# Effects of season, altitude and daylength on floral initiation of two contrasting genotypes of Trifolium repens L.

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# Effects of season, altitude and daylength on floral initiation of two contrasting genotypes of *Trifolium repens* L.

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## SUMMARY

The effects of season and altitude on floral initiation of two *Trifolium repens* L. cultivars, Haifa and California Ladino, were tested in the area of Ayacucho, Peru (2730 m, 13° S), with regard to their seed production potential. Specific effects of daylength at a constant temperature (20 °C) were examined in growth chambers.

In Ayacucho, the proportion of inflorescence-bearing nodes was found to be lowest between December and June (Haifa 10–20%, Ladino 0–10%) and highest in August (Haifa 30%) and September–October (Ladino 15%). Late in the cool season, floral initiation of Ladino, but not that of Haifa, increased strongly at a high altitude (3250 m). In growth chambers, Haifa initiated few inflorescences and Ladino none in a 10 h daylength. In 16 h, the floral initiation of Haifa was very limited but Ladino formed many inflorescences. Floral initiation of Haifa was most pronounced and lasted longest after a daylength shift from 10 to 13 h.

It is concluded that seasonal and altitudinal variations in low temperature were the main factors influencing floral initiation in the region of Ayacucho. Haifa is considered to be an intermediate-day plant, suited for seed production in the region because of its marked and early flowering. Ladino was classified as a quantitative long-day plant, unsuitable for seed production at this low latitude because of its retarded floral response to low temperature.

## INTRODUCTION

*Trifolium repens* L. (white clover) is an important pasture legume at high altitude in low latitudes. Seed production in these regions is necessary in order to ensure the continuous supply of seed of locally adapted varieties. However, the potential for seed production of particular genotypes near the equator depends mainly on the presence of floral stimuli as shown for Colombia (Crowder, 1960) and Indonesia (Nurjaya, Nitis & Britten, 1983). In Hawaii, floral initiation of a genotype of *T. repens* was limited by short daylengths, but some compensation was possible through the effect of low night temperatures (8·3 °C) (Britten, 1961).

An accelerated and more profuse flowering in longer photoperiods has been reported for many genotypes of white clover (Laude, Stanford & Enloe, 1958; Beatty & Gardner, 1961; Haggar, 1961). *T. repens* has therefore usually been regarded as a long-day plant (Cooper, 1960). However, some genotypes exhibited characteristics of short–long-day plants (Thomas, 1961*a*). Owing to the different floral response to photoperiod and temperature, Thomas (1982) proposed distinguishing between groups within the species to characterize their responses to floral stimuli. Only one report describes a short-day response (Beatty & Gardner, 1961).

The objective of the present study was to determine the influence of season and altitude at 13° S on floral initiation of two contrasting cultivars of *T. repens* under field conditions. Their potential for seed production was assessed and criteria for the selection of a site suitable for seed production was found. The specific effects of daylength on floral initiations were tested in additional growth chamber experiments.

## MATERIALS AND METHODS

*Plant material.* The cultivars used were Haifa from Australia whose floral response to photoperiod and temperature has not been described

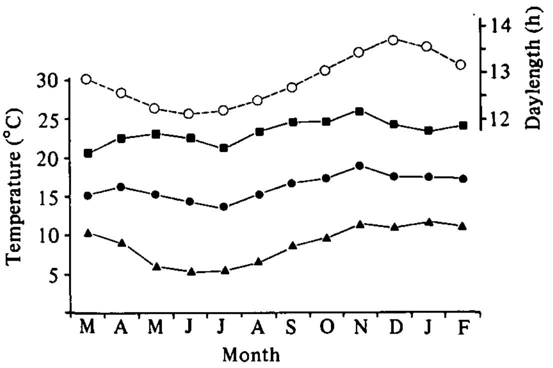


Fig. 1. Daylength (○) and temperature (°C) (average: ●, maximum: ■, minimum ▲) in Ayacucho, March 1979–February 1980.

previously, and Ladino from California. Out of 11 cultivars compared in a preliminary experiment, these two had ranked second and third in the number of inflorescences/m$^2$, with 2600 for Haifa and 250 for Ladino. Although cv. Louisiana ranked first (3400 inflorescences/m$^2$) it was not selected for further experiments because of its poor vegetative growth.

*Sites and climates.* Two experiments were conducted. Experiment 1 examined seasonal effects on floral initiation at Ayacucho, Peru (2730 m above sea level, 13° 8′ S, 74° 13′ W). Experiment 2, concerned with altitudinal effects, was located at Ayacucho, at Wayllapampa (2470 m) and at Huamanguilla (3250 m). The latter two sites were 10 and 20 km respectively from Ayacucho.

