Beiträge zur Tabakforschung International/*Contributions to Tobacco Research* Volume 21 · No. 4 · December 2004
DOI: 10.2478/cttr-2013-0784

Effect of Water Regime and Nitrogen Fertilisation on Growth Dynamics, Water Status and Yield of Burley Tobacco (*Nicotiana tabacum* L.)*

by

Celestino Ruggiero[1], *Giovanna Angelino*[1], *Salvatore Ascione*[2], *and Antonietta Napolitano*[2]

[1] *Department of Agricultural Engineering and Agronomy, University of Naples Federico II, 80055 Portici (Na), Italy*
[2] *Istituto Sperimentale per il Tabacco, via P. Vitello 108, 84018 Scafati (SA), Italy*

SUMMARY

The results of a two-year research project into burley tobacco are reported and discussed. Three irrigation levels (40, 80 and 120% restitution of evapotranspiration (ET)) were factorially combined with four levels of nitrogen fertilisation (0, 80, 160 and 240 kg ha^{-1}). Leaf area, leaf and stem dry matter and root development were measured. We monitored the water status of the 0–90 cm soil layer, the plants and stomatal resistance. Relations were also studied between leaf turgor pressure and plant growth, between the irrigation regime and plant water status, and between root and shoot development. Finally, water use efficiency (WUE) and quality and quantity of cured leaves yields were evaluated. Nitrogen fertilisation did not affect plant water status, although it promoted plant growth, both in terms of leaf area and leaf and stem dry matter, and induced a yield increase in quantity and quality. Our trial showed little interaction between nitrogen fertilization level and water regime. Under such agronomic condition, the margins for increasing plant growth with nitrogen fertilization are limited, which is why application of nitrogen rates in excess of 160 kg ha^{-1} appear inadvisable. The difference in irrigation volumes led to a different soil water content which affected plant water status, stomatal functioning, plant growth, both in the roots and shoots, yield and quality of the cured leaves. The latter did not vary with the increase in water volume, while yield increased. Water use efficiency increased as the irrigation volume decreased and varied during the cropping cycle, increasing until early bloom, then decreasing. Relations between leaf turgor pressure and plant growth highlighted the different response of plants subjected to water stress compared with non-stressed plants. [Beitr. Tabakforsch. Int. 21 (2004) 223–233]

ZUSAMMENFASSUNG

Die Ergebnisse eines zweijährigen Forschungsprojektes über Burley Tabak werden präsentiert und diskutiert. Die Bewässerungsmenge (40, 80 oder 120% Wiederherstellung der Evapotranspiration (ET)) wurde mit vier verschiedenen Stickstoffdünger-Konzentrationen (8, 80, 160 und 240 kg ha^{-1}) kombiniert. Die Blattfläche, das Trockengewicht von Blatt und Stengel sowie die Wurzelentwicklung wurden gemessen. Der Wasserstatus der 0–90 cm unter der Oberfläche liegenden Bodenschichten, die Pflanzen und der Stomatawiderstand wurden ebenfalls untersucht. Die Beziehungen zwischen dem Turgordruck im Blatt und dem Pflanzenwachstum, zwischen Bewässerungsform und Wasserstatus der Pflanzen sowie zwischen Entwicklung von Wurzel und Spross wurden ebenfalls untersucht. Schließlich wurden die Wassernutzungseffizienz (WUE) sowie Qualität und Quantität des Ertrages der getrockneten Blätter evaluiert. Die Stickstoffdüngung hatte keine Auswirkung auf den Wasserstatus der Pflanze, das Wachstum der Pflanzen wurde jedoch sowohl in Bezug auf die Blattfläche als auch auf die Trockensubstanz von Blatt und Stengel gefördert und führte zur qualitativen und quantitativen Ertragsverbesserung. Unsere Studie zeigte eine geringe Wechselwirkung zwischen der Höhe der Stickstoffdüngung und Bewässerungsform. Unter diesen agronomischen Bedingungen sind die Möglichkeiten begrenzt, das Pflanzenwachstum durch Stickstoffdüngung zu fördern, deshalb erscheint eine Stickstoffdüngung mit mehr als 160 kg ha^{-1} nicht ratsam. Die verschiedenen Bewässerungsmengen führten zu einem unterschiedlichen Wassergehalt im Boden, der den Wasserstatus der Pflanze, die Stomatafunktionen, das Pflanzenwachstum sowohl in der Wurzel als auch im Spross sowie Ertrag und Qualität der getrockneten Blätter beeinflusste. Die Qualität veränderte sich durch vermehrte Bewässerung nicht, der Ertrag nahm

---

*Received: 26th February 2004 – accepted: 1st August 2004

jedoch zu. Die Wassernutzungseffizienz erhöhte sich bei verringerter Bewässerung und veränderte sich im Vegetationszyklus; sie nahm bis zur frühen Blüte zu und danach ab. Der Zusammenhang zwischen dem Turgordruck im Blatt und dem Pflanzenwachstum verdeutlichte die verschiedenen Reaktionen der Pflanzen auf Wasserstress im Vergleich zu Pflanzen, die keinem Stress unterlagen. [Beitr. Tabakforsch. Int. 21 (2004) 223–233]

RESUME

Les résultats d'une étude menée au cours de deux années sur le tabac Burley sont présentés et discutés. Trois niveaux d'irrigation (40, 80 et 120% de récupération de l'évaporation (ET)) ont été combinés avec quatre niveaux de fertilisation azotée (0, 80, 160 et 240 kg ha^{-1}). La surface foliaire, la matière sèche dans les feuilles et les tiges ainsi que le développement des racines ont été examinés. L'état hydrique de la couche de sol de 0 à 90 cm, les plantes et la résistance stomatale ont été contrôlés. Les relations entre la turgescence de la feuille et la croissance de la plante, entre le régime d'irrigation et l'état hydrique de la plante, et entre le développement de la racine et la tige ont également été examinées. De plus, l'efficacité de l'utilisation de l'eau (WUE), la qualité et la quantité ainsi que le rendement en feuilles séchées ont été évalués. La fertilisation azotée n'a pas affecté l'état hydrique de la plante, bien qu'il ait stimulé la croissance de la plante, du point de vue de la surface foliaire et la matière sèche dans les feuilles et les tiges, et a induit une augmentation qualitative et quantitative du rendement. Nos essais ont révélé peu d'interaction entre le taux de fertilisation azotée et le régime d'irrigation. De telles conditions agronomiques n'offrent que des marges très restreintes d'une augmentation de la croissance de la plante par une fertilisation azotée, ainsi l'application des taux d'azote de plus de 160 kg ha^{-1} semble être défavorable. Les différents volumes d'irrigation induisent une teneur hydrique différente du sol, affectant l'état hydrique de la plante, la fonction stomatale, la croissance de la plante dans la racine et la tige, ainsi que le rendement et la qualité des feuilles séchées. La qualité ne varie pas en fonction de l'irrigation, cependant plus d'irrigation induit un rendement accru. L'efficacité de l'utilisation de l'eau augmente avec une réduction du volume d'irrigation et varie au cours du cycle de culture, augmentant jusqu'au début de floraison et diminuant après. Les relations entre la turgescence des feuilles et la croissance de la plante révèlent les réactions différentes des plantes soumises au stress hydrique comparées aux plantes non exposées à une privation d'eau. [Beitr. Tabakforsch. Int. 223–233]

INTRODUCTION

Tobacco is particularly sensitive to agronomic practices, especially irrigation and fertilisation, given their effect on plant growth, yield and the commercial value of the cured product. Irrigation is not always applied rationally, in some cases leading to waste (it is currently believed that in the world about two-thirds of available water is used for agriculture) and often yield quality reductions. However, if used soundly irrigation is an effective means to achieve good levels of quantity and quality of cured leaf. This is partly in response to more recent agricultural trends that call for a sustainable redefinition of current cropping systems. Water is not only the main component of plant tissues but is also the vehicle of mineral absorption and is present in all biochemical, physical and cell wall processes that occur in the plant. It is therefore clear that the more or less ample availability of this substance is able to greatly affect both leaf structural characteristics, and the presence and concentration therein of chemical components which characterise its intrinsic qualities.

