Agronomie 19 (1999) 591–601

Original article

Foliar senescence in maize plants grown under different water regimes

Arturo Alvino[a*], Sebastiano Delfine[a], Mauro Mori[b]

[a] Dipartimento S.A.V.A., Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy
[b] Dipartimento di Scienze Agronomiche, Università Federico II di Napoli, via Università 100, 80055 Portici-Napoli, Italy

(Received 20 April 1999; accepted 3 August 1999)

Abstract – The leaf ontogeny of potted maize plants subjected to severe water stress was carried out in a greenhouse. The water stress cycle started at the onset of the vegetative stage (five-leaf stage). Control and water-stressed plants received 100 and 50 % of the water evapotranspired, respectively. After 30 days half of the water-stressed plants were fully irrigated to control levels. Water stress lowered osmotic potentials of all leaves along the plant profile as well as their chlorophyll concentration and photosynthetic rate. Leaf area of stressed plants was reduced, while leaf nitrogen concentration was higher than in control plants at the end of the vegetative stage. After re-watering, recovered plants increased their leaf photosynthetic rate and leaf turgor, although they remained lower than in control plants. At the end of the vegetative stage, leaf nitrogen concentration of re-watered plants was similar to that of the stressed plants, while leaf growth was not resumed though relative death rate was slowed to that of the control treatment. This suggests that severe water-stressed plants, when fully re-irrigated, were able to re-establish good physiological processes although at a level lower than in the control, because chlorophyll concentration was not fully recovered. (© 1999 Inra/Éditions scientifiques et médicales Elsevier SAS.)

leaf senescence / leaf photosynthesis / maize / relative death rate / soil water stress

Résumé – Sénescence foliaire chez les plants de maïs cultivés sous différents régimes hydriques. On a étudié en serre la formation des feuilles de plants de maïs en pot soumis à des stress hydriques sévères. Le cycle de stress hydrique a commencé au début du stade végétatif (stade cinq feuilles). Les plants témoins et les plants stressés ont reçu respectivement 100 et 50 % de l'eau d'évapotranspiration. Dix jours plus tard la moitié des plants subissant le stress hydrique ont été irrigués au même niveau que les témoins. Le stress hydrique a diminué le potentiel osmotique de toutes les feuilles le long du plant, ainsi que leur concentration en chlorophylle et le taux de photosynthèse. La surface de la feuille des plants stressés était réduite, tandis que la concentration des feuilles en azote était plus élevée que chez les témoins à la fin du stade végétatif. Après avoir été à nouveau arrosés, les plants ont récupéré et leur taux de photosynthèse foliaire ainsi que la turgescence des feuilles ont augmenté, tout en restant inférieurs à ceux des témoins. À la fin du stade végé-

Communicated by Jean-François Ledent (Louvain-la-Neuve, Belgium)

* Corresponding author
alvino@hpsrv.unimol.it

tatif, la concentration en azote des feuilles des plants ré-arrosés était semblable à celles des plants stressés, mais la croissance foliaire n'avait pas repris bien que le taux relatif de mort soit descendu au niveau de celui des plants témoins. Ceci suggère que les plants ayant subi un stress hydrique sévère sont capables, lorsqu'ils sont arrosés à nouveau, de bien rétablir les processus physiologiques, à un moindre niveau toutefois que celui des témoins parce que la concentration en chlorophylle n'est pas totalement récupérée. (© 1999 Inra/Éditions scientifiques et médicales Elsevier SAS.)

sénescence / photosynthèse / maïs / taux de mortalité / stress hydrique du sol

Abbreviations: WW, ST, RI = treatments: well watered, soil water stressed, re-irrigated, respectively; ψ_p and ψ_o = pressure and osmotic leaf water potential; CER = CO_2 exchange rate ($\mu moles \cdot m^{-2} \cdot s^{-1}$); CERl = CER related to a whole leaf (μ moles$\cdot s^{-1} \cdot leaf^{-1}$), GLA = green leaf area; PFD = photosynthetic flux density; RDR = relative death rate; SLA = senescent leaf area.

1. Introduction

Senescence processes have been widely studied, yet the boundaries between the processes of senescence and ageing are still unclear [16]. Sexton and Woolhouse [21] defined ageing as those changes which occur in time without reference to death as a consequence. In monocarpic plants growing under natural conditions death inevitably follows flowering and fruiting [16]. This suggests that death is a consequence of the exhaustion of the vital resources of the plant as they are diverted to maximize the production of seeds.

Senescence can be defined as those changes which lead sooner or later to the death of an organism [21], and is considered by us to be an acceleration of processes that will ultimately cause the death of the plant. Senescence involves genetically directed metabolic changes, such as loss of chlorophyll, proteolysis and decreased photosynthesis. During senescence there is a net breakdown of many soluble complex molecules and the products are exported to other developing areas, particularly seeds [14]. This movement often involves nitrogenous compounds, and can be considered as a second stage reallocation of reduced N. Nitrogen export from assimilatory organs prevails over the N acquisition during this stage, contrary to what occurs during plant growth. These two phases roughly correspond to the period of vegetative (first stage) and reproductive (second stage) growth [11]. Nutrient diversion from leaves to other developing organs has been proposed as a mechanism of inducing vegetative senescence [19]. Although there is some evidence of accelerated senescence because of abiotic factors, there is scant published research that shows that senescence may occur during the vegetative stage when plants are severely water stressed.

