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EFFECTS OF TEMPERATURE, PHOTOPERIOD AND DEFOLIATION ON FLOWERING TIME OF *LOTUS TENUIS* (FABACEAE) IN BUENOS AIRES, ARGENTINA

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SUMMARY

The phenological development of crops from emergence to flowering time is largely controlled by temperature and photoperiod. Flowering time is a critical phenological stage for subsequent reproductive phase. *Lotus tenuis* management in grasslands, pastures and seed production systems is through defoliation and sowing date; however, yet little is known about their effects on flowering time. The data presented in this study were obtained from experiments conducted with *L. tenuis* during the years 1989 to 2016 under field conditions. Our objectives were to determine if flowering time (a) is affected by sowing date; (b) can be predicted through equations using temperature and photoperiod and (c) is affected by defoliation applied at vegetative stage. Two defoliation intensities were applied, low (LDI) crop height reduced by 54% compared to pre-defoliation crop height and high (HDI), crop height reduced by 75%. The rate of progress from seedling emergence to flowering time (inverse of time from emergence to first flowering, 1/f) was modulated by temperature, photoperiod and photothermal functions. When *L. tenuis* sowing was delayed from autumn to spring, time from seedling emergence to first flowering decreased from 260 to 100 days. 1/f was linearly related to average temperature ($R^2 = 0.75$) and photoperiod ($R^2 = 0.85$) and both variables ($R^2 = 0.92$). Defoliation retarded flowering time. Flower and pod growth periods were shorter under defoliation than in control one. Defoliation did not cause abortion of flowers and pods. Flower production was fitted to quadratic function of photoperiod. Flowering peak was approximately within 15.2 h. The prediction of flowering time using thermal, photoperiod and photothermal models can provide information about crop management decisions, such as optimal environmental regimes for crop growth through sowing date.

INTRODUCTION

Sowing date determines environmental conditions for seedling emergence and vegetative phase that impact on flowering time and seed yield (Summerfield *et al.*, 1991). The phenological development of crops from emergence to flowering time is largely controlled by temperature and photoperiod. Flowering time is a critical phenological stage for subsequent plant reproductive phase of several grain and forage legumes (Craufurd and Wheeler, 2009; Del Pozo *et al.*, 2000; Iannucci

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et al., 2008). Forage legumes of genus *Lotus* are of great interest for sustainable production of agricultural systems. *Lotus tenuis*, *L. corniculatus* and *L. pedunculatus* are extensively used as forage in many temperate grasslands and pastures of different countries (Blumenthal and McGraw, 1999; Escaray *et al.*, 2012; Fairey and Smith, 1999; Vignolio *et al.*, 2016). These species have economic potential because they fix atmospheric nitrogen via symbiosis, improve soil fertility, optimize the forage quality of pasture and grasslands have high nutritive value and voluntary feed intake of beef cattle (Blumenthal and McGraw, 1999; Escaray *et al.*, 2012; Vignolio *et al.*, 2016). *Lotus* spp. are also used in mixture with *Festuca arundinaceae* when it is infested with *Acremonium coenophilum* to dilute forage toxicity (Romano, 2016).

Through sowing date, plants can be exposed to different environmental conditions of temperature and photoperiod that influenced flowering time (Craufurd and Wheeler, 2009; Del Pozo *et al.*, 2000; Egli and Bruening, 2006; Iannucci *et al.*, 2008; Vignolio *et al.*, 2010). When sowing later, long-day plant species need less accumulated degrees-days for flowering than those sown earlier (Del Pozo *et al.*, 2000). In fact, temperature and phototoperiod that prevail during flower production and subsequent reproductive phase determine seed yield (Iannucci *et al.*, 2008; Summerfield *et al.*, 1991). For example, flower abortion occurs if flowering peak takes place when the environmental conditions are not the optimum to assimilate production (Chaichi and Tow, 2000; Iannucci *et al.*, 2002).

Quantitative relationships to predict the rate of progress to flowering time (1/f, where f is days from emergence to first flowering) were proposed in herbages legumes using linear model as function of temperature and photoperiod (Del Pozo *et al.*, 2000; Iannucci *et al.*, 2008; Papastylianou and Bilalis, 2011; Summerfield *et al.*, 1991). Then, the prediction of flowering time using thermal, photoperiod and photothermal models provides information about crop management decisions such as optimal environmental regimes for crop growth and production (Iannucci *et al.*, 2008; Papastylianou and Bilalis, 2011).

