Effect of Plant Growth Regulators on a White Clover *(Trifolium repens* L.) Seed Crop. I. Plant Growth and Development

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ABSTRACT

The effects of plant growth regulators on white clover growth and development were assessed under field conditions over two consecutive years. In the 1988/1989 season, chlormequat chloride (1.5 and 3.0 kg a.i. ha^{-1}), paclobutrazol (0.5 and 1.0 kg a.i. ha^{-1}) and triapenthenol (0.5 and 1.0 kg a.i. ha^{-1}) were applied either during reproductive bud initiation (October) or when reproductive buds were visible (November), and a further plant growth regulator, daminozide (2.0 and 4.0 kg a.i. ha^{-1}), was applied only in November. In the 1989/1990 season, chlormequat chloride at 3.0 kg a.i. ha^{-1}, paclobutrazol at 1.0 kg a.i. ha^{-1} and triapenthenol at 1.0 kg a.i. ha^{-1} were applied either in September, October or November to a second year white clover crop. Chlormequat chloride did not affect plant growth and development. Daminozide reduced petiole and peduncle lengths, but did not increase node production. Triapenthenol did not show a retardation effect and did not increase node or stolon production. However, reproductive nodes along the stolon were increased following application of triapenthenol at 1.0 kg a.i. ha^{-1} in November. Paclobutrazol reduced petiole length but not peduncle length. It also increased node production, presumably due to increased stolon production since nodes along the stolon were not increased. Increased reproductive nodes along the stolon were recorded following paclobutrazol application in November. Total plant dry matter was not affected by paclobutrazol, but its distribution was altered. The implication of all these effects for seed production is briefly discussed.

Additional index words: **plant growth regulators, chlormequat chloride, daminozide, paclobutrazol, triapenthenol, white clover, plant growth and development.**

INTRODUCTION

Seed yield in white clover depends largely on the number of ripe inflorescences per unit area (Clifford, 1987), and is considered to be limited by the intermittent pattern of inflorescence production, i.e. the delayed development of inflorescences along stolons in which there are several vegetative nodes between successive inflorescences (Thomas, 1987). This flowering pattern dictates that inflorescences appear over a period of time during which inflorescence buds, blooming inflorescences, young pods and mature pods ready to dehisce can be present simultaneously on an individual plant (Hollington, Marshall and Hides, 1989). This extended flowering period and the resultant range of flower ripeness categories in the crop makes it difficult to determine optimum harvest date. In addition, since white clover is an indeterminate plant which forms its inflorescences at nodes, inflorescence production is determined by the number of potential sites, i.e. nodes, initiated in stolon apices, by the number of actively growing stolon apices and the ability of these to respond to the floral stimulus (Thomas, 1987). However, efforts to increase stolon production, such as the provision of additional mineral nutrients (Clifford, 1987; Clifford and Rolston, 1990) and irrigation (Deschamps and Wery, 1988), may result in excessive vegetative growth which subsequently can reduce inflorescence density and fertility by the shading effect of competitive leaves. Therefore, methods that can be used to manipulate this species to both increase and concentrate inflorescence production without necessarily producing excessive vegetative growth, can be expected to increase seed yield.

Plant growth regulators have been used successfully to manipulate growth and increase seed yield in a number of herbage legume species, including *Trifolium pratense* L. (Niemelainen, 1987) and *Lotus uliginosus* Schk. (Tabora and Hampton, 1992; Tabora and Hill, 1992). In the United Kingdom environment, plant growth regulators including paclobutrazol, chlormequat chloride, daminozide and ethepon have also been used to try to increase white clover seed yield (Mohamed, 1981; Marshall and Hides, 1986), but the results were unsuccessful. However, further field experiments reported by Marshall and Hides (1991b) showed that paclobutrazol increased the potential seed yield of white clover cvs. Menna and Olwen mainly through increasing inflorescence numbers, although the response was both time– and rate–dependent. Similarly, Hampton (1991) showed that paclobutrazol increased the number of inflorescences and thereby seed yields in four New Zealand white clover cultivars. On the other hand, in the Belgian environment, Rijckaert (1991) reported that paclobutrazol and triapenthenol increased the seed yield of white clover cv. Merwi through a large reduction in vegetative growth, which resulted in the elevation of inflorescences above the plant canopy for better pollination, and hence increased the number of seeds per floret. However, the effects varied between years depending on climatic conditions. Boelt (1991) also reported that the use of paclobutrazol, triapenthenol and daminozide produced inconsistent results in white clover cv. Milkanova in Denmark, while ethepon and Folicur had no effects on seed yield. These inconsistent results suggest that responses may differ depending on the type of plant growth regulator, rate and time of application, cultivar, site and season.