The gradual and steady decrease in protein content is a good indicator of senescence [16]; an accelerated mobilization of leaf and stalk proteins is reported, in particular enzymes involved in the assimilatory process (e.g. ribulose-1,5-bis-phosphate carboxylase/oxigenase). The N stored in these proteins is translocated, through the phloem to the plant sinks [23, 25]. This could cause a premature reduction in photosynthetic rate and a decline in photosynthate production [22].

Many studies have shown a strong association between senescence and a decline in chlorophyll content [7]. Chlorophyll content is proportional to leaf nitrogen content and the ratio between protein and chlorophyll is relatively constant; the average number of chlorophyll molecules (and therefore proteins) associated with each reaction centre does not change during senescence [7]. The reduction in the number of the reaction centres during senescence, however, causes a corresponding reduction in the photosynthetic electron transport activity of the chloroplasts, limiting the photosynthetic activity of the leaf [7].

Furthermore, in a dense canopy, the reduction of leaf nitrogen content is influenced by the light distribution within the canopy. This phenomenon is not observed if leaves do not undergo mutual shad-

ing [15]. Mooney et al. [18], however, noted a decrease of nitrogen with age in leaves. A reduction of N content per leaf area was found to correlate with the relative extinction of light from the top to the bottom of the canopy when maintained in direct sunlight [15].

In the present paper, maize plants (*Zea mays* L., cv. Rossana) were grown in pots maintained under well-watered and water-stressed condition. Plants were widely distributed in order to avoid leaf shading. The stress period was imposed during the vegetative stage, when ageing phenomena are not present [12], in order to clarify the relation between water stress and leaf senescence. A re-irrigated treatment was used to investigate the recovery from water stress. The objective of this study was to detect changes in morphological (green and senescent leaf area) and physiological (leaf photosynthetic rate, leaf nitrogen content, chlorophyll content, leaf water potentials) parameters that might indicate the appearance of senescence during the vegetative stage in maize plants grown under severe soil water stress.

2. Materials and methods

2.1. Plot design and cultural practices

The study was conducted in potted plants. A clay and a sandy peat soil (2:1) was previously fertilized to reach a 0.3 % N content, then moistened several times with a small amount of water to prevent soil structure collapse and covered with a plastic film to prevent evaporation losses. Three days after reaching field capacity (35 % w/w of water) the soil was used to fill 8-L pots. To prevent soil evaporation, pots were sealed with a transparent film soon after emergence and after plant establishment with sand manure.

The weight of each pot was determined every 3 days, and water added to replenish that lost by evapotranspiration, using as reference the weight of pots in the well-watered treatment (WW). After replenishing the water lost to transpiration, no water table was observed at the bottom of the pots; soil may be considered at field capacity, since initial conditions (when pots were filled with soil at field capacity) were re-established. Twenty-seven days after emergence (27 d.a.e.) some plants were water stressed (treatment ST) by irrigating plants with only 50 % of water given to the pots of the WW control. Forty-five days after emergence half of the water-stressed plants (treatment RI) were fully re-irrigated. During the experiment, temperatures ranged between 20 and 25 °C during the day and between 10 and 15 °C at night. PPFD midday values were always over 1 000 $\mu moles \cdot m^{-2} \cdot s^{-1}$.

2.2. Leaf water and solute potentials

Water potentials were measured with the isopiestic thermocouple psychrometer technique [65]. Leaf samples were taken at midday from fully exposed leaves: the discs were cut from the middle portion of the blade and quickly placed on the bottom of the psychrometer cup with a leaf strip used to line the wall of the cup. Cups were sealed immediately, stored in an insulated box, and placed in a constant temperature (29 °C) bath within 1 h of sampling. Three standard sucrose solutions of differing solute potential were used to calibrate the psychrometer for each sample. Leaf solute potentials were determined similarly, after the psychrometer cups were put into an antifreeze solution at about –15 °C and left overnight to freeze and induce cell rupture.

2.3. Leaf area and senescence

Beginning at 33 d.a.e., length (L) and maximum width (W) of fully expanded leaves (full exposure of the ligule) were measured approximately weekly for all leaf positions of all plants. Areas of the leaves that emerged but were not yet fully expanded were derived by the triangle formula. Areas of other leaves were calculated using the formula suggested by Wolfe [24]:

$$\text{leaf area} = 3.3 + 0.642 \times (L \times W) + 0.001 \times (L \times W)^2;\ R^2 = 0.995$$

At the same time, the percentage of senescent leaf area (SLA) was estimated from a loss of green coloured tissue. Leaves with more than 50 % yellow or brown blades were considered fully senescent. The green leaf area (GLA) was the sum of the areas of the fully expanded leaves and of leaves emerged but not yet fully expanded, minus the SLA.

The relative death rate (RDR) is defined as the rate of increase in senescent plant material divided by the amount of living tissue [20]. RDR is calculated as: RDR = 1/GLA * dSLA/dt, where t is time in days. RDR and leaf expansion rate (defined as the area increment of green tissue per day) were computed throughout the

experiment: for each calculated point, the x-value refers to the mean of two sequential dates of measurements.

2.4. Leaf chlorophyll and nitrogen analyses

Leaves at different nodal positions were sampled periodically for chlorophyll and N determinations. Leaf discs (1.65 cm in diameter), sampled from the middle part of each leaf, were ground in 10 mL of a 0.1-M Tris buffer (pH 7.5). Chlorophyll was extracted by mixing 1 mL of leaf homogenate with 9 mL of 90 % (v/v) acetone. The mixture was allowed to stand for 15 min and then centrifuged (600 g) for 5 min at room temperature. Absorbance of the supernatant was measured at 645 and 663 nm spectrophotometerically, and chlorophyll concentration was calculated according to Arnon [1].

