# Leaf appearance rate and final main stem leaf number as affected by temperature and photoperiod in cereals grown in Mediterranean environment

**Ezio Riggi,[1] Danilo Scordia,[2] Concetta Foti Cuzzola,[2] Giorgio Testa,[2] Salvatore L. Cosentino[2]**

*[1]Trees and Timber Institute, National Research Council (CNR - IVALSA), Catania; [2]Department of Agriculture, Food and Environment (Di3A), University of Catania, Italy*

## Abstract

In the present study, a two-year field trial was carried out with the aim to evaluate daylength and air temperature effects on leaf appearance and related rates in two durum wheat (*Triticum durum* Desf.), two bread wheat (*Triticum aestivum* L.) and two barley (*Hordeum vulgare* L.) cultivars, using six different sowing dates (SD). Significant effects of SD on final main stem leaf number (FLN), thermal leaf appearance rate (TLAR), daily leaf appearance rate (DLAR) and phyllochron (PhL) were found. Cultivars resulted inversely correlated to mean air temperature in the interval emergence - fifth leaf full expansion (E-V). Linear response of leaf number over days after sowing was shown for all SD and cultivars, with $R^2$ higher than 0.95. FLN linearly decreased from the first to the last SD for durum wheat, while more variable behaviour was observed in bread wheat. TLAR and DLAR showed a linear increment of the rate from the first to the last SD in durum wheat, while did not for bread wheat and barley. PhL in durum wheat decreased from the first to the last SD. Barley and bread wheat showed the highest values on those SDs which did not reach flowering. The increase of TLAR was affected by photoperiod and photothermal units in durum wheat, while by temperatures only in barley and bread wheat. Present results might find practical application in the improvement of phenology simulation models for durum wheat, bread wheat and barley grown in Mediterranean area in absence of water and nutrient stress.

Correspondence: Danilo Scordia, Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy.
Tel: +39 095 234496 - Fax: +39 095 234449.
E-mail: dscordia@unict.it

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## Introduction

In warm and cool temperate climates of Europe, durum wheat (*Triticum durum* Desf.), bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are the most cultivated crops both for food and forage production (Miralles *et al*., 2001; FAOSTAT, 2010).

The knowledge on the relationship between crop phenology and environmental conditions represents a key factor for the definition of algorithms involved in the elaboration of prediction models. Within the most relevant phenophases, leaf appearance has been particularly stressed, and the relationships with environmental factors have been studied in wheat and barley with the aim to understand the dynamics of the leaf area development intercepting solar radiation (Kirby *et al*., 1985; Kirby, 1990; Hay and Kirby, 1991; Miglietta, 1991a).

Kirby (1990) has shown that apical meristematic tissue development and leaf appearance are strictly related. Both leaf emission rate and final main stem leaf number affect some subsequent phenological events, such as floral induction, heading and blooming, in turn influencing crop yield and quality (Amir and Sinclair, 1991).

The leaf appearance rate (LAR) can be estimated from a potential, maximal rate which is reduced as a function of temperature or other factors. Linear, two-segment, three-segment, truncated quadratic curve and beta function have been proposed (Yan and Hunt, 1999; White *et al*. 2012). However, it has been shown that there is no benefit from using more complex form of two-segment function of temperature, with optimum at 22°C in wheat (White *et al*., 2012).

Relationships between LAR and environmental variables have been mainly studied on bread wheat and barley in both open field and controlled environments. However, only few open field experiments have been reported on durum wheat (Giunta *et al*., 2001; Streck *et al*., 2003; Ferrise *et al*., 2010), the most involved cereal crop in Mediterranean cropping systems.

Under both growth chambers and field conditions it has been shown that the phyllochron (PhL) greatly varies with sowing dates (Baker *et al*., 1980; Kirby and Perry, 1987; McMaster *et al*., 1992), main stem leaf number (Miglietta, 1991a), phenological stage (Baker *et al*., 1986; Boone *et al*., 1990; Jamieson *et al*., 1995), and genotype (Frank and Bauer, 1995). Other variables, such as soil temperature close to the shoot apex, have been suggested to improve prediction of the PhL in wheat (Jamieson *et al*., 1995; McMaster *et al*., 2003).

Under field conditions, rather than controlled environments, air temperature and daylength greatly changes during the growing season, affecting to some extent leaf primordia, LAR, canopy light interception and photosynthesis, time of flowering, accumulation of dry matter and yield (McMaster *et al*., 2003).

In the present work, air temperature and daylength effects on leaf emission and related rates of durum wheat, bread wheat and barley were evaluated. A two-year field experiment was carried out using six different sowing dates in a costal area of eastern Sicily (Italy), characterised by typical Mediterranean environmental conditions with hot, dry summers and cool, moist winters.

## Materials and methods

### Field trial set-up

The field trial was carried out at the Experimental Farm of the University of Catania (37°24' N., 15°03' E., 10 a.s.l.), during 2002-03 and 2003-04 growing seasons on a sandy-loam-clay soil classified as Typic Xerofluvents, keeping the following hydrological characteristics: field capacity (FC) at -0.03 MPa (27% dry soil weight) and wilting point (WP) at -1.5 MPa (11% dry soil weight).