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The present field trials were conducted with the specific aims of evaluating the potential effects of some selected plant growth regulators applied at different rates and times on a white clover seed crop grown in the New Zealand environment. This paper reports the effects on vegetative growth and development. Results for seed yield components and seed yield will be reported in a later paper.

MATERIALS AND METHODS

The trials were carried out on the Frewin's block of the Pasture and Crop Research Unit, Massey University, Palmerston North during the 1988/1989 (Year 1) and 1989/1990 (Year 2) growing seasons. The soil type was a Tokomaru silt loam soil classified as an aeric fragiaqualf (gleyed yellow–grey earth), of moderate fertility. A medium large leaved white clover cv. Grasslands Pitau was used in the trials. The seed source was certified Breeders seed (Grasslands No. C5890) supplied by AgResearch Grasslands, New Zealand. The seed was sown on 23–24 March 1988 at a sowing rate of 3 kg ha^{-1} and inter row spacing of 45 cm. The crop established from this sowing (a first year crop) was used for the first year trial. No fertilizer or irrigation were applied and no grazing was conducted prior to the beginning of treatment application. Inter row cultivation was conducted to control weeds during early growth, and paraquat (2 1 ha^{-1}) was applied on 21 September 1988 to control weeds and to reduce vegetative growth. A randomized complete block design with three replicates was used. Plot size was 7 x 2 m with regularly cut pathways of 2 m between blocks and 1m between plots. The Year 1 trial consisted of 14 treatments and one control. Treatments used were paclobutrazol at 0.5 and 1.0 kg a.i ha^{-1}, triapenthenol at 0.5 and 1.0 kg a.i ha^{-1}, chlormequat chloride at 1.5 and 3.0 kg a.i ha^{-1} and daminozide at 2.0 and 4.0 kg a.i ha^{-1}, applied during reproductive bud initiation (11 October 1988) and when reproductive buds became visible (8 November 1988) for paclobutrazol, triapenthenol and chlormequat chloride, and only at the reproductive bud stage for daminozide. These application rates were chosen on the basis of previously recorded responses from white clover and/or other herbage legume seed crops (Budhianto, 1992).

In Year 2, three of the plant growth regulators (paclobutrazol at 1.0 kg a.i ha^{-1}, triapenthenol at 1.0 kg ha^{-1} and chlormequat chloride at 3.0 kg a.i ha^{-1}) were applied using the same site as for the Year 1 trial (i.e. a second year crop), but avoiding plots previously sprayed with paclobutrazol to eliminate possible soil residual effects. Applications were either during early reproductive initiation (13 September 1989), during peak reproductive initiation (10 October 1989) or when reproductive buds/early flowers were first visible (8 November 1989). Based on a soil test result for the Year 1 trial, K was limiting for the growth of a white clover seed crop (Clifford and Rolston, 1990). Therefore, KCl was applied at the rate of 25 kg K ha^{-1} to remedy this deficiency. Grazing was stopped on 26 August 1989. To control weeds, herbicides were applied on three occasions, i.e. 2,4–DB (1.6 kg a.i ha^{-1}) on 4 September 1989, MCPB (1.6 kg a.i ha^{-1}) and Fusilade (0.25 kg fluazifop–P–butyl ha^{-1}) on 4 October 1989, and 2,4–DB (2.8 kg a.i ha^{-1}) on 20 October 1989.