After sampling for chlorophyll, the remainder of the leaf material was dried (70 °C) for N analysis. Leaf N (expressed as the percentage of dry mass) of each sample was determined by the Kjeldahl procedure [4] and ammonia was determined using an automated conductimetric apparatus [5].

2.5. Photosynthesis

Photosynthesis was determined in the field with a portable system (ADC, Hoddesdone, UK) operating in differential mode. All measurements were made near solar noon, on the mid portion of the leaves that had been exposed to full sunlight for the entire morning. The central vein was not clamped during gas exchange measurements.

3. Results

The number of leaves, their green area and the time of their appearance varied significantly among the treatments (*figure 1*). After 33 d.a.e., ST

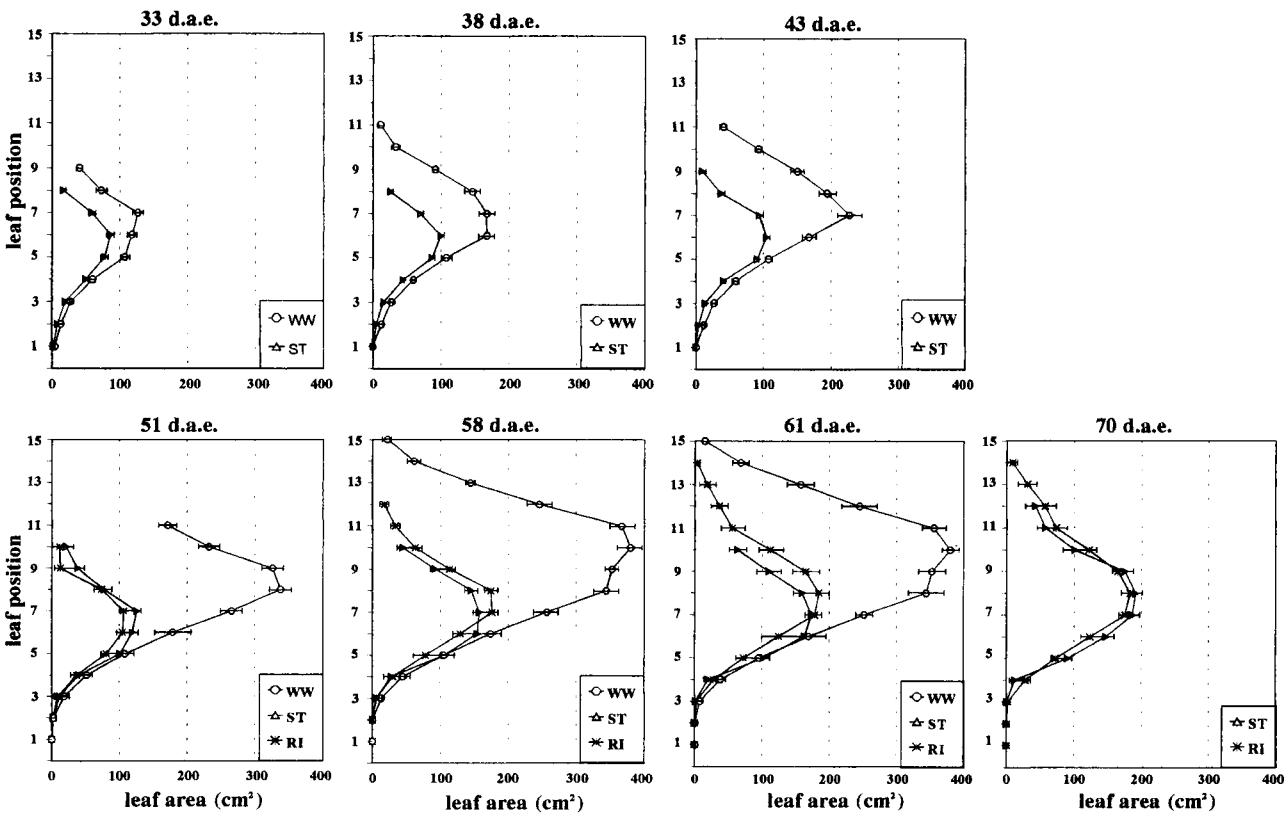

Figure 1. Trend of green leaf surface at different leaf positions for the three water treatments. WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment.

showed a lower green leaf area, starting with the 5th leaf with respect to WW. The stress treatment also delayed (by about 10 days) the appearance of the 9th leaf with respect to the WW treatment. Under the stress conditions, green surface increments of the 5th, 6th, 7th and 8th leaves were small until 51 d.a.e.; from this date onwards new leaves appeared and the increment in green surface of some ST leaves was evident. At 51 d.a.e., 5 days after re-watering, RI did not differ from ST in green leaf area or in leaf number. At 58 d.a.e., RI developed new leaves (11th and 12th leaf) but the increase in green leaf area was negligible with respect to ST. At 61 d.a.e., RI showed two more leaves and had a significantly higher leaf area in the upper leaves (9th and 10th). At 70 d.a.e., ST and RI showed a similar green leaf area at different leaf positions, but RI still had two more leaves than ST.

The maximum green leaf area per nodal position varied with treatments: the 7th leaf at 43 d.a.e. in the WW treatment, then the 8th until 51 d.a.e., and the 10th leaf at 58 and 61 d.a.e. At this last date, the green leaf area of WW plants was about three times that of ST and about two times that of RI.