Much research has addressed the analysis of growth, especially the basic input which regulates this process with the involvement of many physiological parameters such as water potential and its gradients (5,8,28,29,30,31), the role of cell turgor (21,22,46), cell wall extensibility, characteristics and conductivity (9,14,15,16,38), hormones including abscisic acid (ABA) (10,42) and the relations between transpiration and growth (19). Other research has examined the water factor for production purposes, especially the definition of volumes, techniques and the most appropriate times for irrigation (20,33,34,45).

Tobacco is sensitive to soil fertility conditions, whose variations may affect production and the technological properties of the product (24). It has been observed that absorption of nutritive elements follows the pattern of growth, and hence they must be sufficiently available especially in periods prior to and following anthesis. During this period, absorption of each element is about two third of the total present at the end of the cropping season (3,23), with differences in some varieties and different relations in the various phases of the biological cycle.

Of the mineral nutrients, nitrogen is definitely the element that most affects plant growth and conditions product quality (1,7,13,25,26). Given the strict relation between nitrogen fertilisation and irrigation, research has been carried out to determine the joint action of the two factors on development and crop yield (39), although little research has been devoted to examining the influence of the irrigation level on plant water status. Nutrient availability and – to a lesser extent – the reduction in growth processes induced by agronomic factors also remain relatively unexplored.

In the light of factors that regulate the complex physiological reactions typically found in different plants, and the important function played by water and nutrients in the production and features of the cured product, this research aims to evaluate the effect of different water regimes and nitrogen fertilisation rates upon plant water status, plant growth and burley tobacco cured leaf production..

MATERIAL AND METHODS

Research was conducted in the two years, 2001–2002, at Scafati (southern Italy) in the plot of the Tobacco Experimental Institute on a volcanic soil, with a tobacco crop grown the previous year. Soil physical and chemical properties are reported in Table 1.

The experiment involved a factorial comparison between three water regimes and four nitrogen fertilisation levels. A

Table 1. Soil physical and chemical characteristics

Soil properties	Values
Sand	70.5%
Loam	18.6%
Clay	10.9%
Lime	9.6%
Organic matter	1.8%
Total nitrogen	0.1%
Nitrate	15 ppm
Ammonium	27 ppm
P_2O_5 available (Olsen method)	95 ppm
K_2O exchangeable (ammonium acetate method)	540 ppm
Field capacity	22.9% DW [a]
Wilting point	10.9% DW [a]
Bulk density	1.38 t^{-3}
Electric conductivity	0.22 dSm^{-1}
pH	8

[a] DW = dry weight.

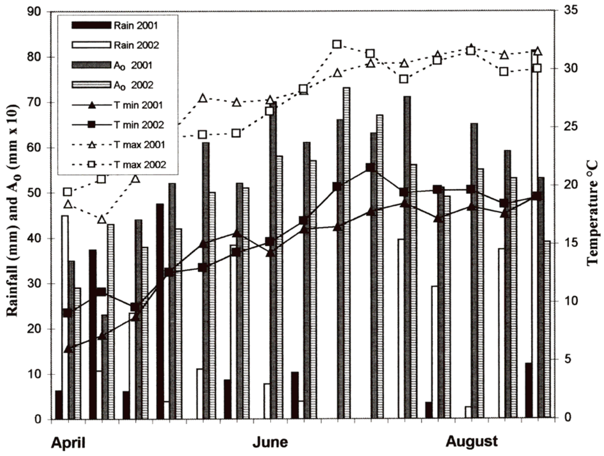

Figure 1. Climatic conditions during the two growing seasons

split-plot experimental design was adopted with three replications, assigning the irrigation treatment to the main plots and nitrogen fertilisation levels to the elementary plot, with a surface area of 73 m^2. Cultivar TN 86, a burley tobacco from the USA, was used for the trial.

Water regimes were applied on a weekly basis, by means of lateral infiltration from furrows, with 40, 80 and 120% restitution of evapotranspiration (ET). This was estimated by class "A" pan evaporation adjusted by applying a pan coefficient of 0.8 and crop coefficients of 0.4 (from transplanting to early growth), 0.7 (from early growth to early bloom) and 1 (from the latter phase to harvest) (12).

Nitrogen was applied after transplanting to the surface at 0, 80, 160 and 240 kg ha^{-1} in the form of ammonium nitrate. Phosphorus and potassium fertilisation were added during soil preparation, distributing to all the treatments 120 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O.

In 2001 transplanting was done on 3 and 4 May, and 12 days later in 2002. In both years rows were spaced 0.90 m apart, with plants arranged every 0.35 m on the row. The subsequent farming technique was that normally used in the zone for tobacco. Parasite control was carried out according to necessity throughout the crop cycle and up to the flowering phase. Half of each elementary plot was used for observations of plant growth through weekly sampling, from the 20th to the 90th day after transplanting (DAT), until topping. Samples were taken at random on sections of rows not disturbed by previous sampling. These plants were used to record leaf number and area, as well as dry weight after oven-drying at 105 ˚C, separately for leaves and stems. Leaf area was measured with a Licor 3100 area meter electronic integrator. Prior to destructive sampling the same plant was used to determine water status (drawing a small leaf disk tissue), stomatal resistance and soil water status. Plant water status was monitored by measuring leaf water potential in its components: total, osmotic and turgor pressure. The measurements were made with a type "B" psychrometer (41), at 29 ˚C, following the isopiestic technique of BOYER and KNIPLING (6). Osmotic potential was measured on the same leaves used for total potential, after freezing at −30 ˚C and with the same technique, while turgor pressure was obtained through the difference between total water potential and osmotic potential, holding the matrix component to be zero (17). Samples were taken at mid-day from leaves that were healthy, young, well-formed and well-exposed to sunlight, and the measurements were done soon after.

Stomatal resistance was monitored on a weekly basis with a ΔT AP34 porometer (Delta-T-Cambridge, UK) at 7:30 h and 12:00 h on leaves of the same type used for water potential. Soil water status was monitored on a weekly basis by gravimetrically measuring moisture at 30 cm intervals to 90 cm of depth, prior to irrigation, taking core samples between the rows at about 20 cm from the plant.

Moreover, development of the root system was measured in length, for the 0–90 cm layer, every 2 weeks from day 23 after transplanting, with the Newmann method (27), and expressed as root density (cm cm^{-3}). The roots were separated from soil by washing and filtration on a 0.2 mm mesh, after treatment with Calgon (85% sodium hexametaphosphate and 15% sodium carbonate, all at 10%), and shaken. Measurements were done on three 30 cm increments to the 90 cm depth.

On the other half of the elementary plot, the plants were topped during bloom at such a height as to retain at least 23–24 usable leaves. After about 18 days the whole plant was harvested and leaves were subjected to air curing, on stalk, in appropriate rooms. At the end of curing, leaf production was determined, converted to standard 19% moisture. The yield was examined separately by trial plot and the leaves were picked in four classes A, B, C and not classified according to physical appearance, such as leaf size, colour, texture and integrity. Data were subjected to analysis of variance (ANOVA) and differences between the means were evaluated with the least significant difference (LSD) test.