Lotus tenuis is a long-day plant spread through seeds (Pomar and Mendoza, 2008). This species is sown in autumn or spring and it is managed under defoliation for forage, seed production or dual purpose (Cambareri, 2010; Vignolio *et al.*, 2010; 2016). Although flowering time is considered critical for plant reproduction, little is known about the effects of sowing date and defoliation on *L. tenuis* reproductive phase (Vignolio *et al.*, 2006; 2010; 2016). *Lotus tenuis* flowers time can be affected by defoliation (Vignolio *et al.*, 2006; 2010; 2016). *Lotus tenuis* and *L. corniculatus* flowering can be synchronized through defoliation; a flush of large numbers of flowers in a short time determines a more uniform seed ripening (Vignolio *et al.*, 2016). However, if most flowers and pods are produced in a shorter time, this can produce abort of these organs, because competition by assimilates increases (Egli and Bruening, 2006).

Our objectives were to determine if flowering time (a) is affected by sowing date; (b) can be predicted through equations using temperature and photoperiod and (c) is affected by defoliation applied at vegetative stage.

MATERIALS AND METHODS

Field site

Data used in this study were obtained from experiments carried out, during the years 1989 to 2016 at the Unidad Integrada Balcarce (Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria Balcarce, Facultad de Ciencias Agrarias, UNMdP, Buenos Aires, Argentina, 37°45′S and 58°18′W, 130 m above sea level). *Lotus tenuis* plants were obtained from seed inoculated with *Rhizobium loti* (N_2-fixing strain 733). The soil was well-drained, Typic Argiudoll and the tests were performed on the upper 0.15 m (Soil Survey Staff-USDA, 1999). Climate is temperate, humid–subhumid. Annual average and median precipitations from 1989 to 2015 were of 908 and 636 mm, respectively. Weather data were provided by the EEA INTA Balcarce meteorological station. Average air temperature increased from 1989 to 2015 and annual average air temperature was 0.84 °C higher in 2015 than in 1989 (Supplementary Figure S1, available online at https://doi.org/10.1017/S0014479717000126).

Description of the experimental design

In one set of experiments, *L. tenuis* was sown in pots of 4 litres (Vignolio *et al.*, 1996) and 2 litres (Vignolio *et al.*, 2002), one plant per pot, with 12 and 10 replications, respectively. According to the soil analysis, pH (soil:H_2O, 1:2.5) was 6.90 and 6.60 and soil had 13.13 and 12.60 ppm P (Bray 1 method) and 5.0 and 2.7% organic matter (Walkley and Black method) (Vignolio *et al.*, 1996; 2002), respectively. Other experiment was done in containers (60 × 40 × 20 cm of length, width and height), 12 replications and 12 plants per container (Romano, 2016). Soil analysis revealed 53 ppm P, 4.3% organic matter and 30.1 ppm $NO_3{}^-N$. Irrigation was according to plant water needs. Pots and containers were kept outdoors.

Field experiments were performed in plots from 2001 to 2016. Table 1 shows details about plant density, rows spacing and soil analysis. The plots were of 6 × 2 m (Cambareri (2010) and 4.0 × 1.4 m (Vignolio *et al.*, 2010, 2016). Crop defoliation data were obtained from plants defoliated at vegetative stage (Vingolio *et al.*, 2010). Two defoliation intensities were applied low (LDI) and high (HDI), reducing crop height by 54 and 75% compared to plant height before defoliation, respectively (Vignolio *et al.*, 2016). Figure 1 synthetizes seedling emergence date, flowering time, photoperiod, air temperature, two defoliation events, data sources and *L. tenuis* cultivars. The experiments were kept free of weeds by hand removal without modifying the crop architecture, protected from herbivores attack and irrigated. The criteria were based on irrigation plus rainfall which replaced evapotranspiration (Vignolio *et al.*, 2016). Symptoms of water deficit and pests were not detected. Pollination was provided by honey bees (*Apis mellifera*). *Lotus tenuis* seedling emergence was during the first 7 days after sowing.

Flower time as function of temperature and photoperiod

Quantitative relationships describing time from seedling emergence date to first flowering (hereafter flowering time) as a function of temperature (T), photoperiod

Table 1. Plant density, rows spacing and soil analysis of different experiments conducted with *Lotus tenuis*. References: pH (soil: H_2O, 1: 2.5); P, phosphorus (Bray 1 method) and O.M., organic matter content (Walkley and Black method).