The leaf expansion rate of WW was higher than the other treatments (*figure 2*), except at 58 d.a.e. when it declined. The leaf expansion rate of ST was about one seventh of that of WW in the time interval 43–51 d.a.e. (corresponding to 47 d.a.e. of *figure 2*). At this point it showed a strong increase, but it was still half that of the WW treatment in the time interval 51–58 d.a.e. (54 d.a.e in *figure 2*). Leaf expansion rate of RI did not differ significantly from ST; around 58 d.a.e., RI showed lower values than ST.

Total senescent area (*figure 2*) of WW was negligible until 43 d.a.e, but it increased significantly later. ST senesced earlier than WW, but at 51 d.a.e. ST and WW were not significantly different. Later, ST had a higher senescent leaf area with respect to WW. RI showed a significant reduction in total senescent area with respect to ST.

Table I. Pressure (Ψp) and osmotic (Ψo) potentials (bars) for the three water treatments at different days after emergence. WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment. Standard error is given in brackets.

	Days after emergence									
Leaf	33		45		54		61		71	
position	Ψp	Ψo	Ψp	Ψo	Ψp	Ψo	Ψp	Ψo	Ψp	Ψo
WW										
4	3.53 (0.2)	−12.7 (0.5)	2.91 (0.3)	−10.7 (0.6)	3.80 (0.2)	−15.7 (0.7)	2.70 (0.2)	−18.7 (0.6)		
6	3.58 (0.3)	−12.6 (0.4)	2.31 (0.2)	−11.8 (0.5)	4.45 (0.4)	−15.2 (0.6)	7.21 (0.3)	−15.3 (0.9)		
8			1.70 (0.3)	−12.2 (0.7)	3.35 (0.3)	−14.2 (0.6)	4.65 (0.3)	−14.7 (0.7)		
10					3.10 (0.2)	−15.2 (0.7)	1.70 (0.2)	−14.3 (0.8)		
ST										
4	1.80 (0.9)	−18.6 (0.2)	2.60 (0.2)	−23.0 (0.8)	0.50 (0.1)	−21.3 (0.8)	1.05 (0.2)	−17.1 (0.6)	1.20 (0.1)	−22.8 (0.9)
6	3.17 (0.3)	−17.7 (0.3)	1.10 (0.4)	−21.0 (0.5)	1.00 (0.2)	−22.3 (0.6)	0.95 (0.1)	−18.4 (0.8)	1.90 (0.3)	−23.4 (0.8)
8							1.00 (0.2)	−18.2 (0.7)	1.35 (0.3)	−22.4 (0.7)
10										
RI										
4					0.49 (0.2)	−21.4 (0.6)	1.55 (0.2)	−13.0 (0.9)	1.25 (0.2)	−20.4 (0.8)
6					1.05 (0.3)	−22.2 (0.7)	2.05 (0.3)	−14.9 (0.7)	2.00 (0.4)	−17.9 (0.7)
8							2.30 (0.2)	−14.8 (0.6)	1.61 (0.1)	−17.5 (0.9)
10									1.25 (0.2)	−17.2 (0.6)

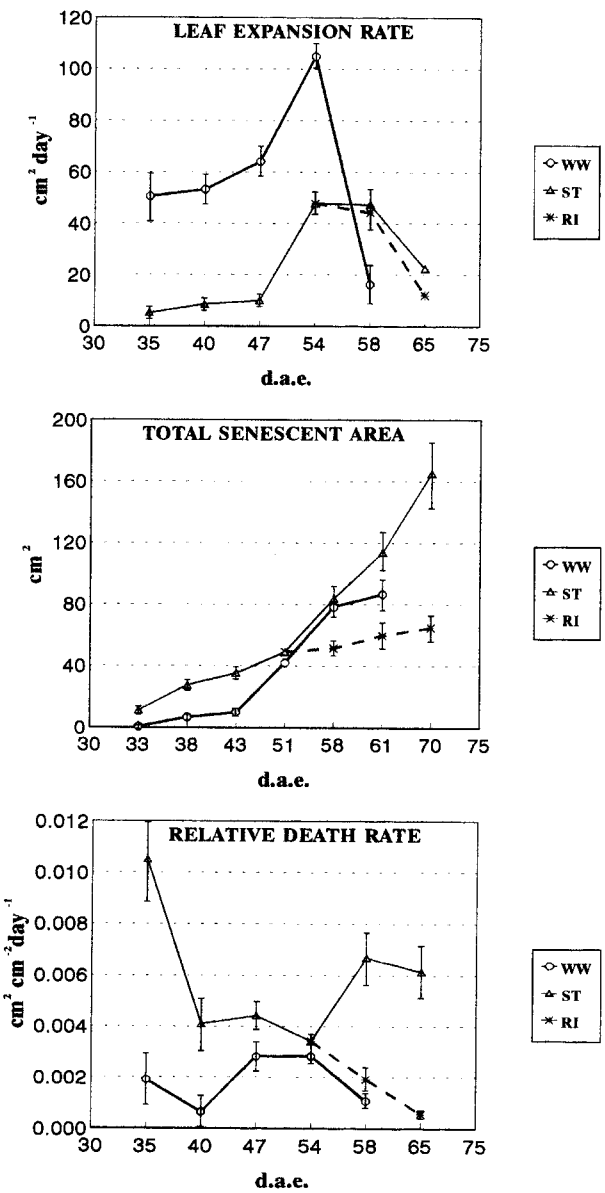

Figure 2. Leaf expansion rate, total senescent area and relative death rate versus time for the three water treatments. WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment.

