Modification of Stolon Growth and Development of White Clover (*Trifolium repens* L.) by Growth Regulators, and its Influence on Flower Production.[1]

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ABSTRACT

A glasshouse experiment investigating the effects of the plant growth regulator parlay on the stolon growth and development of white clover cv. Olwen showed that parlay applied at 0.5 and 1.0 kg a.i. ha^{-1} significantly reduced internode length, stolon length and petiole length and significantly increased the proportion of axillary buds per stolon. The application of parlay did not, however, significantly effect the total number of nodes per stolon, the number of reproductive nodes per stolon or the intermittent pattern of flowering along the stolon.

The results are discussed in relation to the possible use of parlay for increasing the seed production potential of white clover seed crops.

Additional index words: **white clover, growth regulators, stolon growth, flowering, seed yield components, parlay, PP 333**

INTRODUCTION

Flowering in white clover (*Trifolium repens* L.) begins when physiological responses to temperature and daylength are sufficient to induce the development of inflorescence primordia rather than vegetative buds in leaf axils (Thomas, 1962). The pattern of response to these stimuli is typically one in which the first flowering node produced on a stolon is followed by two or three vegetative nodes, another flowering node, a further two or three vegetative nodes and possibly a third flowering node (Thomas, 1981). The stolon then reverts back to the vegetative state, producing only vegetative buds in the axils of the leaf primordia. This intermittent pattern of flower production along the stolon dictates that inflorescences appear over a period of time, with the extended flowering period and the resultant range of flower ripeness categories in the crop making it difficult to optimize the harvest date. While early harvesting can therefore result in unripe heads being gathered, a late harvest can miss the older, earlier produced heads which may have sprouted or fallen below cutting height (Norris, 1984).

Concentrating flower production on successive nodes on the stolon could therefore increase the proportion of ripe heads at harvest and increase seed yield. Both long days (Norris, 1985a) and higher temperatures increase the number of successive nodes producing flowers (Ridley and Laude, 1968) and the number of reproductive nodes per stolon (Norris, 1985b), although there is considerable varietal variation. Clearly one alternative is to produce white clover seed in geographical locations where temperature and daylength are more suitable for optimum flowering. However, white clover seed yields might be increased under United Kingdom conditions, by using plant growth regulators to manipulate the growth, development and flowering pattern either directly by increasing flower numbers and potential seed yields or indirectly by encouraging more stolon production and increasing the potential number of sites for flower production. Such chemicals have been used successfully to manipulate growth and increase yield in a number of species including grasses (Hampton and Hebblethwaite, 1985) and cereals (Hutley-Bull and Schwabe, 1982; Koranteng and Matthews, 1982). One field experiment has identified parlay (PP333) as a potentially useful chemical that could influence crop growth and increase flower production in white clover (Marshall and Hides, 1986). However, results from that experiment did not clarify whether the increased flower production could be attributed to a greater proportion of reproductive nodes on each stolon, which would also influence the proportion of ripe heads at harvest, or simply to increased stolon production per unit area.

The present paper reports the results of a glasshouse experiment designed to study in detail the influence of parlay on stolon development and flower production of white clover cv. Olwen.

MATERIALS AND METHODS

Plants of white clover cv. Olwen were sown on 18 October 1984 in trays containing John Innes No. 3 compost containing 16% grit, 25% peat and 59% soil with a

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50% reduction in nitrogen in a glasshouse maintained at a constant temperature of 16°C. Three seeds sown per pot were singled to one on 24 February 1985 and transplanted into 180 mm diameter pots containing John Innes compost. Plants were cut to stolon level on 15 March 1985 and two actively growing stolons per plant marked with colored wire.

At three stages of development of the first flowering bud on each stolon (Table 1), parlay was applied by foliar spray onto the growing plants at two rates (Table 2). Controls received no growth regulator. At each application date, the marked stolons were tagged with a second colored wire behind the first fully expanded leaf. There were 10 replicate plants, giving a total of 20 stolons per treatment and the pots were arranged in a randomized block design within a glasshouse maintained at 16°C.