Figure 1 shows monthly variation in daylength and temperature at Ayacucho where a mesothermal summer rain climate predominates. The yearly average rainfall is 580 mm. The rainy season December–March) is characterized by regular precipitation and mild nights; the main features of the dry season are the almost complete absence of clouds and low night temperatures. The temperatures of three experimental sites in Expt 2 are compared in Table 1. Maximum temperatures were associated with altitude, while minimum temperatures were strongly influenced by the local topography: the low altitude site had the lowest minimum temperatures because of its location in a wide valley with little air movement and strong outgoing radiation during clear nights. Daylength at all three sites varied from 12 h 7 min in June to 13 h 41 min in December.

Table 1. *Temperature at the three altitudes during the experimental phase from May to October* 1979 (*Expt* 2)

| Altitude (m) | Mean temperature (°C) Average | Minimum | Maximum |
|---|---|---|---|
| 2470 | 15·9 | 1·8 | 26·5 |
| 2730 | 15·6 | 4·8 | 24·2 |
| 3250 | 12·2 | 3·2 | 20·5 |

*Experiment* 1, *seasonal effects.* From March 1979 to February 1980, stolon tips were collected from three 70 × 70 cm plots of each cultivar which had been sown in May 1978. Eight tips per cultivar, containing vegetative and reproductive buds and three unfolded leaves, were harvested at weekly intervals. The stolon tips were dissected under a stereoscopic dissecting microscope and the proportion of inflorescence-bearing nodes was determined. The reproductive axillary buds could be distinguished from the vegetative buds by the rapid increase in size during early growth.

Photoperiod and temperature, particularly minimum temperature, varied sinoidally over a year (Fig. 1). Thus, it was attempted iteratively to fit sine-shaped curves ($y = a + b \sin (t)$, $y$: proportion influorescence-bearing nodes, $t$: time with $2\pi$ radians = 12 months) to the weekly results of Expt 1 in order to test a comparable pattern in the development of floral initiation.

*Experiment* 2, *altitudinal effects.* Plants of five clones of each cultivar, chosen at random from the plots of Expt 1, were grown in a glasshouse at Ayacucho. In May 1979, the 3-month-old plantlets were transferred to the three experimental sites in the field. Single plants, spaced at 30 cm, were planted in four replications. Growing conditions (soil, radiation, watering) were similar at the three sites, with the exception of temperature (Table 1).

The emerging leaves and appearing inflorescences on five stolons per plant were counted fortnightly until October 1979. In July, August, September and October one stolon tip per plant was harvested and the proportion of inflorescence-bearing nodes determined as described under Expt 1.

*Experiments* 3 *and* 4, *daylength effects in growth chambers.* Experiments 3 and 4, with varying daylengths, were conducted in growth rooms (Controlled Environment, Winnipeg, Canada). Leaf temperature was maintained at 20 °C (± 1 °C) in both experiments. Relative humidity was 70% (± 5%) during the day and 85% (± 5%) during the night. Fluorescent tubes provided 92% and incandescent light bulbs 8% of the photosynthetic photon flux density (PPFD) at plant height (400 $\mu$mol/m$^2$/sec in Expt 3 and 500 $\mu$mol/m$^2$/sec in Expt 4). PPFD was measured with a Li-Cor Li190S quantum sensor. Plants grew in perlite and received daily a nutrient solution according to Hammer *et al.* (1978).

Mother plants of one clone of each cultivar were kept in a 10 h daylength. Plantlets produced from mother plants were grown in a 10 h daylength (Expt 3, 10 plants) or in a 16 h daylength (Expt 4, 12 plants) until the imposition of the daylength treatments. At that point, the 35-day-old plants were cut back to all but the three youngest leaves.

In Expt 3, plants grown in a daylength of 10 h were either kept at the same daylength or exposed to 13 h or 16 h for 35 days. In an additional treatment, half of the plants in 10 h received a nightbreak during seven cycles. A period of dark interruption was introduced for 1 h each night with incandescent light bulbs (PPFD 6 $\mu$mol/m$^2$/sec at plant height). In Expt 4, plants grown in a daylength of 16 h were either kept at the same daylength or exposed to a 10 h daylength for 42 days.

At the end of the experiments, the number of emerged leaves was counted on one stolon per plant. The stolon tips were cut off and examined as described under Expt 1. The number of inflorescence-bearing nodes, their position and the number of vegetative nodes enclosed in the stolon tip were recorded. The proportion of plants bearing an inflorescence at corresponding nodes was recorded.