Plant (N m^{-2})	Rows spacing (cm)	pH	P (ppm)	O.M. (%)	NO_3-N (ppm)	Source
5	75.0	5.90	9.46	6.50	6.3	Vignolio *et al.*, 2006
23, 48, 90	17.5	6.50	15.65	6.65	9.53	Cambareri, 2010
28, 38, 83	17.5	6.57	13.30	6.30	9.78	Cambareri, 2010
20	17.5, 35.0	6.53	13.07	6.02	10.51	Vignolio *et al.*, 2010
20	17.5	6.60	28.10	5.90	33.30	Vignolio *et al.*, 2016
20	17.5	7.05	23.20	5.50	22.90	Vignolio not published

(Ph) and accumulated growing degree-days (AGDD) were performed using linear model (Del Pozo *et al.*, 2000; Iannucci *et al.*, 2008; Papastylianou and Bilalis, 2011; Summerfield *et al.*, 1991). Relative contributions of temperature (thermal model, $1/f=a_1+b_1{*}T$), daylength (photoperiod model, $1/f=a_2+b_2{*}Ph$) and with both temperature and daylength (photothermal model, $1/f=a_3+b_3{*}T+c_3{*}Ph$) were determined. Data a_1, a_2, a_3, b_1, b_2, b_3, c_1, c_2 and c_3 are constants to each function and 1/f was calculated as the inverse of the duration in days from emergence to first flowers. Photoperiod was defined as daylength (h) from sunrise to sunset and it was calculated from seedling emergence to flowering time. Mean daily temperature (T) was obtained as

$$T = (((T_{max} + T_{min})/2) - T_b), \tag{1}$$

where T_{max} and T_{min} were the maximum and minimum air temperatures, respectively. T_b is the base temperature of 5 °C (Cambareri, 2010). Thermal time (T_t, °C day) and T_b requirements for flowering were both estimated from thermal model (Iannucci *et al.*, 2008; Papastylianou and Bilalis, 2011) as

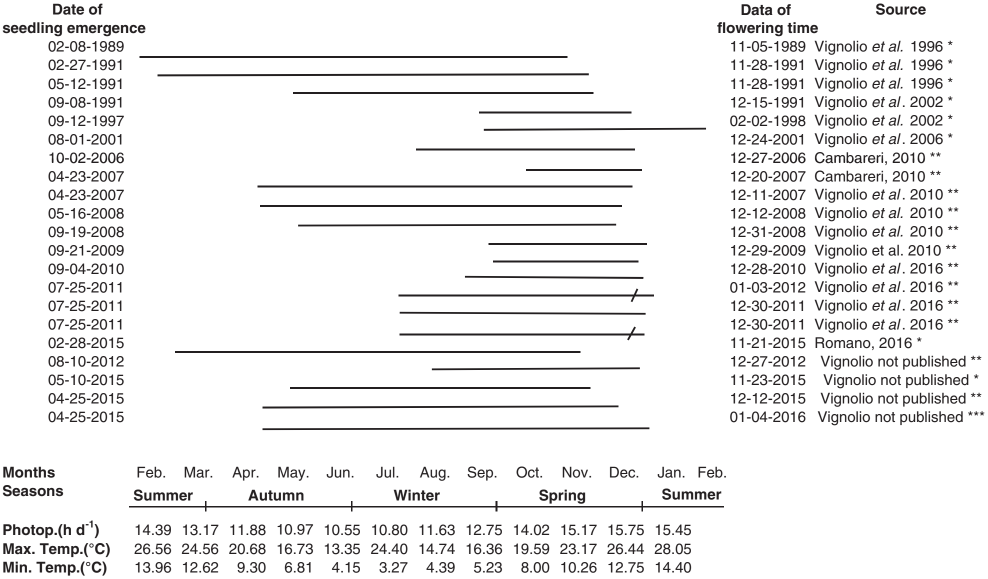
$$T_b = -a_1/b_1, \tag{2}$$

$$T_t = 1/b_1. \tag{3}$$

AGDD between seedling emergence and defoliation, flowering time, pod initiation and harvest time was calculated as the sum of the mean daily temperature. Negative values were not included in the calculus (Papastylianou and Bilalis, 2011). The relationship between photoperiod and percentage of umbels with flowers was evaluated.

