Growth of the white clover plant in relation to seed production

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ABSTRACT. The growth and development at the stolon apex of white clover is described, particularly in relation to inflorescence initiation, and the factors controlling such initiation are reviewed for the range of cultivars which has been studied. The difference between the responses to environment in plants of Mediterranean and high latitude origin are emphasized.

Key words: *Trifolium repens*, growth, flowering, photoperiod, temperature, seed production.

THE VEGETATIVE PLANT

The basic unit of growth of a white clover (*Trifolium repens* L.) plant is the stolon (Fig. 1). In a non-flowering plant the nodes of a stolon each bear a single leaf and an axillary bud. Each leaf normally has three leaflets atop a relatively long erect-growing petiole at the base of which are two stipules. The stipules are fused to form a sheath which surrounds the stem at the apical side of each node and obscures the axillary bud from view. Removal of a stipular sheath invariably reveals the presence of that bud in the leaf axil.

In addition, young nodes each bear two root primordia one of which is on the upper and the other on the lower side of the horizontal stem. When the lowermost primordium contacts a moist substratum it grows out to become a nodal root. Potentially, each node can produce a vegetative lateral shoot (a secondary stolon) from its axillary bud and a nodal root the vascular system of which connects with the lateral shoot. Each rooted node can thus live as an independent unit if it becomes isolated from the rest of the plant.

Increase in stolon length results from the activity of an apical bud consisting of several young leaves enclosed in the stipular sheath of the youngest visible leaf. Dissection of this bud by successively removing leaves reveals the structures shown in Fig. 1b-f. After each step in the dissection the oldest remaining pair of stipules ensheathes all younger leaves.

Each apical bud normally contains 6 or 7 leaf primordia, only very rarely having as few as 5 or as many as 8. This constancy in number results from the rates of leaf initiation and emergence being approximately equal. Each time a new leaf primordium forms at the apical meristem, so does the oldest leaf within the apical bud break through its enclosing stipular sheath to become visible as the youngest "emerged" leaf.

In a vegetative stolon the youngest axillary bud is usually discernible just apical to the point of attachment of the third-youngest leaf primordium, the two youngest leaf primordia subtending no buds.

RATE OF LEAF INITIATION

The rate at which leaf primordia are initiated at the stem apical meristem is strongly influenced by temperature and to a lesser extent by daylength, but is very little influenced by genotype. Thus in the natural environment leaves are initiated at a maximum rate of about two per week under summer growing conditions, and in winter the rate drops to about one every two or three weeks or less, with the rate of leaf emergence paralleling these changes (Fig. 2a). Rates of leaf emergence are very similar, however, in cultivars as different as 'Grasslands Huia', winter-growing 'Tamar' and summer-growing 'Kent Wild White' (Thomas, unpublished).