## RESULTS

*Seasonal effects on floral initiation* (*Expt* 1). Figure 2 shows the percentage of inflorescence-bearing nodes at weekly intervals during the year. For Haifa, it varied between 10–20% (December to June) and 20–35% (July to November). For Ladino, the percentage ranged from 0–10% (December to July) to 10–20% (August to November). The best fitting sinoidal curve ($r$ = 0·73) was found for Haifa. Thus, about 50% of the variation in the proportion of inflorescence-bearing nodes was associated with seasonal effects which followed a sinoidal pattern. The curve for Haifa reached its peak in August, that for Ladino 1 month later.

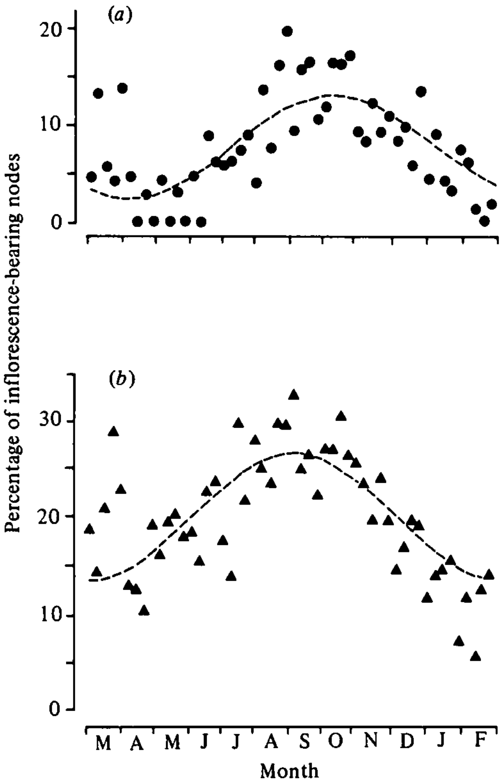


Fig. 2. Seasonal fluctuation in the percentage of inflorescence-bearing nodes in (*a*) Ladino and (*b*) Haifa, grown in Ayacucho. Stolon tips were harvested and examined at weekly intervals from March 1979 to February 1980. Fitted curves are indicated. They are described by

$$\% \text{ inflorescences in Ladino} = 7{\cdot}6(\pm 0{\cdot}52) + 5{\cdot}26(\pm 0{\cdot}73)\cdot \sin(t_{\text{months}}\cdot 30),$$

$$\% \text{ inflorences in Haifa} = 20{\cdot}00(\pm 0{\cdot}62) + 6{\cdot}53(\pm 0{\cdot}87)\cdot \sin(t_{\text{months}}\cdot 30).$$

*Altitudinal effects on floral initiation* (*Expt* 2). Leaf formation influences the number of potential sites for inflorescences because the axillary buds can develop into an inflorescence or a stolon. Therefore the rate of leaf appearance on a stolon basis was calculated. It was highest at the low site (0·97 leaves/week). Haifa developed fewer leaves than Ladino (0·86 as compared with 0·92 leaves/week, average of the three sites). Haifa produced an average of 5·4 visible inflorescences per stolon during the experimental period, Ladino 0·4. For Haifa, the number of inflorescences per stolon decreased with increasing altitude (5·8, 5·2, 5·1; s.e. 0·16), whereas it increased for Ladino (0·1, 0·4, 0·7; s.e. 0·1).

Table 2 shows the percentage of inflorescence-bearing nodes at different altitudes. This proportion was about 35% for Haifa on the first date at all sites but it decreased by October. For Ladino this proportion rose from very low values in July to 20–25% at the highest altitude, remaining below 5% at the lowest altitude.

*Daylength effects on floral initiation* (*Expts* 3 *and* 4). Figure 3(*a*) shows that only a few Haifa plants initiated inflorescences in a 10 h daylength and that this occurred towards the end of the experiment. However, after seven cycles with a low energy nightbreak initiation occurred in a short 'burst'. Following a daylength change from 10 to 13 h (Fig. 3*b*), an average of 3·9 inflorescences was formed on Haifa stolons as compared with 3·0 for Ladino. The daylength change from 10 to 16 h (Fig. 3*c*) led to only 1·7 inflorescences for Haifa but to 7·6 for Ladino. Following a change from 10 to