Data analysis

Pots and containers were randomly arranged. Each experiment in plots were a randomized complete block design. The data were analysed using analysis of variance (ANOVA) at the 5% level of probability. Linear function was used to describe the relationship between umbels with flowers and umbels with pods, 1/f and temperature, photoperiod and both, photothermal.



Date of seedling emergence	Data of flowering time	Source
02-08-1989	11-05-1989	Vignolio *et al.* 1996 *
02-27-1991	11-28-1991	Vignolio *et al.* 1996 *
05-12-1991	11-28-1991	Vignolio *et al.* 1996 *
09-08-1991	12-15-1991	Vignolio *et al*. 2002 *
09-12-1997	02-02-1998	Vignolio *et al.* 2002 *
08-01-2001	12-24-2001	Vignolio *et al*. 2006 *
10-02-2006	12-27-2006	Cambareri, 2010 **
04-23-2007	12-20-2007	Cambareri, 2010 **
04-23-2007	12-11-2007	Vignolio *et al*. 2010 **
05-16-2008	12-12-2008	Vignolio *et al.* 2010 **
09-19-2008	12-31-2008	Vignolio *et al.* 2010 **
09-21-2009	12-29-2009	Vignolio et al. 2010 **
09-04-2010	12-28-2010	Vignolio *et al*. 2016 **
07-25-2011	01-03-2012	Vignolio *et al*. 2016 **
07-25-2011	12-30-2011	Vignolio *et al*. 2016 **
07-25-2011	12-30-2011	Vignolio *et al*. 2016 **
02-28-2015	11-21-2015	Romano, 2016 *
08-10-2012	12-27-2012	Vignolio not published **
05-10-2015	11-23-2015	Vignolio not published *
04-25-2015	12-12-2015	Vignolio not published **
04-25-2015	01-04-2016	Vignolio not published ***

Months	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Photop.(h d^{-1})	14.39	13.17	11.88	10.97	10.55	10.80	11.63	12.75	14.02	15.17	15.75	15.45	
Max. Temp.(°C)	26.56	24.56	20.68	16.73	13.35	24.40	14.74	16.36	19.59	23.17	26.44	28.05	
Min. Temp.(°C)	13.96	12.62	9.30	6.81	4.15	3.27	4.39	5.23	8.00	10.26	12.75	14.40	

Figure 1. Schedule of *Lotus tenuis* growth period from seedling emergence to flowering time. References: monthly means of maximum (Max.) and minimum (Min.) air temperature (Temp.) and photoperiod (Photop.). Temperature data are average from the years 1989 to 2015. Reference: /, indicate defoliation; *, Chaja; **, Pampa INTA and ***, Ruta 226 *Lotus tenuis* cultivars.

RESULTS

There was a great influence of temperature and photoperiod on *L. tenuis* flowering time according to seedling emergence. The number of days from seedling emergence to beginning of flowering decreased with increasing of photoperiod. When seedling emergence occurred between late summer and early spring, the number of days from seedling emergence to flowering decreased. Flowering time was among 100 to 260 days for late (spring) and early (at the end of summer) sowing, respectively (Figure 2). AGDD had a linear and positive effect ($R^2 = 0.67$) on seedling emergence to flowering time (Figure 2), and different AGDD were required when seedling emergence was in early spring (1000 °C day), autumn (1400 °C day), winter (1500 °C days) and at late summer (1900 °C day). The rate of progress from emergence to first flowering (1/f) was linearly related to average temperature ($R^2 = 0.75$, Figure 3a) and photoperiod ($R^2 = 0.85$, Figure 3b). As shown in Figure 4, when temperature and photoperiod were included in the analysis (photothermal model), also significant response was recorded, $1/f = -0.02 + 0.00006{*}T + 0.002024{*}Ph$ ($R^2 = 0.92$; $P < 0.0001$). Base temperature (T_b) and thermal time (T_t) requirements for *L. tenuis* flowering were 3.4 and 909 °C day, respectively.

The beginning and ending of flowering coincided with a photoperiod of approximately 15.8 h d^{-1} (December) and 13.8 h d^{-1} (early March), respectively. Flowering peak occurred between photoperiod of 15.1 and 15.2 h d^{-1} (January) and it was best described by a quadratic function ($R^2 = 0.62$; Figure 5).

