included: those plants received each time 50 per cent of the amount of water that was given to the controls. The plants were harvested 100 days after planting. The stolons and roots that remained attached to the plants upon lifting with a fork were included in the analysis of dry matter production.

Methodology

Stomatal conductance was measured with a Li-Cor 1600 steady state diffusion porometer, attached to the abaxial side of leaves of comparable insertion level near the top of the plant. (Conductance of the adaxial surfaces was invariably very low.) At each date of measurement usually 20 consecutive series of observations were made; in each series one measurement was made per cultivar. The capacity was 30 to 60 measurements per hour.

WUE is defined by the ratio between total dry matter production and cumulative transpiration and is expressed in g dry matter per kg water used.

The carbon isotope composition was determined at the Center for Isotope Research of the University of Groningen. A few milligrams of dry plant material was combusted in a stream of pure oxygen. Combustion gasses, possibly containing organic volatiles and CO, were further oxidized during passage through a furnace containing CuO pellets at 900 °C. Water was trapped at dry ice temperature. After evacuation the CO_2 sample was led through Cu pellets at 600 °C to remove oxygen and nitrogen oxides and collected in a sample tube. The isotopic analyses were carried out on a VG SIRA9 isotope mass spectrometer with corrections for ^{17}O (Mook & Grootes, 1973). The results were expressed in terms of the standard Pee Dee Belemnite (PDB) as defined by:

$$^{13}\delta = (^{13}R_p/^{13}R_{PDB} - 1) \times 1000 \quad (‰) \tag{1}$$

where ^{13}R is the molar abundance ratio $^{13}C/^{12}C$ of a sample.

In this paper we shall not report $^{13}\delta$ values, but $^{13}\alpha$ values which are derived as follows: having defined ^{13}R, the difference in isotope composition of one substance (e.g. plant material) relative to another substance (e.g. CO_2 in air) is given by the fractionation factor:

$$^{13}\alpha = {}^{13}R_p/^{13}R_c \tag{2}$$

where the subscripts p and c designate plant carbon and atmospheric carbon, respectively. In practice the fractionation factor deviates from unity by a small number only, and so it is more convenient to use the fractionation, defined by:

$$^{13}\epsilon = {}^{13}\alpha - 1 \tag{3}$$

From Eqns 1–3 the following relation between $^{13}\epsilon$ and $^{13}\alpha$ can be derived:

$$^{13}\epsilon = {}^{13}\alpha - 1 = (^{13}\delta_p - {}^{13}\delta_c)/(1 + {}^{13}\delta_c) \tag{4}$$

With Eqn 4 the ^{13}C content of plant material is expressed relative to the ^{13}C content of atmospheric CO_2. With our definitions and symbols we follow the established conventions. Farquhar et al. (Farquhar et al., 1982, Farquhar & Richards, 1984, Hubick et al., 1986) use a measure of the change in ^{13}C content that deviates from the conventions: they also apply Eqns 1–3, but calculate Δ values, referred to by the term 'discrimination', obtained by interchanging $^{13}\delta_p$ and $^{13}\delta_c$ in the numerator and the denominator of Eqn 4. After that modification Eqn 4 gives positive values. In our calculations we set $^{13}\delta_{PDB}$ of atmospheric CO_2 to −8.50‰ (Mook, 1988; pers. comm.).

In Expt 1 complete haulms were used for carbon isotope analysis. After harvest these were chopped, dried and powdered. In Expt 3 two leaves of comparable insertion level were picked from each container, bulked, dried and powdered. As a rule two duplicate samples were analyzed. The error of the spectrometer is less than 0.05‰. The SE was 0.02‰ and 0.03‰ in two tests in which four subsamples of a larger sample were analyzed. Two tests with four replicates (containers) in Expt 3 gave SE's of 0.08 and 0.13‰.

Replicas of leaf surfaces were made in two steps. First an imprint was made with Xantoprene (Bayer Dental, Federal Republic of Germany) from which a transparent imprint was made with nail polish. The latter was mounted under a binocular microscope to make counts of stomatal frequency.

Results

Stomatal conductance

Stomatal conductance differed between cvs Désirée and Up-to-Date. Fig. 1 is a representative example of measurements showing that these differences were found at each leaf position at the top of the plant. Before the start of the measurements the plants were temporarily widely spaced to ensure similar illumination of all the leaves. Therefore, the pattern of change in g_s with leaf insertion number can be explained by the dependency of g_s on leaf age (Vos & Oyarzún, 1987).