In both trials, all plant growth regulators were applied with water at a volume equivalent to 500 l ha^{-1} using a knapsack sprayer with four fan nozzles held 25–30 cm above the herbage. Reproductive development was determined by dissecting 10 growing points (stolon apices) under a microscope at 40X magnification every week starting in early September for both trials. Reproductive bud initiation is indicated when bud formation takes place in the axil of the youngest leaf primordium, which forms a so–called 'double ridge structure', as opposed to the axil of the third youngest leaf primordium in the vegetative stage (Thomas, 1987).

Growth analysis in the Year 1 trial was conducted only for paclobutrazol 1.0 kg a.i ha^{-1}, triapenthenol 1.0 kg a.i ha^{-1}, chlormequat chloride 3.0 kg a.i ha^{-1} and daminozide 2.0 and 4.0 kg a.i ha^{-1} treatments, while growth analysis in the Year 2 trial was carried out for all treatments. In the Year 1 trial, samples were taken from one quadrat of 0.125 m^2 per plot at peak flowering (15 December 1988), whereas in the Year 2 trial, samples were taken from one quadrat of 0.15 m^2 per plot on 7 December 1989. All plant material within the quadrat was cut at ground level using electric shears. This material was used to determine the effect of treatments on dry matter composition, i.e. vegetative (stolons and leaves), reproductive (inflorescences and inflorescence buds) and dead material, the number of reproductive nodes (nodes producing inflorescences), the number of vegetative nodes (nodes producing leaves and axillary buds) and the number of growing points (only for the Year 2 trial). Peduncle length was measured from 10 (the Year 1 trial) and 20 (the Year 2 trial) randomly selected inflorescences in full bloom (white) per plot. Petiole length and leaf score were also measured from 10 (the Year 1 trial) and 20 (the Year 2 trial) leaves/plot selected at random. Leaf score measurement was conducted using a clover leaf area estimation chart (Williams, Evans and Ludwig, 1964).

In the Year 2 trial, observations on main stolon growth and development were made by tagging ten main stolons per plot using coloured wire at a position one node behind the first unfolded leaf from the apex on 16 September 1989 and collecting them on 3 January 1990 (110 days later). The sites where the stolons grew were identified with bamboo canes for ease of stolon relocation. Subsequently, the number of vegetative and reproductive nodes, axillary buds and stolon lengths were recorded.

Data analysis of variance was conducted using a randomized complete block design. Treatment mean comparisons were performed using Fisher's LSD tests at a 5 % level of probability.

RESULTS

Morphological characteristics

At peak flowering, plant growth regulators had no significant effect on leaf size in either trial (presented as leaf score in Table 1). Although not always statistically significant, paclobutrazol consistently reduced petiole length in both trials (Table 1). However, it did not reduce peduncle length. The application of daminozide 2.0 kg a.i ha^{-1} in November significantly reduced both petiole and peduncle lengths. Other treatments in both trials did not affect either petiole or peduncle length (Table 1).

Dry matter distribution

Triapenthenol 1.0 kg a.i ha^{-1} applied in October significantly increased total dry matter accumulation at peak flowering in the Year 1 trial, while other treatments did not differ from the control (Table 2). Only chlormequat chloride 3.0 kg a.i ha^{-1} applied in October significantly increased total dry matter in the Year 2 trial. However, there were differences in dry matter distribution in the paclobutrazol treatments in the Year 1 trial and in the triapenthenol treatments in the Year 2 trial. In the Year 1 trial, paclobutrazol 1.0 kg a.i ha^{-1} applied in October significantly increased reproductive dry matter and reduced dead dry matter, whereas paclobutrazol 1.0 kg a.i ha^{-1} applied in November increased vegetative dry matter despite there being no changes in reproductive and dead dry matter (Table 2). In the Year 2 trial, triapenthenol 1.0 kg a.i ha^{-1} applied in either October or November also significantly increased reproductive dry matter compared to the control, despite there being no changes in vegetative and dead dry matter (Table 2).

Table 1 Effect of plant growth regulators on white clover leaf score and petiole and peduncle lengths at peak flowering.