Table 1. Stages of inflorescence development of the first flowering node on stolons of white clover cv. Olwen at the three application dates.

Stage of development of first flowering bud on the stolon	Date of application
Final stage of transition to reproductive phase - but not visible on the stolon	19 March
Appearance of flower bud on the stolon	4 April
Peduncle height of 3 cm	30 April

Biological measurements

On 31 May the position and number of reproductive and vegetative nodes was recorded on each marked stolon. Total stolon length and the internode length and petiole length were measured at each node. Individual plant means of all values were then calculated. For analysis the system of labelling nodes as described by Thomas (1981) was adopted. The first node after the tag produced after chemical treatment was called node 1 and nodes distal to this 2, 3, 4 and so on. The number of nodes that bore an inflorescence or axillary bud at each position was then expressed as a proportion of the total nodes.

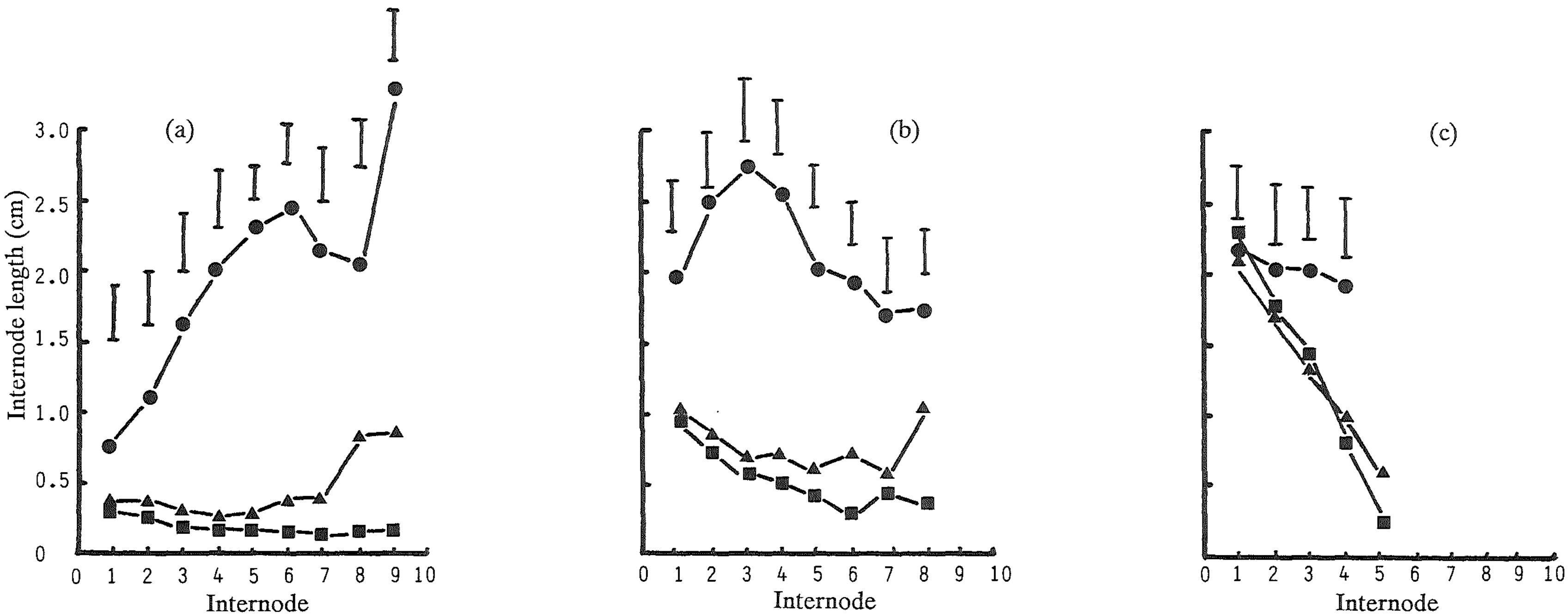
RESULTS

Stolon length was significantly reduced by parlay (applied at both 0.5 and 1.0 kg a.i. ha^{-1}) at both the first and second application dates, but at the third application date was only significantly reduced by the highest rate of parlay (1.0 kg a.i. ha^{-1}) (Table 2). Mean internode length was significantly reduced by parlay (applied at both 0.5 and 1.0 kg a.i. ha^{-1} at all three application dates but the reduction in internode length at the third application date was less than that on the first two dates (Table 2).

At the first and second application dates, parlay significantly reduced the length of the first internode produced after its application, and subsequently reduced the length of all internodes (Figure 1). At the third application date there were fewer nodes formed due to the shorter period of time between applying parlay and sampling, but parlay had less immediate effects on these nodes and only significantly reduced the length of the third and fourth internodes.