Table 2. *Influence of altitude on the percentage of inflorescence-bearing nodes in Ladino and Haifa (Expt 2). (Means of five clones are shown. Standard errors of clones are indicated)*

| Altitude (m) | Harvest date | Inflorescence-bearing nodes (%) Haifa | Ladino |
|---|---|---|---|
| 2470 | 20. vii | 34·0 ± 2·72 | 0·5 ± 0·45 |
| | 17. viii | 30·5 ± 2·54 | 0·0 ± 0·00 |
| | 14. ix | 30·5 ± 3·14 | 3·6 ± 2·59 |
| | 3. x | 29·4 ± 1·90 | 4·0 ± 2·44 |
| 2730 | 20. vii | 35·1 ± 3·39 | 0·0 ± 0·00 |
| | 17. viii | 35·4 ± 0·94 | 4·8 ± 3·14 |
| | 14· ix | 30·3 ± 2·46 | 10·2 ± 6·24 |
| | 3. x | 19·6 ± 4·47 | 6·1 ± 3·16 |
| 3250 | 20· vii | 36·9 ± 3·37 | 2·3 ± 2·05 |
| | 17. viii | 39·3 ± 1·33 | 10·2 ± 5·82 |
| | 14. ix | 34·7 ± 1·15 | 26·8 ± 4·42 |
| | 3. x | 31·7 ± 1·91 | 22·9 ± 4.26 |

13 h, floral initiation in Haifa continued almost to the end of the experiment. A change from 10 to 16 h led to a pattern of initiation similar to that at a low-energy night-break (Fig. 3*a*).

Figure 4 shows that Haifa did not produce any inflorescences in continuous 16 h days. Following a change from 16 to 10 h Haifa initiated 0·7 inflorescences but only towards the end of the experiment. In contrast, Ladino formed 6·3 inflorescences in a continuous 16 h daylength. Following a change from 16 to 10 h, floral initiation decreased strongly and only 1·4 inflorescences were produced.

## DISCUSSION

The increase in the proportion of inflorescence-bearing nodes in Expt 1 (Fig. 2) followed the onset of the cold season and coincided with the shortest days (Fig. 1). The flowering response of *T. repens* to photoperiod was usually found to be positively related to long days (Cohen & Dovrat, 1976; Thomas, 1981). In short daylengths, however, a positive flowering response to low temperatures has been reported (Britten, 1961; Thomas, 1979). The present results support these findings. The increase in the proportion of inflorescence-bearing nodes followed the relatively low temperatures from June owards. This increase was greater for Ladino at the higher altitudes (Table 2).

Many flowers were initiated in Haifa, irrespective

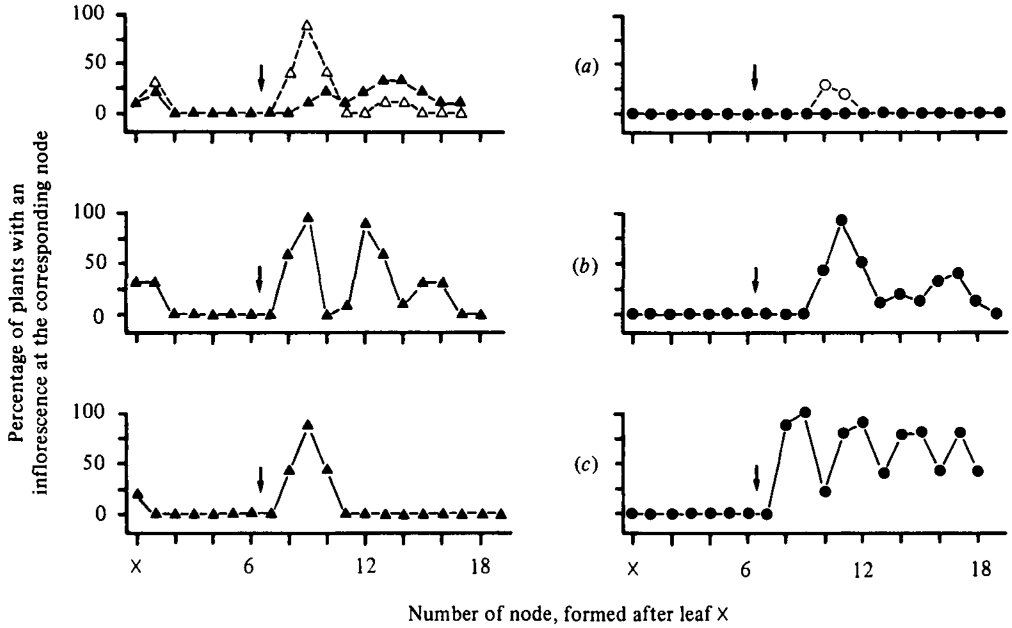


Fig. 3. Experiment 3: effect of three daylengths (*a*) 10 h, (*b*) 13 h; (*c*) 16 h, following a common 10 h daylength, on sequence and intensity of floral initiation in a Haifa (▲) and a Ladino (●) genotype. ×, Youngest fully emerged leaf at the moment of differentiation of the daylength treatments. The arrow (↓) separates nodes formed before (left) from nodes formed after (right) the differentiation of the treatments. Nodes between × and arrow correspond to leaf primordia contained in the stolon tip at the differentiation of the treatments. △, ○, Floral initiation at low-energy night-break during seven cycles after differentiation of the treatments.