Treatments		1988/1989			1989/1990		
		Leaf Score	Length (mm) PTL	PDL	Leaf Score	Length (mm) PTL	PDL[1]
Control		19.9	202.5	244.2	20.8	209.4	251.8
Paclobutrazol	1.0 kg Sept	–	–	–	19.7	167.6	221.3
Paclobutrazol	1.0 kg Oct	20.0	153.1	204.5	20.1	170.3	232.7
Paclobutrazol	1.0 kg Nov	19.7	165.4	224.1	20.0	177.3	222.3
Triapenthenol	1.0 kg Sept	–	–	–	20.8	194.9	244.6
Triapenthenol	1.0 kg Oct	20.2	215.7	262.7	21.0	208.1	256.8
Triapenthenol	1.0 kg Nov	20.6	178.2	255.0	20.9	207.8	273.5
C. chloride	3.0 kg Sept	–	–	–	20.7	214.3	259.3
C. chloride	3.0 kg Oct	20.2	177.7	241.3	20.6	200.6	242.2
C. chloride	3.0 kg Nov	20.8	238.9	220.4	21.1	208.1	261.4
Daminozide	2.0 kg Nov	19.0	136.1	183.9	–	–	–
Daminozide	4.0 kg Nov	20.0	207.5	263.3	–	–	–
LSD (P<0.05)		NS	39.8	45.7	NS	37.9	NS
CV (%)		7	12	11	3	11	8

1 *PTL = petiole and PDL = peduncle.*

Table 2 Effect of plant growth regulators on white clover plant dry matter at peak flowering.

Treatments		Plant dry matter ($g\ m^{-2}$) 1988/1989 VDM	RDM	DDM	TDM	1989/1990 VDM	RDM	DDM	TDM[1]
Control		197	37	51	285	405	58	113	576
Paclobutrazol	1.0 kg Sept	–	–	–	–	381	68	102	551
Paclobutrazol	1.0 kg Oct	160	73	29	262	382	66	95	543
Paclobutrazol	1.0 kg Nov	276	54	39	369	429	52	103	584
Triapenthenol	1.0 kg Sept	–	–	–	–	411	61	135	607
Triapenthenol	1.0 kg Oct	255	60	110	425	425	79	132	636
Triapenthenol	1.0 kg Nov	189	51	64	304	436	99	103	638
C. chloride	3.0 kg Sept	–	–	–	–	413	74	103	590
C. chloride	3.0 kg Oct	133	54	48	235	494	64	116	674
C. chloride	3.0 kg Nov	208	39	58	305	453	67	108	628
Daminozide	2.0 kg Nov	167	44	45	256	–	–	–	–
Daminozide	4.0 kg Nov	249	54	61	364	–	–	–	–
LSD (P<0.05)		77	25	21	101	NS	20	NS	90
CV (%)		22	28	21	191	71	17	20	9

1 *VDM = vegetative dry matter, RDM = reproductive dry matter, DDM = dead dry matter and TDM= total dry matter.*

Node and stolon production

In both trials, paclobutrazol treated plants produced more vegetative nodes than control plants at peak flowering (Table paclobutrazol 1.0 kg a.i ha^{-1} applied in November. In the other treatments, there were no significant differences in vegetative node production compared to the control. There was a tendency for all treated plants to show a higher total number of reproductive nodes per unit area than the control plants, but only triapenthenol 1.0 kg a.i ha^{-1} applied in November in the Year 2 trial differed significantly. No treatment increased the number of growing points (Table 3).

Table 3 Effect of plant growth regulators on white clover vegetative and reproductive nodes, and growing points at peak flowering.