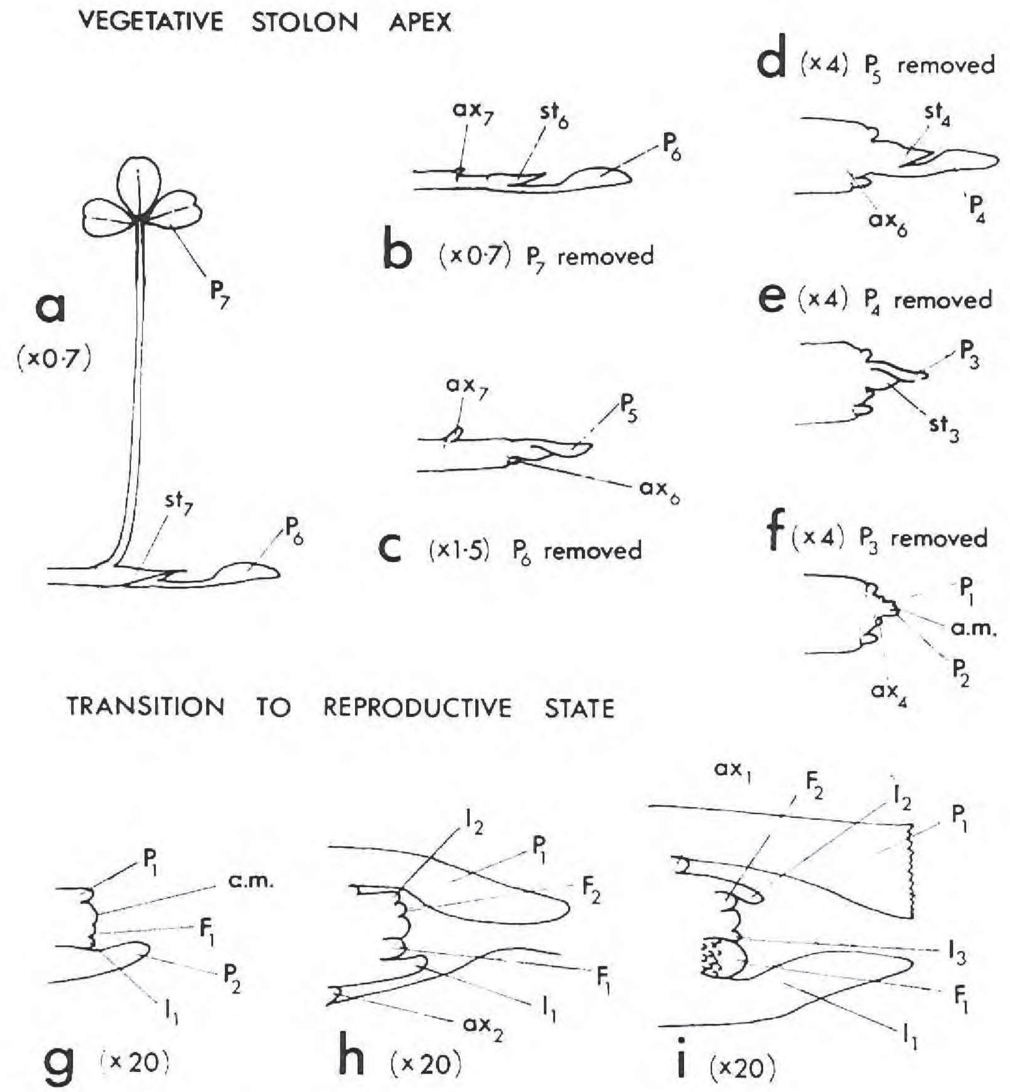

FIG. 1: *Structure of a vegetative stolon (a) and its apical bud as revealed by dissection (b–f); and the changes occurring at the apex of the same stolon during its transition to the reproductive state (g–i). Leaves and their primordia present in (a) are labelled P_1–P_7 in sequence from youngest to oldest; their axillary buds are labelled ax_1–ax_7 and their stipules S_1–S_7. New leaves formed as the apex develops from its original state (f) are labelled sequentially I_1, I_2, etc. Inflorescence primordia are labelled F_1, F_2, and the stem apical meristem a.m.*