Two *Triticum durum* Desf. cultivars Creso and Simeto, two *Triticum aestivum* L., Centauro and Soissons, and two *Hordeum vulgare* L., Amillis and Gotic, were sown on six different sowing dates (SD), in order to explore a wide range of thermal and daylength conditions. Adopted cultivars hold a winter growth habit, and all are suited to the Mediterranean environmental conditions.

Sowing dates were ranked as following: 10/11/03 (I SD), 17/12/02 (II SD), 21/12/03 (III SD), 20/01/03 (IV SD), 04/02/04 (V SD) and 05/03/03 (VI SD). Middle to late autumn is the typical sowing window for winter cereals in this environment.

A randomised blocks design three times replicated was used. Plant densities of 300, 350 and 250 plants $m^{-2}$ for durum, bread wheat and barley were adopted, respectively. All plots were fertilised one month before sowing with 110 kg $ha^{-1}$ $P_2O_5$ as mineral superphosphate and 80 kg $ha^{-1}$ $K_2O$ as sulphate of potash. Nitrogen (100 kg $ha^{-1}$) was distributed in two times (50% before sowing, as ammonium sulphate, and 50% at V-leaf stage, as ammonium nitrate). Weeds were controlled manually throughout the growing seasons.

### Measurements

Air temperatures and rainfall were measured in continuum with sensors connected to a data logger (CR 10, Campbell Scientific Inc., UT, USA) in a meteorological station beside the experimental field. Photoperiod (civil twilight to civil twilight) was calculated adopting Keisling equation (1982) and checked against tabulated values of the US Naval Observatory.

Soil moisture was monitored throughout the growing seasons in order to avoid water stress effect. Soil water content was measured gravimetrically, collecting soil samples at 0-60 cm soil depth in three replicates for each treatment. Soil samples were dried in a ventilated oven at 105°C until constant weight. The available soil water content (assumed as available water stored in the soil between field capacity and wilting point) never approached the 33% of its content, which is considered a threshold after which the plant starts to suffer (Patanè *et al*., 2010).

Ten plants per plot were tagged and weekly observed to record developmental stages. Dates of emergence (10), V-leaf (15), stem elongation start (30) and last leaf (39), were determined when 50% of observed plants reached a specific stage (Zadoks *et al*., 1974). The measurement of number of appeared leaves was determined by scoring main stem leaf development on each plant according to Haun (1973). Each leaf was defined as emerged when the tip of the lamina was visible above the uppermost auricles of the subtending leaves (Kirby *et al*., 1985). The final main stem leaf number (FLN) was registered as flag leaf emerged and reported as mean number of leaves per main stem in ten plants.

The duration between plant emergence and leaf appearance was expressed in number of days and in growing degree days (GDD), calculated as sum of daily difference between mean air temperature and base temperature, according to:

$$GDD=\sum_{i=1}^{n}\left[(T_i\max+T_i\min)/2\right]-T_{base}$$

where $n$ is the number of days from plant emergence, $T_i max$ and $T_i min$ are the maximum and the minimum air temperature at day $i$, respectively, and $T_{base}$ is the minimum threshold temperature when the plant stops its growing. A $T_{base}$ of 0 °C, as proposed by many authors, for durum wheat (Gallagher, 1979; Baker and Gallagher, 1983; Baker *et al*., 1986; Hay and Delècolle, 1989), bread wheat (Friend *et al*., 1962; Bauer *et al*., 1984; Baker *et al*., 1986; Jame *et al*., 1998; Jamieson *et al*., 2008) and barley (McMaster and Wilhelm, 2003) was used. Leaf appearance rate (LAR) was calculated by plotting the number of leaves appeared and cumulated GDD at the same date (TLAR), or number of days from emergence (DLAR). In both methods, the LAR was assessed by the regression coefficient of the fitted linear equation, which represents the fraction of leaf developed for each GDD (leaves °C $day^{-1}$) or for each day (leaves $day^{-1}$).

The phyllochron (PhL), representing the amount of GDD requested for the full expansion of each leaf (°C day $leaf^{-1}$), was calculated using a linear regression where the slope represents the PhL. The effects of maximum (Tmax), mean (Tmean), minimum (Tmin) air daily temperature and photoperiod (h) measured at different phenophase, namely sowing (S), plant emergence (E), fifth leaf full expansion (V) and jointing (J) stages were studied on LAR, PhL and FLN. In addition, the average between intervals (S-E, S-V, S-J, E-V and E-J) and the photothermal index were also considered. Photothermal index was calculated, for each stage or interval, by multiplying air temperature and photoperiod (Tmax*h, Tmean*h and Tmin*h). All these environmental parameters were correlated with TLAR, DLAR, PhL and FLN of each cultivar within the six SDs by using the Pearson's correlation test at 95% confidence level (Minitab 16 Statistical Software).