Treatments		The number of nodes m^{-2}				Growing Points m^{-2}
		Vegetative		Reproductive		
		1988/1989	1989/1990	1988/1989	1989/1990	1989/1990
Control		3679	3280	256	742	1255
Paclobutrazol	1.0 kg Sept	–	3627	–	907	1511
Paclobutrazol	1.0 kg Oct	4971	3916	340	789	1484
Paclobutrazol	1.0 kg Nov	6240	4362	354	811	1469
Triapenthenol	1.0 kg Sept	–	3829	–	931	1616
Triapenthenol	1.0 kg Oct	3792	2789	356	893	1275
Triapenthenol	1.0 kg Nov	3784	3445	342	1113	1398
C. chloride	3.0 kg Sept	–	3033	–	796	1351
C. chloride	3.0 kg Oct	3595	3442	343	769	1447
C. chloride	3.0 kg Nov	2736	3080	267	800	1249
Daminozide	2.0 kg Nov	3939	–	363	–	–
Daminozide	4.0 kg Nov	4055	–	330	–	–
LSD (P<0.05)		1513	1017	NS	234	NS
CV (%)		21	17	27	16	18

Growth and development of main stolons

Plant growth regulators did not affect stolon length when measured at harvest (Table 4). The development of vegetative nodes along stolons was significantly reduced by paclobutrazol and triapenthenol applied in September, while other treatments had no effect (Table 4). However, the number of reproductive nodes along stolons was increased significantly by paclobutrazol and triapenthenol applied in November, while other treatments did not differ from the control (Table 4). The number of axillary buds along stolons was increased by the application of paclobutrazol in November compared to the control, but other treatments were not significantly different (Table 4).

Table 4 Effect of plant growth regulators on the growth and development of a second year white clover crop's main stolons.

		Vegetative nodes (no.$stolon^{-1}$)	Reproductive nodes (no.$stolon^{-1}$)	Axillary buds (no.$stolon^{-1}$)	Stolon length (mm)
Control		9.9	1.1	1.1	110.7
Paclobutrazol	1.0 kg Sept	8.5	1.2	1.1	74.3
Paclobutrazol	1.0 kg Oct	9.7	1.3	1.5	98.9
Paclobutrazol	1.0 kg Nov	10.1	1.6	2.2	116.7
Triapenthenol	1.0 kg Sept	8.4	1.3	1.4	90.6
Triapenthenol	1.0 kg Oct	9.4	1.4	1.6	117.9
Triapenthenol	1.0 kg Nov	10.1	1.7	2.0	143.5
C. chloride	3.0 kg Sept	9.7	1.5	1.9	131.7
C. chloride	3.0 kg Oct	9.5	1.3	1.2	132.8
C. chloride	3.0 kg Nov	9.3	1.5	1.6	119.3
LSD (P<0.05)		1.2	0.4	0.9	39.8
CV (%)		8	19	35	20

DISCUSSION

White clover plant canopy height can be reduced following plant growth regulator application. Provided peduncle length is not decreased, inflorescences would then be elevated above the leaf canopy, resulting in a more favourable micro–environment for pollination as suggested by Marshall and Hides (1986) and Rijckaert (1991). However, three of the four plant growth regulators used in these trials did not achieve this objective. Chlormequat chloride did not retard growth, a result also reported by Marshall and Hides (1986). The development of stolons and also nodes along stolons in white clover was not affected by chlormequat chloride and thus, in terms of the contribution of vegetative growth and development to high seed yield, chlormequat chloride would not be expected to improve white clover seed yield.

Daminozide is generally effective in controlling shoot elongation of most dicotyledonous crops (Davis and Andersen, 1989), and it significantly retarded white clover growth. Petiole length was effectively reduced, but peduncle length was also decreased and thus no elevation of inflorescences above the plant canopy occurred. Daminozide also did not increase the number of nodes. Therefore, it is also unlikely that daminozide would have any direct advantages for improving seed yield.

A retardation effect at peak flowering was not recorded following triapenthenol application. However, under Belgian conditions, Rijckaert (1991) reported that triapenthenol reduced the height of the leaf canopy at flowering. The present trials used rates of 0.5 and 1.0 kg triapenthenol ha^{-1}. These are generally lower than the rates used by Rijckaert (1991) which ranged from 0.7 to 2.8 kg a.i ha^{-1}. It is possible that the rates used in the present trials may have been too low to maintain triapenthenol's retardation effect through to peak flowering. In oilseed rape, triapenthenol is reported to increase branching (Child *et al.* 1987). However, in white clover, it did not increase the development of nodes and stolons. Reasons for this might be either that triapenthenol is species specific or that the rates used in the present trials were too low to show its effect. Nevertheless, from the Year 2 trial, application at the reproductive buds visible stage significantly increased reproductive nodes along main stolons. This effect would clearly be an advantage for improving potential seed yield, as indicated by the fact that although not always statistically significant, the number of reproductive nodes produced at peak flowering following triapenthenol application was higher than the control. However, since the observations were conducted for only one trial, it is not known whether this effect is consistent and further investigation is required.

