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Rate of progress to flowering in annual species of genus *Trifolium* grown for silage in Galicia (NW of Spain) as affected by sowing date

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Abstract. With the objective of gaining insight on the relationship between sowing date and the onset of flowering of annual *Trifolium* species in Galicia (NW Spain), commercial genotypes of arrowleaf clover (5), balansa clover (5), berseem clover (2), crimson clover (6), persian clover *ssp. majus* (2) and *ssp. resupinatum* (3) were sown in small plots from 15th September 2012 to 15th April 2013 at monthly intervals following a split-plot design with two replications. The time from sowing to flowering, measured as days after planting (DAP) was significantly affected by species and sowing dates, ranging from 175 to 124 days for the latest (berseem clover) and the earliest (balansa clover) species and from 218 to 79 days for the September and April sowings dates, respectively, which represented the least and the most inductive flowering conditions. Although a significant effect in DAP was detected for the interaction species x sowing date, the earliness of annual clovers studied ranked in a similar way (balansa clover > persian clover *ssp. resupinatum* and crimson clover > arrowleaf clover > persian clover *ssp.majus* > berseem clover) across sowing dates. Phenological models that related the rate of progress to flowering $(DAP)^{-1}$ with temperature and photoperiod during the growing cycle accounted for 65% and 90% of the variability, showing that in the agroclimatic conditions of NW Spain flowering of annual clovers was more sensitive to photoperiod than to temperature.

Keywords. Development – Phenology – Winter legumes – Rate of flowering.

Influence de la date de semis sur la vitesse de floraison d'espèces annuelles du genre Trifolium cultivées pour l'ensilage en Galice (NO Espagne)

Résumé. *Dans le but d'approfondir la connaissance de la relation entre la date de semis et la floraison de diverses espèces de trèfles annuels en Galice (NO Espagne), des génotypes commerciaux de trèfle vésiculeux (5), trèfle de Micheli (5), trèfle d'Alexandrie (2), trèfle Incarnat (6), trèfle de Perse* ssp. majus *(2) et* ssp. resupinatum *(3) ont été semés mensuellement dans de petites parcelles entre le 15 septembre 2012 et le 15 avril 2013, en adoptant un dispositif aléatoire en blocs avec des parcelles divisées en deux répétitions. Le temps entre la date de semis et celle de la floraison, mesuré en jours après le semis (DAP) a été influencé de manière significative par l'espèce et la date de semis. Ainsi, cet intervalle a varié de 175 à 124 jours pour l'espèce la plus tardive (trèfle d'Alexandrie) à la plus précoce (trèfle de Micheli) et de 218 à 79 jours pour les dates de semis de septembre et d'avril, respectivement, ce qui représente les conditions de floraison les moins et les plus favorables. Bien qu'un effet significatif de l'interaction (espèces) x (semis) ait été observé sur le DAP, la précocité des trèfles annuels étudiés se classe d'une façon similaire pour toutes les dates de semis, à savoir : trèfle Balansa > trèfle de Perse* ssp. resupinatum *et trèfle Incarnat > trèfle vésiculeux > trèfle de Perse* ssp.majus *> trèfle d'Alexandrie. Les modèles phénologiques qui corrèlent la vitesse de progression de la floraison* $(DAP)^{-1}$ *avec la température et la photopériode au cours du cycle de croissance, expliquent jusqu'à 65% et 90% de la variation. Ces résultats démontrent que dans les conditions agro-climatiques du NO de l'Espagne, la floraison des trèfles annuels est plus sensible à la photopériode qu'à la température.*

Mots-clés. *Dévelopement – Phénologie – Légumineuses d'hiver – Vitesse de floraison.*

I – Introduction

Results of the evaluation of annual legumes of genus *Trifolium* in Galicia performed the last four years have yielded promising results showing a high productivity of these species grown as winter crops for silage, indicating that can be fit in an annual rotation with maize (Valladares *et al.*, 2012). Another interesting feature of these species is their good nutritive value along the growth cycle (Pereira-Crespo *et al.*, 2012) but, as indicated by these authors, annual legumes loose quality fairly rapid after the flowering stage is reached. For a given genotype, flowering time is mainly affected by environmental variables like temperature and daylength, while water and light intensity are of secondary importance (Bernier and Périlleux, 2005). In order to support crop management decisions like sowing or harvest dates, it is of interest to study the relationships which describe time to flowering of these species as a function of measurable climatic variables. Simple linear models relating the rate of progress to flowering with temperature and photoperiod during the growing cycle have been described, showing additive effects of both variables (Roberts and Summerfield, 1987). With the aim of gaining insight on the prediction of flowering time of annual legumes sown in Galicia, it is the objective of this work to (a) analyze the effect of genotype and planting date on time to flowering of twenty three commercial cultivars from six annual *Trifolium* species and (b) apply the linear models to quantify the effects of temperature and photoperiod on the rate of progress to flowering of these species.