RESULTS

Climate pattern

Figure 1 summarizes the pattern of rainfall, maximum and minimum temperature and class A_0 pan evaporation for April to August, for both years. Mean decadal temperatures, both maximum and especially minimum, were higher

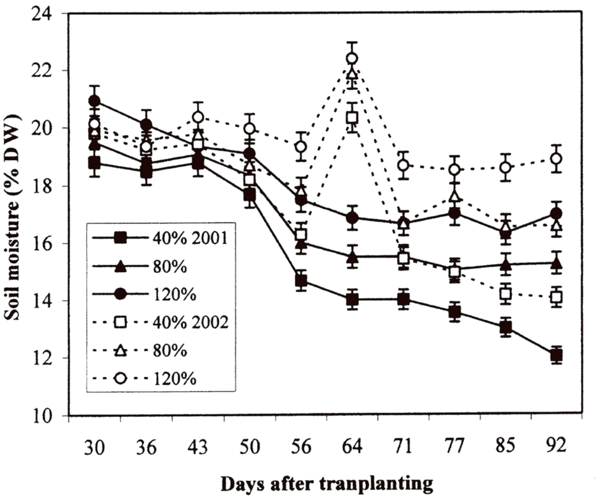

Figure 2. Soil water content during the two growing seasons for the three water regimes. Values are the mean of 36 replications ± standard error or the mean.

Table 2. Values of total (Ψ_t) and osmotic (Ψ_π) leaf water potential for the two years and the three irrigation levels. Values are averages throughout the growing period.

	Ψ_t (MPa)		Ψ_π (MPa)	
Water regime	2001	2002	2001	2002
40% ET	-1.41	-1.35	-1.73	-1.67
80% ET	-1.35	-1.16	-1.84	-1.64
120% ET	-1.33	-1.07	-1.87	-1.63
LSD $P = 0.05$	0.04	0.06	0.05	n.s.[a]

[a] n.s. = not significant.

than the corresponding historical means from 1896–2000, whilst still being suitable for crop production.

Of the two trial years, the first experienced higher minimum and maximum temperatures in May, and lower values, though only for minimum temperatures, in the remaining period of June to August.

Rainfall was much higher in 2002 (334 mm) than in the previous year (132 mm). In 2001 the rain was chiefly concentrated in April and early May, prior to transplanting, while during the crop cycle rainfall was fairly low, also compared with the long-term mean (102 mm) (2), in total 23.2 mm, with two events of a certain significance, though they failed to exceed 10 mm. In 2002 rainfall was also better distributed, with only two dry decades during crop development: the first and the second decades of July.

The same figure shows that evaporation from a class *A* evaporimeter differed in the two years: cumulative values for the whole vegetative period were 624 and 579 mm, respectively for the first and second trial year. Compared to the mean for the period 1980–2000, in 2001 ET demand exceeded 57 mm.

Soil moisture

Figure 2 summarizes the time course of average soil moisture throughout the 0–90 cm profile, and the nitrogen fertiliser treatments, for the three irrigated treatments and the two years. In 2002, given the higher rainfall, moisture levels were higher than in the previous year, and to a greater extent in the second part of the cycle (July to the first ten days of August), with a peak on 18 July coinciding with several rainfall events (33.3 mm) which occurred on the days prior to measurement. In general, during the cycle, soil moisture followed a decreasing trend for all three irrigation treatments, especially in the first half of the cycle and in the treatment with 40% restitution of ET. By contrast, in the other two treatments (80 and 120% ET) in the second phase of the cycle, moisture stayed fairly constant. This pattern was observed first in the 0–30 cm surface layer and later in the deeper layers (data not reported). As regards the level of nitrogen fertilisation, the average across the profile showed no statistically significant differences between the treatments. Only in the treatment irrigated with 40% restitution of ET was slightly higher moisture noted in the non-fertilised treatment (data not reported).

Crop water availability

The irrigation volumes supplied, respectively for the three treatments (40, 80 and 120% ET), amounted to 110, 220 and 330 mm in 2001 and 89, 166 and 243 mm in 2002, while the quantity of water extracted from the soil, calculated by means of the hydrological balance, was respectively 70, 44 and 48 mm in 2001 and 56, 40 and 15 mm in 2002. In all, adding rainfall to the above, water availability was 203, 287 and 401 mm in 2001 and 305, 366 and 418 mm in 2002.

Plant water status

Mean total water potential (Ψ_t) and leaf osmotic potential (Ψ_π), for the three irrigation treatments and for the whole period in question, are reported in Table 2. In 2002 both values were slightly higher than in 2001. In both years in the 40% irrigation treatment total potential was lower than that of the other two treatments. The same differences were also observed with the osmotic potential which, however, in 2002 showed no difference between the treatments. During the cycle Ψ_t and Ψ_π decreased in the 40% treatment, while they appeared to be constant in the other two treatments after an initial reduction (data not reported).

Turgor pressure (Ψ_p) (Figure 3), on average, did not appear different in the two years, although it differed in the three treatments: turgor pressure values tended to decrease with the reduction in water volume supplied. For treatments 80 and 120% ET the differences were not great for either years, but Ψ_p tended to increase during the growing season. In the 40% ET treatment Ψ_p decreased during the season. Fertilisation resulted in no significant differences in plant water status throughout the cycle.

Stomatal resistance

The time course of stomatal resistance (Figure 4), reported as the average of the two measurements made during the day, was affected in the course of the cycle by variations in climatic conditions, especially in 2002, with reductions coinciding with wetter periods. However, on average no significant differences were observed between the two years. Treatment 40% ET showed higher values than treat-

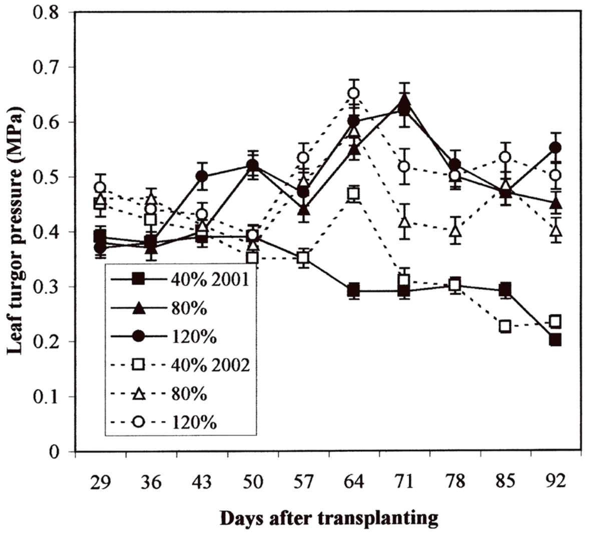

Figure 3. Leaf turgor pressure during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error of the mean.