Data of FLN, DLAR, TLAR and PhL were subjected to a two-way ANOVA according to the randomised block design, with sowing date and cultivar as fixed factors. Before performing the ANOVA, results were previously evaluated according to Bartlett's test for homogeneity of variances. When statistical significance was observed, Student Newman Keuls test was carried out at 95% confidence level. The relationships between main stem leaf number until booting and sowing dates were calculated by linear regressions. The Shapiro-Wilk test was developed to test residuals for normality. Coefficients were considered significant when P-value was ≤0.05. The goodness of fit was assessed by calculating $R^2$ (SigmaPlot11, Systat Software Inc., San Jose, CA, USA).

## Results

### Meteorological data and phenology

Air temperature measured during the first and the second growing season reached its highest value on June 1st (31.0°C) and on May 31st (32.6°C), while the lowest on the last decade of March (6.1°C) and on the first of February (1.2°C) (Figure 1). The mean daily air temperature, within each year in the interval between the first sowing date and the last jointing date, showed higher value in 2002/03 than in 2003/04 (respectively 13.2 and 12.1°C). By comparing the mean air temperature to the past 30-year-period, higher values (+1.5 °C) were recorded in 2002/03, while in the second year mean temperature was in line with the historical data.

Rainfalls were well distributed during the two growing seasons and the available soil water content did not fall below the 33%; hence, no water stress was encountered. Throughout the growing seasons, about 250 mm of rainfall were recorded, which were mainly concentrated in autumn and winter with some events in spring-time (about 50 and 40 mm in 2003 and 2004, respectively). Photoperiod measured at emergence ranged between 10h35' (SD II) and 14h03' (SD VI).

The interval emergence - fifth leaf full expansion (E-V) resulted inversely correlated to Tmean measured in the same period and it was significant for all cultivars, with correlation coefficients ranging from 0.82* (Centauro) to 0.93** (Simeto). The relationships between Tmin or Tmax and phenological stage durations, although negatively correlated, showed more variable trends within species. Photoperiod and phenological stage durations showed significant negative correlations in both cultivars of durum wheat (E-J period), while more variable trends were again observed in bread wheat and barley (Table 1).

Referring to the whole crop growing cycle, including the reproductive phase, it is worth to mention that on the latest SD (VI), barley and bread wheat cultivars did not attain heading stage. Soissons (bread wheat) did not reach heading stage also on IV and V SD. On these SD by genotype combinations, Tmean measured during the E-J interval exceeded 14.0°C, suggesting an influence of air temperature on the reproductive phase of these cultivars.

### Final main stem leaf number

FLN decreased rather linearly from the first to the last SD for both durum wheat cultivars (Table 2). For barley and bread wheat the highest FLN was registered on the IV SD. This unexpected result lead to significantly highest FLN at the IV SD across the average of genotypes; however, it was not significantly different from the I and the II SD. The lowest FLN was at the VI SD. Averaged across SDs, Soissons showed the significantly highest FLN, while both durum wheat the lowest. A SD x cultivar interaction was observed (P≤0.05).

Linear response of leaf number over days after sowing was shown for all the SD treatments in durum wheat, bread wheat, and barley cultivars (Figure 2) with $R^2$ of 0.95 or higher. The slope of the fitted linear regressions varied with SD, suggesting a faster leaf emission as the sowing was delayed. It represents the rate of leaf appearance respect to time (DLAR) and will be discussed later.

### Leaf appearance rate and phyllochron

TLAR (leaves °C day$^{-1}$) was significantly highest at the III and V SD averaged across genotypes, while it was the lowest at the II, IV and VI SD. Averaged across SDs, both durum wheat showed the highest values, while Soisson the lowest (Table 3).

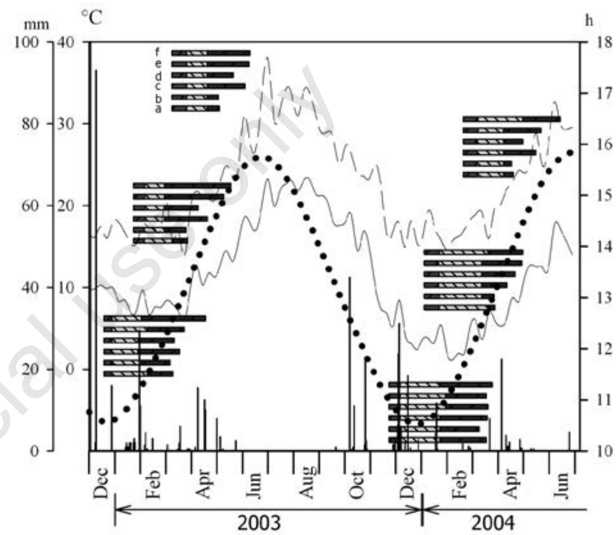


**Figure 1. Maximum (bold line) and minimum (fine line) air temperature, daylength (dotted line) and rainfall (vertical bars) in the two growing seasons. Stacked bars (groups of six elements represent I to VI sowing dates from the bottom to the top of the graph) for each cultivar (a=Simeto; b=Creso; c=Amillis; d=Gotic; e=Centauro; f=Soissons), in S-E (first stack), E-V (second stack) and V-J (last stack) phenological intervals.**