Table 2. Effect of parlay on stolon length and internode length of white clover cv. Olwen.

	Application rate (kg a.i. ha^{-1})							
Date of application	Total stolon length (cm)				Mean internode length (cm)			
	Con	0.5	1.0	Mean	Con	0.5	1.0	Mean
19 March	16.5	4.5	3.0	8.0	2.2	0.9	0.6	1.2
4 April	17.3	7.0	6.8	10.4	2.6	1.1	1.0	1.6
30 April	10.2	9.1	8.0	9.1	2.9	2.3	2.1	2.4
Mean	14.7	6.9	5.9		2.6	1.4	1.2	
LSD (0.05)								
Dates	1.2				0.2			
Rates	1.2				0.2			
Dates x rates	2.0				0.4			



LEGENDS TO FIGURES

Figure 1. **The effect of parlay applied at 0.5 kg a.i. ha^{-1} (▲—▲) and 1.0 kg a.i. ha^{-1} (■—■) on 19 March (a) 4 April (b) and 30 April (c) on stolon internode length (cm). Controls (●—●) received no growth regulator. Vertical bars represent LSD at P = 0.05.**

Table 3. Effect of parlay on stolon development of white clover cv. Olwen.

Date of application	Total nodes/stolon				Axillary buds/stolon				Reproductive nodes/stolon				Mean petiole length (cm)			
	Con	0.5	1.0	Mean	Con	0.5	1.0	Mean	Con	0.5	1.0	Mean	Con	0.5	1.0	Mean
19 March	8.6	6.8	6.9	7.4	2.9	2.6	2.4	2.6	1.9	1.8	2.1	1.9	9.5	4.3	3.3	5.7
4 April	7.6	7.2	7.8	7.5	1.3	3.5	3.7	2.8	1.6	2.0	2.1	1.9	9.2	5.2	5.2	6.5
30 April	4.5	5.0	4.8	4.8	0.6	1.0	1.1	0.9	1.4	1.1	1.3	1.3	5.2	4.5	4.3	4.6
Mean	6.9	6.3	6.5		1.6	2.4	2.4		1.6	1.6	1.8		7.9	4.7	4.3	
LSD (0.05)																
Dates	0.4				0.5				0.2				0.7			
Rates	NS				0.5				NS				0.7			
Dates x rates	0.4				0.8				NS				1.3			

There was no significant overall effect of rates of chemical application on the total nodes/stolon (Table 3), but the number of nodes produced was significantly less at the third application date due to the shorter time between spraying and sampling. The significant interaction between rate and date of application resulted from the fact that at the first application date the total number of nodes/stolon was reduced by both rates of application, while on the other two application dates the application of parlay had no effect on node production.