The relative death rate (RDR) of ST was particularly high at the beginning of stress (*figure 2*), but then it decreased until 54 d.a.e., when its values were similar to those of WW. Later, RDR of ST increased significantly, while it decreased in WW, assuming values not different from those of RI.

The soil moisture regimes influenced leaf water potentials (*table I*). ST lowered the osmotic potential so that pressure potential was maintained at a minimum level. Stressed plants, once re-irrigated, raised their osmotic potential values, so that their ψ_p values became higher than those of ST plants but lower than WW ones. Leaves of WW appeared turgid, and ψ_p was in some cases almost eight-fold higher than the corresponding values of ST plants. At all dates ψ_o values of WW plants were considerably less negative than those of ST plants, excluding the WW fourth leaf at 61 d.a.e.

Leaf nitrogen concentration of the WW treatment (*table II*) was generally above 4 % until 54 d.a.e., and then started to decrease. ST showed the same trend as WW. At 61 d.a.e., nitrogen concentration of WW plants was significantly lower than for the other treatments, irrespective of leaf position. RI nitrogen concentrations were not significantly different from ST at 61 and 71 d.a.e. As in the leaf, the stem nitrogen concentration of ST was constant over time (*table III*). However, stem nitrogen decreased significantly with time both in WW and RI treatments.

Differences in leaf photosynthetic rate (*table IV*) were highly significant among treatments. WW and ST plants showed a lower photosynthetic rate in the basal leaves and higher values in the middle part of the plant. The leaf photosynthetic rate of ST was always significantly lower than that of WW. Lowest values were recorded at 54 d.a.e. after which there was a slight recovery. When fully re-irrigated, the photosynthetic rate of stressed plants soon recovered.

The chlorophyll concentration of control plants increased with time until 45 d.a.e., and thereafter it remained constant (*table V*). ST plants showed a constant decrease in chlorophyll with time. At 71 d.a.e., chlorophyll concentration of ST leaves was half that at 33 d.a.e. At 61 and 71 d.a.e., chlorophyll concentration of RI did not decrease below the values observed at 54 d.a.e. and remained intermediate between ST and WW. In all treatments,

Table II. Trend in leaf nitrogen concentration (percentage on dry weight basis) at different plant heights for the three water treatments: WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment. Standard error is given in brackets.

	Days after emergence														
Leaf		33			45			54			61			71	
position	WW	ST	RI	WW	ST	RI	WW	ST	RI	WW	ST	RI	WW	ST	RI
13										3.15 (0.03)					3.63 (0.18)
12							3.65 (0.07)			3.14 (0.18)					3.41 (0.07)
11				3.32 (0.02)			3.91 (0.21)			3.30 (0.12)		3.39 (0.09)		3.53 (0.33)	3.68 (0.12)
10				3.63 (0.02)			4.13 (0.03)			3.40 (0.05)	4.75 (0.11)	3.67 (0.14)		3.78 (0.35)	3.60 (0.06)
9				3.90 (0.07)			4.02 (0.04)			3.29 (0.03)	4.47 (0.19)	4.15 (0.14)		4.14 (0.16)	4.21 (0.02)
8	3.99 (0.08)			4.02 (0.11)	3.85 (0.05)		4.19 (0.03)	4.35 (0.13)	4.37 (0.11)	3.39 (0.07)	4.02 (0.13)	4.27 (0.07)		4.34 (0.26)	3.93 (0.18)
7	4.44 (0.03)	4.27 (0.03)		4.19 (0.10)	4.32 (0.01)		4.56 (0.16)	4.34 (0.16)	4.20 (0.13)	3.21 (0.20)	4.05 (0.10)	4.21 (0.06)		3.96 (0.11)	3.61 (0.23)
6	4.49 (0.16)	4.34 (0.01)		4.41 (0.07)	4.40 (0.02)		4.69 (0.14)	3.96 (0.26)	3.96 (0.26)	3.28 (0.36)	3.90 (0.06)	3.91 (0.04)		3.59 (0.21)	3.24 (0.16)
5	4.46 (0.05)	4.61 (0.08)		4.65 (0.18)	4.08 (0.11)		4.75 (0.15)	4.25 (0.18)	4.30 (0.18)	3.23 (0.27)	3.89 (0.04)	3.90 (0.08)		3.80 (0.18)	3.22 (0.29)
4	4.72 (0.26)	4.50 (0.05)		4.08 (0.12)	4.21 (0.09)		4.10 (0.16)	4.08 (0.13)	3.99 (0.13)	2.51 (0.18)	3.32 (0.03)	3.30 (0.07)		2.97 (0.05)	3.41 (0.18)
3	4.49 (0.16)	3.73 (0.06)		3.87 (0.18)	3.51 (0.15)		3.45 (0.14)	3.69 (0.11)	3.65 (0.18)	1.80 (0.15)	2.77 (0.02)	2.42 (0.06)		2.22 (0.06)	
2	4.20 (0.12)			3.86 (0.14)										2.66 (0.04)	

Table III. Trend in stem nitrogen (percentage on dry weight basis) for the three water treatments: WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment. Standard error is given in brackets.

	Days after emergence														
Leaf		33			45			54			61			71	
position	WW	ST	RI	WW	ST	RI	WW	ST	RI	WW	ST	RI	WW	ST	RI
				3.81 (0.34)	4.05 (0.44)		3.05 (0.29)	4.11 (0.19)	4.12 (0.21)	1.98 (0.31)	4.58 (0.45)	3.05 (0.37)		4.01 (0.34)	2.22 (0.23)

Table IV. Trend in photosynthetic rate ($\mu moles \cdot m^{-2} \cdot s^{-1}$) of leaves at different nodal positions for the three water treatments: WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment. Standard error is given in brackets.