**Table 1. Correlation coefficients between environmental variables during plant phenological intervals in two durum wheat (Simeto and Creso), two bread wheat (Centauro and Soissons) and two barley (Amillis and Gotic) cultivars.**

| Environmental variables | Phenological intervals | *T. durum* Simeto | *T. durum* Creso | *H. vulgare* Amillis | *H. vulgare* Gotic | *T. aestivum* Centauro | *T. aestivum* Soissons |
|---|---|---|---|---|---|---|---|
| Air temperature | | | | | | | |
| Tmean | E-V | -0.93** | -0.85* | -0.92** | -0.91* | -0.82* | -0.82* |
| | S-V | -0.83* | | -0.85* | | -0.82* | -0.85* |
| Tmax | E-V | | -0.85* | | | | |
| | V-J | -0.87* | | | -0.84* | | |
| | E-J | -0.90* | -0.84* | | | -0.85* | |
| Tmin | S-E | | | | | | |
| | E-V | -0.86* | | -0.87* | -0.85* | | -0.91* |
| | S-V | | | -0.82* | | | -0.94** |
| Daylength | | | | | | | |
| Hours | V-J | -0.83* | | | -0.82* | | |
| | S-J | -0.82* | | | | | -0.84* |
| | E-J | -0.88* | -0.86* | | | | -0.84* |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L.

DLAR (leaves day$^{-1}$) resulted the significantly highest at the VI SD averaged across genotypes, while it was the lowest at the I SD. Averaged across SDs, Simeto showed the highest values and Soissons the lowest (Table 4).

Averaged across genotypes, PhL (°C day leaf$^{-1}$) was the significantly highest at the VI SD, while it was the lowest at the III SD. Averaged across SDs, Soissons showed the highest value and Simeto the lowest (Table 5).

Significant SD x cultivar interactions ($P \leq 0.05$) were observed for TLAR, DLAR and PhL.

It is noteworthy that within each genotype of durum wheat, steady decreasing trends were observed delaying SD. On the other hand, in barley and bread wheat the highest values were reached on those SDs which did not attain flowering. Indeed, in barley and bread wheat cultivars on the last SD, and on the IV and the V SD for Soissons, when jointing stage was not reached, the highest PhL (from 140 to 155.9 °C day leaf$^{-1}$) was observed.

### Environmental variables correlations with leaf appearance rate and final main stem leaf number

As expected, the increments of the TLAR from the first to the last SD for both durum wheat cultivars showed significant positive correlations with photoperiod (Table 6). The highest coefficients of correlation were shown with photoperiod measured at the fifth leaf full expansion stage (V), when photoperiod ranged from 10h40' to 14h03'(0.90* and 0.96** for Simeto and Creso, respectively). No significant correlations were found for barley and bread wheat with photoperiod on each phenological stage or interval.

In spite of what showed for daylength, air temperature appeared strongly and inversely correlated to TLAR in barley and bread wheat. Indeed, significant correlation coefficients were found between TLAR and Tmean during E-V interval (-0.96**, -0.85*, -0.86* and -0.92* for Amillis, Gotic, Centauro and Soissons, respectively). When the Tmean during S-V interval was correlated to TLAR, improvements were reported for barley (-0.98*** and -0.95** for Amillis and Gotic, respectively). In both durum wheat cultivars a positive correlation emerged between Tmax measured during E-V interval and TLAR (0.87* in Simeto and 0.91* in Creso).

When the photothermal units (Tmin*h) and TLAR were calculated in the E-V interval for barley and bread wheat cultivars, significant negative correlations were found, leading to improved correlations for bread wheat as compared with the environmental variables alone (Tmin or daylenght at E-V). Photothermal units (Tmax*h) at E-V interval in durum wheat improved the correlation coefficients as well, reaching 0.94** in Creso.

As far as DLAR is concerned, significant positive correlations were obtained with photoperiod in both durum wheats, particularly on the E-V interval (0.94** and 0.99*** for Simeto and Creso, respectively), as shown in Table 7. The relationship with photoperiod at E and at V (0.88* and 0.94** for Simeto and 0.90* and 0.99*** for Creso, respectively), allowed to emphasise that photoperiod at later stages had a greater influence on DLAR as compared with the photoperiod at emergence.

Photothermal units, although significant for durum wheat, did not improve the correlation coefficients as compared with photoperiod alone.

The relationships between DLAR and air temperature, photoperiods or photothermal units at any phenophase or interval were not significant for both barley and bread wheat cultivars.