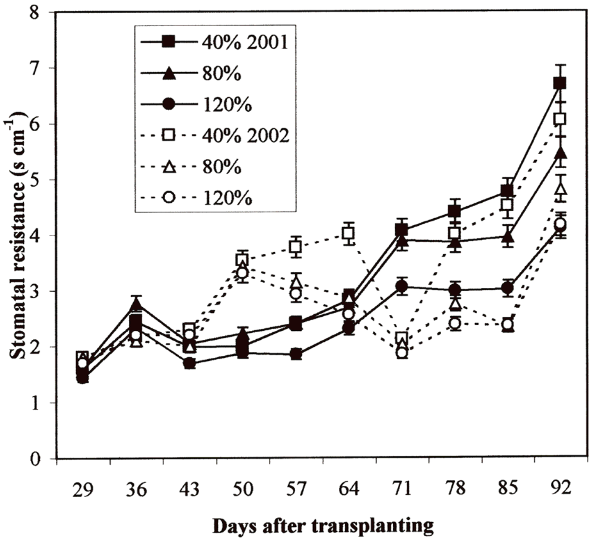

Figure 4. Stomatal resistance during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

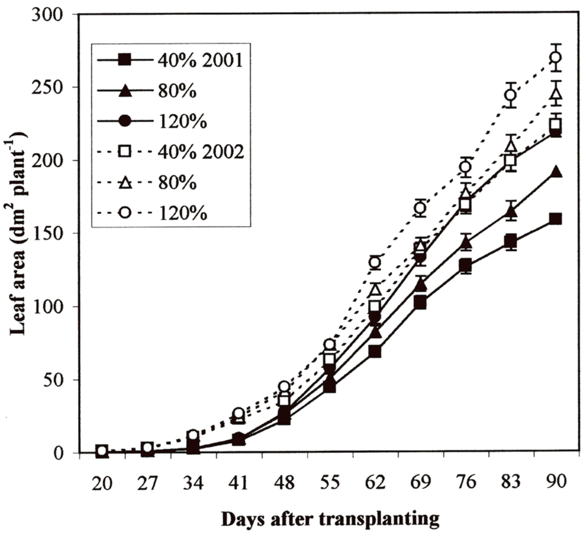

Figure 5a. Leaf area per plant during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

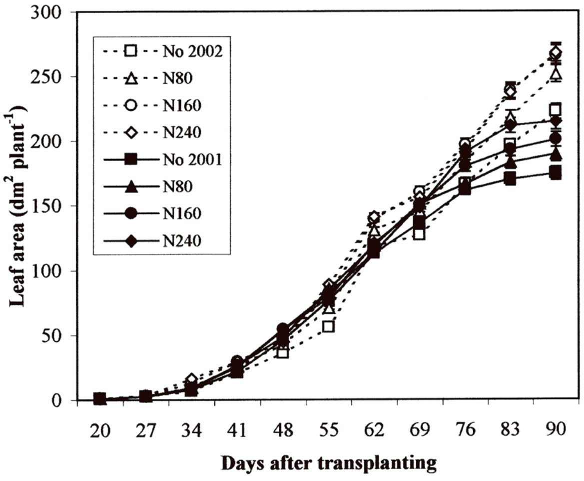

Figure 5b. Leaf area per plant during the two growing seasons for the four nitrogen levels. Values are the mean of 9 replications ± standard error or the mean.

ment 120% and, in 2001 it also exceeded those of treatment 80%, which in 2002 had a stomatal resistance similar to 120%. No statistically significant response were observed for nitrogen fertilisation between the measurements for the four treatments and in the two years, whether during the cycle, or during the day or under the three water regimes.

Leaf area

In Figures 5a and 5b we report the time course in leaf area values per plant, during the growing season, respectively for irrigation and nitrogen fertilisation treatments. In 2002 all the treatments showed more development of leaf surface (+30%) than in the previous year. In both years, as early as 30 DAT, a lower leaf area was noted in the treatment irrigated with less volume (40% ET), compared to the other two. One week later treatment 120% ET had greater leaf area than treatment 80% ET. Such differences increased with time: at the end of the cycle the three treatments had fairly different values between them, in 2001, a 34% increase between 40 and 120% ET and 13% between 80 and 120% ET, while in 2002, increases in leaf area were 20% lower between 40 and 120% ET and 10% lower between 80 and 120%.

There were smaller differences between the nitrogen fertilisation treatments, which also occurred later: at about 60 DAT comparing the non-fertilised vs. fertilised treatments, and 70 days between the other treatments, which had increased leaf area with the increase in nitrogen rate applied. However, such increases at the end of the cycle were lower than those of the irrigated treatments, with an increase of 8.6 and 7% respectively, in 2001 and 13.5 and 1% in 2002. There was little interaction between water regime and nitrogen fertilisation: the nitrogen rate of 240 in treatment 40% ET showed no increase in leaf area over the treatment fertilised with 160 kg of nitrogen.

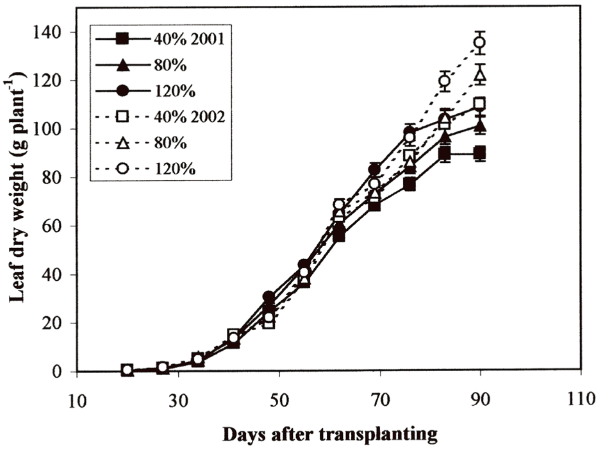

Figure 6a. Leaf dry weight per plant during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

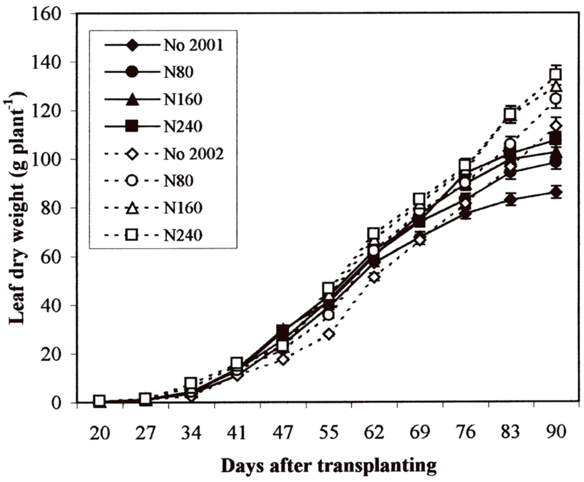

Figure 6b. Leaf dry weight per plant during the two growing seasons for the four nitrogen levels. Values are the mean of 9 replications ± standard error or the mean.

Leaf dry matter

The trend in leaf dry matter (DW) per plant (Figures 6a and 6b) is similar to that of leaf surface, though due to different leaf specific weight (SLW), both in relation to the water regime and to the nitrogen fertilisation rate (Tables 3 and 4), the differences between treatments were not the same. The increase in irrigation volume caused a reduction in weight of the leaf area unit and in proportion to the volume supplied. By contrast, with nitrogen fertilisation an increase was noted in SLW up to 160 kg ha^{-1}. This occurred in both years, with a higher SLW in 2001 than in 2002. Also in this circumstance, interaction between water regime and nitrogen fertilisation was scant, as 240 kg ha^{-1} of nitrogen which in treatment 40% ET had no increase in leaf dry matter compared with the treatment fertilised with 160 kg ha^{-1} of nitrogen.

Number of leaves per plant

The number of leaves per plant in relation to time, 0 to 80 DAT, was fairly linear. In treatments 80 and 120% ET the maximum number of leaves per plant was reached earlier than in treatment 40% ET. Between the start of early growth and full bloom, the non-fertilised treatment had lower leaf numbers. In the two study years the maximum number of leaves per plant was observed with 160 kg ha^{-1} of nitrogen and ranged from 31.7 in 2001 to 37 in 2002. For irrigation regimes, the highest leaf number was reported in the second trial year with treatment 80% ET.