In Expt 2 measurements were confined to the recently expanded leaves, i.e. the stage of development of leaf numbers 10 through 12 in Fig. 1. Five series of measurements were made in the period between 42 and 69 days after planting. Significant differences

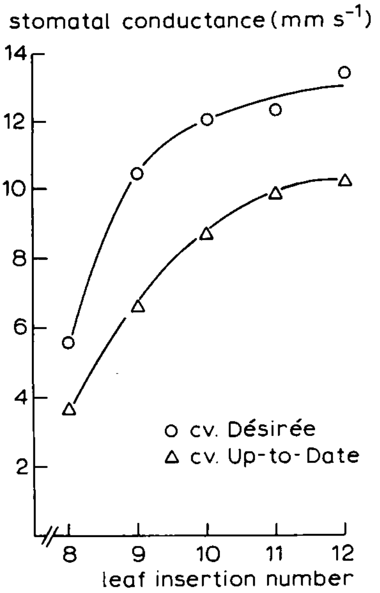

Fig. 1. Stomatal conductance of abaxial leaf surfaces as a function of leaf insertion number (Expt 1; 53 days after planting). The differences between the two cultivars were significant ($P \leq 0.05$) except for leaf 8.

Table 1. Mean stomatal conductance (g_s, mm s^{-1}) of 12 cultivars, averaged over five dates of measurement (Expt 2). The difference between any two means is significant ($P \leq 0.05$) when greater than 1.3 (Tukey's Yardstick).

Cultivar	Stomatal conductance	Cultivar	Stomatal conductance
Cardinal	10.2	Saturna	9.1
Pimpernel	10.2	Hertha	8.8
Désirée	10.1	Arran Banner	8.6
Surprise	9.4	Provita	8.3
Aurora	9.2	Bintje	7.5
Kennebec	9.2	Up-to-Date	7.4

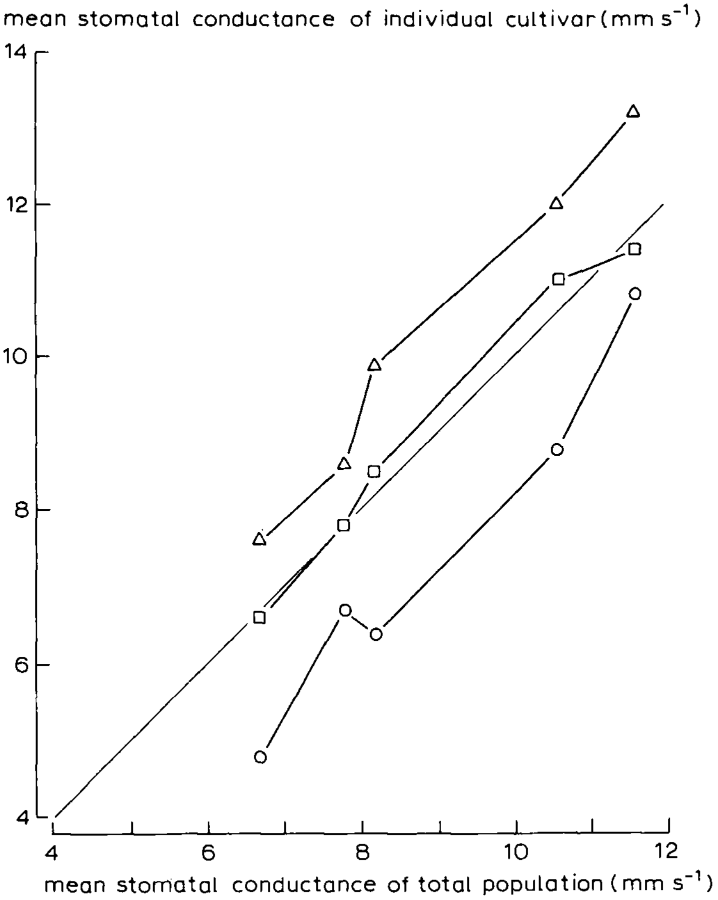

Fig. 2. Mean stomatal conductance of cv. Cardinal (△), cv. Kennebec (□) and cv. Up-to-Date (○) at five dates of measurement plotted against the mean conductance of the total population (12 cvs).

were found between cultivars (Table 1). The difference in conductance between cultivars with a high g_s (e.g. cvs Cardinal, Pimpernel and Désirée) and the cultivars with a comparatively low g_s (e.g. cvs Bintje and Up-to-Date) was about 25 per cent. The overall mean conductance varied significantly between dates of measurement as conductance depends on weather conditions (Vos & Oyarzún, 1987). The ranking order between cultivars was largely maintained, irrespective of weather conditions, i.e. irrespective of the mean g_s of the total population. This is demonstrated in Fig. 2 for three cultivars with the genotype × environment interaction analysis.