Paclobutrazol was the only plant growth regulator used in the present trials which had a direct effect on altering plant growth and development, and therefore could potentially improve white clover seed production. Paclobutrazol reduced petiole length but not peduncle length. This finding agrees with those reported by Marshall and Hides (1986, 1991a), Hampton (1991) and Rijckaert (1991). This morphological change should produce a better micro–environment for pollination, i.e. inflorescences are more accessible to bees, and also reduce shading during seed set and seed maturation. The latter is important for seed production because post fertilization abortion of fertile ovules is increased when shading occurs during seed set and seed maturation due to insufficient production of photoassimilate (Pasumarty and Thomas, 1990).

Paclobutrazol increased the number of nodes produced per unit area. This finding also agrees with the report by Marshall and Hides (1991a). As inflorescence production is determined by the number of potential sites, i.e. nodes (Thomas, 1987), any increase in node number should increase inflorescence production. Since paclobutrazol did not increase nodes along stolons, the increase of nodes per unit area was most likely caused by increased stolon production, as indicated by more growing points m^{-2} and increased axillary buds along stolons. Marshall and Hides (1991a) also found that paclobutrazol increased the number of stolons as indicated by an increase in the number of growing points produced per unit area, while the number of nodes along stolons was either reduced or unaffected. The increase in branching is presumably the result of the removal of apical dominance. Paclobutrazol has been shown to reduce apical dominance, and hence increase branching or tillering, for example in *Lotus uliginosus* Schk. (Tabora and Hill, 1992) and *Lolium perenne* L. (Hampton and Hebblethwaite, 1985).

Results from the Year 2 trial showed that paclobutrazol applied at the visible reproductive bud stage increased reproduction nodes along main stolons. A similar result from a sward trial was also reported by Marshall and Hides (1991a). This effect is certainly an advantage for improving seed yield. However, a detailed study using individual plants (Budhianto 1992) showed that paclobutrazol did not directly induce the initiation of inflorescences, and this was in agreement with results from glasshouse experiments (Marshall and Hides, 1987). The differences might have arisen from the methods used. Firstly, under sward conditions, stolons were indiscriminately selected for observation, while in the individual plant trial, stolon categories were carefully determined. Budhianto (1992) found that not all stolons (primary, secondary, tertiary, quaternary or quinary) could produce inflorescences, although the size of stolons from different categories could be similar. Secondly, in the individual plant trial, node initiation was closely monitored before and after paclobutrazol application, while in the sward trial, observations were made by counting the number of stolon nodes which emerged after the first tagging in the early stage of the trial, without noting which nodes were initiated under the influence of paclobutrazol. A further detailed investigation under sward conditions is therefore required.

Despite the fact that paclobutrazol reduced plant growth, there was no significant reduction of total dry matter when measured at peak flowering. Paclobutrazol is a plant growth retardant which reduces plant growth by inhibiting gibberellin biosynthesis (Hedden, 1990). Its important physiological effect is in reducing cell elongation. Therefore, paclobutrazol retards growth only, and the developmental sequence of the plant continues, although new plant organs which develop are smaller in size (Kaufmann, 1990). As paclobutrazol increased branch production, it is possible that the loss of dry matter due to reduced plant growth, in particular petiole length, was compensated for by increased dry matter from increased branch numbers. Another possibility is that although its length was reduced, the petiole might have become thicker. However, no data were collected. In spite of these unknowns, the results suggest that paclobutrazol did not have a detrimental effect on the assimilate production needed to support growth and development.

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