II – Materials and methods

Field experiment was carried out at the Centro de Investigacións Agrarias de Mabegondo research station farm (Galicia, NW Spain: 43º 15´ N, 8º 18´ W, 100 m above the sea level) from September 2012 to August 2013. The species evaluated were: Arrowleaf clover (*Trifolium vesiculosum* Savi.) cv. Arrowleaf, Cefalu, Vesiculoso, Yuchi and Zulu II; Balansa clover (*T. michelianum* Savi.) cv. Balansa, Bolta, Frontier, Micheliano and Paradana; Berseem clover (*T. alexandrinum* L.) cv. Alex and Akenathon; Crimson clover (*T. incarnatum* L.) cv. Cardinal, Conete, Contea, Dixie, Linkarus and Viterbo; Persian clover (*T. resupinatum* L.) ssp. *majus* cv. Laser and Maral and Persian clover ssp. *resupinatum* cv. Gorbi, Lightning and Nitroplus. The experimental design was a split plot where the sowing date (eight dates, from 15 September 2012 to 15 April 2013, at monthly intervals) was the main plot and the legume cultivar the subplot arranged in a randomized block design with two replications. Each subplot consisted of three rows 3 m long, 30 cm apart, hand-sown with a seed dose of 20 kg ha^{-1} (500 seeds m^{-2}) for crimson clover and 10 kg ha^{-1} (750 seeds m^{-2}) for the rest of legume species. Plots were observed three times per week from the beginning of March onwards and flowering time was recorded as the day in which 5% of the plants of a given plot showed open flowers. Mean air daily temperature (T) was obtained from the records of the automatic Meteorological Station located *in situ*, and photoperiod (P) was calculated as the time in hours from sunrise to sunset corresponding to the latitude of the experimental site using the formulae proposed by List (1971) and Klein (1977). The number of days from sowing to beginning of flowering stage (days after planting, DAP) for each species in each plot was recorded and the rate of plant development (1/f) defined as the inverse of DAP for each species was regressed on T, P, or both using three linear models where the coefficients are constants specific for each species (Roberts and Summerfield, 1987): a thermal model ($1/f = a + bT$), a photoperiodic model ($1/f = a' + b'P$), and a photothermal model ($1/f = a'' + b''T + c''P$). Base temperature (t_0) was calculated as $t_o = -a/b$, and Thermal time for flowering (T_t) was computed as the accumulated mean daily air temperature minus the base temperature between planting and flowering dates for every plot. ANOVA analysis of DAP and regression procedures were performed using Proc GLM of SAS (SAS Institute, 2009).

III – Results and discussion

The time from sowing to flowering, measured as days after planting (DAP) was significantly affected by species and sowing dates (Table 1). DAP period ranged from 218 to 79 days for the September and April sowings dates, respectively, which represented the least and the most inductive flowering conditions. On average, for every month of delay in sowing, DAP was reduced in 19.9±5.5 days in a fairly constant way along the experiment. As an average of planting dates, flowering precocity of clover species ranked as follows: Balansa > Persian *resup.* ≥ Crimson > Arrowleaf > Persian *majus* > Berseem. The interaction sowing date x clover species on DAP was also significantly and, as can be seen in Fig. 1 (left), whilst species relative precocity maintained as exposed above, the absolute differences among species markedly tightened when delaying the sowing date towards spring.

Table 1. Effect of sowing date and clover species on time from sowing to flowering (DAP)

	Sowing date								
	15-SEP	**15-OCT**	**15-NOV**	**15-DEC**	**15-JAN**	**15-FEB**	**15-MAR**	**15-APR**	**p**
DAP	218.7 [a]	195.5 [b]	174.0 [c]	155.9 [d]	136.2 [e]	110.2 [f]	101.3 [g]	79.7 [h]	<.0001
n	46	46	46	46	46	46	46	46	

	Clover species						
	Arrowleaf	**Balansa**	**Berseem**	**Crimson**	**Persian *majus***	**Persian *resup.***	**p**
DAP	157.0 [c]	124.4 [e]	175.0 [a]	143.2 [d]	164.1 [b]	141.4 [d]	<.0001
n	80	80	32	96	32	48	

Means sharing a letter in their superscript are not significantly different (p>0.05).