Stolon nodes can remain dormant or produce axillary buds, leaves or flowers, and the proportion of these are shown in Table 3. Although axillary bud development was significantly reduced at the third application date, the overall effect of application of parlay at both rates was to increase the proportion of axillary buds. The significant interaction between dates and rates shows that there was a significant increase in axillary bud production as a result of chemical treatment on the second application date, there being no significant effect of treatment on the other two dates. The influence of the application of parlay on axillary bud development is shown further in Figure 2, where it can be seen that in general at both rates of application at all three dates, more of the later formed nodes were induced to produce axillary buds compared to the untreated controls. The increase in axillary bud production resulting from the application of parlay on the second treatment date is also clearly shown.

The petiole length was significantly reduced by both rates of application of parlay and was significantly influenced by application date (Table 3). There was also a significant interaction between dates and rates, as application rate had a significant effect on petiole length at the first treatment date, but there were no differences between application rates at the second date, whilst at the third date neither rate of parlay reduced petiole length. Figure 3 shows the effect of parlay on individual petioles. In general, parlay applied at both rates at the first and second application dates had an immediate effect on petioles formed immediately after application. On the third application date, however, parlay had less effect and significant effects were only observed at the highest application rate.

DISCUSSION

A previous field experiment (Marshall and Hides, 1986) suggested that parlay could influence the vegetative and reproductive growth of white clover. The present glasshouse experiment has confirmed some of the results from the field experiment, and some conclusions about the effect of parlay can be made in relation to its possible use for improving seed yield of white clover.

The importance of application date is emphasized by the reduction in number of nodes per stolon occurring at first application but lack of effect at other dates. Parlay also reduced stolon length by shortening the length of all internodes. Similar results have been obtained with parlay from studies on perennial ryegrass (*Lolium perenne*) seed crops in which parlay prevented or reduced lodging, depending upon the application rate, by reducing stem internode length and thereby shortening and strengthening the stem (Hampton and Hebblethwaite, 1985). Although shortening internode length of white clover may not directly increase seed yields, it may do so indirectly if the growth rate of these shortened stolons is unaffected by chemical treatment, thereby reducing the time period over which flowers appear. This could result in an increase in the proportion of ripe heads at harvest and thus an increase in potential seed yields. This, however, needs confirmation from further measurements of stolon growth rates in the glasshouse and the field.

In the present experiment parlay did not increase the number of reproductive nodes per stolon, and had little influence on the intermittent pattern of flowering on the