Plant dry matter

Plant dry matter accumulation was different than leaf area. There was accumulation even at harvest for the irrigation treatments and nitrogen rate. Only for treatment 40% ET was a slackening in the accumulation rate noted at the last sampling. Irrigation volume and nitrogen rate effects were evident at all levels and all rates (Figures 7a and 7b). This was due to a difference in stem dry matter accumulation, which was less affected by the factors tested (Tables 3 and

Table 3. Values of some plant parameters for the two years and for the three irrigation levels. Values are averages throughout the growing period.

Water regime	Plant DW (g)	Stem DW (g)	Leaf DW/Stem DW (g g^{-1})	SLW (mg cm^{-2})
		2001		
40% ET	64.0	23.1	3.6	5.2
80% ET	71.9	26.6	3.7	5.0
120% ET	78.0	28.5	3.7	4.8
LSD P = 0.05	2.5	1.5	n.s.[a]	0.2
		2002		
40% ET	67.5	20.4	3.7	4.9
80% ET	72.7	23.7	4.6	4.8
120% ET	77.7	24.7	4.6	4.7
LSD P = 0.05	2.0	1.2	0.3	0.2

[a] n.s. = not significant.

Table 4. Values of some plant parameters for the two years and for the four nitrogen levels. Values are averages throughout the growing period.

N-rate (kg ha^{-1})	Plant DW (g)	Stem DW (g)	Leaf DW/Stem DW (g g^{-1})	Root density (cm cm^{-3})	Leaf area/Root density (dm^2/cm cm^{-3})	SLW (mg cm^{-2})
			2001			
0	65.9	25.9	3.4	2.3	35.9	4.8
80	70.8	25.5	3.5	2.4	37.9	4.9
160	73.2	25.9	3.9	2.4	38.5	5.0
240	75.5	27.0	4.0	2.4	39.2	5.0
LSD $P = 0.05$	2.1	1.3	0.3	n.s.	3.0	0.2
			2002			
0	63.3	20.5	3.9	2.8	41.2	4.7
80	71.3	22.5	4.1	2.7	45.9	4.8
160	77.1	24.3	4.4	3.0	48.2	4.9
240	78.7	24.5	4.8	2.7	48.8	4.9
LSD $P = 0.05$	2.6	1.6	0.4	0.2	4.0	0.2

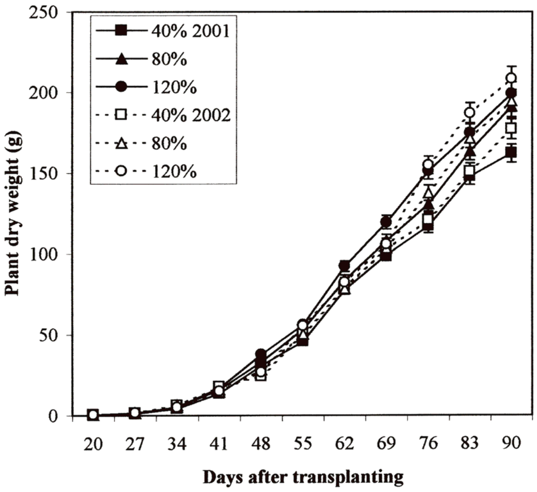

Figure 7a. Plant dry matter per plant during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

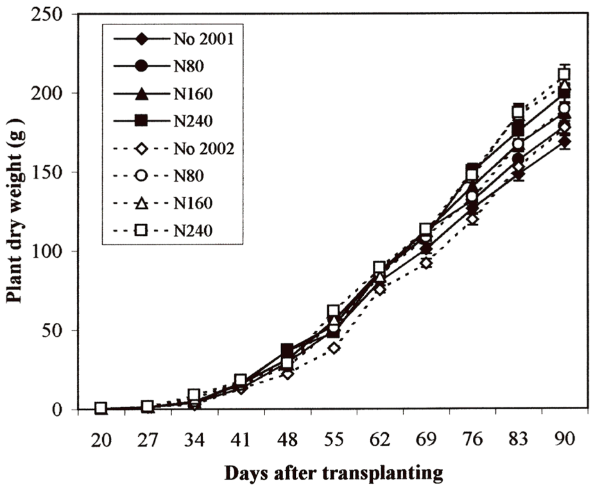

Figure 7b. Plant dry matter per plant during the two growing seasons for the four nitrogen levels. Values are the mean of 9 replications ± standard error or the mean.

4). The ratio between leaf and stem dry matter was higher in 2002 and increased both with irrigation volume and with the rate of nitrogen fertilisation.

Interactions between nitrogen fertilisation and irrigation volume were minimal and were similar to those observed for leaf area and leaf dry matter.

Development of the root system

Development of the root system (Figure 8), for the three irrigation treatments and in the 0–90 cm soil layer, reached a maximum at about 57 DAT, prior to early bloom, for the 40% ET treatment, and 15 days later in treatments 80 and 120% ET. Subsequently, it remained relatively constant. The greatest root development was observed with the least irrigated treatment (40% ET), and the least development with the greatest water volume (120% ET). These differences appeared at about 40 DAT in the 0–30 cm layer, and later in the deeper layer (30–60 cm), where the differences between treatments were greater and more development was achieved by the lowest irrigation volume. In the 60–90 cm layer, only in mid July was the presence of roots found to be appreciable, and to a greater extent in the least irrigated treatment (data not reported). No differences in root development was measured for nitrogen fertilization treatments (Table 4).

Relation between shoots and roots

The ratio of leaf area to root density for the three water regimes is reported in Figure 9. Root development was relatively greater until 43 DAT, but after 43 DAT leaf area increase was relatively greater than root density development. This ratio was greater in 2002 than in 2001, mostly from a higher leaf area than from a lower root density. There is an increased leaf area/root density ratio with increase in the irrigation volume supplied, due both to less root development and to a leaf area increase. Increased nitrogen rates increased the ratio (Table 4), but only because of increased leaf area.

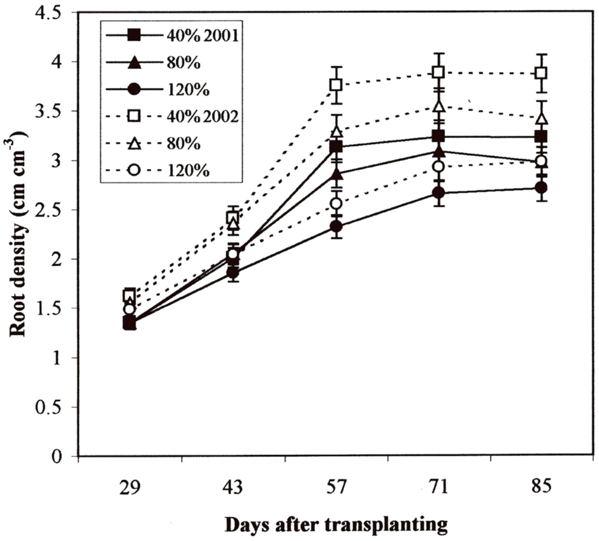

Figure 8. Root density during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

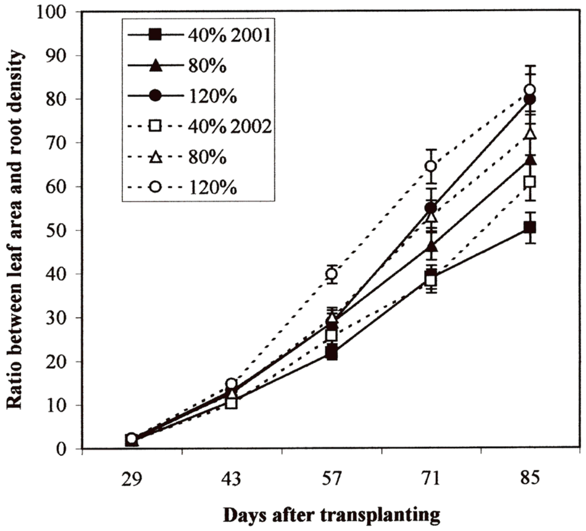

Figure 9. Ratio of leaf area to root density during the two growing season for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

Yield of the cured product

Cured leaf yield (Table 5) was higher in 2002 and increased from the 40% ET irrigation regime to 80% ET, while the difference between the latter treatment and 120% ET was not statistically significant. Yield progressively increased with increased nitrogen fertilization applied. However, increases were slight as the nitrogen rate rose from 160 to 240 kg ha^{-1}. Yield quality (Table 5) did not vary by year or water regime, with leaf grades A and B percentage of cured leaf weight averaging 59% for both years, with small non significant variations between the water regimen and nitrogen fertilizations.