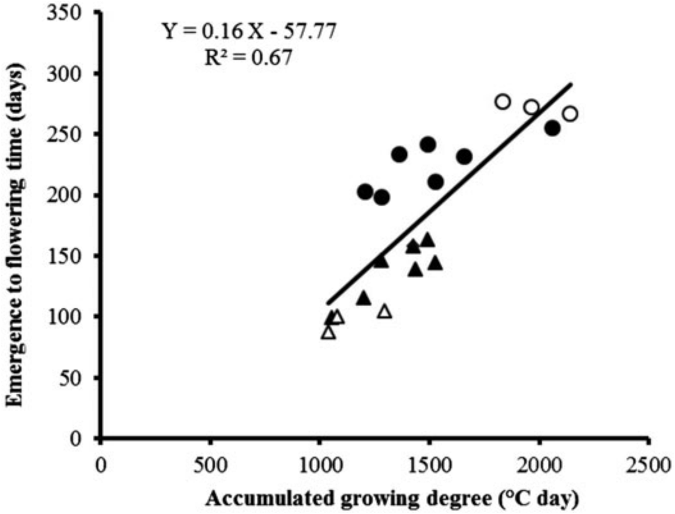


Figure 2. The relationship between accumulated growing degree-day and rate of progress from seedling emergence to flowering time in *Lotus tenuis*. References: seedling emergence in summer, ○; autumn, ●; winter, ▲ and spring, Δ (see Figure 1).

Defoliation intensities retarded flowering time. Floral initiation was earlier in control and LDI (1450 °C day) than in HDI (1490 °C day) treatment (Table 2). Defoliation delayed pod initiation and harvest time (Table 2).

DISCUSSION

The different seedling emergence (or sowing dates) provided a wide range of environmental conditions to examine the performance of *L. tenuis*, which showed phenotypical plasticity in flowering time. As the emergence time was delayed, *L. tenuis* crops were exposed to higher air temperature and longer photoperiod, resulting in a shortening of the number of days for the beginning of flowering. When sown later, plant species of long-day, such as different ecotypes of *Medicago polymorpha*, needed less accumulated growing degree days for flowering that those sown earlier (Del Pozo *et al.*, 2000). With increasing day-length and air temperature during vegetative growth, its development is hastened shortening its cycle (Andrade, 1995; Beuselinck and McGraw, 1988; Papastylianou and Bilalis, 2011).

Temperature and photoperiod are factors controlling plant phenological development (Craufurd and Wheeler, 2009; Del Pozo *et al.*, 2000). Flowering is the most critical stage in different legume crops because it determines pod production and seed yield (Egli and Bruening, 2006; Iannucci *et al.*, 2008; Vignolio *et al.*, 2016). Our studies showed that the threshold photoperiod of *L. tenuis* for flowering time was 15.8 h day^{-1}, approximately the same reported for *L. corniculatus* (Steiner, 2002). Pomar and Mendoza (2008) reported that the optimal flower production of *L. tenuis* requires 16 h day^{-1} photoperiod but 14 h day^{-1} could be sufficient to ensure an adequate production of flowers. Our results showed that flower initiation of *L. tenuis*