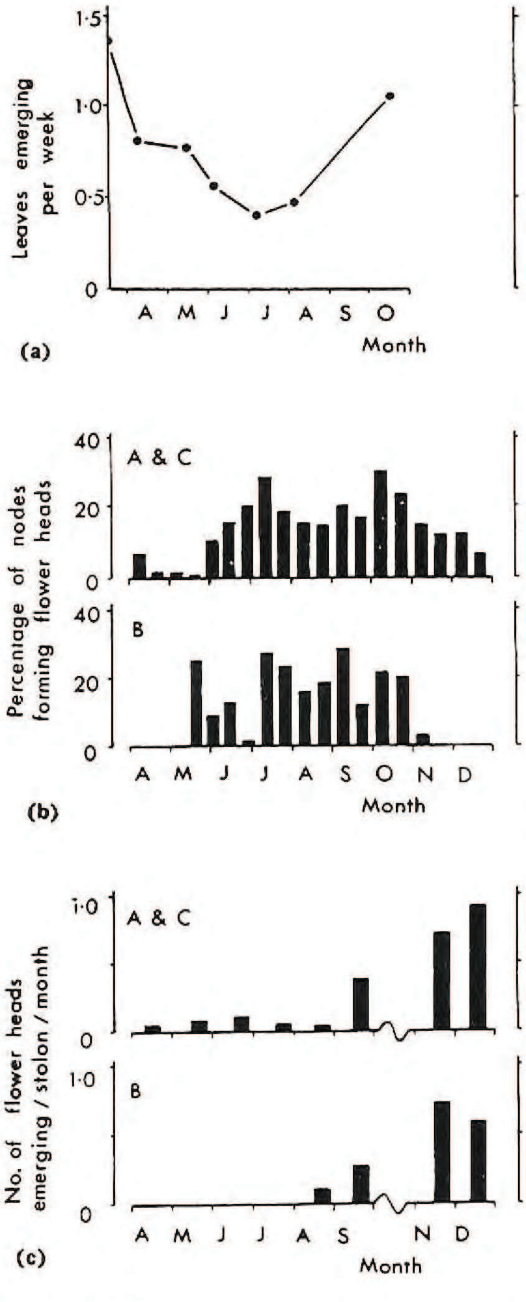

FIG. 2: *(a) Average number of leaves emerging per week in 3 clones (A, B, C) of 'Grasslands Huia' growing outside in Palmerston North. (b) & (c) Time of inflorescence initiation and emergence, respectively, in the same plants of clones A, B and C as in (a).*

THE FLOWERING PLANT

TRANSITION TO FLOWERING

Flower heads form at the stolon apex. Whereas in plants in the vegetative state the youngest axillary bud appears in the axil of the third-oldest leaf primordium at the apex of a stolon, with the transition to the reproductive state a bud forms in the axil of the youngest leaf primordium. This precocious axillary bud then grows into a flower head primordium (Fig. 1g–i) which usually emerges from its surrounding stipular sheath a few days after emergence of its subtending leaf. The subsequent behaviour of the stolon tip after this transition varies with the genotype of the individual plant and with the environmental conditions. In some plants, but not commonly, precocious axillary buds form with every leaf primordium initiated once the reproductive state is reached. More often, though, the formation of precocious axillary buds at one or two nodes is followed by formation of two or three leaf primordia without precocious buds, and this sequence is repeated for three or more cycles. These patterns of reproductive behaviour become apparent after several flower heads have emerged. In all cases, however, flower heads form only in a lateral, axillary, position; the terminal stem apical meristem on a stolon always remains vegetative.

On any given stolon at the time of transition to the reproductive phase, every stem apical meristem is a potential site for the initiation of inflorescence primordia. That is, every vegetative axillary bud at every node of the stolon has theoretically the potential to produce a precocious axillary bud at the time of initiation of its next leaf primordium. This does not happen, however. Initiation of inflorescence primordia occurs only at the terminal apical meristem on stolons and on elongating lateral branches. A correlative influence within the plant seems to prevent axillary buds within the apical bud and at the nodes immediately basal to it from forming inflorescence primordia. Even the youngest axillary buds initiated at the stolon apex just before its transition to the reproductive state always remain vegetative.

EFFECT OF ENVIRONMENT ON INFLORESCENCE INITIATION

The change from purely vegetative growth to flower head initiation is under the control of the environment, the two most important controlling factors being temperature and daylength. All white clover cultivars studied in New Zealand initiate flower heads in the cool conditions of autumn/winter/spring, and in addition some are stimulated to initiate flower heads by long days. Other factors, such as availability of mineral nutrient and defoliation, affect the degree to which plants are able to respond to temperature and daylength.

(a) *Effect of Low Temperature*

In common with many forage legumes, several cultivars of white clover initiate inflorescences under cool conditions regardless of daylength (Thomas, 1979). In plants of 'Grasslands Huia' grown in 12-h photoperiods at day/night temperatures of 12.5/6°C, initiation occurred on 83% of stolons after about 6 weeks, whereas at the same photoperiod plants grown at day/night temperatures of 17.5/10 or 22.5/14°C remained almost completely vegetative (Table 1). Plants of 'Grasslands Pitau' and a Spanish ecotype responded similarly, and similar results have been obtained with ecotypes adapted to high altitudes in Hawaii (Britten, 1960).