Leaf position	Days after emergence 33 WW	33 ST	33 RI	45 WW	45 ST	45 RI	54 WW	54 ST	54 RI	61 WW	61 ST	61 RI	71 WW	71 ST	71 RI
13										10.0 (0.62)					
12							13.7 (0.67)			12.2 (0.67)					9.30 (1.57)
11				10.6 (0.22)			13.8 (0.72)			15.2 (2.37)	2.55 (0.52)				11.6 (5.60)
10				17.6 (0.35)			19.6 (1.00)			14.7 (0.89)	3.35 (0.38)	9.20 (0.56)		2.00 (0.19)	15.6 (1.06)
9				10.2 (0.16)			15.3 (2.00)			18.8 (3.92)	6.34 (2.22)	13.3 (0.64)		2.36 (0.10)	14.2 (1.08)
8				8.89 (3.48)	2.72 (0.11)		11.7 (0.11)	0.53 (0.13)	0.56 (0.23)	20.2 (2.58)	1.29 (0.51)	20.2 (2.33)		3.38 (0.52)	15.5 (1.59)
7				10.5 (2.86)	4.90 (1.62)		16.2 (1.85)	0.77 (0.24)	0.74 (0.33)	16.8 (4.74)	1.49 (0.18)	16.7 (2.33)		3.37 (0.71)	13.2 (2.40)
6	14.3 (2.58)	2.25 (0.34)		15.1 (1.95)	6.56 (2.35)		17.4 (0.83)	0.66 (0.16)	0.68 (0.25)	15.6 (1.10)	1.74 (0.78)	13.4 (1.06)		4.21 (0.75)	11.7 (3.97)
5				6.31 (1.97)	1.06 (0.17)		12.8 (3.88)	0.25 (0.18)	0.27 (0.21)	10.3 (5.19)	1.33 (0.08)	4.71 (0.33)		1.68 (0.17)	7.70 (3.74)
4	10.0 (1.74)	1.75 (0.26)		5.32 (0.58)	0.33 (0.03)		6.94 (1.91)	0.58 (0.16)	0.56 (0.28)	4.55 (0.55)	1.27 (0.29)	3.53 (0.90)			5.44 (0.80)
3				2.44 (0.52)	0.22 (0.02)		4.62 (0.70)	0.80 (0.76)	0.83 (0.56)	5.18 (2.73)					
2				2.51 (0.24)											

Table V. Trend in leaf chlorophyll concentration ($\mu g \cdot cm^{-2}$) at different nodal positions for the three water treatments: WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment. Standard error is given in brackets.

Leaf position	Days after emergence 33 WW	33 ST	33 RI	45 WW	45 ST	45 RI	54 WW	54 ST	54 RI	61 WW	61 ST	61 RI	71 WW	71 ST	71 RI
11							32.3 (1.19)			38.8 (0.86)					30.9 (0.56)
9				39.7 (2.67)			42.9 (2.35)			44.1 (1.62)	16.5 (3.98)	27.8 (4.49)		20.4 (0.77)	33.0 (1.31)
7	36.3 (3.73)	43.3 (1.65)		49.7 (2.62)	37.8 (0.43)		49.9 (1.65)	27.1 (0.45)	27.0 (0.40)	46.6 (1.27)	30.9 (0.40)	33.5 (0.22)		23.9 (1.63)	33.0 (0.47)
5				45.0 (2.39)	38.7 (0.72)		44.0 (0.99)	36.3 (0.49)	36.4 (0.39)	40.5 (1.02)	27.4 (0.60)	34.2 (3.41)		23.0 (1.71)	31.5 (1.09)
4	39.1 (3.29)	46.8 (1.57)		43.8 (1.08)			42.7 (0.21)	36.9 (0.18)	36.5 (0.29)	38.0 (0.98)					

chlorophyll concentration was highest for leaves in the middle part of the plant.

All leaves of WW showed increasing leaf photosynthesis values (CERl) until 54 d.a.e. (*figure 3*); thereafter this increase was observed in leaves above the eighth nodal position. CERl of stressed plants showed a strong decrease from 45 to 54 d.a.e. From that date the photosynthetic rate of the ST leaves increased constantly. At 71 d.a.e. re-irrigated plants showed decreasing values in some basal leaves (6, 7 and 8), whereas upper leaves showed the reverse trend and even a valuable contribution from newly expanded leaves.

4. Discussion

The experiments presented in this paper suggest that water stress induces early senescence in maize during the vegetative stage, when metabolic changes associated with ageing are normally absent. Soon after the beginning of soil water stress, leaf growth of ST was markedly reduced; therefore, its leaf expansion rate was lower than that of the WW treatment, although it showed a parallel trend until 54 d.a.e. Furthermore, leaf emergence was affected by water stress, in contrast to the findings of Dwyer and Stewart [6]. The result was a significant difference in leaf area between ST and WW plants, while the difference in RDR between the two treatments was significant only at 61 d.a.e. Along the plant profiles a higher leaf expansion rate was observed in medial than in basal leaves; this was more evident in WW than in ST, probably because the ST leaves were smaller, and self shading was less of a problem than in WW leaves.