Relation between irrigation regime and plant water status

Figures 10a and 10b illustrate the relationship between the average soil water potential in the 0–90 cm layer and the

Table 5. Values of the total yield and A+B product for the two years, the three irrigation and four nitrogen levels

Source of variations	Yield (t ha^{-1})	A + B Product	
		t ha^{-1}	% of total
Year			
2001	2.81	1.66	59.1
2002	2.98	1.75	58.7
LSD *P* = 0.05	0.08	0.08	
ET %			
40	2.78	1.69	60.8
80	2.93	1.71	58.4
120	2.98	1.73	58.1
LSD *P* = 0.05	0.08	n.s. [a]	
N-rate kg ha^{-1}			
0	2.68	1.44	53.7
80	2.88	1.70	59.0
160	2.98	1.86	62.4
240	3.04	1.87	61.5
LSD *P* = 0.05	0.04	6	

[a] n.s. = not significant.

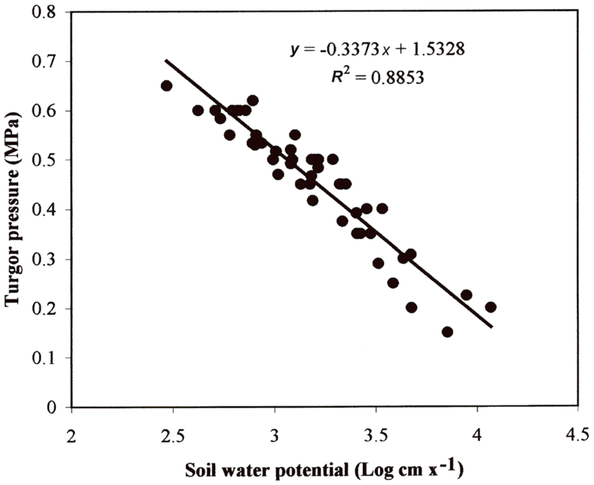

Figure 10a. Relations between soil water potential and leaf turgor pressure

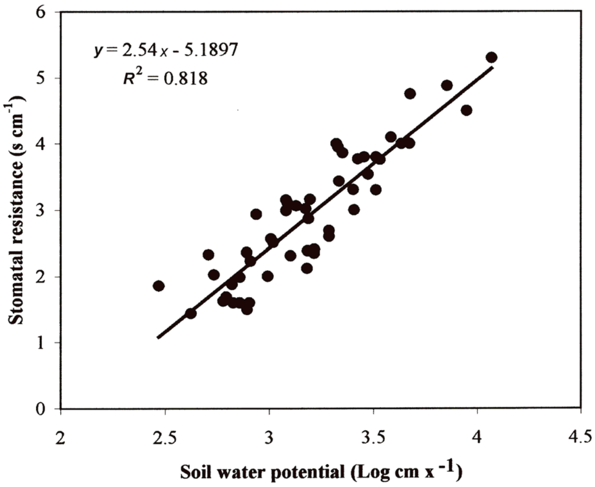

Figure 10b. Relations between soil water potential and stomatal resistance

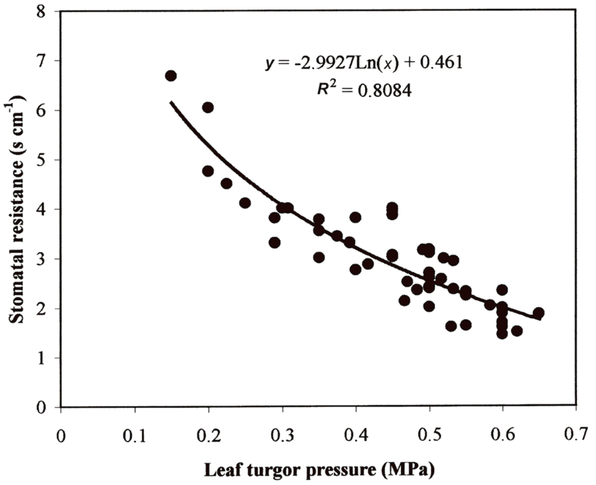

Figure 11. Relations between leaf turgor pressure and stomatal resistance

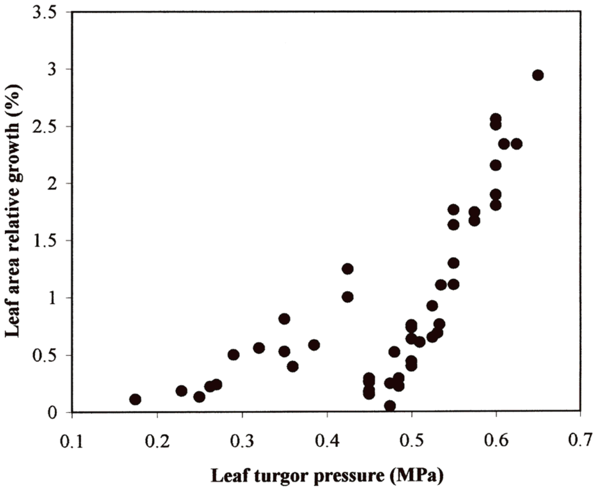

Figure 12a. Relations between leaf turgor pressure and leaf relative growth as leaf area

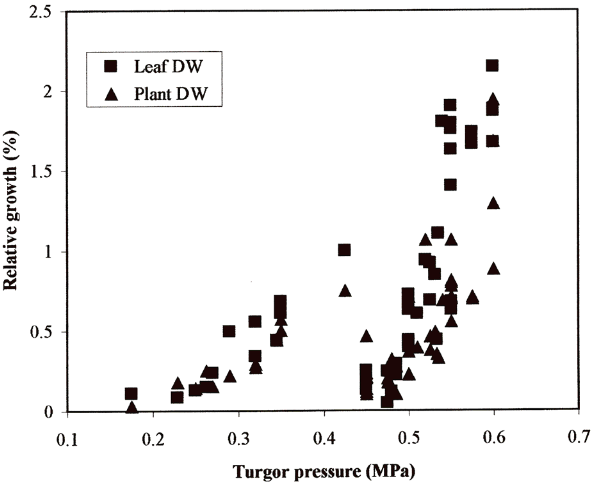

Figure 12b. Relations between leaf turgor pressure and leaf and plant relative growth as dry weight

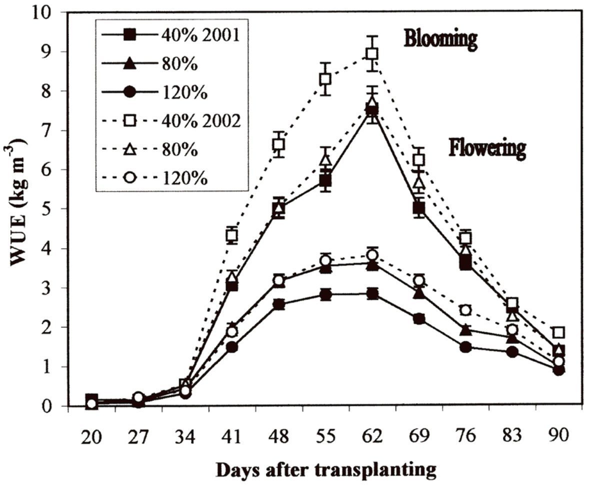

Figure 13. Water use efficiency during the two growing seasons for the three water regimes. Values are the mean of 12 replications ± standard error or the mean.

leaf turgor pressure, and between soil water potential and stomatal resistance. The relations appear very strict and linear. There was also a high correlation between leaf turgor pressure and stomatal resistance, with increased stomatal resistance as leaf turgor decreased (Figure 11), while at high turgor values stomatal resistance tends to remain constant at low values.