Although the PhL was calculated by plotting GDD over leaf number rather than the inverse of TLAR, the relationships between



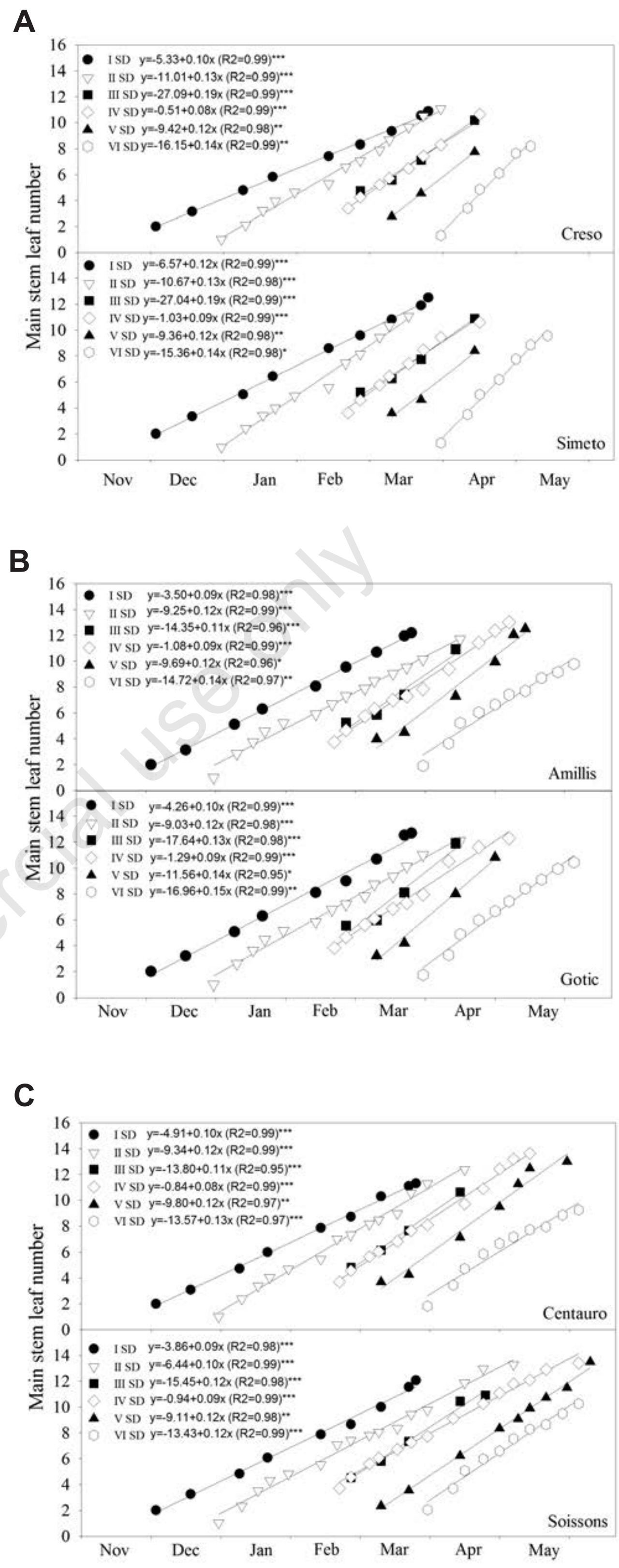


**Figure 2. Main stem leaf number until booting for the six adopted sowing dates in (A) two durum wheat cultivars (Creso and Simeto), (B) two bread wheat cultivars (Centauro and Soissons) and (C) two barley cultivars (Amillis and Gotic). Significance of the fitted linear regressions (*P≤0.05; **P≤0.01; ***P≤0.001).**

PhL and environmental variables or photothermal units assumed similar ranges observed in the relationships involving TLAR, but with opposite sense in all cultivars (Table 8).

## Discussion

For all cereal crops involved in the present experiment, sowing date strongly affected the FLN, TLAR, DLAR and PhL. Many authors reported results of experiments conducted in open field or under controlled conditions, studying the response to air temperature and photoperiod separately or coupled on leaf development in cereal crops (Miglietta, 1989, 1991b; Bassu *et al*., 2009; He *et al*., 2012). According to Miglietta (1989, 1991b) leaf primordia formation in durum and bread wheat, in absence of severe stresses, depends only by the temperature, while it is fully independent by the photoperiod, sowing time, nitrogen and water deficit. Jamieson *et al*. (1995) well predicted the LAR based on temperature near the apical meristem and leaf number without the necessity to include a response to daylength or its rate of change in wheat.

These observations support our findings on TLAR and PhL, in

**Table 2. Final main stem leaf number in different sowing dates of durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Sowing date | *T. durum* | | *H. vulgare* | | *T. aestivum* | | |
|---|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons | Average |
| I | 12.0 | 11.7 | 12.2 | 13.0 | 11.4 | 12.1 | 12.1[a] |
| II | 11.3 | 11.3 | 11.5 | 12.0 | 12.4 | 13.2 | 11.9[a] |
| III | 10.1 | 10.3 | 11.0 | 11.0 | 10.7 | 11.0 | 10.7[c] |
| IV | 10.6 | 10.3 | 13.5 | 12.5 | 13.6 | 13.4 | 12.3[a] |
| V | 9.6 | 9.8 | 11.6 | 11.1 | 12.2 | 12.3 | 11.1[b] |
| VI | 9.1 | 8.2 | 10.5 | 10.1 | 9.3 | 10.3 | 9.5[d] |
| Average | 10.5[c] | 10.3[c] | 11.7[b] | 11.6[b] | 11.6[b] | 12.1[a] | |
| $LSD_{(0.05)}$ | | | 0.748 | | | | |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L.; $LSD_{(0.05)}$, least significant difference at P≤0.05 of sowing date x cultivar interaction. [a-d]Across average, different letters represent statistical significance of P≤0.05 (SNK test).