ST and WW had similar senescent leaf area until 61 d.a.e. though a very different total leaf area. The resulting RDR of ST was much higher than that of WW. The loss in photosynthesizing surface area most likely impaired the CO_2 assimilation rate of ST plants, which appeared to be several times lower then in WW. Another significant cause was probably the lower (50 % with respect to WW) chlorophyll concentration in the ST leaves along the plant profile.

We hypothesize that the higher percentage of N found in ST plants with respect to WW plants at the end of treatment was used for osmoregulation based on leaf water potential data. This hypothesis is supported by Fedina and Popova [9], who reported that proteins and amino acids (such as proline) were involved in an increase in solute potential. As the water stress progressed (71 d.a.e.), the contribution of N compounds to osmoregulation was probably replaced by mineral salts [2]. Evidence in support of this comes from the lack of correlation (not shown) between osmotic potential and N concentration values. On the other hand, nitrogen is one of the resources determining photosynthetic capacity [8, 10], but our data would suggest that this relationship applies only under non-limiting conditions (as in WW). Under stress conditions the amount of nitrogen was not correlated with CO_2 assimilation rate [13].

When ST plants were fully re-irrigated, leaf photosynthesis and leaf turgor recovered, but at a level significantly lower than in WW. Despite the appearance of new leaves, the leaf expansion rate of RI remained unchanged, so that no appreciable increase in leaf surface was observed. However, in contrast to the ST treatment, the senescence rate of leaves in the RI treatment fell to near zero as did the RDR. At 61 d.a.e., ST, WW and RI showed a senescent leaf surface that represented about 13, 3 and 6 % of the total leaf surface, respectively. Furthermore RI chlorophyll remained higher than ST, but significantly and consistently lower than WW. The recovery of RI photosynthetic capacity in comparison with ST plants could be due to higher stomatal conductance values [17].

Changes in fully irrigated plants after a constant severe soil water stress indicate a recovery from stress conditions, which did not fully restore the morphological and metabolic processes to the WW level. In this experiment senescence represented an irreversible break in the metabolic pathways and an irreversible reduction in leaf expansion rate.

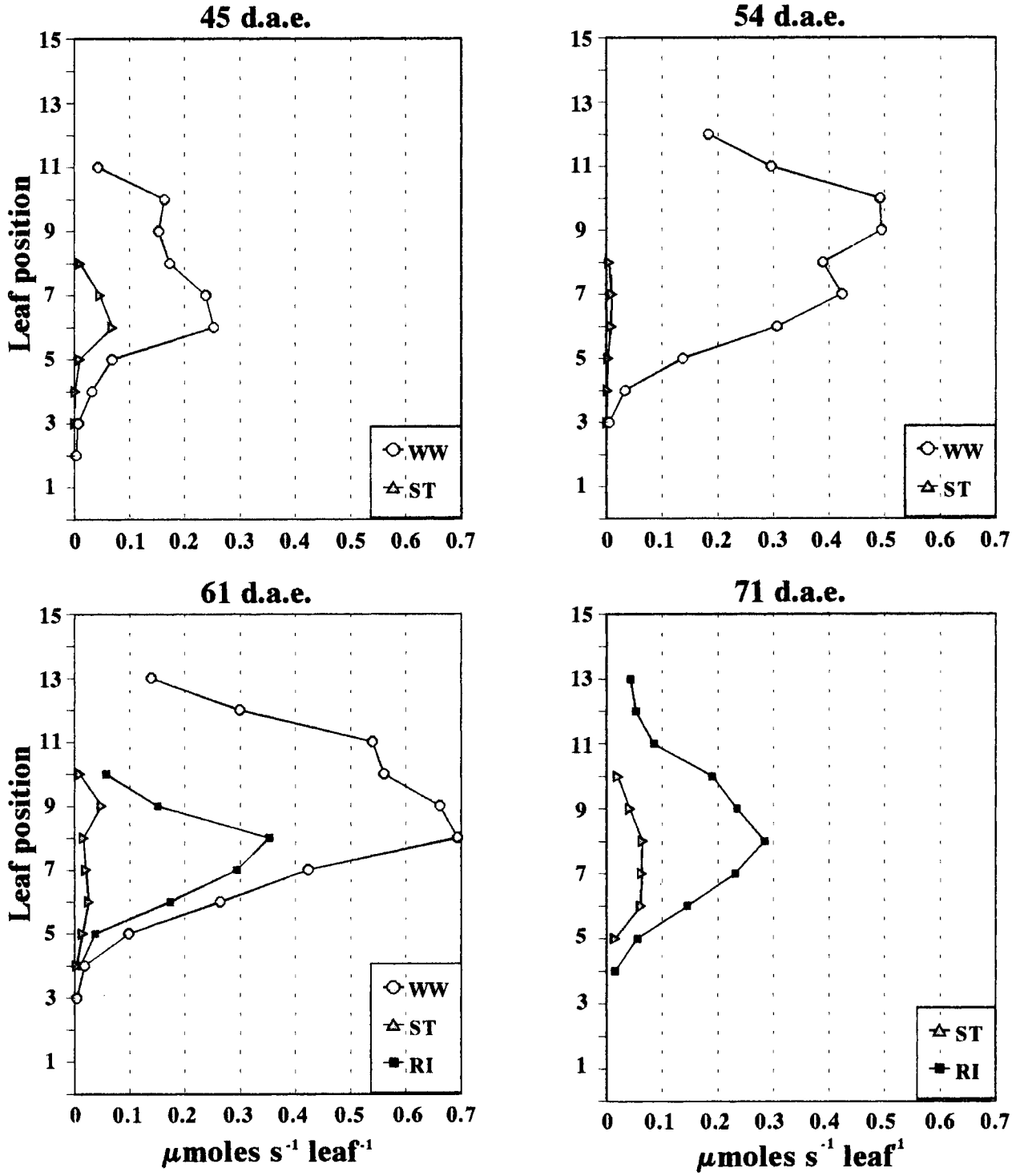

Figure 3. Photosynthesis per leaf at different dates for the three water treatments. WW = well-watered treatment, ST = water-stressed treatment, RI = re-irrigated treatment.