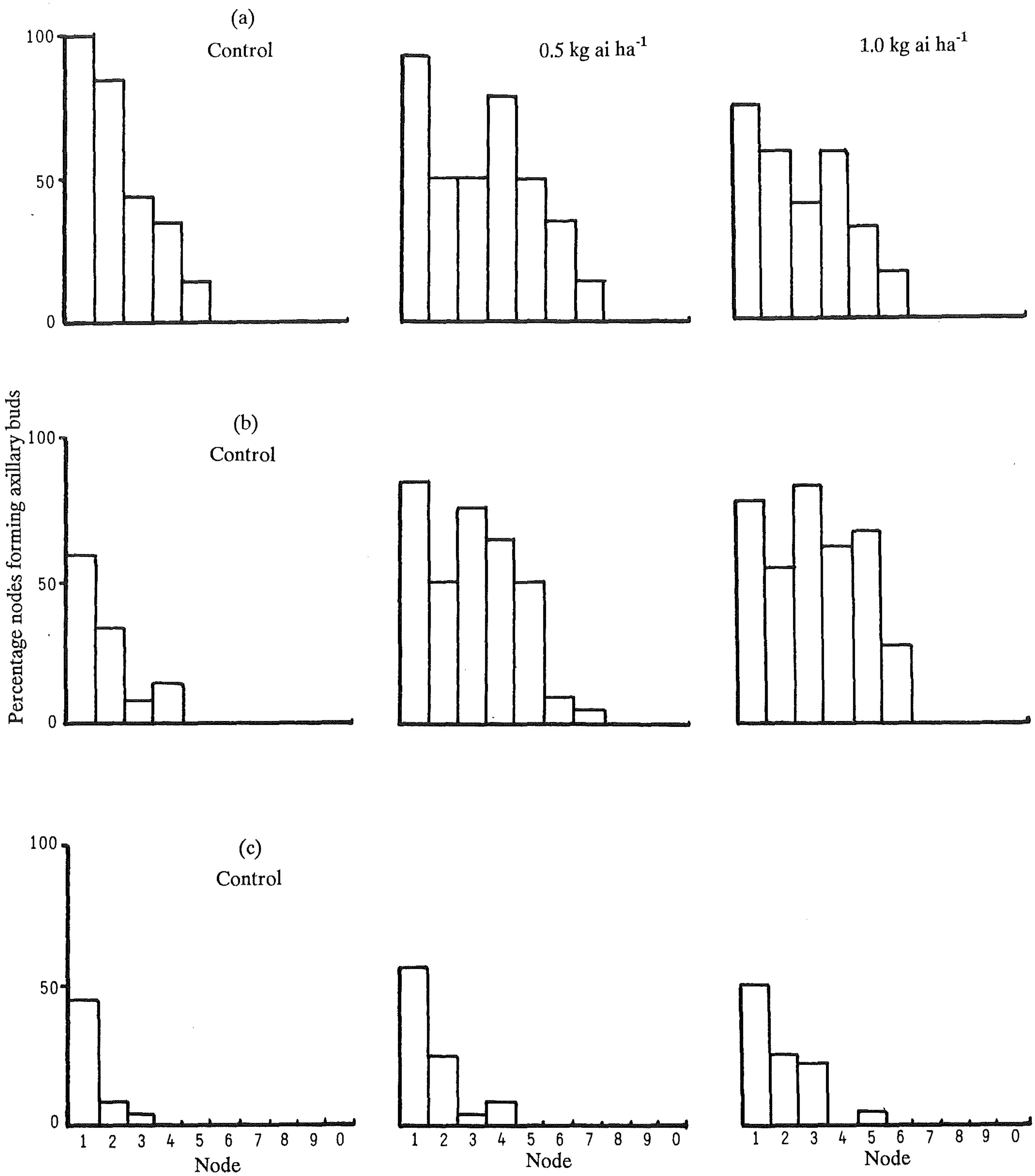


Figure 2. **The effect of rate and date of application of parlay on axillary bud development at nodes in relation to their distance from the stem apex. Node age decreases from left to right, node 1 being the first node formed after application of parlay. Parlay applied at 0.5 kg a.i. ha^{-1} and 1.0 kg a.i. ha^{-1} on 10 March (a), 4 April (b) and 30 April (c). Controls received no growth regulator.**