(b) *Effect of Long Days*

Many, but not all, lines of white clover initiate inflorescences when transferred from warm short days to long days. In 'Grasslands Huia' the critical daylength for initiation is about 14 h at 21°C (Thomas, 1961a). The number of flower heads produced in response to such a transfer increases with temperature (Thomas, in prep.) and with daylength, only reaching a maximum in continuous light (Thomas, 1961a, b). In 'Grasslands Huia', for example, virtually all stolons were found to initiate inflorescences when grown in 16-h days at 27.5/18°C day/night, whereas only 74% did so at 22.5/14°C (Table 1). Ladino clover responds similarly (Ridley and Laude, 1968).

In several cultivars, including 'Grasslands Huia', inflorescence initiation does not continue indefinitely in 16-h photoperiods, the initiation that occurs in response to transfer from short to long days stopping after about 3 weeks. Return of the plants to short days for about 4 weeks with photoperiods between 8 and 12 h renders them capable of initiating inflorescences once again upon transfer to 16-h days. 'Grasslands Huia' is thus a short-long-day plant at warm temperatures (Thomas, 1961a).

(c) *Effect of Nitrogen*

Several glasshouse trials have indicated that low availability of mineral nutrient decreases the intensity of inflorescence initiation in otherwise favourable environmental conditions. The strong interaction between temperature and nitrogen source is shown in Table 1 for two groups of pot-grown plants of 'Grasslands

TABLE 1: EFFECT OF TEMPERATURE AND NITROGEN SOURCE (INORGANIC N OR SYMBIOTIC N) ON PERCENTAGE OF STOLONS PRODUCING INFLORESCENCES IN 'GRASSLANDS HUIA', 'GRASSLANDS PITAU' AND A SPANISH ECOTYPE (C1067) IN 12-H AND 16-H PHOTOPERIODS

		Percentage Inflorescence Initiation					
		Huia		*Pitau*		*Spanish*	
Daylength (*h*)	*Temperature* (*day/night*) °C	*Inorg. N*	*Symb. N*	*Inorg. N*	*Symb. N*	*Inorg. N*	*Symb. N*
12	27.5/18	10	0	10	0	0	0
	22.5/14	0	7	0	9	0	0
	17.5/10	0	0	25	8	15	0
	12.5/6	84	82	69	76	94	94
16	27.5/18	98	86	—	—	—	—
	22.5/14	74	48	63	61	6	6
	17.5/10	70	3	71	34	29	20

Huia' and 'Grasslands Pitau', one group of which received nitrogen solely from a mineral nutrient solution and the other only by symbiosis with *Rhizobium*. The source of nitrogen had little effect on inflorescence initiation in 12-h photoperiods or in warm 16-h days, but in cool long-day conditions initiation was greatly depressed in plants dependent entirely on symbiotic nitrogen-fixing bacteria as a source of nitrogen (Thomas, in prep.).

(d) *Effect of Defoliation*

The cessation of inflorescence initiation in long days can be overcome temporarily by defoliation. This has been shown in controlled environment studies for one clone of 'Grasslands Huia' and in field plots of 'Grasslands Huia', 'Kent Wild White', 'Milkanova', 'Blanca', 'Olwen' and 'S.100' growing at the Grassland Research Institute, Hurley, England (Thomas, in prep.). At Hurley a marked stimulatory effect of defoliation on inflorescence initiation was revealed by comparing non-defoliated plants growing in the long days of mid-July with those defoliated 4 weeks before.

(e) *Inflorescence Initiation in the New Zealand Environment*

Studies of 'Grasslands Huia' grown in pots in the natural environment at Palmerston North have shown that the observed seasonal pattern of inflorescence initiation agrees with predictions based on controlled environment studies (Thomas, 1979). The times of inflorescence initiation and emergence in three clones of 'Grasslands Huia' grown outside for 9 months are shown in Fig. 2 (b & c). All clones initiated inflorescences in the cool short days of winter, starting in May/June; two (A and C) behaved in addition as short-long-day plants, initiating inflorescences in November/December in response to long days but tending to stop when the days were longest; the other (B) did not flower under warm conditions at any daylength.