**Table 3. Thermal leaf appearance rate (leaves °C day$^{-1}$) in different sowing dates of durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Sowing date | *T. durum* | | *H. vulgare* | | *T. aestivum* | | |
|---|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons | Average |
| I | 0.0096 | 0.0081 | 0.0095 | 0.0098 | 0.0087 | 0.0090 | 0.0094[b] |
| II | 0.0103 | 0.0088 | 0.0074 | 0.0080 | 0.0084 | 0.0075 | 0.0083[c] |
| III | 0.0103 | 0.0100 | 0.0105 | 0.0119 | 0.0105 | 0.0095 | 0.0106[a] |
| IV | 0.0101 | 0.0100 | 0.0085 | 0.0084 | 0.0083 | 0.0070 | 0.0087[c] |
| V | 0.0119 | 0.0119 | 0.0091 | 0.0115 | 0.0095 | 0.0068 | 0.0103[a] |
| VI | 0.0117 | 0.0119 | 0.0065 | 0.0076 | 0.0062 | 0.0069 | 0.0086[c] |
| Average | 0.0106[a] | 0.0102[a] | 0.0088[c] | 0.0097[b] | 0.0086[c] | 0.0080[d] | |
| $LSD_{(0.05)}$ | | | | 0.012 | | | |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L.; $LSD_{(0.05)}$, least significant difference at P≤0.05 of sowing date x cultivar interaction. [a-d]Across average, different letters represent statistical significance of P≤0.05 (SNK test).

**Table 4. Daily leaf appearance rate (leaves day$^{-1}$) in different sowing dates of durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Sowing date | *T. durum* | | *H. vulgare* | | *T. aestivum* | | |
|---|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons | Average |
| I | 0.0925 | 0.0776 | 0.0914 | 0.0974 | 0.0844 | 0.0870 | 0.088[f] |
| II | 0.1270 | 0.1035 | 0.0929 | 0.1021 | 0.1060 | 0.0958 | 0.104[e] |
| III | 0.1231 | 0.1189 | 0.1243 | 0.1404 | 0.1248 | 0.1188 | 0.125[c] |
| IV | 0.1324 | 0.1302 | 0.1187 | 0.1179 | 0.1189 | 0.1000 | 0.120[d] |
| V | 0.1448 | 0.1466 | 0.1347 | 0.1537 | 0.1355 | 0.1130 | 0.138[b] |
| VI | 0.1920 | 0.1916 | 0.1148 | 0.1338 | 0.1103 | 0.1216 | 0.144[a] |
| Average | 0.135[a] | 0.128[b] | 0.113[d] | 0.124[c] | 0.113[d] | 0.106[e] | |
| $LSD_{(0.05)}$ | | | 0.012 | | | | |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L.; $LSD_{(0.05)}$, least significant difference at P≤0.05 of sowing date x cultivar interaction. [a-f]Across average, different letters represent statistical significance of P≤0.05 (SNK test).

bread wheat and barley, which were both affected by mean and minimum air temperature rather than by photoperiod (Tables 6 and 7).

Other authors argued on the effect of water stress, nutrients availability, $CO_2$ and salinity to affect LAR and PhL (Maas and Grieve, 1990; Longnecker *et al*., 1993; McMaster *et al*., 2003). It is noteworthy that nitrogen and water stress were not observed in the present study in the different SDs, therefore the relationships were fully dependent by the temperature (minimum, mean and maximum), daylength or the combination of both environmental variables (photothermal index).

The PhL in barley and bread wheat was greater in those SDs which did not attain flowering. This might be associated to a lack of vernalisation of these cultivars at later sowing dates. Worland (1996) reported that both daylength and vernalisation control the final leaf number, explaining the highest PhL observed in barley and bread wheat at SD with increasing daylength associated to an increase of air temperatures. An adaption strategy of wheat when plants emerge too early or too late, or experience a slightly different temperature was hypothesised by Brooking *et al*. (1995); in these conditions, authors proposed that when LAR differs, plants will compensate by experiencing daylength at different times when they set their final leaf number for synchronising anthesis.

The PhL in durum wheat decreased when SD was postponed. In accordance to Giunta *et al*. (2001), the decrease of PhL from early to late sowings was associated to a decrease in final number of leaves. Our results on PhL of durum wheat are in the range reported by Ferrise *et al*. (2010) by using November and January sowings, and Bassu *et al*. (2009) with October and March sowings in Mediterranean environments.