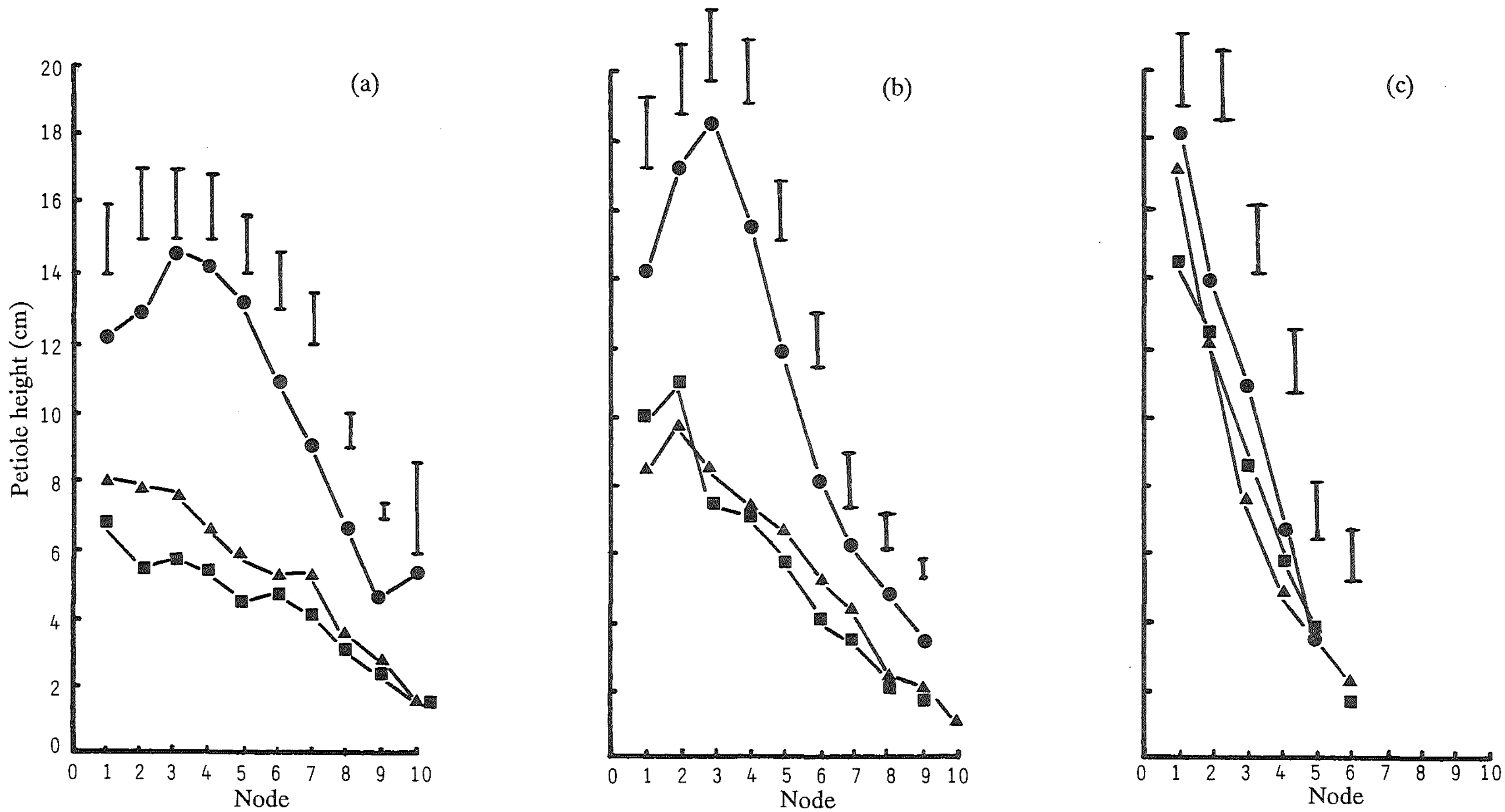


Figure 3. **The effect of parlay applied at 0.5 kg a.i. ha^{-1} (▲—▲) and 1.0 kg a.i. ha^{-1} (■—■) on 19 March (a), 4 April (b) and 30 April (c) on petiole height at nodes on the stolon. Controls (●—●) received on growth regulator. Vertical bars represent LSD at P =0.05.**

stolon, which was similar to that described by Thomas (1981). This suggests that in the field, these chemical treatments are unlikely to increase the total number or percentage of ripe heads at harvest by a direct effect on the number of primary nodes producing flowers. There was, however, a significant increase in the proportion of nodes producing axillary buds after chemical treatment, especially at the second application date. This resulted from an increase in the proportion of later formed nodes producing axillary buds. Similar results have been obtained in other glasshouse experiments by applying ABA to white clover grown under long days (Cohen and Dovrat, 1976). This suggests that in the field, the appropriate application of growth regulators could increase the proportion of axillary buds and thereby stolon branching which could lead to an increase in flower production. In a previous field experiment parlay had no effect on flower numbers (Marshall and Hides, 1986), but its effect on stolon branching was not measured and only one application date was considered. Furthermore, since evidence from field studies has shown that an overdense stand can reduce flower production and seed yield (Hides et al., 1984), this effect of parlay on stolon branching clearly requires further investigation in order to determine its value for improving seed yields.