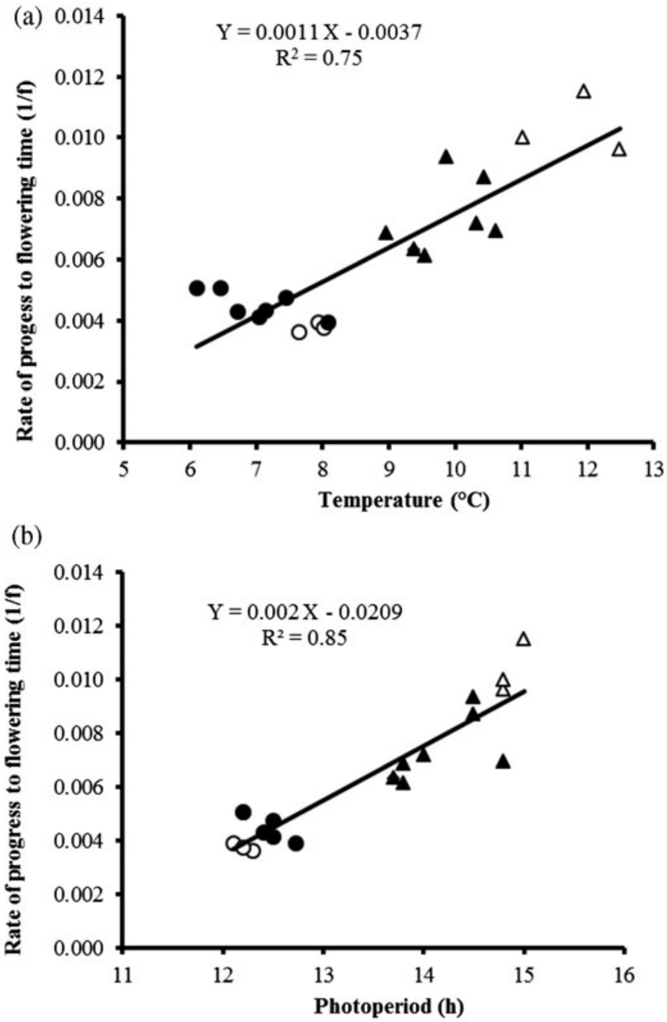


Figure 3. The relationship between (a) average air temperature and (b) photoperiod and rate of progress from seedling emergence to flowering time in *Lotus tenuis*. References: seedling emergence in summer, ○; autumn, ●; winter, ▲ and spring, Δ (see Figure 1).

was also modulated by the temperature. Temperature alone explained 75% of the observed variation in rate of progress to flowering (1/f). Base temperature (T_b) requirement for flowering for *L. tenuis* was 3.36 °C. This value was in agreement with the results reported by Iannucci *et al.* (2008) in *Trifolium alexandrinum* (3.5 °C) and *Hedysarum coronarium* (3.9 °C). Thermal time (T_t) requirement for *L. tenuis* flowering (909 °C day) is in agreement with the results reported by Iannucci *et al.* (2008) in *Onobrychis viciifolia* (880 °C day) and *Trifolium resupinatum* (871 °C day), both long-day Fabaceae species (Papastylianou and Bilalis, 2011). When temperature and photoperiod were both included in the analysis, a significant response was recorded.

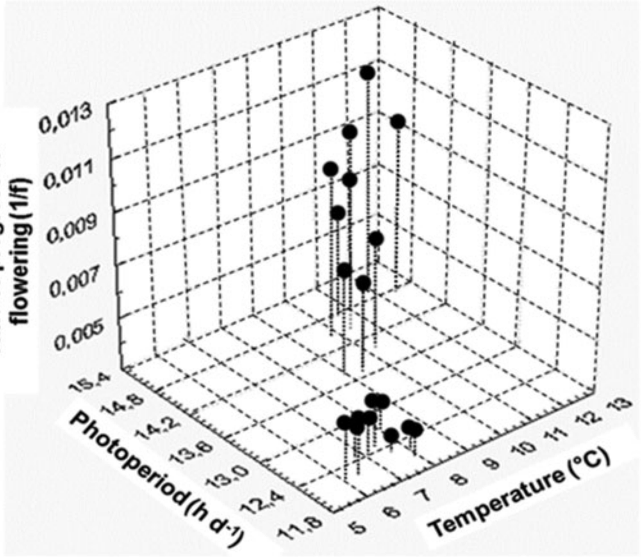


Figure 4. Effects of photoperiod and mean temperature on rates of progress from seedling emergence to flowering time in *Lotus tenuis.*

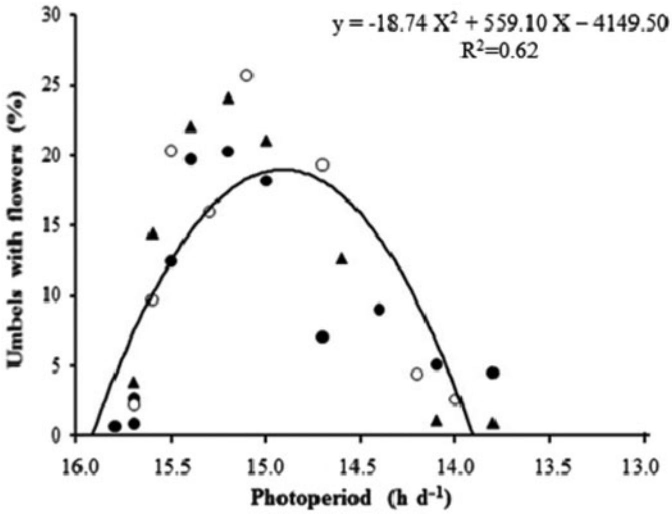


Figure 5. Relationship between the photoperiod and percentages of umbels with flowers produced by *Lotus tenuis* crops. References: control experiment of the years, 2009/2010, ●; 2010/2011, ▲ and 2011/2012, ○.