PhL variation with sowing time, and the impact of the variation of leaf number and PhL on important phenological events, such as flowering, is still a controversial matter. It is generally assumed that measurements of the PhL in wheat using air temperature show a systematic variation with sowing date, with long phyllochrons for crops sown in autumn and winter, but shorter phyllochrons for sowings outside that range (Jamieson *et al*., 2008). It has been suggested that the apparent variation with sowing dates was an artefact, and that the PhL was in fact constant for any particular cultivar (Jamieson *et al*., 1995). Recently, Jamieson *et al*. (2008) showed that air temperature measurements might be the source of error for reported variation of PhL with sowing date. Authors suggested that LAR responds linearly to shoot apex temperature, as long as temperature measurements are made close to the site of perception, providing strong evidence for the concept of a constant PhL. This assumption constitutes an important step forward in prediction models of flowering based on PhL and FLN of determined



**Table 5. Phyllochron (°C day leaf$^{-1}$) in different sowing dates of durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Sowing date | *T. durum* | | *H. vulgare* | | *T. aestivum* | | |
|---|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons | Average |
| I | 104.1 | 124.1 | 105.4 | 98.0 | 114.1 | 110.3 | 109.3$^d$ |
| II | 95.8 | 112.4 | 134.0 | 130.5 | 117.4 | 131.6 | 120.3$^b$ |
| III | 95.8 | 98.7 | 92.5 | 80.5 | 95.4 | 103.8 | 94.4$^f$ |
| IV | 95.2 | 99.4 | 117.2 | 118.5 | 119.7 | 141.5 | 115.3$^c$ |
| V | 83.2 | 83.6 | 107.7 | 86.2 | 104.2 | 142.6 | 101.3$^e$ |
| VI | 84.3 | 83.1 | 145.4 | 155.9 | 150.1 | 140.0 | 126.5$^a$ |
| Average | 93.1$^e$ | 100.2$^d$ | 117.1$^b$ | 111.6$^c$ | 116.8$^b$ | 128.3$^a$ | |
| $LSD_{(0.05)}$ | | | 0.205 | | | | |

*T. durum*, *Triticum durum*; *H. vulgare*, *Hordeum vulgare* L.; *T. aestivum*, *Triticum aestivum* L.; $LSD_{(0.05)}$, least significant difference at P≤0.05 of sowing date x cultivar interaction. [a-f]Across average, different letters represent statistical significance of P≤0.05 (SNK test).

**Table 6. Correlation coefficients between environmental variables, in different phenological stages and sowing dates, and thermal leaf appearance rate for durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Environmental variables | *T. durum* | | *H. vulgare* | | *T. aestivum* | |
|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons |
| Daylength | | | | | | |
| hE-V | 0.87* | 0.93** | | | | |
| hS-V | 0.86* | 0.91* | | | | |
| hV | 0.90* | 0.96** | | | | |
| Air temperature | | | | | | |
| TmeanE-V | | | -0.96** | -0.85* | -0.86* | -0.92* |
| TmeanS-V | | | -0.98*** | -0.95** | | |
| TminE-V | | | -0.97** | -0.92** | -0.88* | |
| TminS-V | | | -0.93** | -0.98*** | | |
| TmaxE-V | 0.87* | 0.91* | | | | |
| Photothermal units | | | | | | |
| Tmin*hE-V | | | -0.97** | -0.86* | -0.92* | -0.83* |
| Tmax*hE-V | 0.89* | 0.94** | | | | |

*T. durum*, *Triticum durum*; *H. vulgare*, *Hordeum vulgare* L.; *T. aestivum*, *Triticum aestivum* L. *(P≤0.05); **(P≤0.01); ***(P≤0.001).

plants as wheat, since an error of one leaf in predicting emergence of the flag leaf can introduce an error of several days in the prediction of anthesis date depending on temperature (Jamieson *et al*., 1998; Xue *et al*., 2004).

Increasing trends for TLAR were reported only for durum wheat cultivars delaying SD, suggesting again a relation with daylength. Findings obtained by Cao and Moss (1989a, 1989b, 1989c, 1991), adopting daily leaf appearance rate (DLAR), substantially confirms the direct influence of air temperature and photoperiod on this rate. According to what observed by these authors, for durum wheat cultivars, the highest TLAR and DLAR values have been obtained on SD with the longest daylength.

The relationship between the LAR or the FLN with air temperatures and photoperiod, measured on different phenophases or intervals, emphasises the sensitivity of these indices to environmental variables and the most sensitive stages. As expected, significant correlations with photoperiod were found for durum wheat. The highest correlations were shown for the relation with photoperiod measured at fifth leaf full expansion stage (V) when photoperiod was from 10h40' to 14h03'(0.90* and 0.96** for Simeto and Creso, respectively). The positive sense of correlations suggest the increase of the efficiency of each GDD in terms of leaf production, when photoperiod raises. On the other hand, no significant correlations were found for barley and bread wheat cultivars with photoperiod on each phenophase or interval evaluated.

Air temperature (min and mean) appeared strongly and inversely related to TLAR in barley and bread wheat. By contrast to what reported for the positive correlation with photoperiod in durum wheat, the negative sense suggests the reduction of the efficiency of each GDD with the increase of air temperature, as also argued by Cao and Moss (1989a).