A general effect of all parlay treatments was to significantly reduce the length of petioles formed along the stolon. This confirms results from field experiments where significant reductions in petiole length but not peduncle height elevated flowers above the leaf canopy (Marshall and Hides, 1986). Although peduncle height was not measured in this experiment, the elevation of flowers above the leaf canopy has been identified as a potentially useful method of improving pollination by making flowers more accessible to a pollinating insect. This is particularly important in growing seasons where less than ideal weather conditions and excess leaf growth may reduce the efficiency of the pollinator and lead to difficulties in drying of seed heads.

Although the general effects of parlay on white clover are evident, some anomalies clearly exist particularly in relation to the effects of application dates. This can be attributed partly to the fact that the effect of application date is confounded with the growth stage of the plant, as on later dates of application there was a shorter period between spraying and data collection. Consequently, parlay appeared to have less effect at the later application dates. This may not be due to the ineffectiveness of the chemical, but in part due to fewer nodes being formed after chemical treatment and to the removal of stolons for assessment. Furthermore, since parlay is soil active (Shearing and Batch, 1982) and its uptake and activity require adequate moisture in the upper part of the soil (Hampton and Hebblethwaite, 1984), soil conditions will influence the effectiveness of this chemical. The different response of some clover growth components at the different application dates suggests that growth stage and chemical activity have been confounded. If any of these treatments are to have beneficial effects on the seed yield potential in the field then the date of application in relation to the growth of the clover plant and soil conditions will need to be clearly defined.

In conclusion, the present experiment has shown the considerable potential of growth regulators to influence stolon growth and development of white clover in ways that could increase seed yields. However, further investigation is required to determine whether chemical treatment can affect the potential seed yield of white clover and whether these effects can be used to give consistent and profitable improvements in seed yield.

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Breeding for Seed Retention in Orchardgrass (*Dactylis Glomerata* L.)[1]

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ABSTRACT

Loss of seed from maturing inflorescence (seed shattering) is common in grasses and constitutes a serious economic problem in many species. Seed shattering was studied in two orchardgrass cultivars which showed high and low shattering ('Hallmark' and 'Marta', respectively) and in their progenies with the aim of improving seed retention in 'Hallmark'.

Morphology and histology of individual spikelets of both cultivars were examined in 1986 to determine the mechanism of seed shattering.

It was observed that shattering is a two-stage process, involving disarticulation of the rachilla, followed by subsequent release of seed from the glumes. No clear-cut morphological and histological differences in spikelet structure were observed between high and low-shattering cultivars. The major differences were found in the development of the two abscission layers: the primary below the caryopsis and the secondary just above the glumes. In Hallmark these layers were identifiable and more evident at an earlier stage of seed development.

Twenty plants of Hallmark were selfed and crossed with a Marta counterpart and the same was done for 20 plants of Marta. Parents and progenies of plants from the two cultivars obtained by selfing or crossing were evaluated for seed shattering as spaced plants in the field. Fifty days after the anthesis date, plants were visually scored for seed shattering. Hallmark and Hallmark selfed had very high seed shattering scores (8.02 and 8.52, respectively), while Marta and Marta selfed had low seed shattering scores (2.53 and 2.04, respectively). The two F1 populations from reciprocal crosses had the same scores (6.60). There was a large difference in seed retention between Hallmark and Marta and the genetic control of this character seems to be simple and well fixed in the two populations.

Italian genotypes such as Marta have genetic resistance to shattering. Combining this resistance to seed shattering has great potential to improve seed yield characteristic of new forage cultivars.

Additional index words: **shattering, cocksfoot, abscission.**

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INTRODUCTION

Orchardgrass is one of the grazing plants of the temperate zones. It is taxonomically classified in the genus *Dactylis*, which is a small genus in the tribe *Festuceae* of the grass family. The best-known species in the genus is *Dactylis glomerata* L., orchardgrass or cocksfoot. The