Again, our results are in agreement with that reported in different herbages legumes, such as *M. polymorpha*, *T. alexandrinum*, *T. resupinatum*, *Vicia sativa* and *V. villosa* (Del Pozo *et al.*, 2000; Iannucci *et al.*, 2008). Therefore, *L. tenuis* flowering analysis should be done in terms of photothermal responses rather than temperature or photoperiod alone (Del Pozo *et al.*, 2000; Iannucci *et al.*, 2008).

Table 2. Accumulated growing degree-days (AGDD) for of *Lotus tenuis* plants defoliated at different intensities and recorded at different phenological phase. Treatments were an uncut Control, a low (LDI) or high (HDI) defoliation intensity (Vignolio *et al.*, 2016). Reference: seedling emergence, S. E.

	Phenological stage			
	S E to defoliation	S E to flowering time	S E to pod initiation	S E to harvest time
Treatment	AGDD (°C day)	AGDD (°C day)	AGDD (°C day)	AGDD (°C day)
Control	–	1450	1490	2480
LDI	1421	1450	1490	2528
HDI	1421	1490	1609	2699

Parameters b_3 and c_3 in the photothermal model used in this study were positive. This is indicative that the rate of progress to flowering was accelerated by warmer conditions and longer photoperiod (Butler *et al.*, 2002; Iannucci *et al.*, 2008; Papastylianou and Bilalis, 2011). When *L. tenuis* was sown early (autumn or winter), plant flowered with a photoperiod and temperature average lower than those plants sowed later (spring). *L. tenuis* sowed later needs less AGDD for flowering than when the crop was sowed early. These results are in agreement with previous experiments where flowering time of *L. tenuis* sown in same year, was earlier in autumn than in spring (Vignolio *et al.*, 2010). With increasing day-length and air temperature during vegetative growth, crop development is hastened, shortening its cycle. Reduction in vegetative and reproductive phases was also reported in *Lotus corniculatus*, *Trifolium subterraneum*, *T. resupinatum*, *Phaseolus vulgaris*, *Medicago sativa*, *Zea mays*, *Glycine max* and *Helianthus annuus* when they were sowed late (Andrade, 1995; Beuselinck and McGraw, 1988; Papastylianou and Bilalis, 2011).

Under defoliation conditions, *L. tenuis* showed plasticity in the production of reproductive organs. Different AGDD requirement was recorded for flowering and pod peaks according to defoliation conditions (Vignolo *et al.*, 2016). For example, flowering peak was later under LDI than HDI condition, however, seed yield was not significantly affected because self-compensation increased harvest index (Vignolio *et al.*, 2016). *L. tenuis* flowering is indeterminate and this attribute confers, through phenotypical plasticity, the capacity to compensate the vegetative and reproductive biomass under different environmental conditions such as defoliation and sowing date (Vignolio *et al.*, 2006; 2010; 2016). As *L. tenuis* plants were irrigated, plants showed phenotypic plasticity in flowering time mainly in response to environmental conditions such as photoperiod and air temperature. The average air temperature from 1989 to 2015 was increasing (Figure S1) and *L. tenuis* phenotypic plasticity in flowering time can provide responses to new environments scenarios associated with global warming.

L. tenuis flowering, pod set, and physiological maturity occur simultaneously. When flowers and pods are using the same source of assimilate, this can increase abortion of reproductive organs (Egli and Bruening, 2006). *L. tenuis* flowers abortion was not affected by treatments. Approximately, 74% of flowers per umbels developed pods.

L. tenuis produces many more flowers than mature pods. Reproductive regulation was principally through of aborting of some flowers per umbel and not through of umbels with all flowers or pods.

CONCLUSION

Lotus tenuis showed differential sensitivity of flowering time to sowing date, being modulated by photoperiod and temperature conditions. Flowering time occurred when a threshold of thermal time of 909 °C day and the photoperiod of 15.2 h d^{-1} was reached and pod production began with 1450 °C day. According to the sowing date, *L. tenuis* growth cycle was bounded among 100 and 260 days. Defoliation intensities in vegetative stage retarded flowering time, which was earlier in control and low defoliation (1450 °C day) than in high defoliation treatment (1490 °C day). Phenotypic plasticity in flowering time in response to both, temperature and photoperiod, provides a convenient management schedules through sowing time.

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SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479717000126

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