Acknowledgement: The authors are deeply grateful to Dr Francesco Loreto (CNR-IBEV, Rome, Italy) for critical review and suggestions.

References

[1] Arnon D.I., Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*, Plant Physiol. 24 (1949) 1–15.

[2] Bernstein N., Silk W.K., Läuchli A., Growth and development of sorghum leaves under conditions of NaCl stress: possible role of some mineral elements in growth inhibition, Planta 196 (1995) 699–705.

[3] Boyer J.S., Knipling E.B., Isopiestic technique for measuring leaf water potential in corn and soybean, Proc. Natl. Acad. Sci. 54 (1965) 1044–1051.

[4] Bremner J.M., Total nitrogen, in: Black C.A. et al. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, Agronomy 9, 1965, pp. 1149–1178.

[5] Carlson R.M., Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide, Anal. Chem. 50 (1978) 1528–1531.

[6] Dwyer L.M., Stewart D.W., Leaf area development in field-grown maize, Agron. J. 78 (1986) 334–343.

[7] Evans J.R., Nitrogen and photosynthesis in the flag leaf of wheat, Plant Physiol. 72 (1983) 297–302.

[8] Evans J.R., Photosynthesis and nitrogen relationships in leaves of C_3 plants, Oecologia 78 (1989) 9–19.

[9] Fedina I.S., Popova A.V., Photosynthesis, photorespiration and proline accumulation in water stress pea leaves, Photosynthetica 32 (1996) 213–220.

[10] Field C.B., Mooney H.A., The photosynthesis-nitrogen relationship in wild plants, in: Givnish T.J. (Ed.), On the Economy of Form and Function, Cambridge University Press, Cambridge UK, 1986, pp. 25–55.

[11] Hanson A.D., Hitz W.D., Whole-plant response to water deficits: water deficit and the nitrogen economy, in: Taylor H.M., Jordan W.R., Sinclair T.R. (Eds.), Limitations to Efficient Water Use in Crop Production, Michigan State University, East Lansing, Michigan, 1983, pp. 331–343.

[12] Jordan W.R., Brown K.W., Thomas J.C., Leaf age as a determinant in stomatal control of water loss from cotton during water stress, Plant Physiol. 56 (1975) 595–599.

[13] Kuppers M., Kock G., Mooney H.A., Compensating effects to growth of changes in dry matter allocation in response to variation in photosynthetic characteristics induced by photoperiod, light and nitrogen, Aust. J. Plant Physiol. 15 (1988) 287–298.

[14] Leffer H.R., Leaf growth and senescence, in: Hesketh J.D., Jones J.W. (Eds.), Predicting Photosynthesis for Ecosystem Models, CRC Press, Boca Raton, FL, Vol. 2., 1980, pp. 133–144.

[15] Lemaire G., Onillon B., Gosse G., Chartier M., Allirand J.M., Nitrogen distribution within a lucerne canopy during regrowth: Relation with light distribution, Ann. Bot. 68 (1991) 483–488.

[16] Leshem Y.Y., Halevy A.H., Frenkel C., Processes and Control of Plant Senescence, Elsevier Science Publisher BV, Amsterdam, The Netherlands, 1986, pp. 250.

[17] Massacci A., Battistelli A., Loreto F., Effect of drought stress on photosynthetic characteristic, growth and sugar accumulation of field-grown sweet sorghum, Aust. J. Plant Physiol. 23 (1996) 331–340.

[18] Mooney H.A., Field C., Gulmon S.L., Bazzaz F.A., Photosynthesis capacity in relation to leaf position in desert versus oldfield annuals, Oecologia 50 (1981) 109–112.

[19] Nooden L.D., Senescence in the whole plant, in: Thimann K.V. (Ed.), Senescence in Plants, CRC Press, Boca Raton, USA FL, 1980, pp. 219–258.

[20] Runeckles V.C., Relative death rate: A dynamic parameter describing plant response to stress, J. Appl. Ecol. 19 (1982) 295–303.

[21] Sexton R., Woolhouse H.W., Senescence and abscission, in: Wilkins M.B. (Ed.) Advanced Plant Physiology, Longman Scientific and Technical, USA NY, 1984, pp. 469–497.

[22] Swank J.C., Below F.E., Lambert R.J., Hageman R.H., Interaction of carbon and nitrogen metabolism in the productivity of maize, Plant Physiol. 70 (1982) 1185–1190.

[23] Wittenbach V.A., Ribulose bisphosphate carboxylase and proteolytic activity in wheat leaves from anthesis though senescence, Plant Physiol. 64 (1979) 884–887.

[24] Wolfe D.W., Water and nitrogen effects on leaf senescence in maize (*Zea mais* L). Ph.D. thesis, Univ. of California, Davis, 1984 (Diss. Abstr. 85–07341).

[25] Woolhouse H.W., Leaf senescence, in: Smith H., Grierson D. (Eds.), The Molecular Biology of Plant Development, University of California Press, Berkeley, Botanical Monographs, USA CA, Vol. 18, 1982, pp. 256–281.