Although strong inflorescence initiation was found to occur during June and July, inflorescences did not emerge until late August because the growth rate during the winter was slow. A flower head does not emerge until 7 or 8 more leaf primordia have formed at the

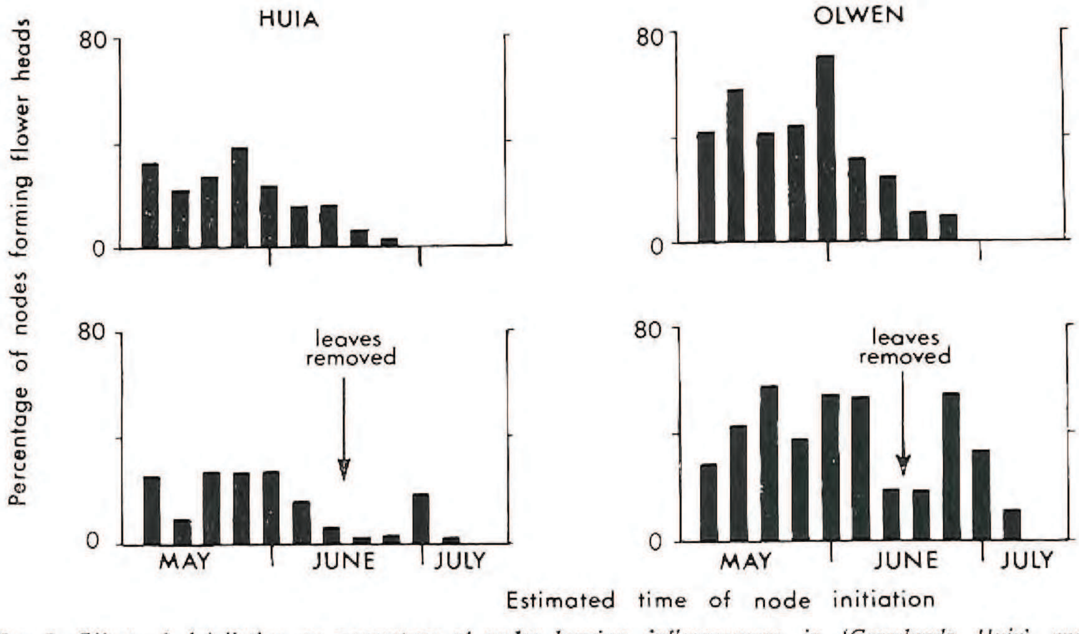

FIG. 3: *Effect of defoliation on percentage of nodes bearing inflorescences in 'Grasslands Huia' and 'Olwen' growing outside at Hurley in July (northern summer). All plants were fully foliated until mid-June.*