Mean temperature during S-V interval was correlated to TLAR, showing significant correlations for barley, but neither for durum nor for bread wheat cultivars.

A positive relationship emerged between maximum air temperature measured during E-V interval and TLAR for durum wheat, however did not for barley and bread wheat. Daily maximum air temperature linearly increased from 16.9 to 18.3°C during E-V and this variation seems to reduce the thermal energy requested to produce leaves.

Photothermal units during E-V interval (Tmin*h) were significant for barley and bread wheat, while did not for durum wheat. When Tmax*h at E-V interval was calculated, durum wheat resulted significantly affected, while bread wheat and barley were not.

In durum wheat, the significant coefficients with photoperiod at E and at V suggested that photoperiod in the later stages influenced



**Table 7. Correlation coefficients between environmental variables, in different phenological stages and sowing dates, and daily leaf appearance rate for durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Environmental variables | *T. durum* | | *H. vulgare* | | *T. aestivum* | |
|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons |
| Daylength | | | | | | |
| hE-V | 0.94** | 0.99*** | | | | |
| hS-V | 0.94** | 0.98*** | | | | |
| hE | 0.88* | 0.90* | | | | |
| hV | 0.94** | 0.99*** | | | | |
| Air temperature | | | | | | |
| TmeanS-E | | | | | | |
| TmeanV | 0.93** | | | | | |
| TmaxE-V | | 0.86* | | | | |
| TmaxV | | | | | | |
| Photothermal units | | | | | | |
| Tmean*hE-V | 0.91* | 0.87* | | | | |
| Tmax*hE-V | 0.91* | 0.97** | | | | |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L. *(P≤0.05); **(P≤0.01); ***(P≤0.001).

**Table 8. Correlation coefficients between environmental variables, in different phenological stages and sowing dates, and phyllochron for durum wheat (Simeto and Creso), barley (Amillis and Gotic) and bread wheat (Centauro and Soissons).**

| Environmental variables | *T. durum* | | *H. vulgare* | | *T. aestivum* | |
|---|---|---|---|---|---|---|
| | Simeto | Creso | Amillis | Gotic | Centauro | Soissons |
| Daylength | | | | | | |
| hE-V | -0.89* | -0.91* | | | | |
| Air temperature | | | | | | |
| TmeanE-V | | | 0.94** | 0.90* | 0.84* | 0.90* |
| TmaxE-V | -0.87* | -0.87* | | | | |
| Photothermal units | | | | | | |
| Tmean*hE-V | | | 0.86* | 0.83* | 0.85* | 0.86* |
| Tmin*hE-V | | | 0.96** | 0.94** | 0.90* | 0.82* |
| Tmax*hE-V | -0.90* | -0.91* | | | | |

*T. durum, Triticum durum; H. vulgare, Hordeum vulgare* L.; *T. aestivum, Triticum aestivum* L. *(P≤0.05); **(P≤0.01).

more strongly DLAR with respect to photoperiod at emergence.

Temperatures (min and mean) coupled or not with daylength at E-V interval strongly affected barley and bread wheat, while durum wheat seems to be more affected by Tmax rather than Tmin or Tmean.

Other experiments, to accurately predict the LAR, introduced the effect of seed reserves and a chronology response function on LAR for winter wheat (Strek *et al*., 2003), or soil temperatures near the crown depth instead of air temperatures above the canopy to predict LAR on bread wheat (Jamieson *et al*., 1995).

Our results strengthen the idea that together with maximum temperatures, photoperiod is one of the main determinant in durum wheat LAR and PhL with changing SD.

Jamieson *et al*. (2008) raised a number of points on daylength and temperature effects to further improve LAR predictions. Authors pointed out that daylength effects on LAR are possibly due to a confounded mechanism involving the actual temperature of the organs, suggesting that the observed response might be caused by the physics of the system rather than the biology.

## Conclusions

Dynamics of leaf development in cereal plants is a key step necessary in order to estimate the radiation intercepted by the crop on the construction of simulation models for crop growth and production. In earlier works, leaf development was calculated using less effective photothermal models (Tuttobene *et al*., 1993; Cosentino *et al*., 2002).

Present findings allow underlining that an increasing trend for TLAR was reported only for durum wheat cultivars by delaying SD; it hints a relation with environmental variables which varied linearly during the studied period, such as the photoperiod. Although this strong modification of the biological cycles did not influence dramatically the final number of main stem leaf, a relevant reduction of the efficiency of the thermal energy involved in leaf production might be supposed.

In spite of what showed for daylength, air temperature appeared strongly and inversely related to TLAR in barley and bread wheat.

According to DLAR findings, significant correlations have been obtained in both durum wheat cultivars for the relationship with photoperiod, mainly at the emergence and the fifth leaf full expansion interval. In accordance with the relation with photoperiod in this phenological interval, it could be concluded that photoperiod at later stages had a greater influence on DLAR with respect to photoperiod at emergence.

Present results might find practical application in the improvement of phenology prediction models for durum wheat, bread wheat and barley grown in Mediterranean area in absence of water and nutrient stress.

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