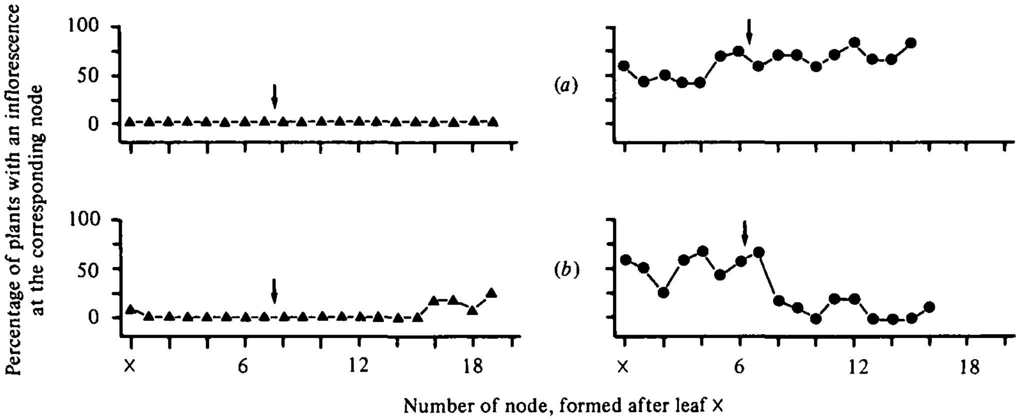


Fig. 4. Experiment 4: effect of two daylengths, (*a*) 16 h; (*b*) 10 h, following a common 16 h daylength, on sequence and intensity of floral initiation in a Haifa (▲) and a Ladino (●) genotype. ×, youngest fully emerged leaf at the moment of differentiation of the daylength treatments. The arrow (↓) separates the nodes formed before × (left) from nodes formed after (right) the differentiation of the treatments. Nodes between × and arrow correspond to leaf primordia contained in the stolon tip at the differentiation of the treatments.

of season and altitude. Ladino's response to season and altitude or, presumably, to low temperature was very marked but retarded. This delayed reaction corresponds to indications of retarded flowering of white clover cultivars from higher latitudes (i.e. Ladino) under cool short-day conditions (Thomas, 1982).

The differences in floral initiation between Haifa and Ladino at Ayacucho may be explained by their distinct photoperiodic requirements for flowering as shown in Fig. 3 and Fig. 4. Haifa showed a short–long-day response, but its floral initiation was most marked and persistent in a 13 h day, i.e. intermediate daylength similar to that prevailing in the Ayacucho region. Ladino showed a quantitative long-day response, forming no inflorescences in a 10 h daylength, few in 13 h and many in 16 h. Other clones of the cultivars Haifa and Ladino showed the same photoperiodic response as the two examined in the present study (von Sury, 1983). Haifa can, therefore, be considered to be an intermediate-day plant and Ladino to be a quantitative long-day plant. The results for Ladino confirm the findings of Thomas (1982) who classified Ladino with *T. repens* lines having a long-day response under warm conditions.

Haifa responded more strongly than Ladino to the seven night-break cycles. Floral initiation of Haifa in a 10 h daylength was also greatly enhanced by an exposure to only one 16 h cycle (data not shown). In other studies, at least two long days were necessary for *T. repens* to exhibit a positive floral response (Booysen & Laude, 1964; Thomas, 1961*b*). Haifa was apparently particularly sensitive to photoperiodic effects in this study.

For seed production at low latitudes, the contrasting photoperiodic characteristics of Haifa and Ladino, together with their responses to low temperature, have to be considered. Seasonal and altitudinal variation in floral initiation were probably related mainly to the corresponding fluctuation and duration of low temperatures. Ayacucho with its intermediate daylength appeared to be suitable for seed production of the intermediate-day cultivar Haifa. Haifa initiated many inflorescences early in the cold season thus enabling a seed harvest before the onset of the rains. Ladino, a long-day cultivar grown at this low latitude, required long-lasting low temperatures at a high altitude site for a marked floral initiation, thus delaying a potential seed harvest until the rainy season.

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