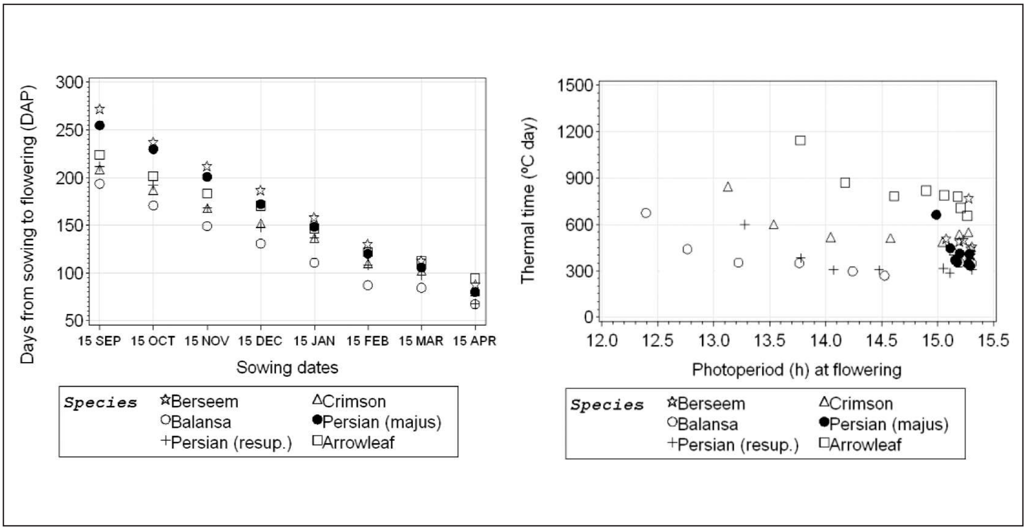

Fig. 1. Interaction between clover species x sowing date on time from sowing to flowering (left) and plot of thermal time in this period (ºC day) vs. photoperiod (h) in the day of flowering (right).

Regression analysis of the data showed that temperature is an important factor controlling flowering time, in accordance with the observations of other authors which studied the flowering behaviour of annual clovers in Mediterranean countries (Iannucci *et al.*, 2008 in Italy and Papastylianou and Bilalis, 2011 in Greece). Explained variance of the thermal model (ranging from 0.53 to 0.80 for Balansa and Berseem clovers, respectively) was markedly lower than that of the photoperiodic model (ranging from 0.86 for Persian *resup*., Crimson and Arrowleaf clovers to 0.95 for Berseem clover), indicating a superior effect of daylenght compared to mean air temperature on flowering developmental rate in the conditions of our study (Table 2). Additionally when temperature and photoperiod are included in the model, temperature effect was non-significant for any species but for the earliest flowering species Balansa clover. This marked effect of photoperiod compared with temperature on the control of flowering time of annual clovers differs from the response observed by the above cited authors which reported the temperature as the main factor determining time to flowering in experiments performed in southern latitudes.

Table 2. Values of constants (x 10^{-4}) and explained variance (R^2) of the linear models based on mean temperature (1/*f*) = a+b*T*, on mean photoperiod (1/*f*) = a'+b'*P*, and on both mean temperature and photoperiod (1/*f*) = a"+b"*T*+c"*P* obtained in the regressions of the rate of progress to flowering (1/*f*)

		Thermal model			Photoperiodic model			Photothermal model			
Clover species	**n**	**a**	**b**	**R^2**	**a'**	**b'**	**R^2**	**a"**	**b"**	**c"**	**R^2**
Balansa	80	-192.4^{***}	26.1^{***}	0.53^{***}	-130.8^{***}	18.7^{***}	0.91^{***}	-80.6^{***}	-9.3^{***}	23.0^{***}	0.93^{***}
Persian (resup)	48	-227.5^{***}	27.9^{***}	0.64^{***}	-145.3^{***}	18.5^{***}	0.86^{***}	-156.4^{***}	2.1^{ns}	17.4^{***}	0.86^{***}
Crimson	96	-105.1^{***}	16.3^{***}	0.58^{***}	-98.3^{***}	14.3^{***}	0.86^{***}	-93.9^{***}	-1.1^{ns}	15.1^{***}	0.86^{***}
Arrowleaf	80	-62.6^{***}	11.4^{***}	0.64^{***}	-86.8^{***}	12.6^{***}	0.86^{***}	-86.6^{***}	-0.1^{ns}	12.7^{***}	0.86^{***}
Persian (majus)	32	-183.0^{***}	22.2^{***}	0.72^{***}	-183.5^{***}	20.2^{***}	0.94^{***}	-180.3^{***}	-1.2^{ns}	21.0^{***}	0.94^{***}
Berseem	32	-151.4^{***}	18.5^{***}	0.80^{***}	-175.4^{***}	18.9^{***}	0.95^{***}	-177.1^{***}	1.7^{ns}	17.4^{***}	0.95^{***}

^{ns} $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Base temperature (t_0), computed from the coefficients of thermal model, averaged 7.3 ± 1.1 ºC, ranging from 6.0 ºC for Arrowleaf clover and 8.5 ºC for Persian *majus* and Berseem clovers. Thermal time for flowering (T_t) averaged 507.2 ± 170.0 ºC day, ranging between 360.5 and 819.3 ºC day for Persian *resup.* and Arrowleaf clovers, respectively. The plot of T_t on photoperiod at flowering (P_i), averaged across varieties of each clover species for the different planting dates (Fig. 1 right), shows that flowering is prevented until a minimum P_i is reached, independently of the thermal time accumulated. This threshold daylength ranks from 12.3 to 15.1 h for the earliest and latest flowering species Balansa and Berseem clover, respectively. Also, there is a minimum number of degree-days for the flowering of a species, ranking from 300 to 650 ºC day for Balansa and Arrowleaf clovers, respectively.

IV – Conclusions

There is a different response in flowering time among annual clover species to temperature and photoperiod conditions. Photoperiod explained most of the variability of the developmental rate to flowering compared with temperature. Flowering was observed to initiate when a minimum requirements of daylength and temperature was reached for each species.

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