Relation between turgor pressure and plant growth

There was no univocal relationship between turgor pressure and leaf area percentage growth (over the preceding value). Indeed, Figure 12a shows two series of values: those common to 80 and 120 ET treatments and which have a very great slope, and another series belonging only to treatment 40% ET. Both series have a linear relation with a very high correlation coefficient (r^2 = 0.92 in both cases) but different regression coefficients (b = 11.9 and 4.6, a = −5.3 and −1.0, for 80 and 120% ET and 40% treatments, respectively). The same type of relation was noted between turgor pressure and dry matter of the leaves and plant (Figure 12b).

Water efficiency

From Figure 13, which reports water efficiency (as the total volume of supplied, rainfall and use of soil water reserves) in terms of plant dry weight accumulation, it may be noted that the efficiency rises until early bloom, and subsequently decreases. The same figure shows that greater efficiency was obtained in 2002 and in the treatment irrigated at 40% ET, while in the other two treatments efficiency declined as the irrigation volume increased.

DISCUSSION AND CONCLUSIONS

Water consumption of the 120% ET treatment (about 400 mm) falls within the range of volumes normally supplied for this crop in the southern Italian region of Campania (33,34). In this circumstance the Food and Agriculture Organization (FAO) method adopted (12) to calculate ET underestimated the actual evapotranspiration. Indeed, although irrigation supplied 120% of ET, soil moisture decreased during the season, though to a greater extent in the first 45 days. This means that the first crop coefficient K_c adopted are to be considered rather low.

Irrigation treatments resulted in different soil water content that affected plant water status, stomatal functioning, the growth in leaf area and plant dry matter. The plant response to decreased irrigation volumes was reduced by the shoot-root ratio and uptake of a greater portion of soil water reserves. However, this was not sufficient to maintain the normal plant water balance. In particular, leaf turgor potential was more affected than total and osmotic potential. As the soil water potential decreased, there was a correlative decrease in leaf turgor, which in turn was correlated with increased stomatal resistance and decreased leaf and plant growth.

Plants, subjected to greater water stress may well have undergone modifications to their tissues with thickening of the cell walls or to their composition, which reduced their elasticity, hence less responsive to hydraulic stimulus, yet maintaining a minimum growth at low turgor pressure. Applying Lockhart's equation (18,21,22) to these data we obtained a turgor pressure threshold of 0.125 and 0.43 MPa and an extensibility of the wall of 0.15 and 0.36, expressed in terms of gram of dry matter per week, respectively for the two populations illustrated in Figures 12a and 12b.

The time course of this relationship highlights the issue of water stress, which occurs differently according to the rapidity with which the plant adapts to changing environmental conditions. Sudden water stress does not allow the plant to adapt to adverse conditions. However, stress imposed slowly allows the plant to adapt to a somewhat xerophytic environment and permits a certain level of development even at low turgor pressures.

Some authors (11,32,35,36,37,40,43,44) have questioned the value of water potential, and likewise the role of cell turgor, as a basic input that regulates growth. Undoubtedly, many physiological parameters are involved in the phenomenon. However, these results show that, other factors being equal, turgor pressure is a good indicator of growth. In agronomic terms, this is very important because of the measured relationship between soil water potential and turgor pressure, as measurement of soil water potential (i.e. soil moisture) is straightforward, easy and has wide applications for plant production.

We infer that reduced water volumes do not allow high overall growth to be achieved, but a higher water use efficiency occurs as may be noted from Figure 13. The same figure shows that water efficiency in the early stages is low, probably because it is the root apparatus that grows more in this phase. Water use efficiency increases considerably during early growth until early bloom, falls sharply during bloom and remains low during leaf maturation. This trend should be explained considering the BHAGSARI and BROWN (4) results, who found that photosynthetic efficiency is linked to leaf size and specific leaf weight. The cropping year had considerable influence on water efficiency (Figure 13). This may be linked not only to the *quality* of the water supplied given that in 2002 the share from rainfall was higher, but also to climatic factors such as temperature and humidity, as in 2002 the minimum temperature was higher and the evaporative demand lower. WUE for cured leaves increased also with decreased irrigation volumes with values of 0.73, 0.89 and 1.03 kg m^{-3}, respectively for 120, 80 and 40% ET treatments. This corresponds to an increase of WUE of 22 and 41%.

Nitrogen fertilisation did not affect plant water status. The reason for this probably was that even without adding nitrogen fertilisers good plant development occurred, but nitrogen fertilisation did increase shoot/root ratio.

The results obtained in this research demonstrate that plants grown in conditions of increased water stress had lower but adequate yields. This suggests that in this environment and given the techniques adopted, the crop did not undergo such water deficit as to substantially affect production and quality.

In conclusion, for this type of tobacco and for areas of Campania, overall water availability ranging from 3500 to 4000 m^3 ha^{-1} is sufficient to obtain good plant growth with satisfying yields and good quality. Under such agronomic conditions, the opportunity for increased plant growth with nitrogen fertilisation are limited which is why application of nitrogen rates in excess of 160 kg ha^{-1} appear inadvisable.

REFERENCES

1. Ascione, S., P. Lombardi, and C. Ruggiero: Influenza della diversificazione colturale e della concimazione fosfo-azotata sulla produzione di tabacco Burley e mais [Influence of crop system and N-P fertilization on yield of Burley tobacco and maize]; Riv. Agron. 34 (2000) 81–91.
2. Ascione, S., M. Niglio: Missing data estimation in precipitation time series; Giornata di Studio: Metodi statistici e matematici per l'analisi delle serie idrologiche; Rome (2003) 259–270, CNR-GNDCI, no. 2812.
3. Atkinson, W.O., L.P. Bush, and Y.L. Sims: Dry matter and nutrient accumulation in burley tobacco; Tob. Sci. 21 (1977) 81–82.
4. Bhagsari, A.S. and R.H. Brown: Leaf photosynthesis and its correlation with leaf area; Crop Sci. 26 (1986) 127–132.
5. Boyer, J.S., A.J. Cavalieri, and E.D. Schulze: Control of cell enlargement: Effects of excision, wall relaxation, and growth-induced water potentials; Planta 163 (1985) 527–543.
6. Boyer, J.S., E.B. Knipling: Isopiestic technique for measuring leaf water potential with a thermocouple psychrometer; Proc. Nat. Acad. Sci. USA 54 (1965) 1044–1051.
7. Bridges, T.C., L.R. Walton, and J.H. Casada: Assessing the quality of burley tobacco. Part 1: Grade index and associated factors; Tob. Sci. 38 (1994) 38–41.
8. Cavalieri, A.J. and J.S. Boyer: Water potentials induced by growth in soybean hypocotyls; Plant Physiol. 69 (1982) 492–496.
9. Cleland, R.E.: The role of hormones in wall loosening and plant growth; Aust. J. Plant Physiol. 13 (1986) 93–103.
10. Davies, W.J. and J. Zhang: Root signals and regulation of growth and development of plants in drying soil; Ann. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 55–57.
11. Davies, W.J., J. Metcalfe, T.A. Lodge, and A.R. Da Costa: Plant growth substances and the regulation of growth under drought; Austr. J. Plant Physiol. 13 (1986) 105–125.