Carbon isotope fractionation

The total range in $^{13}\epsilon$ values is large (Table 2; Expt 1). Cv. Up-to-Date had the highest $^{13}\epsilon$ value (−18.04 ‰) and cv. Cardinal the lowest value (−20.23 ‰) (In Farquhar's no-

Table 2. Carbon isotope fractionation values ($^{13}\epsilon$, ‰) measured in haulm dry matter of 15 cultivars (Expt 1).

Cultivar	$^{13}\epsilon$	Cultivar	$^{13}\epsilon$
Cardinal	−20.23	Kennebec	−19.37
Arran Banner	−20.03	Pimpernel	−19.28
Krostar	−19.72	Woudster	−19.27
Provita	−19.52	Bintje	−19.25
Saturna	−19.51	Katahdin	−19.16
Prévalent	−19.45	Prominent	−19.14
Astarte	−19.40	Up-to-Date	−18.04
Désirée	−19.40		

Table 3. Carbon isotope fractionation values ($^{13}\epsilon$; ‰) and water-use efficiencies (WUE; g kg^{-1}) as dependent on cultivar and watering regime in Expt 3.

Cultivar	Optimal water regime		Reduced water regime	
	$^{13}\epsilon$	WUE	$^{13}\epsilon$	WUE
Hertha	−21.25	8.10 a[1]		
Surprise	−21.09	7.94 a		
Saturna	−21.04	8.15 a	−18.40	11.26 e
Bintje	−20.94	8.95 b	−18.45	11.31 e
Kennebec	−20.59	8.68 b	−18.92	10.60 d
Up-to-Date	−20.15	9.49 c		

[1] Values followed by different letters were significantly different ($P \leq 0.05$).

tation the corresponding Δ's would be 18.37‰ and 20.64‰, respectively.) However, the values of 11 out of 15 cultivars clustered in a narrow range between −19.14‰ and −19.52‰.

In Expt 3 $^{13}\epsilon$-values ranged between −21.25‰ (cv. Hertha) and −20.15‰ (cv. Up-to-Date) for controls with optimal water supply (Table 3); reduced watering resulted in markedly higher (less negative) $^{13}\epsilon$-values.

Water-use efficiency

WUE ranged between 7.94 g kg^{-1} and 9.49 g kg^{-1} in the controls with optimal supply of water and differed significantly between cultivars (Table 3). Limited water supply resulted in significantly higher WUE.

Association between traits

The data from Expts 2 and 3 can be interrelated because planting material and the experimental environment were similar. Fig. 3A indicates an inverse relation between g_s and WUE ($r = -0.85$). Fig. 3B shows a strong positive relation between $^{13}\epsilon$ and WUE. The correlation coefficient was 0.85 for optimal watered controls (n=6) and