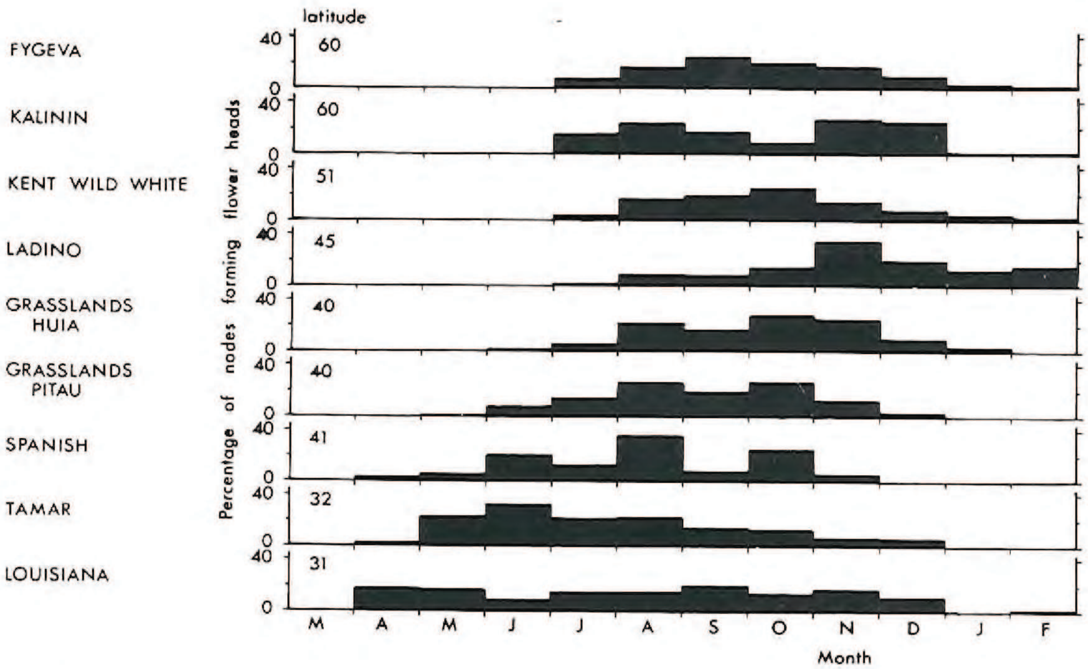

FIG. 4: *Average percentage of new nodes formed each month that also initiated flower heads in nine lines of white clover growing at Palmerston North (20 plants per population).*

same stem apex after its initiation. From the rates of leaf emergence indicated by the graph in Fig. 2a, averaging *ca.* 0.45 per week over June/July/August, an inflorescence developed from a precocious axillary bud initiated on 1 June would not have emerged until *ca.* mid-September. The first of the crop of spring flower heads thus developed from primordia initiated in midwinter.

(f) *Variation in Flowering Responses with Latitude of Origin*

Ecotypes of white clover, and cultivars and lines selected from them, have originated in a range of geographical localities extending from the Mediterranean in the south to arctic Norway in the north. The species also occurs wild in high altitude areas near the Equator, such as Colombia and Hawaii. The variation in response to the Palmerston North environment has been studied by comparing the growth of 20 representative clones from each of nine lines in pots outside from 1977 to 1979 (Thomas, in prep.), and the combined results of several experiments carried out over this period are shown in Fig. 4. This figure shows very clearly that, in general, inflorescence initiation started in the cool days of autumn/winter and declined during late spring and summer, in some cases stopping completely. Cessation of initiation in summer long days occurred similarly in other cultivars examined at Hurley, namely 'S.100', 'Milkanova', 'Blanca' and 'Olwen' (Fig. 3), but the most extreme case of summer cessation observed was found in wild populations growing at 70°N in Norway in which plants produced only one or, at most, two flower heads per stolon per year before initiation stopped in the continuous light of midsummer (Thomas, 1980).

On the basis of latitude of origin and flowering responses, white clover plants fall into two groups: "Mediterranean" types, such as 'Tamar' from Israel, "winter-growing" Spanish ecotypes and 'Louisiana', all originating at low latitudes; and "summer-growing, high-latitude" types, such as 'Kent Wild White', 'Ladino' and two Russian lines ('Kalinin' and 'Fygeva'). In the "Mediterranean" types, initiation in response to cool conditions starts in autumn and continues through the winter, the onset of initiation being earliest in plants from lowest latitudes ('Tamar' and 'Louisiana'). "High-latitude" ecotypes mostly do not begin initiation in response to cool conditions until June or July onwards or, in some individual plants, October. The time of low-temperature-induced initiation in 'Grasslands Huia' is intermediate between that in Mediterranean and high-latitude plants; and in 'Grasslands Pitau', a hybrid between 'Grasslands Huia' and a Spanish ecotype (C1067), low-temperature-induced initiation occurs at a time about midway between that in its two parents.

The difference between "Mediterranean" and "high-latitude" lines also shows, albeit less clearly, in relation to inflorescence initiation in late spring and summer, the former tending to stop initiation earlier than the latter. In 'Tamar' and the Spanish ecotype, both of which show a typical "Mediterranean" response, initiation stops with the onset of warm conditions, regardless of daylength, whereas the high-latitude plants tend to be short-long-day plants (SLDP) in which strong initiation occurs in the long days of November and December but stops in January. 'Louisiana' differs in that it behaves as a typical Mediterranean plant in the autumn with regard to low temperature initiation but reacts as a SLDP in summer; and 'Ladino' clover differs from the other high-latitude plants in that it seems to be a long-day-plant, rather than a SLDP, inflorescence initiation continuing right through the summer. With regard to the long-day response, 'Grasslands Huia' is similar to "high-latitude" plants; and its hybrid derivative, 'Grasslands Pitau' is midway between its two parents.