12. Doorenbs, J. and W.O. Pruitt: Guidelines for predicting crop water requirements; FAO irrigation and drainage paper, 24, Rome, 1977.
13. Evanylo, G.K. and J.L. Sims: Nitrogen and potassium fertilization effects on yield and quality of burley tobacco; Soil Sci. Soc. Am. J. 51 (1987) 1536–1540.
14. Fry, S.C.: The structure and functions of xyloglucan; J. Exp. Bot. 40 (1989) 1–11.
15. Fry, S.C.: Cellulases, hemicellulases and auxin-stimulated growth: A possible relationship; Physiol. Plant 75 (1989) 532–536.
16. Hayashi, T.: Xyloglucans in the primary cell wall; Ann. Rev. Plant Physiol. Plant Mol. Biol. 40 (1989) 139–168.
17. Hsiao, T.C.: Plant response to water stress; Ann. Rev. Plant Physiol. 24 (1973) 519–570.
18. Hsiao, T.C. and L.K. Xu: Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport; J. Exp. Bot. 51 (2000) 1595–1616.
19. Jones, H.G.: Stomatal control of photosynthesis and transpiration; J. Exp. Bot. 49 (1998) 387–398.
20. King, M.J.: Bilateral leaf expansion of tobacco under water stress; Can. J. Plant Sci. 68 (1988) 887–892.
21. Lockhart, J.A.: An analysis of irreversible plant cell elongation; J. Theor. Biol. 8 (1965) 264–275.
22. Lockhart, J.A.: Cell extension; *in* Plant Biochemistry, edited by J. Bonner and J.E. Varner, Academic Press, New York, 1965, pp. 826–849.
23. Massantini, F.: Accrescimento ed esigenze nutritive di alcune varietà di tabacco (*Nicotiana tabacum* L.) [Growth and nutritional supply of some tobacco cultivars (*Nicotiana tabacum* L.)]; Il Tabacco 747(1973) 15–33.
24. Miner, G.S. and J.L. Sims: Changing fertilization practices and utilization of added plant nutrients for efficient production of tobacco; Rec. Adv. Tob. Sci. 9 (1983) 4–76.
25. McCants, C.B. and W.G. Woltz: Growth and mineral nutrition of tobacco; Adv. Agron. 19 (1967) 211–265.
26. Mylonas, V.A., V.N. Athanasiadis, and X.A. Perakis: Effects of nitrogen and potassium on yield, value and chemical composition of burley tobacco; Coop. Cent. Sci. Res. Relat. Phitopatol. Stud. Group. Agron. Stud. Group. Rep.: 70-83, October 1979, Tob Abstr., 1981, 1, 39.
27. Newman, E.I.: A method of estimating the total length of root in a sample; J. Appl. Ecol. 3 (1966) 139–145.
28. Nonami, H. and J.S. Boyer: Origin of growth-induced water potential: Solute concentration is low in apoplast of enlarging tissues; Plant Physiol. 83 (1987) 596–601.
29. Nonami, H. and J.S. Boyer: Turgor and growth at low water potentials; Plant Physiol. 89 (1989) 798–804.
30. Nonami, H. and J.S. Boyer: Primary events regulating stem growth at low water potentials; Plant Physiol. 93 (1990) 1601–1609.
31. Nonami, H. and J.S. Boyer: Wall extensibility and cell hydraulic conductivity decrease in enlarging stem tissues at low water potentials; Plant Physiol. 93 (1990) 1610–1619.
32. Passioura, J.B.: Response to Dr P.J. Kramer's article, 'Changing concepts regarding plant water relations', Plant Cell Environ. 11 (1988) 565–568; Plant Cell Environ. 11 (1988) 569–571.
33. Postiglione, L.: L'irrigazione del tabacco nella Piana del Sele [Tobacco irrigation in the Sele plain], Nota II; Ann. Fac. Sci. Agrarie, Università di Napoli, Portici, serie IV, vol. VII, 1973, 254–265.
34. Ruggiero, C.: Irrigazione del tabacco. Influenza del volume stagionale sulla resa nella piana del Volturno [Tobacco irrigation. Influence of seasonal water volumes on the yield in the Volturno plain]; L'Italia Agricola Anno 115- N.10 (1978) 121–127.
35. Schulze, E.D.: Whole-plant responses to drought; Austr. J. Plant Physiol. 13 (1986) 127–141.
36. Schulze, E.D., J. Cermak, R. Matyssek, M. Penska, and F. Zimmerman: Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil; Ann. Rev. Plant Physiol. 37 (1986) 247–274.
37. Schulze, E.D., E. Steudle, T. Gollan, and U. Shurr: Response to Dr P.J. Kramer's article, 'Changing concepts regarding plant water relations', Plant Cell Environ. 11 (1988) 565–568; Plant Cell Environ. 11 (1988) 573–576.
38. Shackel, K.A., M.A. Matthews, and J.C. Morrison: Dynamic relation between expansion and cellular turgor in growing grape (*Vitis vinifera* L.) leaves; Plant Physiol. 84 (1987) 1166–1171.
39. Sifola, M.I., L. Berritto, and L. Postiglione: Effetto di dosi crescenti di azoto in differenti regimi idrici su produzione e qualità di tabacco (*Nicotiana tabacum* L.) tipo Burley [Effect of increasing nitrogen rates and different water regimes on yield and quality of burley tobacco (*Nicotiana tabacum* L.)] Riv. Agron. 30 (1996) 607–616.
40. Sinclair, T.R. and M.M. Ludlow: Who thought plants thermodynamics? The unfulfilled potential of plant water potential; Austr. J. Plant Physiol. 12 (1985) 213–217.
41. Slavik, B.: Methods of studying plant water relations; Ecological Studies, no. 9., Acad. Sci. Prague, Springer Verlag, Heidelberg New York, 1974, pp. 449.
42. Tardieu, F., T. Lafarge, and T.H. Simonneau: Stomatal control by fed or endogenous xylem ABA in sunflower: Interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species; Plant Cell Environ. 19 (1996) 75–84.
43. Termaat, A., J.B. Passioura, and R. Munns: Shoot turgor does not limit growth of NaCl-affected wheat and barley; Plant Physiol. 77 (1985) 869–872.
44. Turner, N.C.: Crop water deficit: a decade of progress; Adv. Agron. 39 (1986) 1–51.
45. Yoon, K.E.: Effects of simulated rain on yield and quality of oriental tobacco; Korea Tob. Res. Inst. Res. Bull. 2 (1980) 7–14, Tob. Abstr., 1981, 5, 1014.
46. Zhu, G.L. and J.S. Boyer: Enlargement in Chara studied with a turgor clamp: Growth rate is not determined by turgor; Plant Physiol. 100 (1992) 2071–2080.

Corresponding author

Celestino Ruggiero
Department of Agricultural Engineering and Agronomy
University of Naples Federico II
80055 Portici (Na)
Italy