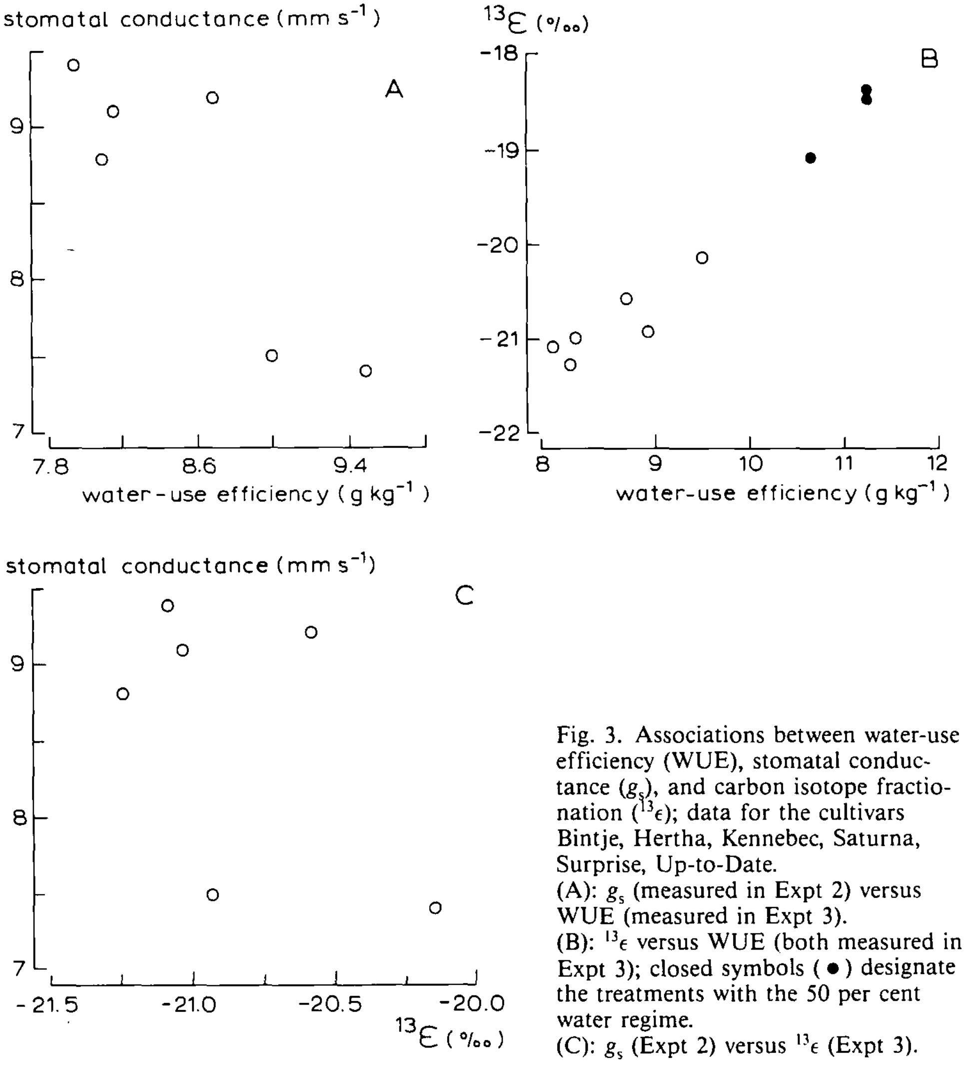

Fig. 3. Associations between water-use efficiency (WUE), stomatal conductance (g_s), and carbon isotope fractionation ($^{13}\epsilon$); data for the cultivars Bintje, Hertha, Kennebec, Saturna, Surprise, Up-to-Date.
(A): g_s (measured in Expt 2) versus WUE (measured in Expt 3).
(B): $^{13}\epsilon$ versus WUE (both measured in Expt 3); closed symbols (●) designate the treatments with the 50 per cent water regime.
(C): g_s (Expt 2) versus $^{13}\epsilon$ (Expt 3).

increased to 0.98 when the points from the treatments with reduced water supply were included. A positive relationship between $^{13}\epsilon$ and WUE means that the fractionation of ^{13}C is least, i.e. ^{13}R is highest, in those cultivars that fix the most carbon per unit amount of water transpired. From an inverse relationship between g_s and WUE and a positive relationship between $^{13}\epsilon$ and WUE, one would expect an inverse relation between g_s and $^{13}\epsilon$. However, our data (Fig. 3C) are too scattered to ascertain the relationship.

The lower stomatal conductance of cv. Up-to-Date in comparison to cv. Up-to-Date was not explained by a lower stomatal frequency on the abaxial leaf surface, rather the reverse was true (Table 4).

Table 4. Stomatal frequency (mm^{-2}) of a basal leaflet and the terminal leaflet of leaf number 11. Measurements made at 60 days after planting in Expt 1.

Cultivar	Stomatal frequency			
	terminal leaflet		basal leaflet	
	adaxial	abaxial	adaxial	abaxial
Désirée	33 a[1]	180 c	51 b	212 d
Up-to-Date	34 a	201 d	38 ab	239 e

[1] Values followed by different letters were significantly different ($P \leq 0.05$).