The vigour of initiation of high-latitude plants in December in Palmerston North is dependent on the critical daylength for the long-day reaction. In 'Grasslands Huia' this is approximately 14 h and long-day initiation starts in early November (Thomas, 1979), but in many individuals within populations from higher latitudes, such as 'Kent Wild White' and Russian lines, the critical daylength exceeds the length of the longest photoperiod, with the result that initiation does not occur. Such plants not showing long-day initiation in late December in Palmerston North can be readily induced to flower by transference into continuous light (Thomas, in prep.).

The Effect of Flowering on Vegetative Growth

White clover is unusual as a forage legume in that there is no reduction in leaf size associated with the production of flower heads; in fact a slight increase in the rate of growth has been recorded in association with the start of flower initiation (Thomas, 1961c). Internodes immediately basal to first-formed inflorescences are longer than average, and those immediately apical tend to be shorter (Booysen and Laude, 1964).

Under normal growing conditions the only clear effect of flower production on growth is a reduction in the number of vegetative axillary buds because flower heads develop in axillary bud sites. Each node can thus produce one vegetative axillary bud or one flower head, but never both. In a plant in which every node on a stolon produces a flower head, the only potential for regrowth from lateral branches after flowering stops is from the base of the plant, whereas a plant in which many nodes do not initiate flower heads has in theory much more capacity for regrowth from the younger regions of its stolons. Gibson (1957) has shown persistence in Ladino clover to be related to flowering intensity and duration of inflorescence initiation. More vigorously flowering plants persisted less well than weaker flowerers, and those individuals that stopped inflorescence initiation earlier in the growing season persisted better than those that continued to initiate freely right into the hottest and driest period of summer. On this basis, from a comparison of the data for 'Grasslands Huia' and 'Olwen' in Fig. 3, the latter might be expected to have a higher yield of seed heads per unit area but to have less potential for regrowth than 'Grasslands Huia'.

CONCLUSIONS

The seed production capacity of white clover is the combined result of the number of flower heads emerging per unit time, the number of florets per head, the number of ovules per floret and the fertility of pollen and embryo sacs (Thomas, 1961b). There is much genetic variability within the species, both in the average number of flower heads produced per node and in the conditions affecting their initiation. Maximum head emergence per week is attained under conditions in which the rate of leaf emergence and the percentage of nodes forming precocious axillary buds are both at their highest.

In plants of Mediterranean type the proportion of nodes initiating inflorescences is highest from late autumn to early spring, and these emerge from September to December. Plants of high-latitude origin tend not to begin to initiate till late winter or early spring, and the peak in flower-head emergence thus occurs a few weeks later than in Mediterranean plants. This difference between Mediterranean and high-latitude lines in times of emergence of the first crop of flower heads in the spring is much less than might be expected from the times of initiation (Fig. 4) because low temperatures in winter slow the rate of emergence of inflorescences formed in autumn and the increase in warmth as spring progresses hastens the emergence of late-formed heads.

Inflorescence initiation also tends to continue for longer in high-latitude plants than in lines of Mediterranean type, the duration of the period of initiation depending upon whether the plants are short-long-day plants, stopping in long days, or long-day plants in which initiation continues. Most plants of Mediterranean origin stop initiation well before Christmas, whereas some high-latitude cultivars will continue through the summer. In all cases, however, under New Zealand conditions a peak of blossoming occurs in spring/early summer.

The overriding controls of initiation and blossoming time, apart from the genetic constitution of the plants, are temperature and daylength. Once initiation has occurred, the rate of progression to blossoming can be slowed by low temperatures and drought, and the number of heads produced at the time of initiation can be influenced by the level of mineral nutrition and by defoliation treatments in some plants; but given good cultural conditions of high available nutrient and moisture, management treatments can only be expected to influence timing and intensity of flower-head production to a limited degree.

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