Discussion

This study consolidates the earlier reports on genetic differences within C_3-species in stomatal conductance (Dwelle et al., 1981; Levy, 1983; Vos & Groenwold, 1988), water-use efficiency (Farquhar & Richards, 1984; Bodlaender, 1986; Hubick et al., 1986) and carbon isotope fractionation (Farquhar & Richards, 1984; Hubick et al., 1986), and confirms the strong relationship between WUE and carbon fractionation, regardless whether variation in WUE is due to genetic factors or drought (Farquhar & Richards, 1984; Hubick et al., 1986).

Carbon fractionation depends on the ratio between the intercellular and external CO_2 concentration, which in turn is determined by the balance between photosynthetic capacity and stomatal conductance (Farquhar et al., 1982). Although substantial variation in g_s was shown (Table 1), $^{13}\epsilon$ was not clearly correlated with g_s (Fig. 3C), leaving room for the hypothesis that variation in photosynthetic characteristics contributes to variation in carbon fractionation between potato cultivars. Data from a larger number of cultivars are needed to ascertain the relation between g_s and $^{13}\epsilon$.

Genetic differences in WUE are now well established and, physiologically, are interesting. High WUE is an advantageous property in conditions where water is a limiting factor. WUE could be enhanced by breeding. Carbon isotope analysis and porometry show potential as indirect screening methods. The former is an almost ideal screening test in a breeding programme: the plant is not sacrificed because only a small amount of plant material is needed and the result of the measurement is independent of conditions during sampling. Further research on the applicability of these methods as screening techniques requires the well articulated interest of plant breeders to breed for improved water-use efficiency.

Acknowledgements

The authors thank Prof. W. G. Mook and Mr H. G. Jansen at the Center for Isotope Research of the University of Groningen for the carbon isotope analyses and particularly for the guidance in the conversion of $^{13}\delta_{PDB}$ values into $^{13}\epsilon$ values, Ir L. J. M. Hendrickx (former student Wageningen Agricultural University) and Mr G. Versteeg for their help, and Mr J. Engelsman for drawing the figures.

References

Bodlaender, K. B. A., 1986. Effects of drought on water use, photosynthesis and transpiration of potatoes. 1. Drought resistance and water use. In: A. G. B. Beekman, K. M. Louwes, L. M. W. Dellaert & A. E. F. Neele (Eds), Potato research of tomorrow. Pudoc, Wageningen, pp. 36–43.

Dwelle, R. B., G. E. Kleinkopf, R. K. Steinhorst, J. J. Pavek & P. J. Hurley, 1981. The influence of physiological processes on tuber yield of potato clones (*Solanum tuberosum* L.): stomatal diffusive resistance, stomatal conductance, gross photosynthetic rate, leaf canopy, tissue nutrient levels, and tuber enzyme activities. *Potato Research* 24: 33–47.

Farquhar, G. D. & R. A. Richards, 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* 11: 539–552.

Farquhar, G. D., M. H. O'Leary & J. A. Berry, 1982. On the relation between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9: 121–137.

Hubick, K. T., G. D. Farquhar & R. Shorter, 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology* 13: 803–816.

Levy, D., 1983. Varietal differences in the response of potatoes to repeated short periods of water stress in hot climates. 1. Turgor maintenance and stomatal behaviour. *Potato Research* 26: 303–313.

Mook, W. G. & P. M. Grootes, 1973. The measuring procedure and corrections for the high-precision mass-spectrometric analysis of isotopic abundance ratios, especially referring to carbon, oxygen and nitrogen. *International Journal of Mass Spectrometry and Ion Physics* 12: 273–298.

Tanner, C. B. & T. R. Sinclair, 1983. Efficient water use in crop production: research or re-search? In: H. M. Taylor, W. R. Jordan & T. S. Sinclair (Eds), Limitations to efficient water use in crop production. ASA/CSSA/SSSA, Madison, pp. 1–27.

Vos, J. & P. J. Oyarzún, 1987. Photosynthesis and stomatal conductance of potato leaves – effects of age, irradiance, and leaf water potential. *Photosynthesis Research* 11: 253–264.

Vos, J. & J. Groenwold, 1989. Characteristics of photosynthesis and conductance of potato canopies and the effects of cultivar and transient drought. *Field Crops Research* 20 (in press).

Wit, C. T. de, 1958. Transpiration and crop yields. Verslagen van Landbouwkundige Onderzoekingen no. 64.4. Institute of Biological and Chemical Research on Field Crops and Herbage, Wageningen, 88 pp.